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Epididymal Functions and their Hormonal Regulation*

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Abstract

The epididymis is a complex organ which maintains a specific intraluminal environment thought to be important for effecting sperm maturation in proximal regions and sperm storage in distal regions of the duct. The composition of the internal milieu is achieved both by transport between blood and lumen (and vice versa) and by synthesis and secretion into the lumen. Several low-molecular weight organic molecules achieve high concentration in the epididymal lumen, but their functions in the events of sperm maturation and storage still remain unclear. Metabolic processes occurring within epididymal tissue and the absorptive and secretory activity of the epididymal epithelium are regulated by androgens. The synthesis of some, but not all, secretory proteins is also androgen-dependent. In addition to androgens, other hormones and local testicular factors may influence epididymal function. There is now increasing evidence that epididymal-specific and androgen-dependent secretory proteins play a fundamental role in modifying the surface characteristics of sperm in preparation for the events of fertilization.

Introduction

The mammalian epididymis is one of the longest single ducts in the body with an estimated length of 2, 6 and 50 m in the rat, human and bull respectively. It is derived from the primitive mesonephric duct (Wolffian duct) of the embryo, and in the adult it retains some of the characteristics of a nephric duct, particularly with respect to absorption of sodium and water and secretion of potassium. However, its primary function is to lead sperm from the testis to the urethra and in so doing it allows post-meiotic gamete development to continue with the result that the immature testicular sperm progressively acquire the potential to fertilize eggs. Sperm maturation takes place principally within the caput and corpus regions of the epididymal duct whereas the caudal region serves more as a storage area where mature sperm are maintained in a quiescent condition prior to ejaculation.

The phenomenon of sperm maturation within the epididymal duct has been recognized from the early 20th century (e.g. Tournade 1913; Benoit 1926; Young 1929). The preponderance of early studies of the epididymis were concerned with descriptive anatomy but within the last 20 years there has been an upsurge of interest in the physiology and biochemistry of the organ. The initial stimulus for this renewed interest was probably brought about by a desire to find a reversible male contraceptive which would be effective at a post-testicular site.

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This article will examine some of the recent developments in our understanding of epididymal functions and their hormonal regulation. Space limitations dictate that the coverage be highly selective and some topics will be treated only in summary, but in these instances an attempt will be made to direct the reader to more comprehensive reviews. The article will begin by outlining the changes associated with sperm maturation, followed by an examination of the chemical composition of the luminal environment in which these maturational changes take place. Current concepts concerning the mechanism of action of androgens in the epididymis will then be presented followed by some better-studied examples of androgenic regulation of particular epididymal functions.

Sperm Maturation in the Epididymis

Testicular sperm are both immotile and infertile. The capacity for motility develops as sperm pass through the proximal regions of the epididymal duct. In biochemical terms, the acquisition of the capacity for motility may be unrelated to the development of fertilizing capacity. Indeed, capacity for motility develops much earlier in epididymal transit than does fertilizing capacity (Fig. 1). Nevertheless, motility is unquestionably essential for fertility.

The term 'capacity for motility' has been chosen carefully since it is generally considered that sperm remain immotile within the epididymis and display motility only when removed from the epididymal environment. The pattern of motility alters as sperm move distally through the epididymal duct. At first sperm display only vibratory movements, then they begin to swim in tight circles, and finally they become capable of forward progression (e.g. Gaddum 1968).

The mechanism by which sperm acquire the capacity for full motility has been under active investigation (Hoskins *et al.* 1978). Two contributing factors seem important for full progressive motility. The first requirement is for raised levels of intracellular cyclic AMP. This leads to a vibratory type of movement. Conversion of this vibratory movement to forward progression has been thought to require a specific protein factor of epididymal origin termed 'forward motility protein'. More recent evidence indicates that forward progression can be largely attributed to a non-specific effect of protein in preventing sperm from sticking to the glass surfaces of the viewing chamber (Stephens *et al.* 1981). Whether the epididymis does produce specific motility-promoting proteins has therefore become an open question.

Assessment of the development of fertilizing capacity as sperm pass through the epididymis has been carried out by recovering sperm from sliced epididymal tissue, inseminating them into the uterus or oviducts of females and scoring eggs fertilized or number of animals becoming pregnant (see Orgebin-Crist *et al.* 1975). In some instances penetration of the zona-free hamster egg *in vitro* has been used to assess fertility (Hinrichsen and Blaquier 1980). In all species studied, progressive acquisition of fertilizing potential at more distal sampling sites has been demonstrated (e.g. Fig. 1).

