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# MECHANISM OF THE SPERMATOPHORIC REACTION IN THE GIANT OCTOPUS OF THE NORTH PACIFIC, *OCTOPUS DOFLEINI MARTINI*

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## INTRODUCTION

In the giant octopus of the North Pacific, *Octopus dofleini martini*, the male reproductive tract contains up to 10 spermatophores. Each is about a metre long. It has a tube-shaped body surrounded by a tough, thin membrane, the 'outer tunic'. The body consists of two parts. The thicker part, which during copulation emerges first from the 'penis', contains the sperm rope with $10^8$–$10^{10}$ tightly packed spermatozoa, surrounded by a viscous, colourless fluid, the spermatophoric plasma. The thinner part, which emerges second, consists of the rod-shaped, hyaline ejaculatory apparatus. The space between the sperm rope and ejaculatory apparatus is filled with the so-called cement liquid, usually amber coloured and even more viscous than the spermatophoric plasma. Attached to the thin distal end of the body is the 0·5 m long filamentous cap thread; this is the portion of the spermatophore, which is the last to emerge during copulation from the 'penis'.

Copulation lasts about 2 h, during which time one or two spermatophores undergo the so-called spermatophoric reaction. As a result of this reaction the ejaculatory apparatus everts and the sperm rope advances the entire length of the spermatophore, to be finally ejected into the 'spermatophoric bladder', a portion of the tunic which assumes the size and shape of a hen's egg. Subsequently, the 'bladder' bursts inside the female's reproductive tract, releasing the spermatozoa. Fertilization then takes place, and the fertilized eggs are deposited in the sea water where further embryonic development follows.

Some of the outstanding morphological and chemical characteristics of spermatozoa, and the main phases of the spermatophoric reaction in *Octopus dofleini martini*, have been described in the earlier papers (Mann, Martin & Thiersch, 1966, 1970; Martin, Thiersch, Dott, Harrison & Mann, 1970; Brooks, Lutwak-Mann, Mann & Martin, 1971; Mann, Karagiannidis & Martin, 1973; Martin, Lutwak-Mann, McIntosh & Mann, 1973).

Under *in vitro* conditions, that is, in a spermatophore extracted manually from the male's reproductive tract and transferred for observation into sea water, the start of the spermatophoric reaction is indicated by swelling of the spermatophore due to influx of sea water. The sperm rope begins to advance and uncoil, while the ejaculatory

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apparatus moves towards the distal (*in vivo* female-oriented) end of the spermatophore. Next, usually within ½ h of placing the spermatophore in sea water, the cap, located at the distal end of the spermatophore's body, suddenly ruptures, and this is followed immediately by the extrusion of the ejaculatory apparatus. Sea water continues to enter the intraspermatophoric space. Consequently, the volume of fluid in that space increases steadily, while the ejaculatory apparatus is pushed still further, undergoing progressive evagination. This phase of the spermatophoric reaction usually lasts over 1 h until evagination is completed and the distal end of the spermatophore is transformed into the spermatophoric bladder.

As a result of the influx of sea water during the spermatophoric reaction, the spermatophoric plasma undergoes an approximately fivefold dilution. Consequently, its dry weight and protein and potassium concentrations decline, while the sodium and chloride concentrations increase. In addition to these changes, however, there are a number of other changes, both physical and chemical, which occur in the course of the spermatophoric reaction. The two most remarkable ones, which form the subject of the present paper, are a characteristic alteration in the hydrostatic pressure within the spermatophore, and the diffusion of nitrogenous organic material and carbohydrate, originally bound to protein, across the outer tunic, into the surrounding sea water.

## MATERIAL AND METHODS

*Animals.* The males, 12–37 kg in weight, were trapped in the Puget Sound–San Juan Archipelago area during the period January–May. They were brought alive to Seattle in plastic bags filled with sea water, and on arrival in the laboratory were transferred to tanks filled with constantly aerated sea water, maintained at 8–10 °C. In these tanks the animals survived well for several months. From such animals the spermatophores were pulled out either by direct manipulation of the 'penis', that is, the end portion of the terminal spermatophoric duct, or, after dissection, from the spermatophoric sacs (Needham's sacs).

*Spermatophoric reaction.* The course of the spermatophoric reaction was followed *in vitro*, that is, in spermatophores placed in trays filled with sea water; the procedure employed for that purpose, and the terms used in designating the main steps in the spermatophoric reaction were as described by Mann *et al.* (1970). In some of the pressure-recording experiments, the initial phase of the spermatophoric reaction was accelerated, either by tugging at the cap-thread or in addition pressing the spermatophore gently with a finger about 1 cm proximally to the cap, thus creating just enough pressure to cause the cap to break, and the ejaculatory apparatus to be extruded.

*Pressure recording.* Pressure inside spermatophores was measured in 27 experiments: in 13 spermatophores throughout the whole period of the spermatophoric reaction, and in the remaining ones during the early stages only.

