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Testicular Changes in Adult Rat following Bilateral Partial (Caput) Epididymectomy

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Adult Wistar rats were either partial (caput) and bilaterally epididymectomized or bilaterally efferentectomized, as controls of duct obstruction. The effects on testicular germinal epithelium were studied at 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 days after surgery. No abnormalities were detected in sham-operated animals. Epididymectomized animals showed different levels of alterations with progressive disruption of the seminiferous epithelium, emergence of multinucleated bodies and some tubules obliterated by degenerated cells and cellular debris. Half way through the experiment there were tubules lacking their epithelia, as well as the Sertoli cells. On the 25th day degeneration was so important that it affected not only the epithelium (missing in almost all tubules) but also the tubular morphology. Eventually efferentectomized animals showed a progressive alteration, but its level was much lower than that observed after partial epididymectomy, indicating a possible specific function of the caput epididymidis in the control of testicular function.

Key words: Epididymectomy, Efferentectomy, Testicular, Caput epididymidis, Rat.

The epididymis has secretory and absorptive functions that are believed to be related to the maturation and storage of sperm (9), and a large number of studies about the epididymal physiology having been carried out on this topic (31).

Several studies have been performed about the epididymal function by means of surgical technics: experimental criptorchidism (21, 35); orchidectomy (6, 30); ligation or cutting of the ductuli efferentes (1, 24, 25, 27); ligation at different levels of the epididymal duct (2, 17, 18); or vasectomy (7, 32).

Less effort has been spent in the comprehension of the proper epididymal function, especially its possible role on the control of the male sexual physiology (12, 13). Thus, the only references found

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about, total or partial epididymectomy were those mentioned by LE LANNOU and CHAMBON (11).

The present study examines the testicular morphological changes after bilateral partial (caput) epididymectomy (PCE) as a new approach to the problem of the epididymal significance into general male sexual physiology.

Materials and Methods

Fifty adult Wistar rats (150 days old) were randomly divided in partial caput epididymectomized (PCE) group (twenty animals); efferentectomized (EDL) group (twenty animals); and sham-operated group (10 animals). The animals were mantained under natural light-dark schedule and received standard laboratory diet and water *ad libitum*.

Animals from PCE and EDL groups were anesthetized by ether inhalation and the epididymal caput was exposed through a little incision of the scrotum of each side. In the EDL group the efferent ducts of each testis were ligated close to epididymal caput and cut at the epididymal side of ligature. In the PCE group the same procedure was carried out and, moreover, the epididymal duct at the border between the caput and corpus was ligated on each side and both caputs were removed (fig. 1). Testis and remaining epididymis were returned into the scrotum. The other animals were sham-operated and used as controls.

Testis from two animals of the PCE group, two of the EDL group, and one of the control group were removed under ether anesthesia and fixed in 4 % formaldehyde for two hours on the 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25th days after surgery. A longitudinal 3 mm slice from each testis was made and post-fixed in 10 % formaldehyde for other 24 h. The slices were dehydrated through graded alcohols and embedded in parafine. Sections were cut at 6 μm and stained with haematoxylin and eosin (for conventional light microscopy) or with Acridine Orange (for fluorescence microscopy). A Nikon Fluophot microscope, equipped either with normal illumination or with epifluorescence illumination systems (set at wavelengths of 420-490 nm excitation) was used for the observations.

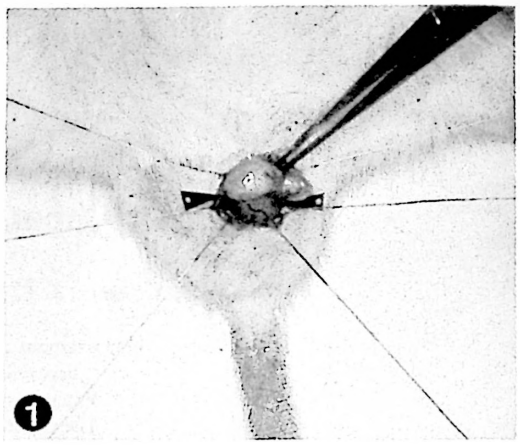

Fig. 1. *Scrotal incision and exposure of caput epididymidis for its extirpation.*
The pointers indicate the sites of ligatures and cutting.

Results

No abnormalities were detected in tissues from sham-operated animals in all the periods considered (fig. 2A).

The germinal epithelium in the PCE animals showed different levels of alteration, ranked in periods as follow:

7-11 days (fig. 2B): several tubules showed disruption of the seminiferous epithelium with loss of spermatids in all steps of development, as well as occasional presence of multinucleated bodies into the tubular lumen (fig. 4). These alterations were more frequent in the peripheric zone and apical pole, near the *rete testis*.

