Pflügers Arch. 318, 225—243 (1970)
© by Springer-Verlag 1970

The Composition of Fluid Collected by Micropuncture and Catheterization from the Seminiferous Tubules and Rete Testis of Rats*

R. R. Tuck, B. P. Setchell**, G. M. H. Waites***, and J. A. Young

Department of Physiology, University of Sydney and C.S.I.R.O., Division of Animal Physiology, N.S.W., Australia

Received January 9, 1970

Summary. Fluid and electrolyte secretion in the rat testis has been studied by micropuncture and catheterization. Three fluids have been collected and analyzed:

1. *Rete-testis fluid,* obtained by catheterization of the rete testis.
2. *Free-flow fluid,* obtained by micropuncture of seminiferous tubules.
3. *Primary fluid,* also obtained by micropuncture but after first filling a tubule segment with oil and allowing new secretion to form and break up the oil column into droplets.

Primary fluid contained:

[K] = 112 ± 2 (S.E.M.) mEq/l; [Na] = 38 ± 3 mEq/l; [Cl] = 62 ± 3 mEq/l. The trans-epithelial potential difference was -1.2 ± 0.3 mV (lumen negative).

Free-flow fluid contained:

[K] = 50 ± 5 mEq/l; [Na] = 108 ± 7 mEq/l; [Cl] = 120 ± 5 mEq/l; osmolality = 337 ± 12 mOsm/Kg $_{H_2O}$. The trans-epithelial potential difference was -7.4 ± 0.5 mV.

Rete-testis fluid contained:

[K] = 14 ± 1 mEq/l; [Na] = 143 ± 4 mEq/l; [Cl] = 140 ± 2 mEq/l; [HCO_3] = 21 ± 5 mEq/l; osmolality = 328 ± 23 mOsm/Kg $_{H_2O}$, and high concentrations of free myo-inositol 33.5 ± 1.1 mg-% and of some amino acids, e.g. glycine, proline, lysine, alanine and aspartic acid.

It is proposed that the seminiferous tubules secrete a potassium-rich primary secretion, probably by active potassium and bicarbonate transport, and that the

---

* A preliminary report of this work was presented at the Autumn Meeting of the Australian Physiological and Pharmacological Society held at Monash University, Melbourne, in May 1969.

** Present Address: A.R.C. Institute of Animal Physiology, Babraham, Cambridge, England.

*** Present Address: Department of Physiology and Biochemistry, The University of Reading, England.

rete testis or tubuli recti produce a sodium-rich secondary secretion in relatively larger volumes. The origin, fate and role of these fluids is discussed.

Key-Words: Seminiferous Tubule — Rete Testis — Micropuncture — Electrolyte Secretion — Fluid Secretion — Potential Difference — Amino Acids — Myoinositol.

Schlüsselwörter: Samenkanälchen — Rete Testis — Mikropunktion — Elektrolytsekretion — Flüssigkeitssekretion — Potentialdifferenz — Aminosäuren — Myoinositol.

Introduction

Recently a fluid of unique composition has been collected from the testes of conscious rams by catheterization of the efferent ducts [43,44]. It flowed out at a constant rate and contained all the immature spermatozoa from the cannulated testis. From further studies, it was concluded that this fluid originated in the seminiferous tubules and bathed the cells of the germinal epithelium [34,36,46]. When the sperm cells were removed, the fluid was found to be potassium-rich and isosmotic with plasma, but it contained only very low levels of protein. Testosterone was present in concentrations similar to those in testicular lymph, and amino acids and inositol in concentrations quite different from those of plasma [32,33].

The present study was undertaken with the aim of characterizing the testicular secretion further by analyzing seminiferous tubule and rete testis fluid separately. It proved relatively simple to place a cannula in the rete testis of the rat, the flattened space under the tunica albuginea from which the efferent ducts drain, and to collect testicular secretion in adequate volumes.

Micropuncture and microanalytical techniques were originally developed to study kidney function [24,41,48,51] and have subsequently been used for studies of the function of sweat glands [28], salivary glands [16,17,49,50], pancreas [22,29], and thyroid acini [9,38]. In view of the structural similarities between the testis, when viewed as an exocrine gland, and these other secreting organs, it was thought that a micropuncture study of the composition of seminiferous tubule fluid would yield information about its mode of formation.

This paper, therefore, reports an analysis of the fluid collected from the rete testis of rats and a comparison of its composition with that of fluid collected from the seminiferous tubules by direct micropuncture. Furthermore, it was seen that when seminiferous tubules were filled with oil, fluid was secreted into the oil column and broke it up into discrete droplets which could then be aspirated for analysis. This fluid has been called "primary fluid" secretion and its composition has been compared with "free-flow" and rete testis fluids.

Materials and Methods

Albino rats (age 70—100 days) weighing 220—300 gm were used. For collection of rete testis fluid and for micropuncture experiments, animals were anaesthetised with Inactin® (sodium 5-ethyl-5-(1-methyl propyl)-2-thiobarbiturate) given intraperitoneally (100 mg/kg body weight). The animals were given a tracheostomy and a catheter was inserted into the jugular vein for collection of blood samples. The body temperature of the rats was maintained at 37.5° C.

Collection of the Rete Testis Fluid

Cannulation of the rete testis proved difficult unless it was distended by ligating the efferent ducts (EDL) 12—18 hours previously. EDL was done with the rats under ether anaesthesia, the testis being temporarily exposed through an abdominal incision. The testis was then returned to the scrotum, the wound cleansed and closed, and the animal allowed to recover.

Twelve to eighteen hours later the animals were again anaesthetised (with Inactin) and the ligated testis exposed through a scrotal incision. The testis, now turgid, was positioned so that its posterior surface was visible. A 23-gauge hypodermic needle, carrying a polyvinyl catheter (0.2 mm I.D., 0.5 mm O.D.) plugged into its lumen [10] was inserted through the tunica albuginea at the small avascular area on the cranial pole of the testis, next to the point where the efferent ducts leave the organ (Fig. 1). The needle was advanced just below the tunica on the epididymal surface parallel to the testicular artery and finally emerged about one third of the way down. The catheter was carefully pulled through after the needle until the side hole lay within the rete. When the needle was cut off the catheter fluid usually flowed out rapidly. The testis was covered with gauze and bathed with warm saline (30—35° C).

