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The Movement of Fluids and Substances in the Testis*

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Abstract

Three aspects of the control of movements of fluids and substances into, out of and inside the testis are discussed: the tubular barrier, the interstitial extracellular fluid and the testicular blood vessels. The functional basis for the tubular barrier is twofold; there are significant differences in the concentration of many substances inside and outside the tubules and marker substances enter or leave the tubular fluid at widely different rates, depending on lipid solubility and the presence of specific carrier systems. The anatomical basis for this barrier appears to be the specialized junctions between adjacent pairs of Sertoli cells. The barrier develops only at puberty, as the first cells undergo meiosis, but the development may not be as sudden as previously believed. The barrier breaks down after efferent duct ligation when spermatogenesis is disrupted. Techniques for measuring the volume, the turnover rate, the composition and fate of the interstitial extracellular fluid are described, and the unsatisfactory features of the presently available techniques for collecting this fluid for analysis are emphasized. There is a relationship between the fluid in the testis and lymph from vessels in the spermatic cord and lymph may be important for the transport of hormones to the general circulation in some circumstances and to other organs close to the testis. The testicular blood vessels display certain unusual features, a very high susceptibility to the toxic effects of cadmium salts, a high level of alkaline phosphatase activity in all endothelial cells but only after puberty and a high level of gamma-glutamyl transpeptidase in the endothelial cells of the arterioles and the testicular artery. These same cells are the site for a specific transport system for leucine and phenylalanine, with kinetic characteristics similar to the system in brain. Flow of blood may limit hormone secretion by the aspermatogenic testis, but diffusion limitation may also be important under some circumstances. A fuller understanding of the ways in which substances move around in the testis, particularly how they cross the endothelial cell layer or penetrate into the tubules, will be important for a better appreciation of testicular function.

Introduction

In this lecture, I propose to describe some features of the movement of fluids and substances within the testes of mammals. That such a topic merits consideration is perhaps surprising, but I hope to show that the testis has many curious features which influence the free movements of substances both within the tissue, and between the tissue and the rest of the body. As the testis is an important endocrine tissue, and hormones are important factors in the production of spermatozoa, this discussion will concentrate on hormones, although not exclusively.

It is well known that most of the mammalian testis consists of the seminiferous tubules, which contain the Sertoli cells and the various germinal cells; these tubules

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are avascular and no nerves penetrate through their walls. The rest of the tissue (between 10 and 30% depending on the species) is usually referred to as the interstitial tissue; this includes all the blood vessels and lymphatics, the nerves, the Leydig cells and a significant population of macrophages.

I propose to divide this paper into three portions. First, I would like to describe the current state of knowledge concerning the permeability barrier inside the seminiferous tubules; this is the barrier that is usually referred to as the 'blood-testis barrier', but in this paper I will use the term 'tubular barrier'. Second, I will consider the fluid in the interstitial tissue, its volume, composition, rate of turnover and fate, and its relationship with testicular lymph. Last, I would like to present some recent data which suggests that the blood vessels of the testis and spermatic cord possess many unusual features which may be of considerable importance in testicular function, and to discuss how the entry and egress of substances from the testis may be limited by diffusion or flow.

The Tubular Barrier

During the last 20 years, a formidable battery of evidence has been accumulated for the existence of a permeability barrier in the seminiferous tubules. This evidence is both functional and structural. The functional evidence is of two types, involving data on the composition of the fluids inside the seminiferous tubules and the rete testis as well as studies on the rate at which radioactively labelled and other markers move from the blood stream into these fluids or from the lumina of the seminiferous tubules into the blood stream. Although there are important differences in composition between rete testis fluid (RTF) and seminiferous tubule fluid (STF) which will not be further considered here, both fluids contain appreciably more potassium and less sodium than blood plasma or testicular lymph from a lymphatic vessel in the spermatic cord. RTF and STF also contain considerably more of some organic compounds, such as inositol and some amino acids, and much less of others, such as glucose, protein and particularly immunoglobulins than blood plasma or lymph (see Setchell and Waites 1975; Waites 1977; Setchell 1980; Waites and Gladwell 1982, for review). This would suggest that these molecules do not pass readily into or out of the tubules, otherwise these differences would be dissipated.

Measurement of the entry rates of markers into RTF and STF confirms that there are considerable differences in the values for different compounds. In general, the entry rate is high when the lipid solubility of the substance is high, and vice versa (see Setchell and Waites 1975; Setchell 1980, for review). However, there are two definite exceptions to this generalization, 3-*O*-methylglucose and testosterone. Both enter both STF and RTF more rapidly than one would expect from their lipid solubility, in the case of testosterone more rapidly than dihydrotestosterone which is actually marginally more lipid-soluble (Middleton 1973; Cooper and Waites 1975; Setchell and Main 1975; Setchell *et al.* 1978). The finding with 3-*O*-methylglucose is not unexpected, as this substance is transported in many other tissues by the same saturable facilitated-diffusion type carrier as glucose but is not metabolized. This system can also be studied using isolated seminiferous tubules *in vitro* and the kinetic characteristics of the carrier have been determined (Middleton 1973). However, the situation with testosterone is different. Entry of this substance into the tubules *in vitro* does not show saturation kinetics, and consequently, the system has proved to be difficult to study.

The structural basis of the tubular barrier has been well characterized and has been shown to reside mainly in the specialized junctions between pairs of Sertoli cells. The peritubular myoid cells and the associated non-cellular material offer some restriction to movement, but this is believed to be secondary in rodents and primates

