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Morphological examination of epididymal epithelium in the mule (*E. hinnus*) in comparison with parental species (*E. asinus* and *E. caballus*)

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Summary. Following previous studies about the ultrastructure of male genital tract in parental species, a comparative study of epididymis of one of the possible hybrids, the mule, has been undertaken.

Apart from small differences, general features of epididymal epithelium in the mule are similar to those of parental species. However, extension of our studies from the donkey to the horse to the hybrid permits a deeper insight into the morphology of this tract of excurrent duct. In the meantime, it is possible to evidence some features, sometimes shared with other species if taken separately, which in the whole characterize the epididymis in Equidae: the presence in principal cells of intranuclear inclusions and peculiar small granules in the basal cytoplasmic edge; the organization of groups of cells, likely to be principal ones, in such a way as to constitute intraepithelial crypts; a cumbersome presence of lipofuscinic matter all along the epithelium. Another interesting observation is the presence in the mule epididymis of well recognizable macrophages.

All these data are discussed in comparison with parental species and with other species described in literature. Beyond any other consideration, it can be outlined that the complex morphology of the epithelium lining ductus epididymis in the mule is unaffected by the absence of spermatozoa, which are normally the target of the manifold functions of the epithelium itself.

Key words: Epididymis, Equidae, Mule, Ultrastructure

Introduction

The man-made breeding of jackass and mare produces a sturdy and fatigue-resistant hybrid, the mule. About the history and the character of such an animal a lot of legends and a rich anecdotage exist since the mists of times (Savory, 1970; Page, 1978).

The possible fertility of male and female Equine hybrids has largely drawn the attention of biologists, since it has been reported that gametes can be produced in both sexes (Trujillo et al., 1969; Taylor and Short, 1973; Short et al., 1974; Short, 1975; Chandley, 1981). With regard to the offspring, especially of female hybrids, several reports exist in literature, some of them seeming cytogenetically well documented (Short et al., 1974; Rong et al., 1985; Ryder et al., 1985).

Anyway, the mule is an interspecific hybrid, therefore overmentioned cases of fertility must be considered sporadic, sterility being the normal condition. Thus, a study about morphological aspects of epididymis in the mule has been undertaken to see if modifications could take place in the epithelium, linked to the presumed absence of spermatozoa. It is in fact well known that morphology of male excurrent duct and maturation of spermatozoa are intimately correlated.

The aim of this work is also to complete a morphological investigation concerned with excurrent ducts of domestic Equidae (Arrighi and Romanello, 1984; Aureli et al., 1984; Romanello et al., 1985; Arrighi et al., in press).

Materials and methods

Epididymides were collected at surgical castration from 4 healthy mules aged respectively 18, 18, 24 and 36 months, whose origin from interspecific breeding (jenny X stallion) was undoubtedly known.

The organs were dissected free from testes, gently distended to easily distinguish caput (including ductuli efferentes), corpus and cauda. From every region fragments were obtained and then subdivided into specimens for light and electron microscopy. Samples for light microscopy were fixed in Helly's fluid and 10% buffered formalin, those for electron microscopy were trimmed and fixed in the following solutions:

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1) Karnovsky's mixture (Karnovsky, 1965).

2) 2.5% Glutaraldehyde solution in phosphate buffer 0.1 M pH 7.4.

3) 1% Osmium tetroxide in phosphate and cacodylate buffers.

Specimens fixed in n.1 or n.2 chilled mixtures for 2 hours at 4° C, were postfixed in n.3 solution, dehydrated in ethanol and embedded in Epon 812 (Luft, 1961). Sections were obtained with a LKB ultramicrotome; semithin sections were counterstained with a mixture of Azur II and Methylene Blue, and thin sections were counterstained with uranyl acetate and lead citrate (Reynolds, 1963). Micrographs were taken with a Zeiss EM 109.

A morphological light microscopical study was also undertaken on testes to avoid any possible doubt regarding the breakdown of the germinal line. The normal morphology of donkey and horse epididymis, including ductuli efferentes, was reported in previous publications, to which the reader can refer also for technical details (Aureli et al., 1984; Arrighi and Romanello, 1984; Romanello et al., 1985; Arrighi et al., in press).

Results

Morphological examination of *testes* (Fig. 1) confirmed the absence of spermatozoa, while earlier elements of the germinal line were present, sometimes undergoing degeneration. Sertoli cells were present as well as Leydig cells, both showing a quite normal appearance.

In the epithelium lining *ductuli efferentes*, ciliated and non-ciliated cells were present as well as wandering lymphocytes (Fig. 2a), macrophages and very few lipofuscin-rich cells. The apical specializations (Fig. 2b) concerned with absorbing activity, such as microvilli, caveolae, vesicles and apical canaliculi, were well developed in the non-ciliated cells, as well as other organelles (mitochondria, Golgi apparatus and endoplasmic reticulum); lysosomes were very prominent over and below the nucleus; in general they were roundish with a more or less homogeneous and electron-dense core. Also peroxisomes were easily detectable in the cytoplasm. In ciliated cells the absorbing apparatus was less developed and lysosomal population seemed

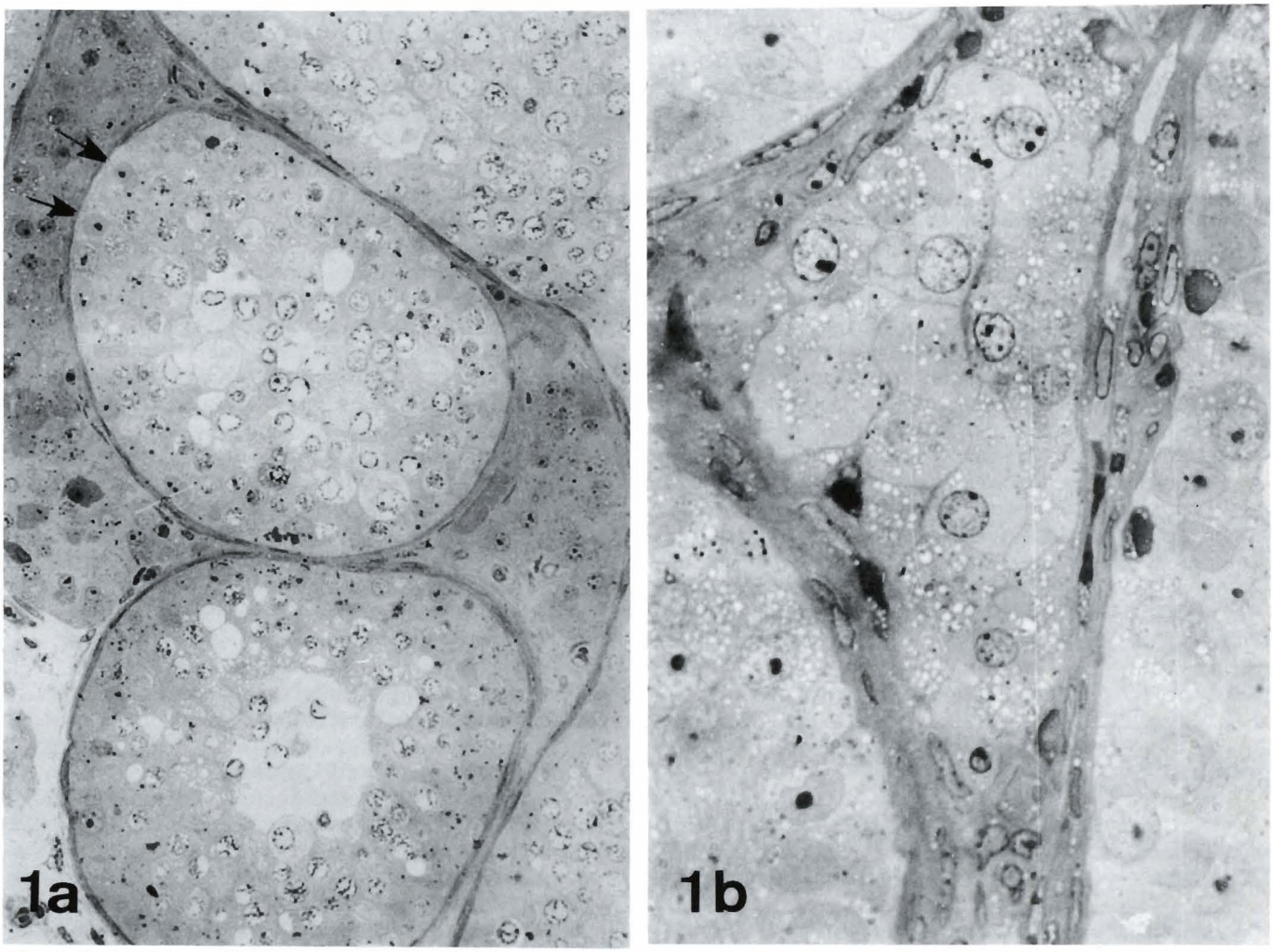


Fig. 1. Testis of the mule. Semithin sections. **a.** Spermatozoa are completely lacking in the seminiferous tubules, while Sertoli cells (arrows) are normally developed. Original magnification: × 250. **b.** Interstitial tissue: Leydig cells show a normal appearance. Original magnification. × 800

