THE PHYSIOLOGY OF ECHINUS ESCULENTUS SPERMATOZOA

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(With Four Text-figures)

INTRODUCTION

Ripe sea-urchin spermatozoa are motionless in undiluted semen and intensely active when shed or artificially diluted. This activity is of short duration, presumably because the amount of energy-producing material within the spermatozoon is small, and because in pure sea water all of the endogenous substrate may not be available for combustion (Gray, 1928a, b, 1931). If semen is diluted with sea water which has been in contact with ripe eggs of the same species, the rate of oxygen consumption, the total O₂ consumed, and the mechanical activity of the spermatozoa increase (Gray, 1928c), though in certain conditions this effect is not observed (Carter, 1931; Hayashi, 1946). The substance responsible for the activating effect of egg water, as it is called, is said by Hartmann, Schartau, Kuhn & Wallenfels (1939) to be a substituted naphthoquinone, echinochrome, though this claim has been contested by Tyler (1939) and Cornman (1940, 1941). Carter (1932) has shown that the sperm-activating effects of egg water can be reproduced by thyroxine and certain allied substances, while Clowes & Bachman (1921) showed that propyl, allyl and cinnamyl alcohol, and related compounds also activate spermatozoa. Hartmann and Schartau (1939) found that a mixture of Naphthopurpurin and Juglone partly reproduced the effects of egg-water, both as regards activation and agglutination.

There is little information about the nature of the material within the sea-urchin spermatozoon which provides the energy for motion, the class of enzyme system or systems which contributes to the production of this energy, or the respiratory quotient during sperm metabolism. Such information would be of intrinsic interest and also might shed some light on the fertilization reaction, by indicating what, apart from paternal hereditary material, a spermatozoon brings into the egg at fertilization.

The position is not the same in the case of mammalian semen. In mammals fertilization is internal; the spermatozoon must continue to be viable for hours, if not days, to achieve fertilization, and it is therefore not surprising that the energy for motion is normally provided outside the spermatozoon, in the form of substrates in the seminal plasma. Furthermore, there is considerable information about the metabolism of mammalian spermatozoa (Mann, 1945a, b, c). The existence of

* Ball & Meyerhof (1946) detected spectroscopically the bands of cytochrome in the spermatozoa of Arbacia punctulata, and Mann (1945b) identified cytochromes a, b, c and COa in ram and bull spermatozoa.
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semen plasma as a normal environment for mammalian spermatozoa, and its content of glycolysable substrate, recently recognized as fructose by Mann (1946), may be responsible for certain differences between the behaviour of mammalian and sea-urchin spermatozoa. On the other hand, mammalian spermatozoa can survive and move in the absence of external substrates, under aerobic conditions, for considerable periods of time.

To throw some light on some of these metabolic problems, the $O_2$ consumption of suspensions of *Echinus esculentus* spermatozoa was investigated manometrically, in the presence of CO. The superficially more simple experiment of measuring the respiration in the presence of cyanide was not attempted because of certain difficulties encountered in the measurement of $O_2$ consumption in the presence of cyanide, if the evolved $CO_2$ is absorbed by KOH (Robbie, 1946). There are other difficulties, discussed later, when the respiration is measured by the indirect method of Warburg (1925).

Respiration through the cytochrome system is inhibited by CO in the dark, the inhibition depending upon the ratio of CO to $O_2$ and not upon the partial pressure of CO alone (Warburg, 1926). CO forms a compound with ferrocytochrome $a_3$, which is spectroscopically identifiable (Keilin & Hartree, 1939). If, therefore, the respiration of spermatozoa is photo-reversibly inhibited by CO, it follows that their metabolism is mediated through the cytochrome system.

**MATERIAL**

Ripe *E. esculentus* testes were dried with filter-paper and squeezed through fine-mesh bolting silk. 0·5 ml. of this viscous semen was mixed with 20 ml. of sea water or egg water. This sperm suspension was tested for activity and fertilizing power before use.

**METHOD**

The $O_2$ consumption of the spermatozoa was measured in Warburg manometers fitted with conical vessels of about 13–15 ml. capacity. The vessels contained 3·0 ml. of sperm suspension and 0·3 ml. 10% KOH in the inner cup. The latter also contained a roll of filter-paper (Whatman no. 40) protruding 0·5 cm. over the edge of the cup and 'flowered' to increase the absorbent surface.

Experiments were done at 15° C. The shaking rate was 96–100 cycles min.$^{-1}$ The stroke was 3·5 cm.

The gas space contained air, 90% $N_2$ and 10% $O_2$, or 90% CO and 10% $O_2$.

Suspensions were kept in the dark by means of lined black bags tied over the manometer vessels round the capillary tubing above the manometer stoppers. For illumination of the suspensions the bags were removed and a 200 or 500 W. lamp was switched on, some distance away from and above the water-bath. The light did not cause any heating effect as the bath was effectively cooled.

The number of spermatozoa in the suspensions was estimated by diluting a known amount of the original suspension with sea water and then doing haemocytometer counts on samples of this latter suspension, after the spermatozoa had become inactive.
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**RESULTS**

*Manometric experiments.* Fig. 1 shows the results of three simultaneous experiments on samples from the same sperm suspension, one being carried out with air, one with 90% N₂ and 10% O₂, and one with 90% CO and 10% O₂, in the gas phase. All three vessels were kept in the dark for 40 min. (indicated in the graph by a black line under the time axis), after which the bags were removed and the vessels illuminated. The graph shows, first, that light and darkness have no effect on the spermatozoa with air in the gas phase; secondly, that spermatozoa, while showing a slight decrease in O₂ uptake with 90% N₂ and 10% O₂ instead of air in the gas phase, are unaffected by the alternating dark and light periods; and thirdly, that in 90% CO and 10% O₂ the sperm respiration is inhibited by about 60%. This inhibition is completely reversed by light, the slopes of the O₂ uptake curves in the N₂-O₂ mixture and the illuminated CO-O₂ mixture being parallel.

