STRUCTURAL CHANGES, RELATED TO REPRODUCTION, IN THE HYPOTHALAMUS AND IN THE PARS TUBERALIS OF THE RHESUS MONKEY

PART I. THE HYPOTHALAMUS
PART II. THE PARS TUBERALIS

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[Plates 63 to 76]

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Certain cells lining a circumscribed area of the III ventricle of the rhesus monkey differ from those cells which constitute the characteristic ependymal lining of the brain. The specialized cells studied comprise a number of types which differ in their structure, ultrastructure and staining affinities; all demonstrate features which are generally associated with active secretion and/or absorption. A group of such cells, which form a limited area of the latero-ventral walls of the anterior hypothalamus, have long processes which extend to the walls of the blood vessels in the median eminence. The evidence indicates that many of these cells, here described as Type B or tanycyte cells, secrete their products into the primary capillary network of the pituitary portal system. Another group of cells, here described as Type C and C′ cells are found in a slightly more posterior position lining the floor of the ventricle; as yet there are no indications that these may secrete into blood vessels in the median eminence.

Some of the specialized cells lining the III ventricle (Types B and C′) showed changes in relation to reproductive activity: No such changes were observed in Type C cells nor in the characteristic ependymal cells (Type A) found elsewhere.

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Studies on normal and experimental male and female monkeys showed that Type B tanycyte cells differed in males and females and altered during the menstrual cycle in the female. Following ovariectomy these cells showed regressive changes but returned to a normal appearance after a single injection of oestradiol. In view of the close spatial relationship of the tanycyte ependyma to cells of the pars tuberalis it was interesting to note that pars tuberalis cells also altered in relation to the menstrual cycle.

The evidence presented accords with the view that certain cells which line the III ventricle of the brain and have prolongations extending to pituitary blood vessels, thus linking the cerebrospinal fluid and the blood system in the region of the pituitary, may play a role in the regulation of pituitary function and thereby constitute an important neuro-endocrine system.

PART I. THE HYPOTHALAMUS

1. Introduction

There are indications that the anterior hypothalamus affects pituitary function, but the exact nature of the pathway by which this may be effected has not yet been finally determined. Many authors have pointed to certain monoamine-containing neurons in nuclei of the anterior hypothalamus (e.g. arcuate, tubero-infundibular nuclei) as possible centres for gonadotropic control (Szentágothai, Flerkó, Mess & Halász 1968), and indeed there are some indications that these neurons may alter in relation to the reproductive activity of the rat (Fuxe & Hökfelt 1967; Lichtensteiger 1967): fibres originating in these anterior hypothalamic nuclei terminate in close relation to a capillary network in the median eminence.

Attention has also been drawn to a possibility that modified ependymal elements may play an important part in pituitary regulation. This suggestion, that some specialized ependymal cells might affect pituitary function, is based on morphological observations, some of which have been related to function. Certain ependymal cells described as tanycytes in a circumscribed area of the mammalian hypothalamus have long processes extending to the primary capillary networks of pituitary portal vessels (Schachenmeyer 1967; Leonhardt 1966; Wittkowski 1967). Anand Kumar & Knowles (1967) have drawn attention to the fact that these tanycytes link the III ventricle with blood vessels of the median eminence, and with the pars tuberalis in the rhesus monkey, and that preliminary studies have indicated that they alter in relation to reproductive cycles (Knowles, Anand Kumar & Jones 1967). The ependymal cells in question are so situated that they could detect substances circulating in the cerebrospinal fluid (c.s.f.) and thereafter play some part in the regulation of pituitary gonadotropic activity. It has been suggested that they might thus form a link in a process of gonad-pituitary feedback control (Knowles 1967, 1969).

This concept of a neuroendocrine control which involves the cerebrospinal fluid derives from earlier observations which indicated a functional relationship between ependymal cells of the infundibular recess and neurosecretory tracts in the eel (Knowles & Vollrath 1966). Under certain conditions of pituitary pars nervosa activity specialized ependyma, innervated by neurosecretory fibres originating in the preoptic nuclei, appeared to secrete into the III ventricle. In the eel, as in many other lower vertebrates, dendrites of the perikarya of the preoptic nuclei project into the ventricle and are therefore so situated that they could detect any substance secreted by the ependymal cells into the c.s.f.

During recent years structural modifications in ependymal cells lining the III ventricle have been the subject of intensive study, but for the most part the functional significance of these specialized ependyma is far from clear. It is therefore interesting to observe correlations between

neuroendocrine activity and changes in ependymal cells, which might indicate that these and the c.s.f. may be concerned in hypothalamic control of the pituitary.

