Anaerobic heat production of bull spermatozoa

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A micro-calorimeter, suitable for detecting rates of heat production of the order of 1 mcal/h in about 2-3 ml. fluid, is described.

Measurements have been made with it of the anaerobic heat production of bull spermatozoa in semen. The result, for the first hour, 220 ± 15 mcal/10^9 live spermatozoa/h at 37 °C, is consistent with the hypothesis that, in the first hour of incubation, the heat produced is derived from the enthalpy change associated with the breakdown of fructose to lactic acid.

In the second hour of incubation the heat produced by the spermatozoa, 111 ± 18 mcal/10^9 live spermatozoa/h at 37 °C, was sometimes greater than that associated with fructolysis.

Measurements were also made of the heat production of spermatozoa killed or inactivated by various methods, of seminal plasma, and of the heat of partial neutralization of seminal plasma by lactic acid.

Introduction

Sperm heat production has rarely been measured and when it has, the experiments have, with one exception, been of a preliminary nature. Meyerhof (1911) made ten measurements of the heat production of the spermatozoa of Paracentrotus lividus in sea water, the sperm density, which was noted in six experiments, ranging from 3-8 x 10^7 to 2-5 x 10^8/ml. The average heat production in these was 428 mcal*/10^9 spermatozoa/h and in all ten experiments 45.5 mcal/ml sperm suspension/h at 19 °C. These values may give a false impression of the heat production of fully active sea-urchin spermatozoa for two reasons: first, because in the absence of aeration, the spermatozoa quickly use up all the oxygen in the sea water surrounding them; and secondly, because at such sperm densities, the CO₂ evolved by the spermatozoa will cause a fall in the pH of the sea water, which markedly reduces sperm activity (Rothschild 1957).

Rogers & Cole (1925), in a paper on the heat production of sea-urchin eggs, said they did one experiment on the heat production of the spermatozoa of Arbacia punctulata, the semen having been diluted about 1/120 with sea water. After 50 min the heat production was approximately 250 mcal/10^9 spermatozoa/h at 21 °C.

The only other measurement of sperm heat production, on bull spermatozoa, was described in a recent letter to Nature by Bertaud & Probine (1956), who kindly sent us a report, R. 213, issued in 1953 by the Department of Scientific and Industrial Research, New Zealand, in which their experiments were described in detail. The experiments were of a preliminary nature and suffered from certain unavoidable deficiencies, which were:

1. Consistent results were only obtained with one sample of semen.
2. Measurements of heat production were made on the day after collection of the semen, which was kept undiluted at about 0 °C until the experiments were made.

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* It may be convenient to recall the following conversions relating to the millicalorie (meal): 1 meal = 4.1840 x 10^-3 absolute joules (Rossini 1956). 1 meal/h = 1.1622 micro-watts.
started. Estimates of the proportion of live spermatozoa in the semen were made immediately after collection, which would be unlikely to provide a reliable estimate of the number of live spermatozoa in the semen at the beginning of the experiment.

(3) The experiments were done under approximately anaerobic conditions. The extent to which anaerobiosis is not achieved will have an important effect on sperm heat production, which may be significantly greater in aerobic than anaerobic conditions.

(4) Allowances were not made for the following possible sources of ‘extraneous’ heat: (a) heat of partial neutralization of lactic acid produced during the anaerobic breakdown of fructose; (b) heat production of dead spermatozoa; (c) heat production of seminal plasma; (d) heat of dilution of fructose (because it disappears from the seminal plasma); (e) heat absorption due to the ‘concentration’ of lactic acid; (f) heat of dilution of lactic acid. Needless to say, there are difficulties in making the allowances which should ideally be made for these factors.

(5) After the semen was put into the calorimeter, there was a delay of 1 h, for temperature equilibration, before measurements could be started.

In these circumstances, the good agreement between Bertaud & Probine’s figure of 170 mcal/ml./h at 37° C for undiluted bull semen and heat production estimates based on average fructolysis figures (Mann 1954, p. 47) may have been fortuitous.

