

THE MIGRATION OF SPERMATOOA IN THE FEMALE OF *RHODNIUS PROLIXUS* STAL.

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INTRODUCTION

In *Rhodnius*, as in many insects, a spermatophore containing the semen is deposited in the bursa copulatrix of the female during copulation, and the spermatozoa then migrate to the spermathecae where they are stored until they are used in fertilization. In some insects this migration may be very complex, as in the Lepidoptera (Musgreave, 1937; and many others), and in seeking to explain these movements many authors have assumed that the spermatozoa play an active part and some have invoked chemotaxis (Heberdey, 1931). Evidence to be presented in this paper will demonstrate that the migration in *Rhodnius* is a result of contractions in the female ducts set up by a secretion from the male acting through a peripheral nervous system and that the spermatozoa play no active part in the migration.

The spermatophore of *Rhodnius* has been described by Khalifa (1950a), and its mode of formation and the events at copulation will be examined in a later paper. All that concerns us here is that the spermatophore is placed in the bursa copulatrix so that it fills the bursa and the slit containing the semen encloses the lip-like vestibulum (see Fig. 1). By killing the females at various times after copulation, and sectioning the reproductive ducts, it was ascertained that the spermatozoa travelled to the spermathecae via the lumen of the common oviduct and that they began to arrive at the spermathecae within 5–10 min after the termination of mating. There was no particular orientation of the spermatozoa in the oviduct or spermathecae.

THE OPAQUE ACCESSORY SECRETION

In the male reproductive system of *Rhodnius* there are accessory glands consisting of four finger-like lobes on each side. Three of the four lobes contain a transparent, viscous material which forms the spermatophore; this material is of little importance in this study. The fourth lobe, containing an opaque, granular secretion, is responsible for the movements of the spermatozoa in the female. If both of the opaque glands are removed from a male, and if, after a recovery period of about a week, the male (deprived of its opaque glands) is mated to a virgin female, a normal spermatophore will be produced; but the spermatozoa, also apparently normal, will not leave the bursa. The spermathecae are found to be empty when the female is dissected 5 hr. after copulation. If, on the other hand, the male is operated on one side only, the spermatozoa ascend the oviduct to the spermathecae in the normal way.

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In an attempt to ascertain the site of action of the secretion, the opaque accessory glands of several males were injected with dyes and these males were mated to females which were dissected immediately after copulation to see whether the dye could be detected. Only twice did the dye (in both cases methylene blue) appear; in each case it was found between the dorsal wall of the bursa and the spermatophore just posterior to the mass of semen and the vestibulum. A more

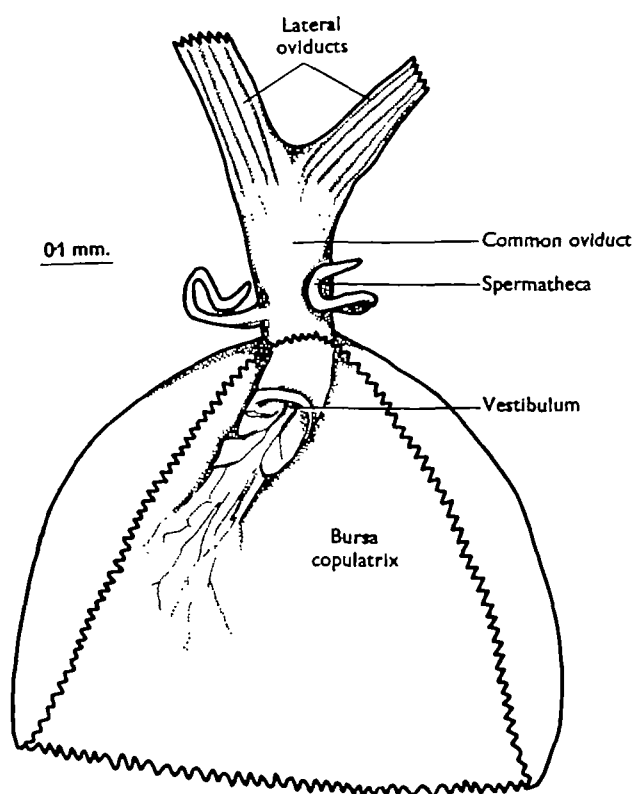


Fig. 1. Ventral view of the female reproductive ducts of *Rhodnius*. The cement gland opening into the bursa has been removed and the floor of the bursa has been slit.

convincing observation is that a faint cloudiness just posterior to the slit can be discerned on the dorsal surface of the normal spermatophore, and that this cloudiness is absent from spermatophores produced by males which have been deprived of their opaque glands. Nonidez (1920) suggested that a secretion in *Drosophila* may activate the spermatozoa, causing them to swim into the spermathecae. The opaque secretion in *Rhodnius* does not appear to function in this way, for in spermatophores produced by males from which the seminal vesicles have been removed (i.e. spermatophores without semen), the opaque secretion is confined to the area posterior to the slit which would normally contain the semen.

