TIME RELATIONS AND THEIR SIGNIFICANCE IN THE OVULATION AND PENETRATION OF EGGS IN RATS AND RABBITS

By C. R. AUSTIN* and A. W. H. BRADEN*

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Summary

Observations have been made on a total of 577 mated adult rats and 52 mated rabbits.

The mean number of eggs recovered from 362 rats killed between 10.00 a.m. and 5.00 p.m. on the day of ovulation was 9.6 per rat (S.D. 2.2, range 2-20 eggs), or 4.8 per fallopian tube (S.D. 1.9, range 0-13 eggs).

In rats mated under normal colony conditions, ovulation occurred between 12.00 midnight and 4.00 a.m. The ovulation of six eggs in any one rat took about 1 hr. Penetration by sperms occurred 2-4 hr after ovulation. The penetration of all eggs in any one rat took a mean time of 3½ hr. Pronuclei were formed about 3 hr after sperm penetration.

In rats kept under conditions of controlled illumination (light from 3.00 p.m. to 5.00 a.m.; dark from 5.00 a.m. to 3.00 p.m.), ovulation occurred between 11.00 a.m. and 3.00 p.m. The duration of the ovulation period (4 hr) and the time between the beginning of darkness and the commencement of ovulation (about 6 hr) were the same as in the rats mated under normal colony conditions. Similar also were the mean time for ovulation of six eggs in any one rat (¾ hr), the interval between ovulation and sperm penetration (2½-4 hr), the time required for penetration of all eggs in any one rat (3 hr), and the time between the penetration of the eggs and the formation of pronuclei (4 hr). Penetration of all the eggs in any one fallopian tube took about 2 hr. In the course of penetrating the eggs, sperms spent a mean time of ½ hr in the perivitelline space.

Groups of rats were mated at 3.00 a.m. (when ovulation was still in progress), at 7.00 a.m., or at 11.00 a.m. In each instance sperm penetration did not begin until at least 2 hr later. Such a period for the "capacitation" of the sperms is therefore probably normal and does not reflect merely a post-ovulatory alteration in the functional state of the tubes.

In rabbits, sperm penetration began about 10 hr after mating, and all eggs were penetrated by the thirteenth hour. With coitus at the time of induced ovulation, penetrated eggs were not found until 5 hr later, although sperms were present at 4 hr. It appears therefore that capacitation of sperms in the rabbit takes about 5 hr.

The delay of 2-4 hr between ovulation and sperm penetration in normally mated rats suggests that a change, possibly a final stage of maturation, must take place in the egg membranes of the adult rat before sperms can enter the eggs. As no delay was observed in the rabbit, it seems that maturation of the rabbit egg is complete at ovulation.

* Division of Animal Health and Production, C.S.I.R.O., McMaster Animal Health Laboratory, Sydney.

I. Introduction

Evidence has been produced which indicates that, in the rat and rabbit, the sperm must spend some time in the female tract, presumably to undergo a process of preparation or "capacitation," before it is capable of penetrating the zona pellucida of the egg (Austin 1951; Chang 1951). This evidence was obtained in experiments that involved manipulation of the sperms and operative interference with the animals—procedures that could be held responsible, in part at least, for some of the effects observed. Confirmatory data were reported by Austin (1952), who found that if rats were permitted to mate after ovulation the eggs were not penetrated until 2 hr or more later, although sperms could be demonstrated at the site of fertilization 1 hr after mating. This delay, together with the rate at which penetration of the eggs then proceeded, seemed to point unequivocally to a need for capacitation of the sperms. The avoidance of operative interference in the experiment lent force to the conclusions drawn, but the objection could be raised that the mating of the rats had taken place several hours after ovulation, whereas under normal circumstances mating occurs about 10 hr before ovulation (Boling *et al.* 1941). It was therefore possible that the delay observed by Austin (1952) may only have been a feature of the abnormal time relations and have indicated a deterioration of the conditions within the fallopian tube that are required for normal fertilization.

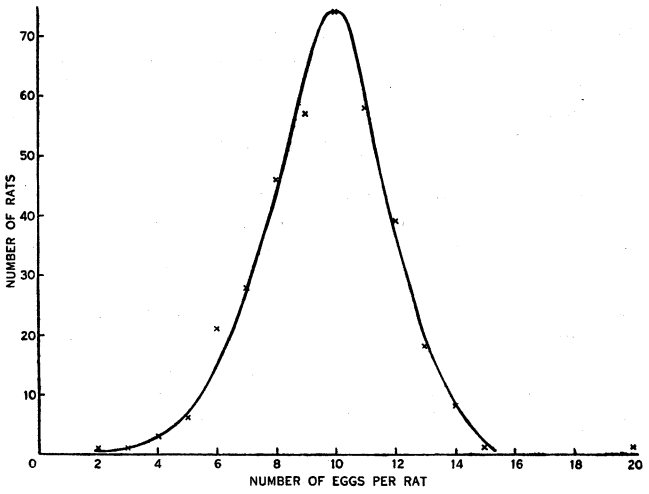

Fig. 1.—Frequency distribution of eggs in 362 rats.