In addition, Bertaud & Probine used the value 23.0 kcal/mole for the enthalpy change, $-\Delta H_{f,1}$, associated with the breakdown of fructose to lactic acid. Unfortunately, $-\Delta H_{f,1}$ cannot be stated with anything like this precision. The following table shows how a probable lower limit for $-\Delta H_{f,1}$ may be obtained; but because of discrepancies in the free energy data for lactate (Burton & Krebs 1953), $-\Delta H_{f,1}$ may be 5 kcal/mole greater than 19.9. We can, therefore, only say at present that $-\Delta H_{f,1}$ probably lies between 19.9 and 24.9 kcal/mole.

**Table 1**

<table>
<thead>
<tr>
<th>reaction</th>
<th>$-\Delta H$ (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-fructose (s) + 6$O_2$ → 6$CO_2$ + 6$H_2O$</td>
<td>671.70</td>
</tr>
<tr>
<td>$\beta$-fructose $\rightarrow$ $\beta$-fructose (s)</td>
<td>2.16</td>
</tr>
<tr>
<td>6$CO_2$ + 6$H_2O$ → 2 lactic acid (l)</td>
<td>-651.44</td>
</tr>
<tr>
<td>2 lactic acid (l) → 2 lactic acid (aq)</td>
<td>-2.52</td>
</tr>
<tr>
<td>$\beta$-fructose (aq) → 2 lactic acid (aq)</td>
<td>19.9</td>
</tr>
</tbody>
</table>

In this paper we are concerned with simultaneous measurements of the heat production and fructolysis of live and ‘dead’ spermatozoa in undiluted bull semen at 37° C. Visual motility scores were made during each experiment, together with sperm counts and live-dead counts. An attempt has been made to allow for the factors listed under (4) above.

Measurement of sperm heat production is of interest from several points of view, some of which are: for comparison with the energy required for sperm movement; for comparison with the enthalpy changes consequent upon fructolysis; as a continuous index of sperm activity under varying environmental conditions; and, possibly, for comparison with the fertilizing capacity of the semen sample. The
most comprehensive account and critique of the various indirect methods of estimating the fertilizing capacity of bull semen will be found in a paper by Bishop, Campbell, Hancock & Walton (1954), where it is made clear that none of the existing methods provides an entirely satisfactory estimate. Measurements of sperm heat production may well have the same deficiencies as the methods reviewed by these workers.

![Diagram](http://rspb.royalsocietypublishing.org/)

**Figure 1a.** Calorimeter. A, Araldite; B, brass tube; C, compensating chamber; G, glass tube; Ga, leads from thermopile to galvanometer; P, Polythene collar; S, stainless-steel tube; S.C., semen container; T, Teflon cork.

**Figure 1b.** Part of the thermopile unrolled. Ga, lead to galvanometer; Co, silk-covered constantan wire, 34 gauge; Cu, silk-covered copper wire, 42 gauge; L, junctions round lower, compensating chamber; S, soldered connexions; U, junctions round upper, experimental chamber.

**METHOD**

**Calorimeter**

The calorimeter is similar in principle to that described by Clarke (1954), though certain modifications have been made to improve the zero stability and, therefore, sensitivity, and to make manipulations, e.g. filling and cleaning, easier. The calorimeter, figure 1a, consists of two cylindrical glass chambers, one above and the
other below an insulating partition. The thermopile, part of which is shown in figure 1b, consists of eighty copper-constantan couples with a resistance of 82 Ω. It is wrapped round the chambers, one set of junctions being in contact with the outer surface of the upper chamber and the other set with the outer surface of the lower chamber. The two chambers and the thermopile are embedded in a metal tube containing a synthetic resin, Araldite 103, supplied by Aero Research Ltd, Duxford, Cambridge. The calorimeter was designed to be as symmetrical as possible about its horizontal axis. The experimental material is placed in a glass tube with a Teflon (polytetrafluoroethylene) cork, through which passes a stainless-steel tube for the introduction of reagents or a heater coil. This tube terminates above the lower surface of the Teflon cork. The glass tube fits snugly into the upper chamber and is inserted through a Polythene collar. A second glass tube, of the same size, is filled with water and inserted into the lower chamber.

