

# DISTRIBUTION OF SPERMS IN THE GENITAL TRACT OF THE FEMALE RABBIT AFTER COITUS

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## Summary

At each of the intervals, 1, 2, 3, 4, 6, 10, 13, 16, 20, and 28 hr after coitus, six rabbits were killed to provide data on sperm distribution.

The mean number of sperms in the cranial half of the vagina at 1 hr was  $8.5 \times 10^6$ . Between 1 and 4 hr it decreased to  $1.9 \times 10^6$ , after which there was no significant change.

The mean number of sperms in the caudal halves of the uterine horns was  $0.9 \times 10^6$  at 1 hr and did not alter significantly up to 28 hr. The mean number of sperms in the cranial halves of the uterine horns was  $0.05 \times 10^6$  1 hr after mating, and rose to  $0.42 \times 10^6$  at 4 hr, after which there was no significant change.

Sperms were not found in the fallopian tubes until 3 hr after mating. The mean number in the entire tubes rose to about 5000 at 6 hr, after which it remained relatively constant. The mean number of sperms in the ovarian third of the tubes rose from 0 at 3 hr to 38 at 4 hr, 249 at 10 hr, and 478 at 28 hr. The increase from 3 to 10 hr was significant but that from 10 to 28 hr was not. In some animals, killed at 10-28 hr, the tubes were divided into thirds and it was found that 57 per cent. of the total number of sperms in the entire tubes were present in the uterine third, 35 per cent. were in the middle third, and 8 per cent. were in the ovarian third.

From this evidence, it is concluded that in rabbits the utero-tubal junction and the cervix are the most important barriers in limiting the passage of excessive numbers of sperms to the site of fertilization.

Above a certain level, increase in the number of sperms deposited in the vagina at coitus apparently has no effect on the total number of sperms entering the fallopian tubes.

The location and state of the eggs and their membranes were noted in the rabbits killed 10-28 hr after coitus. The eggs were mainly found in the middle third of the tubes, indicating rapid passage through the upper third. Ovulation had begun in all animals by 10 hr after mating, but was apparently not complete. Nevertheless, five out of eight eggs examined by histological methods at that time had been penetrated by sperms. The site of fertilization in the rabbit probably includes the whole ampulla, which constitutes about one-half of the fallopian tube.

## I. INTRODUCTION

It has long been known that although very large numbers of sperms are normally deposited in the vagina or uterus at coitus in mammals, very few

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subsequently attain the site of fertilization (Tafari 1889; Sobotta 1895; Sobotta and Burckhard 1910; Hammond 1925). More recently, counts have been made of the actual number of sperms present at this location after mating. Hammond and Walton (1934) recovered 18, 27, 75, and 1600 sperms from the periovarial bursae of four ferrets, and Blandau and Money (1944), Austin (1948), and Blandau and Odor (1949) found 5-100 sperms in the rat ampulla. The ampulla is thought to be the site of fertilization in most mammalian species. For the entire fallopian tubes, numbers of the order of 2000-8000 sperms have been recorded in the ewe (Warbritton *et al.* 1937) and the rabbit (Austin 1948; Chang 1951a, 1951b).

The hypothesis that an important and overt function of the female genital tract is to prevent excessive numbers of sperms gaining access to the eggs during their penetrable life has been put forward recently (Austin and Braden 1952). The investigations to be reported in the present paper supply information on the control of sperm numbers in the rabbit. Studies on the distribution of sperms in the female genital tract after coitus have previously been reported for two other species. In sheep, Warbritton *et al.* (1937) found that the mean number of sperms in the vagina, uterus, and tubes of 20 ewes, killed 2 and 11 hr after mating, was 920,000, 76,000, and 6500 respectively. In the rat, in which intra-uterine insemination apparently occurs (Hartman and Ball 1930), the mean number of sperms recovered from the uterine horns was  $58 \times 10^6$ , whereas mean values as low as 860 and 24 sperms respectively were recorded for the isthmus and ampulla of the tubes (Blandau and Odor 1949). The present study shows that in the rabbit the utero-tubal junction, the cervix, the uterine horns, and the isthmus of the tubes, all operate, though with varying degrees of effectiveness, in limiting the number of sperms which reach the ampulla. The most important of these structures in the rabbit are the utero-tubal junction and the cervix, whereas in the rat the utero-tubal junction and the isthmus are entirely responsible for the enormous reduction in sperm number between the uterus and ampulla. In the ewe, on the other hand, it appears that the cervix plays the major part in limiting the numbers of sperms passing up the tract.