Numerous other changes occur to sperm during epididymal transit. These include modification to sperm dimensions (Bedford 1963*a*), migration and loss of the cytoplasmic droplet (Redenz 1924), increased susceptibility to cold shock (Quinn and and White 1967), increased surface negative charge (Bedford 1963*b*), reduction in whole-cell isoelectric point (Hammerstedt *et al.* 1979), increased disulfide cross-linking (Bedford *et al.* 1973), changes in lipids (Scott *et al.* 1967), proteins (Voglmayr

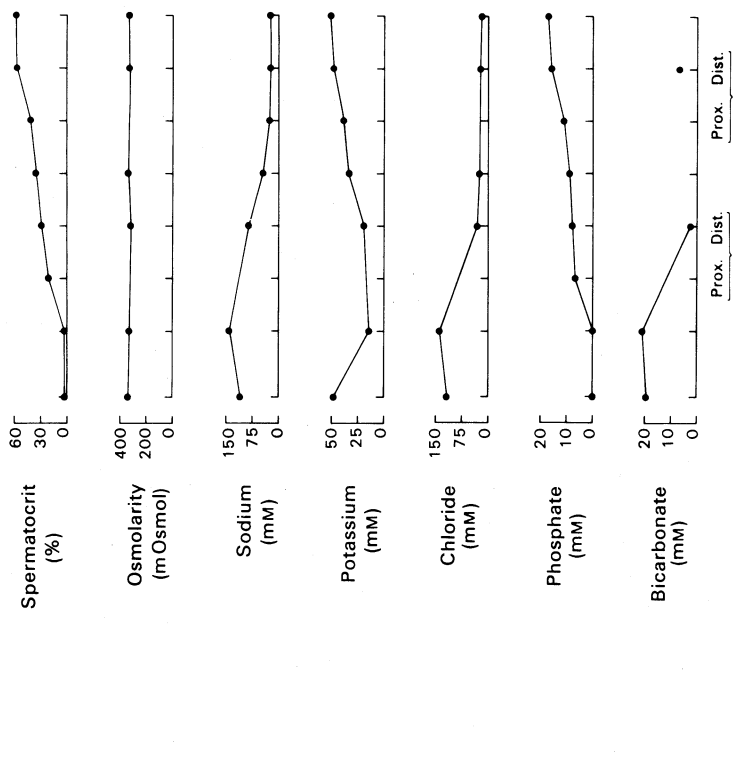

Fig. 1. Changes in luminal fluid composition and sperm characteristics during passage through the testicular excurrent duct system of the rat. Data was obtained from the following sources: spermatocrit, phosphate, glycerylphosphorylcholine (GPC), phosphorylcholine (PC) (Hinton and Setchell 1980); osmolarity, chloride, bicarbonate (Tuck *et al.* 1970; Levine and Marsh 1971); sodium, potassium (Tuck *et al.* 1970; Levine and Marsh 1971; Back *et al.* 1974; Turner *et al.* 1977; Jones 1978; Pholpramool and Sornpaisarn 1980); pH (Levine and Marsh 1971; Levine and Kelly 1978); carnitine (Hinton *et al.* 1979*b*); inositol (Hinton *et al.* 1980); motility (area change frequency; Hinton *et al.* 1979*a*); fertility (Dyson and Orgebin-Crist 1973). A mean value is plotted where data were available from several sources.

et al. 1980) and antigenic composition (Killian and Amann 1973), modified enzymatic activity (Purvis *et al.* 1982), and altered binding of lectins to the cell surface (Nicolson *et al.* 1977). Which, if any, of these changes are of fundamental importance to the process of sperm–egg recognition and fertilization remains to be established.

At present, the most attractive hypothesis is that alteration of the sperm surface by the addition or subtraction of proteins, or by modification of existing proteins, leads to the exposure of the correct surface receptors to allow species-specific recognition between sperm and egg which is integral to the process of fertilization. It has been clearly established that the epididymis does produce tissue- and species-specific proteins (e.g. Garberi *et al.* 1979), and that some of these become associated with the sperm surface (Lea *et al.* 1978). Further support for the importance of sperm receptor modification during epididymal transit as a prerequisite for egg recognition has been provided by the recent demonstration of an increased capacity for zona binding during epididymal transit (Saling 1982). Moreover, treatment of sperm with antibodies against surface proteins can adversely affect fertilization (Moore 1981*a*).