A steady production of heat in the upper chamber makes its temperature rise until the heat lost equals the heat produced, the rise in temperature being directly proportional to the rate of heat production. As any heating of the lower chamber by the upper one is a linear function of the rise in temperature, the e.m.f. developed across the thermopile is, in the steady state, proportional to the heat produced in the upper chamber.

The water bath was designed to reduce random temperature fluctuations to a low level. The calorimeter is situated within three concentrically arranged water baths, figure 2, and is surrounded by a metal tube through which water is drawn, downwards, by a constant-speed propeller. The middle bath is stirred by another propeller and acts as a heat-capacity buffer which insulates the inner bath from temperature changes in the outer one. The latter, whose surfaces are lagged, contains heaters, the thermoregulator, and a rotary water pump with suitably placed outlets.

**Measurement of heat production**

The thermopile is connected via a reversing switch to a Kipp & Zonen Zernike galvanometer, internal resistance 35 Ω, sensitivity $1.6 \times 10^{-8}$ V/mm at 4 m. Stray potentials in the galvanometer circuit are, of course, cancelled out by the use of the reversing switch.

**Stability**

Figure 3 is a record of room temperature (a), outer bath temperature (b), and (c), of the difference in temperature between the upper and lower chambers during some 6½ h. The scale on the left of the lower record, c, is based on the assumption that a temperature difference of 1°C between the hot and cold junctions of one couple will produce an e.m.f. of 42 μV.

**Calibration**

Figure 4 shows the response of the galvanometer to known amounts of heat liberated by a heater coil inserted in the experimental container, figure 5 the response to a rectangular heat pulse, and figure 6 the response to a rectangular heat pulse of short duration. As the heat of ionization of water is known with considerable accuracy (Pitzer 1937), heat losses from the heater coil can be estimated by
Figure 2. Water bath and calorimeter. A, Araldite; B, brass tube; Be, Beckman thermometer; C, compensating or lower chamber; C.P. Perspex cylinder; G, glass container; Ga, leads from thermopile to galvanometer; G.C., granulated cork; H, heater; L, pivoted Perspex lid, in two halves; M, metal container; P, Polythene plug; P.C. 1–P.C. 2, Perspex containers; Pr., propellers; P.W., pulley wheel; R, rubber stoppers; S, stainless-steel tube; S.C., semen container; S.M., stirrer motor; T, thermoregulator; Th, leads to thermopile; W, paraffin wax covering water; W.D., water deflector plate; W.P. 1–W.P. 4, water pump outlets; W.P.M., water pump motor.
noting the difference between the heat theoretically liberated in the calorimeter by the coil and that produced when a known amount of strong acid is added to strong alkali in the calorimeter.

There are several ways in which the calorimeter and associated equipment could be improved; some of these are (a) three or four calorimeters, instead of one, would

Figure 3. Fluctuations (a) in room temperature, (b) in outer bath temperature (at approx. 37°C) and (c) in the difference in temperature between the upper and lower chambers of the calorimeter.

Figure 4. Response of system to known quantities of heat liberated in calorimeter.

Figure 5. Response of system to a rectangular heat pulse switched on at \( t = 0 \) and off at \( \theta \).
enable experiments to be replicated, controls to be run at the same time, etc.; (b) a d.c. amplifier and recording voltmeter, or some other system of automatic recording, would effect considerable economies in manpower; (c) ground-glass joints would be preferable to Teflon corks in the control tube and that containing the biological material. It is hoped to incorporate these improvements in future models.

**Figure 6.** Response of system to a rectangular heat pulse of short duration, 15·6 meal. The area under the curve is 15·8 meal.