In the present paper these problems receive further attention, and supporting evidence is brought forward to show that a period for capacitation is a normal preliminary to sperm penetration. During the course of investigations, data were obtained on several different aspects of the time relations of ovulation and sperm entry. As this appeared to be a promising field of enquiry, the range of the observations was extended and the material thus gained forms the basis for the greater part of this paper.

II. Methods

The rats used were of both albino and "hooded" strains, and were all adult animals, 3 months of age or more. The rabbits were crossbreds, aged between 6 and 15 months.

The time of ovulation was based upon the finding of eggs in the fallopian tubes. Eggs were recovered by dissecting the tubes under normal saline solution and were examined in the fresh state with a phase-contrast microscope, as described by Austin and Smiles (1948).

Ovulation was induced in the rabbit by the intravenous injection of 40 I.U. of chorionic gonadotrophin ("Gonan," B.D.H.).

Some of the rats were kept in a specially constructed cabinet in which the light was controlled. The only light was provided by 25-watt tungsten-filament lamps, which were switched on at 3.00 p.m. and off at 5.00 a.m. each day by means of an electric clock relay. The rats were kept under these conditions during the period of observations and for 3-4 wk beforehand. The practicability of controlling oestrous phenomena in the rat by artificially changing the times of light and dark was first clearly demonstrated by Hemmingsen and Krarup (1937).

III. Observations

(*a*) *Number of Eggs Recovered from Adult Rats*

To determine the number of eggs produced at spontaneous ovulation in mated adult rats, data were collected from a total of 362 animals that had been kept under normal colony conditions and killed at different times between 10.00 a.m. and 5.00 p.m. on the day of ovulation.

The frequency distribution of the numbers of eggs is illustrated in Figure 1. The total number of eggs counted was 3477, and the mean was found to be 9.6 eggs per rat, with a standard deviation of 2.2 and a range of 2-20 eggs. In 94 per cent. of the rats, the number of eggs per rat ranged from six to 13. The frequency distribution of the number of eggs per fallopian tube was similar in shape. The mean number of eggs was 4.8, standard deviation 1.9, and range 0-13 eggs.

(*b*) *Time Relations of Ovulation and Sperm Penetration in Rats*

(i) *Under Normal Colony Conditions.*—To determine the times of ovulation and sperm penetration in animals kept under normal colony conditions, female rats were placed with males during the afternoon and those found to have copulation plugs when examined at 11.00 p.m. were put aside. Groups of these animals were killed at hourly intervals from midnight to 5.00 a.m. and at 7.30 a.m. The fallopian tubes were dissected to determine whether eggs were present, and the eggs were examined for evidence of sperm penetration. Observations were based on a total of 115 mated rats (Table 1).

At midnight, eggs were found in only two out of 15 rats and these provided a total of three eggs, none of which contained sperms. At 1.00 a.m., a higher proportion of the rats (12 out of 30) yielded eggs, one of which showed sperm

TABLE 1

TOTAL NUMBERS OF EGGS AND NUMBER OF PENETRATED EGGS IN RATS MATED UNDER NORMAL COLONY CONDITIONS AND KILLED ABOUT 5½-13 HR AFTER SUNSET

Time of Killing	No. of Rats Killed		No. of Eggs Recovered		Mean No. of Eggs per Rat Killed		Eggs Penetrated (%)
	Total	With Eggs	Total	Penetrated	Total	Penetrated	
Midnight	15	2	3	0	0·2	0	0
1.00 a.m.	30	12	57	1	1·9	0·03	1·6
2.00 a.m.	20	13	89	5	4·5	0·25	5·6
3.00 a.m.	15	12	95	13	6·3	0·9	13·8
4.00 a.m.	11	11	104	27	9·5	2·5	26·3
5.00 a.m.	10	10	84	34	8·4	3·4	40·5
7.30 a.m.	14	14	138	124	9·9	7·9	89·8

penetration. From then on, progressively more rats were found to have eggs and an increasingly larger proportion of the eggs had been penetrated by sperms. Ovulation was completed in the rats as a group by about 4.00 a.m. On the other hand, the incidence of penetrated eggs continued to rise for the rest of the period of observations, although most of the eggs were penetrated by 7.30 a.m.