The present studies extend this concept by investigating in detail a relationship between tanycyte ependyma and reproduction in the rhesus monkey.

2. Materials and methods

Twenty-three sexually mature female rhesus monkeys and ten sexually mature males were used in a study of the normal hypothalamus and pars tuberalis of the adult rhesus monkey: four immature females and two immature males were also examined. A further eleven female monkeys and five males were used in experimental studies. The two juvenile males were colony-born in Birmingham; the remaining animals were imported and, after 6 weeks in quarantine, were examined and found to be free from disease. The menstrual cycles of the female monkeys were normal and at least six normal menstrual cycles had been recorded in most of the monkeys used in the studies.

Normal intact adult female monkeys were examined at different stages of the menstrual cycle. Some were killed at mid-cycle (between the 11th and 23rd day) and others during menstruation (see table 1). By examining the ovarian histology and the flushings of the Fallopian tubes of the female monkeys killed at mid-cycle it was possible to classify these as being either in the pre-ovulatory (follicular) stage or the post-ovulatory (luteal) stage of the menstrual cycle; in the latter case ova were obtained by flushing the Fallopian tubes.

The animals used in the injection experiments had previously been bilaterally ovariectomized, under Nembutal (Abbot Laboratories) anaesthesia, and subsequently kept for a period of 2 months before treatment (see table 1). They were then injected intramuscularly in the left hind limb with either a solution containing 5 mg of oestradiol monobenzoate in 0.1 ml Arachis oil or a solution containing 5 mg of testosterone in 0.1 ml Arachis oil. The injected animals were killed 72 h later.

Another series of gonadectomized animals were examined without further treatment at intervals of 1 to 3 months (see table 1). In order to prepare the hypothalamus and pars tuberalis for observation each animal was anaesthetized with an injection of Nembutal and its brain was then perfused through the carotid arteries with approximately 500 ml of a balanced salt solution (Palay, McGee-Russell, Gordon & Grillo 1962) to replace blood drained from the circulation. When this had been done a further 500 ml of fixative was perfused through the brain. For light microscopy Susa's or Bouin's fluid was employed as a fixative: For electron microscopy a 7.5% solution of glutaraldehyde at 5 °C buffered with sodium cacodylate at pH 7.4 was used. After perfusion with fixative the hypothalamus and attached pars tuberalis were dissected out and immersed in the fixative for a further period of 24 h at 5 °C.

The hypothalami fixed for light microscopy were, after dehydration, embedded in paraffin wax and sectioned at either 5 or 10 μm thickness; eleven were sectioned coronally, three sagitally and two in the horizontal plane. Representative sections from different regions of the hypothalamus and the pars tuberalis were stained by one or other of the following methods: aldehyde fuchsin (Gabe 1953), Bodian's silver method (Bodian 1936), chrome alum haematoxylin (Gomori 1941), Crossman's modification of Mallory's triple stain (Crossman 1937), Marsland & Glees's modification of Bielschowski's silver method (Culling 1963), periodic acid Schiff method, periodic acid Schiff-Alcian blue method (Adams & Pearse 1959).

For electron microscopy small pieces of tissue from the wall of the third ventricle, the infundibular stalk or the pars tuberalis were postfixed in a 1 % solution of OsO_4 buffered with Michaelis solution at pH 7.4 for 1 h at room temperature. Then, after dehydration in ethanol, they were embedded in Vestopal W. or Araldite and sectioned on a Porter-Blum microtome, using glass knives.

Sections were mounted on carbon-coated grids, stained with a saturated solution of uranyl acetate in redistilled methanol, and examined in a Siemens Elmiskop I electron microscope. For histological and topographical comparisons by light microscopy thicker sections were cut at 0.5 to 2.0 nm and stained by a toluidine blue-borax solution.

TABLE 1. NUMBER OF ANIMALS EXAMINED

status	intact		experimental	
	histology	E.M.	histology	E.M.
sexually immature juvenile females	4 sexually immature females. Body weight < 205 kg: age not known	none	none	none
sexually mature adult females	pre-ovulatory 5	6	(ovariectomy only and killed 2 months later) 2	4
	post-ovulatory none	3	(ovariectomy—2 months followed by injection of 1 dose of 5 mg oestradiol given intramuscularly) 2	3
	menstruation 6	3	none	
sexually immature juvenile males	2 months old, 2 years old (2 animals)	none	none	none
sexually mature adult males	5	5	gonadectomy only animal 1 (1 month) animal 2 (2 months) animal 3 (3 months)	none
			gonadectomy, followed by injection 1 adult male castrated for 2 months and then given 5 mg oestradiol by intramuscular injection.	none
			1 adult male castrated for 2 months and then given testosterone 5 mg by intramuscular injection.	none

3. TYPES OF EPENDYMA IN THE VENTRAL HYPOTHALAMUS

In the present studies three areas of ependyma (A, B and C) have been distinguished. For convenience the ependymal cells of these areas will be designated Types A, B and C in the present account (figure 4, plate 63). In addition some cells, described here as C′ cells, were seen in Area C; the ependymal nature of these C′ cells is open to question.

