Journal of Andrology, Vol. 25, No. 1, January/February 2004

Postnatal Development and Regulation of β-Hexosaminidase in Epithelial Cells of the Rat Epididymis

LOUIS HERMO,* HUZAIFA I. ADAMALI,* AND JACQUETTA M. TRASLER†

*From the *Department of Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada; and the †Departments of Pharmacology and Therapeutics, Human Genetics and Montreal Children's Hospital Research Institute, McGill University, Montreal, Quebec, Canada.*

ABSTRACT: β-Hexosaminidase (Hex) catalyzes the hydrolysis of terminal sugar residues from a number of substrates such as GM2 gangliosides, glycoproteins, glycolipids, and glycosaminoglycans. As an enzyme present in lysosomes of epithelial cells of the adult rat epididymis, it serves to degrade substances endocytosed from the epididymal lumen. In this way, it modifies and creates a luminal environment where sperm can undergo their maturational modifications. In this study, the postnatal developmental pattern of expression of Hex was examined in animals from days 7–56. In addition, the role of testicular factors on Hex expression in the different cell types and regions of the epididymis of adult rats was examined in orchidectomized and efferent duct–ligated rats. Both parameters were examined on Bouin-fixed epididymides in conjunction with light microscope immunocytochemistry. At postnatal day 7, the epithelium of the entire epididymis was unreactive for anti-Hex antibody. By day 21, narrow and clear cells of their respective regions became reactive, whereas basal cells became reactive only by day 29. Principal cells displayed only an occasional reactive lysosome at day 21, several by day 29, and numerous reactive lysosomes by day 39, comparable to the region-specific distribution noted for 90-day-old animals, and at an age when high androgen levels are attained. Thus, postnatal onset of Hex expression varies according to the different cell types of the epididymis, suggesting different regulatory factors. This finding was confirmed from studies employing adult orchidectomized and efferent duct–ligated adult rats. Indeed, in all experimental animals, Hex immunostaining in narrow, clear, and basal cells was intense and comparable to control animals. In contrast, there was a notable absence of lysosomal staining in principal cells at all time points after orchidectomy, which was restored, however, following testosterone replacement. No effect on Hex expression was observed in efferent duct–ligated animals. Taken together, the data suggest that Hex expression in lysosomes of principal cells is regulated by testosterone or one of its metabolites. However, the expression of Hex being independent of testicular factors in narrow, clear, and basal cells of adult animals, but occurring at different time points during postnatal development, suggests that different regulatory factors are responsible for onset of Hex expression in these cell types during development.

Key words: Principal, narrow, clear, basal cells, lysosomes, orchidectomy, efferent duct ligation.

J Androl 2004;25:69–81

Among its numerous functions in creating a luminal environment conducive to the maturation of sperm, the epididymis is actively involved in the endocytosis of various substances, including proteins, from the lumen (Hoffer et al, 1973; Hamilton, 1975; Moore and Bedford, 1979; Robaire and Hermo, 1988). The removal of these substances would serve to unclog the lumen of unwanted proteins in each epididymal region and thus allow a more efficient interaction of the sperm surface with recently secreted proteins as sperm traverse the entire length of the duct. The endocytosed proteins, after their receptor-mediated uptake by coated pits, are internalized into an endocytic apparatus consisting of endosomes, multivesicular bodies, and lysosomes, whereupon the end products of endocytosis are ultimately degraded by lysosomal enzymes (Hermo et al, 1994a). Lysosomes are plentiful in the different epididymal epithelial cell types (Hamilton, 1975; Robaire and Hermo, 1988). They contain numerous lysosomal enzymes, including cathepsins, β-glucuronidases, β-galactosidases, and prosaposin (SGP-1), to name a few (Abou-Haila et al, 1996; Hermo et al, 1994a; Tomomasa et al, 1994; Igdoura et al, 1995), including region-specific differences of their integral membrane proteins (Suarez-Quian et al, 1992). A consistent finding among the different lysosomal enzymes, and a recurring theme of the epididymis in many functional parameters, is that expression of a given protein can at times be cell type– and even region-specific (Cornwall et al, 2002; Hermo and Robaire, 2002).

β-Hexosaminidase (Hex) is an example of a lysosomal enzyme showing cell type and region specificity in its expression along the epididymis. In an earlier study, quantification of mRNA expression of the α and β subunits of Hex in the adult rat epididymis indicated that the

Supported by grants from the Canadian Institutes for Health Research.

Correspondence to: Dr Louis Hermo, McGill University, Department of Anatomy and Cell Biology, 3640 University St, Montreal, Quebec, H3A 2B2 (e-mail: louis.hermo@mcgill.ca).

Received for publication June 12, 2003; accepted for publication August 19, 2003.

β subunit is prominent in the epididymis, with highest expression in the corpus region (Hermo et al, 1997). This finding correlated with high levels of mRNA expression (Stirling et al, 1991) and enzyme activity (Conchie et al, 1959; Beccari et al, 1988) in the epididymis and higher specific activity in the corpus region compared with other regions (Hall and Killian, 1987; Hall et al, 1996). By light microscope (LM) immunocytochemistry, although Hex was intensely expressed in narrow and clear cells, principal cells revealed region-specific differences, with numerous lysosomes being intensely reactive in the caput and proximal corpus, followed by the initial segment, distal corpus, and cauda regions (Hermo et al, 1997). The prominent cell and region specificity of Hex suggested an important role for this enzyme in the epididymis, as well as substrate specificity with respect to substances internalized by principal cells (Hermo et al, 1997).

Functionally, Hex catalyzes the hydrolysis of terminal β-linked *N*-acetylgalactosamine or *N*-acetylglucosamine residues from a number of substrates, including GM2 ganglioside, as well as glycoproteins, glycolipids, and glycosaminoglycans (Gravel et al, 1995). There are 2 major isoenzymes of Hex: Hex A, composed of an α and a β subunit, and Hex B, a homodimer of β subunits (Mahuran et al, 1988; Mahuran, 1990; Gravel et al, 1995). To better understand the role of Hex in the epididymis, mice were made deficient in Hex A through targeted disruption of the *Hexa* gene (encodes the Hex α subunit) or in Hex A and B through disruption of the *Hexb* gene (encodes the Hex β subunit) (Phaneuf et al, 1996). In the *Hexa*−/− mice (Hex A–deficient), major abnormalities were restricted to the initial segment and intermediate zone of the epididymis, whereas in *Hexb*−/− mice, extensive abnormalities were found throughout the entire epididymis (Adamali et al, 1999a and b). The abnormalities were seen as dramatic changes to the lysosomes of all epithelial cells, as a consequence of their inability to degrade appropriate substrates internalized via endocytosis. In Hexa−/− and Hexb−/− mice, significant quantities of 2 non-GM2 gangliosides, G2 and G3, accumulated in the epididymis (Trasler et al, 1998). Such an accumulation resulted in a dramatic increase in the appearance, size, number, and distribution of lysosomes within the cytoplasm of the epithelial cells. Taken together, these studies underline the importance of Hex in the lysosomes of the epididymal epithelial cells.

In the context of regulation of epididymal lysosomal enzymes, various studies have revealed that expression of such enzymes can be under different regulatory factors. In fact, several lysosomal enzymes such as acid phosphatase, *N*-acetyl-β-D-glucosaminidase, β-glucuronidase, and arylsulfatase showed decreased enzymatic activity in the epididymis after orchidectomy (Mayorga and Bertini, 1982; Gupta and Setty, 1995; Abou-Haila et al, 1996). In comparison, prosaposin and cathepsin A, as revealed by immunocytochemistry, appeared to be unaffected in their expression in all epithelial cell types in the complete absence of testicular factors (Luedtke et al, 2000; Hermo and Andonian, 2003). On the other hand, regulation of cathepsin D revealed cell type and region-specific differences involving testicular factors (Hermo and Andonian, 2003). Thus, different regulatory factors appear to come into play for the different lysosomal enzymes and between the different epithelial cell types.