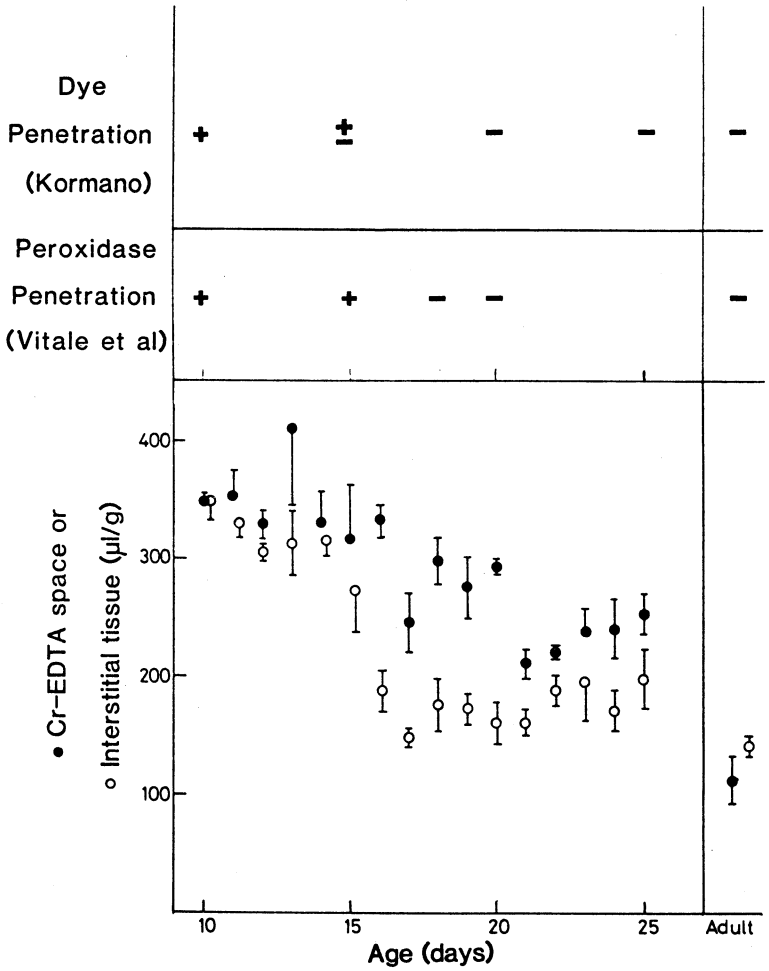

Fig. 1. The Cr-EDTA spaces and the volume of the interstitial tissue in rats of various ages (data of Setchell *et al.* 1981), with a summary of the results of Kormano (1967) and Vitale *et al.* (1973). The + indicates that the marker penetrated into the seminiferous tubules, the – that it did not. Note that there is a sudden fall in the relative volume of the interstitial tissue at about 15 days of age, and that the Cr-EDTA space falls more gradually, remaining greater than the interstitial tissue volume even in rats 25 days old, in contrast to the situation in adult rats where the Cr-EDTA space is about 80% of the interstitial tissue volume.

(Dym and Fawcett 1970; Dym 1973). However, the situation may be rather different in farm animals in which the non-cellular part of the peritubular tissue is much better developed (see Setchell 1970*a*). The Sertoli cell junctions divide the germinal epithelium into a basal or peripheral compartment and an adluminal or central compartment (Dym and Fawcett 1970; Bellve 1979). There is also sound evidence that the tubular

barrier to the electron-opaque markers develops only at puberty as the first cells reach pachytene, in the rat at about 18 days (Vitale *et al.* 1973; Bergmann and Dierichs 1983), which agrees with earlier evidence for the time at which dyes are excluded from the tubules (Kormano 1967).

However, the markers used in these studies are large, unphysiological substances and the results are necessarily only qualitative. An attempt has been made to quantitate barrier function by measuring the 'space' of distribution of the chromium salt of ethylenediamine tetra-acetic acid (Cr-EDTA), which distributes readily throughout the extracellular fluid in most tissues and is filtered by the glomeruli of the kidney, but is virtually excluded from the tubular fluid (Setchell *et al.* 1969). The space is determined by infusing the marker intravenously, allowing time for equilibration and then determining the amount of marker in unit weight of tissue and unit volume of blood plasma. The space, in μl/g, is then determined by dividing the tissue radioactivity in dpm/g by the plasma radioactivity in dpm/μl. In the testes of adult rats, the Cr-EDTA space is about 120 μl/g, about 80% of the interstitial tissue volume as calculated from measurements made on frozen sections. In very young rats, the Cr-EDTA space actually exceeds the volume of the interstitial tissue, indicating that the tubular barrier is not operative, but the changes at puberty are complicated because there are also changes in the relative size of this portion of the tissue. Nevertheless, it is clear that at about 18 days, the Cr-EDTA space falls sharply, relative to the volume of the interstitial tissue (Fig. 1), but even at 25 days of age when electron microscopy indicates that the barrier is fully formed, the Cr-EDTA space is still considerably greater than the interstitial tissue volume (Setchell *et al.* 1981). Clearly then the barrier is not fully functional at this age but studies with rats between 25 days of age and adulthood have not yet been completed. The later development of a fully functional barrier is supported by studies on the development by the testis of the capacity for fluid secretion, which is dependent on the presence of a functional barrier. Secretion rates, as assessed by the gain in testis weight after efferent duct ligation, attain adult levels in rats only at about 40 days of age, and there is a sudden rise in the rate of secretion at about 30 days (Setchell 1970*b*). Before this age, the evidence for secretion is less certain.

Jegou *et al.* (1982) claim that fluid secretion can be demonstrated in rats between 15 and 20 days old, but there is no indication in their data for secretion in 15 day-old animals, and even in the 20 day-old ones there was an average increase in weight of only 10 mg in testes weighing about 200 mg. This apparent increase is of the same order as the variability in weight between the two testes of one animal, and in any case only five animals were used in this age group. Even at 25 days of age where groups of four animals were studied 6, 12, 18, 26, 36, and 48 h after ligation, the rate of fluid accumulation was only about 20 mg per testis in 16 h, compared with the earlier values (Setchell 1970*b*) of about 90 mg in 30 day-old rats. Furthermore, the earlier values were substantiated by measurements of water content, as well as weight of the testes, and this is a much more precise indicator of low levels of fluid secretion since it is much less variable in unligated testes than their weight and in a group of 32 rats, 24 days old, water content was only 2·3% greater in the testis ligated 20 h earlier, whereas testis weight was 10·7% greater (Setchell *et al.* 1973).

A lumen first appears in the seminiferous tubules at about 20 days of age, but continues to enlarge beyond 40 days. It is also relevant that androgen-binding protein

(ABP) concentrations in the testes of rats peak at 20 days of age, and then fall away again as fluid secretion begins; adult levels are not reached until 40 days. In one study (Tindall *et al.* 1975) no ABP could be detected in the epididymis before 18–20 days and before 25 days in another (Danzo and Eller 1985). However, the epididymal concentrations rose by more than twice in the former study and by about 12 times in the latter between 30 days of age and adulthood and the total amount of ABP in the epididymis rose by more than 100 times over that period while testis weight increased only by about four times, emphasizing the increase in fluid secretion which occurs after puberty.

