THE PHYSIOLOGY OF SEA-URCHIN SPERMATOZOA
LACK OF MOVEMENT IN SEMEN
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(With Two Text-figures)

INTRODUCTION

The eggs and spermatozoa of many organisms contain and secrete substances with fairly well-defined physiological properties. A knowledge of the chemical and physiological characteristics of these substances may lead to a more detailed understanding of the fertilization reaction. In addition, they are of interest from other points of view. On the one hand, they may contribute to our knowledge of the mechanism of vibratile movement; on the other, they may shed some light on immunochemistry, as the work of Tyler (1948) has shown.

The secretions of eggs, particularly in the case of marine worms and sea-urchins, were originally called Fertilizin. Eggs were also said to contain Antifertilizin, and so were spermatozoa. At other times, the words Agglutinin, Iso-agglutinin, and Hetero-agglutinin have been used to describe these secretions. Although on historical grounds it might be held that the names Fertilizin and Antifertilizin have a prior claim, they are somewhat cumbersome, if not confusing. Fertilizin may include separate substances with different properties, while Antifertilizin is obtained from both eggs and spermatozoa. Hartmann & Schartau (1939) have suggested that the secretions of eggs and spermatozoa should be called Gynogamones (G.) and Androgamones (A.) respectively. These names might be thought to prejudge several issues, for it is not certain that all the gamete secretions are hormones in the usual sense (gamone = gamete hormone); nor is it yet clear that all the secretions have a gamete significance. Nevertheless, the nomenclature is convenient and often used in Sweden, Germany and Italy. In Table 1,* which summarizes existing gamete secretion data, the new nomenclature is adopted, but the old names are included for comparison and identification.

Table 1 contains two new and debatable features. First, Hetero-agglutinin is included as a separate substance from Iso-agglutinin. This is based on Just's observation (1930) that Hetero-agglutinin can be removed from Arbacia egg water by Nereis spermatozoa, though the full amount of Iso-agglutinin is left in the egg water. Secondly, a new substance, Gynandrogamone I (Antifertuizin), has been

* Individual papers have not been cited in Table 1. For these, reference should be made to Hartmann et al., Runnström et al. and Tyler in the References.
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added to the list. The reasons for the name will be clear from Table 1. The properties of this substance are strikingly similar to those of A. II, but according to Tyler (1939), GA.I can be separated from A. II. In our present state of knowledge, it therefore seems advisable to consider GA.I and Hetero-agglutinin as separate substances from G.I, G. II, A.I, A. II and A. III; but it may well be that reductions in the number of chemically separate gamones will be effected in the future, though the actual list of functions may remain the same.

Table 1

<table>
<thead>
<tr>
<th>Modern name</th>
<th>Former name</th>
<th>Biological properties</th>
<th>Chemical characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gynogamone I (G. I)</td>
<td>Fertilizin</td>
<td>Activates and/or stimulates spermatozoa; exerts chemotactic influence on spermatozoa</td>
<td>Heat resistant; claimed to be echinochrome, or to have echinochrome as prosthetic group, in Arbacia pustulosa</td>
</tr>
<tr>
<td>Gynogamone II (G. II)</td>
<td>Fertilizin or iso-agglutinin</td>
<td>Specifically agglutinates homologous spermatozoa; derived from jelly round eggs, not from eggs</td>
<td>Protein; heat labile</td>
</tr>
<tr>
<td></td>
<td>Hetero-agglutinin</td>
<td>Non-specifically agglutinates spermatozoa</td>
<td>Protein (?)</td>
</tr>
<tr>
<td>Androgamone I (A.I)</td>
<td>—</td>
<td>Paralyses spermatozoa and neutralizes G.I</td>
<td>Soluble in methanol; diffuses through cellophane; not inactivated by trypsin</td>
</tr>
<tr>
<td>Androgamone II (A. II)</td>
<td>—</td>
<td>Dissolves or precipitates jelly round eggs; neutralises G.II.</td>
<td>Protein; properties somewhat similar to those of Hyaluronidase</td>
</tr>
<tr>
<td>Androgamone III* (A. III)</td>
<td>—</td>
<td>Action similar to that of honeybee venom and detergents on surface of sea-urchin eggs</td>
<td>Soluble in methanol; diffuses through cellophane; not inactivated by trypsin</td>
</tr>
<tr>
<td>Gynandrogamone I (GA.I)</td>
<td>Antifertilizin</td>
<td>Combines with G.II; forms membrane on surface of jelly; obtained from eggs and spermatozoa</td>
<td>Protein; can be separated from A. II</td>
</tr>
</tbody>
</table>

* Also called Sperm Lysin (Runnström, 1946).

