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Function of the epididymis in bulls and rams

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Introduction

Spermatozoa undergo a series of remarkable transformations during the 5–6 weeks between when they originate as spherical spermatids in the germinal epithelium and, assuming natural mating, encounter an oocyte(s) within the female reproductive tract. Reproductive biologists tend to consider spermatozoa from the perspective of their own narrow interests such as epididymal function, characteristics of ejaculated spermatozoa, in-vitro fertilization, or sperm transport within the female reproductive tract. This approach ignores the fact that events occurring at any point during the 5–6-week life-span of the spermatid/spermatozoon must influence subsequent events. Since the epididymis has a crucial role in controlling sperm function, an understanding of epididymal function is important for all gamete biologists.

At a first level of consideration, functions of the epididymis are: (a) maturation of spermatozoa so they have a maximal potential for fertilizing oocytes with a minimal loss of embryos, (b) maintenance of mature spermatozoa in optimum condition until ejaculated or voided from the excurrent duct system, and (c) transport of spermatozoa through the epididymal duct. Available evidence (Lino & Braden, 1972; Amann *et al.*, 1974) supports the conclusion that the epididymis is not a major site for dissolution or removal of spermatozoa from the excurrent duct system in a normal male; most spermatozoa produced by the testes are voided during ejaculation or micturition.

Testicular spermatozoa of bulls and rams (Amann & Griel, 1974; Fournier-Delpech *et al.*, 1979), are infertile whereas spermatozoa from the distal cauda epididymidis have a fertilizing potential equivalent to that of ejaculated spermatozoa. Spermatozoa should not be considered as mature until they have acquired normal motility, fertility, and ability to induce normal embryonic development. However, spermatozoa from the cauda epididymidis, which apparently are functionally mature, are further altered by admixture with seminal plasma (Killian & Amann, 1973).

The sites within the epididymis where different aspects of sperm maturation occur probably are similar for rams (Fig. 1) and bulls. Spermatozoa from the distal caput epididymidis bind to about 50% of sheep oocytes during in-vitro incubation, but are immotile and do not fertilize oocytes after artificial insemination into the uterine horn. However, for samples from the distal corpus epididymidis both the motility and fertilizing ability of spermatozoa are markedly greater (Fig. 1); the majority of fertilized oocytes result in lambs (69%) whereas oocytes fertilized by spermatozoa from the central corpus epididymidis invariably underwent early embryonic death. There is an improvement in sperm function between the distal corpus and the proximal cauda epididymidis and cells from either the proximal or distal cauda are equivalent to ejaculated spermatozoa (Fournier-Delpech *et al.*, 1979). The unusually high embryonic death rate associated with oocytes fertilized by spermatozoa from the distal corpus epididymidis is a consequence of delayed early cleavage divisions (Fournier-Delpech *et al.*, 1981a) which presumably is an intrinsic cause of embryo death or leads to improper synchrony between embryo development and the environment afforded by the oviduct and uterus. From Fig. 1, it is obvious that sperm maturation must involve a series of complex changes in the spermatozoa, some of which are discussed by Hammerstedt & Parks (1987), which result from a sequence of events occurring at different points within the caput and corpus epididymidis.

Epididymal function is androgen-dependent, although extensive data are not available for domestic ruminants. The electrophoretic profile of proteins in fluid taken by micropuncture at several sites of the epididymis is different in intact and orchidectomized rams (Fournier-Delpech

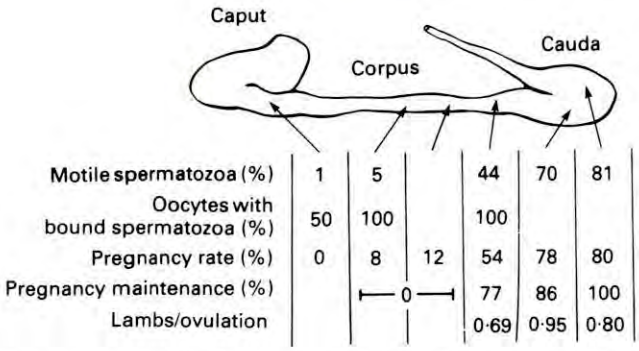

Fig. 1. Sites in the ram epididymis of progressive changes in sperm function associated with sperm maturation. Data for binding of spermatozoa to oocytes are based on in-vitro studies with sheep oocytes and pregnancy data are for spermatozoa inseminated into the uterine horn. From data reported by Fournier-Delpech *et al.* (1979, 1983).

et al., 1981b). Also, differentiation of the initial segment of bulls and rams (Abdel-Raouf, 1960; Nilnophakoon, 1978) does not occur until testicular development has progressed to a stage when the rete testis fluid probably has normal concentrations of androgens and androgen-binding protein (Carreau *et al.*, 1984b). Benoit (1926) noted that castration altered morphology, and presumably function, of the epididymal epithelium in mice and guinea-pigs. He postulated that epididymal secretions were needed to permit the acquisition of motility and maintain the vitality of spermatozoa. Although sperm maturation requires < 4 days (Amann, 1981), data for laboratory animals (Bedford, 1975; Orgebin-Crist *et al.*, 1975) support the conclusion that ageing spermatozoa in a specific location in the epididymis for several days is insufficient to induce maturation. The sequential exposure of spermatozoa to specific environments produced in progressively more distal sites within the epididymal duct is necessary. Furthermore, the process of sperm maturation is dependent upon secretions of the epididymal epithelium, some of which are androgen dependent (Orgebin-Crist & Jahad, 1978; Brooks, 1981, 1983; Jones *et al.*, 1982).

Maintenance of fertile spermatozoa in the cauda epididymidis also is androgen-dependent. For orchidectomized rams given 0, 60 or 300 mg implants of testosterone, the function of spermatozoa taken 9 days after orchidectomy from the distal cauda epididymidis was dependent on androgen concentration in blood (Fournier-Delpech *et al.*, 1984).

This paper focusses on epididymal function in rams and bulls. It is likely that the underlying processes are similar in other domestic ruminants and laboratory animals. However, comparisons will emphasize the fact that certain functions may be located in different regions of the epididymis, depending on the species.

Morphology of the epithelium

The epididymis consists of three distinct compartments which are: (a) the ductal lumen, containing spermatozoa and epididymal plasma; (b) the epithelium lining the lumen; and (c) the connective tissue, vascular elements, smooth muscle and nerves which comprise the extraductal tissue. Emphasis will be focussed on the epithelium and interpretation of analyses of the luminal fluid to deduce epithelial functions; the spermatozoa and extraductal tissue will not be considered.

Cell types

Throughout the epididymis, the ductal epithelium consists of tall, narrow columnar cells, termed principal cells, and small spherical cells, termed basal cells. As discussed below, the cytoplasmic

characteristics of principal cells differ in different regions of the epididymis (Nicander, 1958; Tröger, 1969; Sinowatz, 1981; Wagley *et al.*, 1984; Goyal, 1985). Basal cells have a scant cytoplasm containing only a few organelles, and are similar throughout the duct. Their function is unknown. The epithelium in the caput epididymidis (regions M_1 and M_2 described below) also contains apical cells. The basal half of these columnar cells is very narrow, but the apical half is of normal width and contains the oval nucleus, many mitochondria, and some ribosomes. Apical cells are rarely found in other parts of the ductus epididymidis (Sinowatz, 1981; Goyal, 1985). In addition, macrophages are found in the basal area of the epithelium and lymphocytes (although these may be monocytes) are found both basally and apically; both of these immunocompetent cell types are found along the entire length of the epididymal duct (Sinowatz, 1981). Detailed morphometric analyses of the bovine or ovine epididymis have not been reported, so the relative abundance of principal cells and basal cells is unknown.

In contrast to bulls and rams, the epididymal epithelium in rodents and rabbits also contains clear cells which are found in increasing numbers throughout the duct distal to the initial segment (Robaire & Hermo, 1987). Since rodent sperm typically lose their cytoplasmic droplet while in the cauda epididymidis, and membranous elements similar to those within a sperm cytoplasmic droplet are found free in the lumen of the epididymal duct and in vesicles within the clear cells, it has been proposed (Robaire & Hermo, 1987) that clear cells internalize elements of sperm cytoplasmic droplets. The majority of bull and ram spermatozoa retain their cytoplasmic droplet in the cauda epididymidis (Ortavant, 1953; Amann & Almquist, 1962), and neither bulls nor rams have clear cells within the epididymal epithelium.