During postnatal development, the expression of different proteins often correlates with different events taking place at specific time points. For instance, by day 21, the lumen of many testicular seminiferous tubules is already formed, allowing the entry of Sertoli cell–secreted substances into the epididymal lumen (Tindall et al, 1975; Russell et al, 1989), and as such, these substances might regulate expression of several epididymal proteins (Hermo et al, 1994a). By day 39, androgen levels become high (Scheer and Robaire, 1980), at a time when principal cells become structurally differentiated and many proteins are expressed in an adultlike staining pattern (Sun and Flickinger, 1979; Hermo et al, 1992a; Hermo and Robaire, 2002). By day 49, sperm begin to appear in the caput epididymidis and by day 56 in the cauda region (Robaire and Hermo, 1988). Their entry into the epididymis has been suggested to be important for expression of various proteins (Garrett et al, 1991). Thus, from various postnatal studies, it has been demonstrated that different proteins are expressed at different time points and often in a cell type– and region-specific manner, leading to complex patterns of regulation during development (Hermo et al, 1994b and c; Hermo and Robaire, 2002).

In the rat epididymis, total Hex activity has been shown to increase more than 10-fold from infancy to sexual maturity and be dependent on androgens (Conchie et al, 1959). However, no information exists on the role of androgens on Hex expression in the specific cell types and different regions of the epididymis. Data on the cell type– and region-specific expression of Hex during postnatal development is also lacking. Considering the importance of Hex in the epididymis and its role in the degradation of endocytosed proteins, we undertook to examine the effects of testicular factors on the expression of Hex during postnatal development and in adult animals.

The purpose of this study was to examine the role of testicular factors, including androgens, on the expression of Hex in the different cell types and regions of the adult epididymis, employing efferent duct–ligated rats and orchidectomized rats supplemented or not with testosterone. Studies of rats at different postnatal ages were also performed to determine whether or not a correlation exists between the onset of Hex expression in the different epithelial cell types and regions of the epididymis and

known events occurring in this tissue during postnatal development.

Materials and Methods

Adult Animals and Experimental Protocols

Adult male Sprague Dawley rats (350–450 g, 3–4 months of age) were obtained from Charles River Laboratory Ltd (St Constant, Quebec), maintained on a 14-hour light/10-hour dark photoperiod, and provided with food and water ad libitum. They were divided into 5 groups. The first group consisted of 4 adult control animals. Bilateral ligation of the efferent ducts constituted the second group. After an intraperitoneal injection of sodium pentobarbital (Somnitol, MTC Pharmaceuticals, Hamilton, Ontario), the testes and epididymides of adult rats were exposed through an incision of the anterior abdominal wall. With the help of a dissecting microscope, a ligature was placed around both right and left efferent ducts at a site close to and further removed from the rete testis, with care being taken to avoid interference of the blood vessels entering the testis. The interval between the 2 ligatures was then excised to ensure that no sperm or fluids would enter the epididymis from the testis. The animals (4 per interval) were sacrificed at 3, 7, 14, and 21 days following surgery. Bilateral orchidectomy constituted the third group. After anesthesia, both testes of each rat were removed after a ligature was placed around the efferent ducts and testicular blood vessels. The animals (4 per interval) were sacrificed at 3, 7, 14, and 21 days after surgery. Bilaterally orchidectomized rats that received three 6.2-cm testosterone-filled implants constituted the fourth group. Testosterone-filled polydimethyl-siloxane (Silastic) implants were prepared according to the method of Stratton et al (1973) and have well-characterized steroid release rates (Brawer et al, 1983). Subsequent to anesthesia, both testes were removed from each rat, and the implants were placed subcutaneously immediately after orchidectomy. The rats (4 per interval) were sacrificed at 3, 7, 14, and 21 days after surgery. The fifth group consisted of 4 sham-operated animals, 2 of which received 3 empty 6.2-cm-long implants, with all rats being sacrificed 14 days after initiation of the experiment. The success of orchidectomy with or without testosterone implants was assessed by gross differences in the size of the epididymides as compared with control untreated animals.

All experimentation was carried out with minimal stress and discomfort being placed on the animals both during and after surgery, as set up by the guidelines and approval of the McGill University Animal Care Committee.

Tissue Preparation for Light Microscope Immunocytochemistry

At the end of each experiment, the epididymides of each rat were fixed by perfusion with Bouin's fixative via the abdominal aorta for 10 minutes. Following perfusion, the epididymides were removed and cut along their long axis, so that given sections would include all of the major regions of the epididymis (ie, the initial segment, intermediate zone, caput, corpus, and cauda) (Hermo et al, 1991). The tissue was then immersed in Bouin's fixative for 72 hours, after which it was dehydrated and embedded in paraffin.

Postnatal Studies

For postnatal developmental studies, timed pregnant female Sprague-Dawley rats were obtained from Charles River Laboratory Ltd. A number of litters, out of which 36 male pups were chosen, were maintained on a 14 hour dark/10 hour light photoperiod. They were provided with food and water ad libitum. After birth, assessing body weight gain and palpating their testes and epididymides, we monitored the normal development of the male pups. Only those pups showing normal trends in development as reported by Hermo et al (1992) were used, which consisted of 6 rats at each of the following intervals: postnatal days 7, 21, 29, 39, 49, and 56. At each age, 2 rats were used to obtain the weights of the paired testes and epididymides, and the other 4 were used to prepare the tissue for light microscope immunocytochemical analysis. The body weights of all animals used, as well as the testicular and epididymal weights of the 2 unfixed animals, were within the normal range obtained previously (Hermo et al, 1992). The size and appearance of the testes and epididymides of the 4 chemically fixed animals were similar to those of the 2 unfixed animals.

The epididymides of all animals were fixed with Bouin fixative through the heart (days 7 and 21) or via retrograde perfusion of fixative through the abdominal aorta (all other ages) for 10 minutes. Following perfusion with either fixative, the epididymides were removed and cut so that given sections would include all of the major regions of the epididymis (ie, the initial segment, intermediate zone, caput, corpus, and cauda) (Hermo et al, 1991). The tissue was then immersed in Bouin fixative for an additional 72 hours, after which it was dehydrated and embedded in paraffin.

Light Microscope Immunostaining

Light microscope immunoperoxidase staining of the epididymides of all animals was carried out with a rabbit polyclonal anti-Hex A antibody, raised against human placental Hex A. Dr Don Mahuran provided the antibody to us; details of the specificity and characterization of the antibody are described in one of our earlier publications, where we examined Hex expression in the epididymis of normal adult rats (Hermo et al, 1997).

Sections 5 μm thick were cut and mounted on glass slides. They were then deparaffinized with xylene and hydrated in graded concentrations of ethanol (from 100% to 50%). During hydration, immersing the tissues in 70% ethanol containing 1% lithium carbonate for 5 minutes neutralized residual picric acid. In order to inactivate any endogenous peroxidase activity, the tissue sections were incubated for 5 minutes in 70% ethanol containing 1% (vol/vol) hydrogen peroxide. Following hydration, the sections were incubated (5 minutes) in a 300-mM glycine solution in order to block free aldehyde groups. The tissue was then blocked with 40 mL of 10% goat serum and diluted in TBS (20 mM Tris-HCl saline containing 0.1% bovine serum albumin) at pH 7.4 for 25 minutes at room temperature. The slides were then washed with Tween buffer solution (TWBS, TBS with 0.1% Tween-20). A dilution factor of 1:100 in TBS was used for the affinity-purified polyclonal anti–β-Hex A an-

tibody. Tissue sections were incubated at 37°C in a moist chamber for 1 hour with 50 μL of the anti-Hex primary antibody.

After incubation, the sections were immersed in 4 consecutive wells of TWBS for 2 minutes each. The sections were then blocked with 40 mL of 10% goat serum for 15 minutes and subsequently incubated for 1 hour at 37°C in a humidified incubator with goat anti-rabbit IgG conjugated to peroxidase (Sigma Chemical Co, St. Louis, Mo) at a dilution of 1:250 in TBS. After incubation with a secondary antibody, the tissue was washed by immersion in 4 wells of TWBS for 2 minutes each.

The final reaction product was obtained by incubating the slides for 10 minutes in 250 mL of TBS containing 0.03% hydrogen peroxide, 0.1 M imidazole, and 0.05% diaminobenzidine tetrahydrochloride, pH 7.4. The sections were washed in distilled water, counterstained with 0.1% methylene blue (2 minutes), and then dehydrated in a graded series of ethanol solutions (30 seconds each) and xylene (3 minutes). Cover slips were mounted onto glass slides using Permount.

Incubation of epididymal tissues with normal rabbit serum at a dilution of 1:100 in TBS and in secondary antibody alone, without primary antibody, served as negative controls. We have already published such images in our previous Hex article (Hermo et al, 1997).