Information on the rate of passage of sperms through the female genital tract in the rabbit has also been gained. Heape's (1905) observation that sperms take about 4 hr to reach the ovarian end of the fallopian tube in the rabbit is confirmed, and it is shown that the numbers of sperms present here increases considerably in subsequent hours.

## II. METHODS

Seventy-five virgin female rabbits, 4-12 months of age, and principally of the albino type, were used. Each was allowed to copulate once or twice with vigorous males. The does were killed at 1, 2, 3, 4, 6, 10, 13, 16, 20, and 28 hr after coitus and their genital tracts removed. Each tract was divided into nine parts: the upper or cranial half of the vagina, the upper and lower halves of each uterine horn, and the upper, or ovarian, third and the lower, or uterine,

two-thirds of each fallopian tube. In some instances each tube was divided into upper, middle, and lower thirds. The caudal half of the vagina was not examined. The portions of uterus and vagina were flushed separately with 3.5 ml distilled water and the total sperm content of each washing was estimated by means of a haemocytometer. The sections of fallopian tube were each flushed with approximately 1 ml normal saline solution, and the washings were centrifuged at about 2,500 r.p.m. for 10 min. This procedure was employed to concentrate the sperm suspension as the number of sperms in the tube is relatively small. The bulk of the supernatant fluid was drawn off and the remainder was agitated vigorously with a fine pipette. An aliquot of the centrifuged suspension was placed on a slide, a coverslip with greased edges placed over it, and the total number of sperms present was counted.

The efficiency of the method of concentrating the tubal washings was low unless a drop of wetting agent ("Teepol," diluted 1 in 5000) was added to the washings before centrifugation. The use of centrifuge tubes treated with silicone was also found to be advantageous. With these modifications, the recovery, when a known number of sperms was used, was consistently between 40 and 60 per cent. A correction factor of 2 was therefore applied to the estimates of sperm number in the tubes.

### III. RESULTS

The mean numbers of sperms in the various segments of the female genital tract 1-28 hr after coitus, together with the range of values, are shown in Table 1, and the changes in the distribution of sperms with time are illustrated in Figure 1. The mean value at each interval is based on six rabbits.

The mean number of sperms found in the upper half of the vagina 1 hr after mating was  $8.52 \times 10^6$ . In subsequent hours the number decreased from  $7.83 \times 10^6$  at 2 hr to  $1.86 \times 10^6$  at 4 hr, after which no significant change took place up to 28 hr. The mean value for the number of sperms in the lower or caudal halves of the uterine horns in each rabbit 1 hr after coitus was  $0.93 \times 10^6$ ; estimates for subsequent intervals did not vary significantly from this figure. The number of sperms recovered from the upper or cranial halves of the uterine horns 1 hr after mating was  $0.052 \times 10^6$ . The number at 2 hr was similar but between 2 and 4 hr it rose to  $0.42 \times 10^6$ , after which there was no significant change up to 28 hr.

Sperms were not found in the fallopian tubes until 3 hr after mating, when the mean number present was 56 per animal. These sperms were confined to the lower or uterine segment of the tubes. The mean number of sperms in the entire tubes reached a level of 5158 at 6 hr, after which it remained relatively constant up to 28 hr. In the upper or ovarian third of the tubes, the mean number of sperms increased from 0 at 3 hr to 38 at 4 hr, 84 at 6 hr, and 249 at 10 hr, which is the approximate time of ovulation in the rabbit. From 10 to 28 hr a further rise in the mean sperm number to 478 occurred, but was not significant, owing to the high variation between animals (Table 1). On the other hand, the increase in sperm number from 3 to 10 hr was significant.