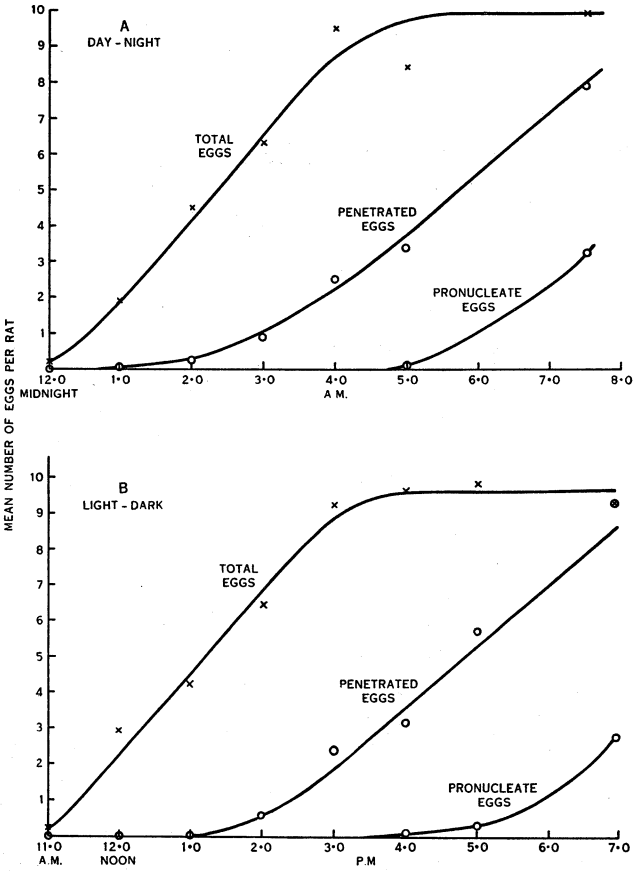

Fig. 2.—Progressive changes in the mean numbers per rat of total eggs, penetrated eggs, and eggs with pronuclei. *A*, rats kept under natural illumination; sunrise was between 4.30 and 5.00 a.m. and sunset between 6.00 and 7.00 p.m. *B*, rats kept under controlled artificial illumination; light was turned on at 3.00 p.m. and off at 5.00 a.m.

The number of eggs recovered at each hour (mean for all rats killed) was 0.2 at midnight, and increased progressively to a mean of 9.5 per rat at 4.00 a.m. (Table 1). Thereafter there was no significant change. The trend of the results is illustrated by the curve for "total eggs" in Figure 2*A*. It is apparent that, for the rats as a group, ovulation took about 4 hr, and was confined almost entirely to the period between midnight and 4.00 a.m. This work was done

during November and December when sunrise was between 4.30 and 5.00 a.m. and sunset between 6.00 and 7.00 p.m.; the days thus varied from 13 to 14½ hr in length.

An approximate assessment of the time required for ovulation to occur in any one rat can also be made. From the data on the number of eggs recovered (Section III (*a*)), it can be shown that 97 per cent. of rats produced six or more eggs. When the curve representing the proportion of rats with eggs was compared with that for the proportion of rats with six or more eggs, an interval corresponding to about 1 hr was observed between them. This is interpreted to signify that in any one rat a mean time of about 1 hr was required for six eggs to gather in the fallopian tubes. Similarly, the mean time required for three eggs to arrive in any one fallopian tube was found to be about ½ hr.

A measure of the rate of penetration of the eggs by sperms is obtained by determining the mean number of penetrated eggs per rat killed, at each interval; this was zero at midnight and 0.03 eggs per rat at 1.00 a.m., and increased to 7.9 eggs per rat at 7.30 a.m. (Table 1). These results form the data for the curve marked "penetrated eggs" in Figure 2*A*. It appears that the eggs are penetrated at a rate that is a little slower than the rate at which they are ovulated. There is an interval corresponding to 2-4 hr between the curves for the mean number of eggs per rat and the mean number of penetrated eggs per rat. Evidently penetration of the eggs in the rats as a group occurs about 2-4 hr after ovulation, the interval being slightly longer for the later than for the earlier times of penetration.

The number of penetrated eggs may also be expressed as a percentage of the total eggs obtained, viz. 1.6 per cent. at 1.00 a.m., increasing to 89.8 per cent. at 7.30 a.m. (Table 1). These results are shown as curve *N* in Figure 3. It can be seen that the increase in the proportion of penetrated eggs is very slow initially, but becomes rapid after 4.00 a.m.

As records were kept for individual rats, it was possible to calculate the proportion of rats with one or more penetrated eggs as well as the proportion of rats in which all eggs were found to contain sperms. Thus, at 1.00 a.m., 2.00 a.m., 3.00 a.m., and 4.00 a.m., the number of rats found with penetrated eggs was one (3.3 per cent.), four (20 per cent.), four (27 per cent.), and seven (64 per cent.) respectively. In none of these rats, however, were all eggs penetrated. Among the animals killed at 5.00 a.m., nine (90 per cent.) were found with penetrated eggs and in one rat (10 per cent.) all the eggs contained sperms. At 7.30 a.m. all of the 14 rats killed carried penetrated eggs (100 per cent.) and in nine rats (64 per cent.) all the eggs were penetrated. These results are shown as curves N_1 and N_2 in Figure 4. The curves are separated by an interval which corresponds to about 3½ hr. This is interpreted as indicating that penetration of all the eggs in any one rat after normal mating takes about 3½ hr from the penetration of the first egg.