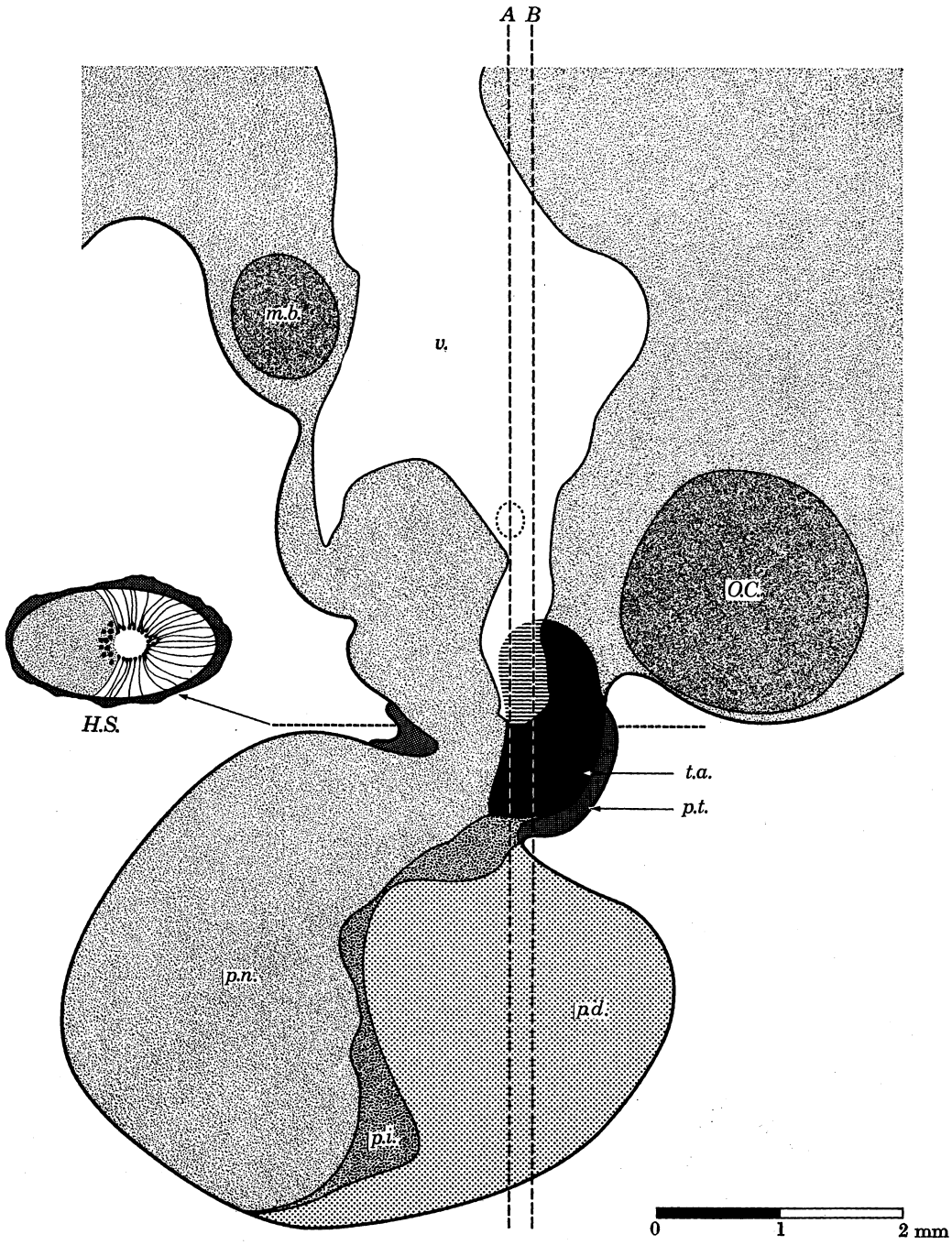

FIGURE 1. A semi-diagrammatic view of a median sagittal section through the hypothalamus and pituitary of a rhesus monkey. Those areas of specialized ependyma described in the text lie in that region depicted in solid black. (The adjacent area, shaded by horizontal lines, shows the extension of the specialized area in the lateral wall of the III ventricle.) The vertical dotted lines *A* and *B* pass through the planes of section depicted in figure 4, plate 63, and figure 7, plate 65, respectively. The horizontal dotted line passes through the plane of a coronal section (*H.S.*) illustrated semi-diagrammatically to show the anterior and antero-lateral distribution of the Type B tanycytes and the posterior position of the C′ cells. *m.b.*, mamillary body; *O.C.*, optic chiasma; *p.d.*, pars distalis; *p.i.*, pars intermedia; *p.n.*, pars nervosa; *p.t.*, pars tuberalis; *t.a.*, tanycyte area; *v.*, III ventricle. The oval area, outlined by dotted lines, in the ventricle delineates the position of the paraventricular 'organ'.

Type A may be termed typical ependyma in so far as it resembles the characteristic ependyma found elsewhere in the ventricles of the brain; it constitutes the greater part of the lining of the III ventricle. Types B and C were found in clearly circumscribed areas in the antero- and latero-ventral walls of the anterior hypothalamus (see figure 1). The three areas of ependyma were distinguished by their structure, ultrastructure and affinity for stains as follows:

Area A (plates 63 and 64, figure 5)

The ultrastructure of Type A cells resembles the descriptions given by Tennyson & Pappas (1962), Brightman & Palay (1963) and Klinkerfuss (1964) in the rabbit, rat and cat respectively though with some small differences of detail. Like the typical ependymal cells described by these authors the Type A ependymal cells in the rhesus monkey bear cilia and microvilli on their juxta-ventricular borders, though the size and number of the microvilli appear to be greater than those described in the other species (figure 5, plate 64). The ependymal cells are linked by terminal bar junctions, though these seem relatively few in number when compared with the descriptions given by the above-named authors.

Many mitochondria were present, especially between the nucleus and the juxta-ventricular border of the cell, and lysosomes also were observed. Ribosomes associated with cytomembrane systems (probably endoplasmic reticulum) were seen. Type A ependymal cells are columnar and do not send processes into the underlying white matter. They are arranged in a single layer and tightly packed, without conspicuous intercellular spaces. They did not stain with silver methods, but were coloured by the connective tissue stains used.

The appearance of Type A ependyma suggests a relatively unmodified ciliated epithelial tissue capable of some synthesis, secretion and absorption, though not apparently very actively engaged in these functions. No marked differences between animals of different age groups, or sex, were observed.

Area B (plates 65 to 73)

Type B ependymal cells showed considerable variation in relation to age, sex and reproductive activity, which will be considered in detail later. For the present only the main distinguishing features, shared by all Type B cells, will be considered. Type B ependymal cells were mainly restricted to the antero- and latero-ventral walls of the anterior hypothalamus (see figure 1). Under the optical microscope they seemed to comprise more than one layer of cells and had processes extending into the white matter to varying depths, often to the surface of the median eminence (figure 8, plate 65); for this reason they have been described as tanycytes (stretch cells). The staining affinities of Type B ependymal cells resembled those of Type A inasmuch as they stained with connective tissue but not with silver stains; their affinity for PAS was however greater than Type A ependyma.

Under the optical microscope the juxta-ventricular border of Type B ependyma could be clearly distinguished from that of Type A (plate 63), for Type A ependymal cells bear numerous