Mitosis of principal cells in the epididymal epithelium of domestic ruminants has not been studied using [^{3}H]thymidine-labelling of cells in the S-phase, followed by radioautography. However, mitosis is rare (Sinowatz, 1981). Based on the labelling indices of cells in the rat epididymis, Clermont & Flannery (1970) concluded that the epithelium was static. This is generally interpreted as evidence that the epithelial cells rarely divide. However, based on several observations [Clermont & Flannery (1970) for rats, Pabst & Schick (1979) for rabbits, and Wagley *et al.* (1984) for cultured ram principal cells], it is likely that principal cells in the epididymal epithelium have a life span of 2–3 months. There is no evidence to support the concept that basal cells are precursors of principal cells. Indeed, from studies of the ontogeny of the epididymal epithelium in bulls and rodents, Abdel-Raouf (1960), Benoit (1926) and Sun & Flickinger (1979) concluded that columnar cells differentiate into principal cells and basal cells before puberty; Nilnophakoon (1978) reported similar data for rams, but felt that origin of the basal cells was obscure.

Regions of the epididymis

Regional differences in morphology of the epithelium lining the epididymal duct of bulls, and the concept that these reflected differences in function, were described by von Lanz (1924) and Benoit (1926). Benoit (1926) introduced the term 'initial segment'. He noted that there were few spermatozoa in the lumen and that the epithelium had histological characteristics which he, and others, incorrectly interpreted as evidence of secretory activity. The detailed description by Nicander (1958) of the regional histology and cytochemistry of the bull and ram epididymis is generally used to demarcate regions (Fig. 2a).

From a combined morphological and physiological perspective, it is advantageous to consider the epididymis as an initial segment involved in adsorption, a middle segment in which sperm maturation occurs, and a terminal segment in which fertile spermatozoa are stored (Glover & Nicander, 1971). Therefore designation of the middle and terminal segments as a maturation region and a fertility region respectively is appropriate. The designations in Fig. 2(b) are based on this concept, sperm function (Fig. 1), and the electron microscopic observations summarized below. The term initial segment has been retained and the maturation region has three morphologically distinct regions (M_1, M_2 and M_3). Region M_3 is subdivided into regions M_{3A} and M_{3B} which,

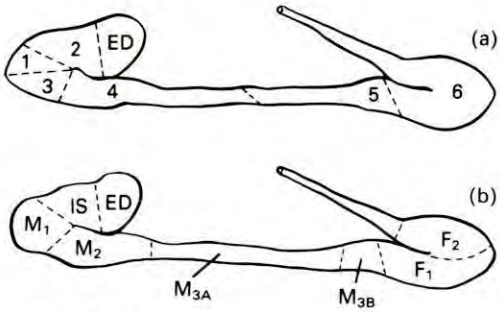

Fig. 2. Approximate demarcations between regions of the bull or ram epididymis (a) as defined by Nicander (1958) or (b) as proposed and defined herein.

although morphologically similar, differ in that spermatozoa from region M_{3A} rarely are motile and are infertile whereas many spermatozoa from region M_{3B} are fertile and result in offspring. The distinction between regions F_1 and F_2 of the fertility region is only in diameter of the duct, since the epithelium is similar throughout the cauda (Sinowatz, 1981; Goyal, 1985) and functional ability of the spermatozoa is similar (Fig. 1). The functional and morphological regions depicted in Fig. 2(b) are appropriate for the rabbit except that region M_{3B} probably should start more proximally.

Ultrastructure of principal cells

This description of regional differences in principal cells of the ram and bull is based on ultrastructural studies reported by Nicander (1979), Sinowatz (1981), Wagley *et al.* (1984) and Goyal (1985), although their descriptions are consistent with earlier light microscopic observations of von Lanz (1924), Benoit (1926), Nicander (1958) and Tröger (1969). General reviews by Hamilton (1972, 1975) and Robaire & Hermo (1987) on the mammalian epididymis should also be consulted. The epididymal epithelium is apparently similar in morphological characteristics and regional distribution in rams and bulls. Figure 3 depicts a synopsis of these data.

Principal cells in the initial segment are characterized by extensive long narrow, or dilated, cisternae of endoplasmic reticulum and a paucity of vesicles between the Golgi apparatus and their apical face. The apical face is covered by long, narrow microvilli among which are coated pits underlain by coated vesicles and a few large membrane-bound vesicles. In the central zone of the Golgi apparatus, small vesicles and cisternae of smooth endoplasmic reticulum are found and on the cis face of the Golgi there are occasional arrays of rough endoplasmic reticulum. The supranuclear area contains a few dense bodies. The oval nucleus is in the lower one-third of the cell and is surrounded by rough endoplasmic reticulum. Mitochondria are concentrated in the basal region of the cell.

Principal cells in region M_1 also have long cisternae of endoplasmic reticulum, with few ribosomes, in the apical portion of the cell, but there are more membrane-bound vesicles, vacuoles, and multi-vesicular bodies present than in principal cells in the initial segment. The apical face has many coated pits and apical vesicles and the microvilli are shorter. Below the prominent Golgi apparatus, vesicles, dense bodies and a few multi-vesicular bodies fill much of the cytoplasm. The nucleus tends to be invaginated and is associated with rough endoplasmic reticulum. The infranuclear region contains vesicles, and most of the mitochondria. In contrast to cells in the initial segment, principal cells in region M_1 have more apical vesicles, a larger Golgi apparatus, more supranuclear dense bodies and lipid vacuoles, and far more basal vesicles.

In region M_2, the apical face of the principal cells has numerous coated pits and underlying vesicles, and they appear to be less prevalent than in region M_1. The apical two-thirds of the cells are filled with large and intermediate sized multi-vesicular bodies, vesicles that vary in size and

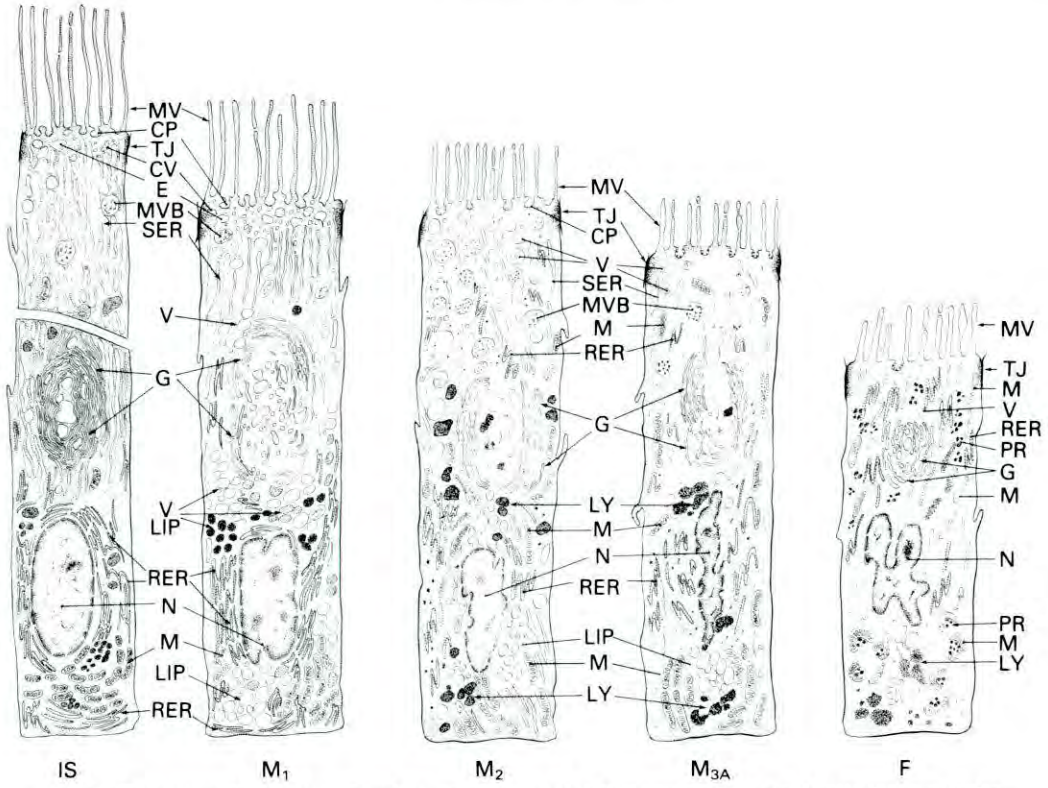

Fig. 3. Drawings illustrating the characteristics and differences in principal cells from specific regions (see Fig. 2) of the ram or bull epididymis. The cells (from left to right) typify principal cells in the initial segment (IS) and regions M_1, M_2, M_{3A} and F. Principal cells are 100–110 μm tall in the initial segment, 65–70 μm in regions M_1 and M_2, and progressively shorter distally to about 50 μm in region F. Structures are identified as: CP, coated pit; CV, coated vesicle; E, endosome; G, Golgi apparatus; LIP, lipid droplet; LY, lysosome; MV, microvilli; MVB, multi-vesicular body; N, nucleus; M, mitochondria; P, polyribosomes; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; TJ, tight junction; V, vacuole. Based on unpublished observations and those reported by Goyal (1985), Nicander (1979), Sinowatz (1981) and Wagley *et al.* (1984).