Pronuclei were observed in one egg (0.1 eggs per rat) from the rats killed at 5.00 a.m., and in 45 eggs (3.2 per rat) from the rats killed at 7.30 a.m. These results are represented by the curve marked "pronucleate eggs" in Figure

2A. It would appear that the pronuclei were formed at a mean interval of about 3 hr after penetration of the eggs.

(ii) *After Delayed Mating.*—The rate of penetration of eggs after delayed mating was studied in a total of 259 mated rats. Rats were selected for oestrus by the nature of the vaginal smear and placed with males for 10-15 min from 3.00 to 3.30 a.m., from 7.00 to 7.30 a.m., or from 11.00 to 11.30 a.m. Coitus was nearly always seen to occur immediately the animals were put together. For reference purposes the times of mating are stated as 3.00 a.m., 7.00 a.m., and 11.00 a.m. respectively.

Forty-five rats that mated at 3.00 a.m. were killed 2 (15 rats), 3 (10 rats), 5 (10 rats), and 7 hr (10 rats) after mating. The numbers of penetrated eggs recovered were none, 25 (28 per cent.), 66 (73 per cent.), and 88 (100 per cent.) respectively. These results are the data from which curve 1 in Figure 3 is drawn.

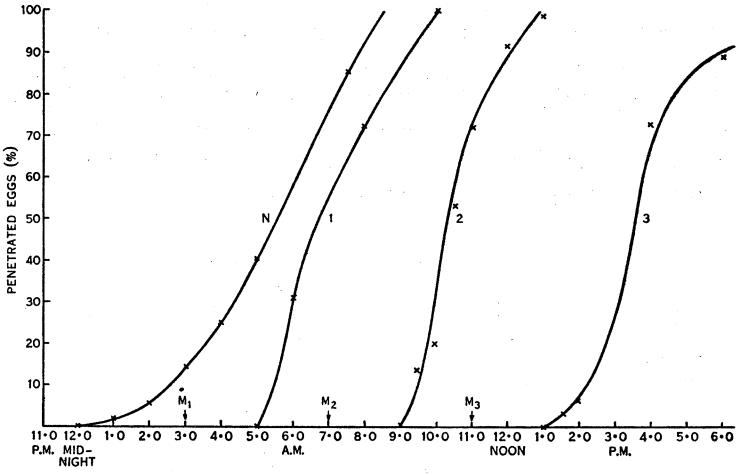

Fig. 3.—Percentages of penetrated eggs in rats at various hours after coitus. *N*, normal mating. 1, 2, and 3, mating at 3.00 a.m., 7.00 a.m., and 11.00 a.m. respectively (M_1, M_2, and M_3).

The numbers of penetrated eggs obtained from 65 rats mated at 7.00 a.m. have been reported in an earlier paper (Austin 1952), but since then some further observations have been added. Twelve more rats have been killed at 2½ hr, 66 at 3 hr, and 31 at 3½ hr after coitus, making a total of 22 rats for 2½ hr, 76 for 3 hr, and 31 for 3½ hr. The total number of eggs recovered is now 221, 752, and 303, and the number of penetrated eggs 14, 154, and 162 respectively. All this material forms the basis for curve 2 in Figure 3.

The 50 rats that were not permitted coitus until 11.00 a.m. were killed 2, 2½, 3, 5, or 7 hr after mating. The numbers of penetrated eggs were none, five (4 per cent.), seven (7 per cent.), 64 (67 per cent.), and 81 (89 per cent.) respectively; these are represented by curve 3 in Figure 3.

Curves 1, 2, and 3 show clearly that penetration of the eggs commences only after about 2 hr after mating. The delay is of the same duration in each

instance although the hour of mating varied from 3.00 to 11.00 a.m. (as indicated by the letters M_1, M_2, and M_3). When penetration of the eggs does begin, it proceeds initially much more rapidly than that observed after normal mating (curve N).