In a good preparation, flow continued uninterrupted for more than 12 hours. Fluid was collected under oil in glass capillary tubing. Sample volumes were estimated by weight and flow rate calculated. All samples were centrifuged for 15 min at 12000 G to remove the spermatozoa. The cell-free fluid was then stored frozen under oil until it was analysed. In 7 other rats rete testis fluid was collected directly into 0.9 % NaCl containing 1 % formalin and 0.1 % Triton X-100 and the spermatozoa counted with a haemocytometer after appropriate dilution.

Sampling of Seminiferous Tubule Fluid

The testis was exposed through a scrotal incision as for rete cannulation and immobilized in a small perspex cup (cf. kidney cup: [41]), with a gap of sufficient width to accomodate the testicular vessels. The cup was filled with paraffin oil to prevent dehydration of the testicular surface.

Micropuncture of the tubules was difficult unless the tunica albuginea was opened where the tubules were to be punctured but it was possible to decapsulate a large area without disturbing the blood supply to the tubules. Although this procedure caused the tubules to bulge out through the tunica, they still remained in close apposition to one another. The appearance of the tubules following decapsulation is shown in Fig. 2.

When the testis was decapsulated and immersed in oil, a fluid exudate accumulated over the surface of the tubules. A similar accumulation is also observed under these conditions on the surface of the kidney, submaxillary gland and thyroid gland [38]. This fluid was a mixture of plasma exudate and fluid which escaped from the seminiferous tubules following puncture. It represents a possible source of

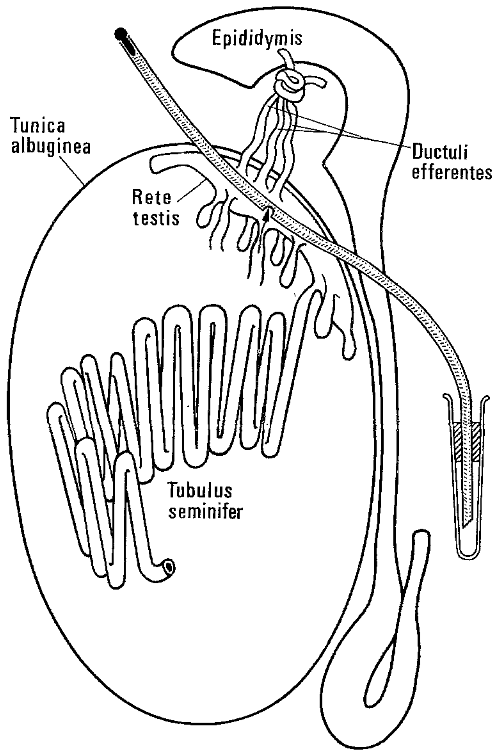

Fig. 1. Schematic diagram of the rat testis showing the anatomy of the duct system and the method of collection of fluid from the rete testis. There are about 20—30 seminiferous tubules in the testis and both ends of each tubule drain into the rete testis. Fluid leaving the rete flows into the head of the epididymis via the efferent ducts (which are shown as tied off in this diagram). Rete testis fluid is collected by passing a polyvinyl catheter with a small side-hole through the rete after first ligating the efferent ducts for 12—18 hours. (Diagram after Y. Clermont and C. Huckins, 1961)

contamination and was collected and analysed at the end of our initial experiments. We have called this fluid surface exudate [cf. 38].

Micropuncture of the exposed seminiferous tubules was done using standard techniques [41]. Sharpened glass micropipettes (tip diameter 10—40 microns) filled with paraffin or castor oil (B.P specification) stained with Sudan-black, were used in all experiments. Tubules were punctured with the aid of a Leitz micromanipulator and the position of the capillary tip in the tubule lumen was verified by injection of a small drop of oil. Samples (200 nanolitre) of normal tubular fluid ("free-flow" fluid) were aspirated for analysis. These samples were drawn up into fine glass capillaries and centrifuged (at 12000 G) under oil to remove the sperm.

In some micropuncture experiments, relatively large segments of tubules were filled with paraffin oil. After 2—20 min the fluid which was secreted into the oil

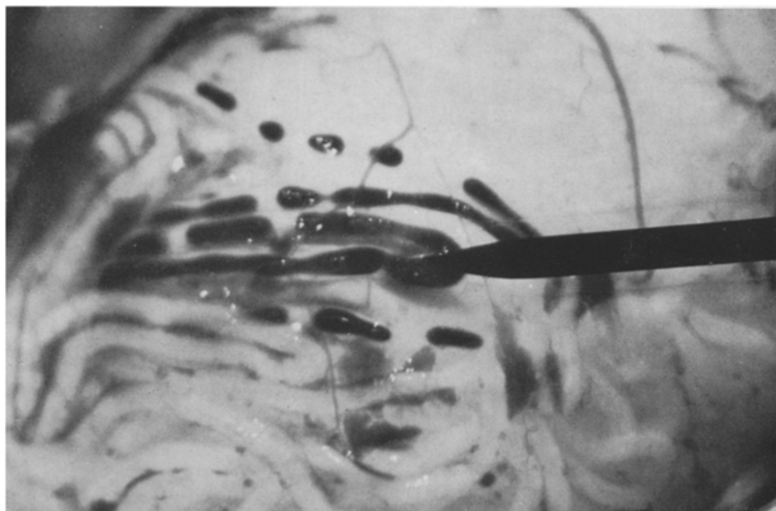

Fig. 2. Photograph of a decapsulated region of the surface of the testis. Paraffin oil, stained with Sudan-black, has been injected from the glass capillary at the right into several loops of a seminiferous tubule. The oil column has been broken up by fluid secreted by the tubules. This "primary fluid" had a composition quite different from that of freely flowing tubule fluid ($\times 20$)

column, breaking it into discrete droplets (Fig. 2), was aspirated in the usual way; this fluid was called "primary" fluid.

Tests of Vascular and Tubular Function

a) After EDL. Eight rats were anaesthetized with i.p. pentobarbitone sodium 12 hr (in 4) and 24 hr (in 4) after unilateral EDL and control manipulations of the contralateral testis.

b) During Micropuncture. Eight rats were anaesthetized with Inactin® and prepared with the left testis immobilized in a perspex cup, as for micropuncture. In addition, the immobilized testes of four rats were decapsulated as before.

