THE ACTIVITY OF RAM SPERMATOZOA

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(With Plate 4 and Four Text-figures)

INTRODUCTION

The object of this paper is to describe a possible new method of measuring the activity of ram spermatozoa. The existing methods may be classified as follows:

Visual estimation of motility, under the microscope.

Manometric determination of O₂ consumption or glycolysis.

Measurement of dehydrogenase activity.

Measurement of fructolytic activity (Mann, 1946; 1948).

Measurement of pH changes (Laing, 1945).

The limitations of the first method, due to its subjective and relatively unquantitative nature, are obvious. The other methods also suffer from certain disadvantages. Undiluted semen is not suitable for manometric experiments, while its dilution may introduce complications unless certain precautions are taken (Tosic & Walton, 1947); moreover there is no a priori reason for assuming that O₂ consumption is a direct measure of motility, particularly as immobile spermatozoa consume oxygen (Gray, 1928) and spermatozoa are motile in anaerobic conditions. Anaerobic CO₂ production may be a measure of acid production, but proof that glycolysis is directly proportional to motility depends on comparing CO₂ or acid production data with some other quantitative, but unrelated, method of assessing sperm motility. Lardy & Phillips (1941) showed that bull spermatozoa retain their motility for some time when glycolytic and oxidative mechanisms are blocked simultaneously, which may cast doubt on the value of oxidation or glycolysis measurements as indicators of sperm motility. For similar reasons, some misgivings might be entertained about the methylene-blue reduction test (dehydrogenase activity). All these methods involve the removal of samples from the original semen specimen: all, except visual estimation of motility, involve measurements or manipulations which cause a fairly considerable delay before a motility assessment is available.

Active ram semen exhibits a striking phenomenon, sometimes known as ‘wave formation’, which is due to the spontaneous reversible aggregation of spermatozoa in the suspension. These aggregations form and disrupt throughout the semen, while the spermatozoa are active. It seemed possible that such macroscopic changes in the ‘structure’ of the sperm suspension might be associated with variations in its electrical properties.
The experiments recorded in this paper are a description of electrical changes observed in suspensions of ram spermatozoa. Their relationship with the 'waves' referred to above, and the relationship of the waves with sperm motility, are discussed in detail later.

Throughout this paper, such phrases as 'active spermatozoa', 'feebly active spermatozoa', and 'intensity of waves' will be found. The fact that these expressions, which reflect an unsatisfactory situation in the field of sperm physiology, must still be used, is one of the reasons for doing these electrical experiments.

**MATERIAL**

Ram semen was supplied from the Animal Research Station, Cambridge, at 15° C. This was placed in a water bath at 37° C., the temperature at which experiments were done.

**APPARATUS**

Electrical measurements were made by placing the semen in a conductivity cell in the unknown arm of an a.c. bridge. The cell was an ordinary test-tube, with two platinized platinum electrodes dipping into the semen in the test-tube. The bridge was of conventional design, except that the detector was an oscilloscope instead of the more usual telephones. The bridge was energized by an oscillator working at 5000 eye. and its sensitivity was such that a 0.01% change in the unknown arm resistance could be detected without difficulty.

**EXPERIMENTAL PROCEDURE**

The sperm suspension in the unknown arm of the bridge is approximately balanced by particular values of the resistances and condensers in the standard arm. If the electrical properties of the unknown arm remain constant, the bridge will remain balanced. If not, the form and frequency of any deviations from balance, i.e. impedance changes, can be observed or photographed. Provided that we are interested in the frequency rather than the precise wave-form of any impedance changes that may occur, it is convenient to study such changes unilaterally in the envelope curve; that is, just outside the region of bridge balance. The advantages and difficulties in doing this have been discussed elsewhere (Hubbard & Rothschild, 1939).

**RESULTS**

**Absence of impedance changes in inactive and diluted suspensions.** Plate 4a, v is a bridge record of a sperm suspension which exhibits no wave formation under the microscope. The electrical record shows no change in impedance and is, in effect, a straight line. Lack of wave formation may be caused by the spermatozoa being motionless or feebly active, which occurs in undiluted semen that has been kept at 37° C. for more than 60 min.; or it may be due to the semen having been diluted, for example, with glucose Ringer in the proportion of 9 parts of Ringer to 1 part of semen. The spermatozoa are very active in such a suspension, but no waves form.

