

Functional Relationships of the Mammalian Testis and Epididymis*

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Abstract

The development of knowledge in four areas of research in male reproductive physiology of particular interest is reviewed. The concept of the *blood-testis barrier* (BTB), which arose following dye exclusion from the seminiferous tubules, has now been established as the differential transfer from interstitial fluid to tubular and rete testis fluids of molecules of physiological importance. The composition of fluid collected mostly from the rete testis of several species, not only reflects the nature of the barrier, but also the secretory capacity of the *Sertoli cell*. The functional significance of the transfer of molecules into testicular fluid and the composition of the fluid flowing into the epididymis are discussed. Sertoli cells establish the structural basis of the BTB during puberty and divide the seminiferous epithelium into basal and adluminal compartments. The Sertoli cell is the prime target for follicle stimulating hormone (FSH). The responses evoked by FSH are discussed, including special mention of androgen binding protein (ABP) and the protein hormone, 'inhibin', with FSH-suppressing properties. The *control of FSH* in the lamb is mentioned including new evidence to support a tubular source of a feedback agent with significance during the impuberal stage. Finally, some of the biochemical properties of the *epididymis* and its fluid contents are reviewed and the epididymal sperm are identified as the site of the antifertility action of the 6-chloro-6-deoxy sugars.

Introduction

Some areas of male reproductive physiology, in which there have been significant advances since 1969, are reviewed under the following headings: the *blood-testis barrier*, its various manifestations and its significance for testicular function; the *Sertoli cells*, once believed to form simply a supportive and nutritive epithelium, but now known to be the site of secretion of an array of proteins and other compounds in a fluid of unique composition; *puberty*, still to be adequately defined in the male, but which is preceded by complex endocrinological and cytological events with lasting significance for the achievement of full testicular potential; and, finally, the *epididymis*, a once neglected structure now receiving proper attention, which is not only an excretory duct, but also the site of sperm maturation and of some intriguing biochemistry.

Whenever possible, each aspect is covered by reference only to recent reviews (distinguished from references to research papers by the prefix 'see'), and, therefore, some key research papers may not be referred to directly.

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The Blood-Testis Barrier

This term was given by analogy with the term blood-brain barrier and early manifestations of the blood-testis barrier (BTB) were accidental. When dyes were injected into adult animals to study their exclusion from the brain and other tissues, it was observed that a similar exclusion from the seminiferous tubules occurred. Since then, many studies with a wide variety of marker substances have confirmed the existence of the barrier and have shown that it develops at puberty around the time when the seminiferous tubules develop their lumen (see Setchell and Waites 1975).

This lumen contains fluid so that three principal fluid compartments exist within the testis: the capillary blood plasma; the interstitial fluid and testicular lymph; and the seminiferous tubular and rete testis fluids. Since testicular lymph closely resembles blood plasma in spermatic veins draining the capillaries and since substances introduced into the blood come rapidly into equilibrium with testicular lymph, it appears that, unlike the brain, the capillary endothelium and associated structures are not acting as the barrier, but that this is associated with the tubules themselves (see Waites 1977).

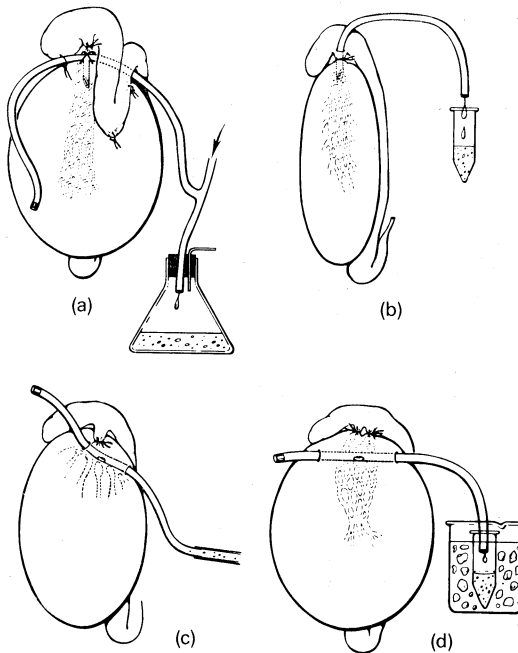


Fig. 1. Collection of rete testis fluid by catheterization. (a) Ram, direct collection through a catheter in the extratesticular rete; the fluid flows down one arm of the catheter to outside the scrotum from where it is collected (Voglmayr *et al.* 1966). (b) Rabbit, collection after prior efferent duct ligation (Cooper and Orgebin-Crist 1975). (c) Rat, collection through a side-hole catheter in the rete testis after prior efferent duct ligation (Tuck *et al.* 1969). (d) Monkey, collection as in (c) (Waites and Einer-Jensen 1974).