The indwelling catheters used for registering the pressure changes consisted of 20–25 cm long polyethylene tubes with a 0·3 mm inside diameter, and a 0·6 mm outside diameter. After filling with sea water each catheter was attached to a Statham P23De pressure transducer (Franke, 1966) by means of a 27-gauge hypodermic needle on a three-way stopcock. The pressure transducer was connected by means of appro-

riate strain-gauge couplers to either a Type-R Beckman-Dynograph or to a model-60 Sanborn recorder. The accuracy of the transducer and of the recording system was checked by means of a water manometer, both before and at the end of each experiment. A syringe connected to the three-way stopcock permitted flushing of the catheter to remove all bubbles of air from the stopcock and catheter.

Prior to insertion into a spermatophore the end of the catheter was cut at an angle of 45° and a strand of 000 surgical silk thread was tied firmly at a distance of 1–2 cm from the bevelled end. Insertions were made at two points. In all spermatophores that had been pulled out freshly from the animals the catheter was inserted at a point located about 2 cm from the proximal end, as shown in Pl. 1 *a*, but in addition in some experiments, after completion of the spermatophoric reaction, a second catheter was inserted into the spermatophoric bladder, as shown in Pl. 1 *b*.

In early experiments the spermatophores were transferred straight into the trays filled with sea water, and then the catheters were inserted. Later, so as to prevent any influx of sea water which might have occurred before a catheter could be introduced, spermatophores were placed on paper towels which were dampened only slightly with sea water, the catheters were inserted, and only then were the spermatophores immersed in sea water. In order to insert the cut end of the catheter into the spermatophores in such a way as to minimize loss of spermatophoric plasma, the following procedure was developed. First, a point on the outer tunic was selected, located at a distance of about 2 cm from the proximal end of the spermatophore, and marked with a small drop of methylene blue. Then, a round taper-point surgical needle was pushed through the marked area, straight into the space filled with spermatophoric plasma, thereby creating a hole about 0·5 mm wide. Next, the needle was withdrawn and in its place the bevelled end of the catheter was inserted with the greatest possible speed (so as to prevent leakage of spermatophoric plasma) but in such a way as to leave the strand of the silk thread within a few millimetres outside the hole. Immediately afterwards the thread was wound around the spermatophore and secured tightly enough to prevent the internal pressure from rejecting the catheter, but not so tightly as to deform or injure the outer tunic. Finally, a small square of Teflon tape was put over the site of entry of the catheter, and tied in place with a silk thread to help keep the catheter inside the spermatophore during the whole period of observation.

*Osmolality determinations.* Osmolality was determined by measuring the freezing-point depression in the Fiske Osmometer (Fiske Association Inc., Bethel, Conn.) and the results were calculated according to Geigy's Scientific Tables (Geigy Pharmaceutical Co. Ltd., Manchester).

*Analysis of sugars, aminosugars, total nitrogen and protein.* Total carbohydrate was determined by the orcinol method (Vasseur, 1948); and total aminosugar, after hydrolysis in 2N-HCl for 24 h at 100 °C, according to Stewart-Tull (1968). Glucose was determined by the glucose oxidase-tolidine method (Middleton & Griffith, 1957), fructose by the resorcinol method (Roe, 1934; Mann, 1946), galactose by galactose dehydrogenase (Baronos, 1971), and pentose according to Mejbaum (1939).

Paper chromatography of carbohydrates was carried out with ethyl acetate:pyridine:water (8:2:1) as solvent (Mann & Rottenberg, 1966), and the reducing sugars were located according to Trevelyan, Proctor & Harrison (1950); prior to chromato-

graphy, the samples were de-ionized in ion-exchange columns containing Amberlite IR-120[$H^+$] at the bottom, and Dowex AG-1[$OAc^-$] on top.

Nitrogen was determined by the micro-Kjeldahl procedure using Markham's distillation apparatus (King & Wootton, 1956).

## RESULTS

### *Changes in turgidity and intraspermatophoric pressure, associated with the spermatophoric reaction*

A spermatophore freshly pulled from the male is already in a state of such high turgidity that a small injury to the outer tunic may cause its entire contents, about 10 ml, to erupt violently. This turgidity corresponds, as Table 1 shows, to a mean transmural pressure of 143 cm $H_2O$, but individual spermatophores differ greatly in that respect. As soon as a spermatophore has come in contact with sea water, its pressure and turgidity increase due to the influx of sea water. The fact that it is the influx of sea water which provides the prime mechanism for these changes, and thereby initiates the spermatophoric reaction, is based on a variety of observations, the most convincing one being that pressure begins to rise only after a spermatophore has been brought in contact with sea water, and this does not occur at all for as long as it is kept on the damp towel in air. In some experiments periods of up to 2 h were allowed prior to the transfer of a spermatophore to sea water, but in none of such experiments did the pressure change during these periods.

Supporting evidence for the conclusion that the spermatophoric reaction is triggered off by sea water was obtained in the following series of experiments.

