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Characteristics of Testicular Spermatozoa and the Fluid which Transports them into the Epididymis

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Significant advances often depend on the development of a technique, and this applies to the study of testicular spermatozoa. Although spermatozoa could be obtained from the testes of dead animals, the purity of the preparation was questionable, the numbers obtained were insufficient for a critical evaluation of their metabolism, and there was always an uncertainty whether or not spermatozoa collected *post mortem* were metabolically normal. Most of the work described in this paper stemmed directly from the development of a technique for implanting a catheter (Fig. 1) into the rete testis of rams (99, 102). This technique enabled testicular spermatozoa to be collected in abundant numbers and under physiological conditions in the fluid which carries the spermatozoa out of the testis and into the epididymis. This fluid is unique in many respects. It is the "milieu" which normally bathes the spermatozoa, and probably most of the cells of the germinal epithelium, and so a knowledge of its composition is vital to an understanding of the process of spermatogenesis. Most of this fluid is resorbed selectively in the epididymis so that its composition changes progressively during its passage through the epididymis (16). The resorbed portion provides a possible pathway for the feedback of information to the rest of the body from the otherwise isolated seminiferous tubules.

Transport of Spermatozoa out of the Testis into the Epididymis

Histological evidence dating from the 19th century suggested that the centers of the seminiferous tubules are filled with a fluid which flows through the rete testis and the efferent ducts into the epididymis (28, 54). The magnitude of this process was apparent when it was observed that fluid flowed from catheters in the rete testis of rams at a rate of between 0.5 and 1.5 ml/100 g/hr or about 2 ml/hr from an average-sized ram testis. This should be contrasted with the blood flow through the testis of 1200 ml/hr (92, 93) and with the testicular lymph flow of 10 ml/hr (15, 46). The goat testis produces fluid at about the same rate (90) and the bull testis at a slightly lower rate (0.4 ml/100 g/hr) (98).

Estimates of the rate of fluid secretion can also be made from the increase in weight or water content after ligation of the efferent ducts and these estimates agree fairly well with the direct observations in these three species (29, 85). In the rat, fluid secretion leads to an increase of water content from 6.4 g/g dry weight to 9.0 g/g in 24 hr after efferent duct ligation, which is equivalent to a secretion of about 2 g fluid/100 g testis/hr. Fluid secretion measured in this way began

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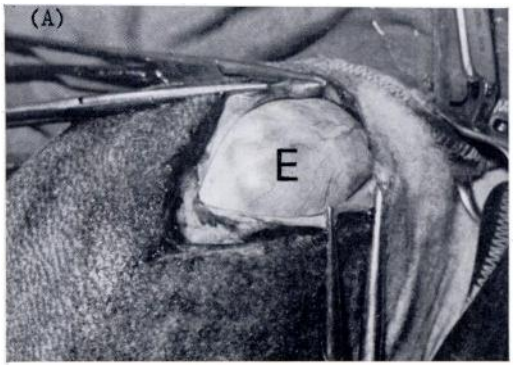


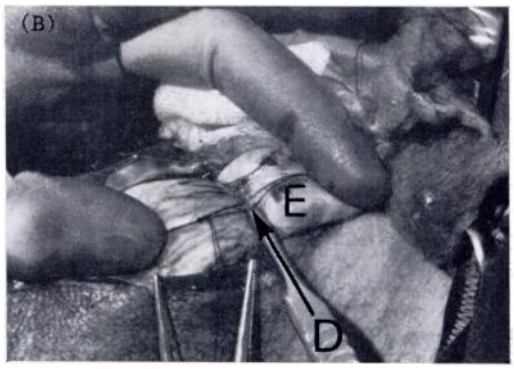
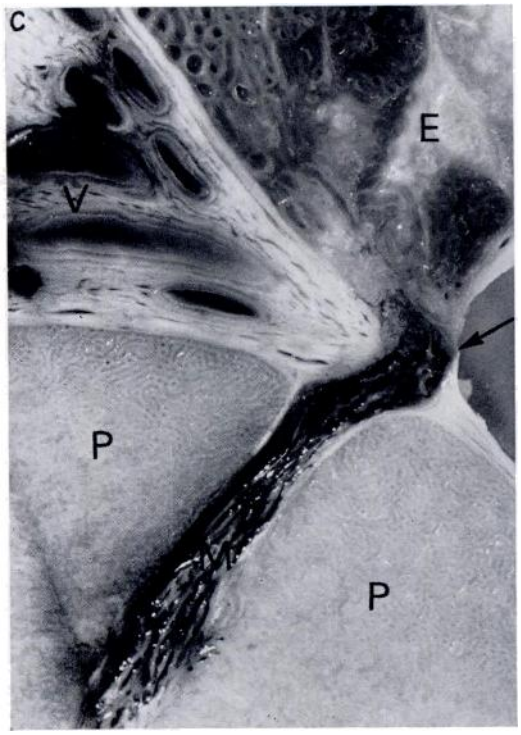
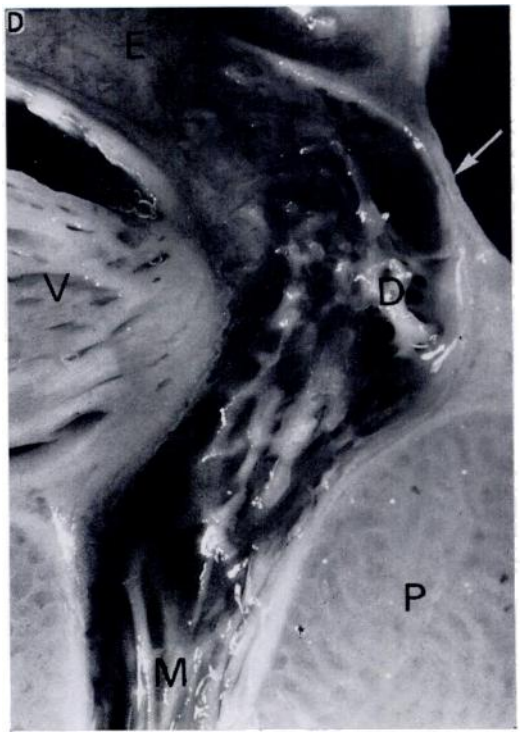


FIG. 1. Cannulation of the rete testis of rams. (A) Step 1—exposure of head of epididymis (E). (B) Step 2—reflection of head of epididymis and passing ligature behind efferent ducts (D).



(C) Sagittal section of the testis showing parenchyma (P); mediastinum (M), here injected with India ink; epididymis (E) and vascular cone (V). The arrow indicates the point of entry of the catheter.



(D) Magnified view of the rete testis and efferent ducts (D).

in rats at the age of about 30 days (85). This is the time when the first generation of spermatids are becoming spermatozoa, the microvasculature of the testis is taking its adult form (36), the tubules are becoming impermeable to acriflavine and other substances (37, 38, 40), the rectum-testis temperature difference reaches its maximal value (39), and when the injection of FSH ceases and the addition of glucose begins to have an effect on the *in vitro* incorporation of amino acids into protein (52), (Fig. 2).

Fluid secretion in mature rats is not affected immediately by hypophysectomy or by returning the testis to the abdomen, but both these operations do decrease fluid secretion after several days (Fig. 3). However, secretion of fluid by the testes of hypophysectomized rats is no less than that by testes of the same size from younger rats, suggesting that the effect of hypophysectomy is to return the testes to an immature condition. This suggestion is supported by the similarity in histological appearance between the testes of hypophysec-

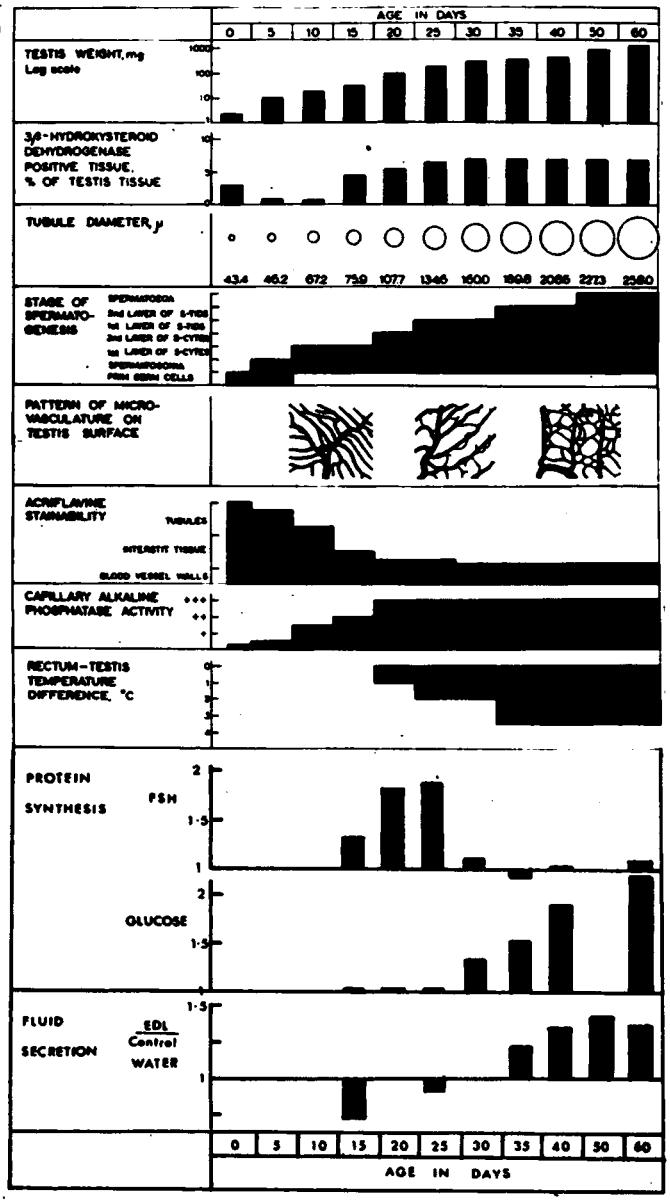


FIG. 2. Changes in the rat testis with age. The top eight panels are by kind permission of Dr. M. Kormano, University of Helsinki, Helsinki, Finland. Panels 9 and 10 are data replotted from Ref. (52). Panel 11 are data from Ref. (85).

tomized and immature rats. In contrast, the secretion of fluid by testes which had been cryptorchid for 4 or 7 days was less than scrotal testes of the same size, suggesting a specific, but not immediate, effect of cryptorchidism (85). The rate of fluid flow is decreased in rams by acetazolamide, an inhibitor of carbonic anhydrase, in doses which decrease the formation of cerebrospinal fluid and aqueous humour. Atropine and pilocarpine have no effect on the flow of rete testis fluid, in contrast to their effect on prostatic secretion in the dog. Oxytocin has no effect on the flow of rete testis fluid (89), although it has been shown to stimulate the expulsion of spermatozoa from the epididymis (23) and the contraction of isolated seminiferous tubules (58). However, oxytocin does appear to increase the concentration of spermatozoa in the fluid, presumably by facilitating their release from the walls of the seminiferous tubules (97). Fluid secretion is apparently unrelated to sperm production whether variations in the latter are due to seasonal variations, or increase in testicular temperature due to scrotal edema or heating (83, 101).

In the isolated perfused testis, the flow of fluid depends on keeping the period of anoxia to an absolute minimum, and maintaining an adequate concentration of glucose in the perfusing fluid (Fig. 4). Moreover, the flow of fluid is independent of perfusion pressure, except for short term changes in flow immediately after the alterations in pressure (48).

