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Review Article: Structure and Function of the Epididymis

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Summary. Testicular spermatozoa are functionally immature in that they cannot fertilize ova. It was first demonstrated by Young [171, 172] that spermatozoa undergo certain changes as they migrate through the epididymis. He proposed that spermatozoa ripen during epididymal transit. It is now known that specific maturational changes occur in spermatozoa during epididymal transit which result in their developing the ability to fertilize ova. Concomitant with this functional maturity are changes in spermatozoal morphology, motility, chemistry, permeability, density and metabolism. It is apparent that in some way not understood these changes are necessary for sperm to achieve the ability to complete the fertilization process. When these mechanisms are understood, we may be able to effectively treat conditions such as necrospermia or abnormally low sperm motility. Furthermore, with the development of the hamster-egg penetration test a "new" type of male infertility has become evident in recent years; the inability of otherwise normal sperm to penetrate an ovum. It is during epididymal transit that this ability is normally acquired. Thus, any insight into how sperm attain the capacity to penetrate an ovum could lead to an effective treatment of patients whose sperm do not have this ability. In addition, the epididymis holds significant promise as the site of action for a male contraceptive. Thus, it is the purpose of this review to describe the structure and function of the mammalian epididymis with particular emphasis on the factors regulating sperm maturation.

Key words: Epididymis, Sperm maturation, Sperm transport, Male contraception, Epididymal plasma.

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Transport of Spermatozoa

The epididymis is a highly convoluted duct which receives spermatozoa from the ductuli efferentes of the testis and conducts them to the more distal vas deferens. It is some 3 to 4 meters long in man [159] while in the bovine it may cover a total length of 40 meters [94]. The epididymis is generally divided into the caput epididymis (head), corpus epididymis (body), and cauda epididymis (tail). However, it is often subdivided into many regions possessing their own distinct epithelial cell types [94, 141]. Detailed examinations of epididymal histology have been extensively described elsewhere [11, 16, 141] and will not be addressed here.

Sperm motility is minimal or non-existent in the testis and proximal epididymis, thus it is apparent that spermatozoa do not contribute to their movement through these structures.

Contractions of the testicular capsule (tunica albuginea) appear to play an important role in the transport of sperm out of the testis [38, 59]. Autonomic innervation to the testicular capsule has been described in the rat by Bell and McLean [17], the rabbit by Hodson [87], and the cat and dog by Leeson and Cookson [115]. Indeed, some investigators [39, 76, 78, 147] have demonstrated that catecholamines stimulate rabbit testicular capsule contractions both in vivo and in vitro. Furthermore, the α-adrenergic blocking agent, dibenamine inhibited this response to catecholamine in vitro. However, since this later response was not seen in vivo and since dibenamine did not alter the spontaneous capsular contractions in vitro, the role of adrenergic innervation of the testicular capsule is still unclear. This suggests another mechanism controlling the spontaneous capsular contractions.

Endogenous prostaglandins of both the F and E series have been shown to exist in the testicular capsule of rats [66] with $PFG_{2\alpha}$ in predominance and in the testes of rabbits [77]. While $PGF_{2\alpha}$ causes a marked increase of rat [59] and rabbit [78, 79, 147] testicular capsule contractions, it was found that PGE abolishes this

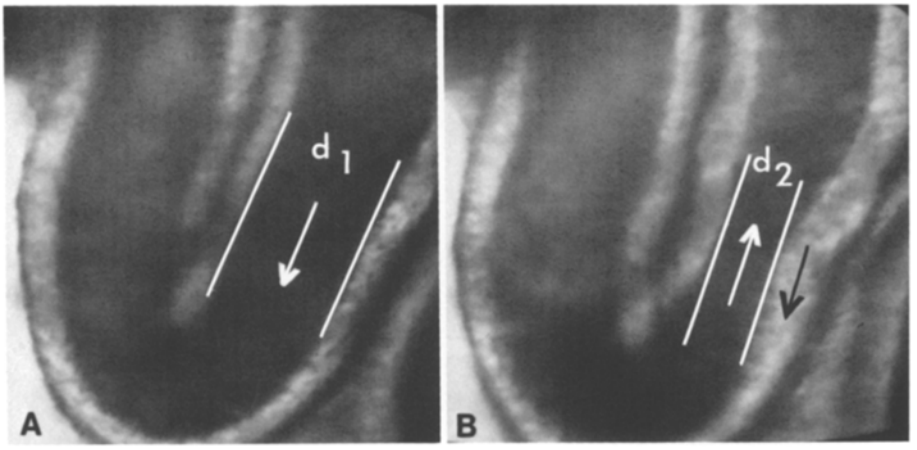

Fig. 1A, B. A segment of rat caput epididymis as seen on the monitor of a videomicrography system (135 x). The tubule is seen in the relaxed state (A) and with a wave of contraction passing through it (B). The resting luminal diameter was determined by measuring distance d_1 (A). The amplitude of contraction was determined by subtracting d_2 (B) from d_1 (A). *White arrows* indicate direction of luminal content movement at the time of photographs. *Black arrow* indicates direction of peristaltic wave movement (From Cosentino et al. 1984c)