This paper is concerned with Androgamone I (A.I) which, according to Hartmann, Schartau & Wallenfels (1940), is responsible for spermatozoa being motionless in the testes of the sea-urchin, and for their senescence after they are mixed with sea water. Many explanations for the lack of sperm movement in non-mammalian semen have been put forward, though the variety of these explanations seems to have escaped the notice of several workers in this field. According to Hartmann et al. (1940), the hormone A.I is the responsible agent in the semen of Arbacia pustulosa and of the Rainbow Trout (Hartmann, 1944); the same conclusion was reached by Schartau & Montalenti (1941) in the case of the Lamprey, while von Medem (1945) appears to have confirmed the existence of A.I in certain Mollusces. No reference is made by Hartmann and his colleagues to an observation of Gray (1928) that sea-urchin spermatozoa are as motile in seminal plasma, obtained by centrifuging semen, as in sea water: nor is any reference made to the work of Schlenk, who concluded (at different times) that inactivation in trout semen is due to hydrogen ions, phosphate, or potassium ions (Schlenk, 1933; Schlenk & Kahmann, 1938). Runnström, Lindvall & Tiselius (1944) express some doubts about the role of A.I in sea-urchin semen, and
tentatively suggest that the absence of movement in this case is due to CO₂ narcosis or O₂ lack. They assert, however, that in salmon semen A.I is responsible for sperm inactivation before dilution. Some support for Hartmann's views on the role of A.I in sea-urchin semen is found in the observations of Southwick (1939) on *Echinometra subangularis*. Southwick states that sperm of this species are made motionless by seminal plasma obtained by centrifugation. This result is in contradiction to that of Gray (1928) already mentioned, and those of Hayashi (1945, 1946), who also found that seminal plasma has no adverse effect on the viability and fertilizing power of spermatozoa of the same species of sea-urchin.

In this paper the cause of sperm immotility in sea-urchin semen is established. In a subsequent paper the alleged action of Androgamone I in inducing senescence of spermatozoa after dilution in sea water will be examined, together with certain allied problems.

**MATERIAL**

The semen of *Echinus esculentus* was obtained in the usual way (Gray, 1928).

**METHOD**

*Gas chamber.* Visual observations on semen were made in a gas-tight chamber (Fig. 1). The top of the chamber was sealed with a cover-slip, on the underside of which was a thin film of semen. The chamber was kept at a constant temperature by water circulating round but not through it. Different gases, such as commercial nitrogen, partially purified by bubbling through alkaline pyrogallol towers, could be passed through the chamber, as shown in Fig. 1.

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**Fig. 1.** Gas chamber. 1, circulating water inlet; 2, gas inlet; 3, water outlet; 4, gas outlet; 5, circulating water chamber; 6, support for cover-slip at top of gas chamber; 7, upper cover-slip; 8, lower cover-slip.
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Oxygen electrode. The possibility of using platinum electrodes for measuring the \( O_2 \) tension in solution was first noted by Danneel (1897). Details of the theory of the Pt \( O_2 \) electrode and its application to biological material can be found in the papers of Davies & Brink (1942) and Hill (1948). The principle of the electrode, very briefly, is as follows: if a Pt electrode and some non-polarizable electrode, such as silver silver chloride wire, are immersed in an aqueous solution of an electrolyte, and a potential is applied between them, the Pt electrode being negative, the current through the system will remain almost constant over a fairly wide range of applied potential provided the solution is not too acid. The reaction at the Pt electrode (Hill, 1948) is probably

\[
O_2 + 4H^+ + 4e \rightarrow 2H_2O.
\]

The current remains constant in spite of changes in the applied potential because the supply of \( O_2 \) to the electrode is limited by the maximum rate at which \( O_2 \) can diffuse from the bulk of the solution to the electrode surface. The current which flows for a given potential across the electrode is therefore directly proportional to the \( O_2 \) tension in the solution. When the voltage across the system reaches a certain level, in the region of 0.8 V., hydrogen ions are reduced to molecular hydrogen at the electrode. This secondary reaction causes the current to rise again, and the system no longer functions as an \( O_2 \) electrode. There are two other conditions in which the system may not function as an \( O_2 \) electrode, and in which, therefore, the current will not be constant over wide ranges of applied voltage. These are first, if the solution whose \( O_2 \) tension is being measured is too acid, in which case hydrogen may be reduced at the Pt electrode; secondly, it would seem possible that there might be other substances in the solution which could react at the Pt electrode within the voltage range at which normally \( O_2 \) reacts according to the equation given above. These points are mentioned because some difficulties were experienced in the measurement of the \( O_2 \) tension of semen.

