**Brief Communication**

**Fixation of Carbon Dioxide by Ram Spermatozoa**

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(Received 23rd May 1967)

As the effect of carbon dioxide on mammalian spermatozoa is of current interest (Lodge & Salisbury, 1963; Hamner & Williams, 1964; VanDemark, Koyama & Lodge, 1965; Wales & O'Shea, 1966; Wales & Restall, 1966), the possibility of its fixation by ram spermatozoa has been investigated.

Aliquots (0.4 ml) of ram semen or twice-washed spermatozoa (5.4 x 10^8 cells/flask) were incubated at 37°C with [14C]sodium bicarbonate (1 μmole and 25 μc) in phosphate-buffered saline containing 1 mM-potassium chloride, 2 mM-magnesium chloride, 10 mM-sodium pyruvate, 30 mg/100 ml penicillin and 50 mg/100 ml streptomycin. After 15 or 60 min, 0.5 ml of the incubated semen was removed and stored at -15°C for subsequent chromatography. The remaining 0.5 ml was acidified (0.1 ml of 6 N-H2SO4) and incubated a further 60 min with the addition of 20% KOH to the centre wells of the Warburg flasks. The semen was centrifuged and the supernatant and the plug assayed for radio-activity in a toluene–triton X100 scintillation mixture (Patterson & Greene, 1965). Aliquots of medium containing [14C]sodium bicarbonate were treated in a similar fashion to the samples containing spermatozoa and used as background for the radio-active counts. In addition, spermatozoa immobilized either by the addition of 4% (v/v) neutral formalin or by standing in solid carbon dioxide for 5 min were compared with active samples.

Evidence that the fixation of carbon dioxide occurs in ram spermatozoa is presented in Table 1. More accumulation of radio-activity from bicarbonate occurred with diluted semen than with washed spermatozoa (P < 0.05). This is presumably due to endogenous substrates in semen, such as lactate, acting as metabolic traps. However, fixation by seminal plasma has not, as yet, been ruled out. The decrease in the rate of accumulation of radio-activity after the first 15 min is most likely due to the utilization of the labelled compounds formed.

Paper chromatography was carried out on trichloroacetic acid extracts of reaction mixtures in a descending system using (i) butanol–acetic acid–water (4:1:5), and (ii) ethanol–ammonium hydroxide (15 N)–water (80:5:15). A large proportion of the accumulated radio-activity ran with authentic lactate on two-dimensional chromatography.

Recently, there has been a brief report of the occurrence of malic enzyme and carbon dioxide fixation in cod spermatozoa (Mounib & Eisan, 1967).
Table 1
INTEGRATION OF $^{14}C$ FROM $[^{14}C]SODIUM BICARBONATE BY RAM SPERMATOZOA

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Washing</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Active cells</td>
<td>Formalin killed cells</td>
</tr>
<tr>
<td>15</td>
<td>–</td>
<td>1.94</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.61</td>
<td>0.0</td>
</tr>
<tr>
<td>60</td>
<td>–</td>
<td>2.70</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.31</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Values are expressed as nc/10⁸ spermatozoa over the experimental period and are the means for two ejaculates.

The present results are consistent with a similar mechanism in ram spermatozoa, and although the predominant metabolic reactions in mammalian spermatozoa are catabolic, carbon dioxide fixation may be added to the synthetic reactions shown to be possible (Mann, 1964). On the other hand, the physiological importance of such a reaction remains to be assessed.

The authors are indebted to Professor C. W. Emmens for interest and criticism. This work was aided by grants from the Rural Credits Development Fund of the Commonwealth Bank of Australia and the Australian Wool Board.

REFERENCES


