ULTRASTRUCTURE OF EPITHELIAL CYTODIFFERENTIATION IN THE INITIAL SEGMENT OF THE RAM EPIDIDYMIS*

การเจริญและเปลี่ยนแปลงของเซลบุผิวในท่อ เอพิดิไดมีสแกะบริเวณส่วนต้นของท่อ

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Abstract

The postnatal cytodifferentiation of the initial segment of the epididymal duct in ram lambs was studied by electron microscopy at ages from 1 week to 18 weeks. Up to 10 weeks, the epithelium showed some growth and general maturation of cell organelles only. At 12 weeks, growth increased and cytodifferentiation of regional characteristics started. Almost complete maturation seemed to be reached at 18 weeks, when numerous spermatozoa were seen in the epididymis. This late differentiation of the initial segment is further evidence of an ascending pattern of regional development

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in the ram epididymis. The Golgi apparatus of the initial segment was less well developed than in the other segments during early differentiation, which might be correlated with the low number of lysosomes found in this region.

Introduction

The term "initial segment" was introduced by Benoit (1926) for the first part of the epididymal duct in mammals. Many studies on the initial segment of the epididymis in various adult mammals have been published (Benoit, 1926; Nicander, 1958; Holstein, 1969; Nicander and Glover, 1973; Hoffer et al., 1973; Edmonds and Nagy, 1974; Suzuki and Racey, 1976). Nicander (1979) has also studied its fine structure in the mature ram. The epithelium is varying in height but mostly higher than that of any other segment. The nuclei are located basally and the cytoplasmic area between the nucleus and the Golgi complex is full of endoplasmic reticulum with many attached ribosomes. The Golgi complex is very large with many cisternae and is localized far above the nucleus. In many cells, numerous small and large vesicles with slightly opaque, flocculent or thread-like conteuts are seen in and above the Golgi area and in the apical cytoplasm, possibly indicating merocrine secretion (Nicander and Malmqvist, 1977).

The fine structure of postnatal differentiation in the epididymis has been studied in the rat only, by Leeson and Leeson (1964), Fahrmann and Schuchardt (1966) and Flickinger (1969), and no information on regional features was included in these papers. The regional fine structure of the ram epididymis durigng postnatal development has recently been described for the terminal segment (Nilnophakoon, 1980). Epididymal

differentiation in various mammals has sometimes been reported to be descending, starting in the caput and then descending into the cauda epididymidis (Reid, 1959; Glover and Gaddum, 1965; Leeson and Leeson, 1970). On the other hand some papers have described an ascending pattern, with a start in the cauda and then a slow progress up into the caput epididymidis (Abdel-Raouf, 1960; Gaddum, 1964; Wrobel and Fallenbacher, 1974). Nilnophakoon (1978) found the histological differentiation of the ram epididymis to proceed in an ascending pattern. Differentiation of the fine structure was also very early in the cauda (Nilnophakoon, 1980). The initial segment has now also been studied with the electron microscope during postnatal development, to find out to what extent the differentiation of fine structure runs parallel to the histological development.

Material and Methods

Two ram lambs of each age at 1, 2, 4, 12 and 15 weeks, and one lamb of each at 6, 8, 10, 13 1/2 and 18 weeks were obtained from a single farm. Samples for electron microscopy were taken from an area well proximal to the anterior bend of the epididymis, corresponding to the central area of the initial segment in most rams. The procedures of epididymal fixation and electron microscopy were the same as for the other regions (Nilnophakoon, 1980) of the same lambs and were described earlier.