In one experiment two catheters, instead of one, were inserted near each other into the proximal portion of a spermatophore lying on a damp towel. By means of a syringe sea water was injected into the spermatophore through one catheter while the other was used to record the pressure changes. The events during this experiment resembled the course of a normal spermatophoric reaction: extrusion of the ejaculatory apparatus occurred when pressure reached 230 cm $H_2O$, and a spermatophoric bladder was formed after evagination of the ejaculatory apparatus at pressures no higher than 70 cm $H_2O$. There was, however, one important difference between the spermatophoric reaction induced by the injection of sea water from a syringe, and one that occurs normally when a spermatophore is placed in a sea-water bath: namely, the time interval between the extrusion of ejaculatory apparatus and bladder formation was only 12 min in the injected spermatophore, compared with 53–99 min in spermatophores undergoing a normal reaction (Table 1).

In another experiment the injection of sea water into a spermatophore was interrupted in advance of the expected moment of bladder formation. This brought the spermatophoric reaction to a halt. But as soon as injection was resumed, the reaction resumed its expected course, culminating in the formation of the spermatophoric bladder. Obviously, the rate at which sea water enters the spermatophore constitutes an important factor in regulating the speed of the spermatophoric reaction.

A complete record of a typical experiment, in which pressure changes have been followed throughout the whole course of a spermatophoric reaction, is presented in Text-fig. 1. As can be seen, in this particular experiment the pressure initially re-

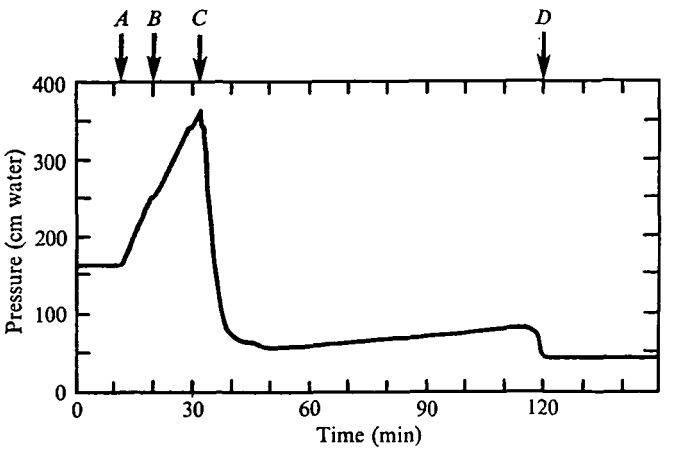
Table 1. *Changes in transmural pressure of* Octopus dofleini martini-*spermatophore during the spermatophoric reaction* in vitro, *at* 10 °C

(The onset of spermatophoric reaction is defined as the moment of placing the spermatophore in sea water. The time interval between the onset of the reaction and the rupture of the cap (which immediately precedes the extrusion of the ejaculatory apparatus) was shortened in Expts 1–5 by pulling the cap thread and in Expts 6–15 by compressing the cap as well. Evagination of the ejaculatory apparatus is defined as the event which immediately precedes the formation of the spermatophoric bladder.)

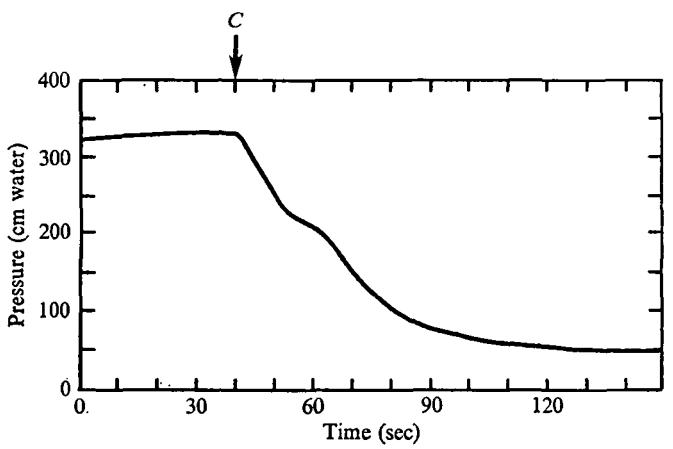
| Expt no. | Pressure prior to onset of reaction (cm $H_2O$) | Rate of pressure rise prior to rupture of cap (cm $H_2O$/min) | Peak pressure just before cap bursts (cm $H_2O$) | Time elapsed during evagination of ejaculatory apparatus (min) | Mean pressure during evagination of ejaculatory apparatus (cm $H_2O$) | Pressure at end of reaction (cm $H_2O$) in: Proximal portion of spermatophore | Pressure at end of reaction (cm $H_2O$) in: Spermatophoric bladder |
|---|---|---|---|---|---|---|---|
| 1 | 145 | 8 | 360 | — | 80 | — | — |
| 2 | 155 | 10 | 355 | 87 | 70 | 40 | 5 |
| 3 | 195 | 7 | 345 | 67 | 45 | 30 | — |
| 4 | — | 7 | 320 | 83 | 60 | 40 | — |
| 5 | 100 | 5 | 280 | 87 | 65 | 60 | — |
| 6 | — | 7 | 320 | 96 | 60 | 30 | 13 |
| 7 | — | 9 | 315 | 56 | 55 | 15 | — |
| 8 | — | 8 | 310 | 73 | 70 | 45 | 10 |
| 9 | 180 | 10 | 240 | 53 | 60 | 15 | — |
| 10 | 140 | 6 | 235 | 99 | 75 | 45 | 14 |
| 11 | — | 12 | 225 | 81 | 60 | 20 | — |
| 12 | 165 | 6 | 210 | 79 | 65 | 35 | — |
| 13 | 125 | 9 | 200 | 88 | 80 | 45 | 2 |
| 14 | 90 | 4 | 200 | 94 | 65 | 40 | 7 |
| 15 | 130 | 8 | 185 | 92 | 95 | 50 | — |
| Mean ± 1 S.D. | 143 ± 31 | 8 ± 2 | — | 81 ± 14 | 67 ± 12 | 36 ± 13 | 9 ± 4 |