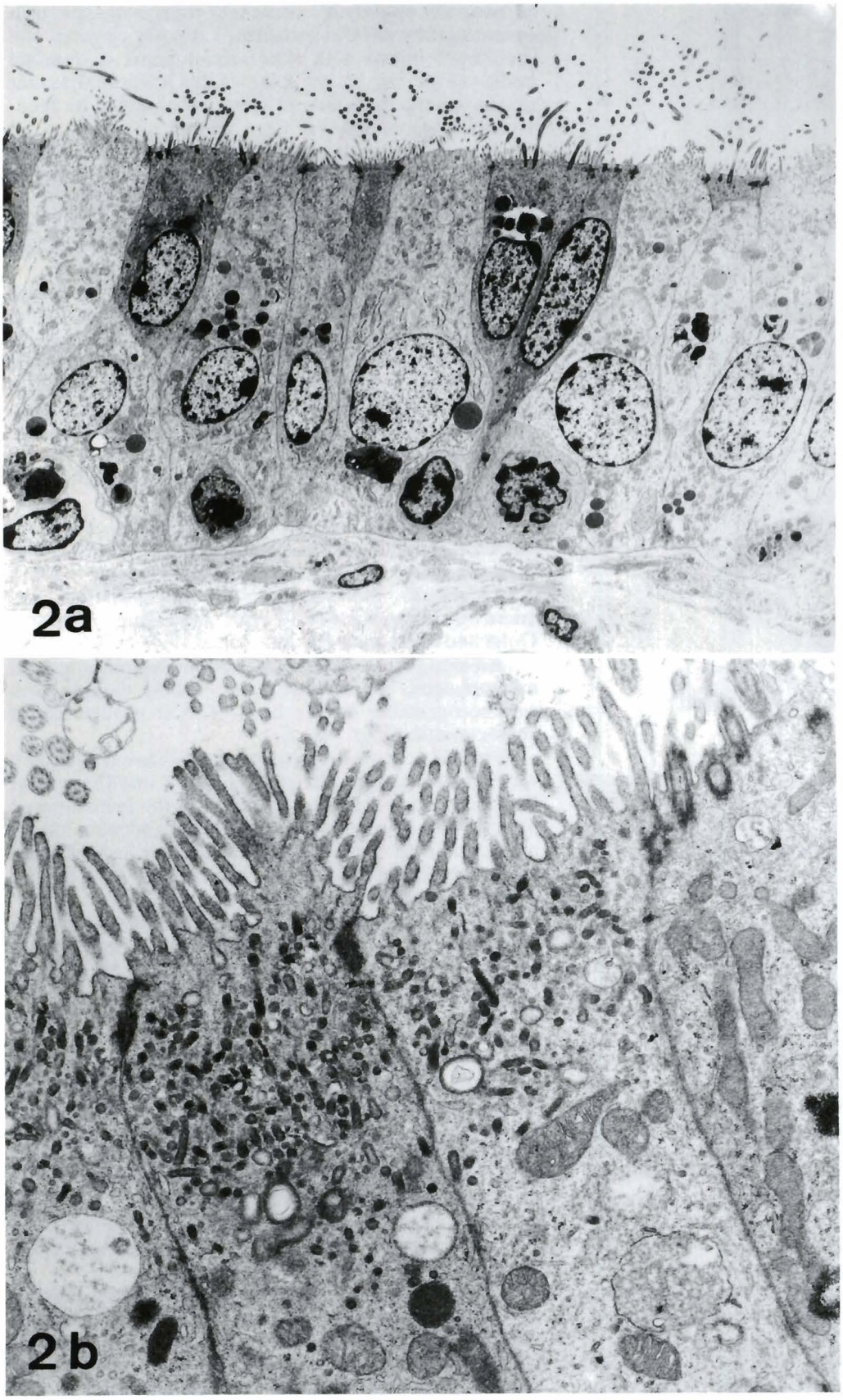


Fig. 2. Epithelium lining ductuli efferentes in the mule.
a. Ciliated and non-ciliated cells can be seen, together with wandering elements. Original magnification. × 1,000.
b. Apical aspect of the epithelium. Original magnification. × 7,000

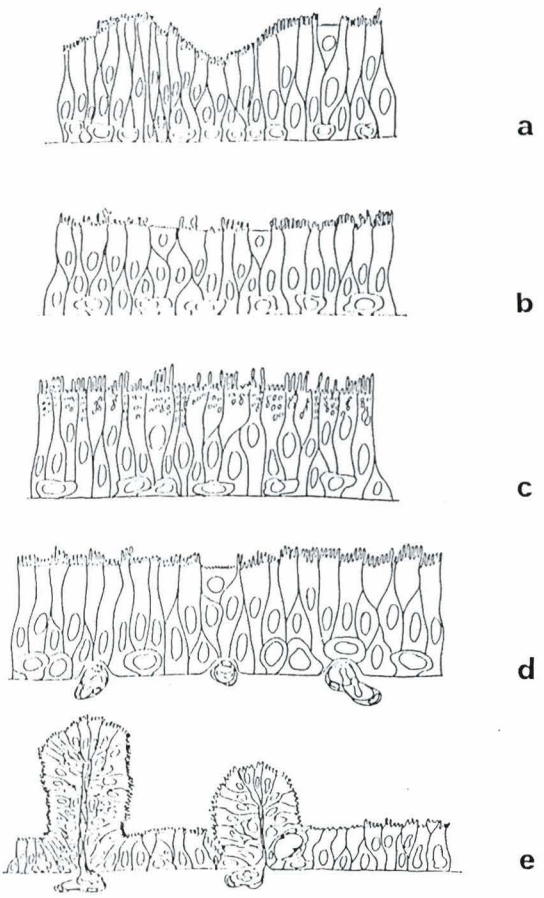
quite different from that of non-ciliated cells, being constituted by confluent, more osmiophilic and heterogeneous bodies sometimes having a conspicuous lipidic component and a floccular matrix.

Epithelial lining of *ductus epididymis* was constituted by principal and apical cells reaching the lumen, and basal cells. Migrating lymphocytes and macrophages could also be seen, together with sporadic lipofuscin-rich cells, mostly situated in the basal compartment.

Light microscopical observations allowed us to distinguish subtle and progressive variations from proximal to distal parts (Table 1).

Epithelial lining of the «initial segment» was characterized by the different height of the cells: groups of very tall and shorter cells alternated all around a narrow star-shaped lumen (a). More distally, after a long tract devoid of any peculirity, and almost corresponding to the whole caput, in which the cells were uniformely tall (b), a lipid-rich zone (c) could be evidenced, in which the apical compartment of principal cells was filled with lipid droplets. Descending towards the distal corpus, a further tract could be seen on the basis of the morphological behaviour of basement membrane: at regular intervals it showed invaginations where capillaries were located (d). This

Table 1.



situation preluded the one of the cauda where the lumen was wider, epithelial thickness decreased and folds were present; in the connective axis of such folds capillaries were present (e). All along epididymal epithelium, with particular emphasis in distal corpus and cauda (Table 1, e), intraepithelial crypts were detectable, lined by flattened cells.

At ultrastructural level the cytoplasmic domain of principal cells was occupied by those structures concerned with the intense absorbing and synthetic activities. Absorbing machinery widely occupied the adluminal third of the cell (Fig. 3a) and the synthetic apparatus, constituted by the large Golgi (Fig. 3b) and the typical sparsely granulated endoplasmic reticulum (Figs. 3a, b), was present over and below the nucleus. Intranuclear inclusions were frequent in the form of crystalloid lamellar needles (Fig. 4a), as well as membrane-bound groups of several electron-dense globules (Fig. 4b). The occurrence of such inclusions was frequent, but in some thin sections they could be seen in almost all the nuclei, and in some others they were completely absent, apparently in a random way.

Small membrane-bound granules of variable shape and size were located in the most basal rim of the cell, together with a lot of mitochondria (Fig. 5).

The lysosomal population was highly peculiar, abundant and heterogeneous; most of the dense bodies all over the epididymis contained polygonal structures constituted by parallel lamellae, variously oriented and sometimes mixed with other components of residual bodies (Figs. 6a, b). In some places a finely floccular matrix emerged, in others clear zones could be seen, especially where lamellae were scarcer.

Apical cells were very rare and interspersed among principal cells, their frequency decreasing from caput to cauda. Their funnel-like shape and the richness in mitochondria were the only salient features (Fig. 7). Also in this cellular type the dense bodies and secondary lysosomes might be prominent.

