LETTERS TO THE EDITORS

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Measurement of Sperm Activity before Artificial Insemination

THE successful application of artificial insemination largely depends on the possibility of making a quick assessment of sperm activity in the sample of semen to be employed. The existing methods of assessing sperm activity suffer from certain disadvantages. They are either subjective, depending on the judgment of an individual who has often examined semen under the microscope, or they involve chemical treatment which may take some time to complete. The most satisfactory is the fructolytic test, which not only gives a combined measure of sperm motility and density[1], but also provides an indication of the hormonal (testosterone) activity of the bull[2]. On the other hand, samples have to be taken from the ejaculate and incubated at 37° C. for one to three hours.

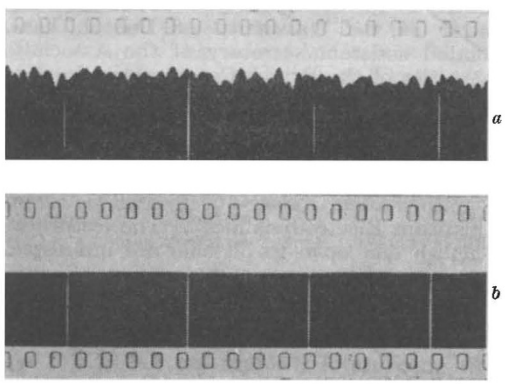

Fig. 1(*a*). Impedance of ram semen containing active spermatozoa. (*b*) Impedance of the same semen after spermatozoa have become motionless. Time-marker, 30 sec.

These difficulties are avoided to a considerable extent by an electrical method of assessing sperm activity. The method is based on the observation[3] that ram semen, when active, exhibits well-defined periodic changes in electrical impedance (Fig. 1). As a result of the experiments on ram semen it was predicted that analogous electrical changes in the semen of other mammals might be too small to observe, or non-existent, unless the density of the sperm were augmented by centrifugation. It has, however, been possible to observe and measure the electrical changes in bull semen with a portable apparatus. Experiments to determine the relative merits of this and other methods of assessing sperm activity have been started; but as these may involve correlating conception-rates with assessments of sperm activity determined in different ways, the results may be considerably delayed.

The apparatus for measuring the electrical changes is mains-operated and consists of a conventional alternating current bridge, energized by a 5-kc. oscillator. The detector is an oscilloscope with amplifier. The method of presenting the data on the oscilloscope is largely a matter of personal choice. For visual observation the electrical changes are usually made to tilt or widen an elliptical trace on the screen. A pair

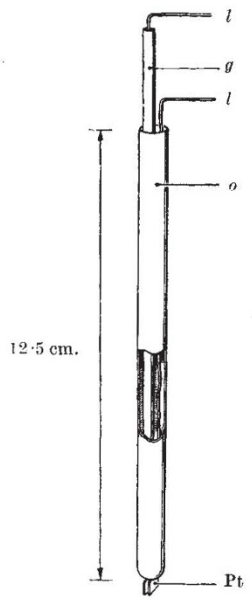

Fig. 2. Electrodes for use with bridge. Pt, platinized platinum plates; *l*, leads to bridge; *g*, insulating glass tubing; *o*, outer glass tubing

of platinized platinum electrodes (Fig. 2) dip into the semen, in a test-tube or centrifuge tube, preferably in a 'Thermos' flask containing water at 37° C. This forms the unknown arm of the bridge, the voltage across it being about 50 millivolts. Trials are in progress to determine whether this treatment has any influence on the fertilizing capacity of the spermatozoa. At the beginning of an experiment, the resistance and capacity of the unknown arm are approximately balanced. If the semen is fresh and the sperm both active and sufficiently dense, periodic impedance changes, which have the following characteristics, are observed: (1) The frequency is maximal immediately the semen has been collected (Fig. 1*a*). (2) When the sperm are dead or only feebly motile, the frequency is zero (Fig. 1*b*). (3) The frequency is reduced by lowering the temperature, and vice versa. (4) The initial frequency can be maintained by adding phosphate buffer to the semen; this treatment also maintains the fructolytic activity at a high level[1]. (5) The changes can be markedly amplified by increasing the sperm density of the sample.

These impedance changes are unlikely to be caused by alterations in the electrical characteristics of individual spermatozoa, such as take place in a contracting muscle fibre; they are probably due to periodic variations in the positions of sperm in the measuring system. The changes are believed to be connected with the 'turbulent wave formation' that is characteristic of active and dense suspensions of bull or ram spermatozoa (Fig. 3). This wave formation represents a periodic aggregation of spermatozoa, the tails of which probably beat synchronously in the aggregations.

The electrical method of measuring sperm activity has the following advantages: (1) Measurements can be made on the semen while still in the glass receptacle at the end of the artificial vagina, or, if necessary, on a separate ½ c.c. sample. (2) Measurements take about five minutes, after which an 'impedance

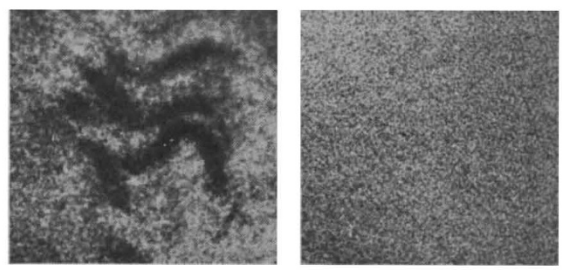

Fig. 3. (*a*) Active suspension of bull spermatozoa showing wave formation. Magnification, approx. 40. (*b*) The same suspension of bull spermatozoa after activity has ceased. Magnification, approx. 40

number' (number of spikes/min.) can be assigned to the sample. (3) The method is quantitative and non-subjective. Photographs can be taken for record purposes. (4) The apparatus is portable and the method of operation can be learnt in a few hours.