There is no doubt that fluid flow is important in carrying the immotile spermatozoa out

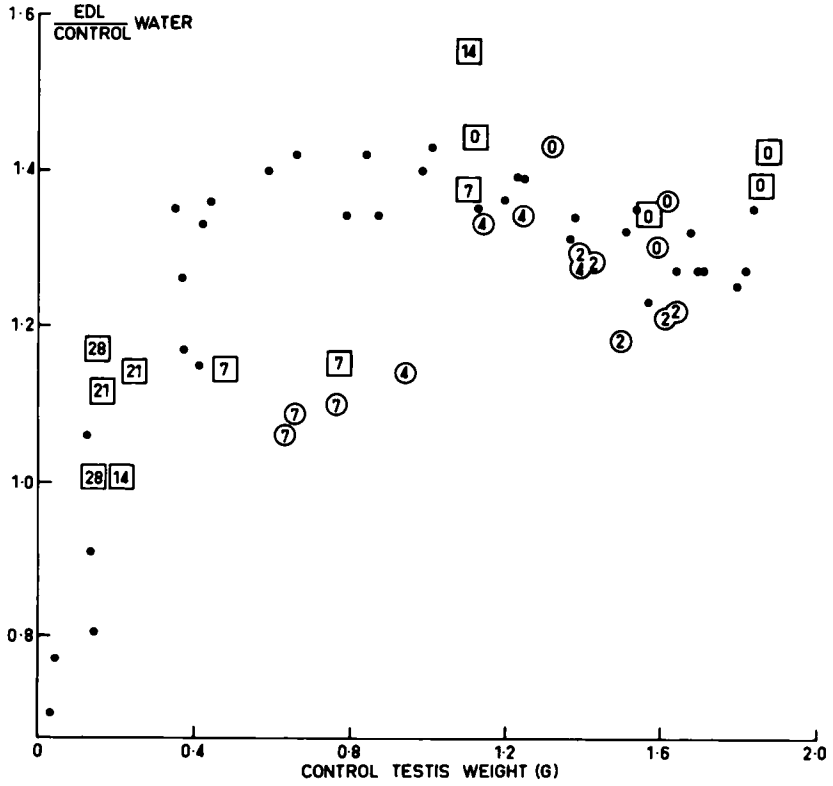


FIG. 3. Effect of hypophysectomy and cryptorchidism on fluid secretion by the rat testis, measured as the ratio of water content of the testis 24 hr after efferent duct ligation (EDL) to that of the other control testis. (●) control rats; (□) hypophysectomized; (o) cryptorchid; number inside square or circle indicates the time (in days) of the first operation before efferent duct ligation. Data from Ref. (85).

of the seminiferous tubules into the epididymis, as can be seen from the patterns formed by the tails of the spermatozoa in the tubules (73) and epididymis (41). It is probably not the only mechanism as the seminiferous tubules are capable of contractile activity (12, 58, 74), presumably by the action of the cells which resemble contractile cells elsewhere in the body and which are arranged concentrically in the boundary tissue of the tubules (12, 26, 34, 42, 43, 76, 77).

In the efferent ducts, ciliary movements may also be important (109), but the flow of fluid from the testis affects movement through

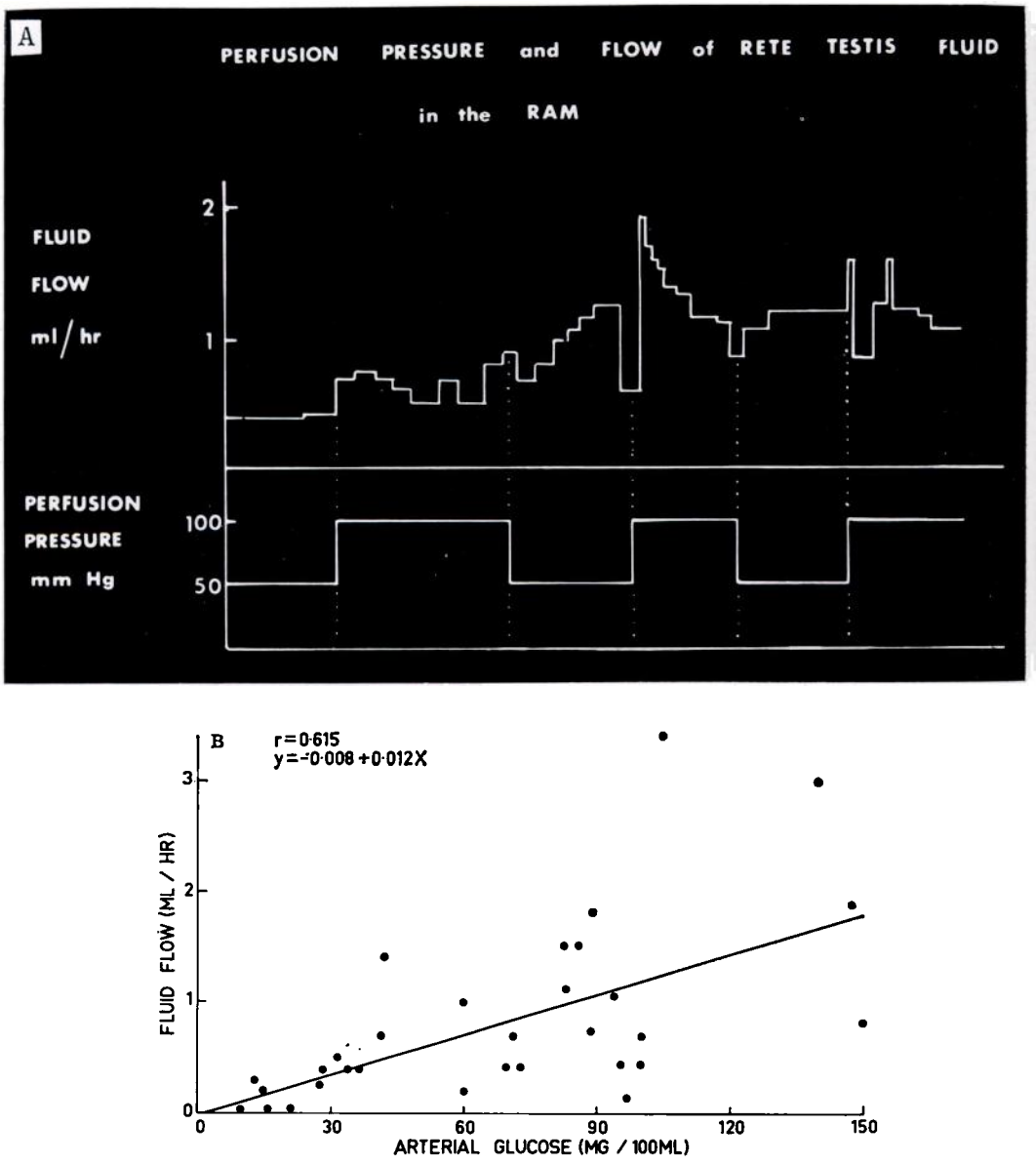


FIG. 4. Effect of perfusion pressure (A) and arterial glucose concentration (B) on the flow of rete testis fluid from the isolated perfused ram testis. Data from Ref. (48).

the epididymis in some species, e.g. guinea pigs (95) but not others, e.g. rats (49-51) and sheep (101).

The Blood-Testis Barrier

The distinction between testicular lymph and rete testis fluid must be borne in mind. Testicular lymph is presumed to arise from the extracellular fluid in the interstitial tissue between the seminiferous tubules. Rete testis fluid is presumably derived from the fluid in the lumina of the seminiferous tubules and thus the extracellular fluid of the seminiferous epithelium (Fig. 5). Some substances do not pass readily between these two fluids, or between blood plasma and rete testis fluid (84, 91) and this is presumably due to a permeability barrier in or around the seminiferous tubules. This barrier would have the effect of maintaining concentration differences between rete testis fluid and testicular lymph, of isolating the seminiferous epithelium immunologically, and of regulating substrate and hormone entry into the seminiferous tubules (91). The wall of the seminiferous tubules consists of four distinct concentric layers, two noncellular and two cellular (12, 42, 75, 76); the inner noncellular layer in the ram is made up of many electron dense lamellae (Fig. 6). However, if the fluid is secreted through the Sertoli cells by an active process (see later), this would also restrict the entry of certain substances into the tubule.

Composition of Rete Testis Fluid

Spermatozoa. The fluid collected from a catheter in the rete testis of rams and bulls is a dilute suspension of spermatozoa in a fluid isosmotic with, but different in composition from, blood plasma or testicular lymph. In rams, the concentration of spermatozoa during the breeding season is normally about 1×10^8/ml (about 1/40 of that in ejaculated semen) (99, 102), but can vary from as high as 2×10^8/ml to as low as 0.2×10^8/ml with season in England (83) or even lower (0.01×10^8/ml) after scrotal heating (101). In bulls, the concentration of spermatozoa is about 0.9×10^8/ml (98).

Ions. Although the total ash is comparable

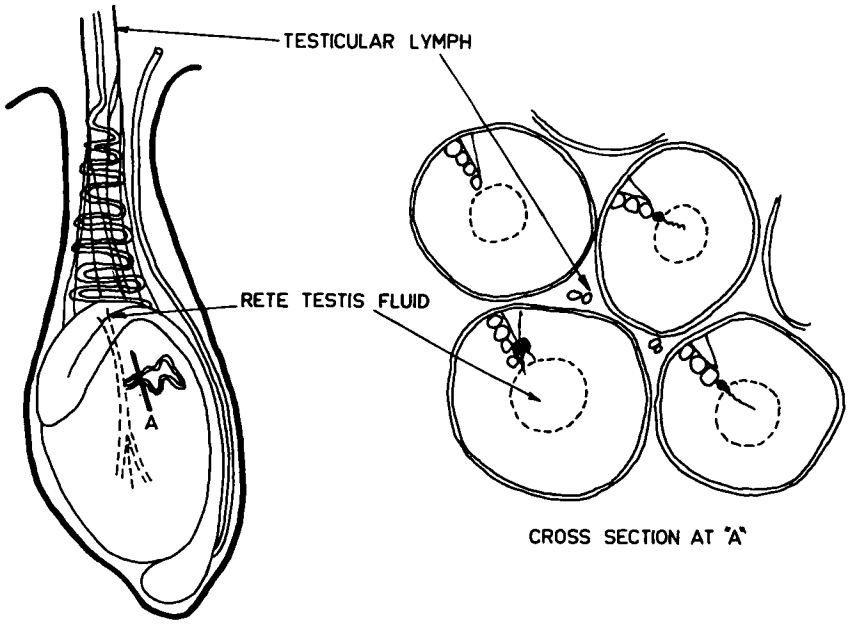


FIG. 5. Diagram of site of rete testis and lymphatic catheters and of the sites of origin of rete testis fluid and testicular lymph.

to that of plasma, the ionic composition of rete testis fluid is quite different. There is less sodium, about three times as much potassium, more chloride, only about half as much bicarbonate, calcium, and magnesium, and very much less phosphate (91). Similar differences in composition are seen between blood plasma and rete testis fluid of bulls (98) (Table 1).

Carbohydrates. Under normal conditions there is practically no glucose or fructose in rete testis fluid (102) but about 100 times as much inositol as in blood plasma (86). There is about half as much lactate in rete testis fluid as in blood plasma (91).

Protein. The total nitrogen of rete testis fluid is very much less than that of blood plasma or testicular lymph, but this difference is largely due to the differences in protein concentration. All the individual serum proteins are present in rete testis fluid but in much lower concentrations. The ratio of the concentration in plasma to that in rete testis fluid is less for albumin than for all the larger proteins, except α-macroglobulin. There is also a specific protein which migrates between α_1 and α_2 globulin on cellulose acetate electrophoresis (33) and a significant concentration of a protein smaller than albumin, with a molecular weight between 20,000 and 30,000 (84). Of particular interest are the very low concentrations of γ-globulin (33) which may provide one explanation why the germinal epithelium is not affected by a complement fixing antibody which is cytotoxic to normal spermatozoa, and which is present in the sera of normal animals of several species (22, 32).

Amino Acids. There are striking differences in concentration in some of the free amino acids. Most are present in lower concentration in rete testis fluid than in testicular lymph or in blood plasma from the internal spermatic centrations up to ten times as high as in lymph vein. However, glycine and alanine and probably glutamine and asparagine are present in appreciably higher concentrations, and glutamic and aspartic acids are present in con- or blood plasma. These amino acids appear to be synthesized within the seminiferous tubules from glucose, because if radioactive glucose is infused into a ram, the specific radioactivity of the amino acids in the rete testis fluid rises to about one half of that of the glucose, but if radioactive glutamate is infused, very little radioactivity appears in the amino acids in rete testis fluid (Fig. 7) (88).

Ram testicular spermatozoa are also able to synthesize glutamate and other amino acids from glucose (88), but bull testicular spermatozoa are less active (98).

Other Nitrogenous Compounds. Urea is present in similar concentrations to those in blood plasma (91) or testicular lymph, and there are no appreciable concentrations of free purine or pyrimidine bases (31).

Steroids. Testosterone can be found in rete testis fluid in concentrations comparable to those in testicular lymph and only slightly less than in blood plasma from the internal spermatic vein (30, 102). It is not yet established whether the steroid arises from the interstitial tissue or within the tubules, and more detailed examination is needed of the identity of the steroids present in rete testis fluid and their concentrations under various conditions.

The Composition of Rete Testis Fluid and the Concentration of Spermatozoa

Over the range of sperm concentrations seen at different times of the year in England, there was no correlation of sperm concentration with the concentration of any of the ions,

FIG. 6. Electron micrograph (×30,000) of the boundary tissue of a seminiferous tubule from the testis of a ram. Fixative: Osmium tetroxide. Embedded: Durcupan. Stain: Uranyl acetate and Millonig's lead. Microscope: Philips EMV200, Sections unsupported. LM—Inner noncellular layer; PC—peritubular contractile cells of inner cellular layer; Coll.—collagen fibers of outer noncellular layer; F—fibroblast of outer cellular layer; N—nucleus and Cy—cytoplasm of cells inside the seminiferous tubule (S); J—junction between two adjacent cells. Kindly supplied by Dr. B. Morris, Department of Experimental Pathology, Australian National University, Canberra.