response. Furthermore, indomethacin, a potent inhibitor of PG synthesis strongly inhibited spontaneous capsular contractions. Thus, strong evidence for prostaglandin control of testicular capsule contractions exists. It is probable that PGs and the autonomic nervous system in these species work together to control the tonus of the testicular capsule. Indeed it has been shown that sympathetic denervation of the rat internal genitalia affects PG levels in those tissues probably by altering activity of the enzymes responsible for PG biosynthesis [30].

Passage of spermatozoa through the ductuli efferentes into the caput epididymis is thought to be due to both contraction of the myoid cells surrounding these structures and to the activity of the motile cilia which extend into their lumens [143, 144]. Similarly, transport through the epididymis is probably due to contractile activity in the ductal wall. It has been shown that epididymal transport of spermatozoa takes place even in castrated animals or those with ligated ductuli efferentes [120]. This same study noted that in the proximal and middle regions of the rat epididymis, localized contractions in vivo have the ability to transport radiopaque materials. These contractions occur rythmically at a rate that is higher in the proximal regions than it is in the more distal regions [155] and first appear in the caput epididymides of 48 day old rats but do not show up in the cauda epididymides until three or four days later [143]. Specifically, the amount of time required for spermatozoa to pass through the length of the epididymis has been found to be 3–15 days depending on the species [2, 4, 5, 50]. Until recently nothing was known regarding factors that control contractions of the proximal epididymides and thus about sperm transport through this organ. One of the obstacles to finding these regulators was the lack of a technique to quantitatively assess proximal epididymal tubule contractions. However, we have recently utilized a videomicrographic system to measure the meachnical activity of rat caput epididymidis in vitro [33]. Using this system we noted the effects of PGs and of aspirin on caput epididymidis contractility by measuring the frequency of contraction, luminal diameter and amplitude of contraction at various concentrations of each test compound in vitro (Fig. 1). In these studies we noted that $PGF_{2\alpha}$ stimulated contractility of the tubules at physiological concentrations, while PGE_2 reduced contractility. Furthermore, aspirin which inhibits the biosynthesis of PGs, strongly inhibited the spontaneous contractility of the epididymidis. Thus, PGs appear to be important regulators of sperm transport throught the rat caput epididymidis.

It is well documented that the smooth muscle in the wall of the epididymal tubule increases in thickness from proximal to distal regions [12, 55]. Furthermore, regional differences in the sympathetic innervation of the tubule walls occur [132, 153]. In the proximal portion of the epididymis adrenergic neurons are sparsely distributed and terminate only on blood vessels. However, these same studies showed a high density of adrenergic neurons terminating on the smooth muscle cells surrounding the cauda epididymis and vas deferens. It is generally believed that this arrangement allows for a selective emptying of the cauda epididymis through the vas deferens at the time of ejaculation. In support of this, Amman [2] showed that in many species epididymal transport of sperm from the caput through the corpus was independent of the frequency of ejaculation. Recently, the contractions of the cauda epididymis have been found to be under the influence of not only sympathetic innervation [83, 113, 153] but also of vasopressin [98], oxytocin [84, 123] and PGs [36, 37, 85]. Indeed, the stimulating effects of norepinephrine, acetycholine and testosterone on distal epididymal contractions appears to be modulated by PGs. Thus, the control of epididymal contractions is partially neurogenic but is also under the influence of various other factors.

Role of the Epididymis

The epididymis has four basic functions a) it serves as a sperm reservoir; b) it disposes of old and superfluous sperm; c) it is the site of spermatozoal maturation; and d) it is responsible for the composition of the fluid surrounding epididymal spermatozoa.

The epididymis serves as a highly efficient storage and preservation site for sperm. Extra-testicular spermatozoa are primarily stored in the epididymis with approximately two-thrids located in the cauda epididymides of man [4] where they remain for 2 to 5 days. Similarly, in the rabbit Robb et al. [145] have shown nearly 60% of the extra-testicular sperm were present in the cauda with approximately 40% in the caput-corpus region. Furthermore, it has been found [46] that the storage capacity of the boar epididymis is sufficiently large to accommodate spermatozoa that have been produced in the testes over a period of three weeks.