The process of sperm maturation within the epididymis is dependent on the normal provision of androgens (Orgebin-Crist *et al.* 1975). The androgen dependence of sperm maturation can be most clearly demonstrated in organ culture systems. Thus sperm maturation can be induced in isolated epididymal tubules from the corpus epididymidis *in vitro* by addition of dihydrotestosterone to the culture medium (Orgebin-Crist *et al.* 1976). The effect of dihydrotestosterone can be blocked by the antiandrogen cyproterone acetate and by inhibitors of RNA and protein synthesis (Orgebin-Crist and Jahad 1978). Control experiments established that the inhibitors did not have a direct effect on the sperm themselves and this led to the conclusion that androgens promote sperm maturation by regulating protein synthesis within the epididymal tissue itself. Androgens are also required to maintain the fertility of mature sperm stored within the cauda epididymidis (Orgebin-Crist *et al.* 1975).

Composition of Epididymal Luminal Fluid

The epididymis receives its luminal fluid in the form of rete testis fluid which passes from the testis to the epididymis through the efferent ducts. The composition of the rete testis fluid (see Setchell 1970) is altered substantially by the absorptive and secretory activity of the epididymal epithelium. Techniques for *in vivo* cannulation of the rete testis (Voglmayr *et al.* 1967; Tuck *et al.* 1970; Free and Jaffe 1979) coupled with cannulation of the ductus deferens (Amann *et al.* 1963; Volgmayr *et al.* 1977) have allowed a direct comparison to be made between the composition of fluid entering and leaving the epididymis. Another approach has been the use of micropuncture (Levine and Marsh 1971; Howards *et al.* 1975; Hinton *et al.* 1979*a*, 1979*b*). This technique has enabled samples to be taken at many points along the epididymis and has thereby provided details of regional changes in fluid composition. A summary of such changes in luminal fluid from the rat epididymis is shown in Fig. 1.

Several important trends can be seen from Fig. 1. The spermatocrit steadily increases at more distal sampling points, indicating a progressive removal of fluid. Water removal is probably a consequence of active transport of sodium chloride out of the epididymis (Wong and Yeung 1978). Sodium ions are replaced in part by potassium ions. However, osmotic pressure is maintained principally by the addition of certain organic molecules, rather than inorganic ions. These organic molecules

include glycerylphosphorylcholine (GPC), phosphorylcholine, carnitine and inositol. These compounds can attain extraordinary concentrations of up to 60 mM and the mechanism of their accumulation has been an active area of investigation.

GPC is a synthetic product of the epididymis itself, with the caput being the most active region in this regard. Some debate has surrounded the actual pathway of GPC synthesis (see Brooks 1979*a*). Some workers have favoured direct synthesis from phosphate and choline without their prior incorporation into lecithin, whereas others have favoured lecithin as the immediate precursor. More recent studies have supported the latter suggestion with good evidence that blood lipoproteins are the ultimate source of epididymal lecithin (Hammerstedt and Rowan 1982). Phosphorylcholine probably arises as a hydrolytic product of GPC (Bjerve and Reitan 1978).

In contrast to GPC, carnitine is not synthesized by the epididymis, but is actively transported from the bloodstream (Brooks *et al.* 1973) to establish a final concentration gradient of over 2000:1 (Brooks 1980). Whereas GPC is added to epididymal luminal fluid principally in the caput, carnitine is added in the distal caput, corpus and proximal cauda (Brooks *et al.* 1973).

Although inositol is present in epididymal fluid of all species studied, very high concentrations are a particular feature of the rat, hamster and monkey (Hinton *et al.* 1980). Some inositol enters the epididymis by way of the rete testis fluid (Tuck *et al.* 1970; Hinton *et al.* 1980). Additional inositol may arise by transport from the bloodstream (Lewin *et al.* 1979; Cooper 1982) and by direct epididymal synthesis (Robinson and Fritz 1979; Hasegawa and Eisenberg 1981).

Reducing sugar is generally absent from epididymal fluid, but this may be more apparent than real since any sugar which does enter the epididymis may be used up immediately by the spermatozoa. In fact, in the absence of spermatozoa, glucose can be shown to accumulate in the epididymal lumen (Cooper and Waites 1979; Cooper 1982).

The functions of many of the intraluminal constituents described above remain problematic. Spermatozoa probably utilize any glucose, lactic acid, glycerol or fatty acids which enter the epididymal lumen (Brooks 1979*a*), but they are unable to utilize inositol or GPC as an energy substrate (Dawson *et al.* 1957; Voglmayr and White 1971; Storey and Keyhani 1974). It is possible that some of these substances may play a role in suppressing sperm metabolism and thereby contribute to the longevity of sperm within the epididymal environment. There is some evidence that high concentrations of GPC and carnitine can act in this manner (Brooks 1980; Hinton *et al.* 1981; Turner and Giles 1981). Carnitine may play a dual role in sperm function. Low concentrations of carnitine promote the ability of immature sperm to become motile, whereas high concentrations inhibit the motility of activated sperm (Hinton *et al.* 1981).