Observations on the rats mated at 7.00 a.m. included both the total number of eggs and the number of penetrated eggs in individual rats, and from these data the proportion of rats with one or more penetrated eggs and of rats with all eggs penetrated has been calculated. At 1 hr after mating, no rat carried penetrated eggs; at 2 hr and 2½ hr one rat (7 per cent.) and 10 rats (46 per cent.) respectively carried penetrated eggs, but in no rat were all eggs penetrated. Among the rats killed at 3, 3½, 4, 5, and 6 hr after coitus, 39 (51 per cent.), 26 (84 per cent.), nine (90 per cent.), 10 (100 per cent.), and 10 (100 per cent.) respectively had one or more eggs penetrated by sperms. At the same hours, the numbers of rats in which all eggs were penetrated were one (1.5 per cent.), seven (23 per cent.), four (40 per cent.), six (60 per cent.), and eight (80 per cent.) respectively. Curves D_1 and D_2 in Figure 4 are based upon this information. The curves are separated by an interval corresponding to 1-2 hr, and it is concluded that, once the penetration of the eggs had begun, the mean time required for penetration of all the eggs in any one rat was about 1-2 hr. In the same way, it can be shown that the mean time required for penetration of all the eggs in any one fallopian tube was about 1 hr.

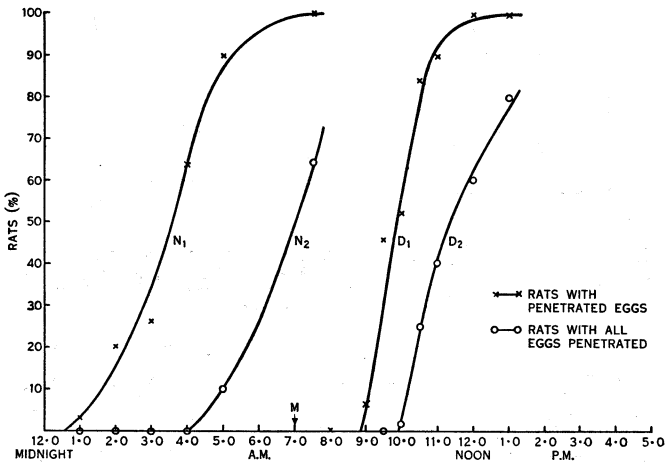

Fig. 4.—Percentage of rats with penetrated eggs (N_1 and D_1) compared with the percentage of rats with all eggs penetrated (N_2 and D_2) at various hours after normal matings (N_1 and N_2) and after mating at 7.00 a.m. (D_1 and D_2).

(iii) *Under Conditions of Controlled Illumination.*—Adult female rats kept with males under conditions of controlled illumination in the special cabinet were examined each morning for signs of mating, and rats with copulation plugs were killed at each hour from 11.00 a.m. to 5.00 p.m. and at 7.00 p.m. The

eggs were recovered and studied in the manner already described. Results were obtained from a total of 100 animals (Table 2).

Of the rats killed at 11.00 a.m. only one had eggs (two eggs), and of those killed at noon and 1.00 p.m. five and 12 rats yielded 29 and 88 eggs respectively. None of these eggs was penetrated by sperms. At 2.00 p.m. 12 rats had a total of 97 eggs, of which seven were penetrated by sperms. Thereafter all rats yielded eggs, and the proportion of penetrated eggs increased progressively. When the results are expressed as the mean number of eggs per rat killed, the values increase steadily from 0.2 at 11.00 a.m. to 9.2 at 3.00 p.m. After this the figures show no significant change. The mean number of penetrated eggs per rat killed is zero for 11.00 a.m., 12.00 noon, and 1.00 p.m., and 0.5 for 2.00 p.m.; for the subsequent hours there is a progressive increase in the values, which reach 9.2 penetrated eggs per rat at 7.00 p.m. These results are illustrated by the curves in Figure 2*B*. For the rats as a group, ovulation apparently took about 4 hr and occurred almost entirely in the period 11.00 a.m. to 3.00 p.m. The time taken is very similar to that observed in the rats mated under normal colony conditions. An estimate of the mean time required for six eggs to gather in the fallopian tubes of any one rat is about ¾ hr, and for three eggs in any one fallopian tube ½ hr. These results are also similar to those obtained from the rats mated under normal colony conditions (Section III (*b*) (i)).

The interval between the curves for total eggs and penetrated eggs in Figure 2*B* corresponds to a period of about 2½-4 hr. This estimate for the time between ovulation and sperm penetration in the rats as a group is virtually the same as the 2-4 hr observed for the rats mated under normal colony conditions.

The number of penetrated eggs, expressed as a percentage of the total eggs recovered, was zero for 11.00 a.m., 12.00 noon, and 1.00 p.m., and 7.2 per cent. for 2.00 p.m., and then increased to reach 100 per cent. at 7.00 p.m. (Table 2). The results show a trend similar to that shown by the corresponding data for the rats mated under normal colony conditions (see curve *N* in Fig. 3).

Comparison of the curves representing the proportion of rats with one or more penetrated eggs and the proportion with all eggs penetrated led to a similar conclusion to that drawn from the data for the rats mated under normal colony conditions: once penetration had begun, the mean time required for penetration of all the eggs in any one rat was about 3 hr. By the same method it was shown that about 2 hr was needed for all the eggs in any one fallopian tube to be penetrated by sperms.