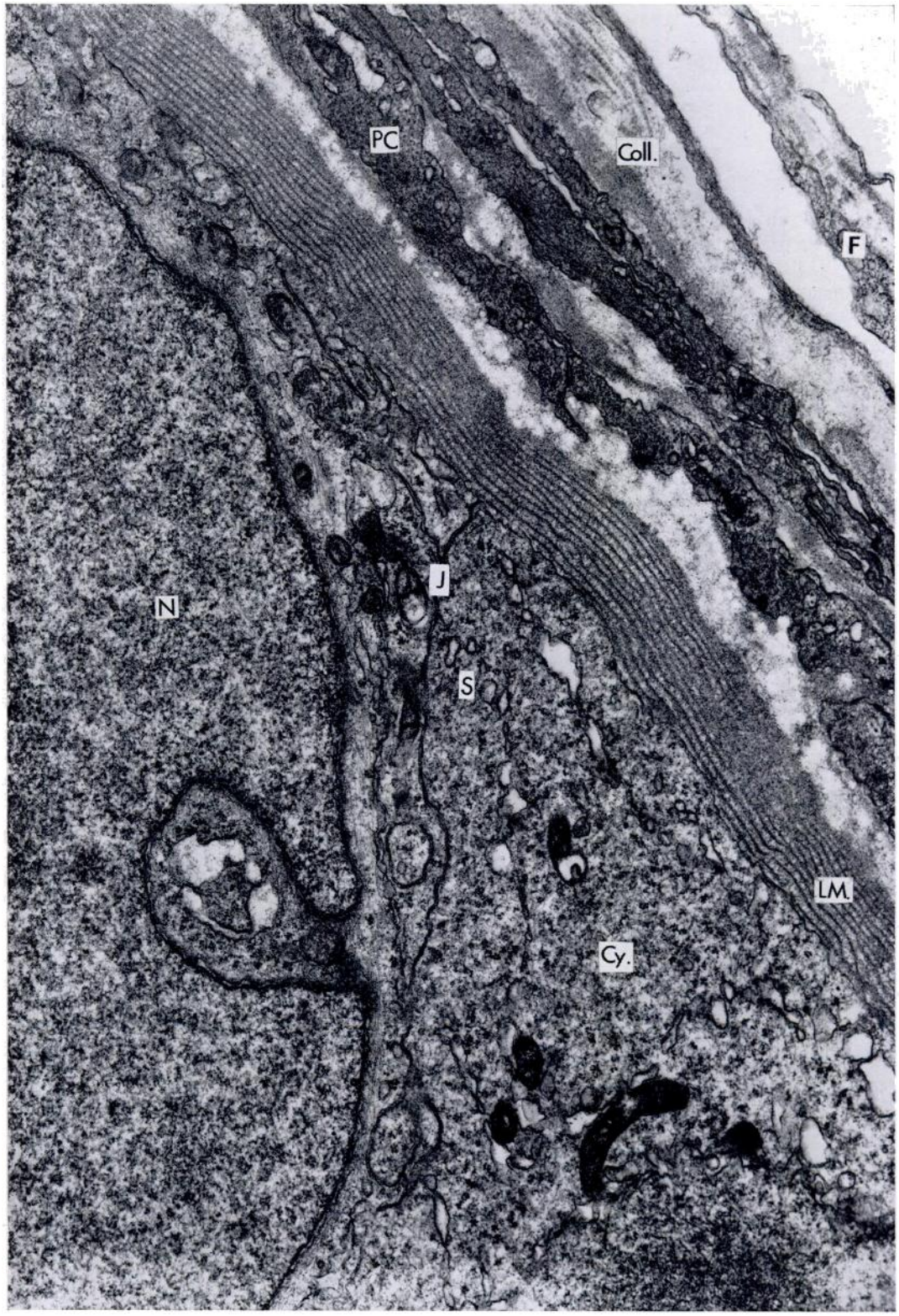


TABLE 1

The concentration of various substances in rete testis fluid, and blood plasma from rams and bulls, and the ratios R_{RTF} and R_{TL} between rete testis fluid or testicular lymph and blood plasma from the same animals. Except where stated otherwise, concentrations are given in meq or mmole/liter. For simplicity, only mean values are given but standard errors are given in the original references.

	Ram (from Refs. 86, 88, 91, 99, and 102.)				Bull (from Ref. 98)	
Substance	Rete testis fluid	Blood plasma from internal spermatic vein	R_{RTF}	R_{TL}	Rete Testis fluid	Blood plasma from jugular vein
Ions						
Sodium	118	145	0.91	1.00[a]	134	138
Potassium	12.5	4.26	3.16	1.00[a]	9.0	4.3
Calcium	2.09	5.50	0.38	0.91[a]	0.95	4.0
Magnesium	0.67	1.87	0.36	0.89[a]	0.83	1.75
Chloride	128	112	1.20	1.03[a]	122	93.2
Bicarbonate	8.1	26.3	0.33	1.09[a]	—	—
Phosphate	0.075	1.54	0.049	1.06[a]	0.05	4.12
Total mg/ml	8.79					
Ash mg/ml	8.19					
Osmolality (mOsm/kg)	291	297	0.98		280	282
Organic material						
Protein g/100 ml (Bierut)	0.28	7.5	0.037	0.66[a]		
Protein g/100 ml (Total *N*)	0.34					
Protein g/100 ml (E_{210})	0.19	8.8	0.022			
γ-Globulin (mg/100 ml)	5	2,000	0.025			
Urea	4.8	4.9	0.98		5.8	6.0
Glutamic acid (enzymatically)	1.93				1.33	0.49
Amino acids by Column chromatography						
Glutamic acid	1.86	0.26	7.2	1.07		
Aspartic acid	0.32	0.035	9.2	0.66		
Glutamine + Asparagine + Serine + Threonine	0.49	0.22	2.2	1.16		
Alanine	0.44	0.21	2.1	0.79		
Glycine	1.58	0.84	1.9	0.62		
All other amino acids	0.22	0.54	< 1			
Testosterone (μg/100 ml)	2.78	6.9	0.40	0.61[b]	2.3	
Glucose	<0.1	3.0		0.76[a]	<0.1	4.5
Fructose	<0.1	<0.1			<0.1	<0.1
Inositol	7.3	0.083	88			
Lactic acid	0.65	1.20	0.52	0.53[a]	0.74	1.45
Volatile fatty acids	0.29	0.56	0.54			
Total mg/ml approx.	4.2					
Total organic matter by ashing	4.68					

[a] From Ref. 105.

[b] From Refs. 46, 47.

protein, glutamate (83) or inositol (86). Even when the sperm concentration was reduced to very low values by heating the scrotum, there was no correlation between sperm concentration and the concentration of sodium, chloride, calcium, or magnesium. However, the concentration of potassium and glutamate decreased with spermatozoal concentrations and at the same time glucose appeared in the fluid when the spermatozoal concentration was less than 20×10^6 cells/ml (101).

The Origin and Fate of the Rete Testis Fluid

Rete testis fluid is assumed to arise within the seminiferous tubules and to flow thence through the mediastinum into the rete testis and through the efferent ducts into the epididymis (14). The first suggestion for the origin of the rete testis fluid was that it came from the dissolution of a special type of daughter cell arising from spermatogonia (28). Later it was suggested that the fluid was formed by filtration (73). The evidence now available suggests that the fluid is actively secreted. There is more chloride in rete testis fluid than can be accounted for by filtration; large water soluble molecules such as inulin and ^{51}Cr-EDTA do not enter the fluid (91); fluid secretion continues when the outlet is blocked by ligation of the efferent ducts, even to the extent of causing atrophy of the germinal epithelium; (see 104 for references); fluid secretion by the isolated perfused testis is independent of perfusion pressure but dependent on adequate supplies of glucose and a minimal

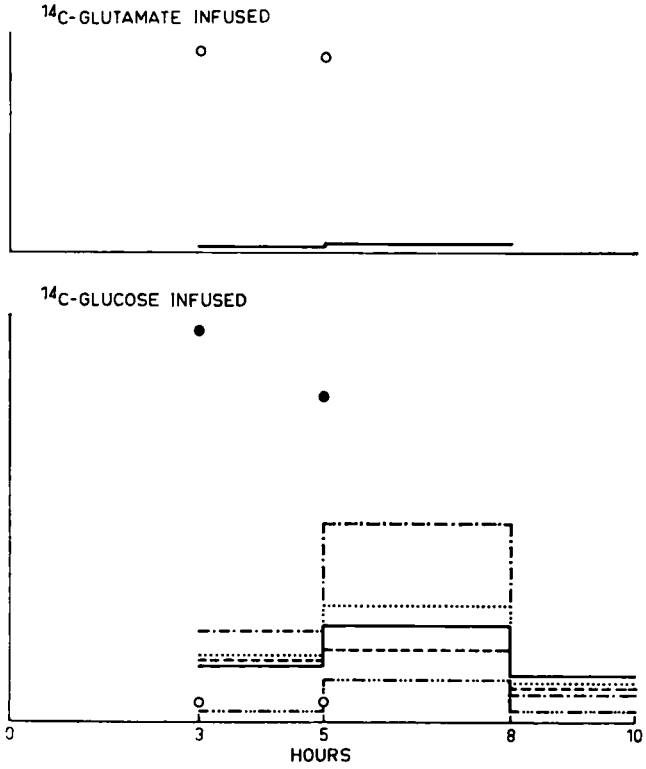


FIG. 7. Synthesis of amino acids from blood glucose by the seminiferous tubule of rams. Specific radioactivity of blood glutamate (o); blood glucose (●); rete testis fluid glutamate (———); aspartate (. . . .); alanine (–·–·–); glutamine (– – – – –) and glycine (——···——···) during infusions of ^{14}C glucose (lower panel) or glutamate (upper panel) from 0 to 5 hr. Data from reference (88).

period of anoxia (48); fluid secretion is decreased by acetazolamide, an inhibitor of carbonic anhydrase (83).

The secretion of rete testis fluid is thus similar in many ways to the formation of cerebrospinal fluid by the choroid plexus, aqueous humor by the ciliary body (17, 18, 19), and the primary secretion by the acini of the salivary glands (107), and there are a number of similarities in composition between rete testis fluid, cerebrospinal fluid and aqueous humor (91). It will be important to determine whether fluid is secreted by tubules at different stages of the cycle of the seminiferous epithelium and which cells are involved.

It is possible that the narrow junctions between the basal parts of adjacent Sertoli cells (10, 25, 56, 57) may be involved. Then, if solute is pumped by the Sertoli cells from the interstitial extracellular fluid into the intercellular spaces (Fig. 8) above the narrow junction so as to make the fluid there hypertonic, water would be drawn in to make the intercellular fluid isosmotic. Fluid would then flow along the intercellular spaces into the lumen of the seminiferous tubules. This process would thus be similar to that suggested to explain the resorption of fluid from the gall bladder (20, 35) and from the proximal convoluted tubules of the kidney (94) except that in these two organs the direction of fluid movement is from lumen to blood vessel and the narrow junctions are at the luminal end of the intercellular spaces. Sertoli cells have been shown to be the first tubular cells to take peroxidase from blood (65), lending support to the idea that the Sertoli cells are capable of pumping solute. Furthermore, Sertoli cells in the toad testis develop large fluid-filled vacuoles and the water content of the whole

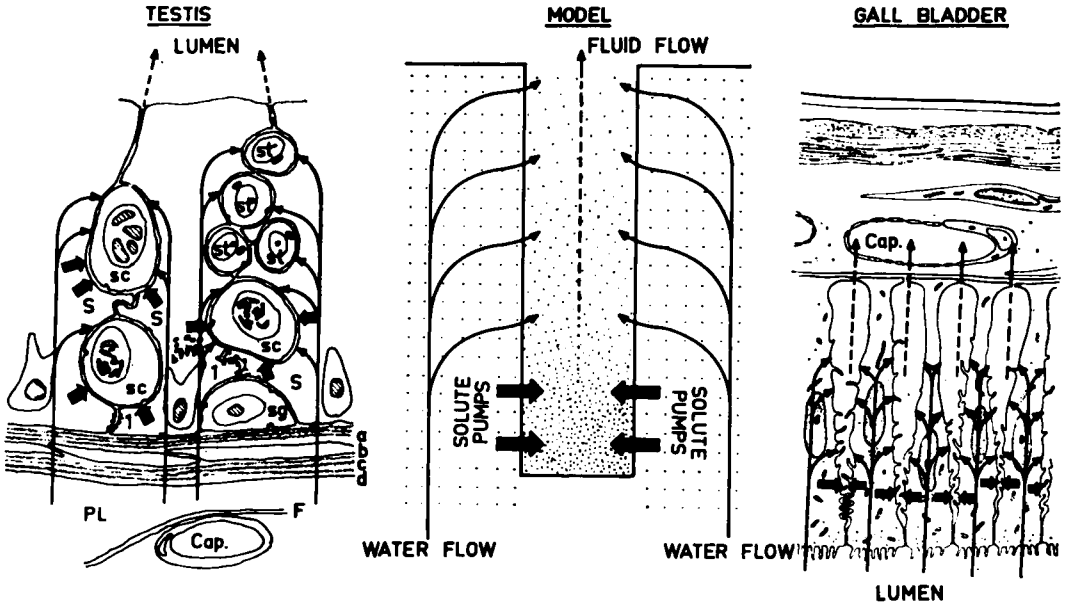


FIG. 8. A diagram illustrating the suggested analogy between fluid secretion in the seminiferous tubules, fluid resorption in the gall bladder and the standing gradient model of Diamond and Tormey (20). In the testis note the four-layered boundary tissue of the tubule (abcd), the narrow junctions (1) between adjacent Sertoli cells (S), the spermatogonia (Sg) on the boundary tissue and the spermatocytes (Sc) and spermatids (St) isolated between adjacent Sertoli cells. Cap; capillary. PL paralymphatic space bounded by fibroblasts (F and d). Note that in the gall bladder the narrow junctions are at the luminal end of the intercellular space and that there are no other cell types between the active cells. The symbols for solute pumps, water flow and fluid flow are common to all three diagrams. Testis modified from a drawing by Nicander (57), gall bladder from a drawing by Kaye, Wheeler, Whitlock, and Lane (35).

testis increases under the influence of LH; eventually these vacuoles rupture, causing liberation of the attached spermatozoa (11).