electron density, and lysosomes in addition to a large Golgi apparatus. Mitochondria, rough endoplasmic reticulum, and free ribosomes are found throughout this region. The nucleus is narrow and invaginated and associated with mitochondria and rough endoplasmic reticulum. The basal portion of the cells contains numerous lipid droplets, mitochondria and smooth endoplasmic reticulum. In contrast to Sinowatz (1981) and Wagley *et al.* (1984), Goyal (1985) considered the principal cells in regions M_1 and M_2 to be similar, but distinct from those in the initial segment and region M_3.

Principal cells in regions M_{3A} and M_{3B} are similar in morphology, although there is a slight diminution in height progressing distally. The apical face contains a few coated pits and there are coated and uncoated small vesicles in the apical region of the cytoplasm. In all portions of the cells, multi-vesicular bodies, vesicles and lysosomes are less prevalent than in region M_2. The Golgi apparatus is relatively small and the nucleus is elongated and invaginated. Small dense bodies are found above the nucleus. Clusters of lipid droplets and dense bodies are found below, and occasionally above, the nucleus. Although mitochondria are found throughout the cells, orderly arrays of mitochondria are conspicuous near the basal membrane.

Principal cells are similar in morphological characteristics throughout region F, although they are progressively shorter passing distally. There are few coated pits or apical vesicles, although numerous polyribosomes and short profiles of rough endoplasmic reticulum are found above the relatively small Golgi apparatus. Many mitochondria are found above the irregularly-shaped nucleus and lysosomes are found in the basal portion of the cell. In contrast to mitochondria located above the nucleus of principal cells in region F, or at all locations in principal cells in more proximal regions, mitochondria located below the nucleus in principal cells in region F are cup-shaped and surround a small cluster of ribosomes.

The microvilli become progressively shorter, and broader, passing distally along the epididymal duct, and histochemical studies (Sinowatz, 1981; Goyal & Vig, 1984) revealed that they probably differ in function. In the initial segment and region M_1, the microvilli stain positive for alkaline phosphatase, but only occasionally for Mg/Ca-dependent ATPase. In contrast, the microvilli of principal cells in regions M_2, M_3 and F are devoid of alkaline phosphatase, but stain intensely for Mg/Ca-dependent ATPase. Acid phosphatase is distributed throughout the apical cytoplasm in principal cells in the initial segment and regions M_1 and M_2, and localized with high intensity in the basal portion of cells in region M_3.

Blood–epididymis barrier

A functional blood–epididymis barrier in bulls and rams is indicated by apically located tight junctions between adjacent principal cells (Sinowatz, 1981; Wagley *et al.*, 1984; Fig. 3), differences in the composition of luminal fluid at different sites along the bovine epididymis (Crabo, 1965), and differences between the luminal contents and blood. More extensive and direct evidence for the blood–epididymis barrier in other species is discussed by Hinton (1985) and Robaire & Hermo (1987). The actual site of the blood–epididymis barrier is the tight junctions found at the apical face of the epithelium.

Functions of the epithelium

Major functions of the epididymal epithelium are to maintain an environment appropriate for maturation of spermatozoa in the caput and corpus and for maintenance of fertile spermatozoa in the cauda epididymidis. With respect to the principal cells, however, three functions might be considered: transport of small molecules (e.g. amino acids, ions, sugars, water), transport of large molecules (e.g. proteins), and metabolism or synthesis. Transport of all sizes of molecules involves one, or usually both, of two coupled transport phenomena–adsorption and secretion. Adsorption of components from the lumen of the duct is coupled with secretion of material from the lateral or basal faces of the principal cells (below the blood–epididymis barrier) to allow eventual passage into the blood. Similarly, secretion requires adsorption of components from the extraductal or luminal environment, optional processing of the material by the cells (metabolism or synthesis), and secretion into the duct lumen. In this review, metabolism refers to modification of molecules (e.g. testosterone to dihydrotestosterone) in the principal cells, and synthesis to de-novo proteins or steroids; the terms do not refer to energy metabolism or to synthesis necessary for cell maintenance.

Transport of small molecules

The earliest quantitative evidence of micromolecule transport by the bull epididymis was provided by Crabo (1965) who used micropuncture techniques, and direct biochemical analyses, to study regional differences in the content of the luminal fluid. His data were largely confirmed by Bech & Koefoed-Johnsen (1973). Extensive data for bulls and rams (Amann *et al.*, 1974; Waites, 1977) show that about 40 ml/day enter the epididymis as rete testis fluid but < 1 ml/day leaves as cauda epididymal plasma. Assuming that spermatozoa were neither made nor lost in the epididymis, Crabo (1965) used the concentration of spermatozoa in successive regions of the epididymis to

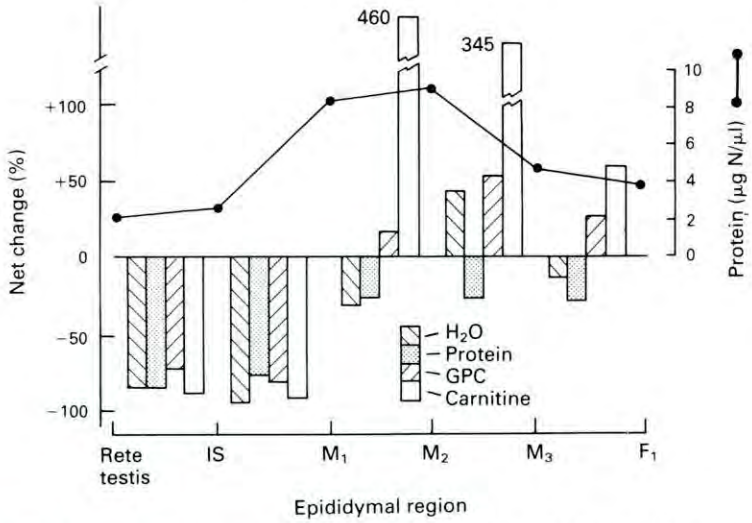

Fig. 4. The concentration of protein in luminal fluid from specific sites of the ram epididymis (●—●) and net changes in the amount of water, protein, glycerophosphocholine (GPC), and carnitine between rete testis fluid and luminal fluid from successive sites (see Fig. 2) in the bull epididymis. Net changes in fluid components, in terms of gain or loss between successive sites, were calculated using (a) the change in sperm concentration to estimate water gain or loss and (b) calculating the total amount of a component per unit volume of fluid at site 1 and the total amount of the component at site 2 for a unit volume adjusted for water gain or loss between site 1 and site 2; the difference was expressed as a percentage of the value at site 1. Calculated from data reported by Crabo (1965) and Besancon *et al.* (1985).

calculate water transport from or to the lumen. Sperm concentration is higher in the initial segment (360×10^6/ml) than in rete testis fluid (60×10^6/ml) and markedly higher in regions M_1 and M_2 (4500 and 6400 $\times 10^6$/ml), although concentrations are lower in regions M_3 and F_1 (4500 and 5200 $\times 10^6$/ml). These data, and the concentrations of components, allowed calculations of changes in electrolyte and protein concentrations along the epididymal duct.