Basal cells had an euchromatic nucleus and scarce organelles, bundles of filaments sometimes being prominent in the cytoplasm all around the nucleus (Fig. 8). Frequently a ciliary bud emerged from the cellular surface and insinuates itself into intercellular spaces (Fig. 8, insert). Residual bodies showing the same complexity as those of principal cells could be seen with moderate frequency.

All these cellular types were connected by normal junctional devices.

Intraepithelial cavities (Fig. 9) were lined by flattened elements, whose general features allowed them to be recognized as principal cells. With respect to other principal cells, their organization was simplified, i.e. microvilli were shorter and rarer, absorbing apparatus, Golgi and RER were less developed (Fig. 9a, b).

All along the epithelium wandering lymphocytes and macrophages, whose appearance widely varied, were well recognizable on the basis of their ultrastructural characteristics (Figs. 10a, b, c).

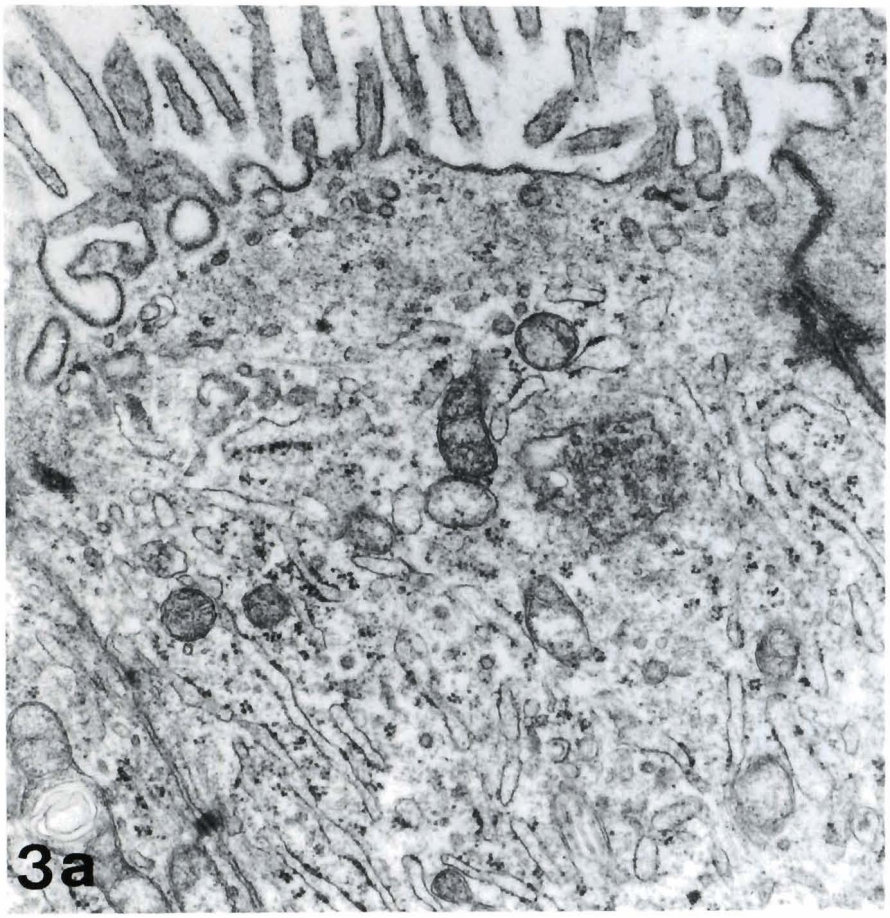
Finally, cells whose cytoplasm was completely filled with residual bodies were detectable in small numbers: they were scattered in the basal half of the epithelium of both ductuli efferentes and epididymis and were hardly recognizable as one of the previously described cell types (Fig. 11). Junctional devices between these cells and epithelial cells have never been observed.

Mitoses were rarely seen, only in animals aging up to 24 months, being more frequent in the caput and located in the upper part of the epithelium. They seem to be concerned only with principal cells (Fig. 12).

Discussion

From our results it can be assumed that epithelium lining ductuli efferentes and epididymis in the mule is quite similar to that of parental species (Arrighi and Romanello, 1984; Aureli et al., 1984; Romanello et al., 1985; Arrighi et al., 1991). Thus, it can be stated that the hormonal supply coming via the blood vessels and the canalicular lumen (Hansson et al., 1976; Moniem et al., 1978; Fawcett and Hoffer, 1979) is sufficient to provide for the normal development of the organ.

Ultrastructure of epithelial cells in both ductuli efferentes and epididymis of mule seemed quite normal, i.e. modifications cannot be detected which might be referred to the lack of spermatozoa. Morphological correlates for absorbing and synthetic activities in cells



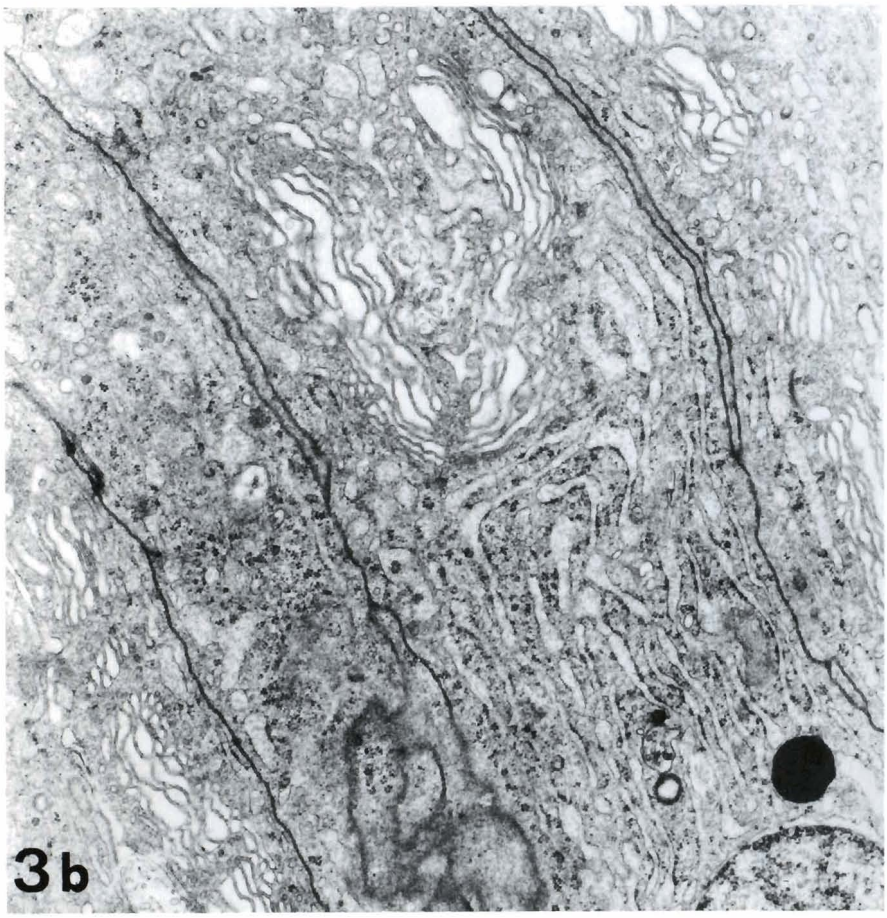


Fig. 3. Epithelium lining ductus epididymis in the mule. Principal cells. **a.** In apical cytoplasm caveolae, vesicles, MVBs and profiles of E.R. can be seen. Original magnification: × 12,000.
b. In the supranuclear area sparsely granulated E.R. and a wide Golgi apparatus are present. Original magnification: × 3,000

of ductuli efferentes and epididymis epithelium were fully developed and not distinguishable from those of parental species.

At this point of our morphological investigations, we can summarize some features, sometimes shared with other species if taken separately, but characterizing in the whole the epididymis of Equidae. Epithelial lining of ductuli efferentes appeared similar to that of other species with respect to ultrastructural details of ciliated and non-ciliated cells, whose absorbing activity is well documented in literature (Yokoyama and Chang, 1971; Aureli et al., 1984; Hermo and Morales, 1984; Hermo et al., 1985). However the presence of peroxisomes in the cytoplasm of non-ciliated cells of ductuli efferentes has never been reported before and will be the subject of further publications.

With regard to the epithelium of epididymis, slight proximo-distal variations were evidenced by light-microscopy observations. Nevertheless, to date there is no reason to believe they are enough to define true morpho-functional specializations. In any case, we substantially agree with some of the topics outlined by Nicander (1958), who described in the horse a well-defined «initial segment» and a somewhat «lipid-rich» zone. A possible interpretation of a lipid-rich zone in the guinea-pig epididymis has been given by Hoffer and Karnovksy (1981), who correlated its presence to the steroid metabolism.