Ligation of the efferent ducts leads to distension of the testis, but ligation further down the epididymis has no effect on the testis (see 104 for references) and therefore it was suggested that most of the fluid secreted by the testis is reabsorbed in the head of the epididymis. This suggestion was confirmed by observations on the sperm concentration in fluid taken from the lumen at various points in the epididymis (16) and from calculations of the amount of fluid leaving the testis and the epididymis during sexual activity (104). It is important to realize that the resorption of fluid in the epididymis is highly selective, leading to increases in the concentrations of glutamic acid but not glycine (88), and increases in potassium but decreases in sodium and chloride (16, 81). There may also be changes in the already high concentration of testosterone, or other substances in the fluid. It is not clear what effects such changes would have on the spermatozoa but it is conceivable that this would be a mechanism for keeping the spermatozoa quiescent in the epididymis during the development of their capacity for motility, and epididymal seminal plasma may be analogous to spermatophore fluid in the squid (1).

Morphology of Testicular Spermatozoa. Leuwenhoek (44) was unable to find spermatozoa in the testes of a ram, but Prévost and Dumas in 1824 (52, 63) described how they found spermatozoa ("une foule d'animalcules") in the testes of all species they examined (polecat, dog, rabbit, cat, hedgehog, guinea pig, Norway rat, stallion, bull, ass but not a mule, various species of birds, frog, toad, and salamander) provided the animals were not too young or too old and in "la saison de leurs amours." With the microscopes at their disposal, they considered that the spermatozoa taken from the testes were identical with spermatozoa from the vas deferens and with ejaculated spermatozoa. As microscopes improved, it was recognized that spermatozoa taken directly from the testis did differ in appearance from ejaculated spermatozoa or spermatozoa taken from the tail of epididymis. The most obvious difference is the presence of the kinoplasmic droplet in testicular spermatozoa of all species so far examined (69, 70, 71). This is a roughly spherical droplet situated just behind the head of the spermatozoa (Fig. 9). During passage of the spermatozoa through the epididymis, the droplet gradually moves away from the head until it reaches the end of the mid-piece, where it detaches. It is interesting that this same movement of the droplet down the mid-piece could be seen in testicular spermatozoa stored at 1 C in the fluid in which they were collected (99) (Fig. 9).

When examined with the electron microscope, changes can be seen in the structure of the kinoplasmic droplet as well as in its position. The droplets on spermatozoa in the testis had many large electron transparent vesicles, but these vesicles were less numerous in spermatozoa taken from the head of the epididymis. Also there were fewer tubular and lamellar structures present in testicular than in epididymal spermatozoa (21).

As the spermatozoa pass down the epididymis, the vesicular and tubular contents of the droplet are considerably reduced, but the migration of the droplet does not modify the structure of the mid-piece (9, 65). Changes can also be found in the acrosome which in testicular spermatozoa surrounds the anterior part of the nucleus in a loose, wavy fashion. The acrosome of spermatozoa from the head of the epididymis was also wavy in appearance, but closer to the nucleus (21). Spermatozoa in the head of the rabbit epididymis show an elongated margin in the rostral part of the acrosomal cap compared with ejaculated rabbit spermatozoa (3, 24). A postnuclear cap was not apparent in testicular spermatozoa but was present in epididymal and ejaculated spermatozoa (21).

Testicular spermatozoa are also significantly

larger than ejaculated spermatozoa from the same ram when measured with a Coulter counter (60). Total dry matter also remains constant during maturation (27) although specific gravity rises, presumably due to progressive dehydration (45).

Cold Shock. Ejaculated ram spermatozoa cannot withstand sudden cooling, but testicular spermatozoa can be rapidly cooled to 1 C with only a small increase in the number of spermatozoa which can be stained with nigrosin-eosin (Fig. 10). This cannot be attributed to an inability of dead testicular spermatozoa to take up stain as testicular spermatozoa killed with formalin were readily stained. However, sudden cooling did have some effects on the testicular spermatozoa, many of which were kinked at the junction of the mid-piece and tail (99).

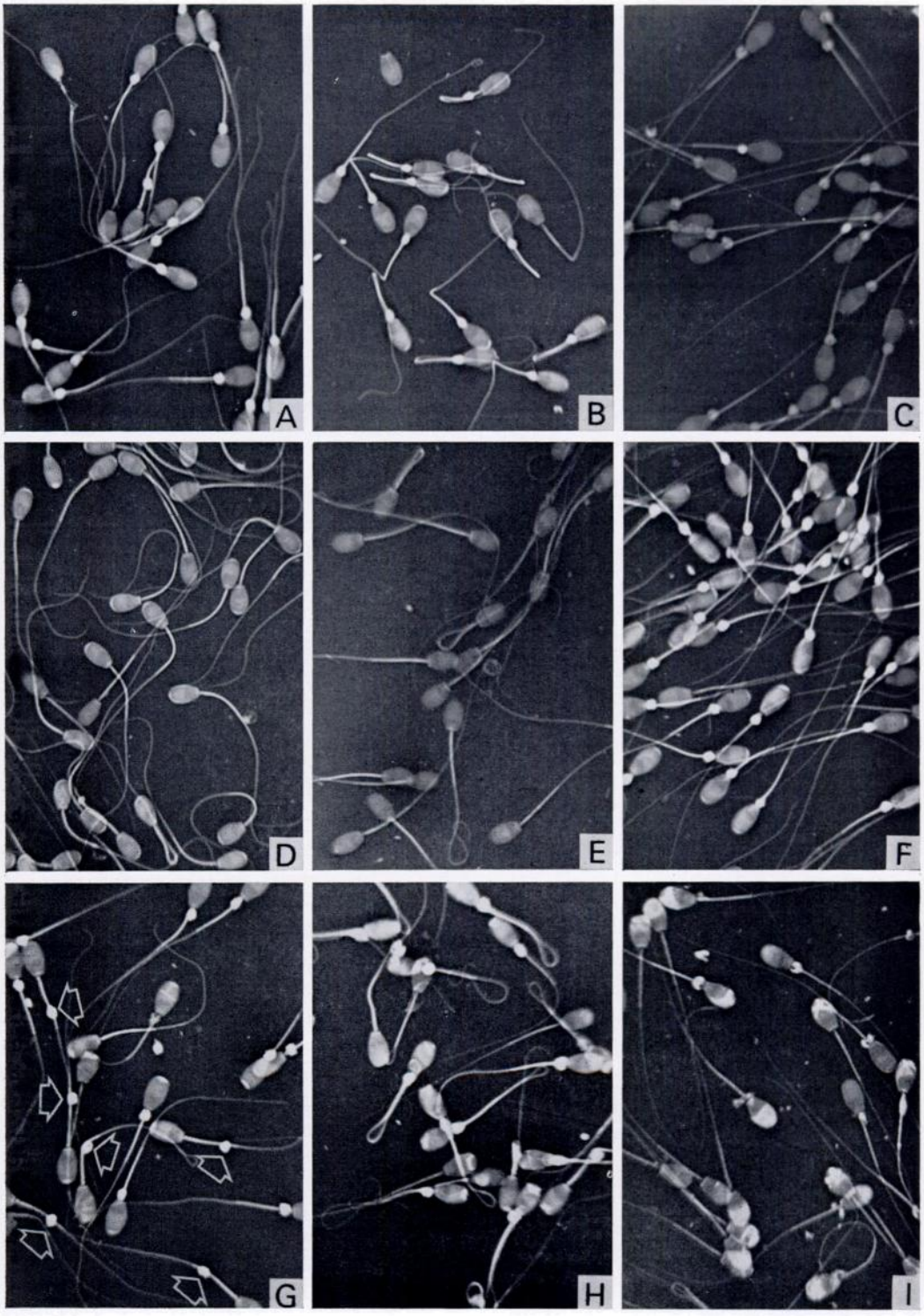
Motility of Testicular Spermatozoa. Prévost and Dumas in 1824 (62, 63) found that when they diluted, with water, fluid expressed from the testis, the "animalcules" were motile for a short time, but especially in the frog, their motility was less than ejaculated spermatozoa. Tournade, 1913 (96) described the motility of sperm from the head of the epididymis as "complètement inertes neuf fois sur dix. Exceptionellement, certains d'entre eux sont animés de faibles oscillations," and many others have since confirmed this observation (2, 6, 13, 53, 55, 56). The same slight motility was seen in ram and bull spermatozoa collected from a catheter in the rete testis (Fig. 11). However, some increase in motility could be induced by storing ram spermatozoa at about 1 C in the fluid in which they were collected (Fig. 12) (99). Increasing the pH to between 8 and 8.5 had a similar effect (66, 67, 68, 99), but the motility achieved was in no way comparable to that of ejaculated spermatozoa.

Fertilizing Capacity. Sperm taken from the testis or the head of the epididymis of laboratory animals were capable of fertilizing only a very small proportion of ova (4, 8, 59, 61, 108). These results have been confirmed with spermatozoa collected from a catheter in the rete testis of rams. A total of 32 ewes were each inseminated with 3×10^8 spermatozoa which had been stored for 3 days at 1 C, but none conceived, although spermatozoa could be recovered from the fallopian tubes (100). It is not yet known whether it is possible to induce fertilizing capacity in testicular spermatozoa in a way analagous to the increased fertility seen in epididymal spermatozoa held in the head of the epididymis by ligation (5).

Composition of Testicular Spermatozoa

Ions. Testicular spermatozoa contain approximately the same concentrations of potassium and magnesium as epididymal spermatozoa but about three times as much sodium and chloride (64). These differences suggest that the increase in specific gravity seen as spermatozoa pass down the epididymis (45) may be due to expulsion from the sperm of a fluid of composition similar to extracellular fluid, i.e. the reverse of the swelling of cells when their metabolism is depressed (72). It is interesting that the composition of epididymal spermatozoa changes abruptly when they are ejaculated, sodium and calcium rise again, and potassium and magnesium fall. This may be due to uptake from and loss to accessory gland secretion, which is much higher in sodium and calcium but lower in potassium than epididymal seminal plasma (64) (Table 2).

FIG. 9. Photomicrograph (×195) of spermatozoal smears after staining with nigrosin-eosin. A—Testicular spermatozoa (Ts) stained at 30 C; B—Ts stained at 1 C; C—Ts killed with formalin and stained at 30 C; D—Ejaculated spermatozoa (Es) stained at 30 C; E—Es stained at 1 C; F, G, H, I—Ts after storage at 1 C in rete testis fluid for 1, 3, 6 and 12 days; stained at 30 C after washing. Reproduced from Ref (99) by permission of the *Journal of Reproduction and Fertility.*



Lipids. The lipid composition of ram testicular spermatozoa is different from that of ejaculated spermatozoa; the content of phospholipid (as amount of phosphorus per cell) is about 30% higher in testicular spermatozoa, although the major phospholipid, choline plasmalogen, is not noticeably different in the two sperm types. There are no differences in the content of sphingomyelin and alkyl ether phospholipid, but all other individual phospholipids are higher in testicular spermatozoa. Among the more abundant phospholipids, the most striking differences are in lecithin, phosphatidylethanolamine and ethanolamine plasmalogen (Fig. 13). Testicular spermatozoa contain 30-40% more phospholipid acyl ester than ejaculated spermatozoa but there were no consistent differences in neutral lipid acyl ester content.

