Interruption of the Luminal Flow in the Epididymal Duct of the Corpus Epididymidis in the Mouse, with Special Reference to Differentiation of the Epididymal Epithelium

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Summary. The efferent duct or the epididymal duct at the border between the caput and corpus epididymidis was cut or ligated in mice at the following stages: birth, 20 days, and 60 days of age. The epididymal duct of the corpus epididymidis was observed at 60 days of age after neonatal or juvenile operation and 4 weeks after the adult operation.

1) After efferent duct interruption, the epididymal duct in the corpus possessed abundant PAS-positive material with no spermatozoa in the lumen, and the principal cells of the epithelium contained PAS-positive inclusions which were considered to be formed due to absorption of the PAS-positive material secreted by the principal cells in the caput epididymidis.

2) Especially after interruption at the border between the caput and corpus epididymidis in juvenile mice, the epithelium of the epididymal duct in the corpus was much taller with peculiar pale, vacuolated cells. These cells were considered as abnormally differentiated forms of the principal cells.

3) The occurrence of the PAS-positive inclusions and peculiar pale cells varied in frequency according to the level and age of interruption of the epididymal duct.

The results suggest that the luminal contents produced in the proximal region of the duct may exert an influence on the differentiation of the principal cells of the epididymal duct.

The mouse epididymal duct can be divided into five segments (I–V): Segments I, II, and III constitute the caput, Segment IV the corpus, and Segment V the cauda (Takano, 1980; Abe et al., 1982a, 1983a) (Fig. 1). After ligation of the efferent duct, PAS-positive inclusions appeared in the principal cells in Segment IV, as spermatozoa disappeared from the epididymal duct (Abe et al., 1982a). Such inclusions did not appear after ligation at the junction between Segments III and IV (Abe et al., 1982a, b). Regarding such inclusions, our experimental studies (Abe et al., 1982a, b; 1983c, 1984) have indicated that the principal cells in Segment II secrete PAS-positive material into the lumen, which, in the absence of spermatozoa, might be absorbed by the principal cells in Segment IV and deposited as intracellular inclusions.

In adult mice which had been subjected to juvenile ligation at the junction between Segments III and IV, the epithelium of the duct in Segment IV contained clusters of
pale, vacuolated cells which were considered as abnormally differentiated principal cells (Abe et al., 1982b). The abnormal, pale cell clusters did not appear when the ligation was performed in adult mice, probably because the principal cells had already been differentiated at the time of operation (Abe et al., 1982b). Therefore, the appearance of the abnormal pale cells is thought to be related to the state of differentiation of the principal cells.

The ligation experiments suggest that the differentiation and function of the principal cells in Segment IV are dependent upon the luminal fluid transported from the caput epididymidis. For the purpose of more fully understanding the relationship between the principal cells and the fluid contained in Segment IV, we reexamined the response of the principal cells in Segment IV to interruption at the efferent duct and at the junction between Segments III and IV. The interruption was also performed on the day of birth when the duct was still undifferentiated.

MATERIALS AND METHODS

In this study, 217 male dd-mice were used. The animals at 20 days or 60 days of age were anesthetized with intraperitoneally injected pentobarbital sodium (Nembutal) and the epididymis and testis exposed through a median incision of the lower abdomen. In each mouse, either the efferent duct or the epididymal duct at the border between the caput and corpus was ligated on one side, while that on the other side was left as a control (Fig. 1). Animals on the day of birth were hypothermally anesthetized by cooling in a freezer for 5 min, and then under the dissecting microscope the epididymis and testis were exposed, and either the efferent duct was cut off or the caput epididymidis was removed.

The animals operated on at birth and 20 days of age were killed at 60 days. The animals operated on at 60 days were killed four weeks later. The epididymis and testis were then removed, fixed in Bouin's fixative for 3 hrs, dehydrated, and embedded in paraffin. Longitudinal sections of the epididymis were serially cut at 10 μm and stained with periodic acid-Schiff (PAS) and hematoxylin.

Quantitative observations: The PAS-positive inclusions appearing in Segment IV were classified into three grades (0, 1, and 2): Grade 0 indicated no recognizable inclusions; Grade 1, a few inclusions in several sections; and Grade 2, many inclusions in every section. The abnormal pale cells were also classified into four grades (0-3) according to the amounts; Grade 0 was normal with no pale cells; Grades 1 to 3 represented the increasing appearance of small to large amounts of abnormal pale cells, as described previously (Abe et al., 1982b).