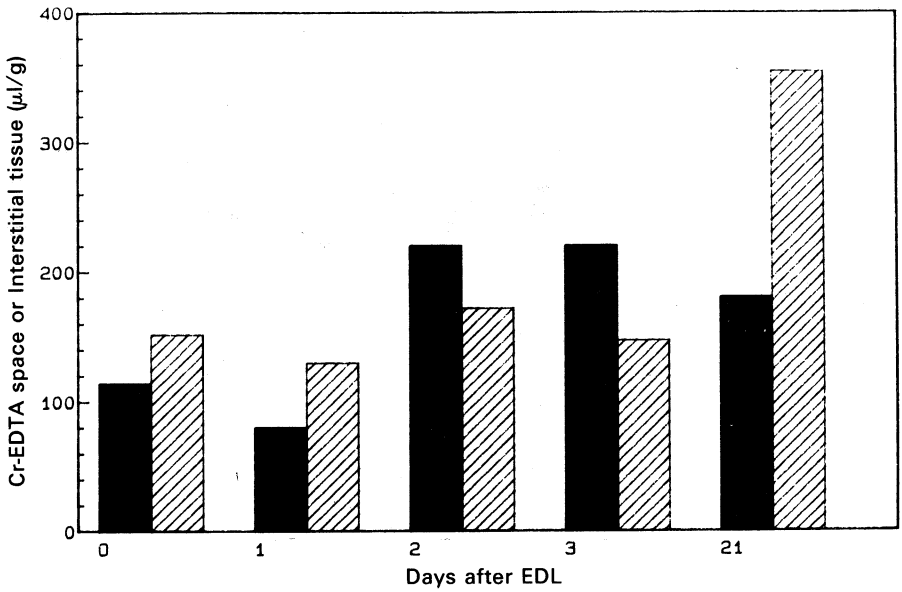

Fig. 2. The Cr-EDTA spaces (in μl/g) determined with ^{51}Cr-EDTA (■) and the volume of the interstitial tissue determined morphometrically (hatching) in testes of rats in which the efferent ducts had been ligated (EDL) 1, 2, 3 or 21 days earlier. Following this operation, the weight of the ligated testis increases linearly for about 36 h due to the accumulation of secreted fluid, then over the next 4 or 5 days, returns to about control levels. Eventually, by 21 days after the operation the seminiferous tubules have degenerated and the testis weighs about half control. Note that 1 day after EDL, the Cr-EDTA space is still less than the volume of the interstitial tissue, whereas 2 and 3 days after EDL, the Cr-EDTA space is appreciably greater. By 21 days after the operation, although the interstitial tissue is appreciably increased due to the degeneration of the tubules, the Cr-EDTA space has fallen below the interstitial tissue volume.

What are the consequences of the tubular barrier? We know that there are effects in the areas of immunology, endocrinology, metabolism, toxicology and in the physiology of fluid secretion, but the relevance of these effects to the spermatogenic or hormone-secreting functions of the testis is not yet certain. One approach would be to study the effects of disruption of the barrier, but it has proved to be extremely resistant to attempts to break it down. The only well-defined example is seen after efferent duct ligation. Because this procedure causes retention of the secreted fluid within the testis, the testis initially swells up and becomes extremely turgid; then quite suddenly it loses its turgor and decreases in size again (Setchell 1970*b*). Once the testis has returned to normal size, measurements of Cr-EDTA space show

that this has increased to values considerably in excess of the measured interstitial tissue volume (Fig. 2), indicating breakdown of the tubular barrier. This is followed by a progressive tubular degeneration so that by 21 days after the operation only Sertoli cells and a few spermatogonia remain inside the tubules. However, by that time, the Cr-EDTA space has fallen again to well below the interstitial tissue volume, suggesting that the barrier has been re-established. The interpretation of these findings is complicated by the observation that the introduction of a plug of non-toxic latex into a single seminiferous tubule produces a very similar lesion to that seen after efferent duct ligation (Pilsworth *et al.* 1981). It is hard to see how the tubular barrier would be affected by a blockage in the lumen, so perhaps the flow of fluid along the tubule is also important for normal spermatogenesis, and the normal flow of luminal fluid would probably be severely disrupted after efferent duct ligation.

However, it appears that normal function of the tubular barrier is intimately concerned with spermatogenesis, and further investigations seem to be warranted in the hope that some light may be shed on some of the so far inexplicable problems of male infertility.

Interstitial Extracellular Fluid and Lymph

I wish now to turn to a consideration of the fluid to be found between the cells in the interstitial tissue. It is always assumed that this fluid is formed by filtration of a fluid of low protein concentration at the arterial ends of the testicular capillaries and resorption of most of the fluid but not the protein at the venous ends of the capillaries, the protein and the rest of the fluid leaving the tissue as lymph. While there is now direct evidence for this process in other tissues, it should be stressed that we can only assume that the system is similar in the testis, where regulation of tissue pressure by contractions of the capsule may be of considerable significance. Interstitial fluid is of considerable interest at the moment since it has been realized that all substances entering or leaving the tubules and probably also the Leydig cells must pass through this fluid. The doubt about the Leydig cells stems from the very close physical association between some of these cells and the walls of the capillaries, so that there may be a short-cut between the Leydig cells and the blood plasma. Be that as it may, a fluid derived at least in part from this compartment has also been shown to be rich in some very interesting peptides (Sharpe 1983, 1984) and therefore it seems relevant to discuss what we know about its volume, its rate of turnover, its composition and its fate.

Volume

The volume of this interstitial extracellular fluid in a testis with a functional tubular barrier can easily be estimated from the Cr-EDTA space, as this marker has been shown to be effectively excluded from the tubules (Setchell *et al.* 1969). It has also been found that the albumin space at equilibrium in the rat testis is approximately equal to the Cr-EDTA space of between 100 and 120 μl/g (Setchell and Sharpe 1981), and the use of albumin has certain practical advantages, although the time required for equilibration is considerably longer (6 h compared with less than 30 min for Cr-EDTA). There is also recent microscopic evidence that albumin is distributed throughout the interstitial tissue, and does not enter the Leydig cells; it does appear to be taken up to some extent by macrophages in the interstitial tissue (Christensen *et al.* 1985), and this may explain the somewhat higher values for albumin spaces

found in normal rat testes 24 h after injection of the marker (Setchell and Wallace 1972). This means that at about 6 h after injection, there may, in fact, be transition from a faster to a slower rate of accumulation of albumin in the testis and it is probably advisable not to use intervals after injection of longer than 6 h.