Results

Developmental Expression of Hex in the Epididymis

In the initial segment of the epididymis, at postnatal day 7, a specific reaction was not noted over the cuboidal undifferentiated epithelium of the initial segment; a lumen was already visible, however, without apparent content by this age (Figure 1a). By postnatal day 29, narrow cells were intensely reactive, as they were at all later ages, but only an occasional lysosome was reactive in the undifferentiated principal cells at day 29 and later ages (Figure 1b through d); similar findings were observed at day 21 (not shown). By day 29, some basal cells were reactive, while others were not (Figure 1b), and this was also observed at all later ages (Figure 1c and d). At postnatal days 39 (Figure 1c) and 49 (Figure 1d), narrow cells and some basal cells were reactive, whereas principal cells, fully differentiated by day 39, continued to reveal only a few reactive lysosomes, as they did at days 56 and 90 (not shown).

In the caput (Figure 2a through c, e, and f), intermediate zone (Figure 2d), and proximal corpus (not shown) epididymidis, the staining pattern of the different epithelial cells was noted to be similar to each other during postnatal development. At postnatal day 7, all the epithelial cells of these regions, undifferentiated at this age, were unreactive (Figure 2a). By day 21, narrow cells, found only in the initial segment and intermediate zone were reactive (not shown) and continued to be so at later ages (Figure 2d). Clear cells found in the caput and corpus regions also became intensely reactive by day 21 (Figure 2b) and continued to be so at all later ages (Figure 2e). In all epididymal regions, although some basal cells were intensely reactive by day 29, others were unreactive (Figure 2c), and this staining pattern persisted at all later ages (Figure 2d through f). Principal cells of the intermediate zone, caput, and proximal corpus epididymidis showed only an occasional reactive lysosome at day 21 (Figure 2b), and although several more became reactive by day 29 (Figure 2c and d), numerous reactive lysosomes were observed at days 39 (Figure 2e) and 49 (Figure 2f), comparable to that noted in normal adult animals (Hermo et al, 1997).

The distal corpus and proximal cauda regions also revealed comparable staining patterns for the different epithelial cells during postnatal development (Figure 3a through d). At postnatal day 7, the entire epithelium of either of these regions was unreactive (Figure 3a). At postnatal days 21 and 29, clear cells present in both of these regions were reactive (Figure 3b and c), and by day 39, numerous reactive clear cells were observed (Figure 3d). By day 29, basal cells revealed reactivity (Figure 3b), and at this age and all subsequent ages, both reactive and unreactive basal cells were noted in each of these epididymal regions (Figure 3d). Principal cells showed only an occasional reactive lysosome at days 21 (Figure 3c), and although several were noted at day 29 (Figure 3b), there was no apparent increase in the number of reactive lysosomes at any later age of development (Figure 3d).

Effects of Orchidectomy and Efferent Duct Ligation on Hex A Expression in the Adult Epididymis

At the different time points after orchidectomy, narrow cells of the initial segment (Figure 4a) and intermediate zone and clear cells of the caput, corpus (Figure 4c), and cauda regions were intensely reactive and expressed Hex at levels comparable to the staining pattern seen in control animals. These cells were also reactive in the orchidectomized animals that received testosterone supplementation (Figure 4b and d). Basal cells of all epididymal regions did not change their staining pattern after orchidectomy, with both reactive and unreactive basal cells being noted (Figure 4a and c), and this was true also for the orchidectomized animals that were supplemented with testosterone (Figure 4b and d). In contrast, principal cells of all epididymal regions showed an absence of reactive lysosomes at all time points after orchidectomy (Figure 4a and c). However, administration of testosterone to orchidectomized rats restored staining to the lysosomes of principal cells of all epididymal regions (Figure 4b and d), such that the number of reactive lysosomes of the different regions became comparable to that noted for control animals. At the different time points after efferent duct ligation, there was no change in the staining pattern of Hex A in narrow, clear, basal, or principal cells (not

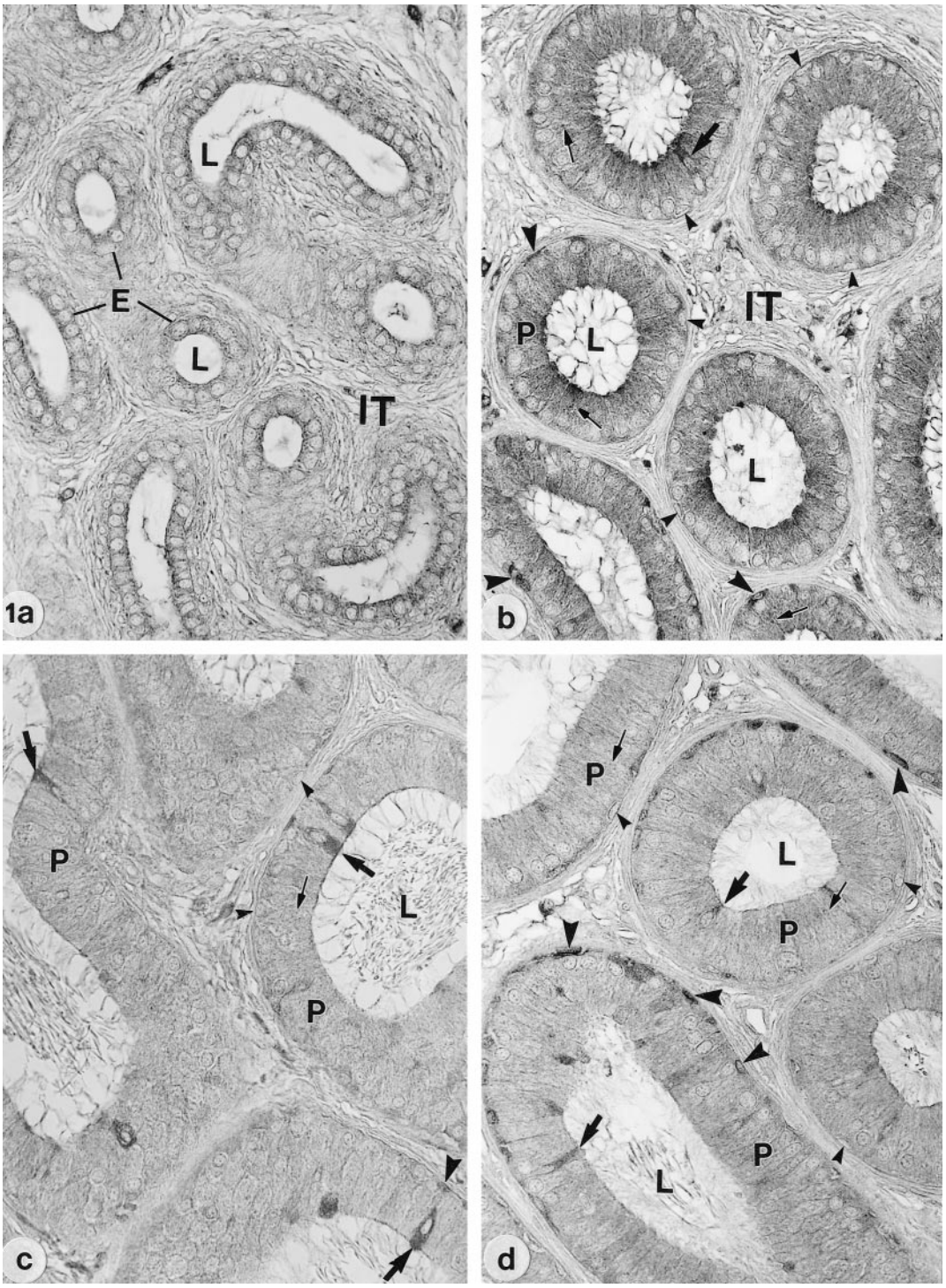

Figure 1. **(a–d)** Light micrographs of tubular cross sections of the initial segment of the epididymis of postnatal 7- **(a)**, 29- **(b)**, 39- **(c)** and 49-day-old **(d)** animals immunostained with an anti–β-Hex antibody. The epithelium (E) progresses from an undifferentiated cuboidal appearance **(a)** to a differentiated columnar appearance with advancing age **(b–d)**. In **panel a**, no reaction of any prominence is noted in the epithelium; in **panel b**, although some basal cells are intensely reactive (large arrowheads), others appear unreactive (small arrowheads), and this staining pattern is maintained **(c, d)**. Narrow cells (thick arrows) are reactive by day 29 and remain so at later ages **(c, d)**. Principal cells (P) show small, few, reactive lysosomes (thin arrows, **b)** and continue to do so at later ages **(c, d)**. Sperm are evident in the lumen (L) at day 39 **(c)** and 49 **(d)**, but not at earlier ages. IT indicates intertubular space. **(a, c, d)** 375×, **(b)** 440×.

