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MICROPUNCTURE STUDIES OF THE ELECTROCHEMICAL ASPECTS OF FLUID AND ELECTROLYTE TRANSPORT IN INDIVIDUAL SEMINIFEROUS TUBULES, THE EPIDIDYMIS AND THE VAS DEFERENS IN RATS

BY NORMAN LEVINE* AND DONALD J. MARSH†

From the Department of Physiology and Biophysics, New York University School of Medicine, New York, New York 10016, U.S.A.

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SUMMARY

1. Micropuncture and micro-analytical techniques were used to study some of the electrochemical aspects of fluid and electrolyte transport in single seminiferous tubules, the epididymis and vas deferens.

2. Seminiferous tubules contain a fluid that is slightly hypertonic to plasma, has a high potassium and chloride ion concentration, a lower sodium ion concentration and is slightly acidic relative to plasma.

3. The lumen of the seminiferous tubule is about 5 mV negative to a Ringers bathing solution.

4. Potassium and chloride ions enter the seminiferous tubule lumen against an electrochemical gradient, while the gradient for sodium ion favours its entry. This does not preclude possible active transport of sodium ion.

5. Between the seminiferous tubules and the beginning of the caput epididymis spermatocrit changes indicate that about 50% of the fluid leaving the testis is reabsorbed. Chloride ion and potassium ion are reabsorbed in concentrations greater than in lumen while sodium ion is reabsorbed in a concentration equal to that in the lumen. This region is also the site of intense hydrogen ion secretion.

6. The region between the seminiferous tubules and the caput is isopotential. Reabsorption of sodium and chloride ions are against electrochemical gradient. Potassium ion reabsorption is favoured by the electrochemical gradient.

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† Career Scientist of the Health Research Council of New York, Contract no. I-391.

7. Osmolar and electrical considerations indicate the probable secretion of organic acids between the seminiferous tubules and the caput epididymis.

8. Between the caput and the vas deferens 50 % of the remaining fluid is reabsorbed. Sodium ion is reabsorbed in concentrations much greater than in lumen, potassium ion enters the lumen and the pH rises. Sodium reabsorption in this region is essentially independent of chloride reabsorption.

9. The corpus epididymis is 20 mV negative to a Ringers bathing medium while the beginning of the vas deferens is 27 mV negative. Reabsorption of sodium ion is against an electrochemical gradient as is potassium entry. Osmolality data and the concentration of sodium in the reabsorbate require further secretion of organic compounds in this region.

INTRODUCTION

Newly formed sperm are suspended in a seminal plasma which carries them out of the testis, through the epididymis, to the vas deferens. During this transit the spermatozoa undergo functional and morphological changes. Since the fluid transporting the sperm also changes as it flows along the reproductive tract, any understanding of what happens to sperm will have to include an account of their interaction with this fluid.

The mechanisms responsible for the formation of the fluid in seminiferous tubules, and its modification in the epididymis and vas deferens have received only limited attention. Studies on the composition and flow rate of seminal fluid collected through a catheter in the rete testis of conscious rams indicate that it is formed by an active secretory process rather than by a filtration mechanism (Voglmayr, Waites & Setchell, 1966; Setchell, Voglmayr & Waites, 1969; Linzell & Setchell, 1969). However, this fluid may have already been modified in the rete testis and there is no information available about the composition of the nascent fluid as it is formed in seminiferous tubules or about the electrochemical circumstances under which it is formed. Mechanisms of fluid and electrolyte transport have been even less well characterized in the epididymis. Studies in this region have been limited to measurements of electrolyte concentrations in fluid scraped from dead tissue. Although they have indicated a pattern of fluid and electrolyte reabsorption (Crabo & Gustafsson, 1964; Wales, Wallace & White, 1966; Scott, Wales, Wallace & White, 1963) the sampling techniques were inadequate to reveal regional variations in the handling of the individual ions. Post mortem changes, contamination with non-luminal fluid and mixing with fluid from adjacent regions are some of the problems associated with this approach. We have applied micropuncture and micro-analytical techniques commonly used in renal physiology to sample and analyse fluids from single seminiferous

tubules, the caput, body, and cauda of the epididymis, and the vas deferens of anaesthetized rats. We have also measured epithelial electrical potential differences and pH of the luminal fluid in these regions. These measurements provide a more detailed account of the transport of major inorganic ions than has heretofore been possible, and also permit some preliminary conclusions about the mechanisms of this transport.