Among the penetrated eggs there were some in which sperms had penetrated the zona pellucida but had not yet entered the vitellus and so lay in the perivitelline space. The number of these eggs and the proportion they represented of the total eggs recovered were as follows: at 11.00 a.m., 12.00 noon, and 1.00 p.m., zero; at 2.00 p.m., four (4.1 per cent.); at 3.00 p.m., eight (6.2 per cent.); at 4.00 p.m., nine (9.4 per cent.); at 5.00 p.m., 10 (10.1 per cent.); and at 7.00 p.m., one (1.0 per cent.).

As all but one of the eggs examined at 7.00 p.m. contained sperms in the vitellus, it may reasonably be assumed that eggs seen earlier with sperms only

TABLE 2

TOTAL NUMBERS OF EGGS AND NUMBER OF PENETRATED EGGS IN RATS MATED UNDER CONDITIONS OF CONTROLLED ILLUMINATION AND KILLED 6-14 HR AFTER THE ONSET OF DARKNESS

Time of Killing	No. of Rats Killed		No. of Eggs Recovered		Mean No. of Eggs per Rat Killed		Eggs Penetrated (%)
	Total	With Eggs	Total	Penetrated	Total	Penetrated	
11.00 a.m.	10	1	2	0	0·2	0	0
Noon	10	5	29	0	2·9	0	0
1.00 p.m.	20	12	88	0	4·4	0	0
2.00 p.m.	15	12	97	7	6·5	0·5	7·2
3.00 p.m.	14	14	129	37	9·2	2·6	28·7
4.00 p.m.	10	10	96	31	9·6	3·1	32·3
5.00 p.m.	10	10	99	59	9·9	5·9	59·5
7.00 p.m.	11	11	101	101	9·2	9·2	100

in the perivitelline space would all eventually have sperms in the vitellus. During the period 2.00-5.00 p.m. the rate of sperm penetration was approximately linear (Fig. 2*B*), so that the frequency with which eggs were seen with sperms in the perivitelline space only should be proportional to the time the sperms spent in the perivitelline space. For the same period, an estimate of the proportion of eggs with sperms in the perivitelline space only is given by the mean of the figures for 3.00, 4.00, and 5.00 p.m., namely 8.6 per cent. The average time (t) spent by the sperm in the perivitelline space may then be calculated by the formula

$$t = \frac{8.6 \times T}{P - p},$$

where T is the period covered by the observations (3 hr), and P and p are the percentages of eggs penetrated by sperms at 5.00 p.m. and 2.00 p.m. respectively (Table 2). The result obtained was 29.6 min, or about ½ hr.

Among the penetrated eggs from the rats killed at 4.00 p.m., one egg (0.1 per rat), and at 5.00 p.m. two eggs (0.2 per rat), had early forms of pronuclei; at 7.00 p.m. 27 eggs (2.5 per rat) had pronuclei in the early and intermediate stages of development. By comparing the curve based upon these figures with that for the mean number of penetrated eggs per rat (Fig. 2*B*), it is seen that a mean period of about 4 hr from penetration was required for the formation of a pronucleus. This is reasonably similar to the estimate based upon the data from rats mated under normal colony conditions (3 hr).

(*c*) *Time Relations of Sperm Penetration in Rabbits*

Observations were made on 14 rabbits mated in the normal manner and killed 10-16 hr later. The eggs were recovered, fixed, embedded in paraffin, and cut in sections 7 μ thick. After staining by the Feulgen technique the preparations were examined to determine whether sperm penetration had occurred. Four rabbits were killed 10 hr after mating and yielded a total of 19 eggs; nine eggs were successfully brought through the histological procedures and five (56 per cent.) of these were found to contain sperms. From four rabbits killed 13 hr after mating, 30 eggs were recovered, and 19 were examined. All these were found to contain sperms. Similarly, all of the 28 eggs examined out of a total of 48 eggs obtained from six rabbits killed 16 hr after coitus showed evidence of sperm penetration (Table 3).

In a second experimental series, 38 rabbits received an intravenous injection of chorionic gonadotrophin for the induction of ovulation and were permitted coitus 10 hr later, i.e. at about the time of ovulation. The eggs from all these rabbits except the last group were examined histologically in the manner just described (Table 3). At 4 hr after mating, none of the 19 eggs examined had been penetrated by sperms. The rabbits killed 5 hr after mating yielded 39 eggs; four of the 26 eggs examined contained sperms. The corresponding figures for the rabbits killed 6 and 8-10 hr after mating were respectively: 27 and 17 eggs examined, and nine eggs (33 per cent.) and 12 eggs (71 per cent.) found to contain sperms. The last group, of 17 rabbits, was killed 15-20 hr after

mating. The eggs were all examined in the fresh state with the phase-contrast microscope; 82 eggs (73 per cent.) showed evidence of sperm penetration. The percentages for the rabbits killed at 8-10 hr and at 15-20 hr did not differ significantly, and it would seem that, when mating occurs at about the time of ovulation, not more than about three-quarters of the eggs can be expected to undergo fertilization.