corded inside the proximal end-portion of the spermatophore was 155 cm $H_2O$, and it remained constant at that level until the spermatophore was brought into contact with sea water. In a series of other similar experiments, listed fully in Table 1, the initial intraspermatophoric pressure varied from 90 to 195 cm $H_2O$; the mean value was 143. However, as soon as contact with sea water was permitted, the pressure began to rise. In the experiment recorded in Text-fig. 1, the pressure rose at a rate of about 10 cm $H_2O$/min, and reached a maximum of 355 cm $H_2O$ at the moment when the cap burst, prior to the extrusion of the ejaculatory apparatus. The events in other experiments followed a closely similar pattern; the lowest peak-value observed for pressure at the moment when the cap burst without manual compression was 280 cm $H_2O$, and the highest 360 cm $H_2O$; the calculated mean value was 330 cm $H_2O$.

As soon as the ejaculatory apparatus begins to be extruded, the pressure falls precipitously. In the experiment recorded in Text-fig. 1 the pressure fell from 355 cm $H_2O$ down to 60 cm $H_2O$. How quickly such a change occurs can best be judged from Text-fig. 2 which represents the record of a similar experiment, but taken at an expanded time scale. From the moment when the ejaculatory apparatus is extruded, and throughout the period of the next 90 min or so, the sea water that enters the spermatophore causes primarily eversion of the ejaculatory apparatus rather than increase of pressure. The pressure inside the spermatophore rises very slowly, and




Text-fig. 1. Changes in transmural pressure (cm $H_2O$) during the spermatophoric reaction *in vitro*, at 10 °C, recorded at the proximal (male-oriented) end of the spermatophore of *Octopus dofleini martini*. Prior to the onset of the spermatophoric reaction, pressure was recorded while the spermatophore was resting on damp towel in air. The arrows indicate the following: *A*, spermatophore has been covered with sea water; *B*, cap-thread was pulled free (when pressure reached 250 cm); *C*, cap has just burst (when pressure reached 355 cm); *D*, evagination of the ejaculatory apparatus has been completed with the resulting formation of spermatophoric bladder.




Text-fig. 2. Changes in transmural pressure (cm $H_2O$) at the moment of the extrusion of the ejaculatory apparatus which follows the rupture of the cap. Conditions as in the experiment depicted in Text-fig. 1 except that the time scale was expanded.

only to a small extent. In the experiment shown in Text-fig. 1, for instance, the pressure rose from 60 to 80 cm $H_2O$ during a period of 60 min. In other experiments (Table 1) the evagination pressures were similarly low; the mean value for the pressure was 68 cm $H_2O$.

The final stage of the spermatophoric reaction, that is, the formation of the spermatophoric bladder, is accompanied by a fall in pressure in the proximal portion of the spermatophore. This fall is not nearly as great as the one which occurred earlier during the spermatophoric reaction at the moment of the cap-break. Nevertheless,

The pressure gradient between the two ends of the spermatophore, which is thereby established, is sufficient to bring the evagination process to sudden completion, and to permit the ejection of the sperm rope into the spermatophoric bladder. In the experiment shown in Text-fig. 1 the pressure in the proximal end-portion of the spermatophore was 70 cm $H_2O$ just prior to the formation of the spermatophoric bladder and 40 cm $H_2O$ after the event; the mean values for the final pressure in the proximal portion of the spermatophores (Table 1) were: 67 cm $H_2O$ before, and 36 cm $H_2O$ just after the formation of the spermatophoric bladder. Considerably lower pressures prevailed in the spermatophoric bladders where the mean final pressure was 9 cm $H_2O$ (Table 1).

Normally, after a spermatophore had been placed in a sea-water bath it took up to 30 min for the cap to rupture and for the ejaculatory apparatus to be extruded. This time interval can be shortened by the two procedures already mentioned in 'Material and Methods', namely, pulling the cap thread or compressing the cap itself. Either one or the other of these two procedures was employed in the experiments listed in Table 1. In addition, however, there were other experiments in which no attempt was made to accelerate the course of the spermatophoric reaction. Under such circumstances the cap sometimes did not rupture at all, but the pressure continued to rise until the outer tunic ruptured some distance from the cap and from the catheter. When that happened the sperm rope erupted and the spermatophoric reaction was thereby brought to an abrupt end. On two such occasions we were able to record the pressure just prior to the rupture of the outer tunic. In one of these two experiments the time interval between placing the spermatophore in sea water and the rupture of the outer tunic was 140 min, and the peak pressure just prior to the break was 450 cm $H_2O$. In the other experiment the corresponding time interval was 58 min, and the peak pressure was 410 cm $H_2O$. Both pressure values are well in excess of those usually encountered in spermatophores just prior to the extrusion of the ejaculatory apparatus; in spermatophores undergoing normal spermatophoric reaction (Table 1) we never encountered a pressure higher than 360 cm $H_2O$. From this we may conclude that the maximum intraspermatophoric pressure which the outer tunic is capable of withstanding without bursting, must be in the region of 400 cm $H_2O$.