Mechanism of Androgen Action in the Epididymis

Steroid hormones reach the epididymis by way of the rete testis fluid and the bloodstream. Testicular lymphatic drainage has been suggested as an additional route, but the extent of this contribution is not known.

There is some evidence that the epididymis can itself synthesize some androgen. The *de novo* synthesis *in vitro* of cholesterol and testosterone from acetate has been demonstrated in all regions of the rat, ram and rabbit epididymis (Hamilton *et al.*

1969; Hamilton and Fawcett 1970; Hamilton 1972). However, in the rabbit epididymis, Frankel and Eik-Nes (1970) were unable to demonstrate conversion of acetate or cholesterol to pregnenolone, testosterone or any of the intervening metabolites whereas conversion of pregnenolone, 17α-hydroxyprogesterone and dehydroepiandrosterone to subsequent products in the steroid metabolic pathway was readily demonstrated. Similar conversion of C_{21} precursors to androgens has been described by Inano *et al.* (1969).

Testosterone is the principal androgen in rete testis fluid (e.g. Vreeburg 1975). Its concentration (approximately 30 ng/ml) is similar to that of the testicular 'androgen-binding protein' which suggests that this protein acts as a transport molecule to carry testosterone from the testis to the epididymis.

Table 1. Characteristics of epididymal androgen receptor and androgen-binding protein

Based on data taken from Hansson *et al.* 1976; Wilson and French 1976; Musto *et al.* 1980; Feldman *et al.* 1981; Tindall *et al.* 1981

Parameter	Epididymal cytoplasmic receptor		Androgen-binding protein
Sedimentation coefficient	4·5–5 S	8–9 S	4·6 S
Molecular weight	117 000	280 000–365 000	85 000
Stokes radius	5·8 nm	8·5–10·6 nm	4·7 nm
Frictional ratio	1·8	1·9–2·4	1·63
K_D for dihydrotestosterone	2×10^{-10} M		$0{\cdot}6 \times 10^{-10}$ M
Dissociation rate of bound androgen ($t_{1/2}$ at 0°C)	40 h		0·1 h
Competitive binding			
(i) Cyproterone acetate	+		−
(ii) Oestradiol-17β	+		−
Stability			
(i) Heating at 50°C, 30 min	−		+
(ii) *p*-Chloromercuriphenylsulfonate	−		+
(iii) Charcoal	−		+

The characteristics of rat androgen-binding protein are shown in Table 1. It has been purified in two laboratories (Musto *et al.* 1980; Feldman *et al.* 1981) and shown to be an acidic glycoprotein of molecular weight 85 000 with a high binding affinity for testosterone and dihydrotestosterone and consisting of subunits of molecular weights of approximately 45 000 and 41 000. Rabbit androgen-binding protein has also been purified (Cheng and Musto 1982). Other species which have an androgen-binding protein include sheep and cattle, but not pigs (Hansson *et al.* 1975). The presence of androgen-binding protein in humans as distinct from the plasma testosterone-binding globulin is still uncertain.

There is a marked change in the androgenic constituents of luminal fluid after it enters the epididymis. Dihydrotestosterone rather than testosterone now becomes the predominant androgen (Vreeburg 1975). This conversion is undoubtedly brought about by 5α-reductase located within the epididymal epithelium. Testosterone arriving at the epididymis by way of the bloodstream would also undergo conversion to dihydrotestosterone. 5α-Reductase is particularly active in the proximal regions of the epididymis (Robaire *et al.* 1981).

The principal metabolites of 5α-dihydrotestosterone are the androstanediols (5α-androstan-3α,17β-diol and 5α-androstan-3β,17β-diol). These conversions are brought about by the cytoplasmic enzymes 3α- and 3β-hydroxysteroid dehydrogenase. In contrast with 5α-reductase, the hydroxysteroid dehydrogenases catalyse reversible reactions and thus androgenic effects of androstanediols are probably due to their conversion to dihydrotestosterone.