Relative blood flow, capillary permeability and ^{86}Rb rubidium transport across the seminiferous tubule were then compared in the operated and control testes by the methods previously described [45, 37].

Analytical Procedures

Sodium and potassium were determined simultaneously on sample volumes of 10^{-10} l using a picomole flame photometer [51]. For measurements in this range (i.e. 10^{-12}—10^{-11} mole) the sample standard error for duplicate determinations was 2—3.5% for sodium and 1—5% for potassium. Chloride was determined with a potentiometric titration procedure [21]. The sample standard error for duplicate determinations on samples containing 10^{-11} mole of chloride was 2%. Bicarbonate was determined by measuring the pH of samples (10^{-7} l) under conditions of con-

trolled temperature and carbon dioxide tension using a bulb-type pH-sensitive glass microelectrode[1]. Sample standard error was 7 %.

Osmolality was determined by measuring melting point using a nanolitre cryostat [51]. Sample standard error for duplicate determinations on 0.5×10^{-9} l was 3.1 %.

Amino acids in rete testis fluid collected from 9 rats were determined on an amino acid auto-analyser (Technicon Instruments Corp. Chauncey, New York). Three samples of the combined collections were compared with the composition of plasma samples collected from the same rats.

Myo-inositol was estimated on 6 samples of rete testis fluid by a spectrophotometric method after paper chromatography with isopropanol-ammonia-water (7:2:1) and elution with perchloric acid [35].

Measurement of Transtubular Potential Differences

Standard Ling-Gerrard glass electrodes (tip diameter about 1 micron; resistance 2—10 megohm) filled with 3 M KCl were used in all experiments. A reference electrode, consisting of 4 mm diameter polyethylene tubing filled with 3 M KCl agar was placed in the subcutaneous tissues of the animal's groin. Both electrodes were connected via calomel half cells to a Keithley model 600 A electrometer (input impedance greater than 10^{14} ohms). The one-volt output of the electrometer was recorded on a low-speed potentiometric pen-recorder (Rikadenki, Kogyo Co. Ltd.).

The electrodes were inserted longitudinally into the tubule lumen for a distance of 1—2 mm. The potential was noted and a small amount of KCl solution was then ejected to ensure that the tip was clear. The potential always returned almost immediately to the initial level. (The volume of ejected KCl solution was minute compared to the volume of even a short segment of the tubule.) This suggested that the electrode tip was not being blocked with cell debris or sperm.

As a guarantee that the electrode tip was sufficiently far from the puncture site, and so uninfluenced by injury potentials, it was then advanced, and the potential did not increase. Finally the potential was monitored as the electrode was withdrawn and it was found that the value remained constant until the tip was retracted at least 1000 microns towards the puncture site. Each recording lasted for 5—20 min, most being 15—20 min; all values were quite stable. The asymmetry potential was measured before and after each recording.

Results

Flow Rate of Rete Testis Fluid

When the catheter was first inserted, the rush of accumulated fluid made any measurements of flow rate meaningless. However by 60 to 90 min, the flow had fallen to a constant level of 0.01—0.06 ml/hr per testis (mean 0.03 ml/hr or 22 µl/g testis/hr). The total volume of fluid collected from a single testis ranged from 0.05—0.5 ml.

Composition of Rete Testis Fluid

There was less sodium, more potassium, more chloride and less bicarbonate in rete testis fluid than in blood plasma although the two

1 Electrode kindly constructed by Associate Professor G. Charlton, Department of Bacteriology, Sydney University.

Table 1. *Values are means ± standard errors of mean*

	Primary fluid collection after injection of		"Free flow" fluid	Rete testis fluid	Surface exudate	Plasma
	Paraffin oil $n = 5$	Castor oil $n = 7$	$n = 14$	$n = 14$	$n = 5$	$n = 14$
Na (mEq/l)	38 ± 3	37 ± 8	108 ± 7	143 ± 4	133 ± 4	160 ± 4
K	112 ± 2	108 ± 2	50 ± 5	14 ± 1	7 ± 0.5	4 ± 0.5
Cl	62 ± 3	67 ± 8	120 ± 5	140 ± 2	108 ± 4	112 ± 4
HCO_3	—	—	—	21 ± 5 ($n = 6$)	—	28, 32
Osmolality (mOsm/kg)	—	—	337 ± 12	328 ± 23	—	319 ± 10

fluids were isosmotic (Table 1). There were much higher concentrations of free myo-inositol in the fluid (33.5 mg/100 ml ± 1.1 S.E.M., $n = 6$) than in blood plasma (1 mg/100 ml). Certain of the free amino acids, glycine, proline, lysine, alanine and aspartic acid were present in higher concentrations while all the other free amino acids were present in lower concentrations in rete testis fluid than in blood plasma (Table 2). The spermatocrit of rete testis fluid (i.e. the percentage of the volume occupied by packed spermatozoa after centrifugation) was about 1% and the fluid contained about $34 \times 10^6 \pm 5.3 \times 10^6$ spermatozoa/ml (± S.E.M. 7 observations).

Composition of Free-Flow Fluid from Seminiferous Tubules

Free-flow fluid collected from seminiferous tubules by micropuncture contained even less sodium and more potassium than rete testis fluid (see above) that is to say, the differences between free-flow fluid and plasma were greater than those between rete testis fluid and blood plasma. However the chloride concentration in free-flow fluid was similar to that of blood plasma and both were less than that in rete testis fluid. Surface exudate was similar to blood plasma suggesting that the fluid collected by micropuncture does indeed come from inside the tubules, not from the surface. Free-flow fluid was isosmotic with the other fluids (Table 1).

The spermatocrit of free-flow fluid was 13% (10 samples) with a range of 6—20%.

