Response of the epididymal duct in the corpus epididymidis to efferent or epididymal duct ligation in the mouse

K. Abe, H. Takano and T. Ito

Department of Anatomy, Hokkaido University School of Medicine, Sapporo, 060 Japan

Summary. Different parts of the epididymal duct were ligated when mice were 90 days old. The mice were killed 1–4 weeks later. PAS-positive materials appeared in the epithelial cells of Segment IV (corpus epididymidis) after ligation of the efferent ducts or at Segment II (middle part of caput) but not when the ligature was distal to Segment II. The inclusions were seen as early as 1 week after ligation and became increased in size and number with time.

Introduction

The epididymis is generally thought to participate in functional maturation and storage of spermatozoa (Hamilton, 1975). The epididymal duct is morphologically divided into several segments which seem to vary functionally, although the functional significance of each segment remains uncertain (Nicander, 1970; Hamilton, 1975; Jones, Hamilton & Fawcett, 1979).

The epididymal duct of the mouse can be divided into five segments (I–V), Segments I–III constituting the head; Segment IV, the body; and Segment V, the tail of the epididymis (Takano, 1980).

It has been well documented that after efferent duct ligation the epithelium of Segment I, generally termed the initial segment, shows significant changes in structure, and that the segment functionally depends on the testicular fluid contained in the lumen (Fawcett & Hoffer, 1979). However, it is not clear whether the ligation induces morphological changes in the other segments. When the efferent ducts of the mouse are ligated the epithelium in Segment IV of the epididymal duct undergoes histological changes which are characterized by the appearance of intracellular PAS-positive inclusions (Takano, Abe & Ito, 1981). We have now examined whether such characteristic changes in Segment IV occur after ligation at various sites of the epididymal duct.

Materials and Methods

Male dd mice were used. At 90 days of age, the 97 animals were anaesthetized with pentobarbital sodium injected intraperitoneally. The epididymis and testis were exposed through a median incision of the lower abdomen. The efferent ducts or epididymal duct were ligated on one side in each mouse, the other side being left as a normal control. The epididymal duct was ligated at Segment II, Segment III, the junction between Segments III and IV or between Segments IV and V (Pl. 1, Fig. 1). The ligatures were of silk thread and avoided damage to blood vasculature.

© 1982 Journals of Reproduction & Fertility Ltd

0022-4251/82/010069-06$02.00/0
The animals were killed 1–4 weeks after operation, and the epididymis and testis were removed. They were fixed in Bouin's or Helly's fixative for 3 h, dehydrated and embedded in paraffin wax. Serial longitudinal sections of the epididymis were cut at 10 μm and stained with periodic acid-Schiff (PAS) and haematoxylin.

Results

The histological characteristics of the 5 segments of the epididymal duct are illustrated in Pl. 1, Figs 1–4. The epithelium of the duct is highest in Segment I, and becomes progressively lower with succeeding segments. In Segment II the cytoplasm of the epithelial cells stains with PAS and the apical portions of the cells are strongly positive; the stereocilia of the epithelial cells are embedded within strongly PAS-positive materials. The cytoplasm of the epithelial cells in Segments I, III, IV and V shows no PAS reaction. Spermatozoa contained in the lumen of the epididymal duct are sparse in Segment I, but they increase in number with their passage through the epididymis. In Segments IV and V, the lumen is extremely distended with abundant spermatozoa. In all the segments but Segment I, PAS-positive materials are present in the lumen in addition to spermatozoa.

After ligation of the efferent ducts, the epithelial cells of the epididymal duct showed striking changes in Segments I and IV. In Segment I, the epithelial cells decreased in height, and the cytoplasm stained with PAS. The lumen also contained PAS-positive materials. Segment I therefore became similar in its morphological features to Segment II. In Segment IV, PAS-positive inclusions appeared in the epithelial cells (principal cells) (Pl. 2, Figs 5–10) and their distribution is summarized in Table 1. The inclusions were usually present in the supranuclear cytoplasm, and occurred as round granules or globules measuring 2–15 μm in diameter. They tended to become confluent, and sometimes occupied the whole cytoplasm. The inclusions were more frequent in the proximal portion than in the distal portion of Segment IV. No spermatozoa were seen in the lumen in Segments I–IV, but a few remained in Segment V. The PAS-positive materials were present in the lumen of Segments I to V, particularly in Segments IV and V.

Table 1. PAS-positive inclusions in the epithelial cells and the luminal contents in Segment IV of the mouse epididymal duct

<table>
<thead>
<tr>
<th>Site of ligation</th>
<th>Efferent duct</th>
<th>Segment II</th>
<th>Segment III</th>
<th>Junction between Segments III and IV</th>
<th>Junction between Segments IV and V</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice studied</td>
<td>38</td>
<td>11</td>
<td>11</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>PAS-positive inclusions in the epithelial cells*</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PAS-positive materials in the lumen</td>
<td>++</td>
<td>++</td>
<td>±</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Spermatozoa in the lumen</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* These inclusions were present (+) or absent (−); there were no exceptions and the inclusions were present by 1 week after ligation.