The current was measured by recording the fall of potential along a resistance in series with the potentiometer providing the e.m.f. across the electrodes. The potential across this resistance must be small compared with that across the electrodes, otherwise the latter will be too low and misleading results will be obtained. A resistance of 5 M\( \Omega \) was found convenient for this purpose. The ohmic drop across this resistance was measured by a thermionic voltmeter (Rothschild, 1937) passing a grid current of less than 10\(^{-13}\) amp. With this apparatus it was possible to measure a current of about 10\(^{-11}\) amp. The recessed type of Pt electrode (100 \( \mu \) diameter), prepared according to the method of Hill (1948), was used, the tip of the Pt wire being about 0.25 mm. within the end of the glass micro-pipette.

Measurements were made on semen in two ways: first, on a thick layer of semen in a specimen tube about 0.5 cm. in diameter, the electrodes being 0.5 cm. below the surface; secondly, on a thin film of semen on a microscope slide, to which a small drop of 40\% formalin had been added to kill the sperm, and which had been equilibrated with air. These latter measurements determined the current flow in semen in which the normal physical barriers to diffusion of \( O_2 \) were obviated. In
some cases bicarbonate was added to the semen to prevent the solution becoming too acid.

pH measurements. These were done with glass electrodes, calomel half cells through agar-sea water bridges, and the thermionic voltmeter already mentioned.

Potassium estimations. Mr R. Milton carried these out by a method involving the oxidation of organic material in the seminal plasma and subsequent determination of the cobaltinitrite (Milton, Hoskins & Jackmann, 1944).

RESULTS

Properties of seminal plasma. Eight determinations of the potassium content of seminal plasma from different sea-urchins were made. The results, in mg./ml., were: 2.18, 1.35, 1.14, 0.84, 1.65, 1.72, 1.78, and 1.75, the average value being 1.55 mg./ml. There is, therefore, considerably more K in seminal plasma than in sea water or E. esculentus perivisceral fluid, in both of which the concentration of K is 0.375 mg./g. water (Robertson, 1939). The high K content of seminal plasma is not, however, responsible for the lack of sperm movement in this medium, as will be shown later.

The pH of E. esculentus semen is between 7.3 and 7.7. The hydrogen ion concentration is not responsible for the lack of movement for the same reasons that apply in the case of K. Apart from this, sea-urchin spermatozoa are active at lower pH's than 7.3.

If semen is gently centrifuged, at 1500 r.p.m. (12 cm. radius), for about 15 min., after which the sperm and seminal plasma separate into distinct layers, and a drop of semen from another sea-urchin is placed in this seminal plasma, the spermatozoa are as active as in sea water. This experiment and the one in the next section dispose of the possibility that potassium or hydrogen ions are responsible for the lack of sperm motility in semen; it also shows that no substance, such as A.I, normally diffuses out of sea-urchin spermatozoa and prevents them moving. However, a substance which does inhibit movement can be separated from spermatozoa by prolonged or more violent centrifugation; its action is unaffected by oxygenation of seminal plasma. There is little doubt that this substance is A.I. The ease with which A.I can be displaced from spermatozoa into the seminal plasma varies greatly from urchin to urchin; this probably explains the conflicting results obtained by Gray (1928), Hayashi (1945, 1946) and Southwick (1939) on the effect of seminal plasma obtained by centrifugation on the motility of spermatozoa of the same species.

While examining the displacement of A.I from spermatozoa into seminal plasma I noticed that the latter has quite a strong catalatic activity, estimated by the method of Bonnichsen, Chance and Theorell (1947); it is about equal to that of human blood diluted 1:50. This catalatic activity is powerfully inhibited by hydroxylamine hydrochloride. The presence of catalase in semen and seminal plasma is interesting as mammalian spermatozoa contain insignificant amounts of this enzyme (Blom & Christensen, 1947), but it has not yet been proved that the catalase in sea-urchin semen is derived from the spermatozoa.