The extent of superfluous sperm elimination by the epididymis is still unclear. Uptake by epididymal epithelial cells and subsequent destruction by macrophages is thought by some to be responsible for the elimination of old or deteriorated sperm, but to what extent this accounts for the difference between daily sperm production and sperm output its unknown [3, 88]. Phagocytosis of spermatozoa by macrophages is commonly seen in sperm granulomas caused by extravasation of semen from a ruptured epididymidal tubule. Similarly, in rams with epididymitis, phagocytosis of whole sperm and sperm parts by polymorphonuclear neutrophils can be seen (personal observations). In a normal epididymis, the epithelium probably phagocytizes very few sperm as this is rarely seen histologically. However, after androgen withdrawal the epididymal spermatozoa disintegrate rapidly by a yet unknown mechanism [106] which may involve the epididymal epithelial cells.

The loss of spermatozoa via the urine (spermaturia) can be significant, especially in the case of prolonged sexual rest. However, various reports on the extent of this loss are not in agreement. For example, in one estimate [118] urine collected from secaully rested rams contained an average of about 6×10^9 spermatozoa/day, while another study [18] showed ram spermaturia to be only 0.2×10^9 spermatozoa/day. A more recent study involving cannulation of the ram urinary bladder has shown the daily loss of spermatozoa into the urine approximates to the data of Bielanski and Wierzbowski [157]. These data indicate that the vesical sphincter, at least in the ram, is a rather ineffective barrier during sexual rest and allows escape of spermatozoa into the urinary bladder.

Sperm Maturation

As sperm migrate through the epididymis, changes occur in both the spermatozoa and the fluids surrounding them. Among the changes occurring in the spermatozoa are increased motility [15] and increased fertility potential [15, 116, 172]. These two important properties of spermatozoa are largely the result of interactions with the epididymal epithelium and the epididymal plasma [68], but, to some extent they also depend on processes inherent in the spermatozoa themselves. This was demonstrated by ligation of the rabbit epididymis at various locations [133] which showed that fertilizing ability is acquired in the distal half of the corpus epididymis. This same study, however, showed that sperm motility was acquired even in sperm retained in the proximal caput epididymis but that this "intrinsic" development was not a forward progressive motility. Thus, it appears that for qualitative changes in motility the spermatozoa must be exposed to the more distal regions of the epididymis. A study of men undergoing vasoepididymostomy demonstrated that when the vas deferens was anastomosed to the proximal epididymis the ejaculated sperm were immotile [58].

Considerable species variation occurs in the attainment of epididymal sperm motility. In the rat, there is no motility of spermatozoa collected from the testis or at any point along the epididymis while in their native fluids. However, when diluted in a buffered solution the motility increases to an extent dependent on their site of collection [158]. Specifically, testicular sperm diluted in this manner show, at most, slight vibration of the tail, while sperm from the caput epididymidis show a disoriented, circular swimming. Those sperm collected from the cauda epididymidis and treated in this manner exhibited forward progressive movement. In humans, it has been shown that caput sperm display no progressive motility while 34% of the cauda sperm do have a forward progressive movement [124]. However, a study by Turner and Howards [159] indicates that human sperm from the cauda epididymidis are not motile, nor do they increase motility if diluted in cauda epididymal fluid. However, if diluted with saline or buffers they exhibit vigorous forward motility. Therefore, epididymal fluid apparently contains a motility inhibiting factor which may serve to conserve the energy stores of the sperm. A similar, more recent study [127] confirms that when diluted in an appropriate buffer (B.W.W.) there was a significantly higher proportion of human spermatozoa showing sustained forward movement in samples recovered from the cauda or corpus epididymidis (Fig. 2). Thus, it is clear that the capacity for progressive forward motility of spermatozoa develops during epididymal transit.

In the bull, it has been demonstrated [64, 65] that cauda epididymal sperm treated with dibutryl cAMP or cyclic nucleotide phosphodiesterase inhibitors (e.g. caffeine or theophylline) exhibited a significantly higher motility. Similarly, spermatozoa from the hamster cauda epididymidis can be changed from immotile to highly motile when diluted in a medium containing cAMP [129]. Under conditions in vivo, an increase in the cAMP content of sperm during passage through the epididymis combined with a forward motility protein (FMP) produced there is believed responsible for the full development of motility [95].

The epididymal spermatozoa of the hamster [91] and the mouse [90] are not able to penetrate eggs until they have reached the cauda epididymis. Typically, in the pig, the fertilization rate using caput epididymal sperm (via artificial insemination) is low but improves significantly when the sperm used is obtained from the cauda epididymidis [89]. In addition, the percent of surviving embryos and