Fig. 1. Diagram of the mouse testis and the excurrent ducts. The epididymis is composed of five segments (I-V) of the epididymal duct. The efferent duct (left) or the junction between Segments III and IV of the epididymal duct (right) was ligated or cut (arrows and thick lines), and the changes occurring in Segment IV (dotted) were observed.
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Fig. 2. Normal epididymal duct. PAS and hematoxylin. ×350  

a. Epididymal duct in Segment II (II) and Segment III (III). Because of its PAS-positive cytoplasm, the epithelium in Segment II appears darker than that in Segment III. The lumen contains spermatozoa and PAS-positive material.  

b. Epididymal duct in Segment IV. The epithelial cells are lower than those in Segments II and III. The lumen is distended with abundant spermatozoa and PAS-positive material.
RESULTS

Normal epididymal duct
The histological features of the five segments of the mouse epididymal duct have been described in detail in our previous papers (Takano, 1980; Abe et al., 1983a). The epithelium of the epididymal duct is mainly composed of principal cells with stereocilia on the luminal surface. The principal cells are highest in Segment I, becoming progressively lower with succeeding segment. In Segment IV, they are about 15 μm high and about 8 μm wide. In Segment II, the cytoplasm of the principal cells stains positively with PAS (Fig. 2a). The apical portion of the cells is distinctively PAS-positive, and the stereocilia are embedded within strongly PAS-positive material. In Segments I, III, IV and V, the cytoplasm of the principal cells shows no PAS reaction (Fig. 2a, b). The lumen distal to Segment II contains PAS-positive material as well as spermatozoa.

 Interruption at the efferent duct
Regardless of the time of interruption, the principal cells in Segment IV contain PAS-positive inclusions (Fig. 3a). These inclusions were present in the supranuclear cytoplasm, and occurred as round granules or globules measuring 2 to 15 μm in diameter. They were usually more frequent in the proximal portion than in the distal portion of Segment IV. Such inclusions were not found in the other remaining segments.

 The PAS-positive inclusions appeared in all the epididymides ligated at 60 days (Fig. 4). Of all the epididymides, 96% were Grade 2. In the epididymides operated on at earlier ages, the inclusions were much smaller in amount. Of all the epididymides neonatally interrupted, 15% were Grade 1 and 60%, Grade 2 (Fig. 4). After neonatal or juvenile interruption, on the other hand, PAS-positive inclusions did not appear in some epididymides. Of the epididymides neonatally interrupted, 24% were Grade 0 and 14% of the epididymides after juvenile interruption were Grade 0 (Fig. 4).

 In Segment IV, which had distinct intracellular PAS-positive inclusions, no spermatozoa were contained in the lumen, which was distended with strongly PAS-positive material. In epididymides with no inclusions, the lumen of the duct contained little PAS-positive material.

 No clusters of abnormal pale cells were seen in the epididymides subjected to the efferent duct interruption.

 Interruption at the junction between Segments III and IV
After interruption at the junction between Segments III and IV, especially at the juvenile age, the epithelium of the epididymal duct in Segment IV became thickened with peculiar epithelial cells, which were usually clustered in the basal portion (Fig. 3b). These cells were rounded, and their cytoplasm appeared pale. They occasionally showed vacuoles measuring 2 to 15 μm in diameter (Fig. 3b). Furthermore, large vacuole-like spaces, 10 to 50 μm in diameter, were seen between the pale cells. The vacuoles and vacuole-like spaces usually had stereocilia on the inner surface. The pale cells appeared to represent abnormally differentiated cells. They were usually more frequent in the proximal than in the distal portion of Segment IV.