Oxygenation of semen. When a drop of semen is placed in the gas chamber in air, the spermatozoa will be found to be motionless. Sometimes a few spermatozoa are
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feebly motile at the edges of the drop, as Runnström et al. (1944) observed, but this is not always the case. If, after establishing that spermatozoa do not move, O_2, unsaturated with water vapour, is introduced into the gas chamber, the spermatozoa in the drop of semen start moving. The translatory movements are slow, presumably because the spermatozoa are so tightly packed. When examining semen in O_2 it is advisable to flush the gas chamber out with O_2, seal off the inlet and outlet tubes, and examine the sample after the gas flow has ceased. This obviates the possibility of the sperm movements being due to convection or other disturbances caused by the flow of O_2 over the surface of the drop. When the spermatozoa in the drop of semen are clearly motile, N_2 is passed through the gas chamber for a few minutes and the chamber is again sealed off. No precautions to remove the last traces of O_2 from the nitrogen are necessary, as, apart from the rapidity of O_2 diffusion out of so small a drop, the spermatozoa in the semen themselves consume any residual O_2 that may be left. In an atmosphere of N_2 the sperm stop moving.

The introduction of N_2 into the gas chamber before the spermatozoa have been activated by O_2 does not cause any movement. This fact disposes of the possibility that activation is due to the removal of CO_2 from the semen, a suggestion put forward by Runnström et al. (1944).

When activated spermatozoa are made motionless by the admission of N_2, the reintroduction of O_2 makes them motile again. Alternating atmospheres of O_2 and N_2 produce alternating periods of movement and inactivity: the process can be repeated frequently.

Oxygen tension in semen. Satisfactory current-voltage curves were obtained with sea water equilibrated with air and with N_2. That is to say, the current remained constant (in the case of sea water equilibrated with N_2, zero) over wide ranges of applied potential. In the case of semen, however, difficulties were experienced. In three out of twenty determinations the current through the semen was zero up to approx. 0.8 V., showing that the O_2 tension was zero. In other cases, however, the current through the semen, though markedly lower than that through the thin film of formalin-treated semen on the microscope slide, was not zero and was about 0.2 of that through the latter, suggesting an O_2 tension of some 30 mm. Hg. In these cases the current was not constant when the potential was varied, and polarization occurred at too low an applied voltage. The same occurred with the formalin-treated drop. The unsatisfactory plateau obtained suggests either that hydrogen ions are reduced to molecular hydrogen at a lower potential than in an alkaline aqueous electrolyte, or that some other unknown substance is reduced at the electrode surface. Although the pH of semen in which the sperm were alive was found to be 7.5, this might not be so in the thin film in which the spermatozoa had been killed by formalin; the thin film and the bulk semen were, therefore, buffered with bicarbonate to obviate this possibility. Fig. 2 is an example of the type of curve obtained in these conditions. The addition of bicarbonate (previously in contact with air) might tend to increase the O_2 tension in the case of bulk semen, so that this alteration in the experimental conditions is unfavourable from the point of view of finding low O_2 tensions. The current flowing between the electrodes in
bicarbonate-treated semen was found to be 0.1 of that in similar semen in which the sperm had been killed by formalin and which had been equilibrated with air. This means that the $O_2$ tension is approx. 15 mm. Hg in semen as compared with 153 mm. Hg in air.

The results are unsatisfactory in the sense that they do not dispose of the possibility that some other substance apart from $O_2$ is reacting at the electrode surface. The material is not perhaps ideal for measurements of $O_2$ tensions as it has the consistency of thick cream, and the seminal plasma may contain a number of unknown organic substances which might interfere with the $O_2$ tension determination.

![Current-voltage curves for Echinus esculentus semen](image)

**Fig. 2.** Current-voltage curves for *Echinus esculentus* semen in a thin film equilibrated with air, I, and in a thick layer, II. Between 0.6 and 0.8 V., where the rate of change of current with voltage is small, the current is approximately proportional to the $O_2$ tension.