Results

Throughout the ages of one week to 12 weeks, the epithelium in the initial segment of the ram epididymis was low columnar with a high nuclear-cytoplasmic ratio and rather primitive cytoplasmic organelles (Fig. 1). The nuclei were elongated with

deep indentations and several nucleoli. The basal cytoplasm contained some small dense bodies (Fig. 1). Distinct age differences mainly concerned an increasing number of cytoplasmic organelles. Glycogen was abundant in both the basal and supranuclear cytoplasm at the earliest age and decreased slowly with age (Fig. 2). Precursors of stereocilia were first seen as very short protrusions at early ages and were clearly differentiated at 10 weeks (Fig. 6). After this period, they increased markedly in length but were always slender. A few mitochondria with few cristae and a light matrix were seen at the age of 1 week and they were more numerous and mature after 4 weeks (Figs. 1, 2). They were located in both the basal and apical cytoplasm. The Golgi complex first was unimportant with a few stacks of short, flattened cisternae and some large, coated vesicles. At the age of 10 weeks it was more conspicuous with some distended cisternal profiles but very few of the large coated vesicles. The endoplasmic reticulum was mostly of the granular type. It was mainly located in the infranuclear area but some profiles were scattered throughout the cytoplasm. At 10 to 12 weeks the cisternae were slender, more numerous, less granular and filled with an opaque substance. Micropinocytotic phenomena were early seen and became prominent after stereocilia were differentiated (Fig. 6). Many coated vesicles were seen in the apical cytoplasm and a few small vacuoles were sometimes observed there. At the ages of 15 and 18 weeks, the cytoplasmic organelles of the principal cells were rather similar to those in the mature epididymis. Moderate amounts of mitochondria were seen under the Golgi complex and in the infranuclear area and a few were still present in the apical cytoplasm (Fig. 5). The granular endoplasmic reticulum had few attached ribosomes and was mostly found in the infranuclear and perinuclear cytoplasm. Agranular endo-

plasmic reticulum was more abundant than in the other segments, especially above the nucleus. It was frequently seen as elongated cisternae close to the lateral cell borders (Figs. 5, 8). A few dense bodies were found, mainly in the basal part of the cell. Free ribosomes were scattered throughout the cytoplasm (Fig. 3). The large Golgi complex showed very long, densely packed parallel cisternae (Fig. 5). A few multivesicular bodies, small coated vesicles, and a few small vacuoles were seen in the Golgi area. Micropinocytotic invaginations were common apically but few pinocytotic vesicles or tubules were seen in the adjacent cytoplasm (Fig. 7, 8). Small coated vesicles were often seen. A few spermatozoa were seen in the lumen at 18 weeks, but the luminal contents were slightly opaque, not clear as in the mature epididymis.

The first few basal cells with large hemidesmosomes were seen in this segment at the age of 10 weeks. Intermediate cells with hemidesmosomes but in other respects similar to ordinary columnar cells were also seen at this age. The number of basal cells slowly increased up to the age of 13 1/2 weeks. Their nucleus showed dense chromatin at the periphery. Deep invaginations of the nuclear envelope were often seen. The cytoplasm had numerous free ribosomes and moderate amounts of granular endoplasmic reticulum. A few mitochondria, small vacuoles and coated vesicles were seen. The Golgi complex comprised a few short, flattened cisternae and sometimes centrioles were found near it. During further development up to the ege of 18 weeks, the number of cytoplasmic organelles increased, but the nuclear-cytoplasmic ratio did not show much change. Free ribosomes became abundant and scattered throughout the cytoplasm. Desmosomes were often present at the border to adjacent cells. Lipid droplets occured at 18 weeks.

Both small and large agranular leucocytes appeared at the same time as basal cells and seemed to be more numerous than in the other segments. The large agranular ones were generally located near the basement membrane. Their cytoplasmic matrix was light and contained very few organelles. Free ribosomes were more common than other organelles. The nucleus showed a very dense chromatin. Macrophages with many large dense bodies were sometimes seen. The small agranular leucocytes were found at any level of the epithelium. In their light cytoplasm there were always numerous ribosomes, and some cells showed many mitochondria (Fig. 4). Other organelles were found in small amounts. There were no desmosomes between the agranular leucocytes and adjacent epithelial cells.