### *Osmolality of the exterior and interior milieu of the spermatophore*

When studying the influence of the spermatophoric reaction on the osmolality of the spermatophoric plasma, Mann *et al.* (1970) noticed that in spite of the increase in NaCl concentration the osmolality of the spermatophoric plasma remained practically unaltered from the onset of the spermatophoric reaction to the time of bladder formation. This finding was confirmed in the present study. In addition to the spermatophoric plasma, however, freezing-point determinations were also carried out in the sea water surrounding the spermatophores. Four spermatophores were used for this purpose, all of them pulled out freshly from the Needham's sac of the same male. They were placed separately, each in 150 ml of sea water, and the difference in osmolality between the external and interior milieu was determined after different time intervals. These determinations showed that the osmolality of spermatophoric plasma does not only remain fairly constant during the spermatophoric reaction but it is also persistently higher than that of the surrounding sea water. The difference,

Table 2. *Passage of organic material from the interior of the spermatophore to the surrounding sea water* (110 *ml*), *during the spermatophoric reaction in* Octopus dofleini martini

(Results are expressed in mg/total volume of external milieu, and mg/total volume of fluid recovered from the spermatophoric bladder.)

| Material | Total nitrogen | Orcinol-reactive carbohydrate | Total aminosugar |
|---|---|---|---|
| Sea water, immediately after the spermatophore had been brought in contact with it | 0·9 | 1·2 | 0·1 |
| Sea water, 23 min later, just prior to the rupture of the cap and extrusion of ejaculatory apparatus | 8·3 | 11·5 | 4·3 |
| Sea water, 1 h 30 min from the onset of spermatophoric reaction, just prior to the appearance of spermatophoric bladder | 112·0 | 33·7 | 23·5 |
| Fluid contents of the spermatophoric bladder (centrifuged supernatant) | 588·5 | 405·7 | 371·0 |

expressed in milli-osmols/kg water, was as follows: just prior to the extrusion of the ejaculatory apparatus (that is, at the peak of intraspermatophoric pressure), 32; immediately after the extrusion has taken place (that is, when pressure has suddenly fallen), 20 in one spermatophore, and 28 in another spermatophore; and just prior to the formation of the spermatophoric bladder, 28.

### *Passage of organic material from the interior to the exterior milieu of the spermatophore*

As reported earlier (Mann *et al.* 1970), some of the bound carbohydrate, aminosugar and amino acids present in the spermatophoric plasma, is in a dialysable form; several glycopeptides, unusually rich in aspartic acid and serine, have been identified in the dialysates. In the present study an attempt was made to determine how much organic material passes from the interior milieu of the spermatophore across the outer tunic into the surrounding sea water during the spermatophoric reaction.

In Table 2 are set out the results of an experiment in which a spermatophore was allowed to undergo the spermatophoric reaction in the presence of 110 ml sea water, and samples of the sea water were withdrawn for analysis: (i) within a few sec of placing the spermatophore in sea water, (ii) 23 min later, just prior to the extrusion of ejaculatory apparatus, and (iii) at 1 h 30 min, immediately after the formation of the spermatophoric bladder. In addition, at the end of the experiment, the contents of the spermatophoric bladder were centrifuged, and the supernatant was taken for analysis.

From the results of this experiment it is clear that some orcinol-reactive carbohydrate and aminosugar passes from the interior to the exterior milieu of the spermatophore during the spermatophoric reaction. But even more striking is the large proportion of total nitrogen that leaves the spermatophore in the course of the spermatophoric reaction and enters the sea water. Some of that nitrogen, especially during the early stage of the reaction, that is, prior to the extrusion of the ejaculatory apparatus, could only have reached the exterior milieu by passing directly through the tunic. More complicated, however, may be the mechanism underlying the

passage of nitrogenous substances subsequent to the extrusion of the ejaculatory apparatus, when owing to the rupture of the cap some fluid may possibly escape into the sea water at the point where the membrane had ruptured. To investigate this possibility we managed to collect four samples of the viscous fluid which oozes out from spermatophores at the very moment of the cap detachment; 10–50 $\mu$l could be collected this way from each spermatophore. In the pooled material from four spermatophores, 0·224 mg N/0·01 ml was found, an amount which is small in comparison with the total amount of nitrogen that passes into sea water during the whole of the spermatophoric reaction, but which, nevertheless, must be taken into account in the final assessment of the results.