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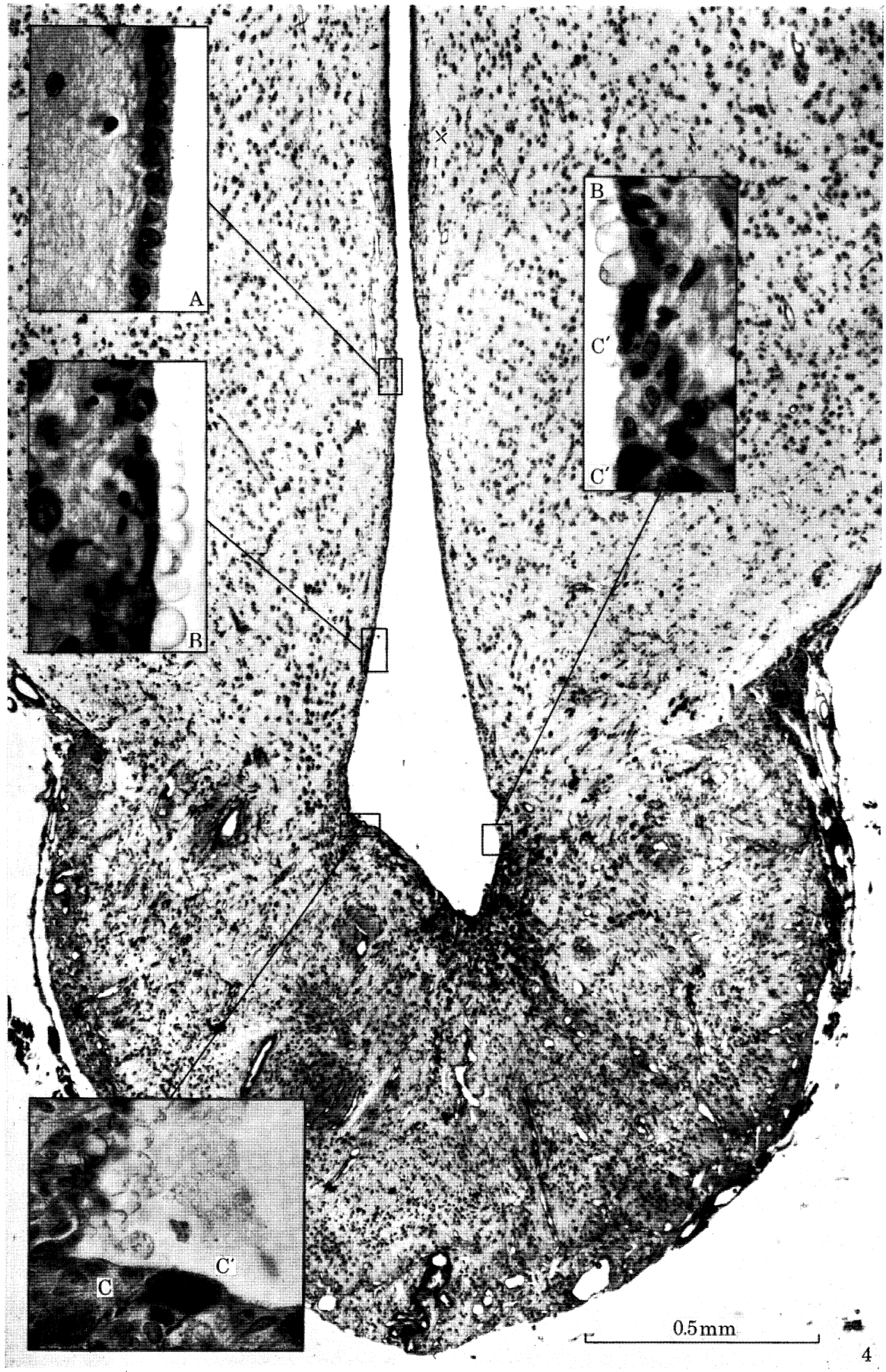
DESCRIPTION OF PLATE 63

FIGURE 4. A coronal section through an anterior region of the hypothalamus of a female rhesus monkey killed at mid-cycle (for exact plane of section, see figure 1). The more highly magnified insets show the distinguishing features of ependymal cell Types A, B, C and C′ as viewed by optical microscopy (for details of ultrastructure, see figures 5 to 9), plates 64 to 65. ×, paraventricular organ. (Magnification of these and subsequent insets × 820. Staining by Gomori method.)