Turnover Rate

If instead of injecting the albumin into the blood stream, it is injected directly into the testis, more than 95% is cleared in the lymph. The clearance is exponential, and from the half-time of the clearance and the volume of distribution of the albumin (usually assumed to be the same as the Cr-EDTA space), it is possible to calculate a lymph flow from

$$Q_{\text{lymph}} = 0{\cdot}693 V_{\text{albumin}} / T_{\frac{1}{2}},$$

where Q_{lymph} is the flow of lymph in μl g^{-1} min^{-1}, V_{albumin} is the volume of distribution of the albumin and $T_{\frac{1}{2}}$ is the half-time of the clearance of albumin from the testis.

The first point to notice is that the lymph flow varies widely between species (Table 1). The flow is so high in the pig that the haematocrit of the blood is measurably increased as the blood passes through the testis (Galil *et al.* 1981), and lymph flow calculated from measured blood flow and this change in haematocrit agrees quite well with the value obtained from the albumin clearance. The lymph flow is much lower in the rat, but in this species, it can be dramatically increased by injecting the animal with hCG 8–24 h earlier (Setchell and Sharpe 1981).

Table 1. Lymph flow from the testes of various species

Lymph flow was calculated from the half-time of clearance of radioactive albumin injected directly into the testis and the volume of distribution (see text). Values for blood flow were obtained by microspheres in rats and by *p*-aminohippurate (PAH) dilution in rams and boars. Data of Galil *et al.* (1981), Laurie and Setchell (1978) and Wang *et al.* (1983)

Species	Lymph flow (μl g^{-1} min^{-1})	Lymph flow (μl min^{-1})	Blood flow (μl min^{-1})
Rat	0·43	0·73	500
Ferret	3·7	12	–
Sheep	0·86	215	25 000
Pig	10·4	2000	30 000

Composition of Fluid

No really satisfactory technique has yet been described for collection of the interstitial extracellular fluid in reasonable quantities for analysis. Micropuncture techniques require removal of at least part of the tunica albuginea and this may disturb the fluid formation and removal. The potassium concentration in samples removed in this way was about 50% higher than blood plasma (Tuck *et al.* 1970) whereas the potassium concentration in lymph from the spermatic cord was equal to that in blood plasma (see Setchell 1970*a*), suggesting that cell contents were contaminating the interstitial fluid sampled in this way. The volumes obtained are also very small, although they were sufficient in one study to yield some testosterone levels

(Table 2). Another technique was originally introduced by Pande *et al.* (1966) and has been used subsequently by Hagenas *et al.* (1978), Sharpe (1979), Sharpe *et al.* (1983) and Turner *et al.* (1984, 1985). This involves incising or removing the capsule of isolated testes then allowing fluid to leak from the tissue in a refrigerator or removing it by centrifugation. This technique has been used extensively in investigations of the androgen milieu of the tubules, but comparatively few attempts have been made to evaluate the purity of the fluid obtained. I believe that the results are not encouraging. Not surprisingly, fluid collected in this way also has considerably more potassium in it than in blood plasma (Pande *et al.* 1966; Sharpe 1979) but also contains approximately 600 times as much lactate, 15 times as much ascorbic acid, only one-fifth as much glucose, five times as much glycogen, up to 400 times as much lactate dehydrogenase and more than 50 times as much acid phosphatase and 5000 times as much glucose-6-phosphate dehydrogenase as blood plasma (Pande *et al.* 1966). Clearly this fluid contains considerable and probably variable amounts of cellular contents and I believe that any statements about its composition should be viewed with profound suspicion. Nevertheless, a physiological technique for the estimation of the composition of extracellular interstitial fluid must be developed if we are to understand how substances cross the endothelium of the testicular blood vessels.

Table 2. Testosterone concentrations in interstitial fluid and venous blood plasma from the testis, and in peripheral blood plasma of rats
Number of observations given in parentheses

Author	Technique	Testosterone concn (±s.e.m.) in interstitial fluid (ng/ml)	Testosterone concn (±s.e.m.) in blood plasma (ng/ml)	
			Testicular vein	Peripheral
Comhaire and Vermeulen (1976)	Micropuncture	150 ± 27 (17)	–	–
Hagenas *et al.* (1978)	Centrifugation	137 ± 25 (10)	–	1·6 ± 0·7 (10)
Turner *et al.* (1984, 1985)	Centrifugation	73 ± 5 (26)	28 ± 5·3 (10)	1·2 ± 0·1 (8)
Sharpe *et al.* (1983)	Gravity			
	Control	315 ± 29 (4)	–	2·7 ± 0·3 (4)
	Control	590 ± 20 (8)	90 ± 20 (8)	–
	LHRH-A	800 ± 30 (8)[A]	175 ± 20 (8)[A]	–

[A] Testes had been injected directly with 1 ng of an agonist of LHRH (see original paper for details).

Different problems are associated with the use of lymph from the spermatic cord as an indication of the composition of the fluid within the testis. The lymphatic vessels are very closely associated with the blood vessels, particularly the veins in the spermatic cord and there is a strong possibility that transfer of material can occur between the various fluids in the cord. Nevertheless, analysis of lymph is probably the best of the available ways of estimating the composition of the interstitial fluid in the testis, particularly if a substance is present in lymph in higher concentrations than in blood plasma. Several years ago, detailed studies of the composition of testicular lymph from the ram were reported (Lindner 1963, 1969; Wallace and Lascelles 1964; Setchell *et al.* 1967) and it appeared that testicular lymph in this species was very similar in composition to blood plasma from the internal spermatic vein; the

testosterone levels were approximately 70% of those in blood and this ratio was maintained when the animals were treated with hCG, either for 8 days previously (Lindner 1967, 1969) or once immediately beforehand (Chandrasekhar *et al.* 1986). A quite different situation applies in the pig. In this species, the lymph resembles testicular venous blood plasma in most regards (Setchell 1982) except for steroids. There are appreciably higher concentrations of free testosterone and oestradiol, but the concentrations of the steroid conjugates, dehydroepiandrosterone sulfate and oestrone sulfate are about 30 times as high as those in blood plasma from the internal spermatic vein (Setchell *et al.* 1983). It is impossible at the moment to say how these enormous concentration differences are maintained across a vascular wall which allows equilibration of albumin from blood to lymph within 1 h, but differences in steroid binding are not involved (B. P. Setchell and R. B. Heap, unpublished observations).