As in other species (Ramos and Dym, 1977; Nicander, 1979; Sinowatz, 1981; Arrighi et al., 1986), the ultrastructural examination of epididymis epithelial lining reveals the presence of principal, apical and basal cells, whose aspect is strongly similar to that of other mammals.

One of the main characteristics in the mule epididymis was the presence of intraepithelial crypts lined by modified principal cells, their significance being unknown. Their presence has been reported in camels (Singh and Bharadway, 1980) and bulls (Nicander,

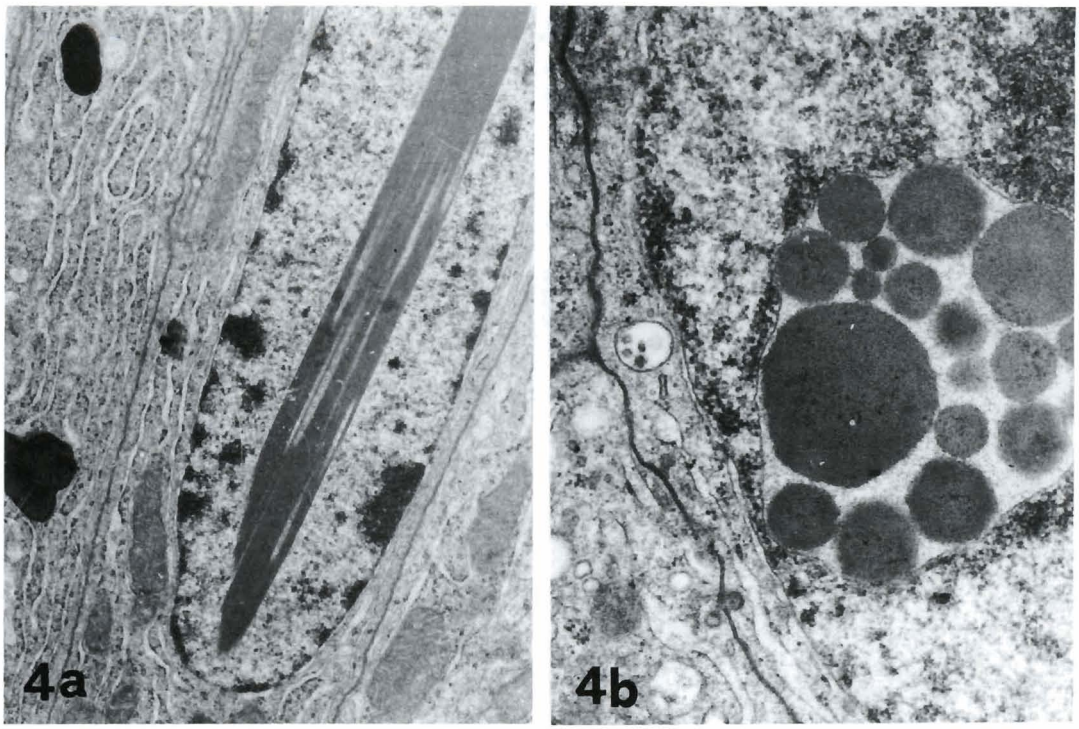


Fig. 4. Epithelium lining ductus epididymis in the mule. Principal cells. **a.** Crystalloid multilamellar intranuclear inclusion. Original magnification × 4,400. **b.** Intranuclear inclusion in form of a membrane-bound group of electron-dense globules. Original magnification × 12,000

1958; Sinowatz, 1981) at L.M. level. Similar cavities have been described in the cat at E.M. level (Arrighi et al., 1986) and it has been suggested they may segregate a microenvironment in which a specific secretory function takes place.

At ultrastructural level basal compartment of

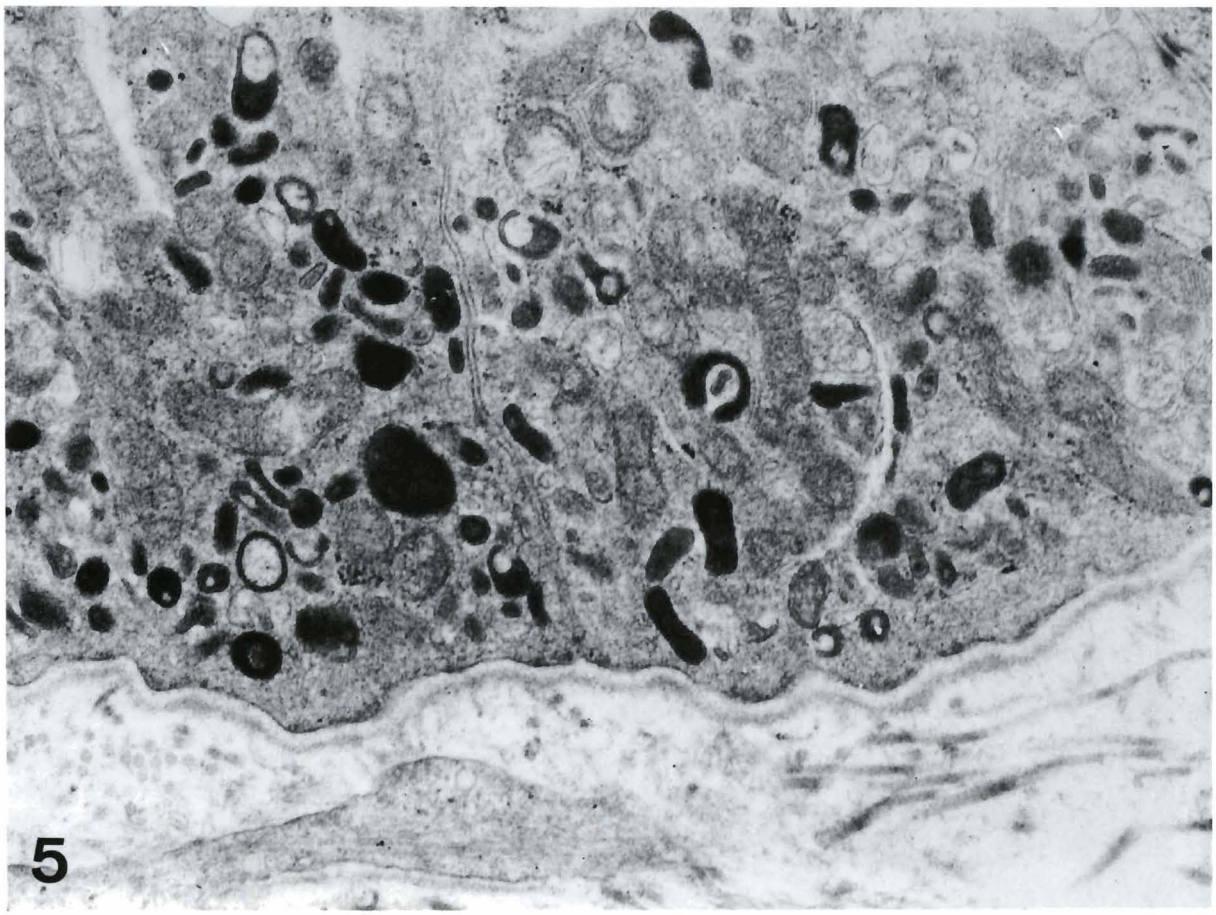
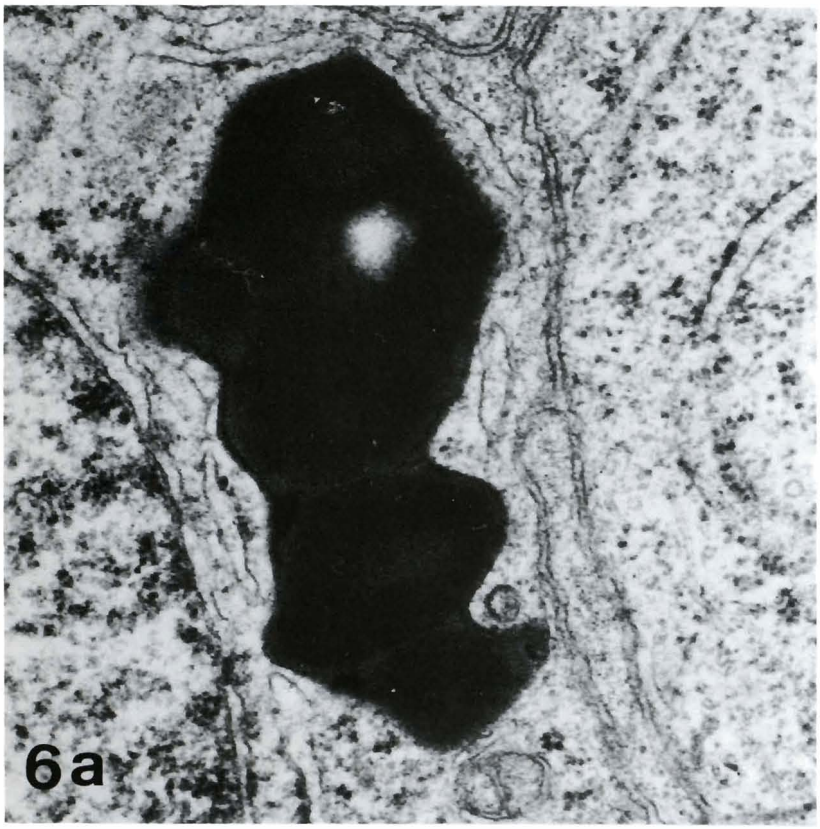