The main shortcomings of the method are, first, that it will not provide an assessment of activity unless the semen is sufficiently dense. This may not be serious, as whenever wave formation occurs in semen, impedance changes are observed. Wave formation is usually accepted as a necessary characteristic of semen for use in artificial insemination[4]. Secondly, although the evidence suggests that the frequency of the impedance changes is proportional to sperm activity, further experiments, and, in particular, parallel measurements of fructolysis, impedance numbers and conception-rates, are necessary before the practical value of this method of assessing sperm vitality can be finally established.

A word of warning is perhaps necessary about the assessment of the fertilizing capacity of sperm samples from measurements of sperm activity. Many other factors, apart from the ability to move fast, go to make up the fertilizing capacity of a spermatozoon. If this were not so, intergeneric crosses would be possible. Equally, metabolism and motility are not necessarily related to each other in a simple way. Mann and Lutwak-Mann[5] have shown that, in ram semen, both motility and fructolysis can be inhibited by sodium fluoride without respiration being affected to the same extent, while I have found[6] that the oxygen uptake of sea-urchin spermatozoa can be greatly reduced by carbon monoxide without any corresponding decrease in motility.

This work has been carried out in close co-operation with Dr. T. Mann of the Molteno Institute, Cambridge, Dr. L. E. A. Rowson of the Artificial Insemination Centre, Cambridge, and Dr. A. Walton of the Animal Research Station, Cambridge. Part of the cost of this work was defrayed by a grant (March 11, 1948) from the Agricultural Research Council to the Department of Zoology, Cambridge, for a laboratory assistant.

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Photosynthesis in Leaves at Very Low Carbon Dioxide Concentrations

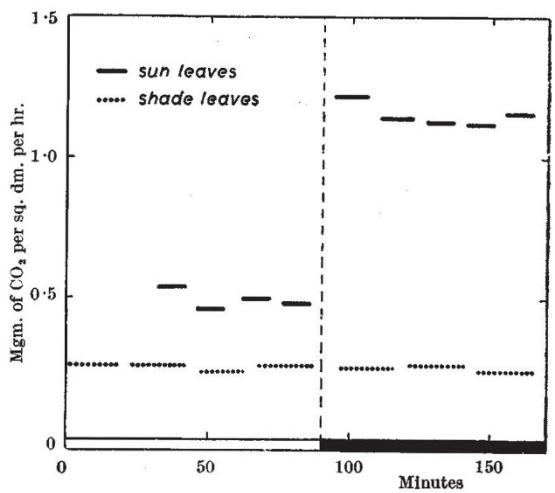
THE photosynthesis of foliage leaves in air free from carbon dioxide is restricted to a re-assimilation of the carbon dioxide which originates from the respiratory process.

Garreau, in the middle of the nineteenth century, demonstrated that green leaves expired carbon dioxide in glass chambers containing small shallow dishes with lime water, or barium hydroxide, when they were exposed to diffuse daylight[1], or even direct sunshine[2,3], that is, the re-assimilation was not complete in surroundings with low carbon dioxide concentrations. F. F. Blackman[4], who, in 1895, placed leaves under similar conditions in a gas stream, could not verify these observations. He concluded that "in bright light a fully green leaf assimilates all the carbon dioxide that it is forming by respiration and none escapes from it". This was the general conception up to 1947.

In this year Audus and Gabrielsen[6], independently of each other, published results which definitely contradicted those of Blackman. Audus performed his experiments in streaming air and exposed the leaves to electric light (intensities up to 3,000 candle-power, or approximately 35,000 lux). He found that the leaves could not re-assimilate more than about nine-tenths of the respiratory carbon dioxide, even at the highest light intensities. Gabrielsen enclosed his leaves in a six-litre flask, illuminated with 10,000 lux, and observed carbon dioxide evolution until a level of about 0·009 vol per cent was reached; these and other static experiments suggest that the photosynthetic process necessitates a concentration of 0·009 vol. per cent carbon dioxide in the surroundings of the assimilation centres before they start working. The rate of carbon dioxide evolution was found to be almost identical for leaves held in light, or in the dark, so long as the carbon dioxide concentration around the illuminated leaves did not exceed 0·006 vol. per cent.

The re-assimilation problem has now been the subject of further studies. Leaves with a total area of 140–170 cm.^{2} were placed in a chamber (150 × 150 × 10 mm.) through which a steady current of carbon dioxide-free air was maintained. The liberated carbon dioxide was absorbed in 0·025 N barium hydroxide in a Boysen Jensen tube and measured for periods of 10–20 minutes duration. The temperature of the mesophyll was controlled by means of thermocouples and kept at the same level ($\pm$ 0·2°) in dark and in illumination experiments.

The results of two typical experimental series (at 20° C. and a flow-rate of 14·8 ml. air/cm.^{2} min. in the leaf chamber) are given in the accompanying graph. Solid lines illustrate results of experiments with sun leaves of *Sambucus nigra* (leaf thickness about 0·23 mm., fresh weight per dm.^{2} 2·51 gm.); dotted lines indicate results of experiments with shade leaves of the same species (leaf thickness 0·13 mm., fresh weight per dm.^{2} 1·34 gm.). The different behaviour of the two kinds of leaves is obvious. In the



Output of carbon dioxide from leaves in light and in the dark. White part of the axis of abscissæ, illumination intensity of 10,000 lux; black part, dark experiments