METHODS

Sample collection. Virgin Sprague–Dawley rats, weighing between 225 and 300 g, were anaesthetized with Inactin (10 mg/100 g body wt.). The scrotal sac was incised, and the testis, epididymis, and vas deferens were exposed and bathed in paraffin oil at room temperature. Care was taken never to occlude the blood supply.

Pipettes for sampling were made from borosilicate glass tubing (1·0 mm outside diameter, 0·2 mm wall thickness). The tubing was made into standard Ling–Gerard micro-electrodes with tip diameters less than 1 μ. The electrode tip was lowered with a rack and pinion onto the surface of a rotating wet stone and ground to give a sharpened micropipette with a tip diameter of 25–50 μ. The micropipettes were filled with paraffin oil coloured with sudan black, and mounted in a pipette holder attached to a Leitz micromanipulator. The sharpened micropipette was inserted into the lumen of individual seminiferous tubules, the head, body or tail of the epididymis or into the vas deferens, and a small droplet of oil extruded to be certain that the micropipette was correctly placed in the lumen. A sample of 50–100 nl. fluid was then aspirated into the tip of the pipette. The collection procedure differed from the usual renal tubular method where the protocol is to aspirate fluid at a rate sufficient to maintain the oil droplet in a constant position just distal to the collection pipette. Since flow velocities are much slower in the reproductive tract, it was sufficient simply to aspirate the desired volume of fluid. From the volume of fluid that was aspirated, and the observed radius of the various regions sampled, it is possible to estimate the length of tubule that contained the sample. In the various regions of the epididymis the length is about 2 mm, which is 1 % or less of the total tubular length. For the seminiferous tubules the length of tubules containing the sample was about 1 cm which is approximately 30–50 % of the tubular length and which is sufficiently small to ensure that the samples came from single tubules.

As the pipette was removed from its sampling site some oil from the pool overlying the tissue was aspirated into the tip to prevent evaporation. After the experiment was completed the samples were transferred under paraffin oil to a long thin siliconized glass tube, also filled with paraffin oil. The sample was aspirated to a distance of at least 1–2 cm from the tip of the tube. The tip was sealed in a gas oxygen flame which provides intense localized heat and does not perceptibly heat the region of the tubing containing the specimen. The tube was then placed in a glass outer jacket and centrifuged for 15 min in an International Model MB clinical centrifuge.

The fractional volume occupied by the sperm (spermatocrit) was measured under a microscope with an eye piece micrometer. The supernatant plasma was then aspirated into another oil-filled micropipette and deposited on a siliconized glass slide under paraffin oil. This seminal plasma was used for the analysis of osmolality, and of sodium, potassium, and chloride concentrations. At all times during collection and subsequent transfer, the sample was surrounded by paraffin oil which had been saturated with water, and was never allowed to come in contact with air.

Sodium and potassium concentrations were measured in samples of approximately 1 nl. volume with an integrating microflamephotometer (Laurence & Marsh,

1970). Chloride concentration was measured with the second coulometric method of Ramsay (Ramsay, Brown & Croghan, 1955) and osmolality was determined by freezing point depression with a commercially available Peltier junction osmometer (Clifton Technical Physics, New York City).

pH measurements were made with antimony electrodes as described by Solomon & Alpert (1969) and by Viera & Malnic (1968). The measurements were made with electrodes having a tip diameter of 20 μ in droplets of whole semen deposited under paraffin oil saturated with 5% CO_2. The samples were collected as previously described for electrolyte measurements except that they were not centrifuged. The reference electrode was a micropipette with a tip diameter of 2 μ filled with 3 M-KCl and connected by a 3 M-KCl agar bridge to a calomel half-cell. This reference electrode was inserted into the semen drop and the potential difference was recorded with a Keithley 603 electrometer (input impedance greater than 10^{14} Ω, grid current less than 5×10^{-14} A). A calibration curve for each antimony electrode was constructed before use by comparing its response to that of glass electrode over the pH range 6·00–8·00 at 0·05 pH intervals. When sample measurements were being made the electrode calibration was checked with a series of three buffers deposited on the same slide as the sample. The interval between these buffers was 1 pH unit and each electrode's calibration was checked after each sample measurement. From these determinations bicarbonate concentrations were calculated using the standard relationship:

$$\mathrm{pH} = 6{\cdot}1 + \log \frac{(HCO_3^-)}{0{\cdot}03 pCO_2}.$$

Electrical potential differences were measured with 3 M-KCl-filled electrodes. The testis and epididymis were exposed as for sampling except that they were bathed in a Ringer solution (NaCl, 135 m-equiv/l.; KCl, m-equiv/l.; $CaCl_2$, 2m-equiv/l.). The potential difference between two calomel half-cells, one connected with a 3 M-KCl bridge to the Ringer bath and the other via a similar bridge to the micro-electrode, was recorded on a Keithley 603 electrometer. The electrodes were bevelled and their outside diameters at the tips were 2–5 μ. These electrodes were used rather than the standard Ling-Gerard electrode with a tip diameter less than 1 μ because the muscular wall of the epididymis and vas deferens invariably broke the fragile high resistance electrodes. Electrodes made in this manner offer two advantages: (1) since they are bevelled, they can be inserted into lumens more readily, with less tearing, and with better sealing than a broken electrode of unknown dimensions and with a jagged tip; (2) the tip of the larger electrode is easily visualized and can be followed during the course of the penetration so that its localization within the lumen can be visually determined. This is a decided advantage in structures such as those we have studied where several cell layers must be punctured and the usual sharp voltage drop associated with penetration of a single cell membrane in such tissues as nerve or muscle is not to be anticipated. A possible objection to the use of these larger electrodes is that the electrolyte filling the electrode's barrel may leak out at higher rates than would be anticipated from Ling-Gerard micro-electrodes, and would alter the potassium and chloride concentrations in the fluid adjacent to the epithelium. Laurence & Marsh (1970) have shown that this problem does not introduce a measurable artifact when 2 μ 3 M-KCl electrodes are used in renal tubules of the hamster. However, linear flow velocities are less in these tissues than in kidney tubules. We therefore imposed the criterion that values would be accepted only if they were stable within $\pm$ 1 mV for at least 1 min after puncture and that they reached these stable values immediately after penetration to the lumen.

RESULTS

Results of electrolyte, spermatocrit, pH and electrical potential measurements are presented in Table 1.

Seminiferous tubules

These tubules elaborate a fluid that differs in several significant respects from blood plasma. It is hypertonic, has a lower sodium concentration, but higher potassium and chloride ion concentrations than does plasma. These differences are all significant at the 0·1 % level. Similar results have been reported by Tuck, Waites, Young & Setchell (1970). The pH of seminiferous tubular fluid equilibrated with 5 % CO_2 is 7·31 ± 0·02, which is more acid than venous blood.

The electrical potential difference across the seminiferous tubule epithelium is 4·8 ± 0·1 mV, lumen negative to the bathing medium. As can be seen from Table 2, potassium and chloride ions both enter the tubule against their respective electrochemical gradients, while sodium entry into the lumen is favoured by the electrochemical gradient.

Epididymis

Head. The sperm in this region comprise 39 % of the total volume, almost double the value found in the seminiferous tubule. If the spermatozoa have undergone no significant volume change this observation suggests that 46 % of the fluid is reabsorbed between the end of the seminiferous tubule and the beginning of the epididymis. The sodium ion concentration does not change significantly during this reabsorption, yet the chloride ion concentration decreases to 31 m-equiv/l. a difference of 87 m-equiv/l. from the seminiferous tubule ($P < 0{\cdot}1\,\%$). The potassium concentration also decreases to 16 m-equiv/l., the lowest value in the regions we have studied ($P < 0{\cdot}1\,\%$). The pH at 6·48 is also the lowest we found and corresponds to a bicarbonate ion concentration of 2·7 m-equiv/l., which is significantly lower than in the seminiferous tubule ($P < 0{\cdot}1\,\%$).

In Table 3 are listed the calculated concentrations of sodium, potassium, chloride and bicarbonate ions in the fluid reabsorbed between the seminiferous tubules and the head of the epididymis. This fluid appears to be hypertonic and the sum of the anions is 65 m-equiv/l. greater than the cations.