Fig. 5. Epithelium lining ductus epididymis in the mule. Principal cells. In the basal compartment small membrane-bound granules can be seen and interspersed mitochondria. Original magnification × 12,000



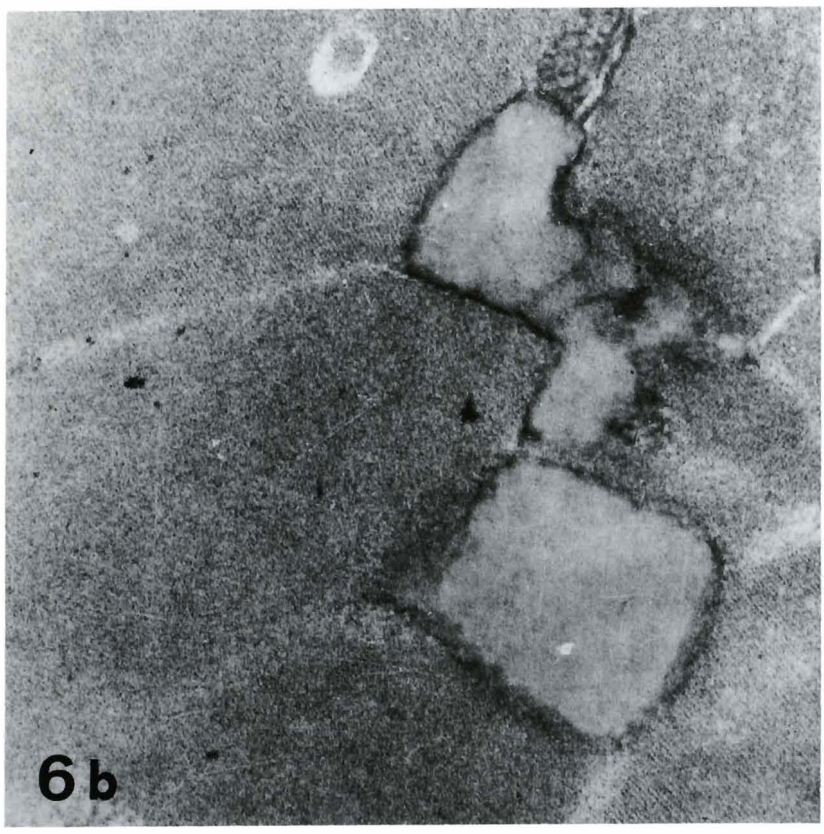


Fig. 6. Epithelium lining ductus epididymis in the mule. Principal cells. **a.** A highly peculiar lysosomal structure with polygonal shape and multilamellar constitution is shown. Original magnification. × 20,000. **b.** Higher magnification. Note that lamellae are variously oriented and mixed to a lipidic component. Original magnification. × 50,000

principal cells exhibited a somewhat peculiar appearance due to the presence of small dense granules interspersed among mitochondria. This feature is typical also of other species such as monkey (Ramos, 1980), bull (Sinowatz, 1981) and cat (Arrighi et al., 1986). The significance of this report is still under discussion. They could perhaps be an unusual form of lysosomes since they have been reported to be positive to acid hydrolases (Sinowatz, 1981).

About the presence of highly peculiar intranuclear inclusions, analogous reports exist (Luthman, 1968; Gouranton et al., 1978) in the horse as well as in other species such as dog (Nicander, 1964; Horstmann, 1965; Sawatzke and Heidger, 1977; Gouranton et al., 1979), monkey (Ramos and Dym, 1977) and man (Horstmann et al., 1966). Their significance as storage forms of oversynthesized material has been discussed previously (Romanello et al., 1985).

Apical cells are not present in every species studied, but in Equidae were a constant finding, scattered among principal cells. They have been classified among mitochondria-rich cells (Brown and Montesano, 1980), whose function is highly specialized in ion exchange; they may thus contribute to the modification of the ionic composition of luminal fluid. The presence in their cytoplasm of typical residual bodies suggests that they are involved in absorbing activity as well (Moore and Bedford, 1979).

After describing some similarities with other species, we now have to point out some differences which can be detected mainly with respect to the latest steps of intracellular digestion, and the cell types possibly involved. The presence of residual bodies, whose appearance and size are extremely varied, is the morphological counterpart of intracellular digestion phenomena, probably related mostly to the absorbing activity of the luminal content, and autophagy (Nicander, 1965). Their general distribution and morphology all over the epithelium may mirror their peculiar composition, related in some way to the

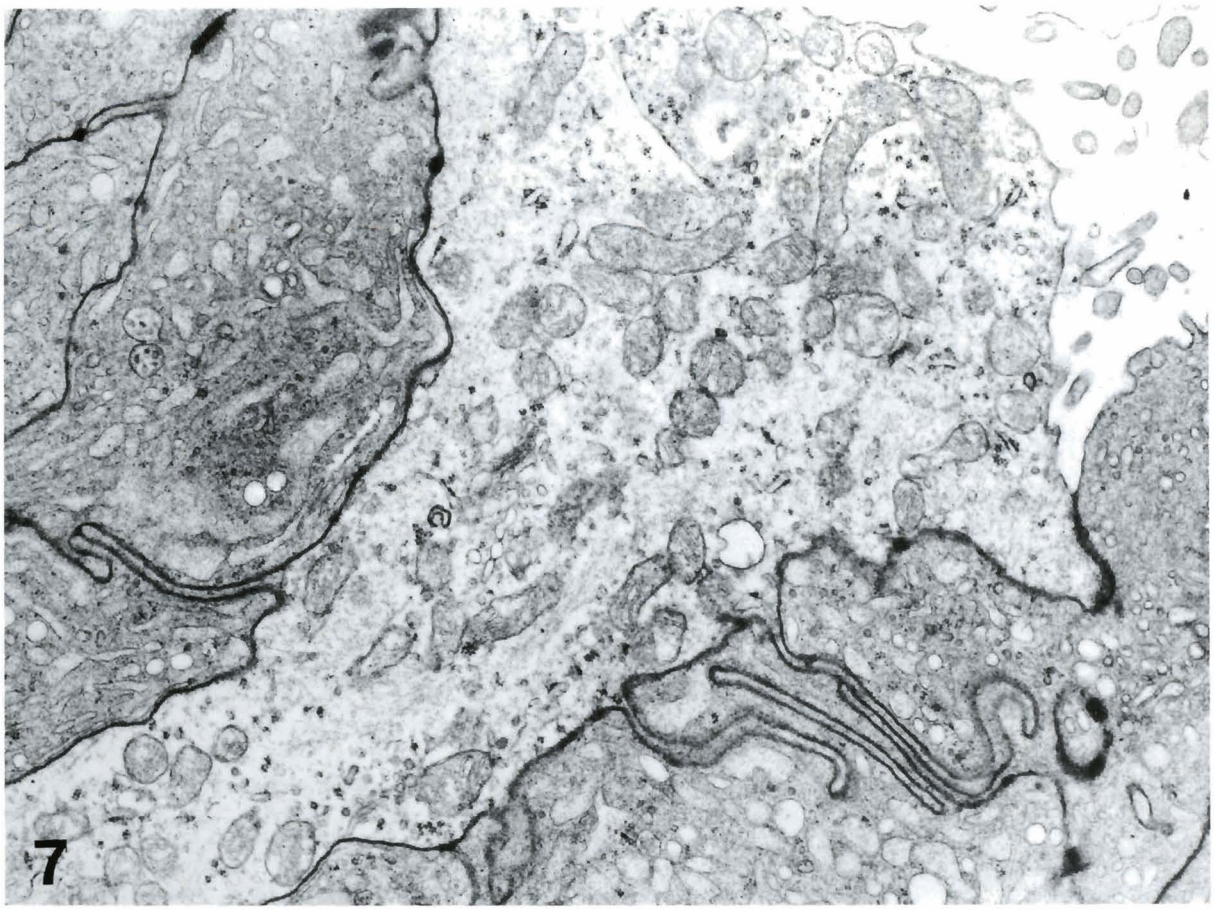


Fig. 7. Epithelium lining ductus epididymis in the mule. Apical cell. Note the crowding of slender mitochondria at the luminal end of the cytoplasm. Original magnification. × 7,000

