COLLECTION AND ANALYSIS OF RETE TESTIS FLUID FROM MACAQUE MONKEYS

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(Received 6th September 1974)

Testicular spermatozoa are transported from the testis through the ductuli efferentes into the caput epididymidis in rete testis fluid (RTF). This fluid was first collected for analysis from conscious rams (Voglmayr, Waites & Setchell, 1966) and then from conscious bulls (Voglmayr, Larson & White, 1970) and anaesthetized wallabies (Setchell, 1970) by cannulation of the extratesticular rete through the ductuli efferentes. Rete testis fluid was then obtained from anaesthetized rats by inserting a side-hole catheter into the rete testis, 12 to 24 hr after ligating the ductuli efferentes (Tuck, Setchell, Waites & Young, 1970). Tuck et al. (1970) suggested that RTF is a mixture of two fluids; one, a high-potassium, low-protein fluid secreted in the seminiferous tubules, and the other, a larger volume of sodium-rich fluid secreted by the rete testis itself.

In addition to its unusual electrolyte composition, RTF contains testosterone at levels which vary according to species (Cooper & Waites, 1974; see review by Setchell, 1974). The rate of appearance of labelled compounds in RTF following their infusion into the circulation has yielded information about the blood–testis barrier to non-steroid (Setchell, Voglmayr & Waites, 1969; Waites, Jones, Main & Cooper, 1973) and steroid molecules (Cooper & Waites, 1975).

None of the previous studies involved primates, and the opportunity arose to collect and analyse RTF from monkeys during the course of another investigation (N. Einer-Jensen and G. M. H. Waites, in preparation).

Seven monkeys (six Macaca mulatta, 7·2 to 10·4 kg; and one M. fascicularis, 5 kg) were anaesthetized with 0·3 to 0·6 ml phencyclidine hydrochloride (Sernylan: Biocentric Labs. Inc; 20 mg/ml intramuscularly). Supplementary anaesthetic doses of phencyclidine (intramuscularly) and sodium pentobarbital (Nembutal: Abbotts; 50 mg/ml intravenously) or halothane (Fluothane: I.C.I.) were given when required. With aseptic precautions, each testis was exposed through a 2- to 3-cm paramedial incision in the anterior aspect of the scrotum. Without removing the testis from the scrotum, the connective tissue attachments of the caput epididymidis were cut to allow it to be gently retracted to expose the ductuli efferentes. These were occluded close to the testicular surface by two 3/0 silk ligatures, taking care not to damage the nearby veins (Text-fig. 1). The tunica vaginalis and skin incisions were then closed.

The monkeys were anaesthetized again 20 to 27 hr later. With aseptic
precautions heparin-saline filled catheters were inserted into a saphenous vein for the infusion of dextrose-saline (1 ml/min) and into a femoral artery and a testicular vein for blood sampling. The upper pole of each, now distended, testis was exposed through the previous incision. A hypodermic needle carrying a side-hole catheter (PVC; 0.5 mm i.d., 0.8 mm o.d.; Dural Plastics, Dural, N.S.W., Australia) was inserted through the capsule to transfix the testis horizontally 5 to 10 mm below the ductuli efferentes; care was needed to avoid the testicular veins where the needle emerged. The needle was cut off, the catheter filled with 0.9% NaCl, and the side-hole pulled inside the testis until the accumulated RTF flowed out. The needle occasionally caused bleeding and sometimes more than one catheter had to be inserted before the correct position was found, but RTF was successfully collected from ten of fourteen testes. Once cannulated, the testis was fully returned inside the scrotum where its temperature was maintained in the range 32 to 34°C. Rete testis fluid was collected in ice-cold glass vials for periods of 2 to 4 hr.