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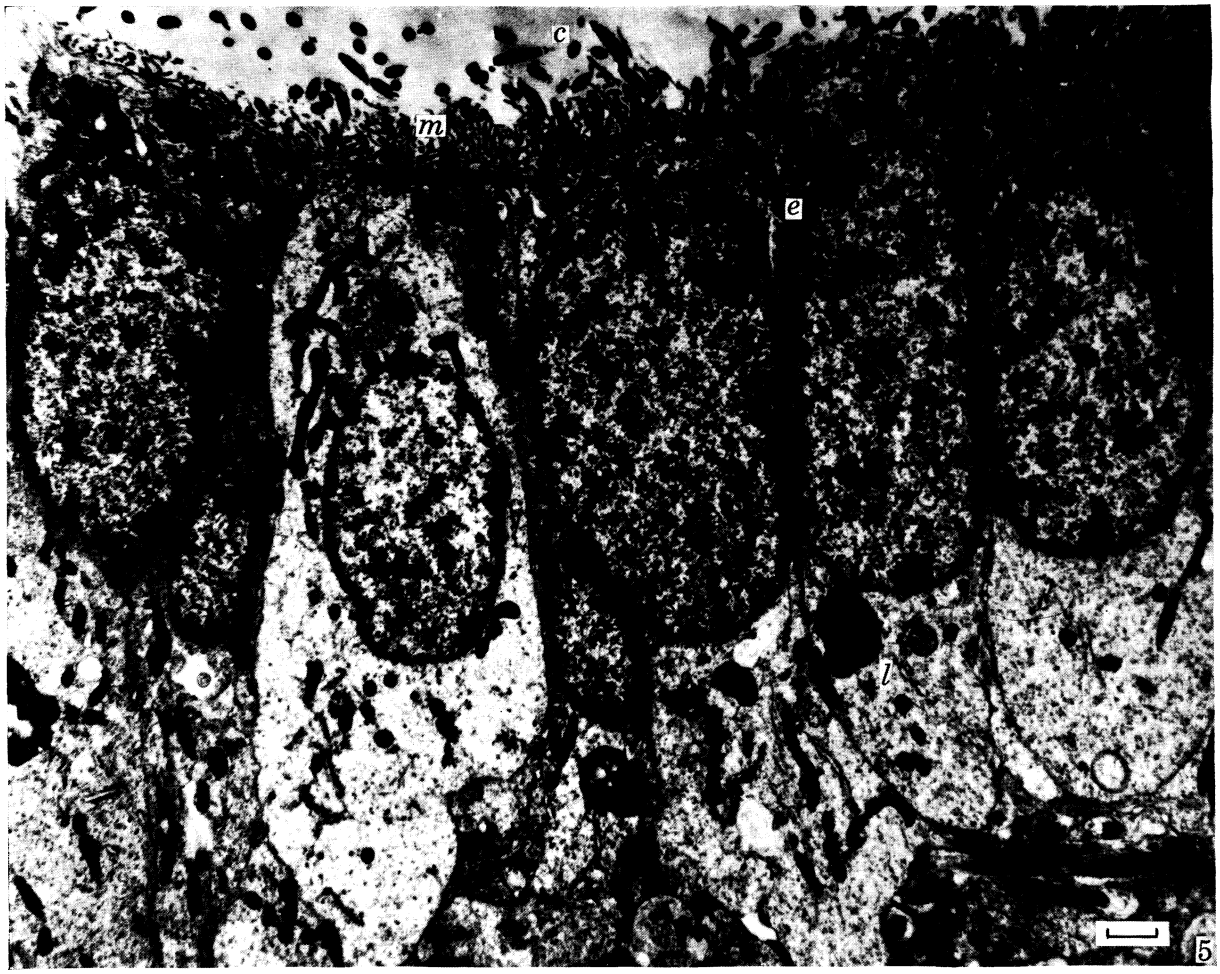
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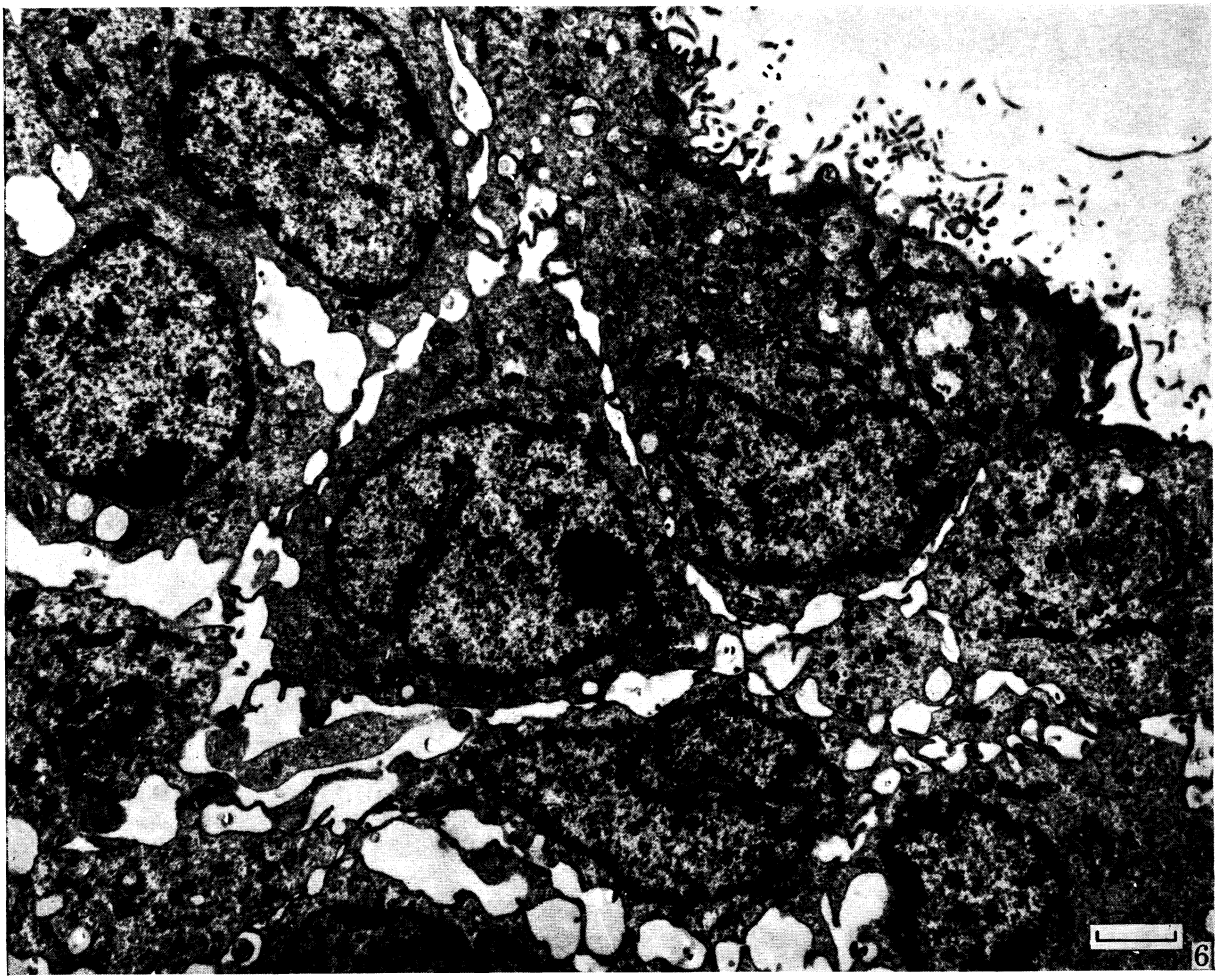
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cilia and Type B do not. Using the electron microscope the following additional distinguishing characteristics of Type B ependymal cells were noted:

(1) Some microvilli were present but most of the juxta-ventricular plasma membrane was extended into numerous bulbous projections.

(2) A narrow electron-dense region, probably representing a terminal web, lay close beneath the juxta-ventricular border.

(3) The cytoplasm contained not only mitochondria fairly evenly dispersed throughout the cell and typical lysosomes, but also numerous spherical electron-dense bodies (plates 68 to 71). Sometimes large vacuoles, containing varying amounts of electron-dense material, were observed (plate 71).

(4) Ribosomes were very abundant, either lying free in the cytoplasm, or associated with cytomembrane systems. Fibrillae were also very numerous and extended throughout the cells including their basal prolongations (plates 66 and 67).

(5) The nuclei of Type B ependymal cells differed from those of Type A ependyma. Characteristically Type A nuclei are oval and uniform, without indentations and little evident nucleolar material (figure 5, plate 64). In contrast, the Type B nuclei were often deeply indented and contained one or more nucleoli (figure 16, plate 69).

(6) Type B cells were attached to one another by terminal bar systems, but intercellular spaces were frequent.

(7) The basal prolongations of Type B cells repeatedly branched and made intimate contacts with one another giving the appearance of a syncytium (see Studnicka 1900; and Kruger & Maxwell (1966) for similar ependymal structures elsewhere). It was not found possible by electron microscopy to decide whether actual anastomoses occurred, but fibres traversing one another, as in figure 10, plate 66, were very abundant and it was usually not possible to distinguish membrane boundaries; instead indications of fusion were seen.

(8) The tanycyte prolongations contained numerous fibrillae, mitochondria with dense contents and thick cristae and electron-dense and electron-lucent small vesicles which were abundant in the preterminal and terminal regions (figures 11, plate 66, and 13, plate 67).

The electron-dense vesicles measured *ca.* 100 nm in diameter and contained a dense core separated from the outer bounding membrane by a narrow electron-lucent space. They therefore resemble, in their form, the Type B neurosecretory vesicles described by Knowles (1965, 1967) which are often associated with the presence of monoamines; as yet we have no information about the chemical nature of these inclusions in the tanycyte terminations; this may be difficult to obtain in view of the presence of aminergic fibres from the arcuate nucleus which intermingle with the tanycyte terminals and subterminal prolongations (see Halász, Pupp & Uhlarik 1962; Szentágothai *et al.* 1968).

This proximity of adrenergic fibres to the tanycyte terminations also makes it difficult to discriminate between these two types of fibre at the level of ultrastructure—a problem made

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DESCRIPTION OF PLATE 64

FIGURE 5. Type A, or typical, ependymal cells, similar to those shown in inset at figure 4, plate 63, and found also elsewhere in the brain (for details see text). *c.*, cilia; *l.*, lysosome; *m.*, microvilli. The scales on this and subsequent plates denote 1 μm.