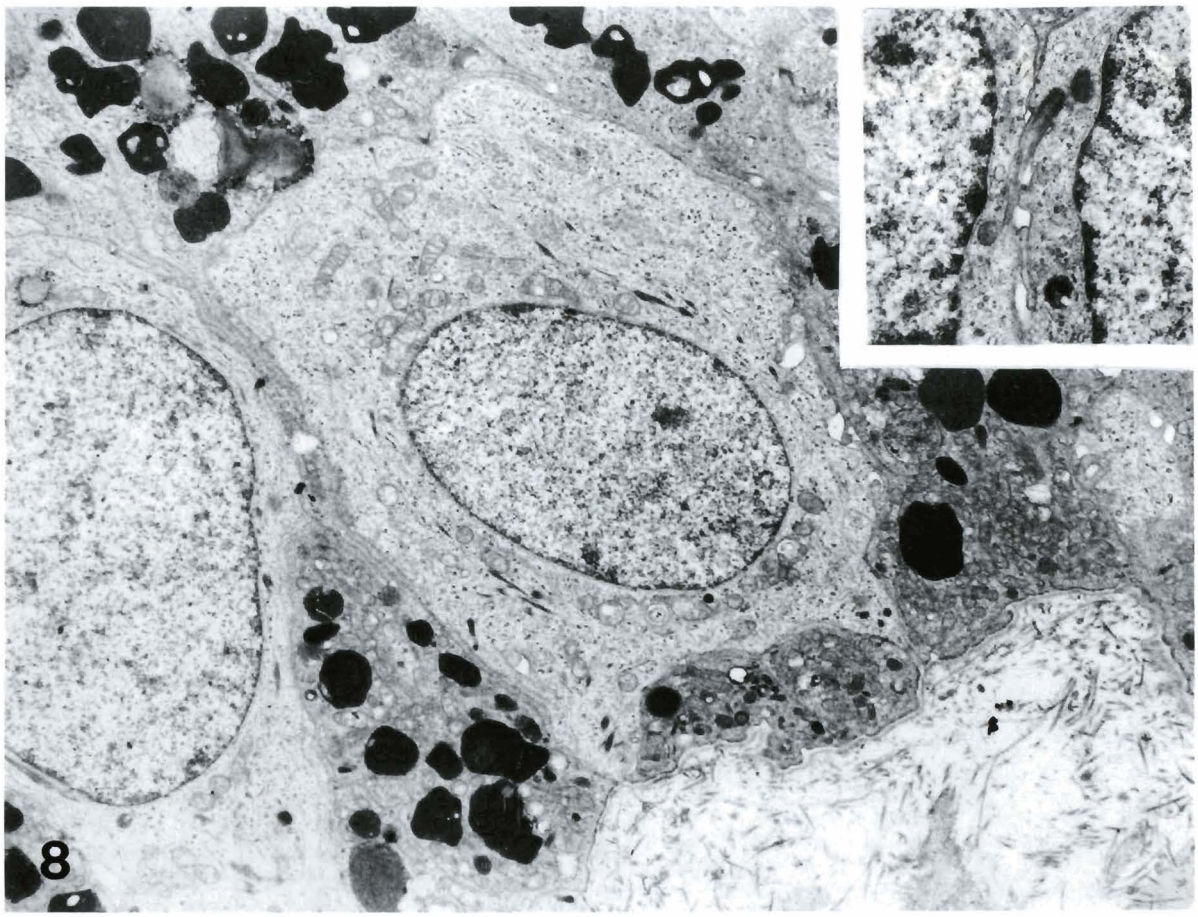
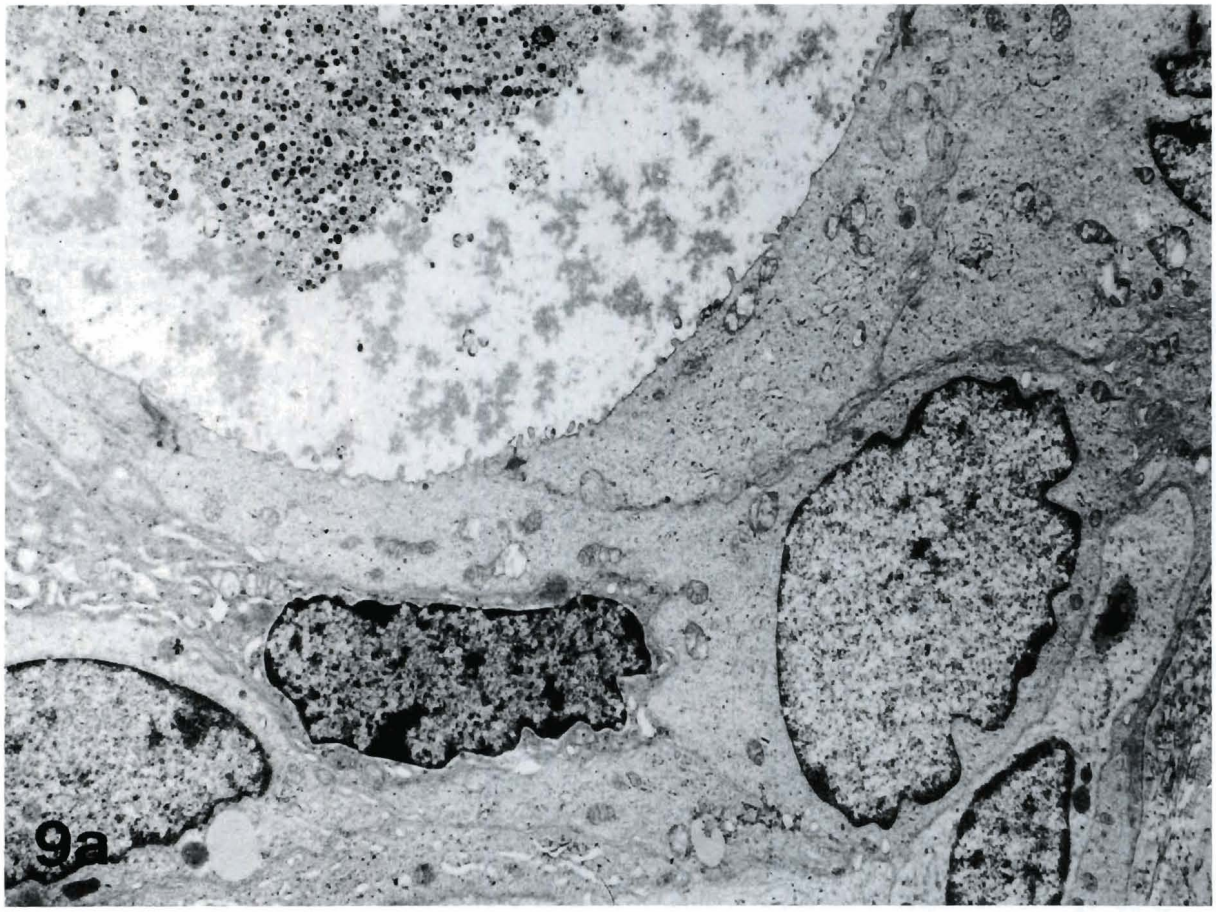


Fig. 8. Epithelium lining ductus epididymis in the mule. Basal cells. Note the presence of filaments all around the nucleus. Original magnification. × 3,000. Insert: ciliary bud insinuating itself two adjacent basal cells. Original magnification. × 12,000