Significant advances often depend on new techniques; the collection of fluid from the testis was made possible by cannulation of the rete testis (Fig. 1). The Australian Merino ram was the first mammal from which such fluid was collected (Voglmayr *et al.* 1966); and the ram remains the most successful source of testicular fluid.

Preliminary ligation of the efferent ducts is not needed, as in most other species, and large volumes of fluid can be collected from conscious rams for relatively long periods. Other procedures have helped to define the nature of fluid secretion by the testis, e.g. weight increment after efferent duct ligation and micropuncture, and recently Free and Jaffe (1979) have managed to collect fluid by direct cannulation of a single efferent duct in rats.

The striking differences between the composition of testicular fluid and that of blood plasma or testicular lymph remain the most compelling pieces of evidence for a BTB. For example, flowing seminiferous tubular fluid has about 13 times as much potassium as in blood plasma, although this difference disappears in fluid from Sertoli cell only (SCO) tubules. The protein concentration is less than 5% of that in plasma, but there are several protein fractions in rete testis and tubular fluids which are not seen in serum or testicular lymph (see Setchell *et al.* 1978). The steroid composition of rete testis fluid is also unique, while the daily steroid output through the efferent ducts to the initial part of the epididymis of the bull is formidable (Ganjam and Amann 1976; Table 1).

Table 1. Daily output of steroids in bovine reproductive fluids
Mean values given in ng/24 h; number of samples given in parentheses.
From Ganjam and Amann (1976)

Steroid	Rete testis fluid (10)	Cauda epididymal plasma (10)	Seminal plasma (3)
Testosterone	1770	4.4	9.8
Dehydroepiandrosterone	1180	3.1	3.6
Androstenedione	397	0.6	2.6
Dihydrotestosterone	69	7.8	2.3
5-Androstenediol	224	1.7	2.4
Progesterone	209	3.0	3.7
Androstenediol-3 α	91	1.5	2.0
Androstenediol-3 β	135	2.5	2.8
Oestrogens	0.63	0.01	0.18

Another fruitful approach to defining the BTB is to examine the differential transfer into testicular fluid of labelled compounds when these are infused into blood. Again, the ram has proved useful, but so also has a rat preparation with cannulated rete testis (Tuck *et al.* 1970; Waites *et al.* 1973). Infused steroids exhibit a wide range of transfer from the rapid equilibration of androgens (testosterone, androstenedione, and dehydroepiandrosterone) to the near or total exclusion of corticosteroids and cholesterol (Cooper and Waites 1975). The entry rates of these steroid molecules may be compared to those of other non-steroid compounds. For example, tritiated water, urea and ethanol are rapidly transferred from blood into rete testis fluid, while albumin is excluded and potassium is slowly, but inexorably accumulated (see Waites 1977). Analysis of the rates of transfer of barbiturates and other drugs from the blood into rete testis fluid led Okumura *et al.* (1975) to suggest that the rates of drug penetration obeyed simple diffusion kinetics and depended on their lipid solubility.

These physiological and biochemical studies attracted the attention of morphologists who were unravelling the complex association between germ cells, and they began to link the presence of specialized tight junctions between Sertoli cells with the BTB. They found that lanthanum and other electron-opaque markers could only penetrate from the blood and between the Sertoli cells for a limited distance, where their passage was blocked by unique junctional specializations (see Fawcett 1975). From such studies, Dym and Fawcett (1970) proposed that the seminiferous epithelium is composed of two compartments; the *basal* compartment, containing the spermatogonia and preleptotene spermatocytes, is separated by the specialized junctions from the *adluminal* compartment, containing the other germ cells (Fig. 2). Once formed, the junctional specializations of Sertoli cells seem virtually indestructible, resisting experimental cryptorchidism, hypophysectomy, heat, osmotic shock, a wide range of drugs or the extreme distension following ligation of the efferent ducts.

While invaluable in visualizing the physical barrier between cells and probably quite accurate in predicting where the passage of macromolecules, such as proteins, might be stopped, the markers used in these studies could hardly be considered physiological in respect of the smaller and the lipid-soluble molecules. Nevertheless, such studies focused attention on the Sertoli cell which, although 'spot-welded' together *in vivo*, was shown to be susceptible to separation into homogeneous cultures *in vitro*, thus enabling them to be more directly studied biochemically.