Discussion

At the earliest ages, the basic ultrastructure of the epithelium in the initial segment is very similar to that in the middle and terminal segment. The nucleus has distinct nucleoli. There is little cytoplasm with few organelles of a simple structure. Free ribosomes are numerous, however, as in all growing cells, and much glycogen is accumulated in some areas. The Golgi apparatus is smaller and shows fewer coated vesicles than in the other segments. The thick-walled tubular structures present along the inner surface of the Golgi stacks in other epididymal regions are not seen in the initial segment. The postnatal differentiation of the Golgi apparatus in this segment is more similar to that described in the rat epididymis (Flickinger, 1969) than to the picture seen in other regions of the developing ram epididymis (Nilnophakoon, 1980). This is remarkable since the Golgi apparatus in the initial segment of the mature

epididymis in the ram is larger than in any other segment. The apparent activity of the Golgi apparatus at early postnatal stages in other regions might indicate the formation of primary lysosomes (Nilnophakoon, 1980). In the mature ovine initial segment lysosomes are less numerous than in other regions (Nicander, 1980; personal communication) and mainly occur basally, where small dense bodies are already present one week after birth. During the period studied here, after 1 week of age, few primary lysosomes were found in these cells. The rapid growth of the Golgi apparatus just before puberty was associated with the appearance of small vacuoles, which have been suggested to be secretory (Nicander and Malmqvist, 1977; Nicander, 1979), but these vacuoles seemed to be fewer than in mature males.

An ascending pattern of postnatal development of the ram epididymis has been observed in histological studies (Nilnophakoon, 1978). Electron microscopical studies on the terminal segment confirmed this at the ultrastructural level. In the terminal segment, differentiation is completed at 6 weeks (Nilnophakoon, 1980). In the initial segment ultrastructural differentiation is not obvious before 10 weeks, and it is not quite complete at 18 weeks, though histological studies (Nilnophakoon, 1978) indicated complete maturation at this age. This means that an ascending pattern of cytodifferentiation is present throughout the ram epididymis, especially as far as early differentiation is concerned. The total time required for the differentiation of regional characteristics is longer for the initial segmant than for the terminal segment, which seems to complete cytodifferentiation in 5 weeks (Nilnophakoon, 1980). The three subdivisions of the epididymis suggested by Glover and Nicander (1971) thus seem to have, as additional characteristics, different patterns of postnatal differentiation.

Fig. 1 Survey of immature epithelium, to show cytoplasm with few organelles. GA, Golgi apparatus, db, basal dense bodies. Lead and uranyl staining. × 3,500. *Inset* : photomicrograph from section of immature initial segment stained with toluidine blue. × 500.

Fig. 2 Top of epithelial cell, 4 weeks, with a small Golgi apparatus (GA), some glycogen (gl) and clear pinocytotic (?) vesicles (arrows). N, nucleus, L. lumen, M, mitochondria. Lead and uranyl staining. × 16,000.

Fig. 3 Supranuclear cytoplasm, 13 1/2 weeks. Elongated cisternae of rough endoplasmic reticulum (ER), a few mitochondria (M) and part of the Golgi apparatus (GA). Lead and uranyl staining. × 14,000.

Fig. 4 Wandering cell (lymphocyte ?) at the supranuclear level of the epithelium. Lead and uranyl staining. × 6,000.

Fig. 5 Supranuclear cytoplasm, 18 weeks. Proximal end of the Golgi complex (GA) with numerous very long cisternae, but without special vesicles. N, nucleus, ER, endoplasmic reticulum, M, mitochondria. Lead and uranyl staining. × 14,000. *Inset* : photomicrograph from section stained with toluidine blue (15 weeks). × 500.

Fig. 6 Parts of two cell tops, 10 weeks, to show immature stereocilia and some pinocytotic invaginations (arrows). L, lumen. Lead and uranyl staining × 9,000.