TABLE 1  
SPERMS RECOVERED FROM THE GENITAL TRACT OF THE RABBIT 1-28 HR AFTER COITUS

Interval Between Coitus and Slaughter (hr)	Vagina, Upper Half (millions)	Uterus		Fallopian Tubes	
		Lower Half (millions)	Upper Half (millions)	Total	Ovarian Third
1	8.52 (1.96-12.2)*	0.93 (0.47-1.79)*	0.052 (0.01-0.10)*	—	—
2	7.83 (4.0-17.1)	1.16 (0.66-1.64)	0.042 (0.01-0.09)	—	—
3	6.03 (0.20-15.0)	1.15 (0.56-1.55)	0.13 (0.04-0.28)	55.7 (0.180)*	—
4	1.86 (0.25-3.1)	0.84 (0.30-1.30)	0.42 (0.10-0.68)	1775 (474-3516)	38 (0.88)*
6	1.41 (0.26-3.5)	1.26 (0.22-2.65)	0.38 (0.12-1.10)	5158 (698-12,560)	84 (0.180)
10	0.64 (0.20-1.03)	1.33 (0.19-3.59)	0.55 (0.09-1.20)	3925 (2000-7608)	249 (96-720)
13	2.76 (0.04-4.48)	1.51 (0.04-2.72)	0.39 (0.01-0.89)	4479 (112-7324)	208 (15-480)
16	0.58 (0.08-1.64)	1.24 (0.37-2.71)	0.41 (0.10-0.71)	3566 (1976-6986)	291 (20-546)
20	2.11 (0.32-6.0)	0.87 (0.11-2.03)	0.31 (0.05-0.62)	4717 (662-8194)	343 (90-736)
28	1.75 (0.36-5.0)	1.18 (0.58-1.86)	0.55 (0.13-1.36)	4353 (1860-7742)	478 (100-956)

\* The mean value and the range of values from six animals.

In five animals figures ranging from 20,000 to 50,000 were obtained as estimates of the total number of sperms present in the tubes. These values were exceptionally high and were excluded when calculating the mean values recorded in the foregoing paragraphs. In two of the five rabbits, the number of sperms recovered from one tube was within the normal range, whereas the number recovered from the other tube was about 10 times larger.