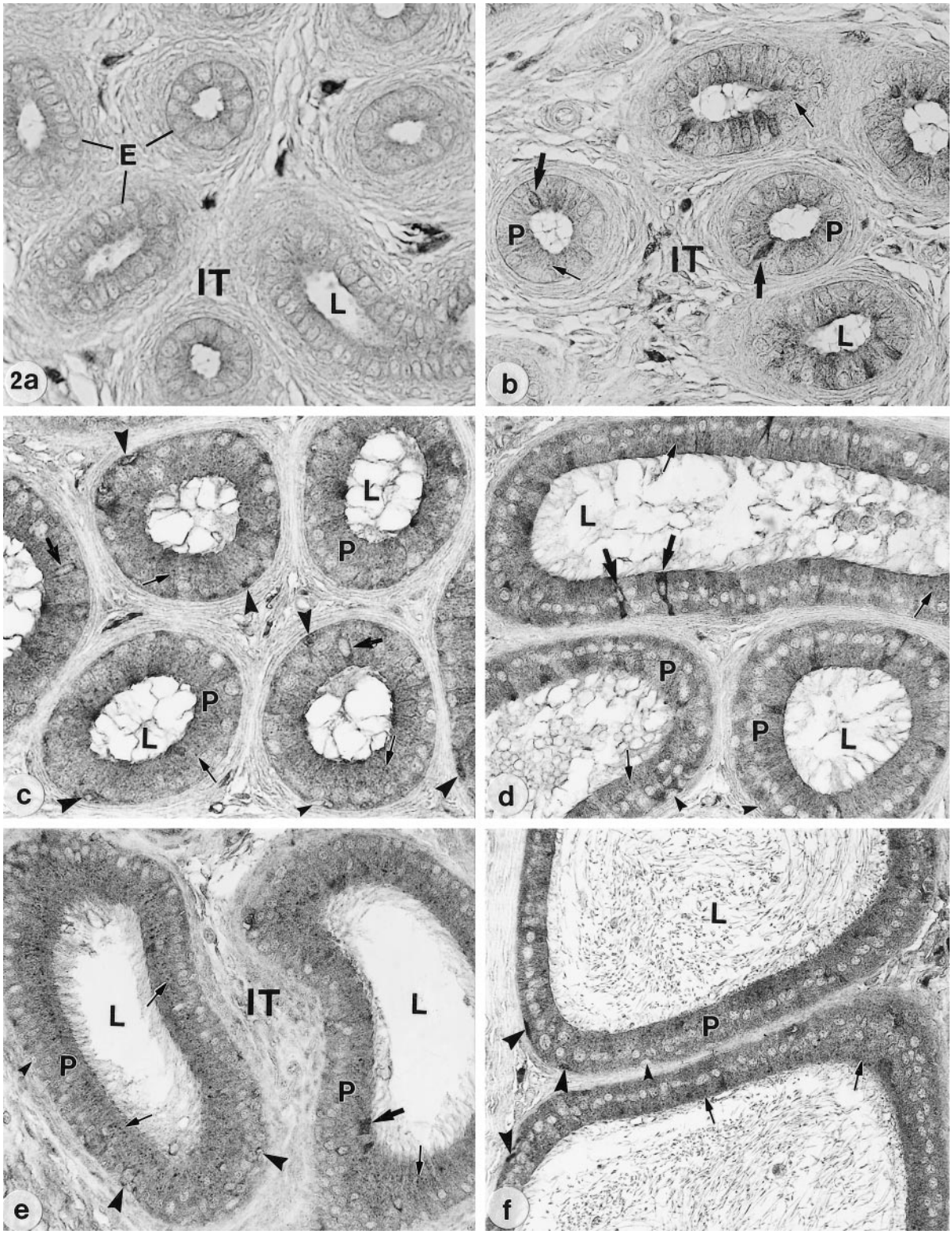

Figure 2. **(a–f)** Light micrographs of tubular cross sections of the caput epididymidis **(a–c, e, f)** and intermediate zone **(d)** of postnatal 7- **(a)**, 21- **(b, c)**, 29- **(d)**, 39- **(e)** and 49-day-old **(f)** animals immunostained with an anti–β-Hex antibody. The intermediate zone, caput, and proximal corpus epididymidis show similar staining patterns during development, and only selected regions are illustrated. **(a)** The epithelium (E) is undifferentiated and shows only background levels of staining. **(b)** Clear cells (thick arrows) are reactive, as is an occasional lysosome (thin arrows) of the undifferentiated principal cells (P). Clear cells of the caput region and narrow cells of the intermediate zone continue to be reactive (thick arrows) at later ages **(c–e)**. Basal cells appear both reactive (large arrowheads) and unreactive (small arrowheads) by day 29. The undifferentiated principal cells (P) reveal few reactive lysosomes in **(c, d)**; however, at day 39 **(e)**, when they become fully differentiated, and day 49 **(f)**, they contain numerous reactive lysosomes. Note absence of sperm in the lumen (L) in panels **a–e**, but their abundance in panel **f**. IT indicates intertubular space. **(a)** 600×, **(b, e)** 350×, **(c)** 375×, **(d, f)** 325×.