EFFECT OF THE OPAQUE SECRETION ON THE FEMALE ORGANS

In females which are carrying spermatophores certain rhythmic contractions of the oviduct can be detected if the female is opened under insect Ringer. These movements take the form of peristaltic contractions beginning with a sharp constriction just above the spermathecae which travels downwards along the oviduct and spreads out over the muscular dorsal surface of the bursa copulatrix. Each wave of peristalsis is accompanied by a longitudinal contraction of the oviduct resulting in that organ being thrust down into the mass of semen held in place by the spermatophore. These contractions continue at a frequency of about four per minute until the preparation dies, usually a matter of 2 or 3 hr. The same contractions have been noted occasionally in females without spermatophores, but they did not persist for more than a minute or two.

In order to test whether a relationship existed between the contractions and the opaque secretion, some of this material was placed in the bursa in the following manner. A flap of cuticle was removed from the last sternite to reveal the membranous floor of the bursa, which was cut, the cut edges being reflected over the cut edges of the cuticle to keep blood out of the bursa. An opaque accessory gland was placed in the bursa and its wall ruptured so as to spill out the contents. Under such circumstances the peristaltic contractions previously described began and persisted until the bursa was rinsed with insect Ringer or the preparation died. After rinsing, the frequency of the contractions gradually declined until, after 15-30 min., the contractions ceased. Persistent contractions were not initiated by the transparent accessory secretion from the male, by fat body, or by insect blood.

Further, the same activation by the opaque secretion occurred in preparations obtained by severing the lateral oviducts and the nerves and tracheae leading to the genital organs and gently pulling the bursa away from the ventral body wall. The preparation, consisting of bursa, common oviduct, and part of the lateral oviducts, was pinned out ventral side up in a drop of Ringer and the bursa was slit open to reveal the vestibulum. This operative procedure often initiated peristaltic contractions which were sometimes quite persistent, but normally they ceased or their frequency fell below two per minute within an hour or two. The application of the opaque secretion either initiated contractions in the quiet preparations or increased their frequency from less than two to five or six per minute in those which were undergoing 'spontaneous' contractions. Transparent secretion, fat body, and insect blood failed to induce persistent contractions in these isolated preparations. It must be pointed out that the preparation is capricious and that considerable patience is required to demonstrate the effect. There can be no doubt that the phenomenon exists; it has been demonstrated in more than fifty preparations.

THE MODE OF ACTION OF THE OPAQUE SECRETION

The following observations suggested that the opaque secretion was acting through the nervous system rather than directly on the muscle. The contractions began at the level of the spermathecae some distance from the point of application of the

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stimulus in the bursa, indicating that some form of conduction is involved. Moreover, the muscular pad surrounding the vestibulum is the only area in the bursa sensitive to the secretion; cauterization of the pad with a loop of electrically heated wire stopped the contractions, whereas cauterization or excision of the ventral or lateral walls of the bursa did not interfere with the effect. Application of the opaque secretion to any part of the outside of the intact bursa failed to elicit a response.

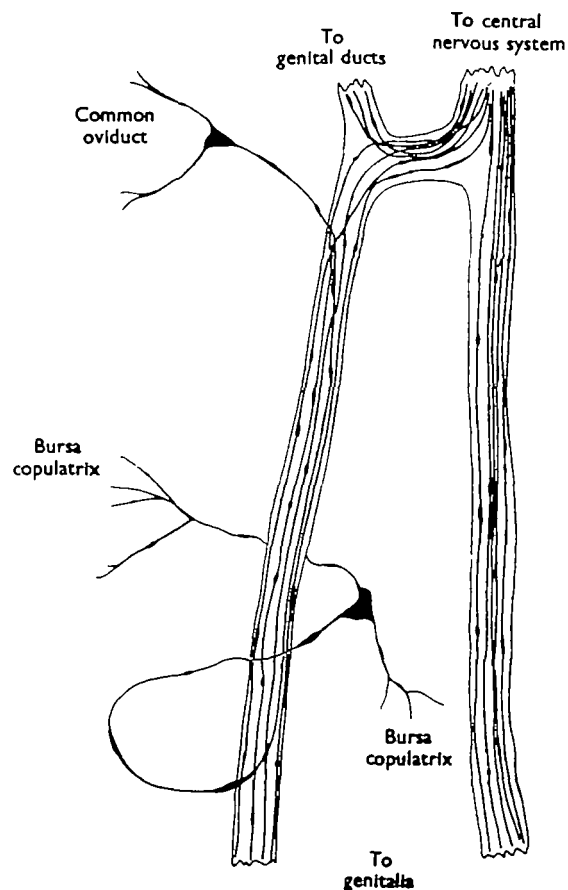


Fig. 2. Diagram of the nerves connecting the bursa and the oviduct as revealed by methylene blue staining in the female of *Rhodnius*.