Fate of the Interstitial Fluid and Lymph

The majority of the lymph in the vessels in the spermatic cord flows through regional lymph nodes and then on to the thoracic duct and the venous return to the heart. The importance of the lymphatic constituents for local effects on the lymph nodes will depend on their concentration and for the significance of this route of secretion to the whole animal on flow and concentration. It is clear that in all species, the regional lymph nodes will be exposed to very much higher steroid concentrations than elsewhere in the body, but the immunological and other consequences of this feature have not been explored. In the rat and the sheep, the comparatively low lymph flows and in the latter, the similar concentrations of steroids to those in venous blood mean that the lymph is an unimportant route of secretion as far as the whole animal is concerned. In the pig, the high rate of lymph flow and particularly the extraordinary concentrations of the conjugated steroids means that the lymph is the major route for secretion of these two compounds from the testis; about 80% of the dehydroepiandrosterone sulfate and about 65% of the oestrone sulfate are secreted in the lymph, compared with about 15% of the testosterone and about 10% of the oestradiol (Setchell *et al.* 1983). In the horse, the values are less but still significant (Setchell and Cox 1983).

Evidence for the direct local transport of water-soluble substances from the testis to nearby organs arose unexpectedly during the measurement of testicular lymph flow by the direct injection of albumin into the testis. At the end of the clearance measurements, the radioactivity in other tissues was measured, and to our surprise, we noticed that the epididymis and fat pad ipsilateral to the injected testis always contained much more radioactivity than the contralateral epididymis and fat pad. A similar difference between the two epididymides was apparent in rams, boars and ferrets. (Jones and Setchell 1986). This transfer was also apparent when radioactive albumin was infused directly into a lymphatic on the surface on the testis. The lymphatic was first made visible by injecting a small volume of the dye pontamine sky blue under the capsule, and then cannulated with a hypodermic needle attached to a polythene catheter. The infusion was continued at a rate of 0·1 ml/min for 1 h and then pieces of tissue and samples of blood and lymph were counted. It was apparent that albumin was transferred to the epididymal tissue not only in the caudal region but also in the corpus and caput (Fig. 3; Jones and Setchell 1986).

The Blood Vessels of the Testis

All fluid and substances entering the testis and most leaving it do so via the blood stream. Is there anything remarkable about the blood vessels of the testis which would indicate that they merit investigation as possible sites of control of testicular function? The capillaries of the testis do appear to be unusual among those of endocrine tissues in the rat in being unfenestrated (see Setchell 1970*a*) and they do show an extraordinary sensitivity to the toxic effects of cadmium salts (Setchell and Waites 1970; Aoki and Hoffer 1978). However, measurements of their permeability–surface area product

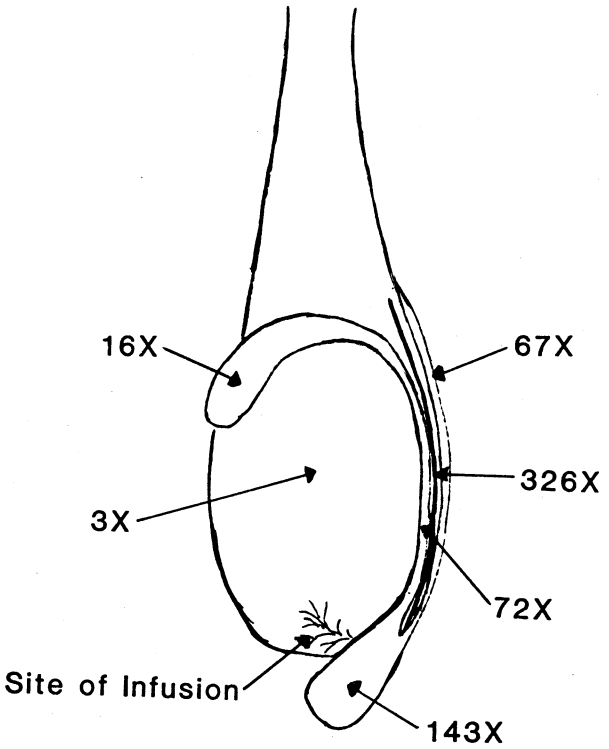

Fig. 3. The concentration of ^{125}I in the testis (3×), the head, body and tail of the epididymis (16×, 72× and 143× respectively) and the ductus deferens (67×) relative to peripheral blood plasma at the end of a 1-h infusion of radioactively labelled albumin into a lymphatic vessel on the surface of the testis as indicated. At this time, lymph from the lymphatic vessel running between the epididymis and the ductus deferens, shown as the heavy line, and sampled at the site indicated, contained 326× as much radioactivity as blood plasma. Data of Jones and Setchell (1986).

(PS) for Cr-EDTA, sodium, vitamin B_{12} (Bustamante and Setchell 1981) and albumin (Setchell *et al.* 1984) suggest that their normal permeability is similar to that in other capillaries in the body. However, the permeability of the testicular blood vessels to albumin is markedly increased if the animal is injected with hCG between 8 and 24 h beforehand (Setchell and Sharpe 1981). The permeability returns to normal between 36 and 48 h after the hCG and remains low during the second peak of testosterone which occurs about 72 h after hCG. The permeability increase does not

involve androgens, prostaglandins, histamine or bradykinin but is mediated to some extent by 5-hydroxytryptamine, which may arise from the abundant mast cells found in the vicinity of the artery on the surface of the testis (Sowerbutts *et al.* 1986).