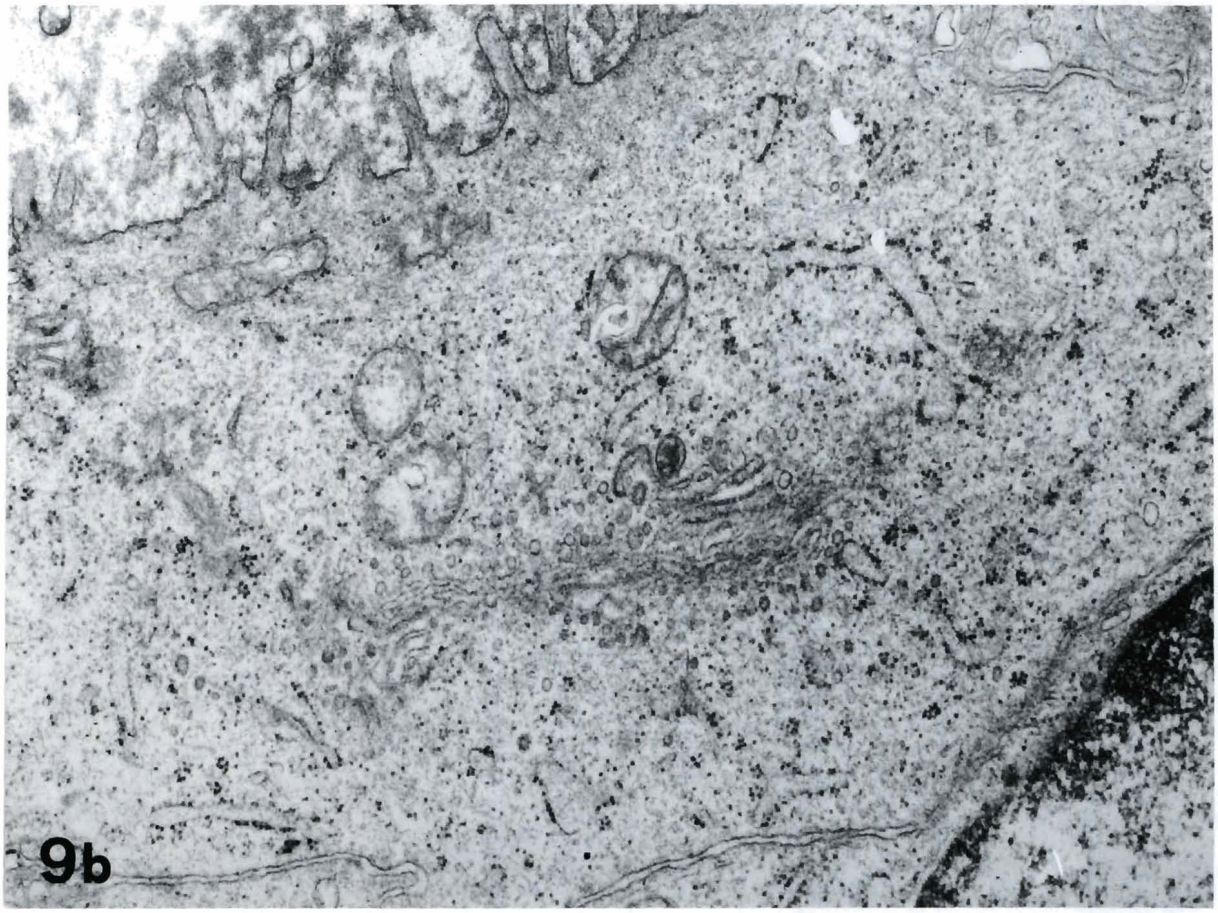


Fig. 9. Epithelium lining ductus epididymis in the mule. **a.** Typical aspect of an intraepithelial crypt. Original magnification. × 3,000. **b.** The general features of elements lining the crypt recall a simplified principal cell. Original magnification. × 12,000

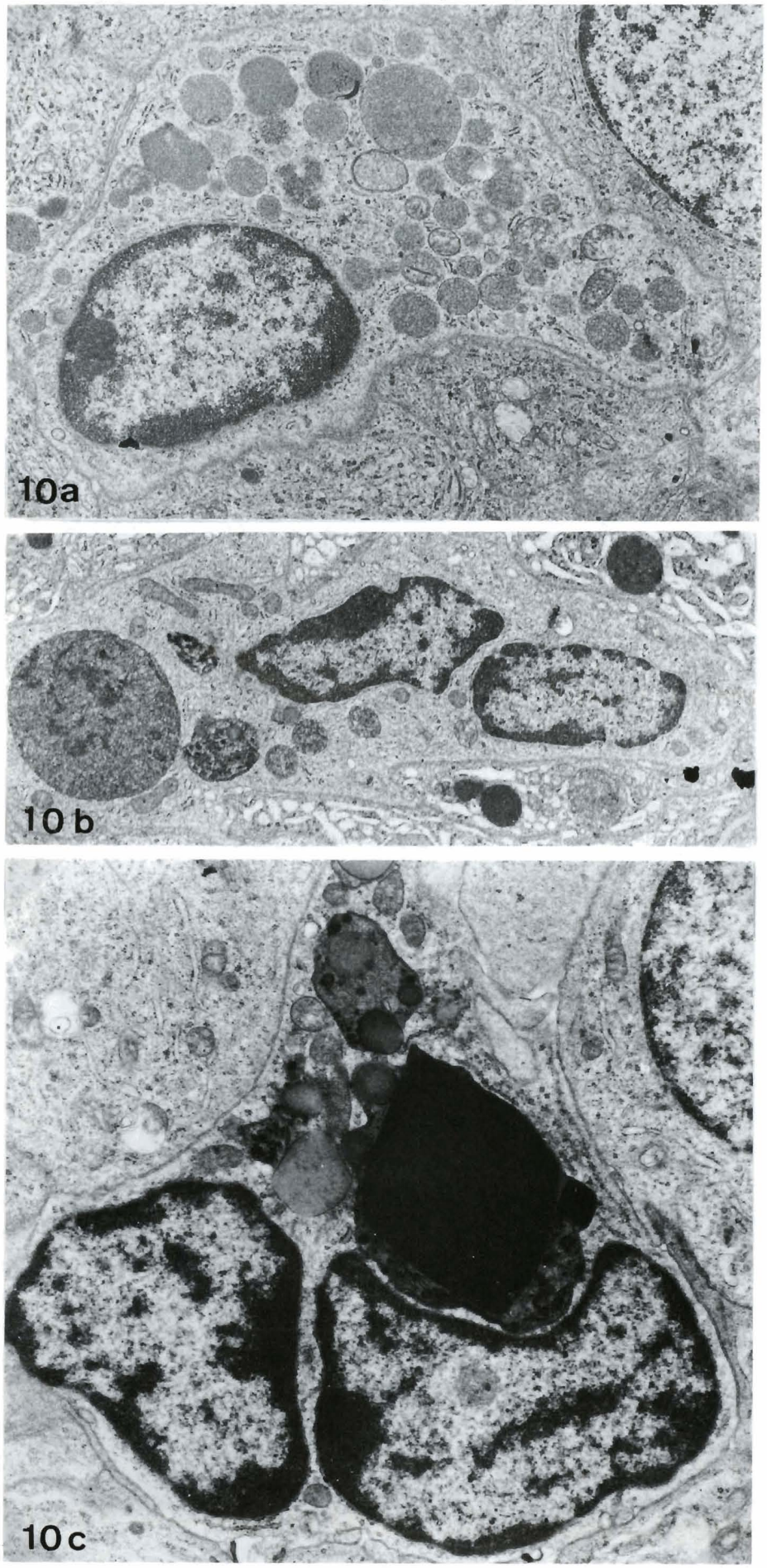


Fig. 10. Epithelium lining ductus epididymis in the mule. Three possible aspects of macrophages, containing different quantities and aspects of residual matter. **a.** Original magnification: × 4,400. **b.** Original magnification: × 4,400. **c.** Original magnification: × 7,000

different substrate on which the heterophagic processes are displayed. Actually, it has been postulated that endocytotic activity of epithelial cells is carried out not only on the luminal fluid, but also on particles derived from luminal fragmentation of spermatozoa (Orgebin-Crist, 1969; Neaves, 1975). In such a way, the lack of spermatozoa could justify the peculiar final aspect of residual bodies in mule epithelial cells. Abe et al. (1984) also describe similar residual bodies in the principal cells of mouse epididymis, after efferent duct ligation.

With regard to the distribution of residual bodies, whose invasive presence in the epithelium seems to be a characteristic of parental species, it must be underlined that differences exist between these and the hybrid: in *Equus asinus* residual bodies in the form of large masses of lipofuscins are confined principally to «lipofuscin-rich cells» so stuffed with debris that no characteristics could be evidenced to hypothesize about their origin from whatever other cellular type (Romanello et al., 1985). On the other hand, in *Equus caballus* (Arrighi et al., 1991) true «lipofuscin-rich cells» are rare and large amounts of lipofuscins are confined in elements easily recognizable as basal cells by their nuclear characteristics and by the presence of electron-opaque filament bundles. Finally in the mule, cells filled with lipofuscinic matter were very rare and residual matter was essentially located in principal and basal cells.