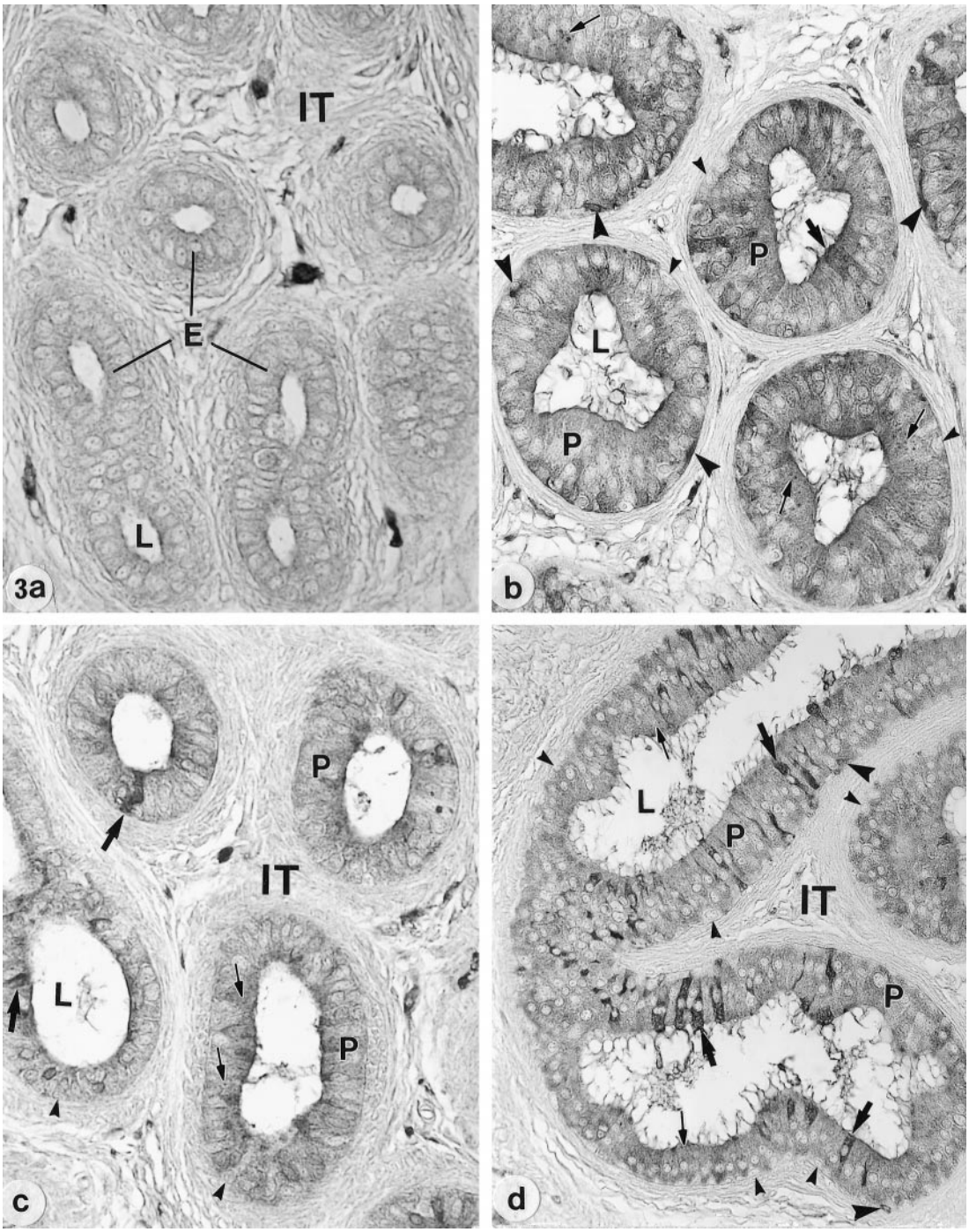

Figure 3. **(a–d)** Light micrographs of the distal corpus **(a, b)** and proximal cauda **(c, d)** epididymides of postnatal 7- **(a)**, 29- **(b)**, 21- **(c)** and 39-day old **(d)** animals immunostained with an anti–β-Hex antibody. **(a)** The epithelium (E) is undifferentiated and unreactive. **(b, c)** Clear cells become reactive (thick arrows) and **(d)** numerous clear cells show intense reactivity, whereas **(c)** basal cells appear unreactive. **(b, d)** Although some basal cells are reactive (large arrowheads), others are not (small arrowheads). The undifferentiated principal cells (P) show only an occasional reactive lysosome (**b, c,** small arrows), and this is also the case when they become fully differentiated **(d)**. There are no sperm in the lumen (L) at these ages. IT indicates intertubular space. **(a)** 600×, **(b)** 400×, **(c, d)** 440×.

shown). Taken together, the data suggest that expression of Hex in principal cell lysosomes is dependent on systemic androgens but that this is not the case for its expression in narrow, clear, or basal cells.

Discussion

Over the past 30 years, a great deal of attention has been devoted to the role androgens play in regulating the struc-

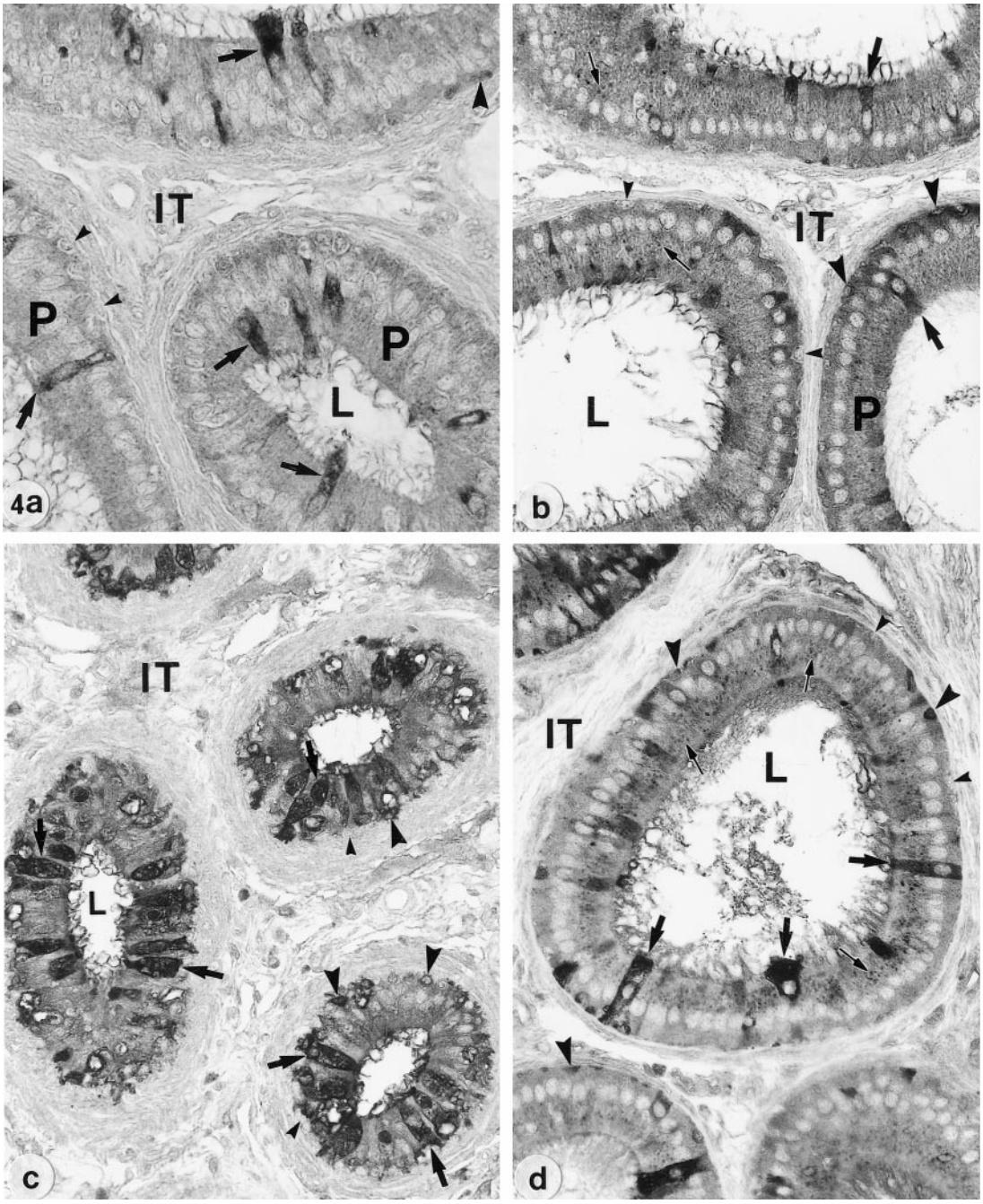

Figure 4. **(a–d)** Light micrographs of the initial segment **(a, b)** and proximal corpus **(c, d)** regions of the epididymis of an adult animal 14 days after orchidectomy without **(a, c)** and with **(b, d)** testosterone supplementation; immunostained with an anti–β-Hex antibody. Narrow **(a, b)** and clear **(c, d)** cells maintain their intense reactivity (thick arrows) after orchidectomy **(a, c)**, comparable to that seen following testosterone supplementation **(b, d)** and controls. Principal cells (P) do not reveal reactive lysosomes (**a, c,** small arrows). However, lysosomes are intensely reactive in these cells **(b, d)**, in numbers comparable to those noted for control animals of these respective epididymal regions. Both reactive (large arrowheads) and unreactive (small arrowheads) basal cells are seen in animals without **(a, c)** and with **(b, d)** testosterone supplementation. IT indicates intertubular space. **(a, c)** 350×, **(b)** 325×, **(d)** 375×.

ture and functions of the epithelial cells of the epididymis. This is of importance because the epididymis is well known to be an androgen-regulated tissue (Orgebin-Crist et al, 1975) and because androgens are a major target for contraceptive developments. Furthermore, because some cases of male infertility can be due to absence or diminishment of androgen action, it would be important to know what functions of the epididymis are compromised under such conditions. Thus, knowledge of Hex expression and its regulation by androgens in the epididymis

Table 1. *Effects of orchidectomy with or without testosterone supplementation and efferent duct ligation on Hex expression in the adult rat epididymis**

Group	Cell Type	Region†			
		IS	IZ/Cap/PCor	DCor	Cau
Control	Prinicipal	+	++	+	+
	Narrow/clear‡	++	++	++	++
	Basal	++	++	++	++
Orchidectomy	Principal	−	−	−	−
	Narrow/clear	++	++	++	++
	Basal	++	++	++	++
Orchidectomy + testosterone supplementation	Principal	+	++	+	+
	Narrow/clear	++	++	++	++
	Basal	++	++	++	++
Efferent duct ligation	Principal	+	++	+	+
	Narrow/clear	++	++	++	++
	Basal	++	++	++	++

* The data were similar at all time points after the different experimental treatments.