**Fructolysis**

This was determined by the method of Roe (1934) as adapted to semen by Mann (1948). The precision of this method is of the order of ± 5% (actual).

**Sperm counts and live-dead sperm counts**

Sperm counts were made by the usual haemocytometer dilution-method, the average standard error of the estimate being approximately 0·5%. Live-dead sperm counts were made by the nigrosin-eosin method, the standard error of the estimates being 3·1 to 4·3% (Campbell, Dott & Glover 1956).

The time between collection of the semen and the start of the experiment was about 3/4 h. The usual precautions were taken to avoid temperature shock.

**Results**

**Rate of heat production**

After the semen in its glass tube had been put into the calorimeter, temperature equilibration was reached in about 20 min. The variation in the rate of heat production with time is shown in figure 7. The general form of this curve was
similar in nine of the ten experiments reported in this paper, though the decline in heat production at $A$ was not always as pronounced as in this case. The one exception, expt. 3, is discussed later. A small but definite increase in the rate of heat production, about $3.2$ mcal/ml./h, was sometimes observed for 20 min before the fall at $A$.

![Figure 7. Anaerobic heat production of bull semen. The figures under the curve to the left and right of the ordinate at $t = 60$ are, the area bounded by the curve, the $t$ axis and the ordinate at $t = 60$; and the area bounded by the curve, the $t$ axis and the ordinates at $t = 60$ and $t = 120$. $A$, see text.]

To obtain an estimate of the heat production of live spermatozoa, the following sources of 'extraneous' heat must be subtracted from the total heat production of the semen.

**Heat production of seminal plasma**

The value was found to be $4.5$ mcal/ml./h in one 20 min run and $5.4$ mcal/ml./h in another run of 78 min. The value $5.0$ mcal/ml./h is used in subsequent calculations. The heat production of seminal plasma would no doubt be higher if it were incubated for a longer time and bacteria were allowed to multiply.

**Heat production of 'dead' spermatozoa**

When bull semen was maintained at $70^\circ$ C for 5 min, all the spermatozoa were killed and the heat production was found to be $0.6$ mcal/ml./h. Semen maintained at $50^\circ$ C for 5 min still contained a significant number of active spermatozoa. The addition of neutral formalin, final concentration $1.7\%$, killed all the spermatozoa. In these conditions, the heat production was $4.1$ mcal/ml./h. We thought, however, that a more realistic method of inactivating bull spermatozoa was by repeatedly
washing the semen by centrifugation and re-suspension in fructose-free Ringer phosphate (Mann 1954). After this treatment the spermatozoa were motionless. This may have been due partly to the centrifugation, which has adverse effects on bull spermatozoa; but the principal reason was the lack of exogenous substrate and the inability of the spermatozoa to utilize endogenous substrates under anaerobic conditions. The heat production of washed bull spermatozoa in fructose-free Ringer phosphate was 1·1 mcal/ml./h, which is used in subsequent calculations.

*Heat of dilution of lactic acid*

The heat evolved when lactic acid is added to seminal plasma includes both the heat of dilution and the heat of partial neutralization. If, therefore, the latter is examined, it is unnecessary independently to consider the heat of dilution.

![Heat of dilution of lactic acid](image)