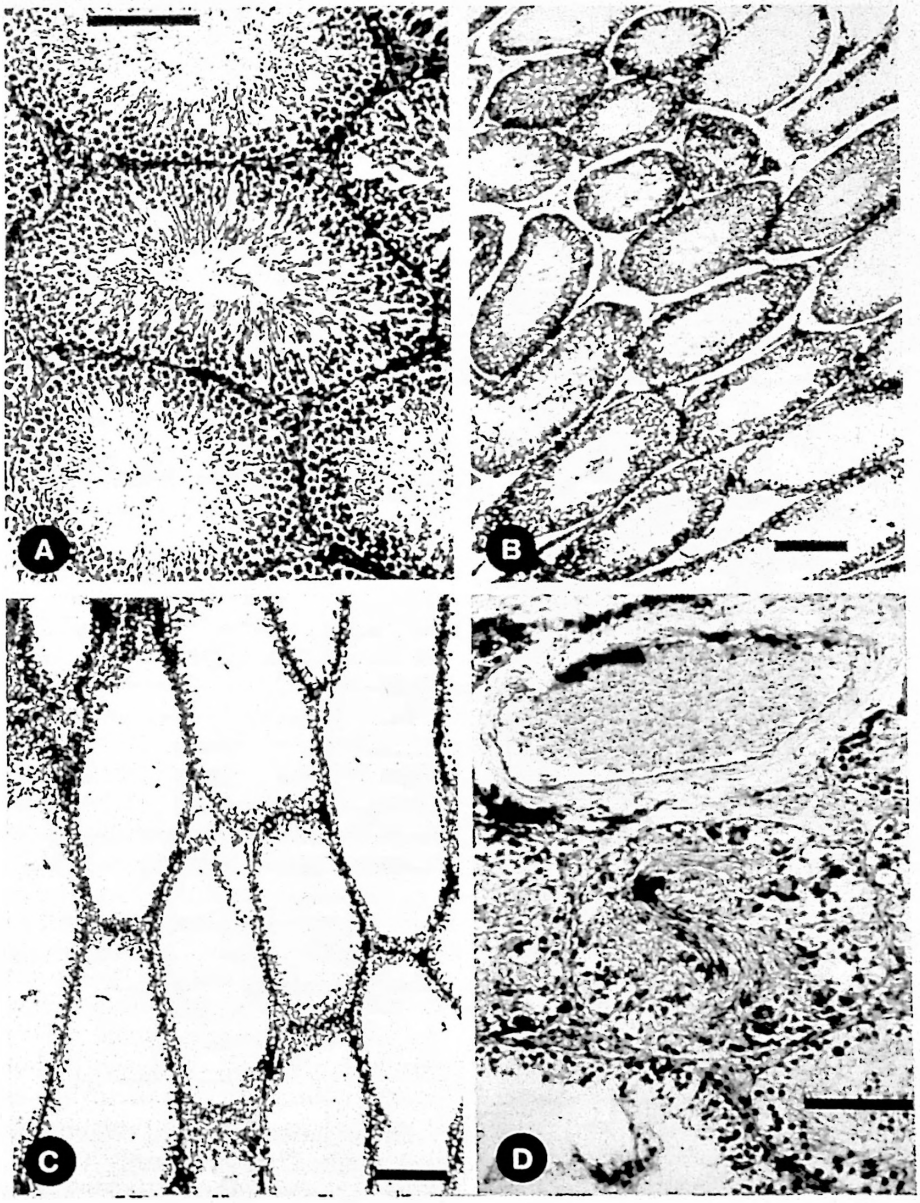

Fig. 2. *Testis of rat.*
A) Control: germinal epithelium seems to be normal. B) Testis 7 days after epididymectomy. Some seminiferous tubules show disruption of spermatogenesis, while others seem to be normal. C) Testis 13 days after epididymectomy. Only spermatogonia and Sertoli cells are present in the seminiferous tubules. D) Testis 15 days after epididymectomy. The germinal epithelium appears disordered and cells have been exfoliated into the lumen. The central tubule is obliterated by degenerate cells and cellular debris. Haematoxylin and eosin. Bar = 50 μm.