Staining by injection of methylene blue into the living animal revealed a direct nervous connexion between the dorsal part of the bursa copulatrix and the common oviduct in a number of whole mount preparations of the genital ducts. Fig. 2 is a diagram showing one of these connexions. Fine branches from the muscular pad in the bursa unite in a nerve fibre which travels anteriorly in one of the bundles of nerves supplying the genitalia. At the level of the spermathecae it leaves this bundle and ends in the muscles of the common oviduct. Only one or two such

connexions were stained in any one preparation. From the physiological evidence one might expect to find ganglion cells in this system, but no structures that could be positively identified as nerve cell bodies were seen.

THE MECHANISM IN THE INTACT INSECT

The foregoing constitutes evidence for a possible mechanism by which the spermatozoa may move into the spermathecae, but is there any evidence that such a mechanism operates in the intact insect? The motility of *Rhodnius* spermatozoa is unaffected by the oxygen deprivation resulting from exposure to nitrogen, but muscular paralysis results under such conditions. Mating pairs of *Rhodnius*, the females of which were previously unmated, were exposed to nitrogen 5 min. before mating normally ended (i.e. 25 min. after its initiation). The pairs usually separated and paralysis ensued within 3 min. The nitrogen was passed slowly over the insects for 5 hr., after which time the females were dissected quickly and the spermathecae examined. Only the results of experiments in which the male subsequently recovered were considered.

Of ten such experiments, the females in eight instances contained no semen in the spermathecae; in the other two it was present in greatly reduced amounts. Normally, of course, the spermathecae would be packed with spermatozoa 5 hr. after mating. Controls consisted of five pairs which were treated as before, but were allowed to recover and after five additional hours were dissected. There were large quantities of spermatozoa in the spermathecae of all five females. Paralysis of the muscles of the intact insect with nitrogen prevents the ascent of the spermatozoa.

These results, however, do not exclude the possibility that the movements of the spermatozoa are partly responsible for the ascent. It was therefore desirable to devise a method for placing motionless spermatozoa in the spermatophore without interfering with the movements of the oviduct. The following technique met with some success. A previously unmated female was mated and the pair gassed as described above. After half an hour the female was operated upon as quickly as possible in the manner to be described. A small piece of cuticle was removed from the ventral posterior abdomen to expose the transparent floor of the bursa through which the subsequent manipulations could be observed. An injection needle mounted on a micromanipulator was inserted through the genital opening and pushed through the spermatophore until the tip of the needle entered the centre of the mass of semen. A 0.01M solution of cetyl trimethyl ammonium bromide (CTAB) in insect Ringer containing a little methylene blue to act as a tracer was injected. CTAB, a surface active agent, is a powerful spermicide but requires some time to act (Koefoed-Johnsen & Mann, 1954). It was therefore necessary to return the female to nitrogen for 2 hr. In the concentrations used, CTAB had no apparent effect on other tissues, but it was necessary to buffer the solution to pH 5.6 to prevent the liquefaction of the spermatophore.

After this 2 hr. period under nitrogen anaesthesia the female was returned to air to recover and 5 hr. later was sacrificed. If the spermatozoa in the bursa were inactive, the spermathecae were examined for the presence of spermatozoa. Out

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of twenty matings, there were only seven in which most of the spermatozoa in the bursa were inactive. In every case there was semen in the spermathecae, but in only three of these were all the spermatozoa in the spermathecae inactive. In the other four a small proportion of the spermatozoa were active, but most of them were quite motionless. Activity of the spermatozoa is not a prerequisite for the ascent.

The opaque secretion will activate the isolated oviducts; but is some degree of nervous control necessary for the ascent in the intact animal? In *Rhodnius* there are no central ganglia in the abdomen, so that removal of the abdomen isolates it from the central nervous system. The abdomen of females from mating pairs which had been anaesthetized with nitrogen as before were removed by ligation and placed in air to recover. After 5-7 hr. the abdomens were dissected and the spermathecae examined. Only those abdomens which were judged to be alive (i.e. gut, heart and oviduct capable of contraction) were included in the results of these experiments. Since the spermathecae in all five such abdomens were packed with spermatozoa, nervous control is not necessary for the ascent of the spermatozoa.