**Figure 8.** Titration of seminal plasma (S.P.) and Ringer phosphate (R) with 0·1 n-HCl.

*Heat of partial neutralization of lactic acid by seminal plasma*

If seminal plasma had no buffer capacity, the addition of acid to it would not be associated with any evolution of heat, apart from that due to the dilution of the acid. Seminal plasma has, however, a considerable buffer capacity, because of its content of protein, amino acids, bicarbonate and citric acid (Mann 1954; Rothschild & Barnes 1954). Figure 8 shows the buffer capacity of seminal plasma and, for comparison, that of the Ringer phosphate often used as a diluent for mammalian semen. To investigate the heat of partial neutralization of lactic acid by seminal plasma, the Teflon cork in the semen container was fitted with a wide stainless-steel tube (internal diameter 1·04 mm), terminating half-way down the cork. A smaller stainless-steel tube whose proximal end was closed with paraffin wax except for a
small hole (area, approximately $5 \times 10^{-3}$ mm$^2$), was connected at its distal end to an Agla syringe. The proximal or delivery end was passed through the outer stainless-steel tube into the semen container which was filled with seminal plasma. Consecutive additions of 0.02 ml. 0.309 N-lactic acid were then made, at appropriately spaced time intervals, the stainless-steel delivery tube being withdrawn after each addition. The very small hole at the delivery end ensured that the lactic acid was squirted into the seminal plasma and, therefore, that quick mixing took place, while the diffusion of seminal plasma into the lactic acid in the stainless-steel tube was minimized. Figure 9 shows the time course of heat evolution during four consecutive additions of 0.02 ml. of 0.309 N-lactic acid. Integration of the curves enables an estimate to be made of the heat produced following each addition of lactic acid. The temperature of the added lactic acid was shown by controls to be satisfactorily close to that of the seminal plasma. The average heat evolution per mg lactic acid added to 2.19 ml. seminal plasma was 10.2 meal, 95% fiducial limits 8.2 and 12.7 meal (thirty-four experiments).

**Heat of dilution of fructose**

This was found to be 0.8 meal/mg fructose. Although this quantity of heat is insignificant within the framework of these experiments, it is greater than might be expected on theoretical grounds. Lange & Gunther (1950) give the integral heat of dilution, $V_c$, of D-glucose as 77.6 cal and 82.1 cal/mole at 15 and 20°C, where $c$, the concentration, is less than 0.3 M. $V_c$ is the amount of heat evolved when a solution containing 1 mole of a substance at concentration $c$ is diluted with an infinite volume of pure solvent. This quantity cannot be measured in an experiment, all ‘real’ heats of dilution being the differences between $V_c$’s for the two concentrations in question. Assuming that $V_c$ for D-glucose is about 90 cal at 37°C
and that a similar value is applicable to fructose, an estimate can be made of the heat of dilution of fructose in expt. 1 (table 3), when at \( t = 0 \) the concentration of fructose was 4.34 mg/ml. and at \( t = 60 \) min, 1.35 mg/ml. If at \( t = 0 \) the semen contains \((x + y)\) moles of fructose and \( x \) moles of fructose are broken down during the first 60 min, the heat of dilution will be

\[
9y(c_1 - c_2) \times 10^4 \text{meal},
\]

where \( c_1 \) and \( c_2 \) are the initial and final concentrations of fructose. Substituting the values obtained in (1), the heat of dilution of fructose is found to be of the order of \( 0.5 \times 10^{-2} \) meal.

### Table 2. Calculations for estimating the anaerobic heat production of live spermatozoa (expt. 5)

<table>
<thead>
<tr>
<th>Sperm density, ( 1.720 \times 10^9 )</th>
<th>vol. of semen in calorimeter, 2.335 ml.</th>
<th>percentage dead, 19.25.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of live spermatozoa, ( 3.243 \times 10^9 )</td>
<td>no. of dead spermatozoa, ( 0.773 \times 10^9 )</td>
<td></td>
</tr>
<tr>
<td>( 10^9 ) dead spermatozoa produce 1.1 meal/h.</td>
<td>( 0.773 \times 10^9 ) dead spermatozoa produce 1 meal/h.</td>
<td></td>
</tr>
</tbody>
</table>

\( 3.243 \times 10^9 \) spermatozoa broke down 4.25 mg fructose and therefore produced 4.25 mg lactic acid in the first hour, \( h_1 \). In the second hour, \( h_2 \), they broke down 2.125 mg fructose and produced 2.125 mg lactic acid. When 1 mg lactic acid is added to 2.19 ml seminal plasma, the heat of partial neutralization is 10.3 meal. The heats of partial neutralization were, therefore

\[
h_1, 44 \text{ meal}; \quad h_2, 22 \text{ meal}.
\]

Using the value 0.8 meal/mg. for the heat of dilution of fructose, the appropriate figures for this experiment are

\[
h_1, 3 \text{ meal}; \quad h_2, 2 \text{ meal}.
\]

The observed heat production in \( h_1 \) was 579 meal and in \( h_2 \), 298 meal.