Figures 4 and 5 show the net changes, in terms of entrance or loss into or from the luminal fluid of a number of molecules. These values for net change in concentration are, however, not equivalent to transport flux within a region of the epididymis. Measurements of flux must also consider residence time (i.e. length of duct and flow rate). Adsorption of water and glycerophosphocholine occurs between the initial segment and region M_1 (Fig. 4), but more distally, especially between regions M_2 and M_3, there is a net increase in water and glycerophosphocholine. Based on data for rabbits (Hammerstedt & Rowan, 1979), glycerophosphocholine is a synthetic product of the epididymal epithelium derived from blood lipoproteins. Although most of the glycerophosphocholine is apparently added between regions M_2 and M_3, some is also added to the ductal fluid in region M_{3B}.

Carnitine is an example of a compound transported directly from blood into the ductal lumen without modification in the principal cells. Transport of carnitine is maximal between regions M_1 and M_{3A} (Fig. 4), even though the concentration is maximal in region F_2 because of water removal (> 10 mM; Hinton *et al.*, 1979; Besancon *et al.*, 1985).

The net change in electrolyte composition of the luminal fluid (Fig. 5) reveals a continuous removal of Na, K, Cl, and P from the luminal fluid in amounts greater than would be equivalent to water loss. However, between regions M_2 and M_3, where water is added back into the ductal lumen (Fig. 4), there is a net increase in all four ions, especially Cl and P (Fig. 5). Nevertheless, as reflected in the ratio of Na to K (Fig. 5), Crabo (1965) found a continuous decline in Na concentration along the ductus epididymidis (from 121 to 33 mequiv./l) whereas the concentrations of K were higher in

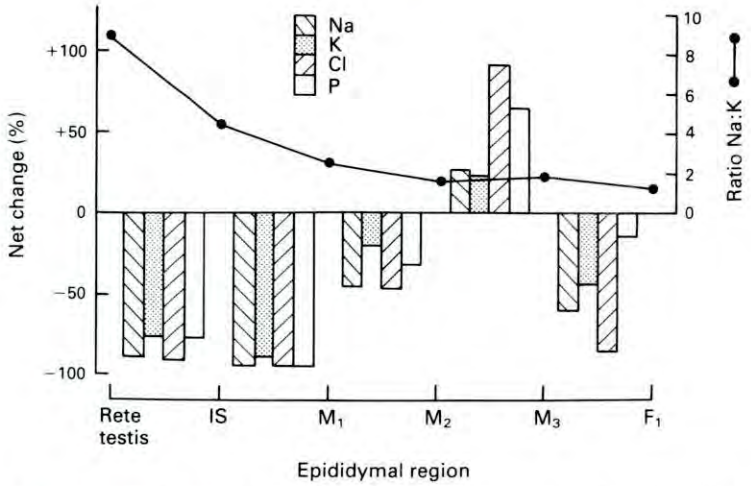

Fig. 5. The Na:K ratio in luminal fluid from specific sites of the ram epididymis (●—●) and net changes in the amount of Na, K, Cl, and P between rete testis fluid and luminal fluid from successive sites (see Fig. 2) in the bull epididymis. Net changes in fluid components were calculated as for Fig. 4 from data reported by Crabo (1965).

regions M_1, M_2 and M_3 than in the initial segment or region F_1 (about 45 vs 28 mequiv./l). Concentrations of Cl were reasonably constant between the initial segment and region M_3, but were much lower in region F_1 (60–80 *vs* 13 mequiv./l) and P concentrations were similar in luminal fluid from each region (38–46 mequiv./l).

The concentration of inositol is probably maximal in region M_1 (>20 mM), based on analyses of bovine rete testis fluid, cauda epididymal plasma and epididymal tissue (Voglmayr & Amann, 1973) and of fluid from several sites of the ram epididymis (Hinton *et al.*, 1980). This probably reflects the concentration of inositol, entering in rete testis fluid, as water is adsorbed (Voglmayr & Amann, 1973).

Transport and synthesis of large molecules

The uptake of carbon particles (India ink) by the epithelium of the proximal ram epididymis (Gunn, 1936) was the first proof of transport of large molecules. The concentration of protein in luminal fluid from the bovine epididymis is higher in region M_1 and M_2 than in more proximal or distal sites (Fig. 4), but there is apparently net loss of protein in all regions of the epididymis. Electrophoretic and immunological analyses of bovine rete testis fluid and cauda epididymal plasma (Amann *et al.*, 1973; Killian & Amann, 1973), and immunofluorescent analyses of the bovine epididymal epithelium (Barker & Amann, 1971), revealed profound changes in the proteins bathing spermatozoa and entering and leaving the epididymis. Many of these changes reflect macromolecular transport into and from the luminal fluid. Cauda epididymal plasma of rams and bulls (Alumot *et al.*, 1971; Killian & Amann, 1973) contains a variety of immunoglobulins as well as some blood serum antigens which were postulated (Killian & Amann, 1973) to enter the luminal fluid via the epididymal epithelium rather than in rete testis fluid. If these immunoglobulins and blood serum antigens are not contaminants of the collection process, their presence is evidence that the blood–epididymis barrier might be 'leaky'. However, most macromolecules transported into the lumen of the epididymal duct are synthesized by principal cells.

Adsorption of specific, identified proteins by the epididymal epithelium has not been demonstrated for rams or bulls, but 60% of the androgen-binding protein entering the epididymis daily in

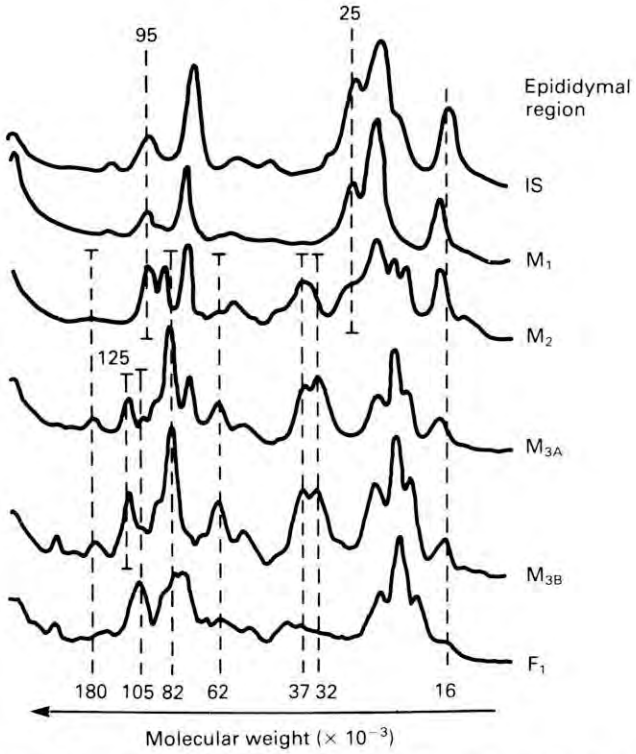

Fig. 6. Electrophoretic profiles of luminal proteins from specific sites (see Fig. 2) of the ram epididymis. The vertical lines designate proteins apparently not present in all fluids. Although not shown, the spectrum of proteins in rete testis fluid is very different from that for fluid from the initial segment (IS). Modified from Dacheux & Voglmayr (1983).

rete testis fluid is undetectable in cauda epididymal plasma. Selective adsorption of androgen-binding protein, α_2-macroglobulin and transferrin, entering the epididymis in rete testis fluid, has been demonstrated in rats. All three proteins are taken up, especially in the caput epididymidis, by receptor-mediated endocytosis via coated pits, and passed through coated vesicles and endosomes to multi-vesicular bodies (see Robaire & Hermo, 1987). The fate of androgen-binding protein has not been established. However, α_2-macroglobulin was transferred to lysosomes while transferrin was recycled back to the epididymal lumen.

The most common approach for study of macromolecule synthesis and transport has been electrophoresis of proteins obtained by micropuncture of the epididymal duct. Huang & Johnson (1975) found at least 6 proteins in the epididymal fluid of rams which were absent in serum. All were detectable in fluid from the initial segment, and one was present in progressively lower concentrations distally. Unfortunately, rete testis fluid or spermatozoa were not analysed and therefore could not be excluded as sources of the detected proteins.