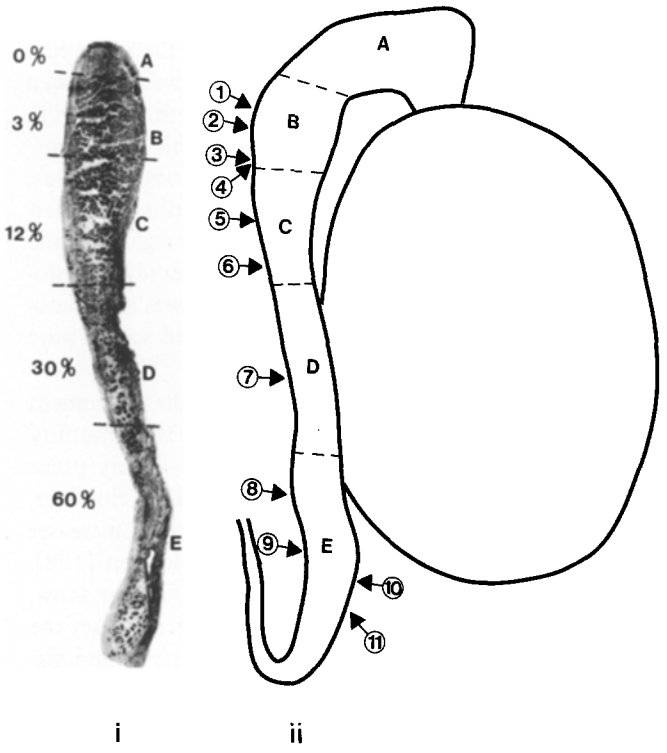

Fig. 2. (*i*) A longitudinal section of a human epididymis, (4.0x). The percentage of motile spermatozoa recovered from each region is indicated. *A*, efferent ducts; *B* caput; *C*, proximal corpus; *D*, distal corpus; *E*, cauda. (*ii*) Diagram showing the most distal site of each epididymal samplex (From Moore et al. 1983 by permission)

litter size is dramatically less when fertilization was induced with sperm from the proximal epididymis as compared with those of the distal epididymidis. This suggests not only an increase in the fertility potential of sperm as they migrate through the epididymis, but also an increased quality to the ensuing pregnancies.

Recently, it has been demonstrated that spermatozoa obtained from the cauda epididymidis of humans were capable of penetrating zona-free hamster eggs while those from the corpus and caput epididymidis were not. This was noted in patients undergoing surgery for testicular cysts or cancer [86] and in fertile men undergoing vasectomy [127]. The development of this functional competency is probably related to the development of progressive development since spermatozoa require a high degree of thrust to penetrate the zona pellucids of an intact ova in vivo [126].

Morphological alterations in spermatozoa during maturation in the epididymis are much less dramatic in man than they are in various other species. The important changes in the physiology of sperm as they pass through the epididymis are not paralleled by much change in sperm morphology. One of the most notable changes in spermatozoa undergoing maturation is that of migration of the cytoplasmic droplet. It represents a cytoplasmic remnant of the residual body from which the spermatid was separated during spermiation [54]. The droplet is most notable in testicular sperm where it is located at the anterior region of the midpiece. By the time sperm have been transported to the cauda epididymidis this droplet has migrated to the more distal annular region and is subsequently sloughed off from the sperm altogether. The role of the protoplasmic droplet and its inttriguing migration is unknown. However, it has been demonstrated [42] that these droplets have strong similarities (biochemical and structural) to lysosomes. It may be that the interactions of the lysosomal-like cytoplasmic droplet with the sperm membrane surrounding the mitochondria prepare these organelles for activity.

The only other significant morphological change to occur in sperm passing through the epididymis involves the acrosome. This was first reported by Mukherjee and Bhattacharya [130] who described the average size of the sperm head in the caput epididymis to be larger than that of ejaculated sperm in sheep, goats and buffalo. More recently, this has been described using electron microscopy as a reduction in the size of the acrosome [101]. One of the species showing more extreme changes in the acrosome during sperm maturation is the guinea pig [53]. In this species the acrosome of testicular sperm appears flattened and large while the mature sperm of the cauda epididymidis takes on a rounded, smaller morphology. In man, however, the shape of the acrosome of a late spermatid is very similar to that of a spermatozoa found in the vas deferens [15].

An additional aspect of sperm maturation is a change in the thermal stability of the cell. This was reported in the work of Mann [121] who showed that mature sperm show a shift of the DNA melting curve to the right as compared to immature sperm. Conversely, however, the susceptibility of spermatozoa to cold shock was found to be greater with the mature epididymal sperm in this same study. That is, the loss of fertilizing ability and motility induced by rapid cooling is a phenomenon attributed to permeability changes in the plasma membrane and acrosome of these cells. Modification of the spermatozoal plasma membrane during epididymal transit have been demonstrated using various techniques including electron spin resonance [73] and the use of plant lectins [131]. It was found that spermatozoa from the caput epididymidis agglutinate much more readily in the presence of wheat germ agglutinin than do those from the cauda epididymidis. This could be related to the decrease of lipoprotein content of epididymal spermatozoa as they mature [114] (see below).

