THE 'SPERM-RISE' TEST

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After centrifugation of a mobile-sperm suspension, the spermatozoa will begin to move upwards from the sediment into the supernatant. This fact constitutes the background to the following method for comparative estimation of the 'amounts of motility' in different test suspensions. The method is simple, adequately objective, and recordings can be made in parallel on several different test suspensions in which the spermatozoa have been in contact with various media for an identical time.

Test suspensions containing 0.5% of cells (see below) were prepared from well-mixed stock suspensions of spermatozoa pooled from the cauda epididymidis of a varying number of gonads from slaughtered bulls. When the effects of different drugs upon sperm kinetic activity were studied, the suspending medium was a Ringer solution in which the 100-ml 0.9% NaCl (0.154 M) component of Mann's Ringer solution (Mann, 1964) was replaced with a 0.191 M-NaCl solution. This replacement rendered the Ringer solution approximately isosmotic with the fluids from the cauda epididymidis, which have a freezing-point depression of -0.66°C (Salisbury & Cragle 1956). When the effects of different ions were investigated, the ionic composition of the 100-ml component of the Ringer solution was altered. All suspending media also contained 0.1% glucose.

Experiments on spermatozoa from different stock suspensions were preceded by a preliminary test, in which a 2.0-ml sample of a control suspension consisting of 0.15 ml of stock suspension + 10.0 ml of medium was centrifuged at room temperature (23 to 24°C) for 15 min at 2000 g. The medium consisted of a mixture of 0.191 M-NaCl and NaF Ringer solution in the proportion of 15:1, in which the sperm motility was immediately blocked by the fluoride (see Lindahl & Wedin, 1963). With the aid of the spermatocrit values then obtained (which usually fell within the range of 0.008 to 0.012 ml), the quantity of stock suspension was calculated which, after mixing with 10.0 ml of a given test medium and subsequently centrifuging in a 2.0-ml sample, would—if the spermatozoa were immotile—give a spermatocrit value of 0.010 ml. This procedure ensured that the 'sperm rises' determined for different test suspensions in different experiments could be related to the same spermatocrit value.

In the calibrated spermatocrit tubes used (AB Grave, Stockholm, Sweden), the lengths of the narrow parts, which were graded in volume fractions from zero to 0.080 ml (Text-fig. 1), were almost identical (range 54.5 to 55.5 mm), i.e. a volume fraction of 0.001 ml had a height of approximately 55/80, or 0.69 mm.

TEXT-FIG. 1. Spermocrit tube of the type used in the experiments.

The time taken for the centrifuge to come to a complete stop after the current had been switched off was 2 min. The cell columns of the different tubes in a given series of test suspensions were then read immediately and again after a further 5, 10 and 15 min. The kinetic activity of the spermatozoa began to induce a visible extension of the cell columns at the 2-min interval (Text-fig. 2). The glucose present in the media ensured that the sperm motility

TEXT-FIG. 2. Increase in a 'sperm rise' with time. 'a₁' shows the spermocrit which gives the zero level of the 'sperm rise'. Reading levels are indicated by arrows.

was maintained by energy provided by glycolysis. With the progress of time and in the absence of sugar, motility was strikingly reduced within the columns, apparently as a consequence of the incipient anaerobic conditions.

The fronts of the extending cell columns became only moderately diffuse within the experimental time and the levels selected for the readings of the 'sperm rises' were easily distinguished (Text-fig. 2, arrows). In practice, since the tubes were graded by volume, the readings of the actual levels were made in millilitres. These values were not converted into millimetres, but the 'sperm rises' were expressed as percentages of the initial spermatocrit value, 0.010 ml.

The asymptotic course with time of a given 'sperm rise' curve (Text-fig. 2) may be explained on the assumption that, parallel with the extension of the cell column, the possibility of random sperm movements will increase. These movements, in combination with the effect of gravity on the spermatozoa, continuously slow the velocity with which the column extends.

The determinations were performed on samples of the test suspensions after storage for 0, 1½ and 3 hr. This revealed if any effects of the media, as initially observed, were of a temporary or long-term nature.

The reliability of the method was satisfactory, considering that it was devised for use in routine experiments. In control studies, four 2.0-ml samples of a given test suspension in each experiment were centrifuged in parallel. The error was found to be less than 2% for readings of the front levels of extending columns within the volume interval of 0.020 to 0.030 ml and less than 3.5% within the interval of 0.030 to 0.040 ml, i.e. in intervals where the 'sperm rises' are 100 to 200 and 200 to 300% greater than the initial spermatocrit value, respectively.

The results of the different experiments will be presented and discussed elsewhere. It may be mentioned here, however, that caffeine in a concentration of 2×10^{-3} M strongly potentiated the kinetic activity of the spermatozoa, and gave rise to large 'sperm rise' values. Results with bromide (Ringer solutions in which NaCl was replaced with NaBr) resembled those with caffeine and the effects of both agents were of a long-term nature.

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