Cholesterol concentration is lower in ejaculated spermatozoa, about one-half of the concentration in testicular sperm. Differences can also be seen in the fatty acid composition of the lipids of the two types of spermatozoa. The proportion of palmitic acid (16:0) was much higher in testicular spermatozoa, whereas the percentage of myristic acid (14:0) was lower (80).

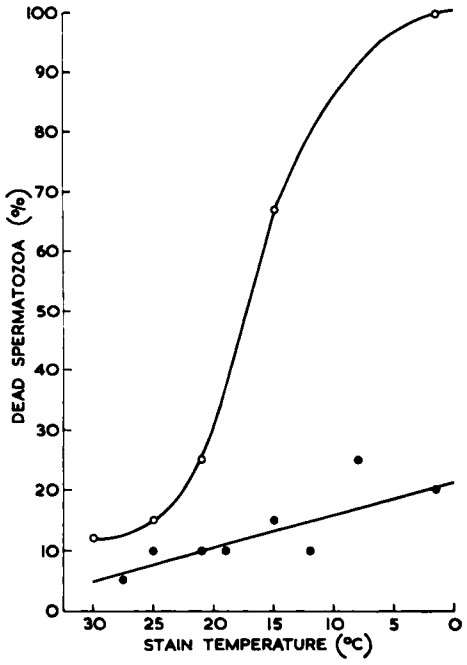


FIG. 10. Effect of sudden cooling on the staining with nigrosin-eosin on testicular (•) and ejaculated (o) spermatozoa. Data from Ref. (99).

TABLE 2
DISTRIBUTION OF MAJOR CATIONS (meq/liter) IN SPERMATOZOA AND FLUIDS FROM THE REPRODUCTIVE TRACT OF RAMS
(Data from Ref. 64.)

	Na	K	Ca	Mg
Testis				
Whole semen	138	14	4.6	2.0
Spermatozoa	69	57	10.2	19.3
Plasma	128	13	5.2	1.3
Epididymis				
Whole semen	26	31	2.4	10.1
Spermatozoa	21	64	3.6	16.9
Plasma	40	24	1.9	2.6
Ejaculate				
Whole semen	88	22	4.1	5.7
Spermatozoa	51	33	5.3	12.4
Plasma	92	17	3.8	4.1
Seminal vesicle				
Fluid	93	37	6.8	6.4

Protein. Testicular spermatozoa contained about 50% more protein than ejaculated spermatozoa. About 40% of the protein of both types of cell was located in the heads (103).

Nucleic Acids. The amount of DNA in the head of bull spermatozoa, determined by absorption of light of wavelength 2650 or 2600 Å, does not change during the passage of the spermatozoa down the epididymis. However, the intensity of the Feulgen stain (measured at 5460 Å) is less in sperm taken from the tail, but the difference is much less than that between round spermatids and spermatozoa from the head of the epididymis. It is thought that these differences are due *not* to a decrease in DNA content per cell, but to changes in the composition and binding of basic nuclear protein to DNA (27).

Adenosine triphosphate and adenosine triphosphatase. Testicular and ejaculated ram spermatozoa contained similar amounts of adenosine di- and tri-phosphate (99), but testicular spermatozoa contained less adenosine

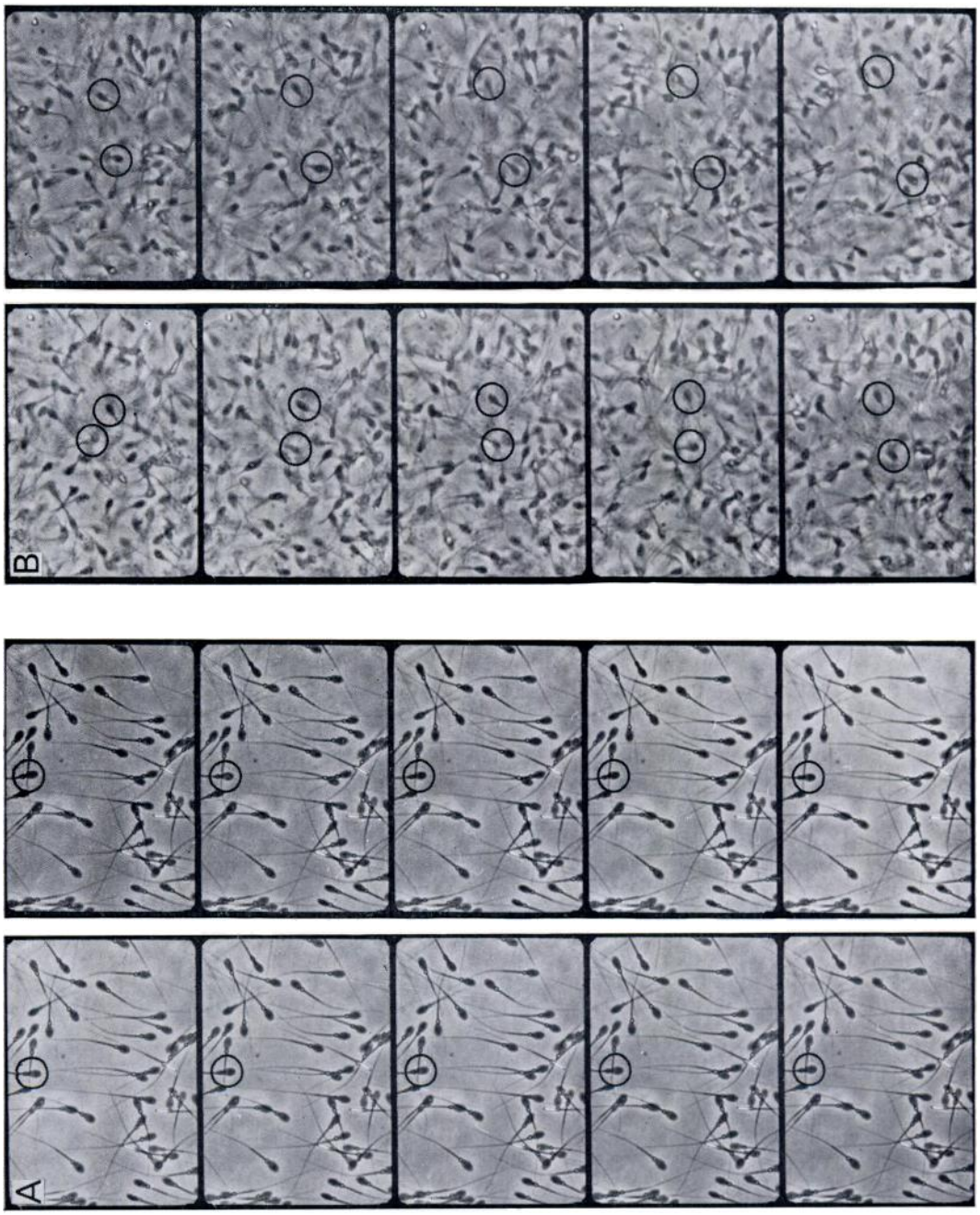


FIG. 11. Motility of testicular and ejaculated spermatozoa; successive frames from a 24 frames per second movie of A—testicular, B—ejaculated ram spermatozoa. Note the immotility of testicular spermatozoa (except for the one circled) and the rapid movement of the two typical ejaculated spermatozoa (circled). Data from Ref. (99).

triphosphatase (ATPase). There was little ATPase in the head of either type of spermatozoa, but the ATPase of the mid-piece of testicular spermatozoa was lower than that in the tail, whereas in ejaculated spermatozoa the mid-piece and tail contained similar activities (103).

Metabolism of Spermatozoa

It is difficult to generalize about the metabolic differences between testicular and ejaculated spermatozoa, as much depends on the exact conditions under which the comparisons are made. However, differences can be demonstrated under almost all conditions of incubation.

Oxygen Uptake. The most consistent feature of the oxygen uptake of washed ram and bull testicular spermatozoa is that the consumption of oxygen remained linear for more than 3 hr, whereas the respiration of ejaculated spermatozoa tended to fall off with time, especially with no added substrate (Fig. 14). Oxygen uptake of ram and bull testicular spermatozoa was stimulated by high phosphate media, but the respiration of ejaculated sperm was depressed (98, 99).

In rete testis fluid, both testicular and ejaculated spermatozoa took up more oxygen than when they were incubated in phosphate buffer (99). The significance of this is difficult to assess at the moment as the two compounds present in greatest concentration in rete testis fluid, glutamate and inositol, do not seem to be oxidized by the sperm (86, 88). It will probably be important to determine whether the increased oxygen uptake is due to an alternative oxidizable substrate or a stimulatory effect of rete testis fluid on spermatozoal metabolism.

When ram testicular spermatozoa were stored at 1 C in the fluid in which they were

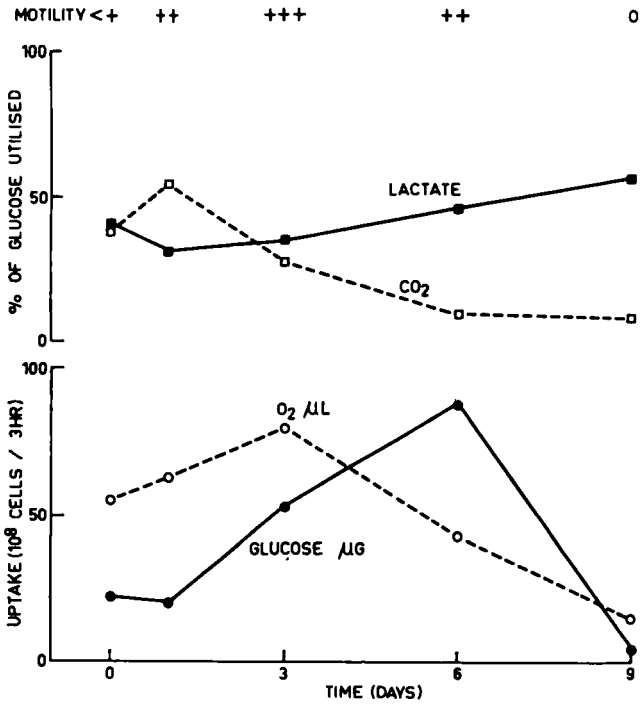


FIG. 12. Effect of storage of testicular spermatozoa at 1 C in the fluid in which they are collected. Motility is given in arbitrary units (+ etc.), but the scale used represents a much lower degree of motility than similar scales used for ejaculated spermatozoa. Data from reference (99).

collected and then incubated, it was found that their oxygen uptake increased with storage for the first 3 days and then fell off (Fig. 12), although even after 6 days storage the glucose uptake was still high and the sperm appeared normal (99).

Carbohydrate Metabolism. There are interesting differences in the dissimilation of glucose by testicular and ejaculated spermatozoa from both the ram and the bull. Ram testicular spermatozoa always utilized less glucose than ejaculated spermatozoa under comparable conditions of incubation. A much smaller fraction of the glucose utilized by testicular spermatozoa appeared as lactate; in contrast, the percentage of glucose converted to carbon dioxide by the two cell types was similar during the first hour of incubation. However, with testicular spermatozoa this fraction continued to increase with time so that by 3 hr, there was a much higher proportion of the glucose converted to CO_2 by testicular spermatozoa (Fig. 15). However, again the extent of these differences depended on the exact conditions of incubation (99).

Bull testicular spermatozoa in low phosphate (20 mM) media also converted a higher proportion of the utilized glucose to carbon dioxide than ejaculated cells, but in high phosphate (80mM) buffer there were no differences between the two types of spermatozoa. However, when testicular spermatozoa were incubated in high phosphate diluent they produced more lactate than ejaculated cells and more lactate accumulated in the media than could have arisen from the glucose utilized, presumably from endogenous metabolism stimulated by the added glucose (98).