Composition of Primary Fluid from Seminiferous Tubules

Primary fluid, collected by aspiration of the fluid which was secreted into a column of paraffin oil, had very low concentrations of sodium and chloride and a very high concentration of potassium (Table 1). Similar

Table 2. *Concentrations (micromole/ml) of amino acids in rete testis fluid and plasma (3 samples of each fluid; each pooled from 3 rats)*

Amino Acid	Rete Testis Fluid			Plasma		
Aspartic acid	0.076;	0.063;	0.027	0.017;	0.039;	0.007
Glutamine	—	—	0.775	—	—	0.582
Proline	1.264;	0.461;	0.495	0.164;	0.127;	0.152
Glycine	2.565;	1.475;	1.820	0.161;	0.262;	0.339
Alanine	1.098;	0.624;	0.720	0.245;	0.569;	0.295
Lysine	0.862;	0.450;	0.420	0.132;	0.317;	0.341
Ethanolamine	—	—	0.037	—	—	0.016
Taurine	—	—	0.070	—	—	0.155
Hydroxyproline	—	—	0.015	—	—	0.031
Threonine	0.267;	0.285;	0.162	0.135;	0.298;	0.173
Serine	0.229;	—	0.216	0.114;	—	0.213
Glutamic acid	0.057;	0.171;	0.052	0.066;	0.255;	0.059
Citrulline	—	—	0.079	—	—	0.064
Valine	0.113;	0.058;	0.059	0.084;	0.252;	0.160
Cystine	0.013;	—	0.009	0.000;	—	0.009
Methionine	0.020;	—	0.021	0.023;	—	0.046
Isoleucine	0.050;	0.026;	0.031	0.036;	0.106;	0.087
Leucine	0.111;	0.058;	0.069	0.070;	0.187;	0.148
Tyrosine	0.042;	0.029;	0.038	0.035;	0.058;	0.052
Phenylalanine	0.050;	0.038;	0.035	0.028;	0.087;	0.071
Ornithine	0.058;	0.029;	0.032	0.044;	0.085;	0.039
Tryptophan	—	—	0.012	—	—	0.048
Histidine	0.061;	0.037;	0.041	0.026;	0.068;	0.068
Arginine	0.160;	0.096;	0.155	0.085;	0.124;	0.159

concentrations were found when the tubules were filled with castor oil instead of paraffin oil (Table 1). Thus there was a progressive fall in potassium concentration and a rise in sodium and chloride concentrations between primary fluid and free-flow fluid and between free-flow fluid and rete testis fluid (Fig. 3).

Transtubular Potential Differences

In all experiments the lumen was negative with respect to the interstitium. Twenty recordings of intraluminal potentials when the tubules were filled with free-flow fluid were made and the mean potential difference (P.D.) was -7.4 ± 0.5 (S.E.M.) mV (lumen negative). Each of these values was stable throughout the recording which lasted from 8—30 min.

In four experiments, when the tubule had been injected with oil and primary fluid had formed, the transtubular P.D. was measured by inserting the electrode tip intraluminally into primary fluid. The mean P.D. under these conditions was -1.2 ± 0.3 (S.E.M.) mV (lumen negative).

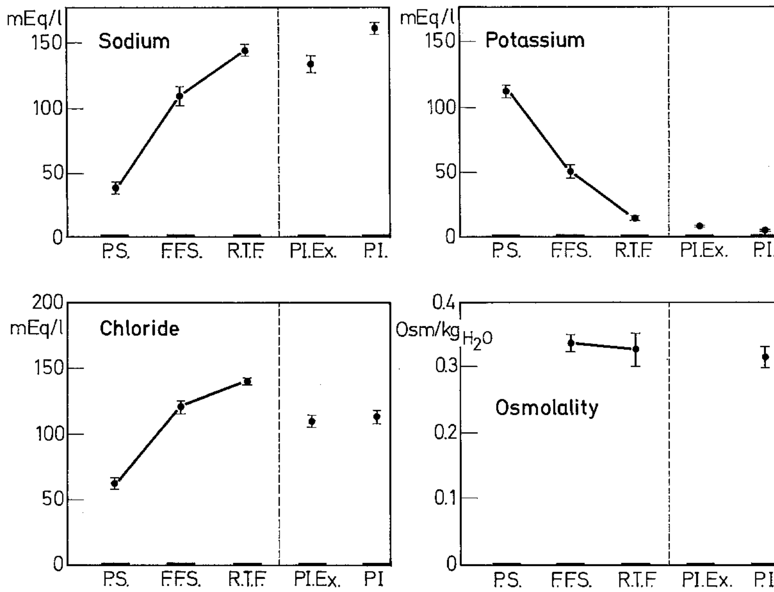

Fig. 3. Sodium, potassium and chloride concentrations and osmolality in testicular fluid samples. *P.S.* primary secretion of seminiferous tubule; *FFS* free-flow secretion of seminiferous tubules; *RTF* rete testis fluid; *Pl Ex* surface exudate; *Pl* plasma. Each point is the mean of 6—14 experiments ± the Standard error of the mean

Table 3. *Effect of experimental manipulations of the testis on relative blood flow, capillary permeability and the uptake of rubidium compared with the contralateral control testis (mean ± S.E.M.)*

Testes	Relative blood flow	Capillary permeability	Rubidium uptake
E.D.L. (12—24 h)	0.783 ± 0.033	0.209 ± 0.013	0.657 ± 0.040
Control	0.895 ± 0.019	0.204 ± 0.011	0.608 ± 0.027
$n = 8$	$P < 0.05$	N.S.	$P < 0.05$
	%	%	%
Into cup v. Control	77 ± 11	179 ± 18	101 ± 5
Decapsulation v. Control	80 ± 3	195 ± 15	110 ± 3
$n = 4$	N.S.	N.S.	$P < 0.05$

Relative Blood Flow, Capillary Permeability and the Blood-Testis Barrier during Collections of Fluid

Twelve to twenty four hours after efferent duct ligation (EDL) there was a 13% reduction in relative blood flow, but capillary permeability remained unaltered and there was an increase of 8% in rubidium

uptake (Table 3). Exposure of the testis and immobilization in the perspex cup as for micropuncture led to a fall of 23% in relative blood flow, a rise of 79% in capillary permeability but no change in rubidium uptake.

Removal of a portion of the tunica albuginea as well as immobilizing the testis in a perspex cup led to no further change in relative blood flow or capillary permeability but rubidium uptake was increased by 10%.

Discussion

Our results lead us to suggest a model of fluid secretion in the testis with the following stages:

1. Secretion of a K-rich primary fluid in the seminiferous tubules.
2. Simultaneous secretion of a Na-rich fluid in the tubuli recti or rete testis.
3. Mixture of these two secretions by an ebb and flow process in the seminiferous tubules.