Dacheux & Voglmayr (1983) compared proteins in epididymal fluid with those in rete testis fluid or on the surface of spermatozoa. Fluid from the initial segment lacked the major proteins of M_r 27 000, 32 000, 40 000 and 56 000 present in rete testis fluid. At least two proteins (M_r 25 000 and 95 000) in fluid from the initial segment (but absent in rete testis fluid), were present at decreasing concentrations through region M_2, but were absent in regions M_3 and F_1 (Fig. 6). At least 5 proteins (M_r 32 000, 37 000, 62 000, 82 000 and 180 000) first detectable in region M_2 were present in higher concentrations in region M_{3A} or M_{3B}, but at lower concentrations in region F_1. In

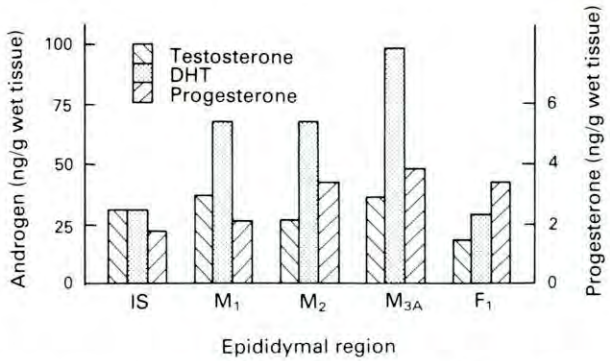

Fig. 7. Concentrations of testosterone, dihydrotestosterone (DHT), and progesterone in whole tissue from specific sites (see Fig. 2) of the bull epididymis. From data reported by Ganjam & Amann (1976).

contrast, a protein of M_r 105 000 first detectable in region M_{3A} was present at increasing concentrations passing distally, and a protein of M_r 125 000 was detectable only in regions M_{3A} and M_{3B}.

Dacheux & Voglmayr (1983) cautioned, as had previous workers (Barker & Amann, 1970; Amann *et al.*, 1973; Killian & Amann, 1973), that apparent changes in the electrophoretic pattern of proteins (or in immunological characteristics) could result from (1) adsorption of specific proteins from the tubule lumen; (2) modification within the lumen of existing proteins by cleavage, congregation, cross-linkage or glycosylation; (3) accumulation to detectable concentration of proteins lost from spermatozoa; or (4) by active secretion of new proteins by the epididymal epithelium. All four of these processes apparently occur.

Data for acid α-glucosidase illustrate the fallacy of making conclusions about luminal concentrations of a compound based on tissue concentration, although the comparison is confounded by species and technique. Besancon *et al.* (1985) measured acid α-glucosidase activity in epididymal plasma from rams and Jauhiainen & Vanha-Perttula (1985) measured the enzyme in bull tissue, using slightly different methods. In ram epididymal plasma, activity of α-glucosidase was low in the initial segment and regions M_1, M_2 and M_{3A}, but was 3-fold greater in region M_{3B}; values in regions F_1 and F_2 were even greater. In whole epididymal bull tissue, however, activity from region M_1 was almost 2-fold greater than for any other region.

Metabolism

Rete testis fluid and cauda epididymal plasma contain a diversity of steroids, including testosterone (> 100 nM), in concentrations much higher than in blood (Ganjam & Amann, 1976; Waites, 1977; Voglmayr *et al.*, 1977). However, based on analyses of epididymal tissue (Fig. 7), dihydrotestosterone must be produced from testosterone in region M_1, and possibly region M_{3A}. Principal cells, but not basal cells, metabolize testosterone to dihydrotestosterone (Klinefelter & Amann, 1980), and *in vitro* (Wagley *et al.*, 1984) testosterone is metabolized most actively by principal cells from region M_1 of rams (Fig. 8). Principal cells from the initial segment are virtually devoid of 5α-reductase activity. This is in stark contrast to data for rat principal cells (Brown & Amann, 1984) or tissue homogenates (Robaire *et al.*, 1981), for which the highest activity of 5α-reductase is in the initial segment. A comparison of testosterone metabolism by cultured principal cells and minced epididymal tissue (Fig. 9) confirmed that the initial segment of the ram epididymis contains little 5α-reductase activity.

Although cultured ram principal cells, especially from region M_1, rapidly metabolize testosterone

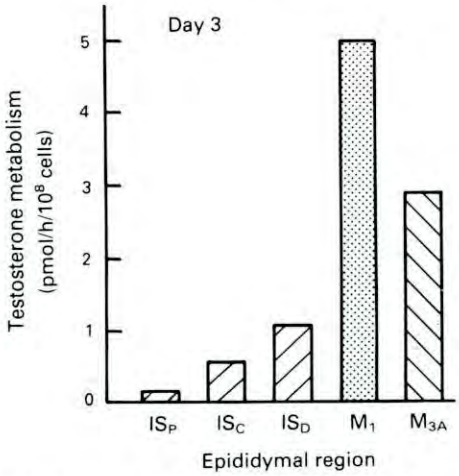

Fig. 8. Metabolism of testosterone to dihydrotesterone and 5α-androstane-3α,17β-diol by intact, cultured ram principal cells from specific sites of the epididymis (see Fig. 2) on Day 3 of culture (n = 3–12; D. V. Brown & R. P. Amann, unpublished data). For this study, the initial segment was subdivided into proximal (IS_P), central (IS_C), and distal (IS_D) portions before isolation of principal cells. Principal cells were isolated and cultured as outlined by Wagley *et al.* (1984) and testosterone metabolism was measured essentially as described by Brown & Amann (1984).

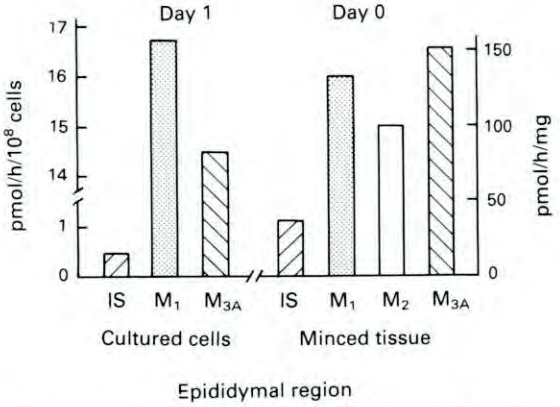

Fig. 9. Metabolism of testosterone to dihydrotestosterone and 5α-androstane-3α,17β-diol by intact and cultured ram principal cells on Day 1 of culture (left) and by fresh minced tissue (right) from specific segments (see Fig. 2) of the same ram epididymides (n = 3; D. V. Brown & R. P. Amann, unpublished data).

to dihydrotestosterone, there is slight (< 10%) metabolism of dihydrotestosterone to 5α-androstane-3α,17β-diol; the 3β-epimer and other 5α-reduced metabolites are produced only in trace amounts (R. P. Amann, S. R. Marengo & D. V. Brown, unpublished data). The 5α-reductase in ram principal cells also metabolizes progesterone to 5α-pregnane-3,17-dione and androstenedione to 5α-androstane-3,17-dione; both products are further metabolized. Consequently, progesterone and androstenedione present in rete testis fluid (Ganjam & Amann, 1976) could serve as competing substrates for epididymal 5α-reductase.

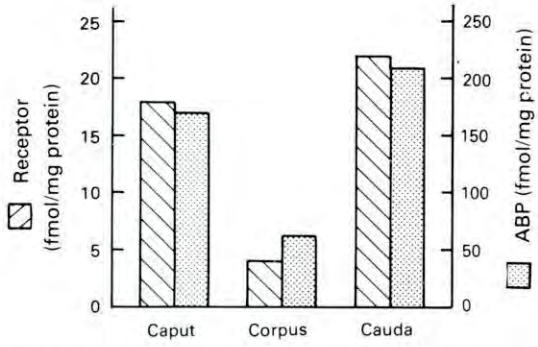

Fig. 10. Concentrations of cytosolic androgen receptor and androgen-binding protein (ABP) in whole tissue from the caput, corpus and cauda of the ram epididymis. From data reported by Carreau *et al.* (1984b).

It is unlikely that ram principal cells synthesize testosterone since, for several regions of the epididymis, they did not convert pregnenolone to progesterone, or 5-androstene-3β,17β-diol or androstenedione to testosterone (R. P. Amann & S. R. Marengo, unpublished data). The androgenic stimulus to the epididymal epithelium must therefore be derived from the blood or the almost 2 μg testosterone entering the epididymis daily in rete testis fluid (Ganjam & Amann, 1976).