The Sertoli Cell

The development of our knowledge of the Sertoli cell is surely one of the principal advances in male reproductive physiology in recent years (see Means *et al.* 1978, 1980) and some of its functional characteristics are summarized in the following tabulation:

<i>A. Architectural</i>	<i>Review references</i>
1. Cell-cell specialized junctions	Fawcett (1975)
2. FSH and androgen receptors	Means <i>et al.</i> (1978)
3. Cytoskeletal	Franke <i>et al.</i> (1979)
4. Phagocytosis	Fawcett (1975)
<i>B. Secretion of:</i>	
1. Foetal anti-Müllerian hormone	Josso <i>et al.</i> (1977)
2. 'Inhibin' protein	de Jong <i>et al.</i> (1979), Steinberger (1979)
3. Androgen binding protein	Hansson <i>et al.</i> (1976)
4. Fluid of unique composition	Waites (1977)
5. FSH-provoked prepubertal oestrogen synthesis	Dorrington <i>et al.</i> (1978)

Indeed the identification of a specific androgen binding protein (ABP) secreted by the Sertoli cell has been itself a major achievement (see Hansson *et al.* 1976). However, its role would now seem to be in need of re-evaluation because, although found in many species, it is absent, or present in only low amounts in primates, including humans. When present, the amount is related to the level of androgens in the rete testis fluid and it undoubtedly assists androgen transport to the epididymis. However, with the evidence for rapid transfer of androgens from blood across the BTB into rete testis fluid (Cooper and Waites 1975), it is difficult to ascribe a transport role to ABP at this point. It might help to maintain a constant testosterone concentration inside the tubules during the fluctuation in its secretion by Leydig cells provoked

by the episodic release of luteinizing hormone (LH; see Comhaire and Vermeulen 1976; Waites 1977).

It is now agreed that the Sertoli cell is a prime target for follicle stimulating hormone (FSH) and there is good evidence that the receptor sites are located mainly over the basal surface of the cell, i.e. outside the junctional specializations (Orth and Christensen 1977). *In vitro* culture systems of Sertoli cells support the evidence from other experiments that FSH provokes the secretion of ABP. Since Gunsalus *et al.* (1978) have developed a radioimmunoassay for ABP and have found significant levels in the peripheral plasma of male, but not female, rats, it may now be possible to monitor a marker protein of Sertoli cell origin as an experimental tool. FSH provokes DNA synthesis, increases cyclic AMP and activates protein kinase presumably to elicit the other cellular responses (see Means *et al.* 1980).

We have had to realize in recent years that the Sertoli cell may change its function with age. There are two clear examples of this. First, Sertoli cells from the gonads of rats 5–10 days old maintained in monolayer cultures could not synthesize steroids *de novo*, but when stimulated with FSH they could aromatize testosterone to oestradiol-17 β (Dorrington *et al.* 1978). This ability diminished with age and was finally lost in Sertoli cells from 30-day-old rats. The significance of this prepubertal synthesis of oestrogen and its possible significance for Leydig cell function or for negative feedback control of gonadotrophin secretion is not yet clear. Secondly, the Sertoli, or 'supporting' cell in early foetal life secretes a protein which inhibits the development of the Müllerian duct (Josso 1973; Josso *et al.* 1977); although small amounts of anti-Müllerian hormone have been detected in rete testis fluid from adult boars (Josso *et al.* 1979).

A review of Sertoli cell function would not be complete without reference to the search for the hypothetical hormone first postulated by Mottram and Cramer (1923) to be secreted by the 'seminal epithelium' and to be responsible for feedback inhibition of secretion by the anterior pituitary. The substance was named 'inhibin' by McCullagh (1932) and several laboratories in recent years have contributed new knowledge about its source, identity and possible function (see Setchell *et al.* 1977; Hudson *et al.* 1979; Main *et al.* 1979; Sheth *et al.* 1979). Rete testis fluid from rams has been shown to contain proteins which, when isolated and fractionated, are capable of dose-related inhibition of FSH secretion by pituitary cells in culture (Franchimont *et al.* 1978). However, high concentrations of these fractions also depress LH secretion and there are indications that ram rete testis fluid contains proteins which may also depress LH secretion when injected into cryptorchid rams which have raised FSH and LH levels (Cahoreau *et al.* 1979).