Fig. 7 Apical cytoplasm of principal cells at 13 1/2 weeks. Well developed stereocilia, some of which are seen in pinocytotic invaginations (double – headed arrows). Many pinocytotic vesicles are seen at the luminal border (arrows).

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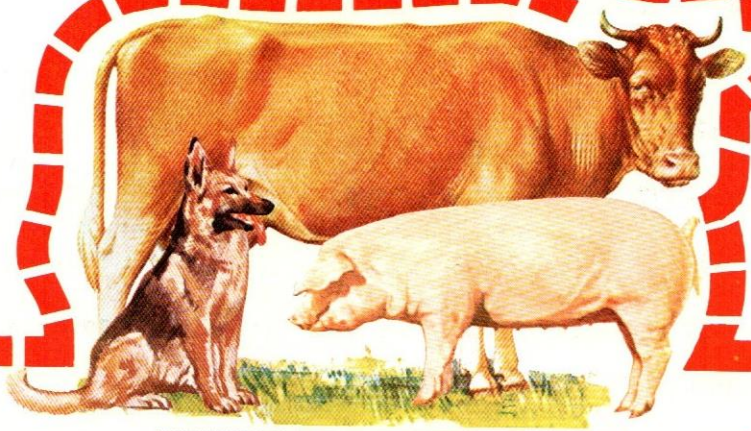

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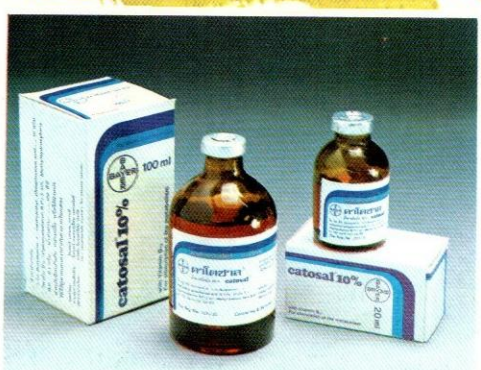

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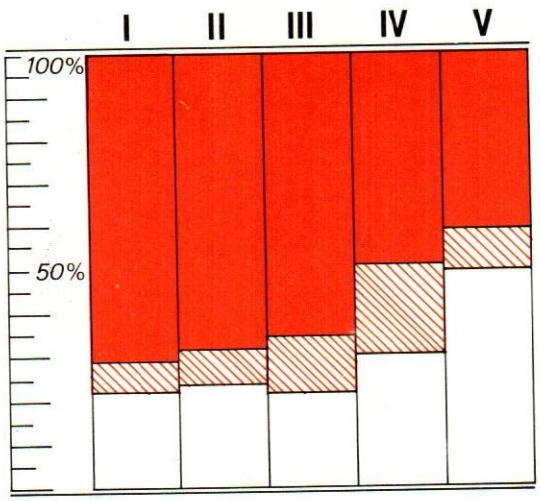

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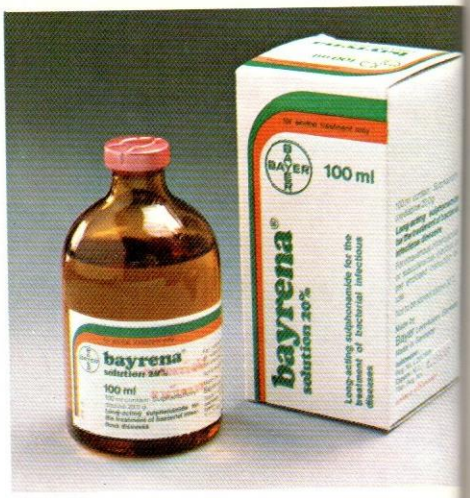