**Presence of impedance changes in active suspensions.** Plate 4a, i–v is a bridge record of a fresh sample of undiluted semen exhibiting wave formation. It will be seen that
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there are 12·5 spikes during the first minute and that the frequency of the spikes declines until after 150 min. they have disappeared altogether. Text-fig. 1 shows the total number of spikes or impedance changes plotted against time, and the corresponding rate curve for a sample of undiluted semen. This sample was

![Graph showing total number of spikes and average spike frequency over time for undiluted semen.](image1)

Text-fig. 1. I, total number of spikes plotted against time, for undiluted ram semen; II, average spike frequency/min., plotted against time, for the same suspension. The broken lines through each point show the time over which the frequency/min. was averaged.

![Graph showing total number of spikes and average spike frequency over time for semen diluted with isosmotic phosphate buffer.](image2)

Text-fig. 2. I, total number of spikes plotted against time, for ram semen diluted with an equal volume of isosmotic phosphate buffer; II, average spike frequency/min., plotted against time, for the same suspension. The broken lines through each point have the same significance as in Text-fig. 1.

markedly more active than the one whose electrical properties are shown in Plate 4a. The total number of spikes plotted against time and the corresponding rate curve for a sperm suspension diluted with an equal volume of isosmotic phosphate buffer are shown in Text-fig. 2.
Effect of phosphate buffer. In Text-fig. 3, the variations in rate of spike frequency in two different suspensions of ram spermatozoa are compared. In I, the semen was diluted with an equal volume of glucose-free Ringer; in II, another sample of the same semen was diluted with an equal volume of phosphate buffer. It is evident, from Text-figs. 1, 2, and 3, that the phosphate buffer has a 'protective' effect on the spermatozoa in that the high rate of spike frequency is maintained in it for longer periods than in undiluted semen or semen diluted with Ringer. The same phenomenon is observed if the disappearance of fructose from the seminal plasma is used as an index of activity (Text-fig. 4). It was, however, noted that the seminal fructose is used up before the impedance changes have stopped.

Effect of temperature changes. Plate 4b, i and ii are records of impedance changes in ram semen at 16.0 and 36.0 °C. There is a marked increase in frequency with temperature, the observed $Q_{10}$ being about 2. This figure is too low, because the same semen sample was used for both experiments and, by the time the low temperature run was carried out, the initial frequency had declined. There are certain practical difficulties in doing $Q_{10}$
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experiments in a more satisfactory way, as they require a series of water baths maintained at different constant temperatures and possibly two or more bridges with the associated apparatus. Nevertheless, for reasons discussed later, such experiments might be important.

DISCUSSION

The nature of the electrical changes. There may be as many as $10^8$ spermatozoa between the electrodes in an experiment. Though the orientation of the spermatozoa is by no means random in such a suspension, it is difficult to believe that the observed electrical changes are in any way similar to the intrinsic variations in potential or resistance found in brain cells, contracting muscle fibres, active nerve fibres, or in Nitella after stimulation. The changes are much more likely to be caused by variations in the position of the biological material in the measuring system (i.e. with respect to the electrodes) than by changes in the membranes of individual spermatozoa. A somewhat analogous situation exists when a trout or salmon egg is placed in a conductivity cell in an a.c. bridge (Rothschild, 1947a, b). The egg is electrically heterogeneous and undergoes spontaneous movements rather like those of a precessing top. Because of this movement and the electrical heterogeneity of the egg surface, there is a periodic variation in the ‘resistance’ encountered by an electric current passing through the egg. Consequently the electrical properties of the system egg in measuring apparatus undergo periodic changes and it was partly because of this effect in fish eggs that similar experiments were tried on ram spermatozoa.