Effects of Various Operative Procedures on Testicular Function

It is important first to assess the possible consequences of the operative procedures which were necessary to obtain fluids from the testis for analysis.

a) Rete Testis Cannulation. Preventing the outflow of fluid from the testis for up to 18 hr in order to collect rete testis fluid might be thought to alter normal secretion. However, this manoeuvre had remarkably little effect on capillary blood flow and permeability, as measures of testicular vascular function, and on rubidium uptake as a measure of tubular function [37]. Blood flow was slightly reduced and rubidium uptake slightly increased by EDL for 24 hr. In other experiments [30], fluid secretion as measured by increases of testicular weight and water content remained linear for 30 hr after EDL and the rate of weight gain was similar to the rate of fluid secretion in the present experiments. Thus, the increase of intra-testicular pressure after EDL did not restrict fluid secretion and in the present study, occlusion periods of only 12—18 hr were used. In addition, there was no progressive change in electrolyte composition of rete testis fluid with time after release from EDL, as would be expected if the arrest of flow were causing abnormal electrolyte exchanges.

b) Micropuncture. Removing the testis from the scrotum and placing it in a perspex cup resulted in a 23% reduction in blood flow and a 79% increase in capillary permeability but was without effect on rubidium uptake, the parameter most indicative of tubular function. Subsequent decapsulation was without added effect on the vascular system, but

there was an increase in rubidium uptake which may indicate some tubular dysfunction.

Some alteration of vascular function results therefore from the manipulations necessary to transfer the testis from the scrotum to the perspex cup and probably reflects interference with venous return from the testis. However, the entry of rubidium into the tubules is only marginally affected, and this would seem to be the parameter most relevant to these studies. Nevertheless, the alterations of the haemodynamic characteristics might have metabolic consequences, and therefore some reservations should be placed on the results. Decapsulation and immobilization is used routinely in micropuncture investigation of the kidney, an organ in which blood flow and capillary permeability are manifestly of great importance.

c) Primary Fluid Collection. Concern has been expressed in studies on the kidney [13] that injection of oil into the tubules might cause damage to the renal epithelium. Thus, paraffin oil has been shown to damage proximal tubule brush border whereas the more viscous castor oil is without this effect [47]. However, primary fluid from seminiferous tubules was of similar composition whether collected after injecting paraffin or castor oil (Table 1). In two further experiments, tubules were flushed with oil, followed by rete testis fluid and then more oil. Despite this treatment, typical primary fluid was again formed and its composition did not change over a two-hour period. In our view, this is evidence against the composition of primary fluid being due to artefacts. We have preferred paraffin oil because it is less viscous and somewhat easier to work with.

Composition of Tubule and Rete Fluids

a) Primary Fluid. The potassium composition of primary fluid was much closer to intracellular fluid than that of plasma. This very high concentration was unexpected; among other mammalian body fluids, only certain salivary secretions [5,50] and cochlea endolymph [1a] show similar concentrations. The source of this K could not have been the spermatozoa themselves as the spermatocrit is only 14%, nor is it likely that damage to the germinal epithelium was great enough to account for the results.

Because of the low concentration of chloride in primary fluid, there is a deficit of anions. If this deficit were made up by bicarbonate, then the fluid would be distinctly alkaline. The significance of an alkaline, potassium-rich primary secretion is still a matter for speculation.

b) Free-Flow Fluid. There was a progressive fall in potassium concentration and rise in sodium and chloride concentration as one moved

from primary to free-flow tubule fluid to rete testis fluid (Fig. 3). It is difficult to imagine how a stationary primary fluid of one composition becomes transformed into a free-flow fluid of quite different composition. We tend to doubt that the composition of primary fluid is the result of experimental manipulation for the reasons given above and because of the lack of variability among animals in its composition. Furthermore, in seven experiments the composition of primary fluid sampled at intervals of up to two hours after its formation showed no change towards that of free-flow fluid.

Primary fluid did not vary markedly in composition when collected from different tubules, and the column of fluid collected for each sample never extended along a length of tubule equal to or greater than that of individual stages of the spermatogenic cycle [20]. Hence if fluid were being secreted by cells in one stage of the seminiferous cycle and modified by the cells of another stage, one would expect much greater variability in composition than was observed.

c) Rete Testis Fluid. Although the potassium concentration of this fluid is low compared with the tubule fluids, it is still 3.6 times the plasma value. The electrolyte composition (Fig. 3) and sperm density in the rat is similar to that collected from conscious rams [43, 44], bulls [42] and anaesthetized wallabies [31]. An estimate has also been made of the electrolyte composition of all the fluids accumulating in the testis of rats after EDL, from the difference in total Na, K, Cl and water between the ligated and the control testes of the same animal. This fluid would presumably be a mixture of tubule and rete fluid and, indeed, its composition was found to be intermediate between those reported here for rete testis and free-flow fluid [30]. Whatever the origin of the potassium in rete testis fluid, it seems unlikely that it leaks from the spermatozoa. If the potassium concentration in the sperm were 150 mEq/l, at most it could only contribute 1.5 mEq/l to the rete testis fluid as the spermatocrit is only about 1%. Furthermore, the high concentrations of potassium persisted when the sperm density was drastically reduced following local heating of the testis of rams [35].

As in rams [32], the pattern of amino acids in rete testis fluid is quite different from that of plasma. The rat differs from the ram in having a high concentration of proline and lysine instead of glutamic acid in the rete testis fluid. It is interesting to recall that proline is formed from glutamic acid by closure of the C ring [8].