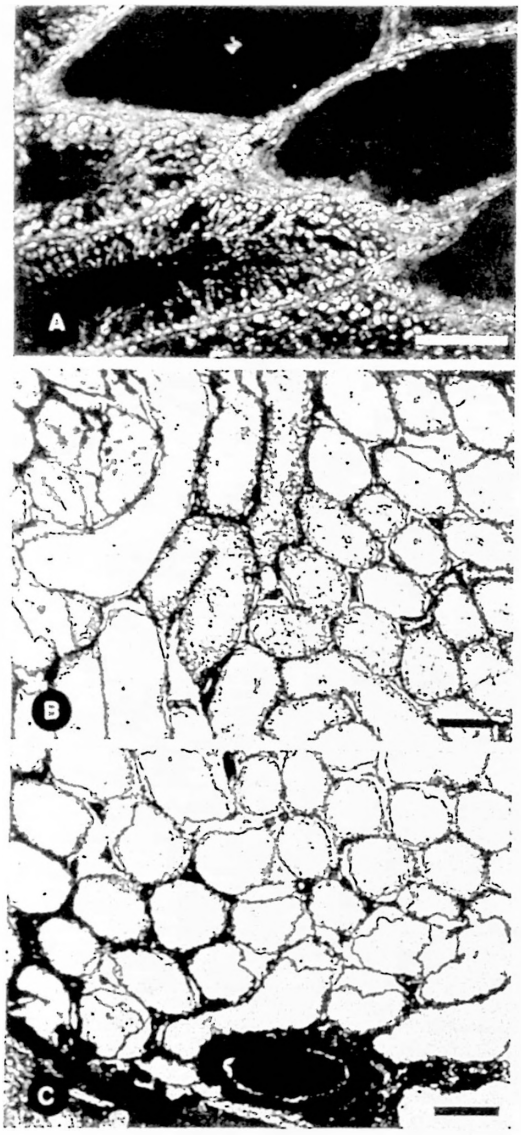

Fig. 3. *Testis of rat after epididymectomy.* A) 17 days: some tubules lack the epithelium, as well as the Sertoli cells, but normal sections of other tubules are in close contact. Acridine Orange. B) 21 days: most of the seminiferous tubules are empty or with only a few isolated germ cells. Heamatoxylin and eosin. C) 25 days: The epithelium are missing in almost all tubules and the tubular morphology was also affected. Haematoxylin and eosin. Bar = 50 μm.

13-17 days: an increase in the number of tubules with disorganization of epithelium (fig. 2C) and multinucleated bodies was observed. The distribution of these alterations was extensible to all the surface of the testicular area. Some tubules, particularly those near the *rete testis*, were obliterated by degenerate cells and cellular debris (fig. 2D). Some tubules with lack of the epithelium, and Sertoli cells, were also detected (fig. 3A).

19-25 days: It the beginning of this period a considerable increase in the number of empty tubules was observed, while normal tubules were very infrequent (fig. 3 B). Since day 23rd the degeneration was so important that it affected not only the epithelium (missing in almost all tubules) but also the tubular morphology (fig. 3C). The number of multinucleated bodies was greater than in the other periods, but those ones were accumulated in the tubules which still had some epithelium (figure 4).

The alterations observed in the EDL animals were very low. So, the major epithelial damage shown in these animals correspond to that observed in PCE animals between 7-11 days. The presence of multinucleated bodies was very rare and it was observed only 13-17 days after surgery. More severe alterations of empty tubules and modifications of tubular morphology were not observed.

Discussion

The practice of partial epididymectomy as a method for the study of the epididymal physiology represents a novelty in the scientific literature about this topic (28). This is comprehensible on the common paradigma about the epididymal function and its relationship to later maturation and storage of spermatozoa (31).

The present results after PCE showed a rapid increase in the degenerative process of testicular epithelium, practically com-

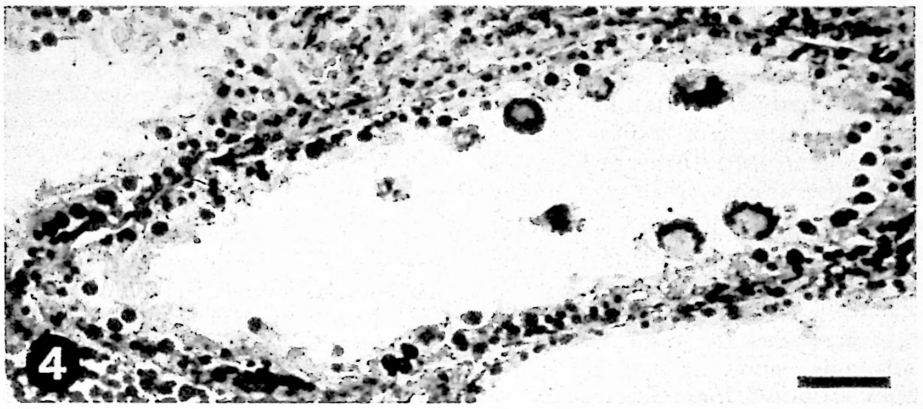

Fig. 4. *Testis 23 days after epididymectomy.*
Multinucleated bodies present a variable number of nucleus with a peripheral distribution. Haematoxilin and eosin. Bar = 20 μm.