**DISCUSSION**

The experiments show that *E. esculentus* spermatozoa do not move in undiluted semen because of the lack of $O_2$. There is about four times as much K in the seminal plasma as in the perivisceral fluid or sea water; the pH of semen is somewhat lower than that of sea water, and centrifugation of semen may cause toxic substances to be released from the spermatozoa. These facts have no bearing on the normal causes of sperm immotility. This result is not unexpected as diluted sea-urchin spermatozoa, unlike those of mammals, cannot move in the absence of $O_2$ (Barron, 1932). This means that the metabolic processes producing the energy needed for movement must be different from those occurring in mammalian spermatozoa. This is confirmed by MacLeod's observation (1941) that cyanide, azide, and carbon monoxide have no
inhibitory action on human spermatozoa, though cyanide and carbon monoxide reversibly inhibit the respiration, and to a certain extent the motility, of sea-urchin spermatozoa (Robbie, 1948; Rothschild, 1948). One can conclude that the cytochrome system is more directly concerned with the chemical changes which produce the energy for contraction in sea-urchin spermatozoa than in mammalian spermatozoa.

On the other hand, the fact that sea-urchin spermatozoa are alive, but motionless, in semen, and that anaerobiosis, though inhibiting motility, does not irreversibly injure diluted spermatozoa for considerable periods of time (Harvey, 1930), shows that the sea-urchin spermatozoon contains a metabolic system which is capable of functioning to a certain extent under anaerobic conditions.

Runnström et al. (1944) state categorically that Androgamone I is responsible for the lack of movement of salmon spermatozoa in a ‘dense suspension’ (p. 285). The same views have been expressed about the semen of various lower organisms, as was mentioned in the Introduction. The possibility of extracting inhibitory substances from spermatozoa is not in dispute. But as such substances are not normally responsible for the lack of sperm movement in sea-urchin semen, it would be important to see whether salmon and trout spermatozoa in semen become active when the O₂ tension is increased. Until such experiments are done, it would perhaps be unwise to assign too important a role in gamete physiology to the unidentified and unspecific Androgamone I.

The possibility that the seminal plasma of sea-urchins contains a substance whose inhibitory action is neutralized by O₂ may be worth mentioning, but it seems unlikely that so remarkable a property would have escaped attention during examination of Androgamone I. In any case, it has been established that oxygenation does not destroy the inhibitory substance found in seminal plasma after centrifugation.

The initiation of sperm movement in semen by O₂ and its subsequent inhibition by N₂ show without reasonable doubt that O₂ lack is the cause of sperm immotility in undiluted semen. It has not, however, been possible to show that the O₂ tension in semen is as low as this result might lead one to expect. If it had been possible to establish that the O₂ tension in semen is 15 mm. Hg, the controversial question would arise as to whether a critical O₂ tension is necessary for the initial activation of spermatozoa. This cannot be considered, as the apparent residual O₂ tension in undiluted semen may not be due to the presence of O₂, but to some unidentified substance reacting at the electrode surface and causing a flow of current in spite of the comparative absence of O₂.

SUMMARY

1. *Echinus esculentus* spermatozoa are normally motionless in undiluted semen. They become active when the semen is diluted with sea water or seminal plasma obtained by gentle centrifugation (1500 r.p.m., 12 cm. radius, for 15 min.).

2. A sperm-immobilizing substance can, however, be obtained in seminal plasma by more prolonged centrifugation of semen.

3. Spermatozoa can be made motile in undiluted semen by increasing the O₂ tension in the atmosphere surrounding the semen.
4. This $O_2$ activation is completely inhibited by $N_2$; the $N_2$ effect is reversible.
5. Measurements of seminal $O_2$ tension were made with an $O_2$ electrode. The $O_2$
tension of semen is low, being at most 15 mm. Hg. It was not possible to decide
whether this residual tension was due to $O_2$ or some other substance reacting at the
electrode.
6. The $K$ content of seminal plasma is about 1.55 mg./ml., which is four times
higher than that of perivisceral fluid or sea water.
7. The pH of semen is lower than that of sea water, being approx. 7.5.
8. Neither $K$ concentration nor pH is responsible for the inactivity of sperm in
semen.
9. A hormone, Androgamone I, is often considered to be responsible for the
inactivity of spermatozoa in non-mammalian semen. No support has been found for
this view and it is concluded that in $E.\ esculentus$ semen the spermatozoa are
motionless through lack of $O_2$.

I am much obliged to the Director and Staff of the Scottish Marine Biological
Station, Millport, where the experiments were carried out.

REFERENCES