During maturation of the epididymal sperm nucleus there is an alteration in the amount of nucelar protein cross-linking [23]. Indeed, by labelling the sulfhydryl groups of proteinbound cysteine in the sperm nucleus, it has been shown that nuclear protein in the rete testis spermatozoa has its cysteine residues predominately in the reduced (SH) from [122]. However, in the cauda, most of it is in the disulfide (S-S) state. These observations agree with the general concept that chromatin is stabilized in the sperm nucleus during maturation in the epididymis and that this stabilization is due to progressive formation of disulfide linkages among the nuclear proteins. This cross-linkage may be the reason that Feulgen's stain is taken up more rapidly by testi-

cular spermatozoa thean epididymal spermatozoa [49]. Furthermore, in the same study, it was noted that Feulgen staining was enhanced in epididymal sperm treated with dithiothreitol which reduces the disulfide linkages.

As previously mentioned an increasing capacity for forward progressive motility is an important feature of sperm maturation in the epididymis. It has been reported that rat spermatozoa obtained from the caput epididymis swims in a circular pattern, while those obtained from the cauda epididymis display a forward progressive movement [58]. This is apparently due to concomitant stiffening of the spermatozoal membrane and, thus, the mature sperm develop a more rigid type of movement. Maturing human spermatozoa do not go through a circular phase of movement. Indeed, sperm from the caput epididymis exhibit motility potentials ranging from immotility to a vigorous, wild thrashing of a highly flexible tail. Rapid forward progression occurs only with a reduction of the tail arch which appears first in the middle corpus epididymis in a few spermatozoa and is the dominant pattern of movement in the cauda epididymis and vas deferens [15]. It if of particular interest in this respect that the sperm tail elements begin to form disulfide linkage as they pass through the epididymis [23]. This may well be the "stiffening" factor responsible for the less flexible tail of cauda epididymal sperm described by Fray et al. [58].

With all the changes noted herein it is difficult to determine whether a given morphological or biochemical change observed in the spermatozoa during epididymal tenure is indeed a part of the maturation process of is merely occurring coincidentally.

Fluid Reabsorption and Ion Transport in the Epididymis

Due to the particularly high reabsorption capacity of the epididymis, much of the water making up the testicular fluid is moved through the epithelial cells into the epididymal interstitial spaces. The mechanism by which this occurs is believed to be the reverse of what occurs in the sertoli cells which produces the rete testis fluid [150]. That is, highly selective ion pumps move sodium out of the epididymal lumen increasing the interstitial osmolarity [34, 47, 105, 164, 166]. Hence, water moves down the resulting osmotic gradient into the interstitium. Specifically, the ion balance of the rete testis fluid is such that a high Na^+ and a low K^+ concentration exists as is the case in blood plasma. However, fluid from the cauda epididymis exhibits the reverse ionic balance [169]. Here, the Na^+ concentration is somewhat less than blood plasma while the K^+ levels are many times greater than that found in blood [149]. These changes in ion concentration and their ratios distal to the testis are believed to be responsible for the osmotic gradient which draws large amounts of water from the epididymal lumen. Indeed, it has been shown [29] that about 34 ml of testicular fluid enters the epididymis of the mature ram daily, while only about 0.9 ml per day enters the vas deferens. This balance of electrolytes and water content in the epididymal lumen has been demonstrated to be at least in part, regulated by aldosterone [167]. Furthermore, since Wong and Lee [168] have also noted that high K^+ levels in fluid surrounding epididymal sperm will inhibit their motility, it would be of interest to examine what effect drugs that alter aldosterone activity might have on the male reproductive capacity. These kinds of studies might lead to the long-sought male contraceptive.

In addition to reabsorbing a great volume of testicular fluid at the proximal caput epididymis, a change in the pH of epididymal fluids is also observed. In the case of the rat, for example, the pH of testicular fluid is 7.4 while that of the proximal epididymis is 6.6 [116]. Similarly, studies in the ram [29] indicate a significant acidification of fluid leaving the rete testis (pH 7.81) upon arrival in the cauda epididymis (pH 6.05) Cohen et al. [27] demonstrated that the epididymal epithilium possesses carbonic anhydrase activity which may be responsible for transport of H^+ or HCO_3^-. Since this enzyme is responsible for luminal acidification in the kidney cortex [67] its presence is consistent with a decrease in the pH of epididymal fluid. In this respect it is interesting that acetazolamide (a carbonic anhydrase inhibitor) decreases the rete testis fluid accumulation in the rat [148] while at the same time, inhibits the acidification of epididymal fluids [8] suggesting that this enzyme is also involved in the secretion and/or reabsorption of fluid in the male reproductive tract. In addition, H^+ ions are known to be released from the spermatozoa during the initiation of motility [170] which may further lower the luminal pH in the cauda epididymis and vas deferens. Even with this knowledge of ion transport and fluid reabsorption in the epididymis the physiological significance of these occurrences remains unclear.