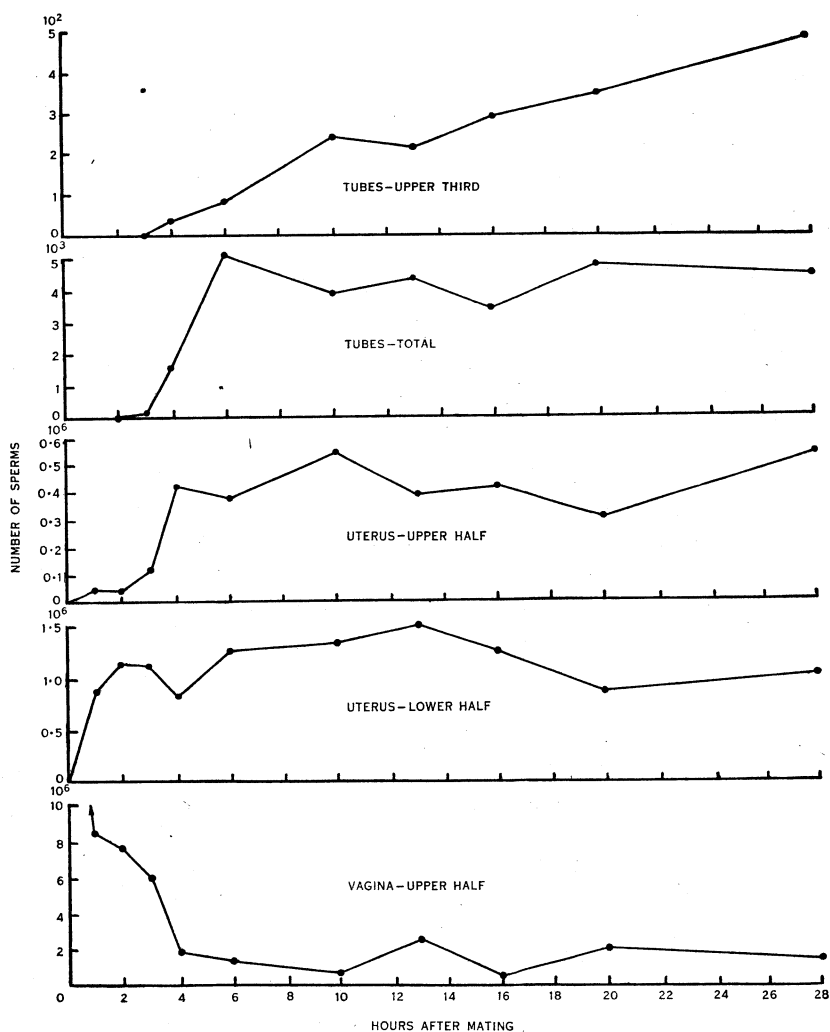


Fig. 1.—Changes in the number of sperms in various sections of the genital tract relative to time.

In some animals, the tubes were separated into ovarian, central, and uterine thirds, and separate sperm counts were made on each portion. The results from these animals are summarized in Table 2. The decrease in the mean number

of sperms in the uterine third, from 4481 at 10 hr to 1806 at 28 hr, is not significant, nor is the increase in the mean number in the ovarian third from 183 at 10 hr to 478 at 28 hr. The results for the period 10-28 hr have therefore been combined to give overall means, which are also included in Table 2. In these animals the mean for the whole tubes was 4620 sperms, of which 2648, or 58 per cent., were in the uterine third, 1614, or 35 per cent., in the middle third, and 358, or 8 per cent., were in the ovarian third. The uterine third of the fallopian tube in the rabbit consists entirely of isthmus, the middle third partly of isthmus and partly of ampulla, and the ovarian third entirely of ampulla.

TABLE 2  
SPERMS RECOVERED FROM THE FALLOPIAN TUBES 10-28 HR AFTER COITUS

Interval Between Coitus and Slaughter (hr)	No. of Rabbits	Fallopian Tubes		
		Uterine Third	Middle Third	Ovarian Third
10	2	4481 (2198-6764)*	1580 (748-2412)*	183 (96-270)*
13	2	4757 (4012-5502)	575 (322-828)	246 (118-374)
16	5	1854 (1054-4394)	1590 (708-2652)	345 (214-546)
20	6	2836 (170-5372)	1539 (402-3664)	343 (90-736)
28	6	1806 (660-3548)	2068 (976-4044)	478 (100-956)
10-28	21	2648	1614	358

\* The mean value and range of values.

Note was taken of the location of the eggs in rabbits killed 10-28 hr after mating, and some of the eggs were fixed, embedded, and sectioned by methods previously described (Braden 1952) to determine whether they had been penetrated by sperms. Twenty-nine eggs were recovered from six rabbits killed 10 hr after coitus, and of these, 10 were found in the ovarian third and 19 in the middle third or, in one animal, the lower two-thirds of the tubes. Eight eggs were examined histologically and five of these had been penetrated by sperms. In one rabbit, two unpenetrated eggs were recovered from the lower two-thirds of the tubes. Five rabbits killed 13 hr after mating yielded 37 eggs, all of which were found in the middle third or the lower two-thirds of the tubes. Only one of the eggs was still embedded in the cumulus mass, four were completely denuded, and the rest had coronae of different degrees of thickness. At 16 hr 60 eggs were recovered from eight rabbits; 56 eggs were found in the middle third, and four eggs in the uterine third of the tubes. A thin layer of mucoprotein (the so-called "albumen" layer) was noticed around all except nine of these eggs when examined in the fresh state. Twenty-four of the eggs were examined histologically and all were found to be penetrated. A total of 51 eggs was recovered from six rabbits killed at 20 hr; nine were in the lower third and 42 in the middle third of the tubes. All had a layer of mucoprotein as thick as, or thicker than, the zona pellucida, but none had reached the 2-cell