The identification of free sugars passing into the exterior milieu during the spermatophoric reaction, met with difficulty on account of the high concentration of NaCl, about 3%, in sea water. For this reason another experiment was carried out, similar in design to the one described in Table 2 except that distilled water was used instead of sea water as the external medium. The total amount of orcinol-reactive carbohydrate (which includes both free and bound sugar) which accumulated under these conditions outside the spermatophore, at the end of the spermatophoric reaction, was 12·6 mg (as compared with 33·7 mg in the experiment recorded in Table 2); and the total nitrogen content of the external medium was 37·6 mg N (as compared with 112·0 mg in the experiment recorded in Table 2). After the medium had been concentrated under reduced pressure, and de-ionized on the Amberlite-Dowex column, it was subjected to paper chromatography. The two main spots revealed by the use of the silver-spray reagent corresponded to $R_F$ values of mannose and ribose; three weaker spots were also present, corresponding roughly in $R_F$ values to inositol, glycerol and glucose. The identity of glucose was confirmed further by the use of glucose oxidase. Fructose and galactose were not detectable. The total pentose content of the external medium corresponded to 2 mg of ribose, when determined by Mejbaum's method.

## DISCUSSION

The spermatophoric reaction as observed *in vitro*, that is, in a spermatophore manually recovered from the male *Octopus dofleini martini*, and placed in sea water at 10 °C, usually requires 1–2 h for completion. This interval corresponds closely to the period of time required by the spermatophoric reaction *in vivo*, that is, in the normal course of copulation (Mann *et al.* 1970). During that period of time, as a result of infiltration by sea water, the volume of fluid inside the spermatophore increases gradually from about 10 ml initially, to about 50 ml at the end of the reaction. As a consequence of the large sea-water influx the whole mass of spermatozoa, initially confined to the proximal (male-oriented) portion of the spermatophore, is propelled over a metre-long distance to the distal (female-oriented) portion, to be finally ejaculated into the spermatophoric bladder. Such a striking performance obviously requires a driving force, and involves, apart from the influx of sea water, a number of factors including limited elasticity of the spermatophore's membrane and the extraordinary ability of the compressed and invaginated ejaculatory apparatus to undergo, during the spermatophoric reaction, first an extrusion and next a complete evagination prior to the formation of the spermatophoric bladder.

As a result of the present study, and in conjunction with the results of previous observations (Mann *et al.* 1970), the following conclusions can now be drawn as regards the most likely mechanism of the spermatophoric reaction.

The increase in the volume of intraspermatophoric fluid, caused by the influx of sea water, is accompanied by a steady rise in the concentration of sodium and chloride ions and a concomitant decrease in dry weight (initially, nearly 30 g dry wt/100 ml) and in concentration of potassium ions and organic matter (made-up largely by glycoproteins). However, in spite of the fivefold dilution and drastic changes in chemical composition, the intraspermatophoric fluid does not markedly change its osmolality throughout the whole period of the spermatophoric reaction, but remains at a level of about 30 mOsm/kg water above that of sea water. A relationship of this kind suggests immediately that the mechanism underlying the spermatophoric reaction is one of osmoregulation, and must be governed by the Donnan distribution law.

In line with the concept that the spermatophore acts as an 'osmotic cell', and its outer tunic as a 'semipermeable membrane', permeable to sea water but not to macromolecules, are a number of additional observations, including those on the behaviour of spermatophoric transmural pressure.

As soon as a spermatophore is brought in contact with sea water its turgidity and transmural pressure, already on the average 143 cm water higher inside than outside (see Table 1), increase rapidly. The strength and elasticity of the outer tunic are such that it can withstand the rise in pressure up to about 400 cm water without bursting. Usually, however, if the cap thread has been pulled, the pressure inside the spermatophore reaches about 330 cm $H_2O$ (Table 1) when the cap of the spermatophore ruptures and permits the extrusion of the ejaculatory apparatus.

The rate at which pressure rises inside the spermatophore *in vitro* during the time interval between the onset of the spermatophoric reaction and the moment of cap rupture has been measured in a number of experiments. It varied around a mean value of 8 cm $H_2O$/min (Table 1). At that rate of progress, a pressure rise from 140 cm $H_2O$ initially, to 330 cm $H_2O$ at the moment when the ejaculatory apparatus is extruded, requires $(330-140)/8 = 24$ min. This means that under similar conditions *in vivo*, a copulating male would have about 24 min to pull out a spermatophore from the 'penis', and to manoeuvre the spermatophore into a position close to the external orifice of the female reproductive tract. Unless this event has taken place by that time, the pressure within the spermatophore would continue to mount, soon reaching a value at which the outer tunic is no longer capable of resisting the influx of sea water and must burst.

As regards the actual events *in vivo*, our observations lead us to conclude that the cap thread is pulled off well in advance of the time limit of 24 min, most probably within less than 1 min after the cap thread has emerged from the 'penis' during copulation. The cap thread, it should be recalled, is the last portion of the spermatophore to emerge from the 'penis'. Once it has emerged, however, it is seized promptly by the male's siphon, and as a result of the movements of the siphon it is pulled away quickly from the hectocotylized arm with the result that the cap ruptures and the pressure is thereby lowered much earlier than during a spermatophoric reaction *in vitro*. The risk of a spermatophore rupturing *in vivo* is therefore very much smaller than under *in vitro* conditions.