Control of epididymal function

If the epididymis contains an androgen-dependent epithelium, the principal cells, or basal cells, should contain androgen receptors which evoke a response. Ram epididymal cytosol binds androgens (Carreau *et al.*, 1984a,b; F. R. Tekpetey & R. P. Amann, unpublished data) and binding proteins for several hormones have been found in rat and rabbit epididymal tissue (Robaire & Hermo, 1987). Although it would be desirable to quantify receptors in principal cells, basal cells and extraductal tissue, only data for whole tissue are available. In mouse tissue, however, [^{3}H]dihydrotestosterone is localized to nuclei of principal cells, especially in the proximal caput and proximal cauda epididymidis (Schleicher *et al.*, 1984). This is consistent with data for the putative androgen receptor in low-salt cytosol from ram epididymal tissue (Fig. 10) and our preliminary data. However, only about 50% of the total androgen receptor per gram of tissue is extracted using low-salt buffer (F. R. Tekpetey & R. P. Amann, unpublished) and so the values reported in Fig. 10 are probably low. Ram epididymal cytosol also contains a protein which specifically binds oestradiol.

The regional distribution of the putative androgen receptor parallels that for androgen-binding proteins (Fig. 10). Androgen-binding protein was quantified by steady-state electrophoresis (Carreau *et al.*, 1984b) and was clearly separated from androgen receptor. Data in Fig. 10, and the 70 nM-dihydrotestosterone present in cauda epididymal plasma (Ganjam & Amann, 1976; Voglmayr *et al.*, 1977), lend credence to the speculation that dihydrotestosterone has a role in function of the cauda epididymidis as well as the distal caput.

Although there are discrepancies in available data (Ganjam & Amann, 1976; Voglmayr *et al.*, 1977; Carreau *et al.*, 1979, 1984b; Jégou *et al.*, 1979) for bulls and rams, the concentration of testosterone plus dihydrotestosterone in rete testis fluid exceeds that of androgen-binding protein (80–155 vs 20–30 nM) so that most of the androgen is presumably transported on the albumin-like protein secreted by Sertoli cells (Amann *et al.*, 1973). In cauda epididymal plasma, however, the concentration of androgen binding protein (>80 nM) is similar to that of testosterone plus dihydrotestosterone. Androgen-binding protein therefore probably conserves dihydrotestosterone, and testosterone, in the epididymal fluid to maintain concentrations exceeding those in blood, as is true in rats (Turner *et al.*, 1984). Concentrations of androgen-binding protein and steroids in the luminal fluid at different sites along the epididymis are unavailable for domestic ruminants.

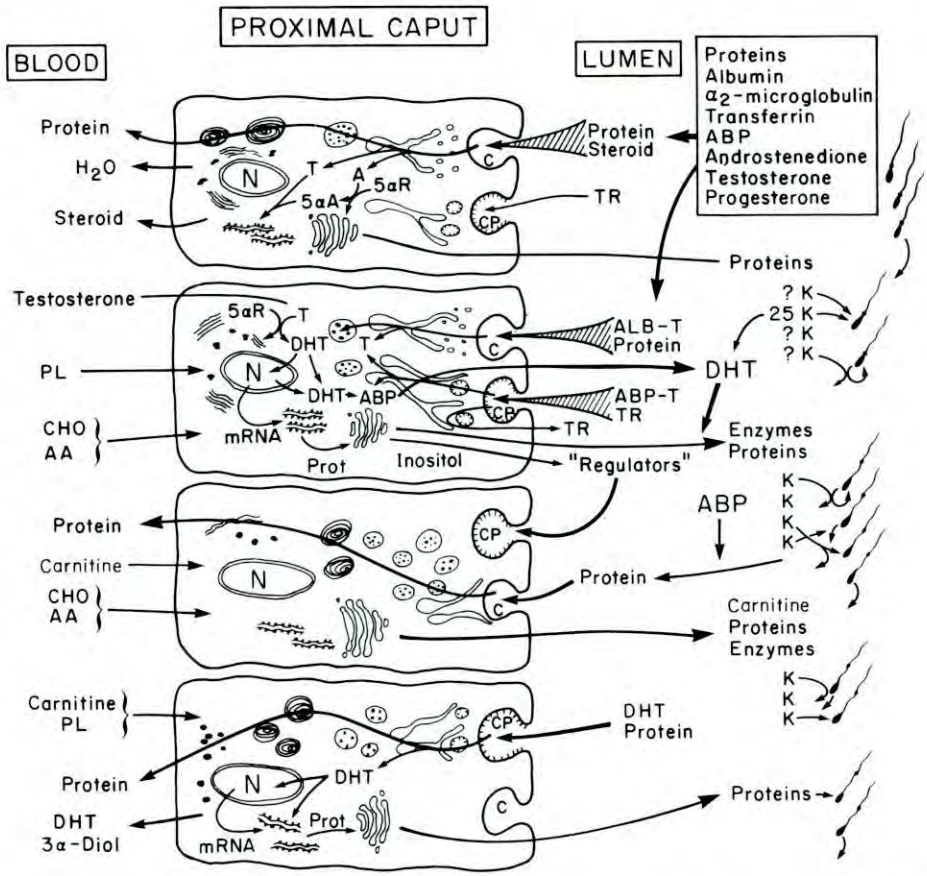

Fig. 11. A hypothetical model depicting the regional and sequential distribution of epithelial functions in the epididymis. See text for explanation.

Integrated hypothesis on regional function

Observations on the morphology of the epididymal epithelium and biochemical analyses of tissue and fluid, together with data for other species (Robaire & Hermo, 1987) are integrated into a conceptualized model (Fig. 11) depicting the possible regionalization of function. It would be naive to assume that all details of the model are correct or that other mechanisms are not involved in regulating epididymal function.

The initial segment is specialized for bulk removal of water, proteins, steroids, electrolytes and other components of rete testis fluid passing through the efferent ducts (Figs 4 & 5). There is a conspicuous difference in the electrophoretic characteristics of proteins in rete testis fluid and luminal fluid from the initial segment (Dacheux & Voglmayr, 1983), and so secretion must also occur in the initial segment (or efferent ducts). These proteins probably initiate sperm maturation. Since the initial segment is proximal to the sites of dihydrotestosterone synthesis (region M_1 and distally), it is unlikely that dihydrotestosterone regulates function of the initial segment in domestic ruminants; a role for testosterone cannot be excluded. The mode of endocytosis in these cells is not established, but principal cells of the initial segment are characterized by unique long arrays of smooth or sparsely-granulated endoplasmic reticulum, which is certainly involved in adsorption.

In region M_1, adsorption of water, proteins, small molecules and electrolytes continues, although the volumes removed by endocytosis are much less than in the initial segment; there is a 30% increase in sperm concentration between region M_1 and M_2 as contrasted with a >90% increase between the initial segment and region M_1. Reduction of the Na:K ratio is almost complete (Fig. 5) and osmotic pressure increases to 370 mosmol/kg (Bech & Koefoed-Johnsen, 1973). There is not a profound change in the spectrum of proteins in luminal fluid between the initial segment and region M_1 (Fig. 6), although the protein concentration is about 3-fold greater (Fig. 4). The principal cells in region M_1 are the most proximal, and primary, site of significant production of dihydrotestosterone (Figs 8 & 9). Thus, adsorption of diverse compounds and especially synthesis of dihydrotestosterone are major functions of region M_1.

Secretion of diverse products into luminal fluid occurs in regions M_2 and M_{3A} (Figs 4, 5 & 6) and may continue in regions M_{3B} or F. Comparing differences between fluids from regions M_1 and M_2, it is evident that the latter contains an extraordinary concentration of carnitine (Fig. 4) and at least five new proteins (Fig. 6). It is speculated that androgen receptor in the caput epididymidis (Fig. 10) actually is localized in region M_2, and present at low concentrations in region M_1. If this is correct, androgen binding protein may help to transport the dihydrotestosterone produced by principal cells in region M_1 to more distal sites such as regions M_2 or F, where it may provide the androgen stimulus necessary for epithelial function. Secretion of specific proteins and glycerophosphocholine, as well as transport of carnitine, are therefore functions of region M_2.