stage. Seven rabbits killed at 28 hr yielded 51 eggs, of which 15 were from the uterine third and 36 from the middle third of the tubes. One of the eggs was undivided, nine were in the 2-cell stage, 37 in the 4-cell, three in the 6-cell, and one in the 8-cell stage.

In order to determine whether an elevation of the number of sperms in the tubes above normal would occur if the number in the uterus was considerably increased, the following test was made: Six rabbits were allowed to copulate with 4-10 males within 10-20 min. The does were killed 16-20 hr later and the sperm numbers counted. The mean number of sperms in the upper half of the vagina was found to be  $7.31 \times 10^6$ , which was about five times larger than the number present when the animals were mated once or twice only (Table 3). The mean number of sperms in the lower half of the uterus in animals mated 4-10 times was  $2.83 \times 10^6$  as compared with  $1.05 \times 10^6$  for animals mated once or twice only. In only three of the animals was the value outside the range previously obtained for animals mated once or twice and killed 16-20 hr later ( $0.11$ - $2.71 \times 10^6$ ). The mean number of sperms in the upper half of the uterus from animals mated 4-10 times was  $0.59 \times 10^6$ , which is little different from the value obtained from animals mated once or twice only ( $0.36 \times 10^6$ ). In only two of the animals mated 4-10 times was the sperm number in the upper half of the uterus above the normal range ( $0.05$ - $0.71 \times 10^6$ ). Sperm counts were made on the tubes of one of these two animals and on the tubes of two other animals, one of which had  $4.35 \times 10^6$  in the lower half of the uterus, though only  $0.52 \times 10^6$  in the upper half. In each instance, the estimate for the total number of sperms in the tubes was less than the mean value of 4142 obtained from animals mated once or twice only. The actual values were 2272, 2640, and 3846, which were well within the normal range (662-8194). The mean number of sperms in the upper third of the tubes after multiple copulation in the three animals was 239, as compared with 317 for animals which copulated once or twice.

The correlation between the number ( $x$ ) of sperms in the various segments of the genital tract was examined for 41 animals killed 10-28 hr after coitus. In place of the actual numbers the quantities  $\log (x + 1)$  were considered as these follow approximately a normal distribution. There was a highly significant correlation ( $r = 0.642$ ,  $n = 82$ , giving  $P < 0.001$ ) between the transformed numbers of sperms in the upper and lower halves of the uterine horns. However, no significant correlation could be demonstrated between the transformed numbers of sperms in the upper half of the vagina and the corresponding value for the lower half of the uterus. Likewise, the number of sperms in a uterine horn appeared to have no relation to the number in the corresponding tube (Fig. 2).

#### IV. DISCUSSION

The investigations reported in this paper lay further emphasis on the fact that, in mammals, only a very small proportion of the sperms deposited in the female tract at coitus subsequently reach the neighbourhood of the eggs. In the rabbit, only one sperm out of every 10,000-100,000 sperms ejaculated attains the ampulla of the fallopian tube, which is the site of fertilization. In the ewe

TABLE 3  
DISTRIBUTION OF SPERMS IN RABBITS MATED 4-10 TIMES AND KILLED 16-20 HR LATER COMPARED WITH THOSE MATED ONCE OR TWICE ONLY

No. of Copulations	No. of Rabbits	Vagina, Upper Half (millions)	Uterus		Fallopian Tubes	
			Lower Half (millions)	Upper Half (millions)	Total	Ovarian Third
1-2	12*	1.50 (0.08-6.0)†	1.05 (0.11-2.71)†	0.36 (0.05-0.71)†	4142 (662-8194)†	317 (20-736)†
4-10	6‡	7.31 (3.55-16.7)	2.83 (1.43-4.35)	0.59 (0.11-1.28)	2919 (2272-3846)	239 (192-326)

\* These figures are taken from Table 1 (16-hr and 20-hr intervals).