The fact that the spermatozoa move up the oviduct while the contractions move down presents some difficulty, and the following is a suggested explanation. At the same time as the oviduct is contracting longitudinally and thrusting the vestibulum down into the mass of semen, the lip of the vestibulum is caused to gape and then close firmly by the peristalsis. In this way the vestibulum makes bite-like motions in the mass of semen. The peristalsis spreading over the surface of the bursa exerts pressure on the spermatophore which prevents each 'bite' from moving backwards. Successive 'bites' must then push the semen up the oviduct. The spermatozoa are prevented from moving beyond the level of the spermathecae in the oviduct by constrictions which form there at the beginning of every contraction and probably also by hair-like projections of the intima in that region.

The spermathecae are blind tubes and the movement of the spermatozoa into them requires either that they be stretched or that some of the secretion which they contain be displaced. The spermathecae of recently mated females appear to be no longer than those of virgin females. However, the spermathecae twitch continuously while the oviduct is contracting, and it is suggested that during these twitchings the lumen of the spermatheca expands momentarily allowing the spermatozoa to enter. When the lumen returns to its normal state, the spermatozoa are prevented from returning to the oviduct by a series of teeth, projections of the intima in the proximal portion of the spermatheca. These teeth act as a sieve, permitting the passage of the secretion and thereby equalizing the pressure. That the spermatozoa are pushed into the spermathecae gains support from the following experiment. The spermathecae of several females were removed and the females were mated to normal males. Upon dissection 5 hr. after mating, masses of spermatozoa were seen projecting from the small holes left by removal of the spermathecae. The spermatozoa had evidently been forced out of these tiny holes by pressure from within the oviduct.

THE MECHANISM IN OTHER INSECTS

In *Rhodnius*, then, the migration of the spermatozoa takes place as a result of contractions set up in the oviduct by the opaque accessory secretion from the male acting through a nervous system. Is there any evidence that a similar mechanism operates in other insects? Of course a number of insects do not produce a spermatophore, but transfer the semen direct to the spermathecae as in *Oncopeltus* (Bonhag & Wick, 1953). Khalifa (1949*a*) has described the spermatophore in *Gryllus* and demonstrated that the migration is brought about by pressure from within the spermatophore, but this type of sperm transfer appears to be unique among the insects. For other species with more typical spermatophores there is in the literature a considerable body of circumstantial evidence that a mechanism similar to the one described for *Rhodnius* may exist. The brief survey to follow presents some of this evidence.

Khalifa (1950*b*) mentions a milky granular secretion associated with the spermatophore of the cockroaches *Blattella* and *Periplaneta*. In *Periplaneta* the milky secretion originates in the complex accessory glands of the male (Gupta, 1947), and in the present study it was found that a suspension of these glands in insect Ringer would activate the oviducts of *Rhodnius* in the same way as the opaque secretion from the *Rhodnius* male. Similarly, the opaque secretions of the reduviid, *Triatoma infestans* causes contractions in the oviduct of *Rhodnius*. Khalifa (1949*b*) mentions a granular secretion in the semen of Trichoptera and describes powerful contractions in the bursa of Neuroptera which also possess an opaque secretion.

Several authors (Omura, 1938; Musgreave, 1937; Norris, 1932) have suggested that the migration of the spermatozoa along a complicated route from the bursa to the receptaculum seminis in Lepidoptera may be a result of contractions in the ducts involved, and Hewer (1934) has seen spermatozoa being propelled in this way along the ductus seminalis of *Zygaena*.

In the Coleoptera, Khalifa (1948) has suggested that the spermatophore in *Coccinella* is emptied by muscular contractions in the bursa and has observed a granular secretion between the spermatophore and the wall of the bursa. Preliminary observations on *Tenebrio molitor* in the present work have revealed rhythmic contractions of the ducts of females carrying spermatophores; these contractions are absent in unmated females. A granular secretion is present in the semen.

SUMMARY

1. Removal of the opaque accessory glands from a male *Rhodnius* prevents the normal migration of the spermatozoa in a female which is inseminated by it.
2. The opaque accessory secretion induces rhythmic contractions in the oviducts, probably by acting through a peripheral nervous system.
3. Paralysis of the muscles of the female without interfering with the motility of the spermatozoa prevents the ascent of the spermatozoa.
4. Killing the spermatozoa in the bursa has no effect on their subsequent migration.

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5. The central nervous system is not essential for the migration.
6. It is concluded that the migration of spermatozoa in the female of *Rhodnius prolixus* is a result of rhythmic contractions set up in the oviduct by the opaque accessory secretion of the male acting through a peripheral nervous system. Evidence in the literature suggests that the mechanism may operate in a number of spermatophore-forming species.

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