Initially, spermatids and other germ cells were proposed as the source of inhibin, but it is now believed to be secreted by the Sertoli cell. The best evidence for this comes from co-culture experiments with Sertoli and pituitary cells in which irrigation of the pituitary cells with media from Sertoli cell cultures gives a dose-dependent and specific depression in FSH secretion (de Jong *et al.* 1979; Steinberger 1979). Like ABP, inhibin must also be secreted into the general circulation either via testicular lymph or directly into spermatic venous blood, because secretion only into rete testis fluid inside the BTB would lead to problems of delivery to the pituitary gland. Indeed, diversion of rete testis fluid out of rams did not lead to an alteration in the concentration of circulating FSH (Walton *et al.* 1978a), indicating that another

route of secretion existed (Fig. 2). There is an urgent need for a reliable assay for inhibin (see Vaze *et al.* 1979).

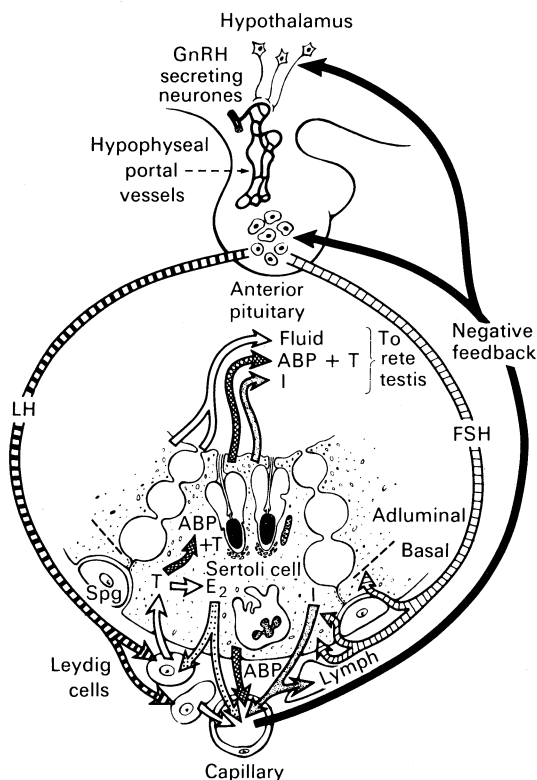


Fig. 2. The Sertoli cell and its possible interrelationships with the Leydig cell and the hypothalamus and anterior pituitary gland. ABP, androgen binding protein; E₂, oestradiol; FSH, follicle stimulating hormone; GnRH, gonadotrophin releasing hormone; I, inhibin; LH, luteinizing hormone; T, testosterone.

Fluid from the testis, therefore, represents a major feature of the functional relationship existing between the testis and epididymis. It transports testicular spermatozoa from the germinal epithelium to the caput epididymis in a fluid containing substrates for sperm survival; the sperm are isolated immunologically by being formed and then released into the fluid inside a barrier to proteins. Secretory proteins with interesting functions are in the fluid; for example, a peptide which inhibits the acrosomal proteinase or acrosin; specific androgen binding proteins; and a protein believed to be of significance for feedback control of FSH secretion. The fluid also transfers an array of steroids into the lumen of the epididymal duct with significance for epididymal secretion.

Puberty

The discussion above has mentioned the control of FSH secretion and the potential of this hormone for the regulation of Sertoli cell function. Among the complex of endocrine changes occurring before puberty in the male, the significance of FSH has

not yet been well described partly because of earlier difficulties in assaying it accurately (see Courot *et al.* 1979; de Kretser 1979; Lincoln 1979). In the past, this would seem to have led to an overemphasis of the importance of the LH-testosterone axis. A major difficulty has been that there are few experimental conditions in the male in which FSH secretion can be increased without simultaneously altering LH secretion. Injections of gonadotrophin releasing hormone (GnRH), experimental cryptorchidism and castration will cause elevated levels of both gonadotrophins, but