† Regions of the epididymis. IS indicates initial segment; IZ, intermediate zone; Cap, caput; PCor, proximal corpus; DCor, distal corpus; Cau, cauda. For principal cells, ++ indicates that many intensely reactive lysosomes are evident in their cytoplasm; +, only a few reactive lysosomes are present; −, absence of reactive lysosomes. For narrow/clear and basal cells, ++ indicates that these cells are intensely reactive. Although qualitative in nature, these data give an overall impression of the status of reactivity based on consistent findings from at least 4 slides for each animal of each experimental group in the case of the different cell types of each epididymal region.

‡ Narrow cells are only present in the IS and IZ regions, whereas clear cells are present in the caput, corpus, and cauda regions.

would provide us with bits of information on a large global response of this tissue to androgens. This type of data will help us better understand the importance of androgens on the functions of the various cell types and how to effectively develop methods of contraception, without compromising the integrity of this tissue.

In a previous study, Hex was localized by immunocytochemistry to principal cells of the normal adult epididymis, where a region-specific expression was noted, and to narrow, clear, and basal cells where no region-specific differences were observed (Hermo et al, 1997). In this study, we examined the regulation of Hex in the various epithelial cell types and regions of the epididymis in adult animals after orchidectomy and efferent duct ligation (Table 1). Adverse effects on expression of a variety of proteins and genes have been shown to occur in the absence of androgens, luminal factors, or both emanating from the testis (Robaire and Viger, 1995; Orgebin-Crist, 1996; Cornwall et al, 2002; Ezer and Robaire, 2002). In fact, several lysosomal enzymes such as acid phosphatase, *N*-acetyl-beta-D-glucosaminidase, β-glucuronidase, *N*-acetylhexosaminidase, and arylsulfatase show decreased enzymatic activity after orchidectomy, which is restored with testosterone supplementation (Mayorga and Bertini, 1982; Gupta and Setty, 1995; Abou-Haila et al, 1996). However, although a role for androgens in regulating epididymal functions has long been recognized (Orgebin-Crist et al, 1975; Robaire and Hermo, 1988), only recently has it been demonstrated that these functions are often regulated in a cell type– and even region-specific manner (Ezer and Robaire, 2002; Cornwall et al, 2002).

In the case of the epididymis, Hex activity has been reported to be androgen dependent (Conchie et al, 1959), and although adverse effects in expression were noted in this study, they were found to be cell specific. For the major epithelial cell type, principal cells, Hex expression was abolished after orchidectomy but restored with testosterone supplementation. Together with the finding that Hex expression was not altered in efferent duct–ligated animals, the data suggest that androgens regulate Hex expression in principal cells. This is of interest because efferent duct ligation has been shown to adversely affect the structural features of principal cells of the initial segment (Hoffer et al, 1973). However, it appears that this procedure has no effect on Hex expression. This is also the case for Y_f-GST, osteopontin and several other proteins expressed by principal cells, which appear to be regulated by androgens (Hermo and Papp, 1996; Hermo and Robaire, 2002; Luedtke et al, 2002). In contrast, prosaposin, visualized by LM immunocytochemistry, was unaltered in its staining pattern in principal cells of the various epididymal regions in the absence of testicular factors (Hermo and Andonian, 2003). Likewise, in the efferent ducts, prosaposin expression in the nonciliated cells was unaffected by absence of testicular factors. However, in hypophysectomized animals, staining was abolished, suggesting that a pituitary factor directly or indirectly affects prosaposin expression in the efferent ducts (Rosenthal et al, 1995). In the case of cathepsin A, various experimental treatments failed to reveal any difference in its expression in lysosomes of the different cell types of the different epididymal regions (Luedtke et al, 2000). This was also shown to be the case for cathepsin D, which, although expressed in lysosomes of principal cells, was

unaffected by absence of testicular factors (Hermo and Andonian, 2003). Thus, although androgens do not regulate expression of some proteins in principal cells, this does not appear to be the case for others (Cornwall and Hann, 1995; Cornwall et al, 2002; Ezer and Robaire, 2002).

In this study, narrow, clear, and basal cells remained as intensely reactive after the different treatments, as noted in control animals, with expression being evident throughout their cytoplasm (Table 1). Such a reaction pattern has been shown to be due to the abundance of lysosomes present in the large clear cells and in the smaller sized narrow and basal cells (Hermo et al, 1988, 1994a; Adamali and Hermo, 1996). Thus, although Hex expression in principal cells is regulated by androgens, this is not the case for narrow, clear, and basal cells, which do not appear to be regulated by testicular factors.

Clear cells, found only in the caput, corpus, and cauda epididymidis are highly active in endocytosis and degrade various substances from the lumen, including the breakdown products of cytoplasmic droplets (Hermo et al, 1988). Although no changes were noted in the expression of Hex in clear cells of this study (Table 1), as was also the case for prosaposin and cathepsin A expression by these cells (Luedtke et al, 2000; Hermo and Andonian, 2003), this was not the case for cathepsin D, which showed cell type– and region-specific differences. In lysosomes of principal cells, cathepsin D expression was unaffected by various treatments; however, clear cells, normally unreactive in all epididymal regions, became intensely reactive after orchidectomy, but only in the corpus and cauda regions. As expression, or a lack thereof, was restored to normal after testosterone administration and not modified in efferent duct–ligated animals, it was suggested that cathepsin D expression in clear cells of the corpus and cauda epididymidis is inhibited by testosterone in control untreated animals (Hermo and Andonian, 2003). Thus, in the epididymis, a great deal of variation based on individual cell types and region specificity is emerging in the regulation of the different lysosomal enzymes. Indeed, expression of different lysosomal enzymes appears to be under the control of stimulatory or inhibitory factors, which can be of similar or different identities.

Narrow cells are located in the initial segment and intermediate zone and are active endocytic cells, expressing a variety of proteins with diverse functions (Adamali and Hermo, 1996; Hermo and Robaire, 2002). Distinct from apical cells of these regions, the 2 differ structurally and functionally from each other (Adamali and Hermo, 1996). However, because of varying planes of section that occur through these 2 cell types, it is often difficult to differentiate one from the other without electron microscope analysis. In addition, because these 2 cell types both express Hex (Adamali and Hermo, 1996), we could not differentiate one from the other in this study. Hence, both narrow and apical cells, showing apically positioned nuclei, were grouped together and simply referred to as narrow cells. After orchidectomy or efferent duct ligation, no difference was noted in the staining pattern of these cells. Narrow cells express various other lysosomal enzymes, which like Hex in the present study (Table 1) are unaffected after orchidectomy (Luedtke et al, 2000; Hermo and Andonian, 2003). Although little is known about the regulation of narrow cell functions, other proteins expressed by these cells, such as Yb_1-GST, appear to be regulated in the initial segment by testicular lumicrine factors (Andonian and Hermo, 2003).

In this study, no changes to Hex expression was noted for basal cells of any epididymal region (Table 1). In the context of other lysosomal enzymes, cathepsin A expression in basal cells was not regulated by testicular factors (Luedtke et al, 2000), as was also the case for cathepsin D and prosaposin (Hermo and Andonian, 2003). Basal cells express various isoforms of GSTs (Papp et al, 1995). In the corpus region, Yb_1-GST expression in basal cells was regulated by testosterone, but in the proximal initial segment, a lumicrine testicular factor appears to regulate its expression (Andonian and Hermo, 2003). These data differ from the Y_f-GST subunit, for which expression in basal cells was regulated by neither a testicular nor pituitary factor (Hermo and Papp, 1996). Expression of metallothionein by basal cells, although detectable in all epididymal regions, was shown to be androgen dependent according to specific regions (Cyr et al, 2001). Thus, even basal cells have complex regulatory mechanisms for expression of proteins of different functions.

Although androgens play a prominent role in regulating a variety of epididymal functions, a role for estrogens has also been implicated in the efferent ducts and epididymis, where estrogen receptors (ER) α and β have been located (Meistrich et al, 1975; Fisher et al, 1997; Hess et al, 2002). Because sperm become absent after orchidectomy and efferent duct ligation and aromatase activity is present in cytoplasmic droplets of sperm, it would be unlikely that estrogen rather than testosterone regulates Hex expression in principal cells. However, this could be eventually tested with the estrogen knockout mouse model systems.