The blood vessels of the testis also display certain histochemical peculiarities. They are second only to brain capillaries in their level of alkaline phosphatase, but this is so only after puberty (Kormano 1967). This enzyme is found in the endothelial cells of all blood vessels in the testis including the capillaries, but virtually no activity is found in other interstitial cells or inside the tubules. In contrast, the enzyme gamma-glutamyl transpeptidase which is also present in high concentrations in brain capillaries, is abundant in the endothelial cells of the arterioles in the testis and of the testicular artery on the surface of the testis and in the spermatic cord, but not in the testicular capillaries (Niemi and Setchell 1986). In other tissues, this enzyme

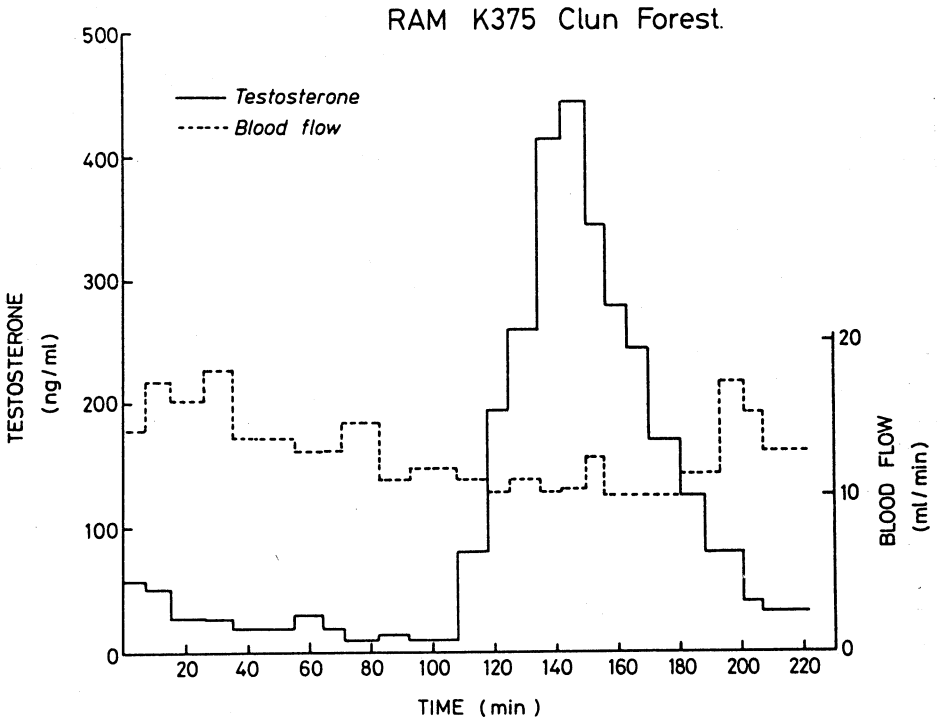

Fig. 4. Testosterone concentration (solid line) in blood from the internal spermatic vein of a conscious ram and blood flow (dashed line) determined by the dilution in the same samples of PAH infused at a constant rate into a vein on the surface of the testis. Data of Laurie and Setchell (1981). Note that there is very little change in blood flow during the spontaneous peak of testosterone, caused by a peak of LH secretion from the pituitary.

is often associated with amino acid transport and this association applies in the testis as well. Using an isolated perfused rat testis preparation, we were able to show that there was a specific, saturable facilitated diffusion type of transport system for leucine and phenylalanine, with kinetic characteristics much nearer to those of the system in the brain than in heart muscle or salivary gland (Bustamante and Setchell 1982*a*, 1982*b*). Furthermore, using autoradiographic techniques, it was possible to localize

the carrier to those same endothelial cells of the larger arterioles in the testis that contain the high levels of gamma-glutamyl transpeptidase (Bustamante *et al.* 1982). The significance of this transport system is not fully understood yet, but it is important to remember that the testis is affected by amino acid deficiencies and that the amino acid composition of the fluid inside the tubules is quite different from blood plasma (Setchell *et al.* 1967), although the differences are not in leucine or phenylalanine, but in other amino acids such as glutamic acid and glycine, which appear to be synthesized inside the tubules from glucose.

One final point is worth mentioning. The vascular system can control the entry or egress of substances into or out of the testis in two ways. For those substances for which there are concentration gradients across the vascular wall, movement can be regulated by varying the rate of diffusion, either passive or facilitated. If diffusion is not limiting, then transport can be regulated by changing blood flow. Flow limitation probably operates for the secretion of testosterone by the aspermatogenic testis, where the concentration is higher than normal in the testis and in the testicular venous blood, but lower in the peripheral circulation; testicular blood flow is reduced in proportion to the reduction in testis weight (Setchell and Galil 1983). Changes in testicular blood flow do not appear to be important in the enhanced secretion of testosterone by the ram testis in response to a spontaneous pulse of LH (Laurie and Setchell 1978; Fig. 4), and the rise in testosterone must either be due to a rapid change in the permeability of the testicular blood vessels or the walls of the Leydig cells, or an equally rapid change in the rate of testosterone synthesis by these cells; at the moment it is not possible to exclude any of these three possibilities, and it should be remembered that there is not even general agreement on how a molecule of testosterone gets out of a Leydig cell. If the concentrations in lymph from the spermatic cord are in fact a good reflection of the situation in the testis of the ram, then the similarities between lymph and venous blood plasma would suggest that diffusion limitation at the vascular wall is not very important in this species, but the striking differences in steroid concentrations between lymph and venous blood plasma in the pig suggest that it is unwise to generalize. The situation in the rat is intriguing. Although, as I have already explained, serious doubts must be cast on the values obtained for the testosterone concentration in interstitial extracellular fluid collected using the present techniques, nevertheless if they are correct, there are concentration gradients of between 3 and 6 from this fluid to venous plasma (Table 2; Sharpe *et al.* 1983, Turner *et al.* 1984, 1985) and furthermore this gradient is appreciably reduced if a small amount of an analogue of LHRH is injected into the testis (Sharpe *et al.* 1983). Even if the absolute value for the gradient is suspect, the reduction is probably an indication that diffusion limitation is operative.

Conclusions

In conclusion, I hope that I have demonstrated that the tubular barrier in the testis is a well-recognized functional and structural entity, although its true significance is still not fully understood. The lack of a satisfactory technique for the collection of interstitial extracellular fluid is a serious limitation to our understanding of the significance of this fluid, but if lymph from the spermatic cord is a reflection of the fluid in the testis, then in some species at least, there are several interesting features which merit further investigation. The transport of hormones to the general circulation via the lymph may be important under some circumstances and the local transfer

by lymphatic channels to nearby organs may be an important means of endocrinological control. Finally, the blood vessels of the testis appear to have several pecularities which may have functional consequences, and the relative importance of diffusion- and flow-limitation of hormone secretion needs to be critically examined.