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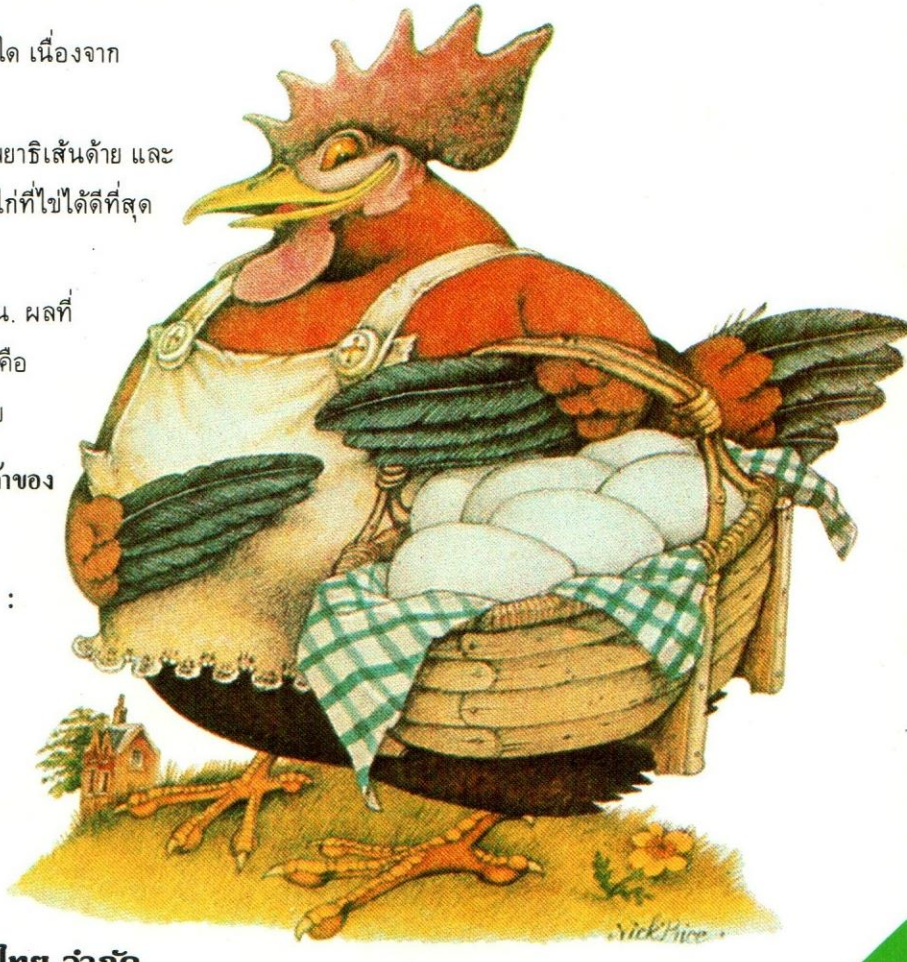