Between regions M_2 and M_3, there is a net loss of protein, but addition of water, electrolytes, glycerophosphocholine and carnitine (Figs 4 & 5). The concentration of protein decreases by almost 50% and osmotic pressure starts to decline. The apical one-third of principal cells in regions M_2 and M_3 contains numerous vesicles of varied electron density, and multi-vesicular bodies, and it is likely that these cells selectively adsorb components from the lumen in addition to their secretory roles. The abundance of lipid droplets in the basal portion of principal cells in regions M_2 and M_3 (Fig. 3) might be related to their uptake of blood lipoproteins which are processed (Hammerstedt & Rowan, 1979) and give rise to secretion of glycerophosphocholine (Fig. 4). Synthesis of dihydrotestosterone occurs in regions M_2 and M_{3A}, but it is also possible that preformed dihydrotestosterone is provided to these cells. Unique proteins appear in luminal fluid from region M_{3A} (Fig. 6), but given the apparent paucity of androgen receptors in the corpus epididymidis secretion of these proteins may not be androgen-dependent. Alternatively, this apparent discrepancy may reflect sub-regional differences in epithelial function and subtle differences in sampling sites in different laboratories.

Between regions M_{3A} and F_1, there is a net loss of water and protein, as well as electrolytes, but some entrance of glycerophosphocholine and carnitine into the ductal fluid (Figs 4 & 5). There may be specific adsorption of selected proteins, since the spectra of proteins in regions M_{3B} and F_1 differ (Fig. 6), or the secretory flux of proteins common with region M_{3B} is sufficient to dilute out some proteins (i.e. proteins of M_r 32 000 and 37 000). The culmination of these events is the maturation of the spermatozoa.

Unique attributes of the environment of the cauda epididymidis which allow prolonged maintenance of fertile spermatozoa have not been identified. The roles of the apparently high concentrations of androgen receptors (Fig. 10), dihydrotestosterone and progesterone (Ganjam & Amann, 1976), and certain proteins (Fig. 6) remain to be established.

Conclusions

During the past 20 years, reproductive biologists have used electron microscopy and microanalytical techniques to improve the descriptions of the epididymal epithelium and its functions, relative to those published 60 years ago. Unfortunately, there has been little improvement in our understanding of the biology of sperm maturation and no attempt to learn how fertile spermatozoa are maintained

in the cauda epididymidis. Although it could be argued that epididymal malfunction is not a major problem in domestic ruminants, it is equally defensible to promote the ram as useful for study of epididymal function with the goal of developing a contraceptive for the human male. Regardless of the goal, future research should focus on control mechanisms and biological endpoints rather than phenomenology and data gathering. Only in this way can we hope to overcome our ignorance concerning how the epididymal epithelium, via its secretions, enables spermatozoa to become mature and remain fertile.

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References

Abdel-Raouf, M. (1960) The postnatal development of the reproductive organs in bulls with special reference to puberty. *Acta endocr., Copenh.* **34,** *Suppl.* **49,** 1–109.

Alumot, E., Lensky, Y. & Schindler, H. (1971) Separation of proteins in the epididymal fluid of the ram. *J. Reprod. Fert.* **25,** 349–353.

Amann, R.P. (1981) A critical review of methods for evaluation of spermatogenesis from seminal characteristics. *J. Androl.* **2,** 37–58.

Amann, R.P. & Almquist, J.O. (1962) Reproductive capacity of dairy bulls. VII. Morphology of epididymal sperm. *J. Dairy Sci.* **45,** 1516–1526.

Amann, R.P. & Griel, L.C., Jr (1974) Fertility of bovine spermatozoa from rete testis, cauda epididymidis, and ejaculated semen. *J. Dairy Sci.* **57,** 212–219.

Amann, R.P., Killian, G.J. & Benton, A.W. (1973) Differences in the electrophoretic characteristics of bovine rete testis fluid and plasma from the cauda epididymidis. *J. Reprod. Fert.* **35,** 321–330.

Amann, R.P., Kavanaugh, J.F., Griel, L.C., Jr & Voglmayr, J.K. (1974) Sperm production of Holstein bulls determined from testicular spermatid reserves, after cannulation of rete testis or vas deferens, and by daily ejaculation. *J. Dairy Sci.* **57,** 93–99.

Barker, L.D.S. & Amann, R.P. (1970) Epididymal physiology. I. Specificity of antisera against bull spermatozoa and reproductive fluids. *J. Reprod. Fert.* **22,** 441–452.

Barker, L.D.S. & Amann, R.P. (1971) Epididymal physiology. II. Immunofluorescent analyses of epithelial secretion and absorption, and of bovine sperm maturation. *J. Reprod. Fert.* **26,** 319–332.

Bech, J. & Koefoed-Johnsen, H.H. (1973) Spermiemorfologi og plasmas sammensaetning i bitestikler fra normale tyre samt to tyre med abnormt saedbillede. *Aarsberetning Inst. for Sterilitetsforskning*, pp. 9–30 Kgl. Vet.-og Landbohøjskole, Copenhagen.

Bedford, J.M. (1975) Maturation, transport, and fate of spermatozoa in the epididymis. In *Handbook of Physiology*, Section 7: *Endocrinology*, Vol. 5; *Male Reproductive System*, pp. 303–317. Eds R. O. Greep & E. B. Astwood. American Physiological Society, Washington, D.C.

Benoit, J. (1926) Recherches anatomiques, cytologiques et histophysiologiques sur les voies excrétrices du testicule chez les mammifères. *Archs Anat. Histol. Embryol.* **5,** 175–412.

Besancon, J., Dacheux, J.-L., Paquin, R. & Tremblay, R.R. (1985) Major contribution of epididymis to α-glucosidase content of ram seminal plasma. *Biol. Reprod.* **33,** 296–301.

Brooks, D.E. (1981) Metabolic activity in the epididymis and its regulation by androgens. *Physiol. Rev.* **61,** 515–555.

Brooks, D.E. (1983) Epididymal functions and their hormonal regulation. *Aust. J. biol. Sci.* **36,** 205–221.

Brown, D.V. & Amann, R.P. (1984) Inhibition of testosterone metabolism in cultured rat epididymal principal cells by dihydrotestosterone and progesterone. *Biol. Reprod.* **30,** 67–73.

Carreau, S., Drosdowsky, M.A. & Courot, M. (1979) Age related effects on androgen binding protein (ABP) in sheep testis and epididymis. *Int. J. Androl.* **2,** 49–61.

Carreau, S., Drosdowsky, M.A. & Courot, M. (1984a) Androgen-binding proteins in sheep epididymis: characterization of a cytoplasmic androgen receptor in the ram epididymis. *J. Endocr.* **103,** 273–279.

Carreau, S., Drosdowsky, M.A. & Courot, M. (1984b) Androgen-binding proteins in sheep epididymis: age-related effects on androgen-binding protein, cytosolic androgen receptor and testosterone concentrations. Correlations with histological studies. *J. Endocr.* **103,** 281–286.

Clermont, Y. & Flannery, J. (1970) Mitotic activity in the epithelium of the epididymis in young and old rats. *Biol. Reprod.* **3,** 283–292.

Crabo, B. (1965) Studies on the composition of epididymal content in bulls and boars. *Acta vet. scand.* **6,** Suppl. **5,** 1–94.

Dacheux, J.-L. & Voglmayr, J.K. (1983) Sequence of sperm cell surface differentiation and its relationship to exogenous fluid proteins in the ram epididymis. *Biol. Reprod.* **29,** 1033–1046.

Fournier-Delpech, S., Colas, G., Courot, M., Ortavant, R. & Brice, G. (1979) Epididymal sperm maturation in the ram: motility, fertilizing ability and embryonic survival after uterine artificial insemination in the ewe. *Annls Biol. anim. Biochim. Biophys.* **19,** 597–605. 597–605.

Fournier-Delpech, S., Colas, G. & Courot, M. (1981a) Observations sur les premiers clivages des oeufs

intratubaires de Brebis après fécondation avec des spermatozoïdes épididymaires ou éjaculés. *C. r. hebd. Séanc. Acad. Sci, Paris* [III] **292,** 515–517.

Fournier-Delpech, S., Pisselet, C., Garnier, D.-H., Dubois, M. & Courot, M. (1981b) Mise en évidence chez le Bélier d'une préalbumine sécrétée par l'épididyme sous l'action de la Testostérone. *C. r. hebd. Séanc. Acad. Sci, Paris* [III] **293,** 589–594.

Fournier-Delpech, S., Hamamah, S., Colas, G. & Courot, M. (1983) Acquisition of zona binding structures by ram spermatozoa during epididymal passage. In *The Sperm Cell*, pp. 103–106. Ed. J. André. Martinus Nijhoff, The Hague.

Fournier-Delpech, S., Hamamah, S., Courot, M. & Kuntz, C. (1984) Androgenic control of zona binding capacity of ram spermatozoa. In *The Male in Farm Animal Reproduction*, pp. 103–107. Ed. M. Courot. Martinus Nijhoff, The Hague.