The osmolality of fluid at the beginning of the head is 315 m-osmole/kg H_2O but the sum of the electrolyte concentrations we measured is only 159 m-equiv/l. If we apply a rational osmotic coefficient of 0·91 to this total ion concentration, there remains 168 m-osmole/kg H_2O unaccounted for (Fig. 1). Moreover, there is also a measured cation concentration of 95 m-equiv/l. greater than the measured anion concentration.

TABLE 1. Results of spermatocrit, osmolality, electrolyte, and electrical potential difference measurements, expressed as the mean ± the S.E. of the mean. Figures in parentheses are the number of animals. Electrical potential differences are given as lumen relative to bathing medium

	Spermatocrit	Osmolality (m-osmole/kg H_2O)	Na^+ (m-equiv/l.)	K^+ (m-equiv/l.)	Cl^- (m-equiv/l.)	pH	HCO_3^- (m-equiv/l.)	Potential difference (mV)
Seminiferous tubules	0·203 ± 0·024 (30)	338 ± 6·7 (30)	109·5 ± 4·5 (30)	46·2 ± 3·9 (28)	118·0 ± 4·0 (30)	7·31 ± 0·02 (10)	19·6	−4·8 ± 0·1 (20)
Initial segment	—	—	—	—	—	—	—	−3·6 ± 0·1 (8)
Caput	0·388 ± 0·015 (23)	315 ± 4·2 (20)	112·1 ± 5·6 (17)	16·0 ± 5·8 (15)	31·0 ± 4·4 (16)	6·48 ± 0·05 (9)	2·7	−5·6 ± 0·1 (26)
Body	0·608 ± 0·038 (21)	340 ± 7·9 (20)	57·9 ± 4·9 (14)	37·3 ± 1·6 (14)	24·4 ± 3·8 (16)	—	—	−20·7 ± 0·4 (18)
Cauda	0·548 ± 0·026 (19)	329 ± 4·6 (17)	20·6 ± 3·0 (14)	55·1 ± 2·3 (14)	23·6 ± 2·8 (15)	6·85 ± 0·03 (10)	6·7	−27·0 ± 0·6 (20)
Vas deferens	0·697 ± 0·023 (16)	339 ± 3·7 (15)	23·3 ± 3·7 (12)	51·9 ± 1·9 (12)	19·3 ± 2·4 (12)			
Plasma	—	311 ± 3·3 (20)	145·5 ± 1·5 (22)	—	98·0 ± 1·9 (20)	7·50 ± 0·02 (6)	30·1	—

The electrical potential at the beginning of the head of the epididymis is $5{\cdot}6 \pm 0{\cdot}1$ mV lumen negative to the bathing medium. This value differs by only 2 mV from the average of a somewhat smaller series of measurements in the initial segment and it appears that the region from the seminiferous tubule to the head of the epididymis is isopotential throughout its length. Potassium ion reabsorption is favoured by the electrochemical potential difference, but both sodium ion and chloride ion are reabsorbed against their gradients.

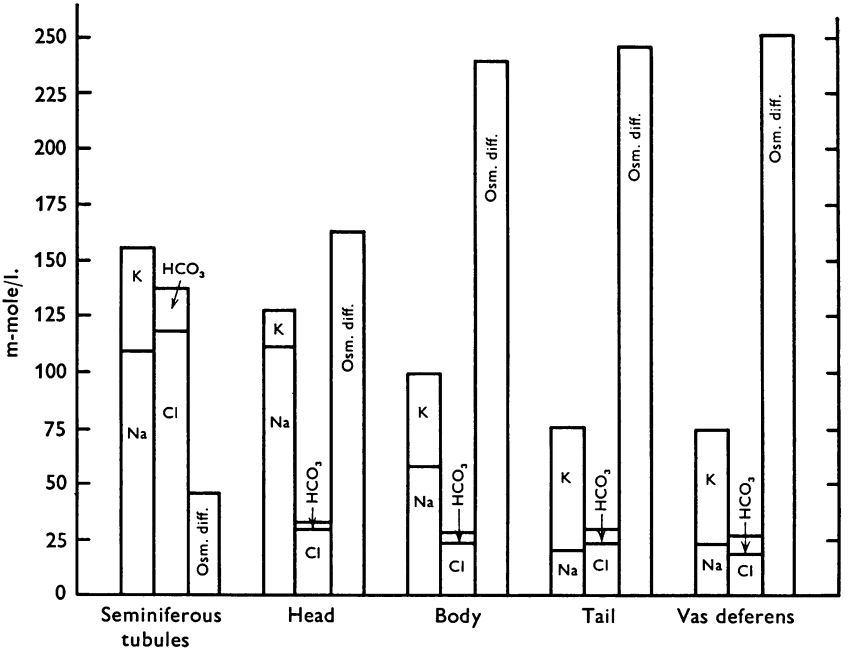

Fig. 1. Luminal concentrations of cations and anions as well as osmolar difference in various regions of the reproductive tract.