Subtracting (1) + (2) + (3) + (4) from these values, we obtain

\[
h_1', 520; \quad h_2', 262.
\]

The \(- \Delta H_{\text{f.f.}}\)'s for 4.25 and 2.125 mg fructose are

\[
\begin{align*}
& h_1', 587 \quad h_2', 293 \\
& 468 \quad 233
\end{align*}
\]

the two values for each hour being appropriate to \(- \Delta H_{\text{f.f.}} = 19.9 \) to 24.9 kcal/mole.

* Mann & Lutwak-Mann (1948) have shown that the anaerobic breakdown of fructose to lactic acid is virtually complete in washed ram spermatozoa.

### Heat absorption due to the ‘concentration’ of lactic acid

As expected, experiments in which sodium lactate in normal saline was added to normal saline showed that this factor was negligible.

Table 2 shows how the heat produced by a known number of live spermatozoa in the calorimeter is compared with \(- \Delta H_{\text{f.f.}}\), in the experiment whose heat production-time curve is given in figure 7. The results of all experiments are summarized in table 3, which shows that, except in the case of expt. 3, discussed later, the anaerobic heat production of live spermatozoa during the first hour of
incubation is consistent with the hypothesis that all the evolved heat is derived from $-\Delta H_{f,1}$ and that, under anaerobic conditions, little if any energy is expended on synthetic activities. In expt. 6, for example, the anaerobic heat production during the first hour, 420 meal, lies well within the two extreme figures for the enthalpy change associated with fructolysis, 396 and 497 meal. Excluding expt. 3, in which the semen was abnormal, the only case which might occasion doubt is that of expt. 8, in which the heat produced by the sample, 502 meal, was 1% more than the maximum amount available from fructolysis, 497 meal. The difference of 1% is not significant; but the experiment suggests either that bull spermatozoa may sometimes be able to metabolize substances other than fructose, or that $-\Delta H_{f,1}$ is in the region of 24.9 kcal/mole and not some figure between 19.9 and 24.9 kcal/mole.

The semen used in expt. 3 was atypical in several respects: its sperm density was unusually low, being 0.370 x 10^9/ml, while the percentage of dead spermatozoa was high, being 35. Fructolysis was low and the rate was nearly the same in both 1 h periods. The motility rating was zero at the beginning of the experiment and the heat production-time curve was atypical. 'Abnormal' semen samples evidently require further investigation.

### Table 3. The anaerobic heat production, $H$, of live bull spermatozoa in semen, during two consecutive 1 h periods $h_1$ and $h_2$, and $-\Delta H_{f,1}$. of the same spermatozoa. The two figures in each row under $-\Delta H_{f,1}$ are based on the values 19.9 and 24.9 kcal/mole. The average pH at $t=0$ was 6.64