Androgen action is now believed to be mediated by specific cytoplasmic receptors (Mainwaring 1977). The androgen receptor has characteristics quite distinct from the testicular androgen-binding protein and these are summarized in Table 1. All target tissues within the one animal species probably share the same receptor (Wilson and French 1979). Complete purification of the receptor from bovine seminal vesicles has recently been reported (Chang *et al.* 1982). Upon binding dihydrotestosterone (or testosterone), the receptor moves to the nucleus where it is presumed to interact with chromatin to induce specific gene transcription; this, in turn, leads to the synthesis of specific protein products which are the manifestation of the 'androgen response'. There is increasing evidence from other systems that 'hormone response' is not restricted to an effect on gene transcription (Darnell 1982). Thus parameters such as processing of the primary gene transcript, mRNA stability and mRNA translation efficiency may also be under hormonal control. However, at present there is little evidence for or against these possibilities in the epididymis.

The epididymis may respond to other hormones besides androgens. For instance there is good evidence that the epididymis contains high-affinity, saturable, binding proteins for oestrogens (e.g. Beurden-Lamers *et al.* 1974; Danzo *et al.* 1981), and a number of studies have demonstrated the presence of prolactin-binding sites (e.g. Orgebin-Crist and Djiane 1979). Moreover, it is also known that proximal regions of the epididymis are regulated by some local factor(s) of testicular origin, distinct from androgen (Fawcett and Hoffer 1979; Robaire *et al.* 1981).

Androgenic Effects on Epididymal Processes

Androgens affect a wide range of epididymal processes. Some of these processes will undoubtedly play an important role in the events of sperm maturation described above. In the following discussion a brief account will be given of some of the androgen-regulated processes which have been studied in more detail.

Intermediary Metabolism

Androgens exert a profound influence on intermediary metabolism in the epididymis. The most notable effect is a switch from complete dependence on carbohydrate as the metabolic fuel in the absence of androgens to a substantial dependence on lipids in the presence of androgens (Brooks 1978). The oxidation of lipids in the androgen-supported state is reflected in the formation of GPC as a metabolic by-product of lecithin (Brooks 1979*a*). GPC accumulates in an androgen-dependent fashion in the epididymal luminal fluid.

The androgen-stimulated change from carbohydrate to lipid oxidation is brought about by induction of lipid oxidation rather than depression of glycolysis. In fact glycolytic rate remains remarkably constant in different endocrine states, despite the fact that androgens specifically stimulate the activity of key enzymes of the glycolytic sequence which are known to perform a regulatory role in other tissues (Brooks 1981*a*).

Resolution of this apparent paradox has been provided by evidence that the rate of flux through the glycolytic pathway is regulated by the very first step of the sequence, namely by the rate at which the substrate, glucose, gains entry to the epididymal cells (Brooks 1979*b*).

Transport Mechanisms

The composition of epididymal luminal fluid is substantially different from blood plasma. This difference is achieved partly by synthesis and secretion of specific epididymal products (e.g. GPC) and also by specific transport systems which move substances from the blood to the epididymal lumen and from the epididymal lumen to the blood.

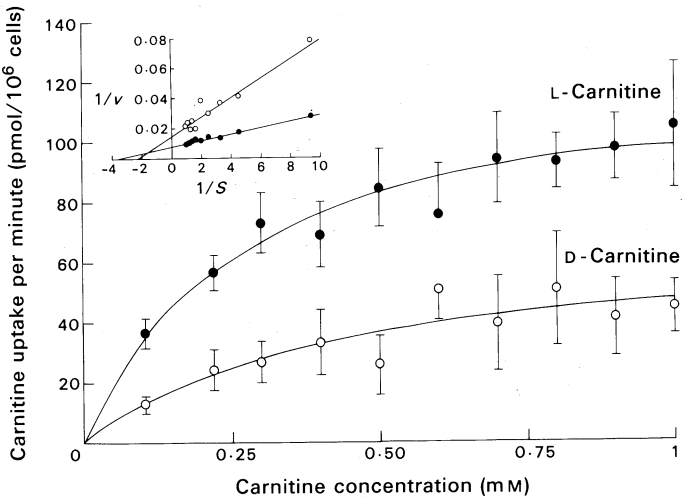

Fig. 2. Kinetics of carnitine uptake by isolated rat epididymal cells. Uptake studies were performed as described by James *et al.* (1981), except that a preparation of cells more highly enriched in epithelial cell types was used. The results are the mean±s.e.m. of five determinations. Kinetic constants for L- and D-carnitine, respectively, were: K_m (mM) = 0·28±0·06 and 0·43±0·22, V_{max} (pmol per minute per 10^6 cells) = 128±9 and 68±15 (L. Chang, A. M. Snoswell and D. E. Brooks, unpublished results).