A Hypothesis to Explain the Different Composition of Fluids in the Testis

The evidence leads us to propose that free-flow fluid is a mixture of primary fluid with another fluid secreted either in the tubuli recti or

rete testis (see Fig. 1). Leeson [14] has suggested from studies of the ultrastructure of rete testis epithelium that it could be involved in fluid secretion or resorption. Although the determination of spermatocrit in tubule fluid was much less accurate than in the larger volumes of rete testis fluid, the difference in spermatocrit between the two fluids (14% and 1% respectively) suggests the occurrence of a secondary dilution of the tubule fluid. The secretion of a fluid high in Na^+ and Cl^- concentrations in the rete testis which then enters the seminiferous tubules could explain the composition of a free-flow fluid intermediate between primary and rete testis fluid. As both ends of the tubules open into the rete testis by way of the tubuli recti such a movement of fluid is possible. Indeed, Clegg and Macmillan [1] observed that Indian ink injected into the rete testis of rats may rapidly move into some of the seminiferous tubules. Furthermore, isolated tubules in saline show peristaltic movements [2, 26, 19] and we have observed that droplets of oil can be carried rapidly from one segment of a tubule to another. In the absence of tubular collapse, such peristalsis would draw rete testis fluid into one end of the tubule, while expelling it from the other. On the other hand, the arrangement of the tails of spermatozoa still attached to the germinal epithelium suggested uni-directional flow [25], and valve-like structures have been described at the opening of the tubuli recti into the rete testis [39, 18, 15, 4, 40, 27]. The two lines of evidence would be reconciled if the fluid always entered at one end of the tubule and left at the other.

It should be possible to calculate, in the light of a knowledge of primary fluid and rete fluid composition, in what proportion the two fluids were mixed. If our admixture hypothesis is correct, the proportion so calculated should be the same for all substances whose concentrations can be determined. The ratios of primary fluid volume to rete testis fluid volume calculated for sodium, potassium and chloride were 0.33, 0.37 and 0.27 respectively. These values are similar and support our hypothesis. On the average, these figures mean that about 65% of free-flow fluid is derived from rete testis fluid.

If, as seems probable, tubule and rete secretions are formed by a process of active electrolyte secretion and passive, osmotically mediated, flow of water, one might expect that the fluids would be isotonic or slightly hypertonic. Thus, the primary secretions of the parotid, pancreas and submaxillary glands have been shown to be isotonic [16, 17, 29, 50] and those of the sweat glands, thyroid acini and terminal tubules of immature submaxillary glands are slightly hypertonic [11, 28, 38]. The mean osmolality of free-flow fluid was 337 $mOsm/kg_{H_2O}$ and of rete testis fluid was 328 $mOsm/kg_{H_2O}$ whereas in plasma the mean value was 319 $mOsm/kg_{H_2O}$. On the small number of samples which we have studied, these differences were found not to be significant. Hence, it

would appear that these testicular secretions are isotonic, or at most, very slightly hypertonic.

Electrochemical Potential Gradients across the Tubule Epithelium

The transtubular potential difference (P. D.), whether the lumen contained primary or free-flow fluid was always small and negative. Recently, studies on the proximal tubule of the rat kidney have shown that the traditional value for the transtubular P.D. of about −30 mV was, in fact, an intracellular potential and the real transtubular P.D. was zero [6, 7]; the correct zero values had always been discarded previously as being the result of injury. (Of course there is no doubt that there are trans-epithelial potential differences across the proximal tubule epithelium of amphibians [7a]). In the present experiments no values have been discarded provided they satisfied the following criteria. 1. The measured P. D. remained stable throughout the recording which was 8—30 min. 2. The potential remained stable when the electrode tip was advanced further along the tubule away from the puncture site. 3. The potential returned to its initial value and remained stable following the injection of small quantity of 3 M KCl through the electrode. 4. The point at which the potential was recorded was at least 1,000 microns from the point at which the potential began to fall as the electrode was withdrawn. In fact, the diameter of the lumen of seminiferous tubules (50—100 μ) is much greater than that of kidney tubules, and stable recordings were easier to obtain so that values were only rarely discarded. For this reason we feel confident that our measured values are true trans-epithelial potential differences. However, as Keynes [12] points out, important potential differences may exist over short parts of the pathways of fluid secretion which are not reflected in an overall P. D.

A knowledge of the transtubular potential difference and the electrolyte concentration gradients should reveal whether ion transport into the tubule is active or passive. It should be pointed out, however, that in primary fluid there is a nett inward movement of both electrolytes and water; i.e. nett secretion is a continuing process and ionic equilibrium is not attained, nor is the composition of free-flow fluid likely to be at equilibrium. Hence it follows that the Nernst equation cannot be applied with certainty. However, active transport has presumably occurred for those ions whose nett flux was against the electrochemical gradient. From Fig. 4, it can be seen that potassium and probably bicarbonate may have entered the tubule against strong electrochemical gradients and therefore these ions were probably transported actively. As the direction of nett flux of sodium and chloride is downhill, we cannot say whether active or passive secretion is responsible for the influx of

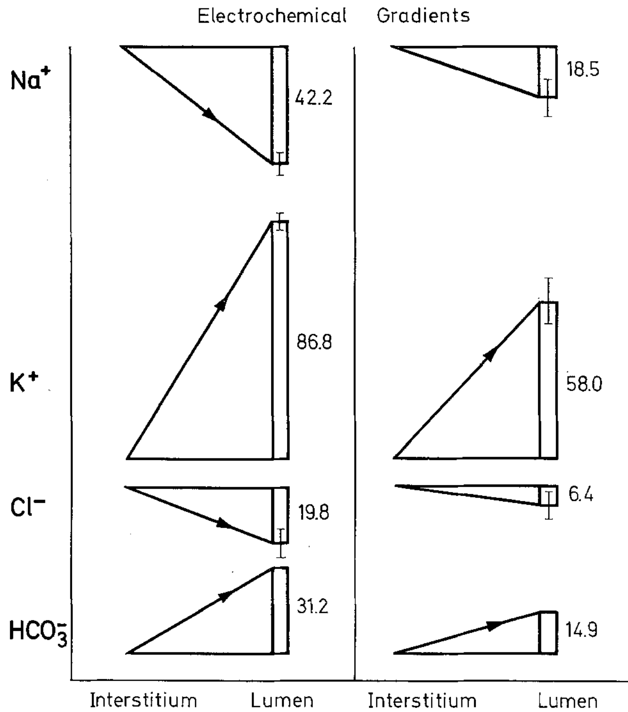

Fig. 4. Electrochemical gradients for Na^+, K^+, Cl^- and HCO_3^- between seminiferous tubule lumen and interstitium under conditions of stopped flow with primary fluid secretion and under conditions of free-flow. The trans-tubular potential difference (*P.D.*) under each condition is given as well as the equilibrium potentials for each ion (i.e. the electrical equivalent of the ionic concentration gradients derived from the Nernst equation). The electrochemical gradient for each ion has been calculated by summing the measured trans-epithelial *P.D.* and the calculated equilibrium potential. These are represented by vertical bars $\pm$ *S.D.* In the case of the primary secretion the direction of nett ionic flux is always inwards and this has been indicated by arrows on the diagram. The arrows point uphill to indicate flux against a gradient and downhill to indicate flux with a favourable gradient. In the case of free-flow fluid, flux directions are not clear since this fluid is believed to be formed by a mixture of primary fluid with rete testis fluid; since (K^+) and probably (HCO_3^+) are lower in this fluid than in primary fluid there is probably still nett inward flux of these ions but the direction of any Na^+ or Cl^- flux, if present, is unknown. The estimates of HCO_3^- are based on the assumption that Cl^- and HCO_3^- together represent the major anionic constituents of tubule fluid since HCO_3^- concentration have not yet been determined in this fluid

these ions. Indeed, one or both of them could be actively resorbed, thus preventing them from attaining the concentration predicted from the Nernst equation.