Initially RTF flowed from the catheters at 20 to 40 μl/min, but the flow fell to 5 to 10 μl/min during the 2nd hour of collection. Assuming the basal value to be near the normal rate of production, then this would be approximately 10 μl/g testis/hr. The fluid from mature monkeys (testis weight 19 to 29 g) had an opalescent grey appearance and contained 130 to 310×10⁶ spermatozoa/ml; occasionally, more concentrated samples were collected containing up to
570 \times 10^6 \text{spermatozoa/ml}. Thus, the estimated daily output from a 25-g testis would be of the order of 1 \times 10^9 \text{spermatozoa.}

The composition of monkey RTF resembles that from non-primates in the components examined so far. The sperm-free fluid was watery-clear, and, like RTF from other species (Johnson & Setchell, 1968; Kormano, Koskimies & Hunter, 1971; Koskimies, Kormano & Lahti, 1971), contained very low amounts of serum and other proteins (M. Dym, personal communication). Electrolyte concentrations, measured by flame photometry, and testosterone and oestradiol-17\beta concentrations, measured by radioimmunoassay (Abraham, 1974), are given in Table 1. As in other species, the concentration of sodium was lower and of potassium was higher in rete testis fluid than in blood plasma.

**Table 1.** Sodium, potassium, testosterone and oestradiol-17\beta concentrations in rete testis fluid and plasma collected from anaesthetized macaque monkeys

<table>
<thead>
<tr>
<th>Component</th>
<th>Retre testis fluid</th>
<th>Plasma*</th>
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<tbody>
<tr>
<td>Na (mequiv./litre)</td>
<td>136 ± 3.9</td>
<td>146 ± 2.5</td>
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<td></td>
<td>(122–154; 9)</td>
<td>(136–152; 7)</td>
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<tr>
<td>K (mequiv./litre)</td>
<td>7.4 ± 0.52</td>
<td>4.4 ± 0.19</td>
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<tr>
<td></td>
<td>(5.3–9.6; 9)</td>
<td>(3.9–5.6; 7)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)†</td>
<td>2.49 ± 0.58</td>
<td>Femoral artery</td>
</tr>
<tr>
<td></td>
<td>(0.85–8.0; 6)</td>
<td>Testicular vein</td>
</tr>
<tr>
<td>Oestradiol-17\beta (pg/ml)</td>
<td>66 ± 30</td>
<td>24.9 ± 2.61</td>
</tr>
<tr>
<td></td>
<td>(14–195; 5)</td>
<td>(18–0–35.0; 6)</td>
</tr>
</tbody>
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Values are expressed as Mean ± S.E.M.; the range and the number of testes or equivalent plasma samples are shown in parentheses.

* From either femoral artery or saphenous vein.
† Two additional rete testis fluid samples contained higher concentrations than the range quoted—14.7 and 30.0 ng/ml; no corresponding plasma values were available.

The plasma concentrations of testosterone and oestradiol-17\beta in Table 1 are similar to those reported in peripheral and spermatic venous blood of four monkeys by Kelch, Jenner, Weinstein, Kaplan & Grumbach (1972). The testosterone concentration in monkey RTF was similar to that in RTF collected from rams, which have a central rete testis, but lower than in RTF of the rat which has a lateral rete testis (Cooper & Waites, 1974). Significant amounts of oestradiol-17\beta were also present in RTF. The fluid concentrations of both steroids were comparable to plasma levels in blood withdrawn from the femoral artery, but lower than in the testicular venous blood of the testes from which the RTF was collected. Monkey testicular spermatozoa are therefore bathed by significant levels of testosterone and oestradiol-17\beta and the caput epididymidis receives about the same concentrations of these steroids in the RTF as arrives in the arterial blood supply.

Finally, if the cannulae were inserted under anaesthesia and with full asepsis, it might be possible to continue collection of RTF from conscious monkeys previously trained to sit in restraint chairs.
We are grateful to Dr M. Dym and the M.R.C. Clinical Research Centre, Harrow, England, for assistance with three monkeys. We also acknowledge the technical assistance of Mr G. Soofi and help with analytical techniques from Mr G. Prater, Mr J. Ward and Mrs K. Connell. The antibodies used in the radioimmunoassays were a gift from Dr K. Sundaram. One of us (G.M.H.W.) was supported by a grant no. M.72.075 from the Population Council, New York.

REFERENCES