The apparatus is very sensitive to vibrations and it therefore seemed possible that the impedance changes might be due to currents of seminal plasma, produced by the waves, flowing past the electrodes. Mytilus gill, whose cilia produce marked currents of sea-water, were placed between the electrodes but no impedance changes were observed.

Can the periodic formation of sperm aggregations be responsible for the observed impedance changes? Provided that the volume concentration of spermatozoa in the seminal plasma is not too high, it is known from Maxwell’s researches on the resistance of suspensions of spheres that the mere alteration in the packing of the spheres will have no effect on the resistance of the suspension, if the total volume concentration remains constant, which is so in the case under consideration. Maxwell’s argument would not hold good if, at the same time as aggregation, there were some preferred orientation of the spermatozoa in the aggregations, coupled with individual electrical anisotropy. Ram spermatozoa are almost certain to be electrically anisotropic because of their shape, and in particular because of the shape of the sperm head, which resembles an elliptical disk. When examined in polarized light, it becomes evident that there is a great deal of orientation throughout the suspension and that it is not restricted entirely to the areas of aggregated spermatozoa.

The case for there being a correlation between the impedance changes and the waves would be made stronger if a strict correlation between the frequency of wave
formation and the frequency of the impedance changes could be found. The difficulty, if not the impossibility, of doing this is almost inherent in the problem. All that can be said at present is that when the waves form frequently, the impedance changes are frequent; when the waves form infrequently, the impedance changes are less frequent; and when there are no waves, there are no impedance changes. As a working hypothesis, it seems reasonable to assume that there is a correlation between the frequency of the waves and the frequency of the impedance changes; but there is as yet no proof that this hypothesis is correct.

Correlation between sperm motility and frequency of wave formation. A high degree of motility is generally agreed to be necessary for successful fertilization. The following quotation, which refers to ram semen, from Anderson’s *The Semen of Animals and its Use for Artificial Insemination* (1945, p. 129), is of interest in this connexion: ‘Undiluted semen, examined microscopically, should show characteristic turbulent wave movements. Only ejaculates which show this very active motility should be used for insemination.’ The writer evidently considers that there is a strict correlation between the degree of wave formation in the suspension and sperm motility, a view which is shared by Dr A. Walton of the Animal Research Station, Cambridge, and Mr L. E. A. Rowson of the Artificial Insemination Centre, Cambridge. The aggregations are not due to local deficiencies of O₂ as in certain protozoan cultures (Munro Fox, 1921), as they occur in anaerobic conditions. Nor is it likely that they are caused by local accumulations of CO₂, the explanation put forward by Lillie (1913) for the spontaneous reversible aggregations of *Nereis* spermatozoa. This explanation must probably be excluded, because wave formation persists when the evolved CO₂ is removed by KOH. There can be little doubt that the views expressed by the above-mentioned authorities are correct and that wave formation depends on motility, but also of course on density. In other words it is a function of collision frequency or the mean free path of each spermatozoon. This could be further investigated by observing the effect of increasing the density of the suspension on the frequency of the waves. There may be a physical explanation of these aggregations, given that, in certain circumstances, sperm heads stick together or remain contiguous. This possibility is not discussed because it is entirely hypothetical; it may, however, be mentioned that one difficulty to be resolved is that the waves often move through the suspension in a direction which tends to be at right angles to the long axis of the wave.

Correlation between impedance changes and sperm motility. If it is agreed that, on general grounds, there is a reasonable case for the hypothesis

\[ \text{Sperm motility} \rightarrow \text{Wave formation} \rightarrow \text{Impedance changes} \]

for a given sperm density, the experiments described earlier on have a certain significance in confirming the hypothesis. When a suspension is fresh, the spermatozoa are very active; consequently they use up the fructose in the seminal plasma at a high rate. The lactic acid they produce has an inhibitory effect on their motility unless the suspension is adequately buffered. As the lactic acid exerts its effect, the rate of fructose disappearance will decrease. The fructolysis curves support this
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interpretation and the impedance rate curves show the same characteristics. If, however, the semen is buffered with phosphate, we should expect the fructose to disappear more quickly, owing to neutralization of the lactic acid inhibition; but, for the same reason, the frequency of the impedance changes should fall more slowly than in unbuffered semen. The phosphate may of course have other functions in the metabolism of the spermatozoa, apart from acting as a buffer. The fact that the seminal fructose is used up before the impedance changes have ceased may be due to the experimental conditions not being completely anaerobic,* in which case lactate might be utilized as a substrate. On the other hand, it may reveal that fructolysis estimations are not an unequivocal index of motility in that after all the external fructose has been used up, some other substrate in the seminal plasma, or endogenous metabolic processes, may provide the energy for motility.