Postnatal Studies of Hex in the Epididymis

During postnatal development, total Hex activity in the rat epididymis has been reported to increase 10-fold from infancy to sexual maturity (Conchie et al, 1959); however, no correlations of Hex expression in the different cell types and regions of the epididymis have been performed during postnatal development. In the present study, the onset of Hex expression varied according to cell type and

Table 2. *Hex expression in the different cell types and regions of the epididymis during postnatal development*

		Region*			
Postnatal age	Cell Type	IS	IZ/Cap/PCor	DCor	Cau
Day 7	Principal	−	−	−	−
	Narrow/clear†	−	−	−	−
	Basal	−	−	−	−
Day 21	Principal	−	−	−	−
	Narrow/clear	++	++	++	++
	Basal	−	−	−	−
Day 29	Prinicpal	−	+	−	−
	Narrow/clear	++	++	++	++
	Basal	++	++	++	++
Days 39, 49, 56	Principal	+	++	+	+
	Narrow/clear	++	++	++	++
	Basal	++	++	++	++

* Regions of the epididymis. IS indicates initial segment; IZ, intermediate zone; Cap, caput; PCor, proximal corpus; DCor, distal corpus; Cau, cauda. For principal cells, ++ indicates that many intensely reactive lysosomes are evident in their cytoplasm; +, only a few reactive lysosomes are present; −, absence of reactivity in lysosomes. For narrow/clear and basal cells, ++ indicates that these cells are intensely reactive. Although qualitative in nature, these data give an overall impression of the status of reactivity based on consistent findings from at least 4 slides for each animal of each experimental group in the case of the different cell types of each epididymal region.

† Narrow cells are only present in the IS and IZ regions, whereas clear cells are present in the caput, corpus, and cauda regions.

age of development (Table 2). Although no cells expressed Hex by day 7, narrow and clear cells showed reactivity by day 21, and although basal cells became reactive by day 29, it was only by day 39 that principal cells of all regions presented their adultlike staining pattern (Table 2). The adultlike pattern in principal cells by day 39 correlates with high levels of androgens attained at this age of development (Scheer and Robaire, 1980) and when principal cells attain their adultlike structural features (Hermo et al, 1992). Taken together, this finding concurs with that obtained from orchidectomized adult rats in this study, both of which reveal the dependence of testosterone or one of its metabolites for Hex expression in principal cells.

The data of this study on postnatal development are also in agreement with the absence of a role for testosterone in Hex expression in narrow, clear, and basal cells (Table 2) because these cells express Hex at time points well before androgen levels reach their peak and when they become fully structurally differentiated (Hermo et al, 1992). Clearly, spermatozoa themselves or proteins derived thereof are not required for Hex expression in these cells because sperm only appear in the initial segment by day 39 (Robaire and Hermo, 1988; Papp et al, 1994), a time point well after the onset of Hex expression in narrow, clear, and basal cells, which occurs at day 21 and 29, respectively (Table 2).

The finding of Hex expression in narrow and clear cells by day 21 correlates with the entry of Sertoli-derived secreted products, which appear in the epididymis beginning at about postnatal day 15, when the seminiferous tubular lumen is formed (Tindall et al, 1975; Russell et al, 1989), and 1 or several of these could be factors regulating Hex expression in these cells. However, onset of expression in basal cells is only by day 29. Because both basal and narrow cells exist in the initial segment, where the Sertoli cell–secreted products would first appear, the data on Hex expression suggest that different factors are involved in regulation of Hex expression in these 2 different cell types.

In the case of other proteins that have been examined in the epididymis during postnatal development, complex patterns of expression have emerged. In all of these cases, onset of expression varied not only according to cell type, but region specificity as well as the time point when onset began and when adultlike staining patterns of expression first appeared (Papp et al, 1994; Hermo et al, 1994a and b, 1999; Badran and Hermo, 2002). Because testicular factors do not play a role in regulating Hex expression in narrow, clear, and basal cells in adult animals, growth factors, hormones, or other stimulatory factors derived from other tissues/organs could be responsible for Hex expression in adult animals and during postnatal development. Clearly more work is required to determine what specific factor or factors regulate Hex expression in narrow, clear, and basal cells of the epididymis.

In this study, expression of Hex was dependent on androgens in principal cells of the entire epididymis, but this was not the case for expression in narrow, clear, and basal cells. The importance of Hex in lysosomes of principal cells has been noted in our earlier studies employing knockout mouse models, in which these cells, with time, become engorged with lysosomes. It is, therefore, suggested that absence of androgen input on the epididymis, as created by anti-androgen contraception, could have deleterious long-term effects on principal cell structure

and functions that could eventually affect the integrity of the blood epididymal barrier.

Acknowledgment

We thank Dr Don Mahuran from the Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada, for his generous contribution of the Hex antibody.

References

Abou-Haila A, Tulsiani DR, Skudlarek MD, Orgebin-Crist M-C. Androgen regulation of molecular forms of beta-D-glucuronidase in the mouse epididymis: comparison with liver and kidney. *J Androl.* 1996; 17:194–207.

Adamali HI, Hermo L. Apical and narrow cells are distinct cell types differing in their structure, distribution and functions in the adult rat epididymis. *J Androl.* 1996;17:208–222.

Adamali HI, Somani IH, Huang J-Q, Mahuran D, Gravel RA, Trasler JM, Hermo L. I. Abnormalities in cells of the testis, efferent ducts and epididymis in juvenile and adult mice with beta-hexosaminidase A and B deficiency. *J Androl.* 1999a;20:779–802.

Adamali HI, Somani IH, Huang J-Q, Mahuran D, Gravel RA, Trasler JM, Hermo L. II. Characterization and development of the regional- and cellular-specific abnormalities in the epididymis of mice with beta-hexosaminidase A deficiency. *J Androl.* 1999b;20:803–824.

Andonian S, Hermo L. Immunolocalization of the Yb1 subunit of glutathione S-transferase in the adult rat epididymis following orchidectomy and efferent duct ligation. *J Androl.* 2003;24:577–587.

Badran HH, Hermo LS. Expression and regulation of aquaporins 1, 8, and 9 in the testis, efferent ducts, and epididymis of adult rats and during postnatal development. *J Androl.* 2002;23:358–373.

Beccari T, Orlacchio A, Stirling JL. Identification of β-*N*-acetlyhexosaminidase A in mouse tissues with the fluorigenic substrate 4-methylumbelliferyl-β-*N*-acetylglucosamine 6-sulfate. *Biochem J.* 1988;252: 617–620.

Brawer JR, Schipper H, Robaire B. Effects of long term androgen and estradiol exposure on the hypothalamus. *Endocrinology.* 1983;112: 194–199.

Conchie J, Findlay J, Levvy GA. Mammalian glycosidases: distribution in the body. *Biochem J.* 1959;71:318–325.

Cornwall GA, Hann SR. Specialized gene expression in the epididymis. *J Androl.* 1995;16:379–383.

Cornwall GA, Lareyre J-J, Matusik RJ, Hinton BT, Orgebin-Crist M-C. Gene expression and epididymal function. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice.* New York: Kluwer Academic/Plenum Publishers; 2002:169–199.

Cyr DG, Dufresne J, Pillet S, Alfieri TJ, Hermo L. Expression and regulation of metallothioneins in the rat epididymis. *J Androl.* 2001;22: 124–135.

Ezer N, Robaire B. Androgenic regulation of the structure and functions of the epididymis. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice.* New York: Kluwer Academic/ Plenum Publishers; 2002:297–316.

Fisher JS, Millar MR, Majdic G, Saunders PT, Fraser HM, Sharpe RM. Immunolocalisation of oestrogen receptor-alpha within the testis and excurrent ducts of the rat and marmoset monkey from perinatal life to adulthood. *J Endocrinol.* 1997;153:485–495.

Garrett SH, Garrett JE, Douglass J. In situ histochemical analysis of region-specific gene expression in the adult rat epididymis. *Mol Reprod Dev.* 1991;30:1–17.

Gravel RA, Clarke JTR, Kaback MM, Mahuran D, Sandhoff K, Suzuki K. The G_{M2} gangliosidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Basis of Inherited Disease.* New York: McGraw-Hill; 1995:2839–2879.

Gupta G, Setty BS. Activities and androgenic regulation of lysosomal enzymes in the epididymis of rhesus monkey. *Endocr Res.* 1995;21: 733–741.

Hall JC, Killian GJ. Changes in rat sperm membrane glycosidase activities and carbohydrate and protein contents associated with epididymal transit. *Biol Reprod.* 1987;36:709–718.