Fig. 2 shows the course of respiration in two identical sperm suspensions, one in air and the other in 90% CO and 10% O₂, illumination in this case being effected by a 500 W. lamp placed inside the water-bath. In these conditions the temperature in the water-bath rises, and this is immediately reflected in the rate of respiration of the sperm suspension in equilibrium with air. The effect of light in reversing the CO inhibition of the sperm suspension in equilibrium with the CO-O₂ mixture is therefore masked by the acceleration of metabolism resulting from the temperature increase.

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Fig. 3 shows the \( \text{O}_2 \) consumption of three identical sperm suspensions, one having the \( \text{N}_2-\text{O}_2 \) mixture (II), and the other two having the \( \text{CO}-\text{O}_2 \) mixture in the gas phase (III and IV). All three vessels were kept for 40 min. in the dark. The \( \text{O}_2 \) consumption rates in the two suspensions with \( \text{CO} \) in the gas phase are practically identical for this period and again show about 60% inhibition. At \( t = 40 \) min., vessels II and IV were illuminated, III being kept in the dark. While the light had
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no effect on the respiration of the spermatozoa in the N₂-O₂ gas mixture (II), it raised the O₂ uptake of the spermatozoa in the CO-O₂ mixture (III) to approximately the same rate as the control, showing that the CO inhibition is completely reversible. The O₂ consumption rate in the vessel left in the dark (IV) remained constant.

Fig. 4 shows the effect of alternating 30 min. periods of dark and light on a sperm suspension with 90% CO and 10% O₂ in the gas phase. The average O₂ consumption per hour for each period is plotted for purposes of comparison. There would seem to be no reason why this procedure should not be repeated for as long as the spermatozoa remain viable.

Some preliminary experiments in the presence of CO₂ using egg water instead of sea water as the medium for the spermatozoa, were carried out. Similar results were obtained.

Spectroscopic examination. Cytochromes a, a₈, b and c were clearly identified. The CO cytochrome compound was formed in the presence of CO.

DISCUSSION
The experiments described in this paper establish that the respiration (and therefore perhaps the motility) of E. esculentus spermatozoa are dependent on metabolic processes involving the cytochrome-cytochrome oxidase system. Egg water has no effect on this part of sperm metabolism. The fact that the cytochromes are spectroscopically identifiable does not necessarily imply that the metabolism will have photo-reversible properties under the influence of CO, though the existence of this effect, demonstrated by the manometric experiments, automatically means that the
cytochrome system is involved. The experiments throw no light on the nature of the endogenous substrate. For this, measurements of R.Q. are required, though their interpretation may be difficult. The 'direct' method of measuring the ratio of CO₂ produced to O₂ consumed (Dixon, 1943) would not seem to be applicable in this case, as the method necessitates the bicarbonate content of the media in the two vessels being different. It is already known (Laser & Rothschild, 1939) that the eggs of sea urchins have different O₂ consumption rates according to the bicarbonate content of sea water. Experiments on the ratio of CO₂ produced to O₂ consumed of sperm, using a modification of Warburg's indirect method, described in the above paper, suggested that this method may not be entirely suitable for measuring the R.Q. of spermatozoa. The difficulties are probably connected with the sensitivity of spermatozoa to changes in the pH of the medium. For measurements of sperm R.Q., a method involving one vessel containing one suspension would be preferable, but this presents some technical difficulties.

Whatever the R.Q. may be, that is, whether protein, fat, or carbohydrate is the endogenous substrate, it is possible to make a rough calculation of the amount of substrate, believed by Lardy, Hansen & Phillips (1945) to be a phospholipid in mammalian spermatozoa, required by each spermatozoon. The O₂ consumption per hour of the uninhibited sperm suspension in Fig. 4 is 80 μl. The number of sperm per ml. was about 4.4 × 10⁸. As there were 3 ml. of sperm suspension in the manometer vessel the O₂ consumption per hour per spermatozoon was 6 × 10⁻⁹ μl. As 1 μl. of O₂ consumed corresponds to 1.34 × 10⁻⁶ g. of carbohydrate completely combusted, each spermatozoon requires 8 × 10⁻¹⁴ g. of carbohydrate per hour to achieve the observed consumption of oxygen.

SUMMARY

1. The O₂ uptake of Echinus esculentus sperm suspensions is strongly inhibited by CO. In equilibrium with a mixture of 90% CO and 10% O₂, the O₂ uptake is 60% less than that of an identical suspension in equilibrium with 90% N₂ and 10% O₂.
2. This inhibition is completely reversed by light.
3. Spectroscopic examination revealed cytochromes a, a₃, b and c. On treatment with CO the spectrum of COₐ₃ was observed.
4. These results establish that the cytochrome system is the catalytic agent which controls the respiration of sea-urchin spermatozoa.
5. The O₂ uptake of sperm suspensions in equilibrium with 90% N₂ and 10% O₂ is about 10% lower than in air.
6. Certain difficulties in measuring the R.Q. of sperm are discussed.
7. The reversible effect of CO on the O₂ uptake of spermatozoa is the same whether they are in egg water or sea water.

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