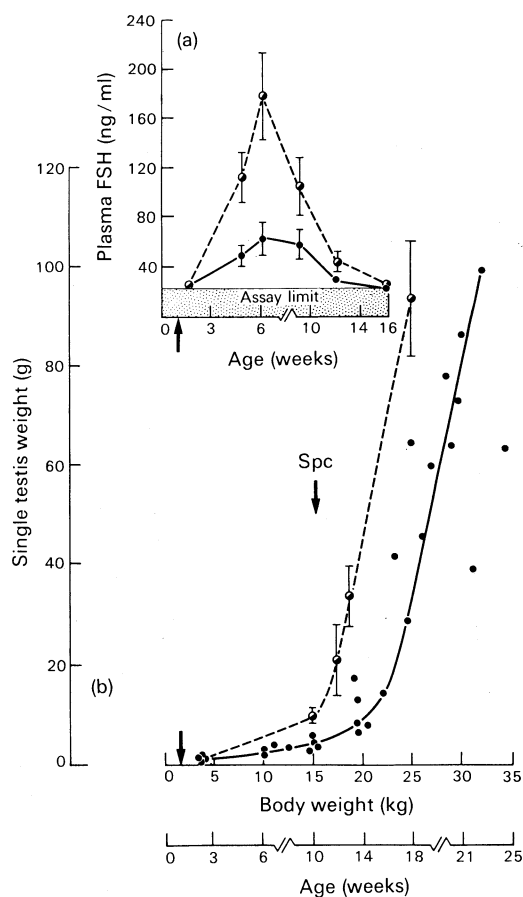


Fig. 3. Effect of removal of one testis from crossbred lambs soon after birth (arrows; hemicastrates, ○) on (a) the subsequent elevation and suppression of plasma follicle stimulating hormone (ng/ml), followed by (b) hypertrophy of the remaining testis compared to controls (●). From Walton *et al.* (1978b, 1980). *Spc*, first appearance of primary spermatocytes.

the removal of one testis soon after birth appears to raise circulating FSH concentrations specifically. Earlier, this surgical manoeuvre had been shown to lead to subsequent hypertrophy of the remaining testis in rats, and, recently Cunningham *et al.* (1978) described significant increases in serum FSH in such rats from 10 to 20 days of age, without any alteration in the intratesticular concentration of testosterone. Independently, the pattern of endocrine changes following early hemicastration in

ram lambs showed a dramatic rise in FSH concentration followed by its subsidence, the whole sequence being completed before the onset of spermatogenesis (Walton *et al.* 1978b, 1980; Fig. 3). The suppression of FSH secretion occurs at a time when the tubules contain only Sertoli cells and gonocytes, and it happens if the gonocytes are damaged by heat. There appears to be no influence of hemicastration on the secretion or onset of pulsatile release of LH or testosterone or on pituitary sensitivity to GnRH injections at 10 weeks of age. We have tentatively concluded that this FSH surge is responsible for the subsequent testicular growth and that, in normal prepubertal ram lambs, it is subject to feedback regulation emanating from the Sertoli cells.

The Epididymis

This is the organ which receives the outflow of fluid from the testis, together with its content of immature spermatozoa, proteins, enzymes, steroids and substrates, e.g. inositol. In the 18th century, William Hunter, by filling human duct systems with mercury, revealed the tortuous ramifications of the human rete, ductuli efferentes, and epididymal duct and, at the same time, taught that 'the whole epididymis (is) one tortuous tube . . . is only an appendage . . . (it) is properly an excretory duct' (Hunter 1762). Yet, over the last 15 years or so, quite simple experiments involving testing the fertilizing ability of sperm removed from various parts of the epididymis have shown that passage through the initial portion leads sperm to acquire the capacity to fertilize on arrival in the more distal portions (see legend to Fig. 4 for references).

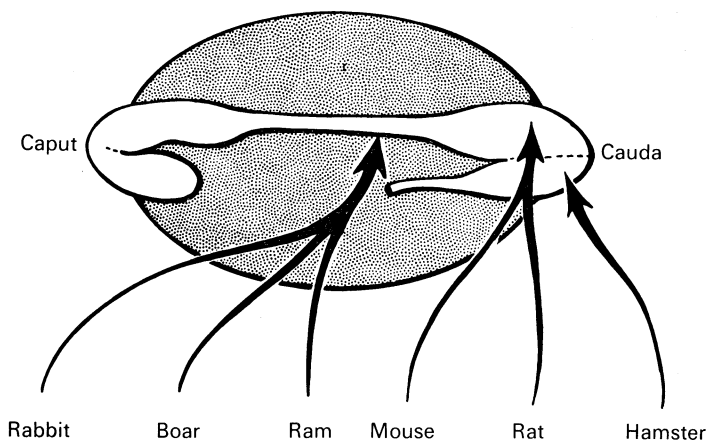


Fig. 4. Approximate regions of the epididymis where more than 50% spermatozoa attain the ability to achieve fertilization. Rabbit, Bedford (1966), Orgebin-Crist (1967); boar, Holtz and Smidt (1976), Hunter *et al.* (1976); ram, Fournier-Delpech *et al.* (1977); mouse, Pavlok (1974); rat, Blandau and Rumery (1964), Dyson and Orgebin-Crist (1973); hamster, Horan and Bedford (1972).