In the light of these observations it is possible to explain the secretion of primary fluid by one type of cell. This would need to have a conventional coupled sodium-potassium exchange pump on the side of the cell nearer the basement membrane with low permeability to sodium and potassium there. The luminal surface of the cell is postulated to have relatively high permeabilities to both sodium and potassium, P_{Na} being slightly greater than P_K. Experiments with ^{22}Na in isolated seminiferous tubules have shown that there are two components of Na-entry [23], but it is not yet possible to say how this observation relates to the cells in the tubule. However a cell with the permeability characteristics suggested would be able to produce a fluid resembling primary fluid and generate a small transtubular P. D. The cell best placed to satisfy these requirements is the Sertoli cell.

We are grateful to Professor E. C. Roosen Runge, University of Washington, who encouraged us to attempt rete testis cannulation in the rat. The project was supported by the Reserve Bank of Australia (Rural Credits Development Fund) and the National Health and Medial Research Council of Australia. Our thanks are also due to the National Health and Medical Research Council for the award of a B.Sc. (Med) studentship to one of us (R.R.T.).

References

1a. Bosher, S. K., Warren, R. L.: Observations on the electrochemistry of the cochlear endolymph of the rat: a quantitative study of its electrical potential and ionic composition as determined by means of flame spectrophotometry. Proc. Roy. Soc. B. **171**, 227—247 (1968).

1. Clegg, E. J., MacMillan, E. W.: The uptake of vital dyes and particulate matter by the Sertoli cells of the rat testis. J. Anat. (Lond.) **99**, 219—229 (1965).
2. Clermont, Y.: Contractile elements in the limiting membrane of the seminiferous tubules of the rat. Exp. Cell Res. **15**, 438—440 (1958).
3. — Huckins, C.: Microscopic anatomy of the sex cords and seminiferous tubules in growing and adult male albino rats. Amer. J. Anat. **108**, 79—97 (1961).
4. Cunningham, J. T.: On ligature of the vas deferens in the cat, and researches on the efferent ducts of the testis in cat, rat and mouse. Brit. J. exp. Biol. **6**, 12—25 (1928).
5. Denton, D. A.: The effect of Na^+ depletion on the Na^+:K^+ ratio of the parotid saliva of the sheep. J. Physiol. (Lond.) **131**, 516—525 (1956).
6. Frömter, E., Hegel, U.: Transtubuläre Potentialdifferenzen an proximalen und distalen Tubuli der Rattenniere. Pflügers Arch. ges. Physiol. **291**, 107—120 (1966).
7. — Wick, T., Hegel, U.: Untersuchungen über die Ausspritzmethode zur Lokalisation der Mikroelektrodenspitze bei Potentialmessungen am proximalen Konvolut der Rattenniere. Pflügers Arch. ges. Physiol. **294**, 265—273 (1967).

7a. Giebisch, G.: Measurements of electrical potential differences on single nephrons of the perfused *Necturus* kidney. J. gen. Physiol. **44**, 659—678 (1961).
8. Greenberg, D. M.: Biosynthesis of amino acids and related compounds. In Metabolic Pathways. Greenberg, D. M., ed. New York: Academic Press 1961.
9. Hayden, L. J., Young, J. A.: Simultaneous determination of picomole quantities of chloride and iodide using an eletrometric coulombometric titration procedure. Aust. J. exp. Biol. med. Sci. **47**, P 14—15 (1969).
10. Herd, J. A., Barger, A. C.: Simplified technique for chronic catheterization of blood vessels. J. appl. Physiol. **19**, 791—792 (1964).
11. Holzgreve, H., Martinez, J. R., Vogel, A.: Micropuncture and histologic study of submaxillary glands of young rats. Pflügers Arch. ges. Physiol. **290**, 134—143 (1966).
12. Keynes, R. D.: From frog skin to sheep rumen: a survey of transport of salts and water across multicellular structures. Quart. Rev. Biophys. **2**, 177—281 (1969).
13. Langer, K. H., Thoenes, W., Wiederholt, M.: Licht- und elektronmikroskopische Untersuchungen am proximalen Tubuluskonvolut der Rattenniere nach intraluminaler Ölinjektion. Pflügers Arch. **302**, 149—165 (1968).
14. Leeson, T. S.: Electron microscopy of the rete testis of the rat. Anat. Rec. **144**, 57—67 (1962).
15. Lohmuller, W.: Die Übergangsstellen der gewundenen in die „geraden" Hodenkanälchen beim Menschen. Z. mikr.-anat. Forsch. **3**, 147—148 (1925).
16. Mangos, J. A., Braun, G., Hamann, K. F.: Micropuncture study of sodium and potassium excretion in the rat parotid saliva. Pflügers Arch. ges. Physiol. **291**, 99—106 (1966).
17. Martinez, J. R., Holzgreve, H., Frick, A.: Micropuncture study of submaxillary glands of adult rats. Pflügers Arch. ges. Physiol. **290**, 124—133 (1966).
18. May, F.: Kurze Mitteilung über den anatomischen Aufbau der Übergangsstellen der Tubuli contorti in die Tubuli recti im menschlichen Hoden. Virchows Arch. path. Anat. **243**, 474—477 (1923).
19. Niemi, M., Kormano, M.: Contractility of the seminiferous tubule of the postnatal rat testis and its response to oxytocin. Ann. Med. exp. Fenn. **43**, 40—42 (1965).
20. Perey, B., Clermont, Y., Leblond, C. P.: The wave of seminiferous epithelium in the rat. Amer. J. Anat. **108**, 47—77 (1961).
21. Ramsay, J. A., Brown, R. J. H., Croghan, P. C.: Electrometric titration of chloride in small volumes. J. exp. Biol. **32**, 822—829 (1955).
22. Reber, H. A., Wolf, C. J.: Micropuncture study of pancreatic electrolyte secretion. Amer. J. Physiol. **215**, 34—40 (1968).
23. Reisin, I., Cereijido, M.: Na fluxes in single isolated seminal tubules of the rat. Biophys. J. **9**, A 164 (1969).
24. Richards, A. N.: Urine formation in the amphibian kidney. Harvey Lect. **30**, 93—118 (1934—1935).
25. Rolshoven, E.: Ursachen und Bedeutung der intratubulären Sekretströmung im Säugerhoden. Z. Anat. Entwickl.-Gesch. **105**, 374—408 (1936).
26. Roosen-Runge, E. C.: Motions of the seminiferous tubules of rat and dog. Anat. Rec. **109**, 413 (1951).
27. — The rete testis in the albino rat: its structure, development and morphological significance. Acta anat. (Basel) **45**, 1—30 (1961).
28. Schulz, I., Ullrich, K. J., Frömter, E., Holzgrove, H., Frick, A., Hegel, H.: Mikropunktion und elektrische Potentialsmessung an Schweißdrüsen des Menschen. Pflügers Arch. ges. Physiol. **284**, 360—372 (1965).