One of the more obvious pumping mechanisms involves the removal of sodium ions from the epididymal lumen. The resorption process is energy-dependent as demonstrated by inhibition with 2,4-dinitrophenol (Wong and Yeung 1977). Transport rate is also dependent on the intraluminal concentration of sodium and appears to be partly coupled to transport of potassium in the reverse direction (Wong and Yeung 1978). Experiments with amiloride and ouabain (inhibitors of passive sodium transport and Na^+–K^+-activated ATPase, respectively) suggest that sodium is transported passively at the luminal surface and actively at the serosal surface of the epithelium (Wong and Yeung 1977, 1978). Movement of these ions is hormone-dependent because transport is drastically reduced by either castration or adrenalectomy (Au *et al.* 1978; Wong and Yeung 1978). Ion transport can be fully restored in castrated animals by the administration of testosterone propionate (Wong and Yeung 1978) but only partial restoration (40%) is achieved by aldosterone in

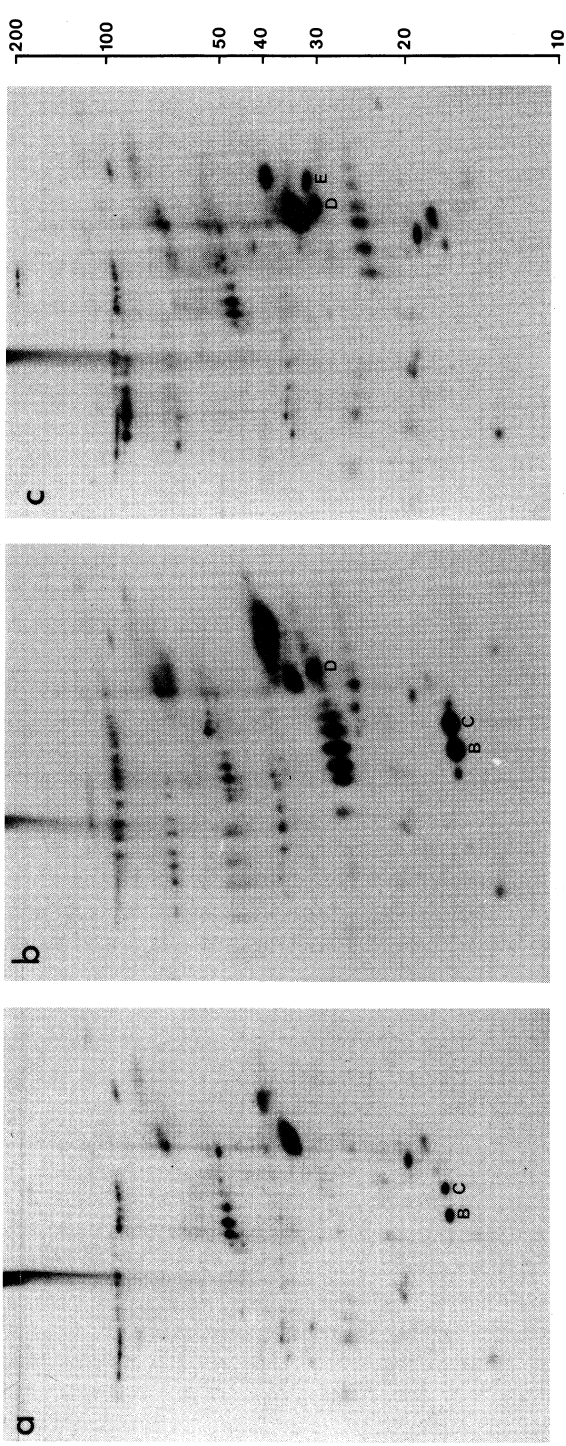

Fig. 3. Secretion of radioactive proteins by initial segments (*a*), caput (*b*) and cauda (*c*) of the epididymis when incubated with radioactive methionine *in vitro*. Details of incubation procedures can be found in Brooks (1981*b*). Isoelectric focusing was used in the first dimension (left to right) and SDS-polyacrylamide gel electrophoresis in the second dimension (top to bottom). $10^{-3} \times$ Molecular weight (M_r) is shown by the scale at the side of the gel. The radioactive secretory proteins were detected by fluorography. The position of previously characterized and androgen-dependent pre-albumin proteins (B, C, D, and E; Brooks and Higgins 1980) is indicated (D. E. Brooks, unpublished results).

adrenalectomized rats (Au *et al.* 1978). The reason for the incomplete restoration by aldosterone may reflect insufficient dosage or length of administration.