No significant pentose cycle activity could be detected in ram or bull testicular spermatozoa (98, 99), and earlier reports of its presence (106) were probably due to contam-

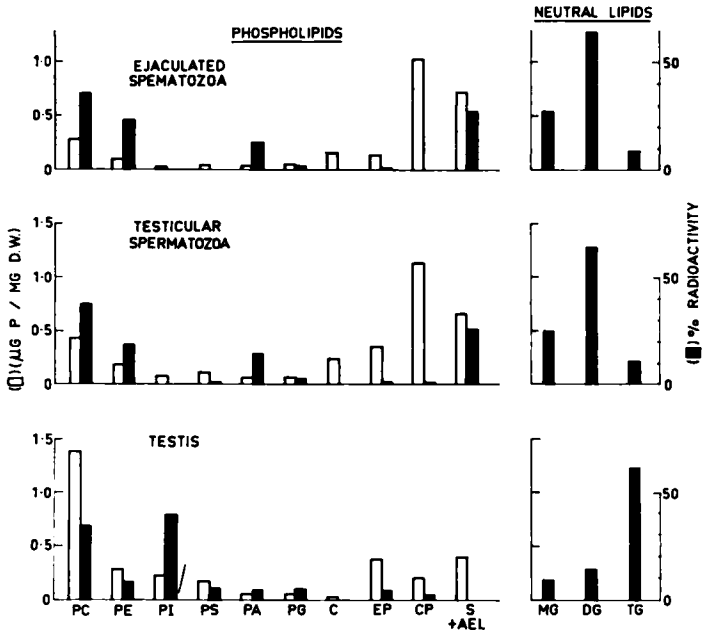


FIG. 13. Lipid composition and metabolism of ejaculated and testicular ram spermatozoa and testis. (□) Phospholipid concentration (μg P/mg dry weight); (■) ^{32}P or ^{14}C radioactivity in individual phospholipids or neutral lipids from testicular and ejaculated spermatozoa and testis. Data from Refs. (79, 80). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid; PG, phosphatidylglycerol; C, cardiolipin; EP, ethanolamine plasmalogen; CP, choline plasmalogen; S + AEL, sphingomyelin + alkyl ether lipid; MG, monoglyceride; DG diglyceride; TG, triglyceride.

ination of the cruder preparations with other testicular cells; testis tissue has quite an active pentose cycle (87). Neither ejaculated nor epididymal spermatozoa show pentose cycle activity (82, 98, 99, 106).

Ram testicular spermatozoa also convert a significant proportion of glucose to glutamic acid, aspartic acid, glycine and alanine; these amino acids were found in higher concentration in rete testis fluid than in testicular lymph (88). In contrast, less amino acids were formed when bull testicular spermatozoa were incubated with glucose, although glutamate is also present in bull rete testis fluid in almost as high concentrations as in the ram (101).

When ram testicular spermatozoa were stored at 1 C in the fluid in which they were collected and then incubated, their metabolism had changed towards that of ejaculated spermatozoa, the glucose utilization and the percentage of the glucose converted to lactate rose, while the percentage converted to carbon dioxide fell (99).

Ram testicular spermatozoa can also utilize fructose both aerobically and anaerobically, although no fructose is present in rete testis fluid. However, fructose utilization by both testicular and ejaculated ram spermatozoa was depressed when the sperm were incubated with an equimolar mixture of fructose and glucose, with only slight effects on glucose utilization (Table 3). Testicular spermatozoa thus show the same effect as ejaculated spermatozoa, of a sparing action of glucose on fructose utilization but not the reverse (102).

Inositol, which is present in such high concentrations in the rete testis fluid of rams, is

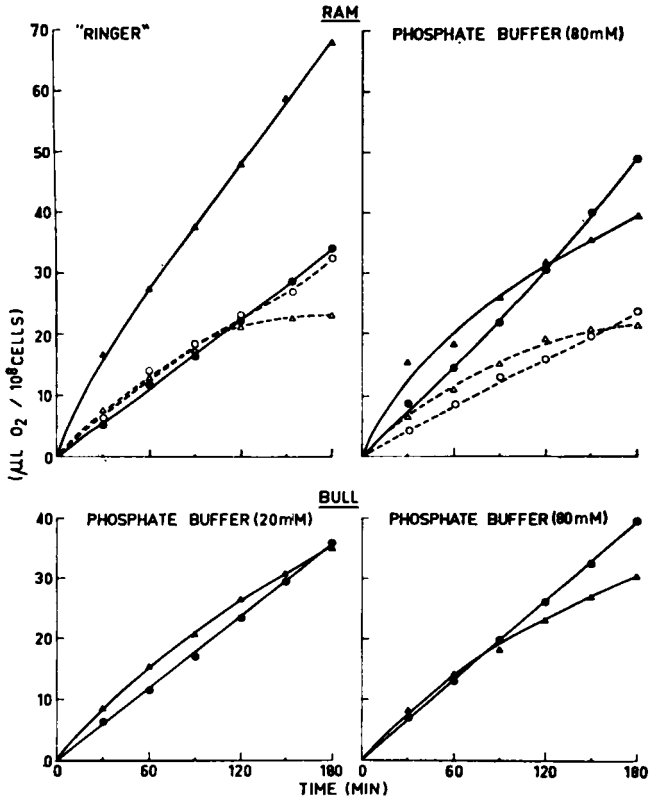


FIG. 14. Oxygen uptake of washed spermatozoa. (▲ △) Ejaculated; (●, o) testicular spermatozoa with (▲, ●) or without (△, o) added glucose. Data from Refs. (98, 99).

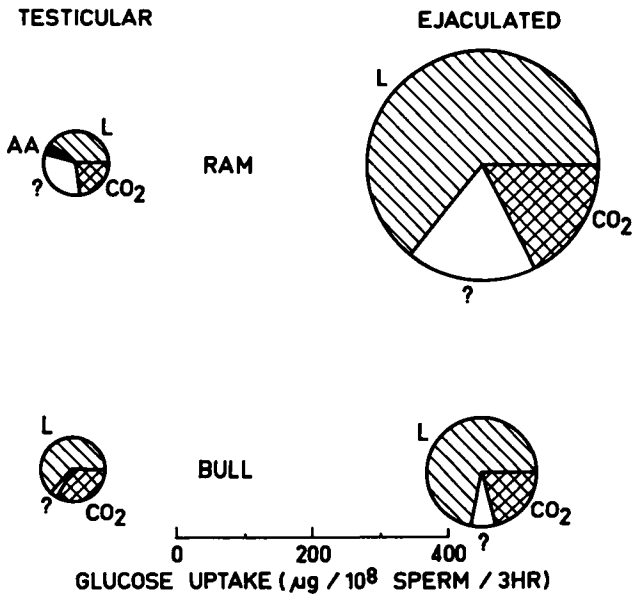


FIG. 15. Dissimilation of ^{14}C glucose by spermatozoa incubated for 3 hr. Data from Refs. (98, 99). The diameter of the circles indicates the glucose uptake and the areas labelled CO_2, lactate and A.A. (= amino acids) indicate the proportion of ^{14}C glucose utilized which appears as these end-products. Ram spermatozoa were incubated in Tris-buffered Ringer and bull sperm in Ringer with 20mM phosphate.

TABLE 3

CARBOHYDRATE METABOLISM OF EJACULATED AND TESTICULAR SPERMATOZOA OF THE RAM[a, b, c]
(Data from Ref. 102.) (Change/10^8 cells in 3 hr.)

Added substrate		Oxygen uptake (µl)		Substrate utilization (µg)		Lactic acid production (µg)	
		Ejac. sp.	Test. sp.	Ejac. sp.	Test. sp.	Ejac. sp.	Test. sp.
—	Aerobic	20	22	—	—	—	—
Glucose	Aerobic	40	59*	262	191	191	54‡
	Anaerobic	—	—	387	473*	368	404
Fructose	Aerobic	37	55*	208	158	177	69‡
	Anaerobic	—	—	265	412+	258	329*
Glucose + Fructose	Aerobic	39	59+	G235 F65	G 202* F64	180	56‡
	Anaerobic	—	—	G360 F119	G430* F154	400	397

[a] The means of six observations on two rams are given. Similar values were obtained in six other rams, but because complete data were not obtained they have not been included.

[b] Significance of differences between ejaculated and testicular spermatozoa: * = $p < .05$; + = $p < .01$; ‡ = $p < .001$.

[c] G and F: glucose and fructose utilization, respectively, from an equimolar mixture.

not utilized to any extent by ram testicular spermatozoa either by conversion to CO_2 or by incorporation into lipid (86).

Lipid Metabolism

(i) *Synthesis.* When testicular spermatozoa leave the testis, they are still capable of synthesizing lipid from exogenous carbon sources. When testicular spermatozoa were incubated with ^{14}C-glucose, radioactivity was incorporated into neutral lipids, phospholipids and volatile fatty acids.

The rate of incorporation into phospholipids was 3-4 times that into ejaculated spermatozoa from the same ram (Fig. 16); most of the radioactivity was present in lecithin but phosphatidylethanolamine and phosphatidic acid were significantly labelled after a 3-hr incubation. No radioactivity could be detected in monophosphatidylinositol and only a very small percentage (2-3%) of the label was found in the plasmalogens which account for about 50% of the phospholipids. The portion stable to alkali and acid hydrolysis contained about 26% of the radioactivity and presumably most of this is in sphingomyelin and alkyl ether lipid. The pattern of labelling was almost identical for the two types of spermatozoa (80). However, infusion of ^{32}P-orthophosphate into the testicular artery resulted in a different pattern of phospholipid labelling in the testis *in vivo*. The radioactivity was highest in monophosphatidylinositol (79).

When spermatozoa are incubated with ^{14}C-glucose, testicular spermatozoa incorporate 25% more label into neutral lipids than do ejaculated sperm. The pattern of labelling is similar for the two cell types, with most of the label in the diglycerides. Saponification of the respective lipid fractions showed that less than 1% of the radioactivity was in the fatty acid fraction (80). The pattern of labelling is again quite different from the pattern in the testis *in vivo* when ^{14}C-glucose was infused into the testicular artery. Most of the radioactivity is then present in triglycerides and only small amounts are found in diglycerides (79).

(ii) *Production of Volatile Fatty Acids from Glucose.* The pattern of incorporation of ^{14}C from glucose into volatile fatty acids is different for testicular and ejaculated sper-

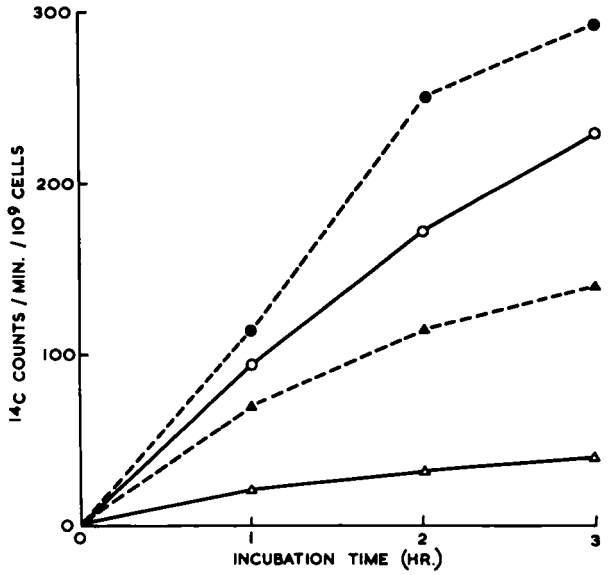


FIG. 16. Incorporation of ^{14}C glucose into neutral lipid (●, o) and phospholipids (▲, △) by testicular (●, ▲) and ejaculated (o, △) ram spermatozoa. Data from Ref. (80).

matozoa. Ejaculated sperm incorporated much more ^{14}C from glucose into volatile fatty acids (1.4% vs. 0.3% of ^{14}C-glucose available) and most of this appeared in acetic acid with smaller amounts in formate, propionate and butyrate. In contrast, most of the radioactivity incorporated into volatile fatty acid by testicular spermatozoa appeared in formate, with smaller amounts in acetate, propionate and butyrate (Fig. 17) (80).

(iii) *Utilization of Lipid.* When testicular spermatozoa were incubated in phosphate-buffered medium without added substrate, there was a decrease in the acyl ester content in five out of six experiments. The mean decrease was 0.51 μeq/10^9 cells in 3 hr, which would require 300 μl of oxygen for its complete oxidation, assuming a chain length of 18 carbon atoms. The value was compatible with the observed oxygen uptake of 385 μl/10^9 cells/3 hr, but there are considerable possibilities for error in these experiments because of the small change in lipid content in relation to the total amounts present. There were no consistent changes in the amount of phospholipid phosphorus but analyses of individual phosphatides before and after incubation suggested a small decrease in lecithin and phosphatidylethanolamine but these were not significant. The free fatty acid content of spermatozoa decreased from 0.86 to 0.67 and 0.86 to 0.49 μmole/10^9 cells during two 3-hr incubations (80).