Body, tail and vas deferens

Between the head and the vas deferens, sodium ion concentration decreases by 88 m-equiv/l. ($P < 0{\cdot}1\,\%$), the potassium ion concentration increases by 35 m-equiv/l. ($P < 0{\cdot}1\,\%$) but the chloride on concentration decreases only 12 m-equiv/l. ($P < 1\,\%$). The spermatocrit rises to 0·697 ($P < 0{\cdot}1\,\%$) indicating that about 45 % of the fluid entering the head is absorbed by the time it has reached the vas deferens. Most of these reabsorptive processes are apparently completed once the fluid has reached the tail, since none of the electrolyte concentrations differ significantly between the tail and the vas deferens.

The pH rises significantly from its lowest value in the head to $6{\cdot}85 \pm 0{\cdot}2$

TABLE 2. Comparison of measured electrical potential difference with equilibrium potential as calculated from the Nernst equation. All values are expressed as lumen relative to blood. A measured potential difference less than the equilibrium potential indicates a free energy difference favouring movement into the lumen, except for chloride ion where just the opposite pertains

	Seminiferous tubule			Head			Tail-vas deferens		
	Measured p.d.	Equilibrium p.d.	Diff.	Measured p.d.	Equilibrium p.d.	Diff.	Measured p.d.	Equilibrium p.d.	Diff.
Na	−4·8	+7·3	−12·1	−5·6	+6·8	−12·4	−27·0	+49·3	−76·3
K	−4·8	−63·6	+58·8	−5·6	−36·0	+30·4	−27·0	−67·2	+40·2
Cl	−4·8	+4·9	−9·7	−5·6	−24·6	+19·0	−27·0	−40·1	+13·1

in the region of the tail and vas deferens ($P < 0{\cdot}1\,\%$) and the calculated bicarbonate concentration is 6·7 m-equiv/l. The ongoing transport processes in the epididymis have exaggerated the osmolar discrepancy so that in the vas deferens seminal plasma, 256 m-osmole/kg H_2O are not accounted for by the measured ions.

The transport processes occurring in the body and tail are associated with a progressive increase in the potential difference across the epithelial wall. It is $20{\cdot}7 \pm 0{\cdot}45$ mV, lumen negative, in the body of the epididymis and $27{\cdot}0 \pm 0{\cdot}6$ mV, lumen negative, in the region of the tail and vas

TABLE 3. Calculated concentrations of inorganic ions in the reabsorbate from two regions. Concentrations in the reabsorbate were calculated from the formula

$$C_R = \frac{C_1 - C_2(S_1/S_2)}{1 - (S_1/S_2)}$$

where C_1 and C_2 are the concentrations at the beginning and end of the segment respectively and S_1 and S_2 are the spermatocrits at the beginning and end of the segment. A negative sign indicates entry into the lumen

	Seminiferous tubule, head	Head, vas deferens
Na (m-equiv/l.)	106·7	225
K (m-equiv/l.)	79·3	−31
Cl (m-equiv/l.)	213·3	43·5
HCO_3 (m-equiv/l.)	38·1	−2·3

deferens. None of the ions appears to be in equilibrium in any region of the epididymis or vas deferens. It can be seen in Table 3 that the sodium concentration in the reabsorbed fluid is about 225 m-equiv/l. This reabsorption proceeds against an electrochemical potential difference. The rising potassium concentration can be accounted for chiefly by removal of water but there also appears to be a contribution from epithelial secretion into the lumen. Potassium is 30–40 mV away from electrochemical equilibrium in the epididymis and vas deferens; attainment of the observed concentrations must therefore involve a more complex process than simple equilibration. There is continuing reabsorption of chloride against an electrochemical gradient (Table 2). However, the reabsorbate is not a simple isotonic sodium chloride solution, since, as is clear from Table 3, the rate of chloride reabsorption is only a fraction of the sodium reabsorption rate in the epididymis so that the net unaccounted anion concentration has increased to 174 m-equiv/l.

