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CHEMOTAXIS OF RABBIT SPERMATOZOA

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INTRODUCTION

According to reviews (Tyler, 1955; Rothschild, 1956), chemotaxis of spermatozoa towards eggs has not been unequivocally demonstrated in animals. However, more recently, Suzuki (1961*a–c*) has claimed that, in several species of Japanese bitterlings, the spermatozoa are attracted to a substance found in the egg micropyle. Suzuki's experiments, as well as earlier ones that aimed to prove or disprove the existence of sperm chemotaxis, were done on animals in which fertilization is external. So far as I am aware, no experiments on sperm chemotaxis in mammals have been published.

In the absence of evidence for chemotaxis, it is assumed that collisions of spermatozoa with eggs, which, in most mammals, take place in the oviduct, occur solely by chance. The factors involved in such collisions are the concentration and movements of spermatozoa and, to a lesser degree, the slight movements of the egg induced by ciliary action and muscular contractions of the oviduct. Apparently the mechanism which brings the gametes together is highly efficient, since normally more than 90% of the eggs are fertilized.

The experiments described below were designed to find out whether sperm chemotaxis, in addition to chance meeting, plays a part in sperm-egg collision.

THEORETICAL CONSIDERATIONS

The rabbit was chosen as the experimental animal because in this species, unlike most mammals, the zona pellucida does not become less permeable to spermatozoa after entry of the first one (Braden, Austin & David, 1954). The continuous penetration of spermatozoa into the zona and the perivitelline space (only one spermatozoon normally enters the vitellus) makes it possible to assess the frequency of sperm-egg collisions by counting the number of spermatozoa within the zona and the perivitelline space. Sperm penetration stops 6–9 hr. after ovulation, when the egg becomes coated with a mucin layer which is impenetrable to spermatozoa (Hammond, 1934).

Assuming that sperm-egg collisions are determined by chance alone, particles which simulate rabbit eggs in size, shape and specific gravity should, when deposited at the site of fertilization, sustain collisions with spermatozoa at the same rate as the rabbit's own eggs. If, in addition, the surfaces of these particles are such that the colliding spermatozoa adhere to them permanently, then the number of collisions can be

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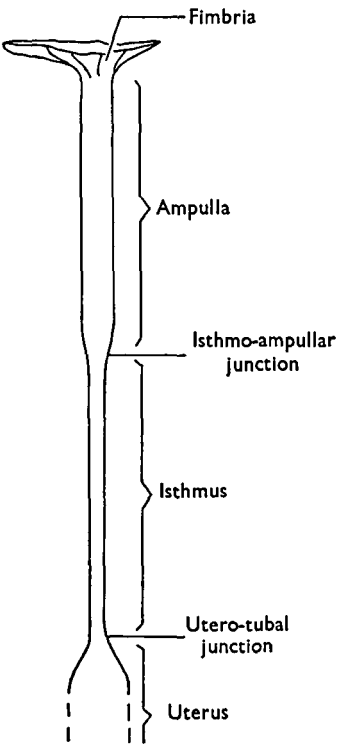
recorded. If collisions occur merely by chance, the number of spermatozoa adhering to a particle will be similar to that collected within an egg, but will be less if chemotaxis is a factor. The 'particles' used in these experiments were rat eggs.

PRELIMINARY EXPERIMENTS

In order to ascertain that rat eggs would be suitable to use as particles which simulate rabbit eggs, two points had to be verified: first, that rabbit spermatozoa adhere permanently to the zonae of rat eggs; and secondly, that when transferred into the



Text-fig. 1. Diagrammatic representation of the rabbit oviduct.

rabbit oviduct, rat eggs are transported at the same speed and to the same location as rabbit eggs. Similarity in transport through the oviduct is necessary to ensure that the eggs of both species will be exposed to the same sperm concentration, which, according to Braden (1953), is highest at the utero-tubal junction and gradually decreases to a minimum at the fimbria (see Text-fig. 1).

Attachment of rabbit spermatozoa to the zonae of rat eggs, in vitro. Rat eggs were recovered shortly after ovulation when they are still in a cumulus mass. They were either left in the cumulus or were denuded, before use, with hyaluronidase, so that the zona would be seen better. In an experiment two or three eggs were mounted in a hanging drop (0·9% NaCl), to the edge of which a very small quantity of freshly ejaculated rabbit semen was added. The preparation was then immediately examined with the microscope. The movement of spermatozoa was random, as determined

visually. When spermatozoa, swimming towards an egg, were 10–30μ from it, the majority continued on and their heads attached to the zona. The attachment was permanent, as spermatozoa were never observed to free themselves from a zona, in spite of the vigorous lashing of their tails. After a few minutes a large part of the surface of the zona became covered with spermatozoa.