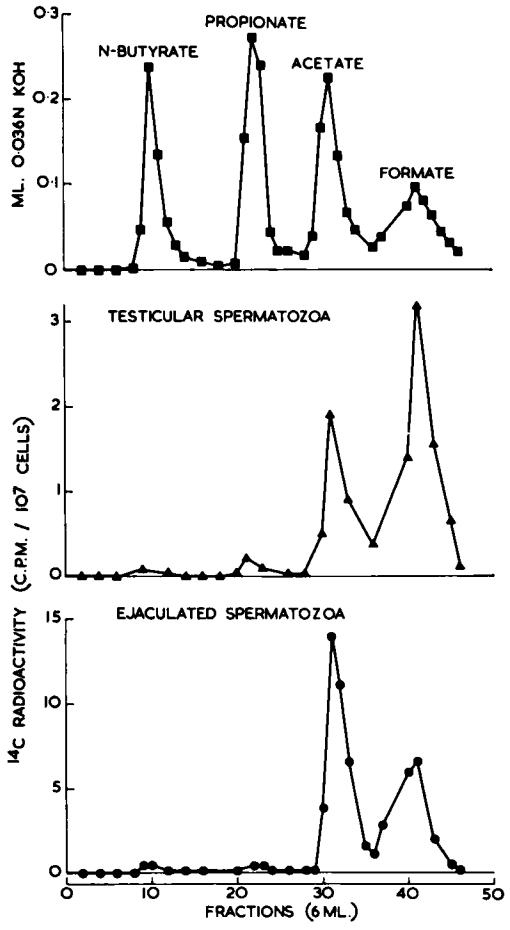


FIG. 17. Separation and distribution of radioactivity in volatile fatty acids (C_1-C_4) isolated from the media after 2-hr incubation of washed testicular and ejaculated ram spermatozoa in diluent containing [U-^{14}C] glucose. Individual acids were separated on silicic acid columns by gradient elution. Data from Ref. (80).

These results, taken with the differences in lipid composition between testicular and ejaculated spermatozoa, suggest that lipids may serve as substrates for spermatozoa during their period of maturation in the epididymis.

(iv) *Metabolism of Exogenous Lipid.* Ram testicular spermatozoa failed to hydrolyze choline or ethanolamine phosphoglycerides when these were added to the incubation media, but both lipids were metabolized by ejaculated spermatozoa and spermatozoa from the ampulla. Glycerylphosphorylethanolamine was the principal water-soluble product formed from the hydrolysis of ethanolamine phosphoglycerides; in addition a lysoethanolamine phospholipid was also produced. The rate of metabolism of added choline phosphoglycerides by ejaculated spermatozoa was much slower than the ethanolamine form; further, no lysolipid was noted and glycerylphosphorylcholine was the main hydrolytic product. In contrast, both testicular and ejaculated spermatozoa, actively metabolized phosphatidylinositol, forming mainly inositol phosphate esters as the water-soluble products (Table 4).

TABLE 4

THE METABOLISM OF EXOGENOUS LIPIDS BY SPERMATOZOA AND FLUIDS FROM DIFFERENT PARTS OF THE REPRODUCTIVE TRACT OF THE RAM (from the data of Ref. 78.)

	Ethanolamine phosphoglycerides			Choline phosphoglycerides		Phosphatidylinositol	
	μg Phospholipid P added/10^8 sperm or ml	Converted to water soluble P (%)	Converted to lysophospholipid (%)	μg Phospholipid P added/10^8 sperm or ml	Converted to water soluble P (%)	μg Phospholipid P added/10^8 sperm or ml	Converted to water soluble P (%)
Ejaculated spermatozoa (washed)	0.37	45	4	0.36	2	0.06	30
Seminal plasma	11.2	37	21	10.8	6	1.8	20
Ampullar spermatozoa	10.6	20	8	16.1	0.3	2.5	45
Testicular spermatozoa	1.9	0.2	0	2.9	0	0.45	39
Rete testis fluid	26.8	0.1	0	40.6	0	6.2	3.5

Seminal plasma (containing no spermatozoa) could also metabolize added ethanolamine, choline, and inositol phosphoglycerides. On the other hand, rete testis fluid showed only a small degree of activity towards phosphatidylinositol (78).

Metabolism of Amino Acids

It was suggested some years ago that ejaculated spermatozoa were still able to synthesize protein from added amino acids (7). However, it would now appear that this result may have been due to contaminating cells as testicular spermatozoa showed no significant incorporation of the amino acids glutamic acid or leucine into protein (88).

Despite the fact that rete testis fluid contains about 25 mg glutamic acid/100 ml, very little conversion of glutamic acid or leucine to carbon dioxide occurred when testicular or ejaculated spermatozoa were incubated with [^{14}C]-glutamate or [^{14}C]-leucine, nor did either amino acid increase the oxygen uptake of spermatozoa above endogenous values (88).

CONCLUSION

Using our technique for cannulating the rete testis, it has been possible to quantitate the exocrine function of the testis, that of producing the fluid to carry the immature spermatozoa out of the testis into the epididymis. This fluid, of unique composition, is produced by active secretion, not filtration, and its rate of production is unrelated to the level of spermatogenesis. Further study of this process is vital to any understanding of testicular function.

The sperm leaving the testis are immotile, incapable of fertilization, and different in appearance, composition and metabolism from ejaculated spermatozoa. Further comparison of testicular and ejaculated spermatozoa should lead to an understanding of the process of maturation which normally takes place in the epididymis. This, in turn, may lead to the preservation outside the body of the spermatozoa of those species in which this has not so far been achieved.

REFERENCES

1. AUSTIN, C. R., LUTWAK-MANN, C. AND MANN, T. (1964). Spermatophores and spermatozoa of the squid *Loligo pealii*. *Proc. Roy. Soc. (London), Ser. B.* **161**, 143-152.
2. BEDFORD, J. M. (1963). Changes in the electrophoretic properties of rabbit spermatozoa during passage through the epididymis. *Nature* **200**, 1178-1180.

3. BEDFORD, J. M. (1965). Changes in fine structure of the rabbit sperm head during passage through the epididymis. *J. Anat.* **99**, 891-906.
4. BEDFORD, J. M. (1966). Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit. *J. Exptl. Zool.* **163**, 319-330.
5. BEDFORD, J. M. (1967). Effects of duct ligation on the fertilizing ability of spermatozoa from different regions of the rabbit epididymis. *J. Exptl. Zool.* **166**, 271-282.
6. BENOIT, J. (1926). Recherches anatomiques, cytologiques et histophysiologiques sur les voies excrétrices du testicule chez les mammifères. *Arch. Anat. Histol. Embryol.* **5**, 173-414.
7. BHARGAVA, P. M., BISHOP, M. W. H. AND WORK, T. S. (1959). Incorporation of [^{14}C] amino acids into the proteins of bull spermatozoa. *Biochem. J.* **73**, 247-256.
8. BLANDAU, R. J. AND RUMERY, R. E. (1961). Fertilizing capacity of rat spermatozoa recovered from various segments of the epididymis. *Anat. Rec.* **139**, 202.
9. BLOOM, G. AND NICANDER, L. (1961). On the ultrastructure and development of the protoplasmic droplet of spermatozoa. *Z. Zellforsch.* **55**, 833-844.
10. BRÖKELMANN, J. (1963). Fine structure of germ cells and Sertoli cells during the cycle of the seminiferous epithelium in the rat. *Z. Zellforsch.* **59**, 820-850.
11. BURGOS, M. H. AND VITALE-CALPE, R. (1967). Mechanism of spermiation in the toad. *Am. J. Anat.* **120**, 227-251.
12. CLERMONT, Y. (1958). Contractile elements in the limiting membrane of the seminiferous tubules of the rat. *Exptl. Cell Res.* **15**, 438-440.
13. COLLERY, L. (1943). Note on the physiology of the mammalian epididymis and spermatozoon. *Proc. Roy. Irish Acad.* **49B**, 213-223.
14. COOPER, A., SIR. (1830). "Observations on the structure and diseases of the testis." Longman, Green, London.
15. COWIE, A. T., LASCELLES, A. K. AND WALLACE, J. C. (1964). Flow and protein content of testicular lymph in conscious rams. *J. Physiol.* **171**, 176-187.
16. CRABO, B. (1965). Studies on the composition of epididymal content in bulls and boars. *Acta Vet. Scand.* **6**, *Suppl.* 5.
17. DAVSON, H. (1956). Physiology of the ocular and cerebrospinal fluids. Churchill, London.
18. DAVSON, H. (1962). The intra-ocular fluids. *In* "The Eye" (H. Davson, ed.), pp. 67-137. Academic Press, London.
19. DAVSON, H. (1963). The cerebrospinal fluid. *Ergeb. Physiol.* **52**, 20-73.
20. DIAMOND, J. M. AND TORMEY, J. McD. (1966). Studies on the structural basis of water transport across epithelial membranes. *Federation Proc.* **25**, 1458-1463.
21. DICKEY, J. F. (1967). An electron microscope study of bovine spermatozoa undergoing maturation in the male reproductive system. *Dissertation Abstr.* **26**, 6274.
22. EDWARDS, R. G. (1961). Complement-fixing activity of normal rabbit serum with rabbit spermatozoa and seminal plasma. *J. Reprod. Fertility* **1**, 268-282.
23. EWY, Z., BIELANSKI, W. AND ZAPLETAL, Z. (1963). Influence of oxytocin on spermatozoa transport in the ductus deferens of the ram. *Bull. Acad. Polon. Sci. Ser. Sci. Biol.* **11**, 145-148.
24. FAWCETT, D. W. AND HOLLENBERG, R. D. (1963). Changes in the acrosome of the guinea pig spermatozoa during passage through the epididymis. *Z. Zellforsch.* **60**, 276-292.
25. FLICKINGER, C. AND FAWCETT, D. W. (1967). The junctional specialization of Sertoli cells in the seminiferous epithelium. *Anat. Rec.* **158**, 207-222.
26. GARDENER, P. J. AND HOLYOKE, E. A. (1964). Fine structure of the seminiferous tubule of the Swiss mouse. The limiting membrane, Sertoli cell, spermatogonia and spermatocytes. *Anat. Rec.* **150**, 391-404.
27. GLEDHILL, B. L. (1966). Studies on the DNA content, dry mass and optical area of bull spermatozoal heads during epididymal maturation. *Acta Vet. Scand.* **7**, 131-142.
28. GRIFFITHS, J. (1895). Three lectures upon the testis. *Lancet* **1**, 791-799 and 916-920.
29. GUSTAFSSON, B. (1966). Luminal contents of the bovine epididymis under conditions of reduced spermatogenesis, luminal blockage and certain sperm abnormalities. *Acta Vet. Scand. Suppl.* 17.
30. HEAP, R. B. AND SETCHELL, B. P. (1967). Unpublished.
31. HINKS, N. T. AND SETCHELL, B. P. (1968). Unpublished.
32. JOHNSON, M. H. (1968). Characterisation of a natural antibody in normal guinea-pig serum reacting with homologous spermatozoa. *J. Reprod. Fertility* **16**, 503-506.
33. JOHNSON, M. H. AND SETCHELL, B. P. (1968). The protein and immunoglobulin content of rete testis fluid of rams. *J. Reprod. Fertility* **17**, 403-406.