 The peculiar pale cells differed in amount according to the age of interruption of the epididymal duct (Fig. 5). After interruption at 60 days, most of the epididymides were Grade 0 (no clusters of abnormal pale cells) or Grade 1. Thus, after interruption in adults, the epithelium had an almost normal appearance. After interruption in
Fig. 3. Epididymal duct in Segment IV after efferent or epididymal duct interruption. PAS and hematoxylin. ×350  

a. After juvenile ligation at the efferent duct. PAS-positive inclusions (arrows) are seen in the principal cells. The lumen contains strongly PAS-positive material but no spermatozoa. 

b. After neonatal cutting at the junction between Segments III and IV. The epithelium is thickened by clusters of peculiar epithelial cells with frequent vacuoles (V). The narrowed lumen (asterisks) contains no spermatozoa and little or no PAS-positive material. Arrows indicate the normal-looking epithelium.
juvenile mice, however, the epididymides showed remarkable morphologic changes. Most of them were Grade 3. The changes in the epididymides were generally less marked after neonatal operation than after juvenile operation. About one third of them were Grade 3.

No PAS-positive inclusions appeared in the principal cells in epididymides ligated at 60 days (Fig. 6). On the other hand, distinctive PAS-positive inclusions (Grade 2) were seen in 20% and 8% of epididymides after neonatal and juvenile interruption (Fig. 6).

In the epididymides interrupted at the juvenile stages or just after birth, the lumen of the duct in Segment IV was usually narrow, with no spermatozoa and little PAS-positive material. However, the lumen of the duct where the principal cells had PAS-positive intracellular inclusions, contained much PAS-positive material.

The appearance of PAS-positive intracellular inclusions and peculiar pale cells in the epididymal duct interrupted at various ages is summarized in Figure 7.

**DISCUSSION**

The epididymal duct is morphologically divided into several segments which seem to
vary functionally (Benoit, 1926; see reviews by Bedford, 1975; Hamilton, 1975). In general, the proximal region of the duct is thought to participate in the functional maturation of spermatozoa, and the distal region, in storage of matured spermatozoa (see reviews by Bedford, 1975; Hamilton, 1975). The morphological and functional differentiation dependent on androgen is completed after puberty (Cavazos, 1958; Alexander, 1972).

It has recently been demonstrated in the rat, hamster and rabbit, that the principal cells in the caput epididymidis secrete specific epididymal glycoproteins which coat spermatozoa in order to provide fertilizing ability (Lea et al., 1978; Faye et al., 1980; Moore, 1980). In the mouse, the epididymal glycoprotein is likely associated with PAS-positive material produced by the principal cells in Segment II and the PAS-positive material might be absorbed by the principal cells in Segment IV (Abe et al., 1982a, 1983c, 1984). The absorptive activity in Segment IV is indicated by the finding that PAS-positive inclusions appear after interruption of the efferent duct (Abe et al., 1982a) or testicular irradiation which induces aspermia in the epididymal duct (Abe et al., 1983c). In Segment IV, the principal cells do not possess the PAS-positive inclusions under normal conditions, but their ultrastructure suggests that even normally these cells are involved in absorbing luminal material in the presence of spermatozoa in the duct (Abe et al., 1983b, 1984). The PAS-positive inclusions are considered to occur in the principal cells which have ingested a large quantity of the luminal material under certain conditions. Thus, appearance of the PAS-positive inclusions suggests a functional state of particular differentiation of the cells (Fig. 8).

As shown in Results, PAS-positive inclusions appeared in the principal cells in Segment IV in all the epididymides after efferent duct interruption at 60 days. However, in the epididymides interrupted at birth, one-fourth of all the cases showed no PAS-positive inclusion; in the epididymides interrupted at 20 days, the inclusions did not appear in 14%. The finding that PAS-positive inclusions did not always appear in the epididymis interrupted at earlier ages suggests two possibilities: 1) the principal cells in Segment IV were not yet fully differentiated to absorb the luminal PAS-positive material and/or 2) the principal cells in Segment II were not differentiated enough to secrete the PAS-positive luminal material. The occurrence of the PAS-positive inclusions in Segment IV appears to depend on the amount of the PAS-positive material accumulated in the lumen; large amounts of the accumulated contents in the lumen caused the inclusions to occur, but smaller amounts of the contents did not. As described above, the luminal PAS-positive material is considered to be secreted from the

Fig. 6. Frequency (%) of the epididymides showing Grades 0, 1 or 2 in the appearance of PAS-positive inclusions in Segment IV after neonatal (Nn), juvenile (J) and adult (Ad) interruption at the junction between Segments III and IV. Bars with no dot, small dots, and large dots represent Grade 0 (no inclusion), Grades 1 and 2, respectively; and numbers on the right side of the bars, the frequencies (%).
principal cells in Segment II. After interruption at the efferent duct, it is probable that the function of the principal cells in Segment II may be depleted and that no PAS-positive inclusions are formed in Segment IV because the luminal contents are so small in quantity.