Transport of rat eggs in the rabbit oviduct. Recent experiments by Harper (1961) have shown that when rabbit eggs, still in their cumulus mass, are deposited in the oviduct just below the fimbria, they are transported by the oviduct to the isthmo-ampullar junction within 5–10 min. The eggs remain for a number of hours at or near this junction, which is most likely the site of fertilization (see Text-fig. 1). Harper's experiments were repeated, using rat eggs in their cumuli instead of rabbit eggs. The transport of rat eggs in the rabbit oviduct was similar to that of rabbit eggs.

MATERIALS AND METHODS

Knowing that rabbit spermatozoa attach themselves readily to the zonae of rat eggs *in vitro*, and that the transport of rat and rabbit eggs in the rabbit oviduct is similar, the next step was to examine the rate of attachment of rabbit sperm to rat eggs which had been transferred to oviducts of previously mated rabbits. In control experiments rabbit eggs instead of rat eggs were transferred.

The egg donors were immature rats 29–34 days old, in which superovulation was induced by subcutaneous injection of 30 i.u. of pregnant mare's serum (Gestyl, Organon Laboratories) followed 52–56 hr. later by 20 i.u. of chorionic gonadotrophin (Pregnyl, Organon Laboratories). One to three hours after ovulation had occurred, the rats were killed, their oviducts removed and the eggs recovered—always surrounded by their cumuli. In a given experiment one of the following media was used: (1) equal parts of rabbit plasma and physiological saline (0·9% NaCl); (2) Ringer's solution; (3) physiological saline. The eggs were kept *in vitro*, at room temperature, for 40–60 min., and were then transferred into both oviducts of the recipient rabbit. Ten to twelve hours before the transfer, the recipient rabbit had been mated to two fertile bucks in rapid succession. Thus the eggs were transferred while ovulation was in progress in the recipient. (Rabbits normally ovulate 10–12 hr. *post coitum*). Seven to eleven hours after the transfer the recipient was sacrificed, her oviducts were removed and flushed to recover the native and the transferred eggs. The rat eggs were then examined to count the number of rabbit spermatozoa which had attached to their zonae. The native rabbit eggs served as controls to check that spermatozoa had reached the site of fertilization.

Since rat eggs are smaller than rabbit eggs, the objective was to transfer to each oviduct twenty rat eggs, which have an approximate surface area of five rabbit eggs. (Five is the average ovulation rate per ovary in the rabbit.) In practice, though, a few more or less than twenty rat eggs were transferred, depending on the number available.

Recently ovulated rabbit eggs in cumulus were similarly transferred to the left oviducts of a different group of recipients whose left ovaries had been removed 24 hr. prior to mating. This procedure overcomes the problem of having native and transferred eggs, which would be indistinguishable, in the same oviduct.

RESULTS

The different transfer media did not affect the rate of sperm attachment.

Of the 468 rat eggs which were transferred into twenty-two rabbit oviducts, 394 were recovered. One rabbit spermatozoon was attached to the zona of each of fifty-five eggs and two spermatozoa were attached to each of three eggs.

The eggs recovered from five oviducts (79 eggs) were not denuded of their cumulus cells, those from six oviducts (112 eggs) were denuded, and those from the remaining ten oviducts (203 eggs) were denuded and coated with a thin layer of mucin. No eggs were recovered from one oviduct into which sixteen eggs had been transferred.

Spermatozoa were not attached to undenuded eggs. One of the spermatozoa which was attached to the zona of a denuded egg is shown in Pl. I, fig. 1, while another (head and part of tail), attached to the zona of a denuded egg which also had a thin layer of mucin, is shown in Pl. I, fig. 2.

To ascertain that a spermatozoon was actually on the surface of the zona and not merely close to it, i.e. in the mucin, the following technique was used. The eggs were mounted in a small drop of physiological saline between a glass slide and a coverslip which was supported by four dots of petroleum jelly. The coverslip was then lightly pressed down so that the eggs touched both the slide and the coverslip. By gently pushing the coverslip horizontally, the eggs could be rolled, which made it possible to examine the whole surface of their zonae for the presence of adhering spermatozoa. When an egg is first examined, a spermatozoon may appear to be in the thickness of the zona, but this is not necessarily its true location, which is determined by rolling the egg over until the spermatozoon reaches its most peripheral point in the horizontal plane. When observed at this point spermatozoa were never within the zona; they were either on its surface (Pl. I, figs. 1 and 2) or a short distance away from it (Pl. I, fig. 3).