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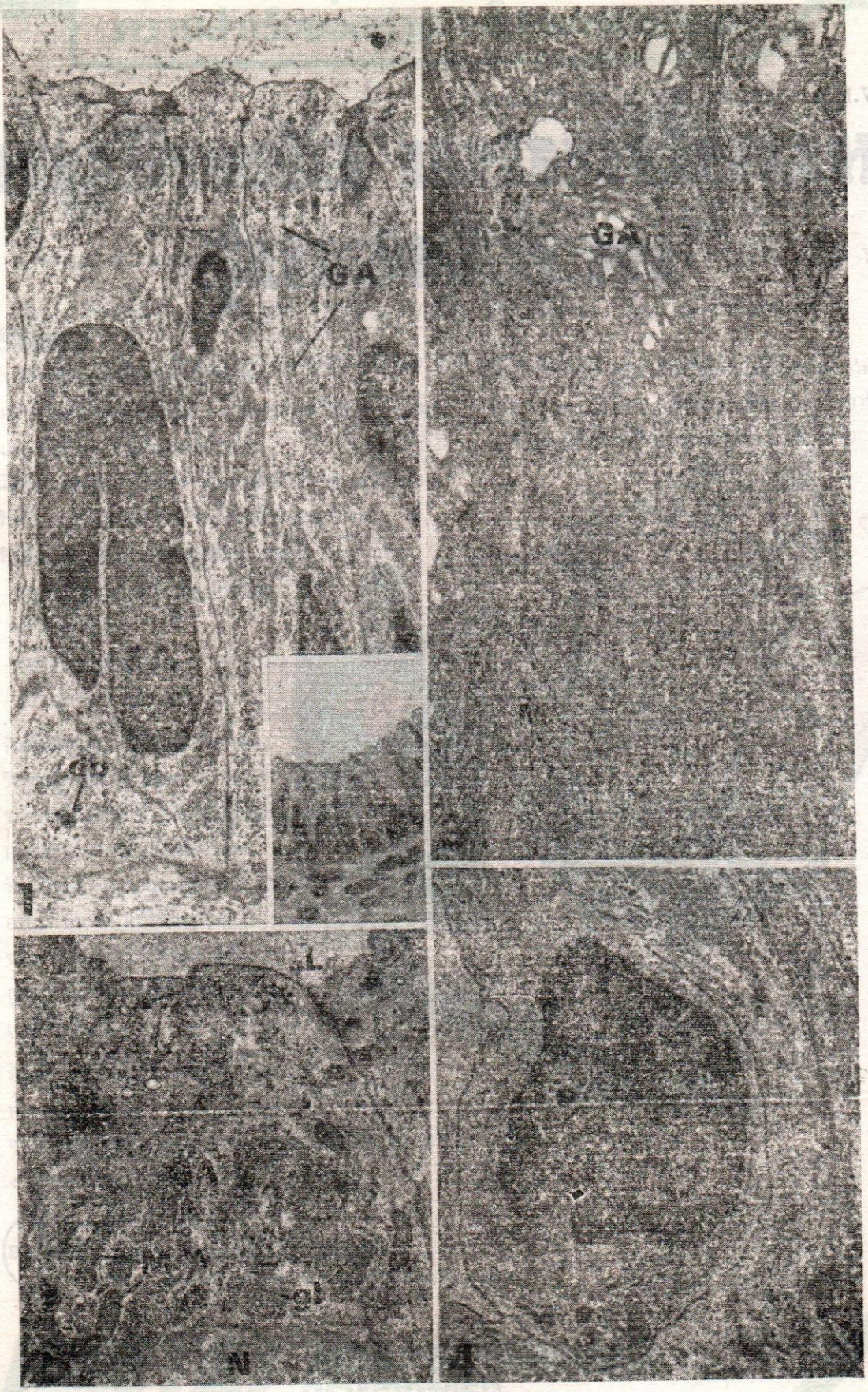
M, mitochondria, GA, Golgi apparatus. Lead and uranyl staining. × 12,000.

Fig. 8 Apex of principal cell, 18 weeks. Apical pinocytotic invaginations (arrows) are less marked. Endoplasmic reticulum (ER) and a few vesicles (V) fill the cytoplasm. L, lumen. Lead and uranyl staining. × 13,000.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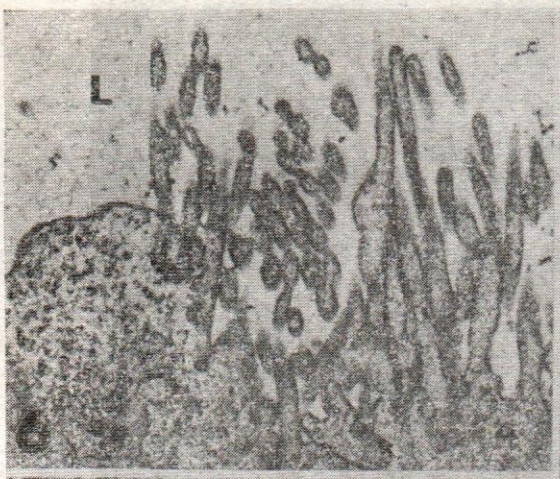

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