<table>
<thead>
<tr>
<th>expt no.</th>
<th>$H_{1}$ (meal)</th>
<th>$-\Delta H_{f,1}$ (meal)</th>
<th>no. of live sperm $\times 10^9$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>424</td>
<td>477-599</td>
<td>1.961</td>
<td>5.70</td>
</tr>
<tr>
<td>2</td>
<td>403</td>
<td>396-497</td>
<td>1.706</td>
<td>5.73</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>144-181</td>
<td>0.562</td>
<td>6.70</td>
</tr>
<tr>
<td>4</td>
<td>331</td>
<td>314-393</td>
<td>2.250</td>
<td>6.60</td>
</tr>
<tr>
<td>5</td>
<td>520</td>
<td>468-587</td>
<td>3.243</td>
<td>5.50</td>
</tr>
<tr>
<td>6</td>
<td>420</td>
<td>396-497</td>
<td>2.654</td>
<td>5.30</td>
</tr>
<tr>
<td>7</td>
<td>431</td>
<td>389-489</td>
<td>2.298</td>
<td>5.61</td>
</tr>
<tr>
<td>8</td>
<td>502</td>
<td>396-497</td>
<td>2.199</td>
<td>5.59</td>
</tr>
<tr>
<td>9</td>
<td>460</td>
<td>382-479</td>
<td>2.054</td>
<td>5.29</td>
</tr>
<tr>
<td>10</td>
<td>484</td>
<td>398-500</td>
<td>2.717</td>
<td>5.29</td>
</tr>
</tbody>
</table>

Average heat production per $10^9$ live spermatozoa: $h_1$, 220 meal, s.e. of m, 15; $h_2$, 111 meal, s.e. of m, 18.
Table 3 shows that the equivalence between live sperm heat production and \(-\Delta H_{f1.1}\) is less clear during the second than the first hour. In expts. 1, 2 and 3, the heat produced during the second hour corresponded almost exactly with the 24.9 kcal/mole value for \(-\Delta H_{f1.1}\); in expts. 4, 7 and 9 more heat was produced than was available from the measured fructolysis; while in expts. 5, 6, 8 and 10, the amount of heat produced lay between the \(-\Delta H_{f1.1}\) values 19.9 and 24.9 kcal/mole, as in the first hour measurements.

**Comparison between sperm motility (visual assessment) and heat production**

Visual assessment of semen is subjective and much less precise than measurements of heat production. In addition, there is often some doubt as to which property of semen is being assessed—the activity level of moving spermatozoa, the proportion of moving spermatozoa, or some combination of both these properties.* Table 4 shows first, the visual rating (in the form of marks out of ten) and the rate of heat production at the beginning of each experiment; and secondly, the rate of heat production when the visual rating had fallen to between 0 and 1, together with the time at which this happened. An interesting feature of this table is the significant level of heat production when virtually all sperm movement had ceased. The visual ratings were made on a drop of semen in a moist chamber containing nitrogen and maintained at 37°C. Motility may have declined more quickly in the moist chamber than in the sample in the calorimeter, as expt. 3 suggests. But unless it ceased much earlier in the moist chamber, Table 4 raises the suspicion that sperm heat production may continue after motility has ceased. Such heat production, if it occurs, is unlikely to be associated with fructolysis, which is closely associated with sperm activity.

**Discussion**

Interpretation of these experiments depends on the semen being oxygen-free during the experimental period. Taking \(a_{O_2} = 0.024\) at 37°C (Dixon 1943) and \(Z_{O_2} = 20\) (Mann 1954, p. 49), it can be shown that all the oxygen in solution in bull semen may be considered to have been utilized by the end of the first hour. Thus, oxygen consumption may be considered to have been negligible during the first hour; and heat production during this hour may be considered to be associated with fructolysis.

* An experienced observer may, however, make good assessments, in the sense that his estimate of the goodness of a semen sample is highly correlated with impedance change frequency measurements on the same sample (Rothschild 1950).
semen will be used up in about $1\frac{1}{2}$ min at 37° C. Drs D. B. Cater and I. A. Silver kindly examined for us the oxygen tension of bull semen in a glass test-tube, internal diameter 10 mm, containing 5 ml. semen at 37° C, with an oxygen electrode. The tension was 2 mm Hg or less everywhere except at the actual surface of the semen. Whether the small observed oxygen tension was due to zero errors in the instrument or to bull spermatozoa being unable to utilize oxygen at such low tensions are questions which require further investigation. But the experiment confirmed that, in the absence of stirring or hydrodynamic flow, essentially all the oxygen in solution in the semen is used up in a very short time.