† The mean value and range of values.

‡ The figures for the tubes in this group are based on only three of these six animals.



and the rat, the proportion is even smaller: only about one sperm out of every  $1.3 \times 10^6$  reaches the site of fertilization (Warbritton *et al.* 1937; Blandau and Odor 1949).

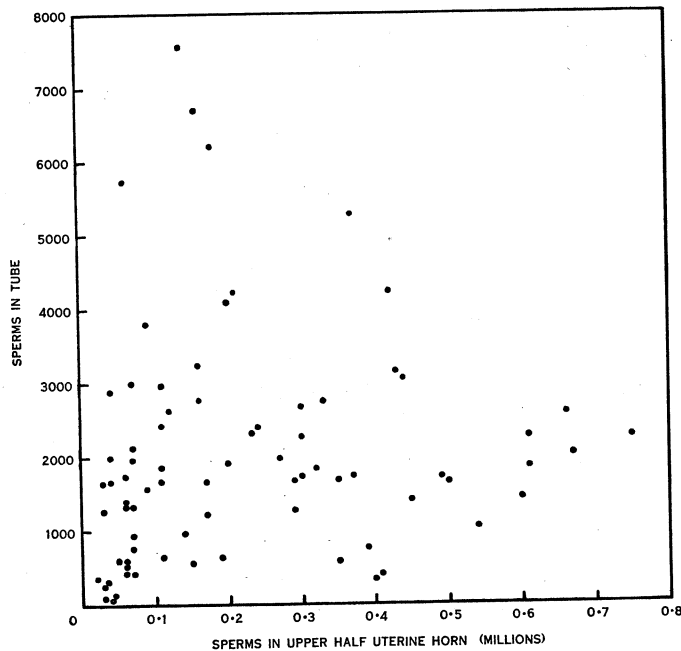


Fig. 2.—Lack of correlation between the number of sperm in the upper half of a uterine horn and the number in the corresponding fallopian tube. The results were obtained from animals killed 6-28 hr after coitus.

Of importance in limiting the passage of sperms up the tract are the cervix, the uterus, the utero-tubal junction, and the isthmus of the tubes. The relative importance of the various structures differs between species. In the rat, the utero-tubal junction plays the major role in restricting the passage of sperms to the ampulla, whereas the isthmus is of less importance. The cervix and uterine horns apparently have no restrictive action. Available evidence indicates that, in the ewe, the cervix is by far the most important structure in this respect; the utero-tubal junction and isthmus are not nearly as effective in restricting sperm passage (Warbritton *et al.* 1937; Braden, unpublished data). In the rabbit the utero-tubal junction and the cervix limit sperm passage most, whereas the isthmus and the uterine horns have only a slight effect. The relative effectiveness of the various parts in the rabbit may be stated as follows: The utero-tubal junction allows only one sperm of every 160 present in the upper half of the uterus to enter the tubes, and only one of every 40 sperms ejaculated into the vagina traverses the cervix. Approximately one-quarter of the number of sperms entering the tubes passes through the isthmus to the ampulla, and

about one-third of the number entering the uterus reaches the upper half of that organ.