plete on the 25th day. Besides, the great number of multinucleated bodies in the tubular lumen is a particular feature observed after PCE, while after EDL these multinucleated bodies are very infrequent. We have preferred to use the term «multinucleated bodies», rather than that used by other authors (16, 34), since the aspect presented by these structures seem to indicate that the nucleus was not surrounded by a cellular membrane (unpublished data).

A similar drastic effect on the testicular germinal epithelium was reported after obstruction of the fluid which flows in a single seminiferous tubule by PILSWORT *et al.* (19). They have postulated that this effect could be due to local build-up of metabolites and/or shortage of some essential substrates in the tubule lumen by alteration of Sertoli cells function. The degeneration of germinal epithelium also could be due to the alterations in the blood-testis barrier, with lack of some necessary compound for normal maintenance of the epithelium (26, 33).

On the other hand, EDL results in an atrophy of the seminiferous epithelium. Before the total epithelium atrophy is produced, serum FSH concentrations are already significantly elevated despite normal serum testosterone concentrations (3, 4, 15). These findings suggest a reduced feedback inhibition of pituitary FSH secretion by testicular inhibine (4, 13, 14). It has been proposed that the route of the inhibine passage to the excurrent duct system where it could be reabsorbed and reenter the general circulation might be blocked (8, 13).

The alterations of the germinal epithelium described here after EDL, according to other autors (4, 5, 10, 23-25, 27) is, however, much lower than that observed after PCE. Thus, the above mentioned explicative hypothesis for testicular atrophy after EDL stands in contradiction with our results. On this basis it can be supposed that the epididymal caput has a specific function, possibly by secretion of some substance or substances, directly or indirectly related to the testicular function. Moreover these substances might be vehicled by blood circulation, only interrupted when epididymectomy was made and never by retrograde ductuli circulation, as PAUFLER and FOOTE postulated (18), since the latter seems theoretically impossible in both cases, PCE and EDL.

Besides, several morphological obser-

vations have suggested a possible endocrine activity located in the epithelial cells of the epididymis. The F-zone of the mouse epididymal caput could absorb Rhodamine 6GO specifically after its subcutaneous application, and this property is common to several endocrine glands (28, 29). According to RAMOS and DYM (20) the many small dense granules at the base of the principal cells near interstitial capillaries resemble endocrine secretion granules seen in the gastrointestinal tract, pancreas, adrenals and other endocrine glands, and this cytologic feature raises the question of a possible endocrine function for the monkey epididymis. As REID and CLELAND (22) said, if we regard the vacuoles of the clear cells as secretory in nature, the fact that these vacuoles accumulate first in the basal cytoplasm facing the blood vessels might suggest an endocrine function in the rat.

Nowadays we are working on the quantization of the degeneration pattern and hormonal analysis after partial epididymectomy in order to approach a better understanding of this problem.

Resumen

En ratas adultas, se estudian los efectos sobre el epitelio germinal del testículo tras epididimectomía parcial (cabeza) o eferentectomía bilateral, como control de la obstrucción ductal, a los 7, 9, 11, 13, 15, 17, 19, 21, 23 y 25 días de practicadas las intervenciones quirúrgicas. No se observan alteraciones en los animales operados ficticiamente. Los animales epididimectomizados muestran diferentes niveles de alteración con una disrupción progresiva del epitelio seminífero, aparición de cuerpos multinucleados y algunos túbulos obliterados por células degenerativas y desechos celulares. A medio plazo aparecen túbulos carentes de epitelio, incluso de células de Sertoli. A los 25 días, la degeneración afecta no sólo al epitelio (ausente en casi todos los túbulos), sino también a la morfología tubular. Los animales eferentectomizados muestran una progresiva alteración con el tiempo, aunque su alteración es mucho menor que la observada tras la epididimectomía parcial, lo que indica una posible función específica de la cabeza del epidídimo en el control de la función testicular.

Palabras clave: Epididimectomía, Eferentectomía, Testículo, Cabeza del epidídimo, Rata.

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