Androgens and the Epididymis

The epididymal epithelium, like that of other secretory epithelia in the male reproductive tract, is dependent on relatively high concentrations of androgens for normal function. This dependence includes, but is not restricted to, such functions as secretory activity, fluid reabsorption and cytological integrity. Indeed, removal of androgens from an organism by castration or hypophysectomy adversely affects these functions [20, 72, 75, 102, 125, 136, 151]. In addition to these functional changes, there are few constituents of the epididymal plasma that are not dramatically altered by androgen withdrawal (see subsequent section). As will be seen, castration reduces the amount of small organis molecules such as prostaglandins and phospholipids along with the large organic constituents (proteins). The only epididymal parameter which does not change after castration is the osmotic pressure of the epididymal plasma. This, apparently, is due to the large post-castration increase

of luminal Na^+ and $Cl^=$ content which compensates osmotically for the dramatic loss of organic molecules from the epididymal plasma [103, 104].

Most of the changes incurred by castration can be readily reversed to normal (or near normal) with androgen administration or in the case of hypophysectomy, with gonadotropin administration. However, the rate and extent of reversal depends on the parameter being studied. An example of this is described by Brooks (1979) who noticed a rapid post-castrational return to normal activity of specific mitochondrial enzymes in the epididymis after androgen supplementation. However, a much longer time was required before epididymal weight reached relatively normal values.

After castration the epididymal spermatozoa are incapable of maturing and rapidly degenerate [134]. It is still unclear if this effect is due to loss of direct contact of androgens with the spermatozoa or an indirect effect of a degenerating epididymal epithelium. The importance of androgens in the epididymal plasma is, however, apparent. It was shown [135] that when the proximal corpus epididymidis was cultured in vitro with 5α-dihydrotestosterone previously infertile sperm became fertile. Furthermore, this response was eliminated in the presence of anti-androgens.

Testosterone enters the epididymis by two principal routes: (1) circulation and (2) testicular fluids. Uptake from the circulation, occurs via the epididymal epithelium. In the cytoplasm of the cells testosterone is primarily metabolized to 5α-dihydrotestosterone (DTH) by the enzyme 5α-reductase. This enzyme has been characterized, in epididymal tissue and in many other androgen sensitive tissues [41, 69]. After conversion to DHT the steroid binds to soluble cytoplasmic receptors in the epithelial cells and is transported to the nucleus where it binds nuclear chromatin, presumably to affect protein synthesis by the classical steroid-receptor mechanism. Some of the intercellular DHT then diffuses into the epididymal plasma where it accumulates in relatively high concentrations [41, 163].

The testosterone which enters the epididymis in the testicular fluid is associated with an androgen binding protein (ABP). This protein is secreted from the Sertoli cells of the seminiferous tubules [60, 74] and appears to be responsive to FSH and testosterone for both its synthesis and release [110, 119]. Secretion of ABP appears to be bidirectional [71]. That is, it is released into both the adlumenal and into the basal compartments established by the specialized junctions of adjacent Sertoli cells. Basal compartment secretion is taken up by the circulation where it has a biological half-life of 18–19 h [14]. Adluminal compartment secretion is released into the seminiferous tubules and makes its way, with the sperm, into the caput epididymidis [137, 156]. Here, immunohistological studies have shown ABP to be transported into the cytoplasm of the caput epididymal epithelium to serve some unknown function [137]. It has been suggested, however, that ABP may play an important role in maintaining the high concentration of androgens necessary for normal function of the proximal epididymidis [9].

Epididymal Plasma

It is beyond the scope of this article to describe all the epididymal plasma constituents. A comprehensive compiltation of its contents in various species may be found in the work of Jones [103]. In general, however, the epididymal plasma contains a wide range of ions in addition to organic constituents such as sialomuco-proteins, lipoproteins, enzymes and lipids. These substances are not all produced in the epididymal epithelium. Indeed, many are absorbed from the circulation or enter the epididymis from the testis.

Of the characteristic constituents of the epididymis the lipids and phospholipids may be of particular interest. The human epididymis has been shown to contain 64% of its total lipids as phospholipids with the remaining portion being made up to neutral lipids [152]. The distribution of these compounds is of particular significance. That is, the phospholipid content of spermatozoa declines as they move through the epididymis not only in the bull [139] but also in the boar [51] and in the ram [35, 52, 149]. Furthermore, bull spermatozoa [139] lose large amounts of arachidonic acid as they move through the male reproductive tract. Similarly, Arares et al. [6] reported a drop in the arachidonic acid-containing phospholipids of spermatozoa as they pass through the epididymis of the Rhesus monkey.