The ability of the cervix to restrict the passage of sperms into the uterus in the rabbit has been demonstrated by the results obtained in the present study. The number of sperms deposited in the vagina by a normal male rabbit is about  $60 \times 10^6$ , though this value is subject to considerable variation (Asdell 1946). In comparison the number of sperms recovered from the upper half of the vagina 1 hr after mating was only  $8.5 \times 10^6$ . Evidently, the great majority of the sperms originally deposited in the vagina by the male are quickly lost to the exterior. Furthermore, the mean number in the remainder of the genital tract was about  $1.5 \times 10^6$  which is considerably less than one-fortieth of the number originally introduced into the vagina. When the number of sperms deposited in the vagina was increased about five times by multiple copulation, the number of sperms found in the rest of the genital tract was increased only 2.5 times, a further indication of the restrictive action of the cervix.

The slight reduction in number which occurs as the sperms pass up the uterus may be imputed simply to the dissemination of the sperms over the relatively large endometrial surface. The strong positive correlation between the numbers of sperms in the upper and lower halves of the uterine horns lends support to this hypothesis. No such simple relation exists, however, between the number of sperms in the upper half of the uterus and the number in the lower segment of the tubes, suggesting that some other mechanism is in operation at the junction between the uterus and the tubes. A mean number of  $0.42 \times 10^6$  sperms was recovered from the upper half of the uterus compared with 2650 from the lower third of the tubes (Table 2): a ratio of about 160:1. In contrast, the number of sperms in the uterus of the rat is about 30,000 times the number in the tubes (Blandau and Odor 1949), and in the ewe the ratio of the number of sperms in the upper part of the uterus to the number in the lower third of the tubes is about 10:1 (Braden, unpublished data).

Morphological studies in a number of species of the region where the uterus and the fallopian tubes are confluent have shown that the utero-tubal junction is well adapted to restrict the passage of sperms (Kelly 1927; Anderson 1928; Lee 1928). The structure has a well-developed circular layer of smooth muscle and numerous polyp-like folds of the mucosa, the latter making the lumen narrow and tortuous. The mucosal folds guarding the tubal ostium apparently act as valves if intra-uterine pressure is elevated.

In five rabbits, the number of sperms recovered from the tubes was exceptionally high—20,000-50,000. This may have been the result of contamination with uterine washings in the recovery technique, though care was taken to avoid such an occurrence. On the other hand, it may be that the utero-tubal junction was not functioning normally in these animals. In two of the animals the sperm number in one tube was about 2500, which is well within the normal range, whereas the number in the other tube was 25,000-50,000.

Some further reduction in sperm number is evidently effected by the isthmus of the fallopian tube in both the rabbit and the rat. In the rat, a mean number of 860 sperms were recovered from the isthmus and only 24 from the ampulla

(Blandau and Odor 1949). The corresponding values for the rabbit found in the present study were about 3500 and 1000. The reduction in sperm number accomplished by the isthmus in the rabbit is not nearly as great as in the rat, and is even less if the number of sperms which actually penetrate the zona of the eggs is also taken into account. The mean number of eggs per animal recovered from rabbits killed 16-20 hr after coitus in the present series was 7.9 and the mean number of sperms which had penetrated them about 490 per rabbit. Corresponding values obtained for the rat were 9.2 eggs and 12 penetrating sperms per animal (Braden, unpublished data). When these sperms are added to the number in the ampulla the ratio of the mean number of sperms in the isthmus to that in the ampulla becomes 24:1 for the rat and 2.3:1 for the rabbit. It must be remembered, however, that these estimates of sperm number in the fallopian tubes are only very approximate, as the technique employed for their recovery is relatively crude.