IV. Discussion

The time of ovulation observed in this laboratory for a group of 115 rats kept and mated under normal colony conditions was between 12.00 midnight and 4.00 a.m. Similar results were obtained by Everett (1948), who killed 28 rats between 12.45 and 4.00 a.m., and noted that ovulation was in progress as early as 1.00 a.m. and was completed (eight or more tubal eggs) in all rats

Table 3

PROPORTION OF EGGS SHOWING SPERM PENETRATION IN RABBITS KILLED AT VARIOUS TIMES AFTER NORMAL MATING AND AFTER MATING AT THE TIME OF INDUCED OVULATION

Mating	No. of Rabbits	Time after Mating (hr)	Total Eggs Recovered	Eggs Examined	Penetrated Eggs	
					No.	Per Cent.
Normal	4	10	19	9	5	56
	4	13	30	19	19	100
	6	16	48	28	28	100
At time of ovulation	6	4	50	19	0	0
	6	5	39	26	4	15
	5	6	36	27	9	33
	4	8-10	44	17	12	71
	17	15-20	112	112	82	73

examined after 2.30 a.m. The narrower range observed by this author may have been owing to the smaller number of experimental animals or to his use of a genetically more homogeneous group of rats. Consistent also is the statement by Pederson (1951) that ovulation in his rats was most likely to occur at 3.00 a.m.

The occurrence of ovulation close to midnight has also been noted in other rodents: about 1.00 a.m. for the hamster (Ward 1946), and between 11.30 p.m. and 1.30 a.m. for the mouse (Runner 1947).

In the present series of experiments the rate of ovulation, and the relationship between ovulation and the times of light and dark, have been found to be very similar whether the animals were kept under natural lighting or in a cabinet under controlled artificial illumination. Ovulation began in the rats as a group about 6 hr after the commencement of darkness and required the last 4 hr of darkness to reach completion. The experiments involving natural light-

ing were made during November and December when the night lasted about 10 hr; the time relationship of ovulation must necessarily vary at other seasons of the year when the nights are either longer or shorter than 10 hr. A close adherence of ovulation to the time relations of artificial "day" (4.00 a.m. to 7.15 p.m.) and "night" was also reported by Snell *et al.* (1940) for mice kept under controlled artificial illumination. They noted that ovulation in mated mice most commonly occurred between "midnight" (i.e. 4¾ hr after the beginning of darkness) and 2.00 or 3.00 "a.m.," but was sometimes as late as 4.40 "a.m." (i.e. 40 min after the end of darkness).

As already mentioned, the occurrence of ovulation is inferred from the finding of eggs in the fallopian tube. Whether the eggs leave the ovary with the same frequency with which they reach the tube is unknown, but is presumed to be so. The impression gained is that the eggs, singly or in pairs, pass rapidly through the first part of the ampulla, to gather in the distended region, where they form the characteristic coherent group. It is therefore considered that an indication of the time required for ovulation in any one rat can be obtained from a comparison of the curves based upon the proportion of rats with eggs, and of rats with six or more eggs, in the fallopian tubes. The figure of six was chosen because the great majority (97 per cent.) of rats produce six or more eggs. The estimates made, both for the rats kept under natural lighting and for those under controlled artificial illumination, were similar, and indicate a period of about ¾-1 hr. This is much shorter than the time required for the rats as a group to produce a mean of six or more eggs, namely 2½-3 hr (from Fig. 2); presumably the longer period for the group is due mainly to variations in the times at which ovulation begins in individual rats. Reliable estimates could not be made for the production of numbers of eggs much in excess of six, because the higher the number the lower the proportion of rats represented and the greater therefore the error in calculation. It is of course likely that, for the ovulation of more than six eggs, proportionately longer periods are involved.

The time between ovulation and sperm penetration, under both natural and artificial lighting conditions, has clearly been shown in the present investigation to be about 2-4 hr. This appears at first to conflict with the statement by Odor and Blandau (1951) that in their experiments 47 per cent. of eggs were found to have been penetrated during the first hour after ovulation, 54 per cent. during the second hour, and 90 per cent. during the third hour. However, Odor and Blandau did not determine ovulation time directly but depended upon the generalization made earlier, that ovulation is proceeding or has been completed in most rats 10 hr after they show a specified copulatory response to handling (Boling *et al.* 1941). Now Boling *et al.* also found that ovulation began in the rats as a group about 6½-7½ hr after the response, so that Odor and Blandau's (1951) "first hour after ovulation" is therefore about the fourth hour after the commencement of ovulation in the rats as a group. Thus Odor and Blandau do not seem to have interpreted their findings quite correctly, but it appears probable that, had their data been adequate, these authors would also have found an interval of about 2-4 hr between ovulation and sperm penetration.