From our results in domestic Equidae, two hypotheses can be put forward about the origin of lipofuscin-rich cells. They could derive from macrophages engulfed by debris from principal cells: this hypothesis is mainly supported by

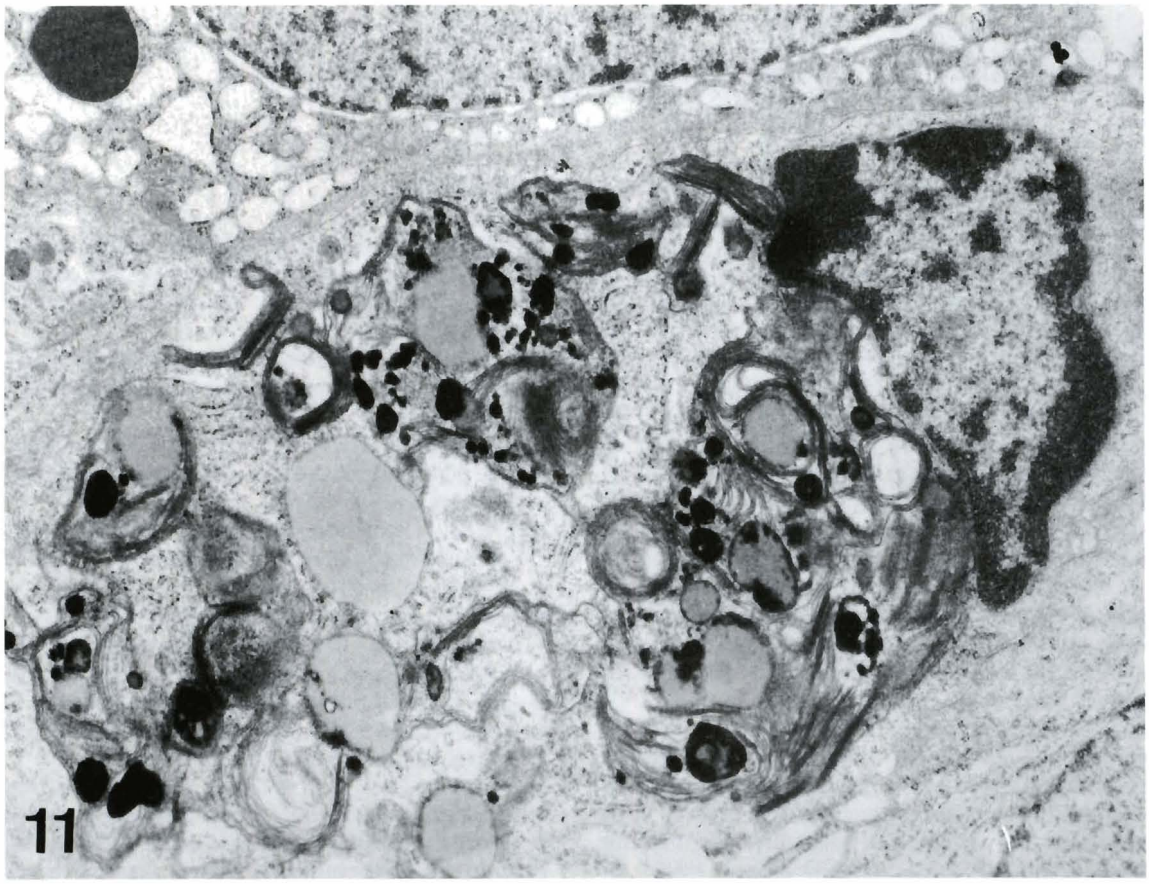


Fig. 11. Epithelium lining ductus epididymis in the mule. A cell whose cytoplasm is completely filled with residual bodies is seen. The cellular type to which it belongs is no longer identifiable. Original magnification. × 7,000

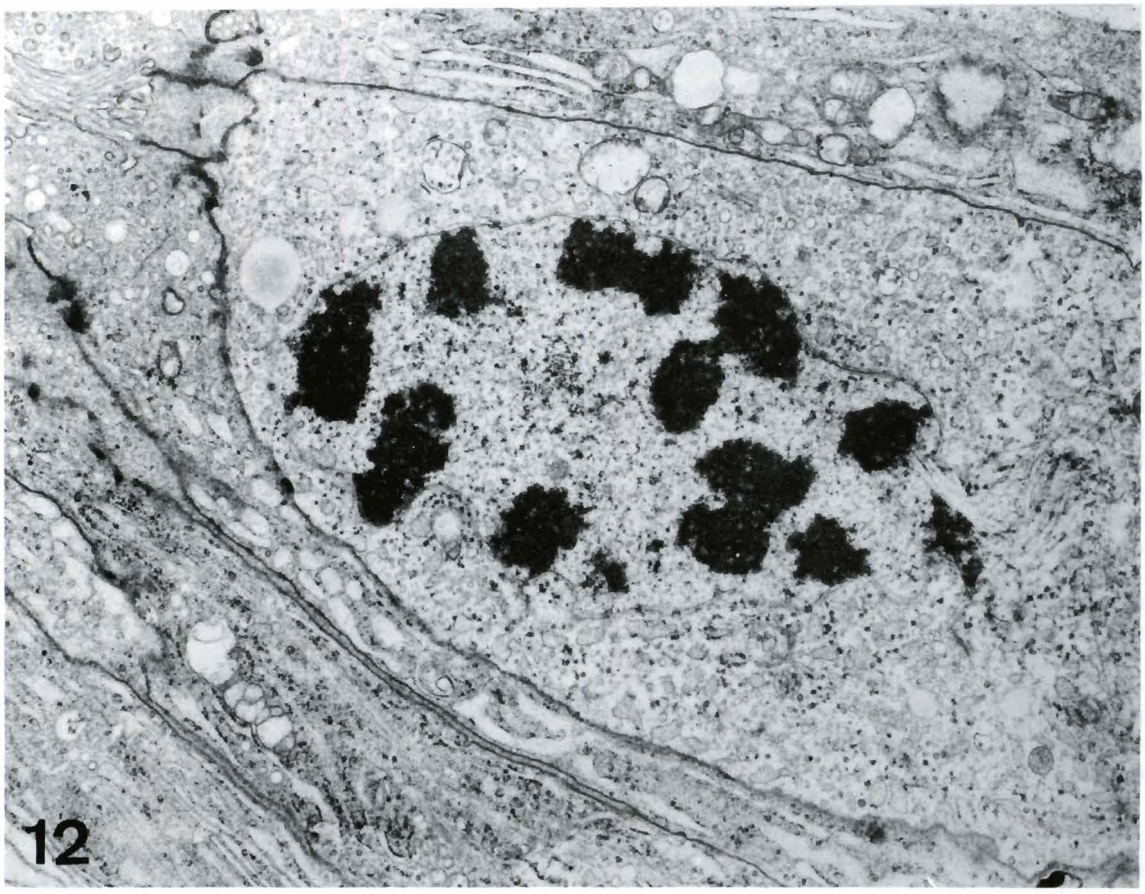


Fig. 12. Epithelium lining ductus epididymis in one of the youngest mules, aged 18 months. In the upper part of the epithelium a cell entering mitosis can be seen, presumably a principal cell. Original magnification: × 7,000

the data obtained in the mule, where, as spermatozoa are lacking in the luminal fluid, absorbing phenomena are quantitatively and qualitatively different; thus it is possible to find «nude» macrophages as well as others whose content in residual bodies is increasing up to the, rare, lipofuscin-rich cell. The presence of macrophages in epithelial lining of epididymis has been reported by several authors (Nicander, 1958; Nilnophakoon, 1980; Sinowatz, 1981; Goyal and Vig, 1984). Intervention of such cells has been reported in those cases in which spermatophagy occurs (Phadke, 1964; Alexander and Tung, 1977; Flickinger, 1982). More generally, it cannot be excluded, as postulated by Sinowatz (1981) in the bull, that macrophages could take over materials reabsorbed and degraded to a certain extent by principal cells. The possibility that macrophages could return to interstitium has also been suggested (Alexander and Tung, 1977; Sinowatz, 1981; Romanello et al., 1984).

Another hypothesis, not excluding the former, can be drawn from the data obtained from horse epididymis: in this species, basal cells also seem to have the possibility of becoming lipofuscin-rich cells after assuming the indigested residues from the cytoplasm of principal cells (Arrighi et al., 1991). If this were the case, a more definite role of these enigmatic cells could be hypothesized since their involvement in only giving stability to all the epithelium (Hamilton, 1975; Ramos and Dym, 1977), as well as their stem function in the turnover of the epithelium (Sun and Flickinger, 1979) seem unlikely. In agreement with other authors (Clermont and Flannery, 1970) we only observed mitoses in the youngest animals, and not involving basal cells. About the possible role played as «scavengers» by basal cells, reports exist concerning the epithelium of the epididymis of the mole after the breeding season during its regression (Suzuki and Racey, 1976) and of castrated golden hamster (Suzuki and Glover, 1973). This hypothesis had also been formulated by us on the basis of data obtained from a case of azoospermia in *Equus asinus* accompanied by a massive involution of epididymal epithelium (Arrighi et al., 1983).

A specific functional role for basal cells may also be postulated considering the studies by Flint et al. (1986) and Burkett et al. (1987), who evidenced peculiar sequences of glycoconjugates in this cell type.

In conclusion, the extension of our studies from parental species to hybrid gives ultrastructural proof that absence of spermatozoa does not affect general morphology of epididymal duct and that this organ shows some peculiar aspects in Equidae. Moreover, from our studies we can achieve a better understanding on some topics of the intracellular digestion exerted on absorbed material in these and other species.

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