The present experiments have indicated that the mean number of sperms in the tubes of the rabbit 6-28 hr after coitus probably lies between 4000 and 5000 and that, above a certain level, increase in the number of sperms inseminated has no significant effect on this value. These findings are thus in agreement with those of Austin (1948) and Chang (1951*b*). Austin estimated that 2000-4000 sperms were present in individual tubes when rabbits were artificially inseminated with  $100 \times 10^6$  sperms. When less than  $1 \times 10^6$  sperms were introduced into the vagina, the number in the tubes was much less. Chang obtained a mean value of 5120 for the number of sperms present in the tubes of five mated rabbits, and 6480 for the tubes of six animals artificially inseminated with  $20 \times 10^6$  sperms. When only  $0.6-1.8 \times 10^6$  sperms were inseminated, the mean number recovered from the tubes of five rabbits was 88. Chang concluded that the number of sperms present in the tubes was relatively constant if  $10 \times 10^6$  or more sperms were inseminated. He postulated that the uterine cervix and the isthmus of the fallopian tube functioned as barriers against the passage of large numbers of sperms. It is worthy of note that the number of sperms in the tubes is essentially the same whether artificial insemination or natural mating is employed. The available evidence, therefore, supports the hypothesis that an important function of the female genital tract is to limit the number of sperms passing to the site of fertilization. This limitation is presumably necessary because the presence of excessive numbers of sperms about the eggs at fertilization may well increase the danger of polyspermy. Furthermore, it is quite possible that even in monospermic eggs very large numbers of supplementary sperms in the perivitelline space would interfere with its normal development.

From the present results it can be seen that passage of the sperms through the cervix of the rabbit is undoubtedly rapid, for maximal numbers were present in the lower half of the uterus within 1 hr after coitus. A few sperms reached the upper half of the uterus within 1 hr, but maximal numbers were not found until 4 hr after mating. There is some difference of opinion on the mode of transport of the sperms through the rabbit uterus: some workers have consid-

ered that the sperms ascend by means of their own motility (Marshall 1922; Parker 1931; Chang and Pincus 1951), whereas others contend that the motility of the uterus is largely responsible for sperm passage (Krehbiel and Carstens 1939; Schilling and Kordts 1952). The present results suggest that both mechanisms may operate; that the sperms which reach the upper half of the uterus during the first hour are transported by the activity of the tract, and that those which arrive at the same location 2-3 hr later do so mainly by their own motility. The findings are in accord with those of Florey and Walton (1932), who reported that increasing numbers of sperms were found near the opening of a uterine fistula 20 min to 2 hr after copulation in the rabbit.

Sperms were not recovered from the tubes until 3 hr after mating, and at this time they were confined to the lower part of the tubes. The sperms began to accumulate in the ovarian third of the tubes at about 4 hr. This confirms Heape's (1905) observation that sperms took 4 hr to reach the infundibulum in the rabbit. Agreement is also found with San Martin (1951), who reported solitary spermatozoa in the upper reaches of rabbit tubes 4 hr after coitus and somewhat larger numbers at this site at 6 hr. Other workers, however, have reported shorter intervals. Hensen (1876) found sperms at the infundibulum  $2\frac{1}{4}$  hr after coitus in one animal, and Parker (1931) recorded finding them there in two rabbits at 3 hr, whereas Chang (1952) claims to have recovered sperms from the upper part of the fallopian tube as early as 1 hr after mating.

Reference has been made to the site of fertilization in mammals, but its location is somewhat ill-defined. Anderson (1927) considered that it was restricted to the upper third of the tube, but the present results suggest that, in the rabbit at least, it probably includes the whole of the ampulla. This does not necessarily mean, however, that fertilization takes place over a considerable interval of time under normal circumstances, for the passage of the eggs through the upper third of the tubes is rapid, as has been shown in the present series, and by Pinner (1880), Assheton (1894), and Chang (1951a). Ovulation was evidently still proceeding in the rabbits killed 10 hr after mating, for the mean number of eggs recovered was 4.8 as compared with 7.7 eggs per rabbit from animals killed at 13-28 hr. Nevertheless, more than half of the eggs recovered at 10 hr were in the middle third of the tubes and five out of eight eggs examined had been penetrated by sperms. Penetration of the zona pellucida by supplementary sperms apparently continues up to about 6 hr after ovulation. About this time a layer of mucoprotein begins to accumulate around the zona, and this probably renders the eggs impenetrable. Eggs were found mainly in the middle third of the tubes at this time, which indicates that penetration of the zona does not normally occur in the lower third of the tubes.

#### V. ACKNOWLEDGMENTS

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