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References

Aoki, A., and Hoffer, A. P. (1978). Re-examination of the lesions in rat testis caused by cadmium. *Biol. Reprod.* **18**, 579–91.

Bellve, A. R. (1979). The molecular biology of mammalian spermatogenesis. *Oxford Rev. Reprod. Biol.* **1**, 159–261.

Bergmann, M., and Dierachs, R. (1983). Postnatal formation of the blood-testis barrier in the rat with special reference to the initiation of meiosis. *Anat. Embryol.* **168**, 269–75.

Bustamante, J. C., and Setchell, B. P. (1981). Measurement of capillary permeability–surface area products in the isolated perfused rat testis. *J. Physiol.* **319**, 16P–17P.

Bustamante, J. C., and Setchell, B. P. (1982*a*). Kinetics of the facilitated diffusion of leucine into the perfused testis of the rat. *J. Physiol.* **324**, 49P–50P.

Bustamante, J. C., and Setchell, B. P. (1982*b*). The effects of L-phenylalanine and D-leucine on the transport of L-leucine across the blood-testis barrier in the rat. *J. Physiol.* **326**, 17P–18P.

Bustamante, J. C., Jarvis, L. G., and Setchell, B. P. (1982). Role of the endothelium in the uptake of amino acids by the isolated perfused rat testis. *J. Physiol.* **330**, 62P–63P.

Chandrasekhar, Y., D'Occhio, M. J., and Setchell, B. P. (1986). Reproductive hormone secretion and spermatogenic function in thyroidectomized rams receiving graded doses of exogenous thyroxine. *J. Endocrinol.* (In press.)

Christensen, A. K., Komorowski, T. E., Wilson, B., Ma, S.-F., and Stevens, R. W. (1985). The distribution of serum albumin in the rat testis, studied by electron microscope immunocytochemistry on ultrathin frozen sections. *Endocrinology* **116**, 1983–96.

Cooper, T. G., and Waites, G. M. H. (1975). Steroid entry into rete testis fluid and the blood-testis barrier. *J. Endocrinol.* **65**, 195–205.

Comhaire, F. H., and Vermeulen, A. (1976). Testosterone concentration in the fluids of seminiferous tubules, the interstitium and the rete testis of the rat. *J. Endocrinol.* **70**, 229–35.

Danzo, B. J., and Eller, B. C. (1985). The ontogeny of biologically active androgen-binding protein in rat plasma, testis, and epididymis. *Endocrinology* **117**, 1380–8.

Dym, M. (1973). The fine structure of the monkey (Macaca) Sertoli cell and its role in maintaining the blood-testis barrier. *Anat. Rec.* **175**, 639–56.

Dym, M., and Fawcett, D. W. (1970). The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol. Reprod.* **3**, 308–26.

Galil, K. A. A., Laurie, M. S., Main, S. J., and Setchell, B. P. (1981). The measurement of the flow of lymph in the testis. *J. Physiol.* **319**, 17P.

Hagenas, L., Ritzen, E. M., and Suginami, H. (1978). Hormonal milieu of the seminiferous tubules in the normal and cryptorchid rat. *Int. J. Androl.* **1**, 477–84.

Jegou, B., Le Gac, F., and de Kretser, D. M. (1982). Seminiferous tubule fluid and interstitial fluid production. I. Effects of age and hormonal regulation in immature rats. *Biol. Reprod.* **27**, 590–5.

Jones, M. R., and Setchell, B. P. (1986). The transfer of albumin injected into the testis or into a testicular lymphatic to the ipsilateral epididymis and fat pad. *J. Reprod. Fertil.* (In press.)

Kormano, M. (1967). Dye permeability and alkaline phosphatase activity of testicular capillaries in the post-natal rat. *Histochemie* **9**, 327–38.

Laurie, M. S., and Setchell, B. P. (1978). The continuous measurement of testicular blood flow in the ram, in relation to the pulsatile secretion of testosterone. *J. Physiol.* **287**, 10P.

Lindner, H. R. (1963). Partition of androgen between lymph and venous blood of the testis in the ram. *J. Endocrinol.* **25**, 483–94.

Lindner, H. R. (1967). Participation of lymph in the transport of gonadal hormones. Exc. Med. Int. Cong. Ser. 132, 821–7.

Lindner, H. R. (1969). The androgenic secretion of the testis in domestic ungulates. In 'The Gonads'. (Ed. K. W. McKerns.) pp. 615–48. (Appleton Century Crofts: New York.)

Main, S. J., Davies, R. V., and Setchell, B. P. (1978). Feedback control by the testis of gonadotrophin secretion: an examination of the inhibin hypothesis. *J. Endocrinol.* **79**, 255–70.

Middleton, A. (1973). Glucose metabolism in rat seminiferous tubules. Ph.D. Dissertation, University of Cambridge.

Niemi, M., and Setchell, B. P. (1986). Gamma-glutamyl transpeptidase in the microvasculature of the rat testis. *Biol. Reprod.* (In press.)

Pande, J. K., Chowdhury, R. R., Dasgupta, P. R., Chowdhury, A. R., and Kar, A. B. (1966). Biochemical composition of the rat testis fluid. *Proc. Soc. Exptl Biol. Med.* **121**, 899–902.

Pilsworth, L., Hinton, B. T., and Setchell, B. P. (1981). Effects of obstruction of the flow of seminiferous tubule fluid on the germinal epithelium in the rat. *J. Reprod. Fertil.* **63**, 347–53.

Setchell, B. P. (1970*a*). Testicular blood supply, lymphatic drainage and secretion of fluid. In 'The Testis'. (Eds A. D. Johnson, N. L. Vandemark and W. R. Gomes.) pp. 101–239. (Academic Press: New York.)

Setchell, B. P. (1970*b*). The secretion of fluid by the testes of rats, rams and goats, with some observations on the effects of age, cryptorchidism and hypophysectomy. *J. Reprod. Fertil.* **23**, 79–85.

Setchell, B. P. (1978). 'The Mammalian Testis.' (Elek Books: London and Cornell University Press, Ithaca, New York.)

Setchell, B. P. (1980). The functional significance of the blood-testis barrier. *J. Androl.* **1**, 3–10.

Setchell, B. P. (1982). The flow and composition of lymph from the testes of pigs with some observations on the effects of raised venous pressure. *Comp. Biochem. Physiol.* **73A**, 201–5.