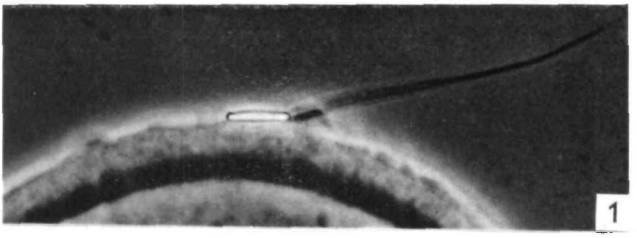
Of the thirty-six rabbit eggs transferred into six left oviducts, twenty-nine were recovered. They contained 598 spermatozoa.

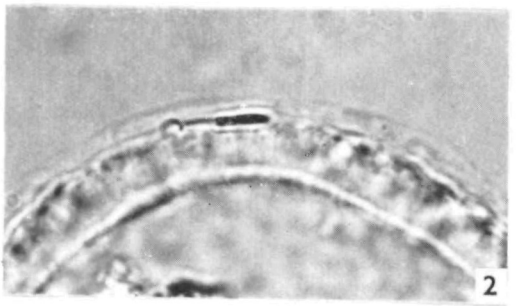
As mentioned earlier, the surface area of the rabbit egg is approximately four times that of the rat egg. Therefore, the mean number of spermatozoa attached to four rat eggs (0·6) was compared with the mean number of spermatozoa penetrating one rabbit egg (20·6), the difference between the means being very striking.

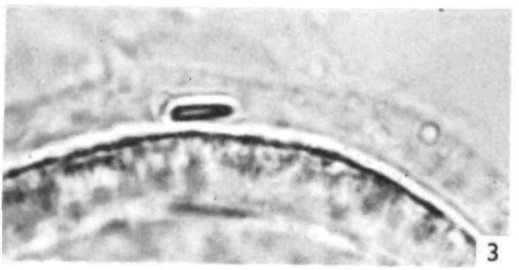
DISCUSSION

The transferred rabbit eggs may appear to have had a slight advantage, from the point of view of sperm attachment, as they were transferred into 'empty' oviducts, whereas the rat eggs were transferred into oviducts which contained a few native eggs. However, pilot experiments disclosed that when rat eggs are transferred into 'empty' oviducts the rate of sperm attachment to their zonae does not increase, and when rabbit eggs are transferred into oviducts which also contain native eggs, the average number of spermatozoa per egg does not decrease.

The experiments described in this paper were designed so that the chances of a rabbit sperm-rabbit egg and a rabbit sperm-rat egg collision were the same. The fact that many more spermatozoa enter rabbit eggs indicates that they attract the sperma-







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tozoa. *A priori*, it is suggested that this attraction is of a chemical nature. In other words, spermatozoa swim preferentially from regions of low to regions of high concentration of a substance secreted by the egg. The highest concentration of the substance, obviously, is at the egg surface. Although the present experiments imply the existence of chemotaxis of rabbit sperm towards homologous eggs *in vivo*, the definitive proof of this hypothesis would rest on the demonstration, *in vitro*, of the substance responsible for attracting the spermatozoa.

SUMMARY

The aim of the study was to determine whether rabbit eggs 'attract' homologous spermatozoa *in vivo*.

Preliminary experiments showed that, *in vitro*, rabbit spermatozoa readily and irreversibly attach to the zonae of rat eggs.

When rat and rabbit eggs were transferred into oviducts of previously mated rabbits, a significantly larger number of spermatozoa collected within the rabbit eggs than on the zonae of the rat eggs. Since the experiments were designed so that rat and rabbit eggs had equal chances of sustaining collisions with rabbit spermatozoa, the great preponderance of spermatozoa in the rabbit eggs suggests that the latter exert a chemotactic influence on rabbit spermatozoa.

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EXPLANATION OF PLATE

Fig. 1. Rabbit spermatozoon attached to the zona of a rat egg. Phase-contrast, × 900.

Fig. 2. Rabbit spermatozoon (head and part of tail) attached to the zona of a rat egg coated with a thin layer of mucin. × 900.

Fig. 3. Head of rabbit spermatozoon (tail is out of focus) embedded in the mucin layer a short distance away from the zona. × 900.