CITRIC ACID, LACTIC ACID AND OXYGEN
METABOLISM OF FROZEN-THAWED SEMEN FROM
FOUR SUBHUMAN PRIMATE SPECIES

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(Received 21st September 1970, revised 6th July 1971)

A series of recent studies has been devoted to the systematic examination of
characteristics of the semen of subhuman primates, with particular reference
to the successful freeze-preservation of these specimens. Roussel & Austin
(1967a) showed that trypsin will liquefy the coagulum which appears in these
ejaculates without harm to the motility of the sperm cells and the survival rates
of cells from five species after 3 days' storage in liquid nitrogen have also been
reported (Roussel & Austin, 1967b). The initial content of fructose, lactic acid
and citric acid in the frozen semen of animals from eleven species was studied
(Ackerman & Roussel, 1968) in order to establish the extent of variability
among species in these aspects of sperm physiology. Metabolic behaviour of the
frozen-preserved spermatozoa of M. mulatta, M. irus, E. patas and C. aethiops are
reported here.

Specimens were obtained from animals located at the Delta Regional
Primate Research Center, Covington, Louisiana. Collections were made by
means of electroejaculation (Weisbroth & Young, 1965); dilution and freezing
procedures have been described (Roussel & Austin, 1967b). Thirty-seven
specimens were collected, and these were stored in liquid nitrogen for 6 to 12
months. The whole semen was assayed for the concentration of citric acid
(Saffran & Denstedt, 1948) and lactic acid (Barker & Summerson, 1941),
before and after a 3-hr incubation at 35° C, in air or in 100% nitrogen. The
respiration of some specimens was followed for 2 hr at 35° C with a polaro-
graphic oxygen sensor (Yellow Springs Instrument Co., Yellow Springs, Ohio).
Sperm-free seminal plasma containing 7.5% v/v glycerol was employed as a
control for these measurements. Absolute values for O₂ consumed were deter-
mined on the basis α = 0.0245.

Table 1 describes the count, motility and eosin-staining characteristics of the
semen specimens immediately upon thawing at room temperature and after
incubation. Table 2 expresses the metabolic performance of the specimens.
The post-thaw motility of all specimens was very low after 6 to 12 months'
storage. In no instance was the rate of recovery as high as the rates reported
after 3 days' storage for specimens of some of the same animals (Roussel &
Austin, 1967b). There were no differences between species in this respect,
long periods of storage in liquid nitrogen being more deleterious than shorter ones (Salisbury & Hart, 1970). The metabolism of citric acid and of lactic acid appeared to be uniform for the species examined here, and similar to that of mammalian semen generally. Where Zo₂ values could be obtained, they were similar for frozen preserved rhesus monkey spermatozoa to those reported

**Table 1**

CHARACTERISTICS OF SEMEN SPECIMENS FROM FOUR SUBHUMAN PRIMATE SPECIES

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of animals</th>
<th>No. of specimens</th>
<th>Months stored at -196°C</th>
<th>Count × 106/ml</th>
<th>% motile pre-incubation</th>
<th>% motile post-incubation</th>
<th>% eosin negative post-incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mulatta</td>
<td>5</td>
<td>15</td>
<td>6.2 ± 0.52</td>
<td>831.9 ± 229.7</td>
<td>2.6 ± 1.1</td>
<td>1.1 ± 0.5</td>
<td>3.4 ± 1.7</td>
</tr>
<tr>
<td>M. irus</td>
<td>3</td>
<td>10</td>
<td>7.2 ± 0.76</td>
<td>1317.5 ± 284.8</td>
<td>8.2 ± 2.7</td>
<td>1.7 ± 0.9</td>
<td>10.8 ± 4.1</td>
</tr>
<tr>
<td>E. patas</td>
<td>3</td>
<td>5</td>
<td>4.9 ± 0.57</td>
<td>655.8 ± 261.2</td>
<td>3.0 ± 0.9</td>
<td>1.0 ± 0.9</td>
<td>7.2 ± 2.6</td>
</tr>
<tr>
<td>C. aethiops</td>
<td>2</td>
<td>7</td>
<td>7.36 ± 1.11</td>
<td>1428.1 ± 818.1</td>
<td>1.1 ± 0.8</td>
<td>0.4 ± 0.3</td>
<td>6.3 ± 2.1</td>
</tr>
</tbody>
</table>

Results expressed as Means ± S.E.

**Table 2**

CITRIC ACID AND LACTIC ACID METABOLISM OF THIRTY-SEVEN PRIMATE EJACULATES, AND O₂ CONSUMPTION OF EIGHT EJACULATES

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of animals</th>
<th>No. of specimens</th>
<th>Gas phase</th>
<th>Citric acid (mg/10⁶ cells/3 hr)</th>
<th>Lactic acid (mg/10⁶ cells/3 hr)</th>
<th>O₂ consumption (ZWyO₂) (µl/10⁶ cells/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mulatta</td>
<td>5</td>
<td>11</td>
<td>Air</td>
<td>-5.52 ± 10.57</td>
<td>-0.99 ± 1.52</td>
<td>14.72 ± 12.03 (N = 3)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>N₂</td>
<td>-24.65 ± 32.19</td>
<td>2.40 ± 1.93</td>
<td></td>
</tr>
<tr>
<td>M. irus</td>
<td>3</td>
<td>6</td>
<td>Air</td>
<td>2.93 ± 3.04</td>
<td>-1.22 ± 0.31</td>
<td>10.52 ± 2.45 (N = 3)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>N₂</td>
<td>0.73 ± 0.68</td>
<td>-1.51 ± 1.78</td>
<td></td>
</tr>
<tr>
<td>E. patas</td>
<td>3</td>
<td>4</td>
<td>Air</td>
<td>-15.69 ± 7.59</td>
<td>3.48 ± 2.88</td>
<td>0 (N = 1)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>N₂</td>
<td>3.06</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>C. aethiops</td>
<td>2</td>
<td>5</td>
<td>Air</td>
<td>0.07 ± 1.14</td>
<td>0.13 ± 1.21</td>
<td>0 (N = 1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>N₂</td>
<td>50.57 ± 35.20</td>
<td>1.06 ± 2.44</td>
<td></td>
</tr>
</tbody>
</table>

for untreated rhesus spermatozoa by Hoskins & Patterson (1968), despite the low motility of the former cells.

This work was supported by Grant No. HD 02592.

REFERENCES

Ackerman, D. R. & Roussel, J. D. (1968) Fructose, lactic acid and citric acid content of the semen of eleven subhuman primate species and of man. *J. Reprod. Fert.* 17, 563.


Studies on frozen-thawed subhuman primate semen