29. Schulz, I., Yamagata, A., Weske, M.: Micropuncture studies on the pancreas of the rabbit. Pflügers Arch. **308**, 277–290 (1969).
30. Setchell, B. P.: The secretion of fluid by the testis measured by ligation of the efferent ducts in rats, rams and goats, with some observations on the effects of age, hypophysectomy and cryptorchidism. J. Reprod. Fertil. (in press) (1970).
31. — Fluid secretion by the testes of an Australian marsupial. Macropus eugenii. Comp. Biochem. Physiol. (in press) (1970).
32. — Dawson, R. M. C., White, R. W.: The high concentration of free myo-inositol in rete testis fluid from rams. J. Reprod. Fertil. **17**, 219–220 (1968).
33. — Hinks, N. T., Voglmyar, J. K., Scott, T. W.: Amino-acids in ram testicular fluid and semen and their metabolism by spermatozoa. Biochem. J. **105**, 1061–1065 (1967).
34. — Scott. T. W., Voglmayr, J. K., Waites, G. M. H.: Characteristics of testicular spermatozoa and the fluid which transports them into the epididymis. Biol. Reprod. Suppl. **1**, 40–66 (1969).
35. — Voglmayr, J. K., Hinks, W. T.: The effect of local heating on the flow and composition of rete testis fluid in the conscious ram. Biol. Reprod. (in press) (1970).
36. — Voglmayr, J. K., Waites, G. M. H.: A blood-testis barrier restricting passage from blood into rete testis fluid but not into lymph. J. Physiol. (Lond.) **200**, 73–85 (1969).
37. — Waites, G. M. H.: Changes in the permeability of the testicular capillaries and of the "blood-testis barrier" after injection of cadmium chloride in the rat. J. Endocr. (in press) (1970).
38. Shagrin, J., Young, J. A.: A micropuncture investigation of the cation content and osmolality of colloid from single rat thyroid acini. Pflügers Arch. **307**, 204–210 (1969).
39. Spangaro, S.: Über die histologischen Veränderungen des Hodens, Nebenhodens und Samenleiters von Geburt an bis zum Greisenalter. Anatomische Heft. Abteilung I **18**, 593–771 (1902).
40. Stieve, H.: Männliche Genitalorgane. In: Handbuch der mikroskopischen Anatomie des Menschen (W. von Möllendorff, ed.), Vol. 7, part 2. Berlin: Springer 1930.
41. Ullrich, K. J., Frömter, E., Baumann, K.: Micropuncture and microanalysis in kidney physiology. In: Laboratory Techniques in Membranes Biophysics (Eds.: H. Passow and R. Stämpfli). Berlin-Heidelberg-New York: Springer 1969.
42. Voglmayr, J. K., Larsen, L. H., White, I. G.: Metabolism of spermatozoa and composition of fluid collected from the rete testis of conscious bulls. J. Reprod. Fertil. (in press) (1970).
43. — Scott, T. W., Setchell, B. P., Waites, G. M. H.: Metabolism of testicular spermatozoa and characteristics of testicular fluid collected from conscious rams. J. Reprod. Fertil. **14**, 87–99 (1967).
44. — Waites, G.M.H., Setchell, B.P.: Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. Nature (Lond.) **210**, 861–863 (1966).
45. Waites, G. M. H., Setchell, B. P.: Changes in blood flow and vascular permeability of the testis epididymis and accessory reproductive organs of the rat after the administration of cadmium chloride. J. Endocr. **34**, 329–342 (1966).
46. — — Some physiological aspects of the function of the testis. In: The Gonads (Ed. McKerns). New York: Appleton-Century-Crofts 1969.

47. Wiederholt, M., Langer, K. H., Theones, W., Hierholzer, K.: Funktionelle und morphologische Untersuchungen am proximalen und distalen Konvolut der Rattenniere zur Methode der gespaltenen Ölsäule (split oil droplet method). Pflügers Arch. **302**, 166 (1968).
48. Windhager, E. E.: Micropuncture techniques and nephron function. London: Butterworths 1968.
49. Young, J. A., Frömter, E., Schögel, E., Hamman, K. F.: A microperfuison investigation of sodium resorption and potassium secretion by the main excretory duct of the rat submaxillary gland. Pflügers Arch. ges. Physiol. **295**, 157—172 (1967).
50. — Schögel, E.: Micropuncture investigation of sodium and potassium excretion in rat submaxillary saliva. Pflügers Arch. ges. Physiol. **291**, 85—98 (1966).
51. — Shagrin, J. M.: Micromethods in biological research. Proc. Aust. Soc. clin. Biochem. **1**, 333—336 (1968).

Dr. J. A. Young
Department of Physiology
University of Sydney
Sydney, N.S.W. 2006, Australia