Our experiments *in vitro* have shown that subsequent to the extrusion of the ejaculatory apparatus the pressure inside the spermatophore falls precipitously (Text-figs. 1, 2), but not below a level of 45–90 cm water. Next it increases once more, but very slowly, until the evagination of the ejaculatory apparatus is nearly complete. Then it drops again, just a few minutes before the formation of the spermatophoric bladder (Text-figs. 1, 2). As the spermatophoric bladder is formed and the sperm rope ejected into it, the pressure inside the proximal portion of the spermatophore continues to decline. When finally the process of the bladder formation has been completed, the pressure inside the bladder is found to be no more than a few cm $H_2O$ higher than in the surrounding sea water. But inside the proximal portion of the spermatophore, now devoid of spermatozoa, the pressure is still about 36 cm $H_2O$ (Table 1). This difference in pressure between the two compartments could not have been established without the development of an effective, physical barrier between them. This barrier, it would seem, is formed as the result of a constriction, and subsequent closure, of one segment of the outer tunic, mainly near the site formerly occupied by the cap. From this and the other observations made in our study we conclude that a pressure gradient constitutes a particularly important factor in the process which leads to sperm transfer during the spermatophoric reaction along the metre-long spermatophore.

*In vivo*, that is during copulation, the time course of the events associated with the evagination of the ejaculatory apparatus and the formation of the spermatophoric bladder appears to be the same as during a spermatophoric reaction proceeding in a tray filled with sea water. The process of evagination *in vivo*, however, takes place along the hectocotylized arm, and the terminal portion of that apparatus is guided and lodged by the hectocotyle into the oviduct before it balloons out into the spermatophoric bladder.

The outer tunic, though essentially a 'semipermeable membrane', is not entirely devoid of permeability to organic substances. It was shown earlier (Mann *et al.* 1970) that molecules of the size of cytochrome *c*, but not much larger, can pass across the outer tunic of spermatophores artificially filled with protein solutions. The present study brings further evidence that carbohydrate and also quite a substantial amount of organic nitrogenous material pass from the interior to the exterior of a spermatophore during the spermatophoric reaction. Since the bulk of organic material in spermatophoric plasma is made up of large-molecular mucoid substances, it would appear that some of that material must be undergoing enzymic breakdown. Particularly relevant in this respect may be the recently discovered presence of several highly active glycosidases in the spermatophores and male accessory secretions of *Octopus dofleini martini* (Mann *et al.* 1973). Much of that activity, particularly as regards $\alpha$-mannosidase and $\beta$-*N*-acetylglucosaminidase, was shown to be concentrated in the so-called cement liquid, that is, the material located within the spermatophore at the junction of the sperm rope and the ejaculatory apparatus. Glycosidases are a group of enzymes well known to catalyse the degradation of glycoproteins. Their involvement in the spermatophoric reaction may well constitute part of the mechanism of osmoregulation which controls the various stages of that reaction.

SUMMARY

1. The spermatophoric reaction in the giant octopus requires 1–2 h, and during that time the large mass of spermatozoa contained in the proximal (male-oriented) portion of the spermatophore, is propelled over a metre-long distance to the distal (female-oriented) end.

2. Osmotic pressure provides the main mechanism for the spermatophoric reaction; and the influx of sea water, as a result of which the intraspermatophoric fluid space increases fivefold, provides the main force for driving the spermatozoa from the proximal to the distal end of the spermatophore.

3. The outer tunic of the spermatophore acts as a semipermeable membrane, permeable to sea water but not to macromolecular substances. Its strength is adequate to resist inside pressure 400 cm water in excess of the external environment.

4. In the normal course of spermatophoric reaction intraspermatophoric hydrostatic pressure increases from about 140 cm water at the onset of the reaction to about 330 cm $H_2O$ at the moment when the cap of the spermatophore ruptures and the ejaculatory apparatus is extruded; the rate at which pressure increases during this phase of the reaction is about 8 cm $H_2O$/min.

5. Immediately after the extrusion of the ejaculatory apparatus the pressure falls precipitously. It then increases once more, but very slowly, reaching 45–95 cm $H_2O$ before the ejaculatory apparatus evaginates. It declines once more during the final stage of the evagination process. At the end of the spermatophoric reaction the transmural pressure is only about 9 cm $H_2O$ in the spermatophoric bladder but approximately 4 times higher in the proximal portion of the spermatophore.

6. In spite of the increase in the concentration of sodium and chloride ions in the intraspermatophoric fluid, the osmolality of that fluid remains largely unaltered throughout the whole spermatophoric reaction, but always at a level distinctly higher than the surrounding water (by about 30 mOsm/kg water).

7. Large-molecular glycoproteins in the spermatophoric plasma are probably undergoing enzymic breakdown by glycosidases during the spermatophoric reaction, as a result of which some carbohydrate, aminosugar and other nitrogenous organic material pass from the interior to the exterior of the spermatophore.