The operation of epididymovasostomy, in which the vas deferens is looped back and anastomosed to the epididymal duct above an obstructive lesion, has yielded evidence in the human also that sperm are more fertile the further they are along the duct (Schmidt *et al.* 1976). The epididymis is now known to have an epithelium with

varying secretory and absorptive properties along its length, with all of its function being heavily dependent on an adequate supply of androgens (see Hamilton 1977).

Reid and Cleland (1957) drew attention to the varying structure of the epididymal epithelium in the rat in which the most numerous cell, called the principal cell (see Hamilton 1975) is now emerging, like the Sertoli cell has, as a polyfunctional structure as indicated in the following tabulation:

<i>Epithelial cell characteristic</i>	<i>Review references</i>
<i>A. Secretion</i>	
1. Proteins	Hamilton (1977)
2. Glycoproteins	
3. Steroids: 5 α -dihydrotestosterone	
4. Glycerophosphocholine	
<i>B. Specific accumulation</i>	
1. Carnitine	Brooks (1979)
2. Metabolite of α -chlorohydrin	Hamilton (1977)
<i>C. Absorption</i>	
1. Particulate material	Hamilton (1975)
2. Proteins	Lea <i>et al.</i> (1978)
3. Fluid and electrolytes	Wong <i>et al.</i> (1978)

Also like the Sertoli cell, reasonably pure preparations of principal cells from the the caput epididymis have been cultured *in vitro* (Killian *et al.* 1976, 1977) and in addition to various secretory functions, these cells contain steroid-5 α reductase enzyme and produce dihydrotestosterone. Although capable of producing its own active androgen from circulating testosterone, the cells of the initial segment of the epididymis need an additional supply derived from the testicular excurrent fluid. Even massive doses of exogenous testosterone cannot recover the atrophy of the initial segment caused by ligation of the efferent ducts (see Fawcett and Hoffer 1979). The caput epididymis probably also receives some androgen reinforcement by exchange in the pampiniform plexus. The evidence for this was derived by infusion of labelled compounds into the testicular venous blood while monitoring their transfer into the ipsilateral tissues (Fig. 5), and also by simultaneous assay of testosterone in testicular venous and arterial blood (Einer-Jensen and Waites 1977; see Free 1977).

Fluid from the lumen of the epididymis has been collected and analysed. Like testicular fluid, it has a unique composition with unexpectedly high concentrations of certain organic compounds and gradients of electrolytes and of water content along the length of the duct (see R. Jones 1978). We are still unclear about the relationship between most of these compounds and sperm maturation, although recently attention has been focused on the secretion of proteins and their accumulation on the sperm membrane (Hamilton 1975). Following the observation by Eliasson and Lindholmer (1973) that immotile human sperm from above an occluded portion of the epididymal duct acquire forward motility when immersed in seminal plasma, forward motility protein (FMP) for bovine sperm was found in cauda epididymal fluid at five times the concentration in seminal plasma (Brandt *et al.* 1978). This protein was more effective on cauda than on caput sperm, and the flagellar activity achieved forward progression when cAMP levels were raised by the simultaneous addition of phosphodiesterase inhibitors (e.g. theophylline—Acott *et al.* 1979). An acidic glycoprotein, possibly secreted by the principal cells in the caput and corpus regions of the rat

epididymis, becomes coated onto sperm during their passage to the cauda region where some is found in the 'clear' cells of the epithelium (Lea *et al.* 1978). Elucidation of the significance of the glycoproteins coating sperm cell surfaces must be one of the more exciting current areas for research.