Another androgen-dependent transport system which has received considerable attention is that associated with the accumulation of carnitine from the bloodstream. It has been proposed that two carnitine transport pumps are operative: an inwardly directed pump in the basolateral membrane of the epithelial cells, and an outwardly directed pump in the apical membrane (Brooks 1980; Yeung *et al.* 1980). The activity of the inwardly directed pump has been studied with preparations of isolated epididymal cells and androgen dependence of the pump has been confirmed (James *et al.* 1981). More recent results in our laboratory, using isolated cells enriched in epithelial cell-types by isopycnic centrifugation in Percoll gradients, have demonstrated a saturable uptake process which transports the L-isomer of carnitine at about twice the rate of the D-isomer, but which shows no significant difference in affinity (K_m) between the two isomers (Fig. 2). We have also studied the rate of efflux from cells pre-loaded with carnitine as a measure of the outwardly directed apical pump. D- and L-Carnitine are both released at a linear rate with a half-time of 100 min. Efflux rate was not altered by reducing external sodium or including choline which does not support the proposal that release is effected by exchange with these ions (Yeung *et al.* 1980). However, release can be stimulated by adding 60 mM L-carnitine to the external medium suggesting the possibility of a carnitine–carnitine exchange.

Protein Synthesis and Secretion

Androgens influence the overall rate of protein synthesis in the epididymis (Brooks and Higgins 1980; Jones *et al.* 1980*b*), but not to the extent observed in other androgen-dependent male accessory glands such as the prostate and seminal vesicles. When calculated on the basis of equal wet weight, androgens promote at most a two-fold increase in synthesis rate (Brooks and Higgins 1980; Higgins *et al.* 1981) and the effect is non-selective in that nearly all proteins are affected to the same degree. It has been suggested that the involution of the gland after castration may reflect androgen regulation of protein degradation more than androgen regulation of protein synthesis (Higgins *et al.* 1981).

Examination of secreted radioactive proteins as distinct from the radioactive cytosolic proteins synthesized during *in vitro* incubations has revealed two important features. First, the epididymis displays marked regionality in the types of proteins which it synthesizes for export (Fig. 3). Second, androgens exert a selective influence on the profile of radioactive secretory proteins. This is illustrated for the caput region of the epididymis (Fig. 4) where it can be seen that androgens regulate the synthesis and secretion of only a limited number of proteins, such as those labelled B, C and D. Apart from the rat, androgen-dependent proteins have now been demonstrated in the epididymis of several species (e.g. rabbit: Jones *et al.* 1981*b*; Moore 1981*b*; hamster: Moore 1981*b*; González Echeverría *et al.* 1982; ram: Jones *et al.* 1982).

Interest in the nature of epididymal secretory proteins and in the control of the secretory process has been aroused by the increasing evidence that these proteins interact with sperm to contribute to the process of sperm maturation in the epididymis (see above). The presence of proteins of epididymal origin on sperm has been demonstrated by direct extraction (Cameo and Blaquier 1976; Brooks and Higgins 1980; Jones *et al.* 1980*a*; González Echeverría *et al.* 1982), immunological techniques