After ligation of the epididymal duct at Segment II, PAS-positive inclusions also appeared in the epithelial cells of Segment IV. However, after ligation of the duct at Segment III or at the junction between Segments III and IV, no PAS-positive inclusions appeared in the epithelial cells in Segment IV (Pl. 2, Figs 5–10), but the epithelial cells increased in height. The lumen in Segment IV was diminished in diameter, contained no spermatozoa, and the luminal contents showed very little PAS reaction. The lumen in Segment V was also less dilated, but contained a
Fig. 1. Longitudinal sections of the testis and epididymis of the mouse. ED, efferent duct; I–V, segments of epididymal duct; DD, deferent duct. Segment II appears dark because the epithelial cells are stained with PAS. PAS–haematoxylin. × 10.

Figs 2–4. Sections through Segments I–V of the mouse epididymis. PAS–haematoxylin. × 70. Fig. 2. Head of the epididymis consisting of Segments I, II and III. In Segment II the epithelial cells are PAS-positive, and their luminal border is dark because the stereocilia are embedded within PAS-positive materials. Fig. 3. Body of the epididymis representing Segment IV. Fig. 4. Tail of the epididymis containing Segment V.

(Facing p. 70)
Figs 5–7. Sections through Segment IV of the mouse epididymal duct. PAS–haematoxylin. × 200. Fig. 5. Normal: the lumen contains spermatozoa and PAS-positive materials. Fig. 6. After ligation at the efferent ducts: the lumen contains strongly PAS-positive materials but no spermatozoa. Arrows indicate PAS-positive inclusions in the epithelial cells. Fig. 7. After ligation at the junction between Segments III and IV: the lumen is diminished in diameter, and contains no spermatozoa and almost no PAS-positive materials.

Figs 8–10. Epithelial cells of Segment IV of the mouse epididymal duct. PAS–haematoxylin. × 600. Fig. 8. Normal Fig. 9. After ligation at the efferent ducts: arrows indicate PAS-positive inclusions in the epithelial cells. Fig. 10. After ligation at the junction between Segment III and IV.
few spermatozoa and PAS-positive materials. In Segments II and III, which were proximal to ligation, the lumen was distended with spermatozoa and PAS-positive materials, but Segment I remained almost unchanged in appearance. After ligation at the junction between Segments IV and V, the lumen of the epididymal duct in Segment IV was distended with many spermatozoa and PAS-positive materials, but no inclusions appeared in the epithelial cells.

Discussion

The present results suggest that PAS-positive materials are produced in Segment II and then transported along the duct. The distribution of acidic epididymal glycoprotein in the normal or ligated rat epididymal duct (Lea, Petrusz & French, 1978) is almost the same as that of PAS-positive materials observed in the mouse epididymis. It is therefore possible that the PAS-positive materials in the epididymal lumen contain the glycoprotein demonstrated chemically.

In the rat, it has been shown that specific epididymal glycoprotein or protein is secreted by epithelial cells of the caput (distal to the initial segment), and spermatozoa become coated with these compounds as they leave the initial segment and remain coated during passage through the organ (Lea et al., 1978; Faye, Duguet, Mazzuca & Bayard, 1980; Kohane, Cameo, Piñeiro, Garberi & Blaquier, 1980). Evidence for the binding of glycoprotein or protein to the sperm membrane has accumulated, and such compounds have been considered to mediate functional maturation of spermatozoa (Barker & Amann, 1970; Köpečný, 1971; Johnson & Hunter, 1972; Köpečný & Pech, 1977; Flickinger, 1979; Moore, 1980). We found that PAS-positive inclusions appeared in the epithelial cells of Segment IV only after ligation of the efferent ducts or Segment II, but not after ligation of Segment III or between Segments III and IV. Ligation of the efferent ducts or Segment II excludes spermatozoa, but allows the luminal contents to flow from Segments II to IV, whereas ligation at Segment III or the junction of Segments III and IV obstructs the flow of the luminal contents from Segment II to Segment IV. It can therefore be inferred that the occurrence of PAS-positive inclusions in the epithelial cells of Segment IV is related to a flow of the luminal contents from Segment II. It is likely, as indicated above, that the PAS-positive materials which are considered to contain compounds binding to spermatozoa are secreted by the epithelial cells of Segment II. However, when the duct is ligated at a level proximal to Segment II and spermatozoa are excluded, the PAS-positive materials would not be utilized for binding to spermatozoa and large amounts of the free PAS-positive materials would flow from Segment II to the distal segments. In such conditions, PAS-positive inclusions appear in the epithelial cells of Segment IV. On the other hand, when the epididymal duct is ligated at the junction between Segments IV and V, the luminal contents of spermatozoa and bound glycoprotein are accumulated within the lumen of Segment IV, and no PAS-positive inclusions appear in the epithelial cells of the segment. Bedford (1978) has suggested that the protein secreted in the proximal region of the epididymis is resorbed in the distal region if not bound by spermatozoa. The ultrastructure of the epithelial cells in the body of the epididymis indicates absorptive activity (Jones et al., 1979). It is therefore likely that the PAS-positive materials produced in Segment II are, in the absence of spermatozoa, reabsorbed by the epithelial cells of Segment IV and deposited as intracellular inclusions.

References


Fawcett, D.W. & Hoffer, A.P. (1979) Failure of exogenous androgen to prevent regression of the
initial segments of the rat epididymis after efferent duct ligation or orchidectomy. Biol. Reprod. 20, 162–181.


Received 2 February 1981