It is well known that the differentiation of the initial segment, Segment I, is dependent upon the testicular fluid flowing into epididymal duct (GUSTAFSSON, 1966; MONIE, 1978; FAWCETT and HOFFER, 1979). The principal cells in Segment I

Fig. 7. Diagram representing the mouse epididymal ducts after neonatal (Neonat), juvenile (Juven) and adult (Adult) interruption. I-V five segments of the duct. Thick lines indicate the sites of ligation or cutting. The first and second lines of the epididymal ducts represent the appearance of PAS-positive inclusions and abnormal pale cells after interruption of the efferent duct, respectively. The third and fourth lines of the epididymides represent the appearance of PAS-positive inclusions and abnormal pale cells after interruption at the junction between Segments III and IV, respectively. The numbers express the approximate frequencies (%) of the epididymides, showing the distinct appearance of PAS-positive inclusions or abnormal pale cells.

Fig. 8. Diagram representing a possible relationship between the principal cells in Segment II (S II) and those in Segment IV (S IV). Squares show the principal cells. The principal cells in Segment II secrete PAS-positive material which binds to spermatozoa, while the remaining PAS-positive material unbound to spermatozoa is absorbed by the principal cells in Segment IV (upper). If no spermatozoa are present in the lumen of the epididymal duct (upper), large amounts of the PAS-positive material is accumulated in the duct lumen, absorbed by the principal cells in Segment IV, and cause the appearance of intracellular PAS-positive inclusions.
significantly decrease in height after efferent duct interruption blocks the influx of the luminal contents from the testis (Gustafsson, 1966; Moniem et al., 1978; Fawcett and Hoffer, 1979; Takano et al., 1981). Similarly, the differentiation of Segment II, in which PAS-positive material coating spermatozoa is thought to be produced, may be influenced by the presence of spermatozoa or fluid produced in the region proximal to the Segment I. If the epithelium in Segment II is not exposed to either spermatozoa or the fluid, Segment II is assumed to fail to differentiate well.

On the other hand, the appearance of peculiar pale cells in Segment IV after interruption at the junction between Segments III and IV seems to be due to abnormal differentiation of the principal cells (Abe et al., 1982b). The pale cells did not appear after interruption in adult mice, though they were distinct after interruption in juvenile mice. Thus, whether the peculiar pale cells appear after interruption or not is probably due to the state of differentiation of the principal cells in Segment IV. The epithelium in Segment IV in adult mice is completely differentiated, while that in juvenile mice remains undifferentiated. The interruption at the junction between Segments III and IV blocks the influx of the luminal contents into Segment IV. As described above, the principal cells in Segment IV are considered to ingest the luminal PAS-positive material produced in Segment II. Thus the differentiation of Segment IV seems to occur in ways varying from the normal if the undifferentiated principal cells are not exposed to the PAS-positive material at early stages. In other words, the PAS-positive material contained in the duct is likely to play a role in the differentiation of the principal cells in Segment IV.

It may be expected that the peculiar pale cells occur more frequently in the epididymis interrupted neonatally than at later age. This was, however, not the case:
the peculiar cells appeared less frequently after neonatal interruption. The PAS-positive inclusions were observed after both juvenile and neonatal interruption, especially frequent in the epididymides subjected to neonatal operation, but they did not appear after interruption at 60 days of age. Thus, it is probable that some of the principal cells in Segment IV become differentiated into the cells that are functionally similar to those in Segment II and produce the PAS-positive material in Segment IV, and that the PAS-positive material thus produced prevents the epithelial cells in Segment IV from differentiation into the pale cells.

The occurrence of peculiar pale cells and PAS-positive inclusions after interruption at the junction between Segments III and IV suggests that the epithelial cells in Segment IV have the potential to differentiate in certain unusual directions early in life, but they lose this potential as they become differentiated with age (Fig. 9). In addition, it is likely that the differentiation of the epithelial cells in the epididymal duct is influenced by the contents transported in the lumen.

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