DISCUSSION

The results presented here provide a more detailed osmotic and ionic balancing than has previously been possible in the male reproductive tract. They reveal a complex pattern of secretion and reabsorption with apparently different mechanisms operating in each of the regions studied.

Seminiferous tubules

There has been considerable interest in recent years about the mechanism responsible for the formation of seminiferous tubular fluid. The studies of Voglmayr *et al.* (1966), Setchell *et al.* (1969) and Linzell & Setchell (1969) demonstrate that the rate of tubular fluid formation is dependent on an adequate supply of oxygen and metabolic substrates, but relatively independent of vascular perfusion pressure. The composition of fluid collected from the rete testis, except for a slightly elevated potassium concentration, appeared to corroborate the hypothesis that the tubular fluid is formed as a typical isotonic sodium chloride solution (Setchell *et al.* 1969).

However, fluid and electrolyte secretion, at least in the rat, appears to be more complex than this simple model suggests. We find that the potassium concentration is 46 m-equiv/l., an order of magnitude more concentrated than in blood plasma and three times the value found by Setchell *et al.* (1969). Because of the prevailing electrical potential difference, potassium ions must cross into the epithelial lumen against a significant electrochemical potential difference and this movement requires the expenditure of metabolic energy. Whether the difference in results can be attributed to the species difference, or to the different sampling sites, is unclear.

The net movement of sodium ion is into the lumen, but while it is true that this secretory flux is favoured by the electrochemical gradient, it would be premature to conclude that the primary work of the epithelium is done on other ions and that there is no active transport of sodium. While nothing in the data excludes this last possibility, there is a molar predominance of sodium and so its passive secretion would be energetically possible only if the epithelium had a very high permeability to sodium. A more likely possibility is that there are a minimum of two transport pathways in parallel, one of which is secreting sodium and anions, and the other potassium and its anions. Active sodium transport down an electrochemical gradient has been demonstrated in the frog skin (Bricker, Biber & Ussing, 1963).

Rete testis, ductuli efferentes, initial segment, and intermediate zone

The function of these regions can be deduced only by noting the changes in fluid composition which occur as fluid reaches the end of these regions and enters into the caput epididymis. Such inferences suggest a substantially different function for these segments than is found in other regions of the male tract. Water, sodium, potassium, and chloride are all removed while hydrogen ion is secreted into the lumen.

About 50% of the fluid leaving the seminiferous tubules has been reabsorbed by the beginning of the head, as estimated from the change in spermatocrit between these two regions. Similar observations have been made by Crabo & Gustafsson (1964). These regions appear well suited to fluid reabsorption because of the more favourable surface to volume relationships than are found elsewhere in the male reproductive tract. The mechanism of this fluid reabsorption is probably osmosis secondary to strong electrolyte transport by processes similar to those found in numerous other reabsorbing epithelia. Sodium reabsorption is against an electrochemical gradient and probably occurs by active transport. Potassium, on the other hand, is moving in the direction favoured by its electrochemical gradient and no active transport need be invoked to explain its movement. The analysis of chloride movement is complicated by the fact that two cations, sodium and potassium, are being reabsorbed in practically equimolar amounts (Table 3) by different mechanisms. It does seem reasonably clear that chloride is reabsorbed against its gradient, but exactly how this apparent active transport is coupled to the reabsorption of the cations is not revealed by these studies.

The region between the testis and the caput epididymis experiences a pH decrease from 7·3 to 6·4; it is the major site of acidification in the parts of the male tract we have studied. The electrolyte and osmolality measurements provide some clues about the mechanism of the acidification process. Fig. 1 shows that inorganic ions account for all but 42 m-osmole/kg H_2O in seminiferous tubule fluid, and are balanced as to charge to within 18 m-equiv/l. Once this fluid has reached the caput epididymis, the sum of inorganic cation concentrations exceeds total inorganic anion concentration by 95 m-equiv/l., and 157 m-osmole/kg H_2O of the total osmolality are due to compounds other than these ions. The secretion of weak organic acids with pKs lower than the seminal plasma's pH could account for both the acidification and the osmotic and electroneutrality discrepancies, particularly if the acid entered the lumen in the unionized form, and then dissociated. Dissociated organic acids generally penetrate cell membranes less well than the uncharged acid forms, so that their net entry into the lumen would continue as long as the luminal fluid remains

more alkaline than the cell interior. This suggestion is analogous to the commonly accepted hypothesis of renal ammonia excretion, where the entry of NH_3 occurs by diffusion of the neutral gas, which then captures a proton (Pitts, 1964).