Glycerylphosphorylcholine (GPC) was first described to be synthesized in the epididymis by Dawson et al. [40]. More recently, it has been shown to accumulate in the distal regions of the organ in relatively high concentrations [22, 142]. Furthermore, it has been demonstrated [61] that 50% of the epididymal GPC is associated with spermatozoa. This is supported by the data of Killian and Chapman [108] and Wang et al. [165] who showed that GPC is synthesized from phosphatidyl-choline incorporated into both the sperm membrane and the principal cells of the epididymis. The role of this highly concentrated glycerophaosphate in the epididymis is still, however, a matter of speculation. One of the more popular theories is that GPC may form a protective coating on the sperm plasma membrane [7] which in turn interferes with denaturation due to thermal or proteolytic insults.

Because of the loss of phosphatidylcholine and arachidonic acid with the concomitant increase in GPC concentration as noted above, one might consider these phenomena to be related. In further support of this interdependency is the data of Wang et al. [165]. These investigators have demonstrated that [^{14}C]-phosphatidylcholine was rapidly converted to [^{14}C]-GPC by both epididymal sperm and epididymal principal cells. They concluded the conversion was likely due to phospholipase(s). Thus, the role of phospholipase may be for the formation of GPC. However, some workers have suggested the role of phospholipase A to be that of providing phospholipid-derived fatty acids as a source of metabolic energy for the sperm [139]. That PLA_2 is not only found in a post mitochondrial preparation of epididymal epithelium but that it is also under

androgen control has been demonstrated [13, 19]. In addition and of particular interest is the role of phospholipase A_2 in providing arachidonic acid for prostaglandin (PG) biosynthesis [56, 112, 161, 162].

Epididymal Prostaglandins

Considerable work has recently been done on PGs in human semen (For review see [31]). The focus on PGs in seminal plasma is due to the fact that human semen is the richest naturally occurring source of these compounds with the primary source being the seminal vesicles. Analyses of the role of PGs in male fertility has been confusing primarily due to variations of extraction techniques and the relatively low numbers of subjects studied. Indeed, seminal PGs are significantly correlated with the seminal concentrations of various divalent metal ions such as Ca^{++} and Zn^{++}. But the only physiological parameter that is interdependent with PGs (i.e. PGE) is sperm motility. This is of particular interest since it is in the epididymis that sperm acquire their capacity for progressive motility.

However, few studies have been done on the levels, or role of PGs in the epididymis. That these levels changed throughout the epididymis of rats and mice was shown by Cosentino et al., [33] and Gerozissis and Dray [66]. Similarly, both PGE and $PGF_{2\alpha}$ have been collected from various regions of the ram reproductive tract including the epididymis [30]. These studies demonstrate a significantly greater quantity of PGE and PGF in the cauda epididymis when compared to the caput area. In addition, an even greater amount was found in the vas deferens. A series of studies [10, 43], have, shown that the levels of PGs in the male reproductive tract are at least in part, under the control of androgens. That PG's appear to be important regulators of sperm transport through the proximal epididymis of rats [33] has been discussed above (see Transport of Spermatozoa) with the physiologically dominant compound being $PGF_{2\alpha}$.

It has been suggested that epididymal PGs are probably formed from the arachidonic acid lost from spermatozoa as they travel through the epididymis [139, 160]. Indeed, that PGs are important for sperm maturation can only be surmised by the increase in that section of the epididymis where sperm become mature. Thus, PGs may play a functional role in spermatozoal maturation [48, 66] in addition to sperm transport.

Cyclic Nucleotides and Epididymal Spermatozoa

It is rapidly becoming apparent that cyclic nucleotides exert a (more or less) direct control over the motility potential of epididymal sperm [93, 95]. Data from a number of observations by various groups have been presented to support the concept of modulation of sperm motility by cAMP [24, 25, 44, 63, 65, 92]. One approach to the analysis of the regulation of motility has been with the use of spermatozoa whose membranes have been male permeable to cAMP [117]. This study showed an increase in spermatozoal motility in the presence of extracellular cAMP. Using disrupted sperm cells, nearly all of the components making up the "second messenger" system have been identified in testicular, epididymal and ejaculated spermatozoa [24, 154]. These include: cyclic nucleotide phosphodiesterase; cAMP-stimulated protein kinase; adenylate cyclase; and, of course, cyclic nucleotides [62].

Another approach to discerning the relationship between cAMP and sperm motility lies in the studies utilizing intact sperm form stages of maturation before and after the natural onset of motility potential. As described above, sperm do not acquire motility potential until they reach the distal regions of the epididymis. In the bull [24] it has been shown that sperm isolated from the testes do not attain motility when incubated in the presence of phosphodiesterase inhibitors even though the cellular cAMP doubles with this treatment. However, when sperm isolated from the distal caput epididymidis [1] and cauda epididymidis [24, 64] are treated to raise their cAMP levels they readily attain motility. In addition, ejaculated bovine sperm motility was both enhanced and maintained by phosphodiesterase inhibitors. Thus, it was concluded that during epididymal transit, sperm undergo changes that subsequently allow them to respond to increased cAMP with progressive motility.