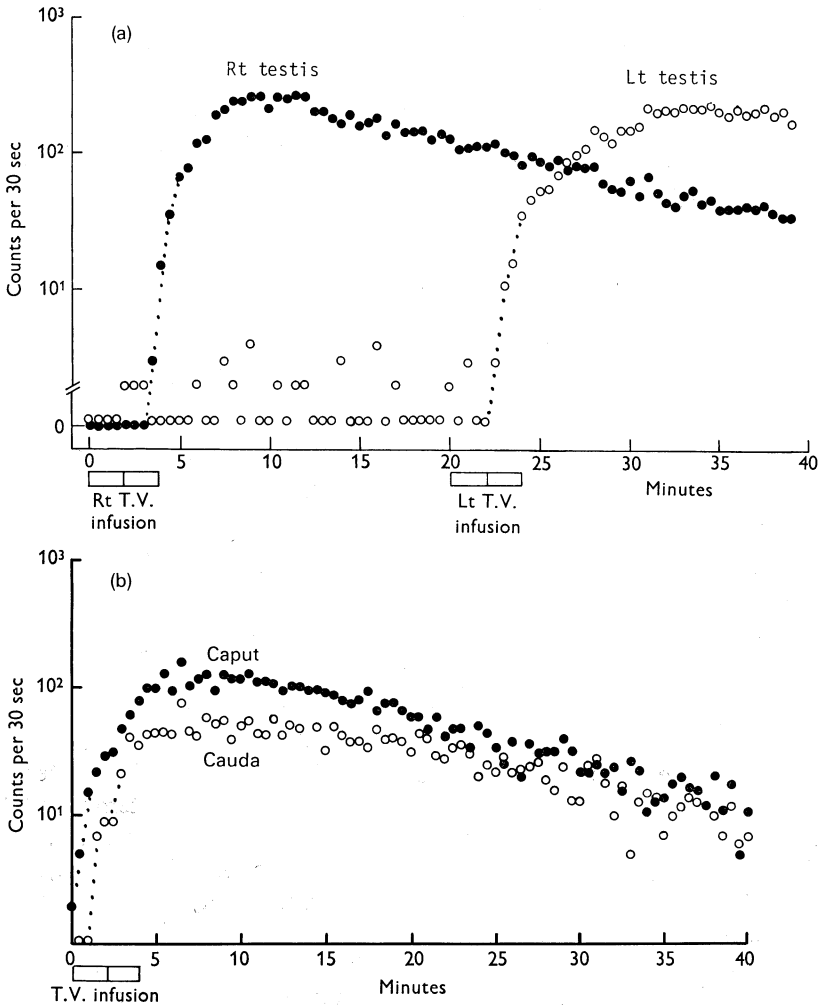


Fig. 5. Transfer of ^{85}K from the testicular veins (T.V.) to the tissue of: (a) ipsilateral testis; (b) ipsilateral caput and cauda epididymis of rhesus monkey (from Einer-Jensen and Waites 1977).

Of the non-protein compounds present in the epididymis, carnitine is of particular interest. Marquis and Fritz (1965) first reported that the rat epididymis contained unusually high concentrations, principally in the luminal fluid (Brooks *et al.* 1974). Carnitine is intimately involved with the oxidation of fatty acids and is formed by the liver, but not by the epididymis. It is concentrated in the epididymal duct by androgen-dependent active transport in the middle segment (Brooks *et al.* 1973) and the sperm themselves accumulate carnitine or its acetylated form, acetylcarnitine,

which could presumably be oxidized by them because there is sufficient oxygen available (see Böhmer and Johansen 1978; Brooks 1979). At Reading, we have used the technique described by Wong and Yeung (1978) to perfuse the lumen of the corpus and cauda epididymis, varying the composition of the perfusate while infusing labelled DL-carnitine into the circulation. As expected from the results of Brooks *et al.* (1973), the uptake of radioactivity is greater in the corpus than in the more distal regions when expressed per unit length of duct and the radioactivity is principally associated with the L-isomer of carnitine. The uptake of carnitine is stimulated by the presence of choline and betaine in the lumen, but unaffected by the