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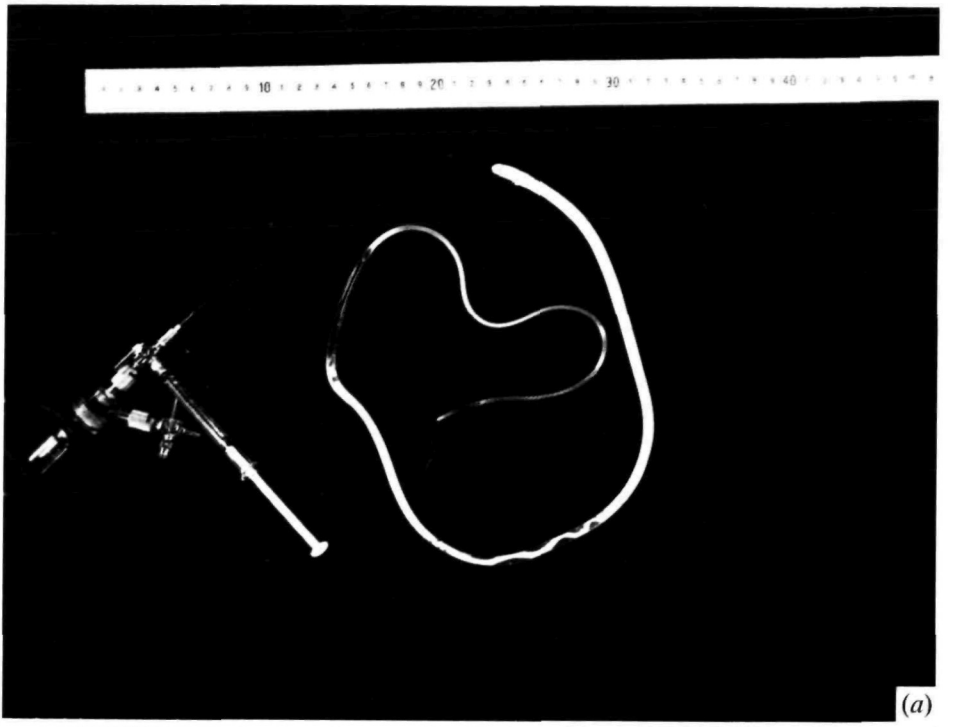
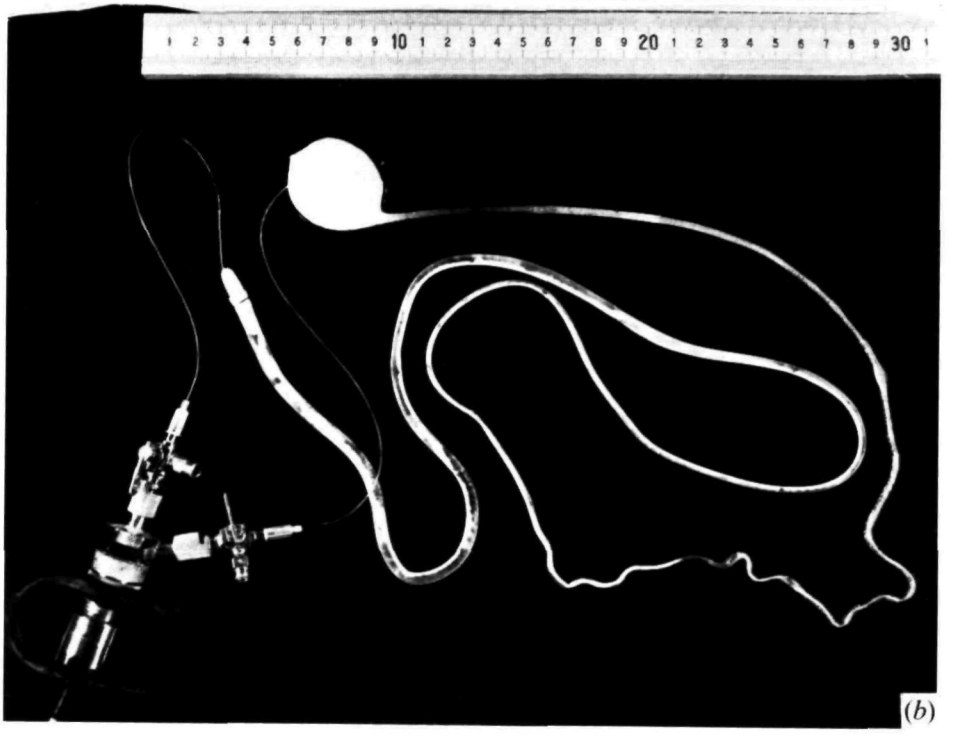

(a)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(b)

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## EXPLANATION OF PLATE

(*a*) Spermatophore of *Octopus dofleini martini* before placing in sea water, i.e. prior to the spermatophoric reaction. A catheter connected with the pressure transducer has been inserted into the proximal (male-oriented) end of the spermatophore. A short fragment of the cap-thread can be seen at the female-oriented end of the spermatophore.

(*b*) Spermatophore of *Octopus dofleini martini* at the end of the spermatophoric reaction. In addition to the catheter in the proximal end of the spermatophore, a second catheter has been inserted at the distal end, that is, into the spermatophoric bladder. Appropriate adjustment of two stopcocks permits pressure to be measured at either end of the spermatophore with the same pressure transducer. In order to perform the experiment syringes filled with sea water had to be attached to the sidearm of each stopcock. The syringes have been removed to simplify the figures.