The mechanism by which cAMP affects sperm motility is still unclear. However, in some tissues, enyzmes that regulate cyclic nucleotide metabolism are known to be regulated by a Ca^{++} dependent regulating protein known as calmodulin [26, 107]. It has been found that Ca^{++} causes an elevation of cAMP concentration in the spermatozoa of hamsters [128] and guinea pigs [97]. Futhermore, the result was found to be due to a Ca^{++} dependent increase in sperm adenylate cyclase activity [96]. This effect of Ca^{++} was eliminated when a known inhibitor of calmodulin was added to the incubate. Thus, it would appear that Ca^{++} bound to calmodulin raises the cAMP level of sperm. It is of particular interest, in this respect, that Ca^{++} uptake into spermatozoa is inhibited by theophylline and exogenous dibutyryl cAMP [138]. Thus, increased sperm levels of cAMP decrease the amount of Ca^{++} taken into the cell. These later two studies taken together suggest a possible local negative feedback mechanism of Ca^{++} uptake into the sperm. That is, increased levels of Ca^{++} binding to calmodulin and stimulating adenyl cyclase would elevate spermatozoal cAMP which, in turn, would regulate the amount of Ca^{++} entering the cell. Another possibility is that PGE mediates the uptake of CA^{++} into a sperm cell.

Notably, Ca^{++} uptake into the prejunctional neurons of adrenergically innervated issue is under the control of PGE in various tissues [80, 81, 99]. Indeed, these studies suggest that the PGE interferes with norepinephrine release by decreasing the availability of calcium for the release mechanism, possibly by closure of the Ca^{++} gates in the axonal mem-

brane. Thus, it may be that PGE (the predominant PG in the male reproductive tract) controls Ca^{++} entry by increasing the sperm cAMP (see below). These proposals are speculative, but raise possible directions for further research.

That there are opposing effects on the control of cellular function by cAMP and cGMP has been known for some time [70]. This Yin Yang hypothesis provides some insight into the different effects of the E and F series of prostaglandins at a given time or on a particular cell type. For example, Dunham et al. [45] demonstrated that $PGF_{2\alpha}$ initiated contraction of both bovine and canine venous tissue and that these contractions were coincidental with an increase of smooth muscle cGMP levels relative to the cAMP levels. In opposition, PGE_2 causes relaxation of the venous smooth muscle with a concomitant rise in cAMP levles. Thus, in at least some tissues PGE_2 is associated with increased cAMP concentrations and a given physiological response, while $PGF_{2\alpha}$ is associated with increased cGMP levels and opposite physiological response (for review see [111]). Indeed, testicular interstitial cells [28] showed, as did whole testes [82, 109] a similar increase of cAMP levels in response to PGE_1 and PGE_2. That his response is mediated by membrane receptors with high specificity and low binding capacity was shown by Sebokova and Kolena [146]. Of particular interest is recent work using washed sperm from various parts of the ram reproductive tract [30]. This study indicated that when incubated in the presence of exogenous PGE_2, only sperm that had passed through the epididymis showed elevation of cAMP levels. This suggests that spermatozoa respond to PGE_2 with an increased cAMP level only after they have undergone epididymal transit, and may define another criterion of sperm maturation.

The Epididymis and Contraception in the Male

Recently, the approach toward a male contraceptive via alteration of epididymal function has become an important area of research. This approach is attractive for two reasons. First, the latent period of the onset of infertility and of its reversal would be relatively short (about 2 weeks) for such an agent acting on the epididymis. Secondly, by not affecting testicular function such an agent has a greater possibility of being reversible and minimizing adverse genetic effects, since all cellular divisions have occurred prior to the time sperm enter the epididymides. Previous testing of several compounds has been undertaken in an attempt to alter epididymal function. Alpha-chlorhydrins were first, then aminochlorhydrins and lastly the chlorinated carbohydrates were examined. The mechanims by which these compounds produce their effect has not been clearly defined, but they probably interfere with the normal carbohydrate metabolism in the epididymal epithelium [57]. Unfortunately, these compounds produce serious systemic toxic effects and no one has produced suitable nontoxic analogues of them [100]. Until the recent discovery that at least one sulfonamide compound (sulfasalazine) probably affects human male fertility at the level of the epididymides in a reversible manner [32], no lead on a preparation to safely alter male reproductive capacity at the level of the epididymides has been found. This new knowledge along with the fact that a number of sulfonamide compounds are already approved for human use with established low toxicity warrants immediate screening of these compounds in the hope of finding the most effective and safe preparation for the purpose of reversible male contraception. Such studies are currenlty underway in many parts of the world.

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