The identity of the secreted acids is not known. Lactic acid has been found in millimolar amounts in ram and bull epididymal fluid (Scott *et al.* 1963; Wales *et al.* 1966). However, its reported concentration is insufficient to account totally for the ionic and osmotic discrepancies. Another organic compound secreted in the early segments of the epididymis is glycerylphosphorylcholine (Mann, 1964). It is present in relatively high concentrations (tens of millimoles per litre) but its pKs are unknown, so its contribution to pH changes and ionic balancing cannot be judged.

Epididymis and vas deferens

The transport behaviour of these regions appears to differ from that of the previously discussed regions. The spermatocrit increases by a factor of 1·8, approximately the same increase as seen between the seminiferous tubules and the caput epididymis, which means that the more proximal region reabsorbs about 46 % of the fluid formed in the seminiferous tubule, and the remainder of the epididymis only 23 %. There are also differences in the transport of the individual ions between this region and the others. For example there is continuing reabsorption of sodium ion which leads to a progressive lowering of sodium ion concentration, but this is largely unaccounted for by inorganic anion transport as there is an excess of 174·5 m-equiv/l. cations in the reabsorbate (Table 3).

Let us treat the unaccounted osmolality as if it were due to a single equivalent solute. The equivalent solute's concentration increases between the caput and the vas deferens, but this concentration increase is more than accounted for by water reabsorption and there is actually net reabsorption of that solute. The equivalent solute's osmolality in the reabsorbate is 55 m-osmole/kg H_2O. Even if all this solute was a completely dissociated anion, it could not account for more than one third of the discrepancy between cations and anions in the reabsorbate. This discrepancy is made still greater when account is taken of the pH changes, since the progressive alkalinization toward the vas deferens requires proton reabsorption in one form or another. These charge and osmolality deficits indicate a significant turnover of organic compounds, the low pK compounds entering the lumen in more proximal regions and being reabsorbed to some extent more distally, aided in part by the increased pH.

The picture that emerges from these and other studies on fluid and electrolyte reabsorption in the epididymis and vas deferens is that of a series of progressive concentration changes which begin in the head and

reach their full extent somewhere near the tail, or in the vas deferens itself. Since these changes develop with distance and therefore with time, it might be natural to suppose that the epithelium is functionally uniform throughout its length, and that the concentration changes are flow-rate limited. There are no tests of this hypothesis available, but it seems improbable because of the very slow linear flow velocity. Rather, we would suggests an analogy with certain experimental conditions commonly met in the kidney. If a renal tubule is perfused with a solution of a non-reabsorbable solute, such as raffinose, the system will relax until a steady state is reached in which concentrations become time invariant and net fluxes of water and all osmotically important solutes are nearly zero. Sodium chloride transport normally causes isotonic fluid reabsorption; when an additional degree of freedom is introduced by adding raffinose, water is restricted from leaving, and the sodium chloride concentration is permitted to decrease from plasma values. The steady-state condition is reached when the active transport of sodium chloride is balanced by a passive leak flux in the opposite direction whose driving force is the concentration gradient (Gertz, 1963; Marsh, Ullrich & Rumrich, 1963). In the epididymis, the role of raffinose is probably taken by the assorted organic acids and bases but in other respects the situations are comparable. The strong electrolyte concentrations at any point in the epididymis would then be determined mainly by the ability of the epithelium to generate and maintain concentration differences across the epithelial wall, and the progressive decline of sodium potassium and chloride concentrations implies a progressive change of the transport properties.

If this view of the rate determining processes is correct, the concentration of a strong electrolyte should also be indifferent to the transport activity of the sperm, since any movement into or out of the sperm would simply perturb the steady state, and the epithelium would respond by changing the transport rate until the steady state is achieved again.

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