Possible applications of impedance measurements to determine sperm motility. Any suggestion that the frequency of the impedance changes in ram semen can be used as a quantitative measure of sperm motility and density would be premature. A number of comparative tests between the electrical measurements and the other existing methods of estimating sperm motility are necessary. Apart from this, the results so far obtained suggest further experiments of an electrical nature. Among the more important of these is to determine the effect of varying electrode size and separation on the observed impedance changes. A further experiment of interest would be to reduce the motility of the spermatozoa artificially, either by chemical (e.g. NaF) or physical means, and examine the results of such treatment on the impedance changes. The marked effect of changes in temperature on the frequency of the impedance changes is suggestive in this connexion.

An electrical method of assessing sperm motility would have certain advantages over the other methods described in outline earlier on. The advantages are:

1. Measurements can be made on the actual semen obtained in the artificial vagina. No samples need be taken from the original one.
2. Measurements can be made in a matter of minutes.
3. The method is quantitative and non-subjective.
4. Permanent photographic records can be kept.
5. The method may provide a standard with which other methods of assessing sperm activity can be compared.

The main disadvantage of the method lies in the fact that its applicability depends on a hypothesis, for which at present there is only partial justification, that the impedance changes are due to periodic sperm aggregation and that the frequency of the latter is a direct measure of sperm motility, for a given density of sperm. The density factor is responsible for another disadvantage. The method could not be used to measure the activity of human, boar, or stallion spermatozoa unless these were concentrated to the required density by centrifugation. Centrifugation has disadvantages in these cases, apart from vitiating some of the advantages claimed for the method earlier on.

* Ram's semen is so viscous that, in the conditions of these experiments, the sperm between the electrodes must be in a virtually O₂-free medium.
SUMMARY
1. Concentrated suspensions of active ram spermatozoa exhibit periodic changes in their electrical properties when these are measured in an a.c. bridge.
2. Suspensions containing immobile or feebly active spermatozoa do not exhibit this phenomenon.
3. The frequency of the changes has the following characteristics:
   (a) It is maximal when the suspension is fresh, and declines to zero after some 60 min. at 37°C.
   (b) It can be maintained for about 20 min. at a high level by the addition of phosphate buffer.
   (c) It is susceptible to changes in temperature, the $Q_{10}$ between 16 and 36°C. being about 2.
4. A tentative hypothesis is put forward that these electrical changes are caused by the spontaneous, reversible aggregation, or 'turbulent wave formation', which is characteristic of active and concentrated suspensions of ram spermatozoa; and that they are due to the position of the spermatozoa in the measuring apparatus rather than to any intrinsic changes in the electrical properties of individual spermatozoa.
5. As the frequency and intensity of the wave formations are thought to be proportional to sperm motility, there is a possibility that the electrical changes might be used as an objective and physical measure of sperm motility.
6. Certain advantages and disadvantages in measuring the activity of the sperm suspensions by this method are discussed.

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REFERENCES

EXPLANATION OF PLATE
Plate 4a. i–v. Undiluted ram semen: variation in frequency of impedance changes with time. The bridge unbalance corresponds to a 0.25% change in the standard arm resistance (0.1 in 50 Ω at 5000 cyc). The vertical white lines are 1 min. apart in all records. The first vertical white line in each record corresponds to 10.5, 30.5, 51.5, 72.0 and 150 min. respectively from the start of the experiment.
Plate 4b. Ram semen diluted 1 : 1 with glucose-free Ringer: variation in frequency of impedance changes with temperature. i, 16-0°C.; ii, 36-0°C.
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