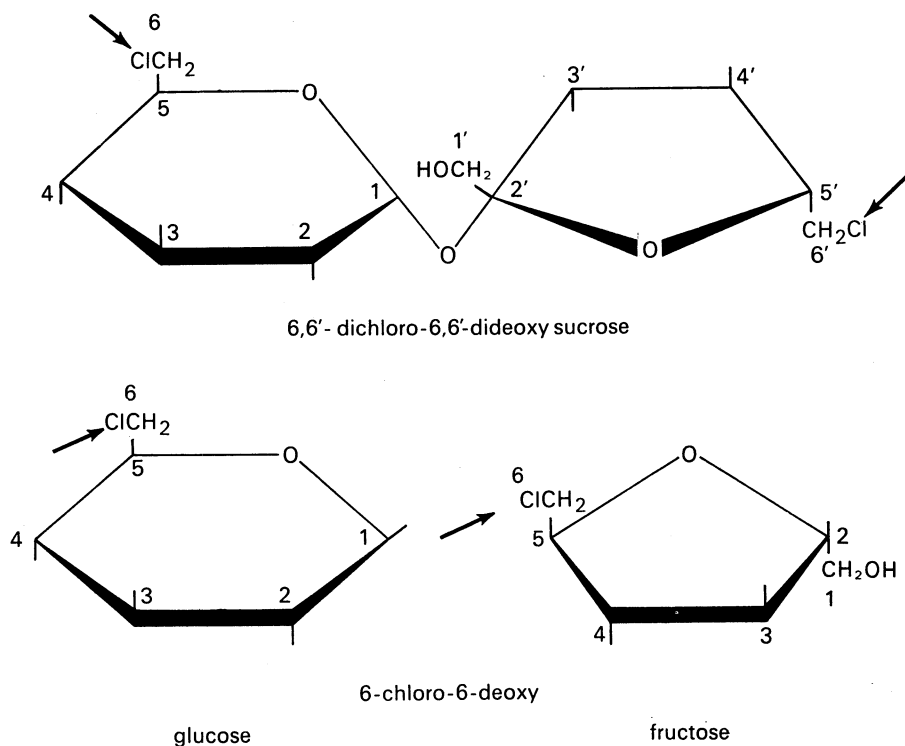


Fig. 6. Structure of three 6-chloro-6-deoxy sugars with contraceptive action on male rats (Ford and Waites 1978a, 1978b; arrows indicate substitution positions of chlorine).

presence of carnitine itself or of butyrobetaine, a precursor of carnitine (Yeung *et al.* 1980). We speculate that a carnitine pump, probably in the principal cells of the middle segment of the epididymis, could exchange choline for L-carnitine to augment its transport. We do not know if rat luminal fluid contains significant levels of choline or if this mechanism is linked to the almost equimolar concentrations of glycerophosphocholine (41 mM versus 63 mM carnitine; see Hamilton 1977). Recent evidence suggests that accumulation of carnitine in the rat epididymal duct may be directly associated with the acquisition of sperm flagellar movement (Hinton *et al.* 1979).

Whatever its physiological function, any mechanism such as a carnitine pump, which is specific to the epididymis, is of immediate interest in the context of the

epididymis as a target for compounds with a contraceptive action which might arise from agents acting in the pre-meiotic stages of spermatogenesis. Metabolic interference of the epididymis or of epididymal sperm would have the advantages of being rapidly acting and rapidly reversible and would minimize the danger of genetic effects.

The chlorine-substituted glycerol, α -chlorohydrin (3-chloropropan-1,2-diol) given in low daily doses to male rats, sheep, pigs, hamsters and guinea pigs causes reversible infertility by an action in the epididymis (see Jackson 1975). Spermatozoa from treated rams or rats had a reduced capacity to metabolize glucose or fructose and a similar effect was observed when normal spermatozoa were incubated with α -chlorohydrin *in vitro* (see A. R. Jones 1978; Ford *et al.* 1979). The reduced glycolytic flux in spermatozoa was caused by inhibition of glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12) (Mohri *et al.* 1975). The α -chlorohydrin was toxic in rhesus monkeys at the doses needed to produce an antifertility effect and was never tested on humans.

However, subsequent research showed that a number of hexoses and sucroses with chlorine substitution, specifically at the 6 and/or 6' position (Fig. 6), are reversible antifertility compounds in the male rat (Ford and Waites 1978*a*, 1978*b*), and 6-chloro-6-deoxyglucose is so in the marmoset monkey (Hearn 1978). As with α -chlorohydrin, the action appears to be by depression of sperm glycolysis, associated with reduced adenine nucleotide content and severely reduced sperm motility. Although these compounds are not without their toxicity problems also, they have a lower acute toxicity than α -chlorohydrin and offer a greater scope for structural modification.

Conclusion

This account illustrates some of the developments in male reproductive physiology in the last decade and has pointed the way towards further advances. However, a new perspective has already opened, that of defining function in terms of cell biology, including the interactions between the cells of the testis and their responses to external stimuli. This will undoubtedly require new techniques, some of which may already exist in neighbouring fields of study. Their application should help us to a better understanding in such challenging areas as: differentiation of the foetal testis; the suppression of meiosis before the onset of spermatogenesis; the initiation of puberty; and all the many factors involved in sperm production and their maturation in the epididymis.

Acknowledgments

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