Setchell, B. P., Bustamante, J. C., and Niemi, M. (1984). The microcirculation of the testis. In 'Progress in Microcirculation Research II'. (Eds F. C. Courtice, D. G. Garlick and M. A. Perry.) pp. 291–6. (Committee in Postgraduate Medical Education, University of New South Wales, Sydney.)

Setchell, B. P., and Cox, J. E. (1982). Secretion of free and conjugated steroids by the horse testis into lymph and venous blood. *J. Reprod. Fertil.* (Suppl.) **32**, 123–7.

Setchell, B. P., Duggan, M. C., and Evans, R. W. (1973). The effects of gonadotrophins on fluid secretion and production of spermatozoa by the rat and hamster testis. *J. Endocrinol.* **56**, 27–36.

Setchell, B. P., and Galil, K. A. A. (1983). Limitations imposed by testicular blood flow on the function of the Leydig cells *in vivo*. *Aust. J. Biol. Sci.* **36**, 285–93.

Setchell, B. P., Hinks, N. T., Voglmayr, J. K., and Scott, T. W. (1967). Amino acids in ram testicular fluids and semen and their metabolism by spermatozoa. *Biochem. J.* **105**, 1061–5.

Setchell, B. P., Laurie, M. S., and Jarvis, L. G. (1981). The blood-testis barrier at puberty. In 'Development and Formation of Reproductive Organs'. (Eds A. G. Byskov and H. Peters.) Exc. Med. Int. Cong. Ser. No. 559. pp. 186–90.

Setchell, B. P., Laurie, M. S., Flint, A. P. F., and Heap, R. B. (1983). Transport of free and conjugated steroids from the boar testis in lymph, venous blood and rete testis fluid. *J. Endocrinol.* **96**, 127–36.

Setchell, B. P., Laurie, M. S., Main, S. J., and Goats, G. C. (1978). The mechanism of transport of testosterone through the walls of the seminiferous tubules of the rat testis. *Int. J. Androl.* (Suppl. 2.) 506–12.

Setchell, B. P., and Main, S. J. (1975). The blood-testis barrier and steroids. In 'Hormonal Regulation of Spermatogenesis'. (Eds F. S. French, V. Hansson, E. M. Ritzen and S. N. Nayfeh.) pp. 223–33. (Plenum Press: New York.)

Setchell, B. P., and Sharpe, R. M. (1981). Effect of injected human chorionic gonadotrophin on capillary permeability, extracellular fluid volume and the flow of lymph and blood in the testes of rats. *J. Endocrinol.* **91**, 245–54.

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.

Setchell, B. P., and Waites, G. M. H. (1970). Changes in the permeability of testicular capillaries and of the 'blood-testis barrier' after injection of cadmium chloride in the rat. *J. Endocrinol.* **41**, 81–6.

Setchell, B. P., and Waites, G. M. H. (1975). The blood-testis barrier, In 'Handbook of Physiology'. Section 7: Endocrinology; Vol. V: Male Reproductive System'. (Eds D. W. Hamilton and R. O. Greep.) pp. 143–72. (American Physiological Society: Washington, D.C.)

Setchell, B. P., and Wallace, A. L. C. (1972). The penetration of iodine-labelled FSH and albumin into the seminiferous tubules of sheep and rats. *J. Endocrinol.* **54**, 67–77.

Sharpe, R. M. (1979). Gonadotrophin-induced accumulation of 'interstitial fluid' in the rat testis. *J. Reprod. Fertil.* **55**, 365–71.

Sharpe, R. M. (1983). Local control of testicular function. *Q. Jl. Exptl Physiol.* **68**, 265–87.

Sharpe, R. M. (1984). Intratesticular factors controlling testicular function. *Biol. Reprod.* **30**, 29–49.

Sharpe, R. M., Doogan, D. G., and Cooper, I. (1983). Direct effect of a luteinizing hormone-releasing hormone agonist on intratesticular levels of testosterone and interstitial fluid formation in intact male rats. *Endocrinology* **113**, 1306–13.

Sowerbutts, S. F., Jarvis, L. G., and Setchell, B. P. (1986). The increase in testicular vascular permeability induced by human chorionic gonadotrophin involves 5-hydroxytryptamine and possibly oestrogens, but not testosterone, prostaglandins, histamine or bradykinin. *Aust. J. Exp. Biol. Med. Sci.* (In press.)

Tindall, D. J., Vitale, R., and Means, A. R. (1975). Androgen binding protein as a biochemical marker of formation of the blood testis barrier. *Endocrinology* **97**, 636–48.

Tuck, R. R., Setchell, B. P., Waites, G. M. H., and Young, J. A. (1970). The composition of fluid collected by micropuncture and catheterization from the seminiferous tubules and rete testis of rats. *Pflugers Archiv. Europ. J. Physiol.* **318**, 225–43.

Turner, T. T., Jones, C. E., Howards, S. S., Ewing, L. L., Zegeye, B., and Gunsalus, G. L. (1984). On the androgen microenvironment of maturing spermatozoa. *Endocrinology.* **115**, 1925–32.

Turner, T. T., Ewing, L. L., Jones, C. E., Howards, S. S., and Zegeye, B. (1985). Androgens in male reproductive tract fluids: hypophysectomy and steroid replacement. *Am. J. Physiol.* **248**, E274–E280.

Vitale, R., Fawcett, D. W., and Dym, M. (1973). The normal development of the blood-testis barrier and the effects of clomiphene and estrogen treatment. *Anat. Rec.* **176**, 333–44.

Waites, G. M. H. (1977). Fluid secretion. In 'The Testis'. (Eds A. D. Johnson and W. R. Gomes.) pp. 91–123. (Academic Press: New York.)

Waites, G. M. H., and Gladwell, R. T. (1982). Physiological significance of fluid secretion in the testis and the blood-testis barrier. *Physiol. Rev.* **62**, 624–71.

Wallace, J. C., and Lascelles, A. K. (1964). Composition of testicular and epididymal lymph in the ram. *J. Reprod. Fertil.* **8**, 235–42.

Wang, J., Galil, K. A. A., and Setchell, B. P. (1983). Changes in testicular blood flow and testosterone production during aspermatogenesis after irradiation. *J. Endocrinol.* **98**, 35–46.

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