In an earlier paper (Austin 1952) it was reported that rats mated after ovulation, at 7.00 a.m., did not yield penetrated eggs until at least 2 hr later. This was put forward as further evidence in support of the idea that the sperm requires to spend a period for "capacitation" in the female tract before it can penetrate the egg. More data have now been obtained from rats mated at 7.00 a.m. and these are consistent with the earlier results. It has also been shown that the same interval of at least 2 hr is evident whether the rats are mated at 3.00 a.m., when ovulation is still in progress, or at 11.00 a.m., which is 7-11 hr after ovulation. In each group the numbers of sperms at the site of fertilization have been counted (Braden and Austin 1954). The numbers are small at 1 hr and increase during the first 2½-4 hr after mating. On these grounds the suggestion may be made that it is the changes in sperm concentration that are responsible for the interval of 2 hr between mating and sperm penetration. The inadequacy of this theory, however, is revealed upon closer study of the data, particularly the regular nature of the rise shown by sperm numbers during the first 2 or 3 hr, and by contrast, the rapidity with which the proportion of penetrated eggs increases for most of the period of sperm penetration. Consideration of these functions leads plainly to the expectation that 2 hr after coitus the proportion of penetrated eggs should be of the order of 10-20 per cent., whereas the observed incidence is less than 1 per cent. There seems to be no doubt therefore that the interval of about 2 hr can be imputed to the need for the sperm to spend some time in the female tract before it can penetrate the egg. The fact that the interval was much the same whether mating occurred during or after ovulation is interpreted as an indication that the same period for capacitation would be required when mating occurs before ovulation, as under normal circumstances.

In the rabbit, sperm penetration evidently commences very shortly after ovulation, for about half the eggs recovered 10 hr after mating were found to be penetrated. That ovulation has in fact not yet been completed at this hour is evident from the relatively low mean of 4.8 eggs per rabbit; at later hours the means varied from 6.5 to 11.0 eggs per rabbit. At 13 and 16 hr after mating, all eggs were penetrated. Data described elsewhere (Braden, Austin, and David 1954) indicate that penetration of the eggs is probably completed well under 13 hr after mating, for the eggs by that time were found to contain a mean of about 14 sperms per egg. In contrast to these results, the penetration of eggs from rabbits mated at about the time of artificially induced ovulation does not begin until 5 hr after mating and then increases only slowly to reach a maximum about 8-10 hr after mating. In separate communications, it is shown that sperms are present at the site of fertilization 4 hr after coitus, both with normal mating (Braden 1953) and with delayed mating (Braden and Austin 1954). Although more sperms were present 10-12 hr after coitus with normal mating than 4-6 hr after coitus with delayed mating, the difference was insufficient to explain either the absence of penetrated eggs at 4 hr with delayed mating or the much slower rate at which penetration later proceeds. The data therefore add further support to the theory that, in the rabbit also, the sperm requires to spend a period within the female genital tract before it can penetrate the egg (Austin

1951; Chang 1951). The duration of the period appears to be at least 5 hr, more than twice the minimum time required by the rat sperm (2 hr).

Because the interval between coitus and sperm penetration in rats and rabbits, mated during or after ovulation, has been put forward as evidence for the capacitation of the sperm, it may be argued that the same interpretation should be placed upon the interval that occurs between ovulation and sperm penetration in normally mated rats. As the sperms from a normal mating spend 9 or 10 hr in the female tract before ovulation, this suggestion would imply that capacitation involves an interaction between the sperm and the egg, or between the sperm and the liquor folliculi which reaches the tube with the egg. Against such a theory are the observations that sperm penetration can occur within the first hour after ovulation when this is induced artificially in immature rats (Austin 1951) and that sperm penetration into rabbit eggs normally takes place within an hour of ovulation. Furthermore, in the rabbit, capacitation may occur in the uterus (Chang 1951). It seems more probable that during the 2-4 hr interval some change occurs in the egg membranes that must be completed before sperms can enter the egg. The change may represent a final stage in the process of maturation, and may well be more rapid in the immature rat, under the influence of gonadotrophic hormones, than in the adult rat after spontaneous ovulation. The need for such a change would account for the slow initial increase in the percentage of penetrated eggs in the normally mated rats (Fig. 3, curve *N*). In the same way, the occurrence of the change probably underlies the shorter time required for penetration of all the eggs in any one rat, observed after mating at 7.00 a.m. as compared with normal mating (Fig. 4).

Since the number of sperms about the eggs during the period when penetration is taking place is much the same with both these mating procedures (Braden and Austin 1954), the difference between the times required for penetration could be attributed to the fact that by 7.00 a.m. all eggs would have completed their maturation. The eggs would then be penetrable to the first sperm that approached them provided it had undergone capacitation.

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