Hall JC, Perez FM, Kochins JG, Pettersen CA, Li Y, Tubbs CE, LaMarche MD. Quantification and localization of *N*-acetyl-β-D-hexosaminidase in the adult rat testis and epididymis. *Biol Reprod.* 1996;54:914–929.

Hamilton DW. Structure and function of the epithelium lining the ductuli efferentes, ductus epididymis and ductus deferens in the rat. In: Greep RO, Astwood EB, eds. *Handbook of Physiology.* Section 7, Vol 5. Washington, DC: American Physiological Society; 1975:303–317.

Hermo L, Adamali HI, Mahuran D, Gravel RA, Trasler JM. β-Hexosaminidase immunolocalization and α- and β-subunit gene expression in the rat testis and epididymis. *Mol Reprod Dev.* 1997;46:227–242.

Hermo L, Andonian S. Regulation of sulfated glycoprotein-1 (SGP-1) and cathepsin D expression in the adult rat epididymis. *J Androl.* 2003;24:408–422.

Hermo L, Barin K, Robaire B. Structural differentiation of the epithelial cells of the testicular excurrent duct system of rats during postnatal development. *Anat Rec.* 1992;233:205–228.

Hermo L, Dworkin J, Oko R. Role of epithelial clear cells of the rat epididymis in the disposal of the contents of cytoplasmic droplets detached from spermatozoa. *Am J Anat.* 1988;183:107–124.

Hermo L, Lustig M, Lefrancois S, Argraves WS, Morales CR. Expression and regulation of LRP-2/megalin in epithelial cells lining the efferent ducts and epididymis during postnatal development. 1999;53:282–293.

Hermo L, Oko R, Morales CR. Secretion and endocytosis in the male reproductive tract: a role in sperm maturation. *Int Rev Cytol.* 1994a; 154:105–189.

Hermo L, Papp S. Effects of ligation, orchidectomy, and hypophysectomy on expression of the Yf subunit of GST-P in principal and basal cells of the adult rat epididymis and on basal cell shape and overall arrangement. *Anat Rec.* 1996;244:59–69.

Hermo L, Papp S, Robaire B. Developmental expression of the Yf subunit of glutathione S-transferase P in epithelial cells of the testis, efferent ducts, and epididymis of the rat. 1994b;239:421–440.

Hermo L, Robaire B. Epididymal cell types and their function. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice.* New York: Kluwer Academic/Plenum Publishers; 2002:81–102.

Hermo L, Wright J, Oko R, Morales CR. Role of epithelial cells of the male excurrent duct system of the rat in the endocytosis or secretion of sulfated glycoprotein-2 (clusterin). *Biol Reprod.* 1991;44:1113–1131.

Hess RA, Zhou Q, Nie R. The role of estrogens in the endocrine and paracrine regulation of the efferent ductules, epididymis and vas deferens. In: Robaire B, Hinton BT, eds. *The Epididymis: From Molecules to Clinical Practice.* New York: Kluwer Academic/Plenum Publishers; 2002:317–337.

Hoffer AP, Hamilton DW, Fawcett DW. The ultrastructure of principal cells and intraepithelial leucocytes in the initial segment of the rat epididymis. *Anat Rec.* 1973;175:169–202.

Igdoura SA, Morales CR, Hermo L. Differential expression of cathepsins B and D in testis and epididymis of adult rats. *J Histochem Cytochem.* 1995;43:545–557.

Luedtke CC, Andonian S, Igdoura S, Hermo L. Cathepsin A is expressed in a cell- and region-specific manner in the testis and epididymis and

is not regulated by testicular or pituitary factors. *J Histochem Cytochem.* 2000;48:1131–1146.

Luedtke CC, McKee MD, Cyr DG, Gregory M, Kaartinen MT, Mui J, Hermo L. Osteopontin expression and regulation in the testis, efferent ducts, and epididymis of rats during postnatal development through to adulthood. *Biol Reprod.* 2002;66:1437–1448.

Mahuran DJ. Characterization of human placental beta-hexosaminidase I_2: proteolytic processing intermediates of hexosaminidase A. *J Biol Chem.* 1990;265:6794–6799.

Mahuran DJ, Neote K, Klavins MH, Leung A, Gravel RA. Proteolytic processing of human pro-β-hexosaminidase: identification of the internal site of hydrolysis that produces the nonidentical β_a and β_b polypeptides in the mature β-subunit. *J Biol Chem.* 1988;263:4612–4618.

Mayorga LS, Bertini F. Effect of androgens on the activity of acid hydrolases in rat epididymis. *Int J Androl.* 1982;5:345–352.

Meistrich ML, Hughes TH, Bruce WR. Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone. *Nature.* 1975;258:145–147.

Moore HD, Bedford JM. The differential absorptive activity of epithelial cells of the rat epididymis before and after castration. *Anat Rec.* 1979; 193:313–327.

Orgebin-Crist M-C. Androgens and epididymal function. In: Bhasin S, Gabelnick H, Spieler G, Swerdloff R, Wand C, eds. *Pharmacology, Biology, and Clinical Application of Androgens.* New York: Wiley-Liss Inc; 1996:27–38.

Orgebin-Crist M-C, Danzo BJ, Davies J. Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In: Greep RO, Astwood EB, eds. *Handbook of Physiology.* Section 7, Vol 5. Washington, DC: American Physiological Society; 1975: 319–338.

Papp S, Robaire B, Hermo L. Immunocytochemical localization of the Ya, Yc, Yb1, and Yb2 subunits of glutathione S-transferases in the testis and epididymis of adult rats. *Microsc Res Tech.* 1995;30:1–23.

Phaneuf D, Wakamatsu N, Huang J-Q, et al. Dramatically different phenotypes in mouse models of human Tay-Sachs and Sandhoff diseases. *Hum Mol Genet.* 1996;5:1–14.

Robaire B, Hermo L. Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil E, Neill J, eds. *The Physiology of Reproduction.* New York: Raven Press; 1988:999–1080.

Robaire B, Viger RS. Regulation of epididymal epithelial cell functions. *Biol Reprod.* 1995;52:226–336.

Rosenthal AL, Igdoura SA, Morales CR, Hermo L. Hormonal regulation of sulfated glycoprotein-1 synthesis by nonciliated cells of the efferent ducts of adult rats. *Mol Reprod Develop.* 1995;40:69–83.

Russell LD, Bartke A, Goh JC. Postnatal development of the Sertoli cell barrier, tubular lumen, and cytoskeleton of Sertoli and myoid cells in the rat, and their relationship to tubular fluid secretion and flow. *Am J Anat.* 1989;184:179–189.

Scheer H, Robaire B. Steroid Δ^4-5α-reductase and 3α-hydroxysteroid dehydrogenase in the rat epididymis during development. *Endocrinology.* 1980;107:948–953.

Stirling JL, Beccari T, Hoade J, Pezzetti F, Calvitti M, Becchetti E, Orlacchio A. Analysis of the patterns of expression of mRNAs for the alpha- and beta-subunits of the lysosomal enzyme beta-N-acetylhexosaminidase in mouse epididymis and testis. *Histochem J.* 1991;23: 490–494.

Stratton ID, Ewing LL, Desjardins C. Efficacy of testosterone-filled polydimethylsiloxane implants in maintaining plasma testosterone in rabbits. *J Reprod Fertil.* 1973;35:235–244.

Suarez-Quian CA, Jelesoff N, Byers SW. Lysosomal integral membrane proteins exhibit region and cell type specific distribution in the epididymis of the adult rat. *Anat Rec.* 1992;232:85–96.

Sun EL, Flickinger CJ. Development of cell types and regional differences in the postnatal rat development. *Am J Anat.* 1979;154:27–55.

Tindall DJ, Vitale R, Means AR. Androgen binding protein as a biochemical marker of formation of the blood-testis barrier. *Endocrinology.* 1975;97:636–648.

Tomomasa H, Waguri S, Umeda T, Koiso K, Kominami E, Uchiyama Y. Lysosomal cysteine proteinases in rat epididymis. *J Histochem Cytochem.* 1994;42:417–425.

Trasler J, Saberi F, Somani IH, et al. Characterization of the testis and epididymis in mouse models of human Tay Sachs and Sandhoff diseases and partial determination of accumulated gangliosides. *Endocrinology.* 1998;139:3280–3288.