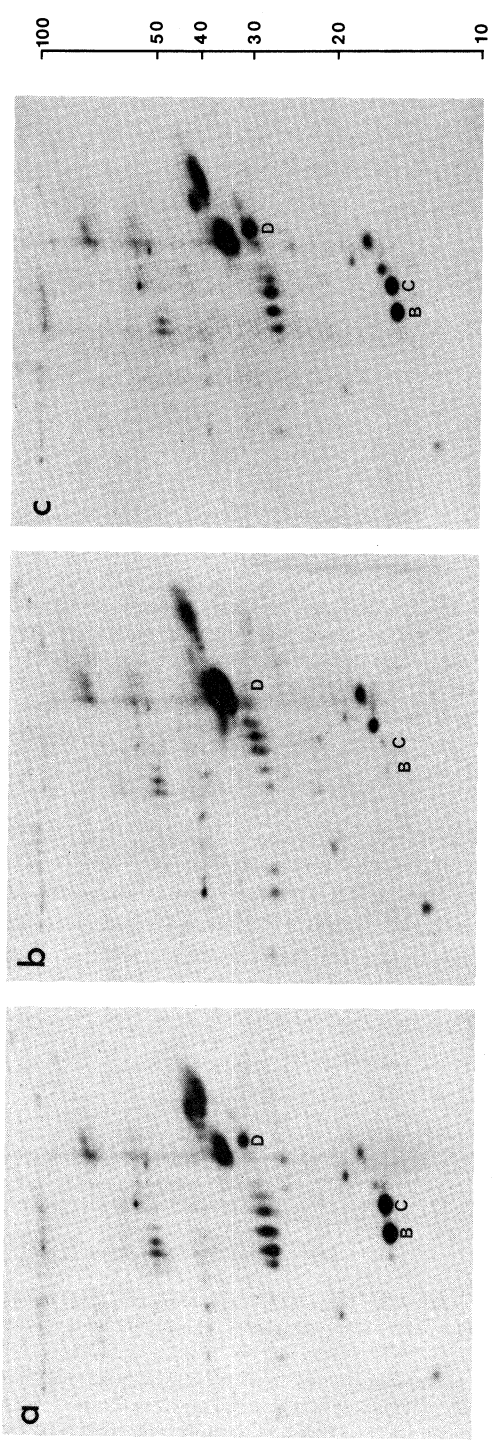

Fig. 4. Effect of androgen status on secretion of radioactive proteins by the caput epididymidis of the rat following incubation with radioactive methionine *in vitro*. Details of incubation procedures and two-dimensional gel electrophoresis are described in the caption to Fig. 3. Scale at right is as for Fig. 3. Tissue was from normal animals (*a*), animals castrated for 3 weeks (*b*), or animals castrated for 3 weeks followed by treatment for 2 weeks with daily injections of 1 mg testosterone propionate (*c*). The position of androgen-dependent proteins B, C, and D is indicated. Because equal amounts, estimated as counts per minute of radioactive protein, were loaded onto each gel, the disappearance of proteins B, C and D after castration has meant that the relative intensity of some proteins has increased (D. E. Brooks, unpublished results).

(Killian and Amann 1973; Lea *et al.* 1978; Kohane *et al.* 1980; Bayard *et al.* 1981; Dravland and Joshi 1981; Moore 1981*a*), and radiolabelling of the sperm surface (Voglmayr *et al.* 1980, 1982; Jones *et al.* 1981*a*).

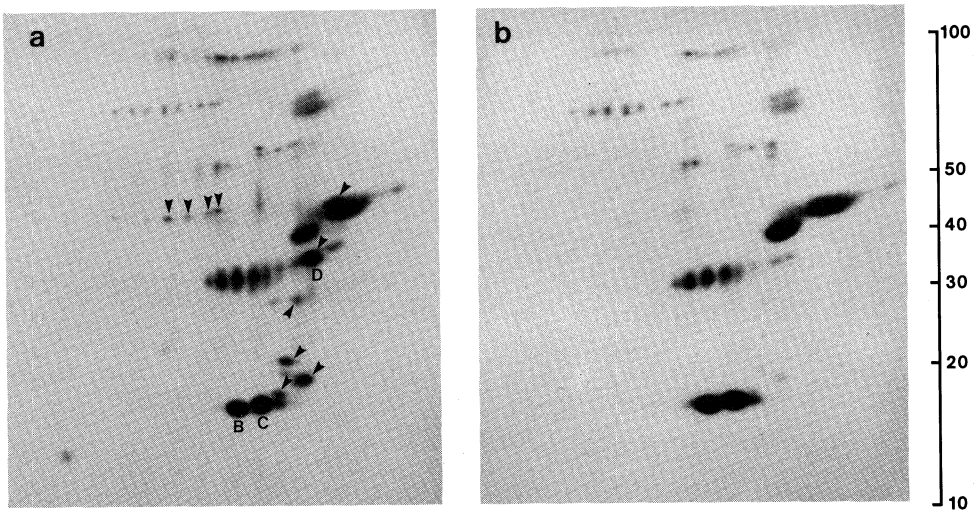

Fig. 5. Selective binding of radioactive epididymal secretory proteins to testicular spermatozoa. Radioactive secretory proteins formed by the caput epididymidis of the rat were incubated with testicular spermatozoa for 1 h. The sperm were then washed, extracted and analysed by two-dimensional gel electrophoresis as described in Fig. 3. Scale at right is as for Fig. 3. (*a*) Radioactive secretory proteins prior to sperm-binding studies; (*b*) radioactive proteins retained by testicular spermatozoa. Many proteins were bound by the spermatozoa, but some (arrowed) were not retained (D. E. Brooks, unpublished results).

The binding of epididymal proteins to sperm is selective in that only some of the secretory proteins remain permanently associated with the sperm (Voglmayr *et al.* 1980; Fig. 5). A particularly intriguing observation is that some of the adsorbed proteins become inaccessible to degradation by externally added protease (Voglmayr *et al.* 1982). This has led to the suggestion that some adsorbed proteins may become internalized. If this is correct, adsorption of proteins during epididymal maturation may affect not only the surface characteristics of the sperm, but also internal structures and metabolic events.

Acknowledgments

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