There are two possible ways in which oxygen might diffuse into the semen and interfere with anaerobiosis. The first is down the stainless-steel tube which connects the semen under examination with the atmosphere outside the calorimeter, figure 2. Bearing in mind the diffusion coefficient of oxygen in water, 0.40 ml. min$^{-1}$ atm$^{-1}$ cm$^{-2}$ μ$^{-1}$ at 37° C (Krogh 1922) and that, before reaching the semen in the experimental container, the oxygen must pass down a column of semen 100 mm long and 0.58 mm in diameter, this source of oxygenation can be ignored. The other possible source of oxygenation is out of the water which surrounds the experimental container, through the Teflon cork and into the semen. If the semen container were stoppered by a cylinder of water of the same dimensions as the Teflon cork, the maximum possible heat evolution due to this leak can be shown to be about 1 mcal/h. If, as is probable, the diffusion coefficient of oxygen through Teflon is of the same order as that through Polythene, 0.01 ml. min$^{-1}$ atm$^{-1}$ cm$^{-2}$ μ$^{-1}$, the figure of 1 mcal/h is reduced to 0.05 mcal/h.

Spermatozoa do not stir the seminal plasma to an extent which vitiates the value of the diffusion coefficient used in the above calculations. The mathematical proof of this statement, by Dr A. A. Townsend, the Cavendish Laboratory, University of Cambridge, has been deposited in the Archives of the Royal Society.

The above observations show that there is no reason to suppose that the observed evolution of heat is due to the aerobic metabolism of the spermatozoa. The average heat production/live spermatozoon/sec during the first hour was $76 \times 10^{-11}$ mcal or $3 \times 10^{-6}$ ergs. Some years ago, one of us (Rothschild 1953) estimated the energy a bull spermatozoon would expend while swimming, using the equations developed by Taylor (1951, 1952). Because of the variability in the parameters of bull sperm movement, these estimates may be very wide of the mark; but it may be worth recording that the values obtained were $2 \times 10^{-8}$ to $4 \times 10^{-7}$ ergs/spermatozoon/sec, i.e. about 10 to 100 times less than the energy actually dissipated in the calorimeter.

The experiments described in this paper raise a number of questions, the answers to which require further work. For example, it may be valuable at a later stage to examine the relationship between fructose consumed and lactic acid produced using the semen sample in the calorimeter. In view of the uncertainty which at present exists about the value of $-\Delta H_{\text{f,1}}$, and the necessity in the first place to obtain basic information about the heat production of mammalian spermatozoa, this experiment has been deferred, as has examination of the increase in the number of dead spermatozoa during incubation.
The use of seminal plasma as the suspending medium for bull spermatozoa has certain disadvantages; first, its rather poor buffer capacity results in the calorimetric experiments being carried out at undesirably low pH's, as can be seen in table 3. Secondly, seminal plasma may be somewhat toxic to bull spermatozoa. Thirdly, the absence of oxygen is harmful to mammalian spermatozoa (Dott & Walton 1956). The problem of oxygenation in experiments involving the measurement of very small amounts of heat is under consideration, though it has not so far been solved.

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References

Burton, K. & Krebs, H. A. 1953 The free-energy changes associated with the individual steps of the tricarboxylic acid cycle, glycolysis and alcoholic fermentation and with the hydrolysis of the pyrophosphate groups of adenosinetriphosphate. Biochem. J. 54, 94-107.
Dott, H. M. & Walton, A. 1956 The measurement of sperm motility in relation to metabolism. J. Physiol. 133, 30P.
Lange, E. & Günther, M. 1950 Über die Verdünnungswärmen einiger Nichtelektrolyte in wässriger Lösung bis in das Gebiet grosser Verdünnung. Z. Elektrochem. 54, 73-76.
Pitzer, K. S. 1937 The heats of ionization of water, ammonium hydroxide, carbonic, phosphoric, and sulphuric acids. The variation of ionization constants with temperature and the entropy change with ionization. J. Amer. Chem. Soc. 59, 2365-2371.