34. KAGAYAMA, M., IRISAWA, S., SHIRAI, M. AND MATSUSHITA, S. (1965). Contractile cells in the boundary tissue of the seminiferous tubules. *Japan J. Urol.* **56**, 842-847. (In Japanese. English Summary).
35. KAYE, G. I., WHEELER, H. O., WHITLOCK, R. T. AND LANE, N. (1966). Fluid transport in the rabbit gallbladder. A combined physiological and electron microscopic study. *J. Cell Biol.* **30**, 237-268.
36. KORMANO, M. (1967). An angiographic study of the testicular vasculature in the post natal rat. *Z. Anat. Entwicklungsgeschichte* **126**, 138-153.
37. KORMANO, M. (1967). Dye permeability and alkaline phosphatase activity of testicular capillaries in the post-natal rat. *Histochem.* **9**, 327-338.
38. KORMANO, M. (1967). Distribution of injected L-3,4-dihydroxyphenylalanine in the adult rat testis and epididymis. *Acta Physiol. Scand.* **71**, 125-126.
39. KORMANO, M. (1967). Development of the rectum-testis temperature differenece in the post-natal rat. *J. Reprod. Fertility* **14**, 427-437.
40. KORMANO, M. (1968). Penetration of intravenous trypan blue into the rat testis and epididymis. *Acta Histochem.* **30**, 133-136.
41. KWIET, K. P. (1966). Uber Strömungsfiguren im Nebenhoden der weissen Ratte. *Gegenbaur. Morph. Jahrb.* **108**, 255-282.
42. LACY, B. AND ROTBLAT, J. (1959). Study of the normal and irradiated boundary tissue of the seminiferous tubules of the rat. *Exptl. Cell Res.* **21**, 49-70.
43. LEESON, C. R. AND LEESON, T. S. (1963). The postnatal development and differentiation of the boundary tissue of the seminiferous tubule of the rat. *Anat. Rec.* **147**, 243-260.
44. LEUWENHOEK. Cited by Prévost and Dumas, 1824.
45. LINDAHL, P. E. AND KIHLSTRÖM, J. E. (1952). Alterations in specific gravity during the ripening of bull spermatozoa. *J. Dairy Sci.* **35**, 393-402.
46. LINDNER, H. R. (1963). Partition of androgen between the lymph and venous blood of the testis in the ram. *J. Endocrinol.* **25**, 483-494.
47. LINDNER, H. R. (1967). Participation of lymph in the transport of gonadal hormones. *Proc. 2nd Intern. Congr. Horm. Steroids, 1966.* Exc. Med. Int. Conf. Ser. No. 132, 821-827.
48. LINZELL, J. L. AND SETCHELL, B. P. (1968). The output of spermatozoa and fluid by, and the metabolism of the isolated perfused testis of the ram. *J. Physiol.* **195**, 25-26P.
49. MACMILLAN, E. W. (1957). The mechanical influence of the vasa efferentia on the transport of radiopaque medium through the ductus epididymidis of the rat. *Proc. Soc. Study Fertility* **9**, 65-71.
50. MACMILLAN, E. W. AND AUKLAND, J. (1960). The transport of radiopaque medium through the initial segment of the rat epididymis. *J. Reprod. Fertility* **1**, 139-145.
51. MACMILLAN, E. W. AND HARRISON, R. G. (1955). The rate of passage of radiopaque medium along the ductus epididymidis of the rat. *Proc. Soc. Study Fertility* **7**, 35-40.
52. MEANS, A. R. AND HALL, P. F. (1968). Protein biosynthesis in the testis. I. Comparison between stimulation by FSH and glucose. *Endocrinology* **82**, 597-602.
53. MERTON, H. (1939). Studies on reproduction in the albino mouse. II. Contributions on maturation of the sperm cells. *Proc. Roy. Soc. Edinburgh* **59**, 145-152.
54. MIHALKOVICS, V. VON. (1873). Beiträge zur Anatomie und Histologie des Hodens. *Ber. Sachs. Ges. (Akad.) Wis.* **24**, 217-256.
55. MUKERJEE, D. P. AND BHATTACHARYA, P. (1949). Study of spermatozoa from different levels of the male reproductive tracts of the sheep, goat and buffalo. *Proc. Zool. Soc. Bengal* **2**, 149-161.
56. NICANDER, L. (1963). Some structural features of mammalian Sertoli cells. *J. Ultrastruct. Res.* **8**, 190-191.
57. NICANDER, L. (1967). An electron microscopical study of cell contacts in the seminiferous tubules of some mammals. *Z. Zellforsch.* **83**, 375-397.
58. NIEMI, M. AND KORMANO, M. (1965). Contractility of the seminiferous tubule of the postnatal rat testis and its response to oxytocin. *Ann. Med. Exptl. Biol. Fenniae* (Helsinki) **43**, 40-42.
59. NISHIKAWA, Y. AND WAIDA, Y. (1952). Studies on the maturation of spermatozoa. I. Mechanism and speed of transition of spermatozoa in the epididymis and their functional changes. *Bull. Nat. Inst. Agr. Sci. Ser. G.*, No. 3, 68-87.
60. O'DONNELL, J. M. (1969). The size distribution of particulate material in mammalian semen and testicular fluid. *J. Reprod. Fertility* (In press)
61. ORGEBIN-CRIST, M. C. (1967). Maturation of spermatozoa in the rabbit epididymis: fertilizing ability and embryonic mortality in does inseminated with epididymal spermatozoa. *Ann. Biol. Animale Biochim. Biophys.* **7**, 373-389.

62. PRÉVOST AND DUMAS (1824). Observations relatives à l'appareil générateur des animaux mâles; Histoire et description des animalcules spermatiques. *Ann Sci. Nat.* Paris. **1**, 10-29.

63. PRÉVOST AND DUMAS (1824). Observations relatives à l'appareil générateur des animaux mâles; Examen des liquides renfermés dans les diverses glandes qui peuvent s'y rencontrer; Histoire et description des animalcules spermatiques. *Ann. Sci. Nat.*, Paris. **1**, 167-187 and 274-293.

64. Quinn, P. J. (1967) Studies of some membrane phenomena of spermatozoa and erythrocytes. Ph.D. Thesis, University of Sydney, New South Wales, Australia.

65. REDDY, J. K. AND SVOBODA, D. J. (1967). Peroxidase transport by Sertoli cells of the rat testis. *J. Cell. Biol.* **35**, 379.

66. REDENZ, E. (1924). Versuch einer biologischen Morphologie des Nebenhodens. *Arch. Mikroskop. Anat. Entwicklungsmechanik* **103**, 593-628.

67. REDENZ, E. (1926). Nebenhoden und Spermienbewegung. *Wurz. Abw. Ges. Med.* **4**, 107-150.

68. REDENZ, E. (1933). Über den Spaltungsstoffwechsel der Säugetier-spermatozoen im Zusammenhang mit der Beweglichkeit. *Biochem. Z.* **257**, 234-241.

69. RETZIUS, C. (1902). Weitere Beiträge zur Kenntniss der Spermien des Menschen und einiger Säugetiere. *Biol. Untersuch. N. F. Jena* **10**, 45-60.

70. RETZIUS, C. (1906). Die Spermien der Amphibien. Die Spermien der Reptilien. Die Spermien der Monotremen. Die Spermien der Marsupialen. Die Spermien der Edentaten. Die Spermien der Vespertilionen. *Biol. Untersuch. N. F. Jena* **13**, 49-94.

71. RETZIUS, C. (1909). Die Spermien der Vögel. Die Spermien von Didelphys. Die Spermien von Bradypus. Die Spermien der Insektivoren. Die Spermien der Nägeltiere. Die Spermien der Huftiere. Die Spermien der Waltiere. Die Spermien der Carnivoren. Die Spermien der Halbaffen. Die Spermien der Affen. Die Spermien des Menschen. *Biol. Untersuch N. F. Jena* **14**, 89-216.

72. ROBINSON, J. R. (1965). Water regulation in mammalian cells. *In* "The state and movement of water in living organisms," *Symp. Soc. Exptl. Biol.* **19**, 237-258.

73. ROLSHOVEN, E. (1936). Ursachen und Bedeutung der intratubularen Sekretströmung im Säugerhoden. *Z. Anat. Entwicklungsgeschichte* **105**, 374-408.

74. ROOSEN-RUNGE, E. C. (1951). Motions of the seminiferous tubules of rat and dog. *Anat. Rec.* **109**, 413.

75. ROSS, M. H. (1967). The fine structure and development of the peritubular contractile cell component in the seminiferous tubules of the mouse. *Am. J. Anat.* **121**, 523-558.

76. ROSS, M. H. AND LONG, I. R. (1966). Contractile cells in human seminiferous tubules. *Science* **153**, 1271-1273.

77. SCHMIDT, F. C. (1964). Licht-und elektronenmikroskopische Untersuchungen am menschlichen Hoden und Nebenhoden. *Z. Zellforsch.* **63**, 707-727.

78. SCOTT, T. W. AND DAWSON, R. M. C. (1968). Metabolism of phospholipids by spermatozoa and seminal plasma. *Biochem. J.* **108**, 457-462.

79. SCOTT, T. W. AND SETCHELL, B. P. (1968). Lipid metabolism in the testis of the ram. *Biochem. J.* **107**, 273-278.

80. SCOTT, T. W., VOGLMAYR, J. K. AND SETCHELL, B. P. (1967). Lipid composition and metabolism in testicular and ejaculated ram spermatozoa. *Biochem. J.* **102**, 456-461.

81. SCOTT, T. W., WALES, R. G., WALLACE, J. C. AND WHITE, I. G. (1963). Composition of ram epididymal and testicular fluid and the biosynthesis of glycerylphosphorylcholine by the rabbit epididymis. *J. Reprod. Fertility* **6**, 49-59.

82. SCOTT, T. W., WHITE, I. G. AND ANNISON, E. F. (1962). Glucose and acetate metabolism by ram, bull, dog and fowl spermatozoa. *Biochem. J.* **82**, 398-404.

83. SETCHELL, B. P. (1967). Fluid secretion by the testis. *J. Reprod. Fertility* **14**, 347-348.

84. SETCHELL, B. P. (1967). The blood-testicular fluid barrier in sheep. *J. Physiol.* **189**, 63-65P.

85. SETCHELL, B. P. (1968). The effect of age, hypophysectomy and cryptorchidism on fluid secretion by the rat testis. *Australian J. Exptl. Biol. Med. Sci.* **46**, 35P.

86. SETCHELL, B. P., DAWSON, R. M. C. AND WHITE, R. W. (1968). The high concentration of free myo-inositol in rete testis fluid from rams. *J. Reprod. Fertility* **17**, 219-220.

87. SETCHELL, B. P. AND HINKS, N. T. (1967). The importance of glucose in the oxidative metabolism of the testis of the conscious ram and the role of the pentose cycle. *Biochem. J.* **102**, 623-630.

88. SETCHELL, B. P., HINKS, N. T., VOGLMAYR, J. K. and SCOTT, T. W. (1967). Amino acids in ram testicular fluid and semen and their metabolism by spermatozoa. *Biochem. J.* **105**, 1061-1065.

89. SETCHELL, B. P. AND LINZELL, J. L. (1968). Effects of some drugs and hormones on the secretion of fluid by the testis of the ram. *J. Reprod. Fertility* **16**, 320-321.
90. SETCHELL, B. P. AND LINZELL, J. L. (1967). Unpublished.
91. SETCHELL, B. P., VOGLMAYR, J. K. AND WAITES, G. M. H. (1969). A blood testis barrier restricting passage from blood into rete testis fluid but not into lymph. *J. Physiol.* **200**, 73-85.
92. SETCHELL, B. P. AND WAITES, G. M. H. (1964). Blood flow and the uptake of glucose and oxygen by the testis and epididymis of the ram. *J. Physiol.* **171**, 411-425.
93. SETCHELL, B. P., WAITES, G. M. H. AND THORBURN, G. D. (1966). Blood flow in the testis of the conscious ram measured with Krypton^{85}—effects of heat, catecholamines and acetyl choline. *Circulation Res.* **18**, 755-765.
94. THORBURN, G. D. AND MOLYNEUX, G. S. (1967). Intercellular fluid pathways in the renal proximal tubule. *Bull. Post-grad. Comm. Med. Univ. Sydney* **23**, 199-208.
95. TOOTHILL, M. C. AND YOUNG, W. C. (1931). The time consumed by spermatozoa in passing through the ductus epididymidis of the guinea pig as determined by means of India-ink injections. *Anat. Rec.* **50**, 95-107.
96. TOURNADE, A. (1913). Différence de motilité de spermatozoides prélévés dans les diverses segments de l'épididyme. *Comp. Rend. Soc. Biol.* **74**, 738.
97. VOGLMAYR, J. K. (1968). Unpublished.
98. VOGLMAYR, J. K., LARSEN, L. H. AND WHITE, I. G. (1969). Metabolism of spermatozoa and composition of fluid collected from the rete testis of conscious bulls. *J. Reprod. Fertility* (In press)
99. VOGLMAYR, J. K., SCOTT, T. W., SETCHELL, B. P. AND WAITES, G. M. H. (1967). Metabolism of testicular spermatozoa and characteristics of testicular fluid collected from conscious rams. *J. Reprod. Fertility* **14**, 87-99.
100. VOGLMAYR, J. K., GILES, J. G. AND SETCHELL, B. P. (1966). Unpublished.
101. VOGLMAYR, J. K. AND SETCHELL, B. P. (1968). Unpublished.
102. VOGLMAYR, J. K., WAITES, G. M. H. AND SETCHELL, B. P. (1966). Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. *Nature* **210**, 861-863.
103. VOGLMAYR, J. K., WHITE, I. G. AND QUINN, P. J. (1969). A comparison of adenosinetriphosphatase activity in testicular and ejaculated spermatozoa of the ram. *Biol. Reprod.* **1** (in press).
104. WAITES, G. M. H. AND SETCHELL, B. P. (1969). Some physiological aspects of the function of the testis. *In* "The Gonads," (K. W. McKerns, ed.). Appleton, New York (in press).
105. WALLACE, J. C. AND LASCELLES, A. K. (1964). Composition of testicular and epididymal lymph in the ram. *J. Reprod. Fertility* **8**, 235-242.
106. WU, S. H., MCKENZIE, F. F., FANG, S. C. AND BUTTS, J. S. (1959). Pathways of glucose utilization in epididymal and testicular sperm cells. *J. Dairy Sci.* **42**, 110-114.
107. YOUNG, J. A. AND SCHÖGEL, E. (1966). Micropuncture investigations of sodium and potassium excretion in rat submaxillary saliva. *Pfluegers Arch. Ges. Physiol.* **291**, 85-98.
108. YOUNG, W. C. (1931). A study of the function of the epididymis. III. Functional changes undergone by spermatozoa during their passage through the epididymis and vas deferens in the guinea pigs. *J. Exptl. Biol.* **8**, 151-162.
109. ZAWISCH-OSSENITZ, C. (1932). Der Flimmerstrom in den Ductuli Efferentes des Hodens und die Bewegung der Spermien. *Z. mikr.-anat. Forsch.* **32**, 84-106.