Ganjam, V.K. & Amann, R.P. (1976) Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions. *Endocrinology* **99,** 1618–1630.

Glover, T.D. & Nicander, L. (1971) Some aspects of structure and function in the mammalian epididymis. *J. Reprod. Fert., Suppl.* **13,** 39–50.

Goyal, H.O. (1985) Morphology of the bovine epididymis. *Am. J. Anat.* **172,** 155–172.

Goyal, H.O. & Vig, M.M. (1984) Histochemical activity of alkaline phosphatase and acid phosphatase in the epididymis of mature intact and androgen-deprived bulls. *Am. J. vet. Res.* **45,** 444–450.

Gunn, R.M.C. (1936) Fertility in sheep. Artificial production of seminal ejaculation and the characteristics of the spermatozoa contained therein. *Bull. Comm. Sci. Industr. Res. Austr.* **94,** 1–116.

Hamilton, D.W. (1972) The mammalian epididymis. In *Reproductive Biology*, pp. 268–337. Eds H. Balin & S. Glasser. Excerpta Medica, Amsterdam.

Hamilton, D.W. (1975) Structure and function of the epithelium lining the ductuli efferentes, ductus epididymidis, and ductus deferens in the rat. In *Handbook of Physiology*, Section 7: *Endocrinology*, Vol. 5, *Male Reproductive System*, pp. 259–301. Eds R. O. Greep & E. B. Astwood. American Physiological Society, Washington, D.C.

Hammerstedt, R.H & Parks, J.E. (1987) Changes in sperm surfaces associated with epididymal transit. *J. Reprod. Fert., Suppl.* **34,** 133–149.

Hammerstedt, R.H. & Rowan, W.A. (1979) Phosphatidylcholine of blood lipoprotein is the precursor of glycerophosphorylcholine found in seminal plasma. *Biochim. Biophys. Acta* **710,** 370–376.

Hinton, B.T. (1985) Physiological aspects of the blood-epididymis barrier. In *Male Fertility and its Regulation*, pp. 371–382. Eds T. J. Lobl & E. S. E. Hafez. MTP Press, New York.

Hinton, B.T., Snoswell, A.M. & Setchell, B.P. (1979) The concentration of carnitine in the luminal fluid of the testis and epididymis of the rat and some other mammals. *J. Reprod. Fert.* **56,** 105–111.

Hinton, B.T., White, R.W. & Setchell, B.P. (1980) Concentrations of *myo*-inositol in the luminal fluid of the mammalian testis and epididymis. *J. Reprod. Fert.* **58,** 395–399.

Huang, H.F.S. & Johnson, A.D. (1975) Comparative study of protein pattern of epididymal plasma of mouse, rat, rabbit and sheep. *Comp. Biochem. Physiol.* **51B,** 337–341.

Jauhiainen, A. & Vanha-Perttula, T. (1985) Acid and neutral α-glucosidase in the reproductive organs and seminal plasma of the bull. *J. Reprod. Fert.* **74,** 669–680.

Jégou, B., Dacheux, J.L., Garnier, D.H., Terqui, M., Colas, G. & Courot, M. (1979) Biochemical and physiological studies of androgen-binding protein in the reproductive tract of the ram. *J. Reprod. Fert.* **57,** 311–318.

Jones, R., Fournier-Delpech, S. & Willadsen, S.A. (1982) Identification of androgen-dependent proteins synthesized in vitro by the ram epididymis. *Reprod. Nutr. Develop.* **22,** 495–504.

Killian, G.J. & Amann, R.P. (1973) Immunoelectrophoretic characterization of fluid and sperm entering and leaving the bovine epididymis. *Biol. Reprod.* **9,** 489–499.

Klinefelter, G.R. & Amann, R.P. (1980) Metabolism of testosterone by principal and basal cells isolated from the rat epididymal epithelium. *Biol. Reprod.* **22,** 1149–1154.

Lino, B.F. & Braden, A.W.H. (1972) The output of spermatozoa in rams. I. Relationship with testicular output of spermatozoa and the effect of ejaculations. *Aust. J. biol. Sci.* **25,** 351–358.

Nicander, L. (1958) Studies on the regional histology and cytochemistry of the ductus epididymidis in stallions, rams, and bulls. *Acta morph. neerl.-scand.* **1,** 337–362.

Nicander, L. (1979) Fine structure of principal cells in the initial segment of the epididymal duct in the ram. *Zentbl. VetMed. C, Anat. Histol Embryol.* **8,** 318–330.

Nilnophakoon, N. (1978) Histological studies on the regional postnatal differentiation of the epididymis in the ram. *Zentbl. VetMed. C, Anat. Histol. Embryol.* **7,** 253–272.

Orgebin-Crist, M.-C. & Jahad, N. (1978) The maturation of rabbit epididymal spermatozoa in organ culture: inhibition of antiandrogens and inhibitors of ribonucleic acid and protein synthesis. *Endocrinology* **103,** 46–53.

Orgebin-Crist, M.-C., Danzo, B.J. & Davies, J. (1975) Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In *Handbook of Physiology*, Section 7: *Endocrinology*, Vol. 5, *Male Reproductive System*, pp. 319–338. Eds R. O. Greep & E. B. Astwood. American Physiological Society, Washington, D.C.

Ortavant, R. (1953) Existence d'une phase critique dans la maturation épididymaire des spermatozoïdes de belier et de taureau. *C. r. Séanc. Soc. Biol.* **147,** 1552–1556.

Pabst, R. & Schick, P. (1979) Proliferation of epithelial cells of vas deferens and epididymis in young adult rabbits. *Archs Androl.* **2,** 183–186.

Robaire, B. & Hermo, L. (1987) Efferent ducts, epididymis and vas deferens: structure, function and their regulation. In *The Physiology of Reproduction*, (in press). Eds E. Knobil & J. D. Neil. Raven Press, New York.

Robaire, B., Scheer, H. & Hachey, C. (1981) Regulation of epididymal steroid metabolizing enzymes. In *Bioregulators of Reproduction*, pp. 487–498. Eds G. Jagiello & H. J. Vogel. Academic Press, New York.

Schleicher, G., Drews, U., Stumpf, W.E. & Sar, M. (1984) Differential distribution of dihydrotestosterone and estradiol binding sites in the epididymis of the mouse. An autoradiographic study. *Histochemistry* **81,** 139–147.

Sinowatz, F. (1981) Ultrastrukturelle und enzymhistochemische Untersuchungen am Ductus epididymidis des Rindes. *Zentbl. VetMed. C, Anat. Histol. Embryol.*, Suppl. **32,** 1–99.

Sun, E.L. & Flickinger, C.J. (1979) Development of cell types and of regional differences in the postnatal rat epididymis. *Am. J. Anat.* **154,** 27–55.

Tröger, U. (1969) Mikroskopische Untersuchungen zum Afubau des Nebenhodenkopfes beim Stier. *Zentbl. VetMed. A* **16,** 385–399.

Turner, T.T., Jones, C.E., Howards, S.S., Ewing, L.L., Zegeye, B. & Gunsalus, G.L. (1984) On the androgen microenvironment of maturing spermatozoa. *Endocrinology* **115,** 1925–1932.

Voglmayr, J.K. & Amann, R.P. (1973) The distribution of free myo-inositol in fluids, spermatozoa, and tissues of the bull genital tract and observations on its uptake by the rabbit epididymis. *Biol. Reprod.* **8,** 504–513.

Voglmayr, J.K., Musto, N.A., Saksena, S.K., Brown-Woodman, P.D.C., Marley, P.B. & White, I.G. (1977) Characteristics of semen collected from the cauda epididymidis of conscious rams. *J. Reprod. Fert.* **49,** 245–251.

von Lanz, T. (1924) Der Nebenhoden einiger Saugetiere als Samenspeicher. *Anat. Anz.* **58,** 106–109.

Wagley, L.M., Versluis, T.D., Brown, D.V. & Amann, R.P. (1984) Culture of principal cells from the ram epididymis. A comparison of the morphology of principal cells in culture and in situ. *J. Androl.* **5,** 389–408.

Waites, G.M.H. (1977) Fluid secretion. In *The Testis*, Vol. 4, pp. 91–123. Eds A. D. Johnson & W. R. Gomes. Academic Press, New York.