FIGURE 6. Type C ependymal cells, similar to those shown in inset at figure 4. Note the many microvilli, absence of cilia, the indented nuclei and the numerous intercellular spaces.

more arduous by the length of the tanycyte fibres (*ca.* 0.5 mm), and their irregular course which cannot easily be followed in ultrathin sections. In the present studies particular attention was directed towards the identification, at the level of ultrastructure, of the tanycyte prolongations, their course, contents and destination. This was achieved by cutting alternately thick and thin sections which were viewed, for comparison, under the optical and electron microscopes respectively. Thus it was possible to determine, with reasonable confidence, that tanycytes, like those depicted at plates 65, 66 and 67, contained electron-dense inclusions and that they terminated in close proximity to blood-vessels in the median eminence.

Area C

Area C is restricted to the floor of the anterior hypothalamus. It contains two distinct types of cell; the first type, here designated as Type C ependymal cells, form a many-layered epithelium of loosely packed cells with few desmosomal junctions and many intercellular spaces (figure 6, plate 64): the juxta-ventricular surfaces of these cells bear microvilli but no cilia; they do not have basal processes; the nuclei are indented and the cytoplasm contains lysosomes, mitochondria, fibrillae and free-lying ribosomes.

Other cells, here termed C′ cells, were found scattered among the C cells from which they were distinguished by their strong affinity not only for connective tissue strains but also for the silver stains (figure 4, plate 63); in this respect they differed from the Types A, B and C ependyma. Basal prolongations were seen but could not be traced any great distance. Many of these Type C′ cells were found at the ventricular surface, but many also were found in deeper layers (Anand Kumar 1968*b*); some were found adjacent to the posterior margin of the infundibular recess (see figure 1). At the level of ultrastructure neither microvilli nor cilia were seen. The ependymal nature of the C′ cells is open to question. They are distinguishable by their shape and argentophilia from the normal and specialized ependymal cells lining the III ventricle.

Age and sex differences

No differences in Type A and C ependyma were detected when animals of different age or different sex were examined: In contrast Types B and C′ cells showed changes in animals of different ages and sexes (Anand Kumar, 1968*a*, *b*).

In juvenile animals of either sex Type B ependyma form a tightly packed epithelium (plate 73) The juxta-ventricular surface bears few and inconspicuous bulbous projections. The nuclei stain densely and there is little cytoplasm.

In adult males the ependymal cells are arranged in two distinct layers, separated by a space (plate 68). The juxta-ventricular border bears small bulbous projections. In contrast the Type B ependymal cells of the adult female are also arranged in layers but these are not separated by

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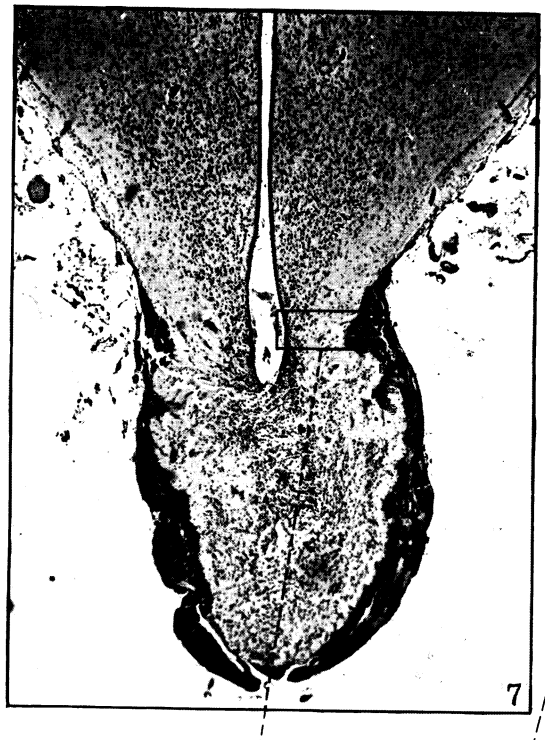
Description of plate 65

Figure 7. A coronal section through a region of the hypothalamus slightly anterior to that depicted in figure 4 (for exact plane of section, see figure 1). At this plane of section the processes of the tanycyte cells are more apparent; fewer Type C′ cells are seen.

Figure 8. The area outlined in figure 7, but more highly magnified and rotated through an angle of 90° (*ab* → *ab*′). Tanycyte processes (*tp*) extending towards the pars tuberalis (*pt*) are shown.

Figure 9. Electron micrographs arranged as a montage showing the cell-bodies of Type C cells and a proximal region of some of their prolongations.



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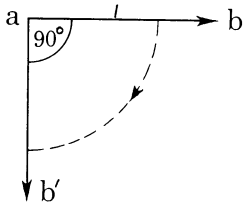

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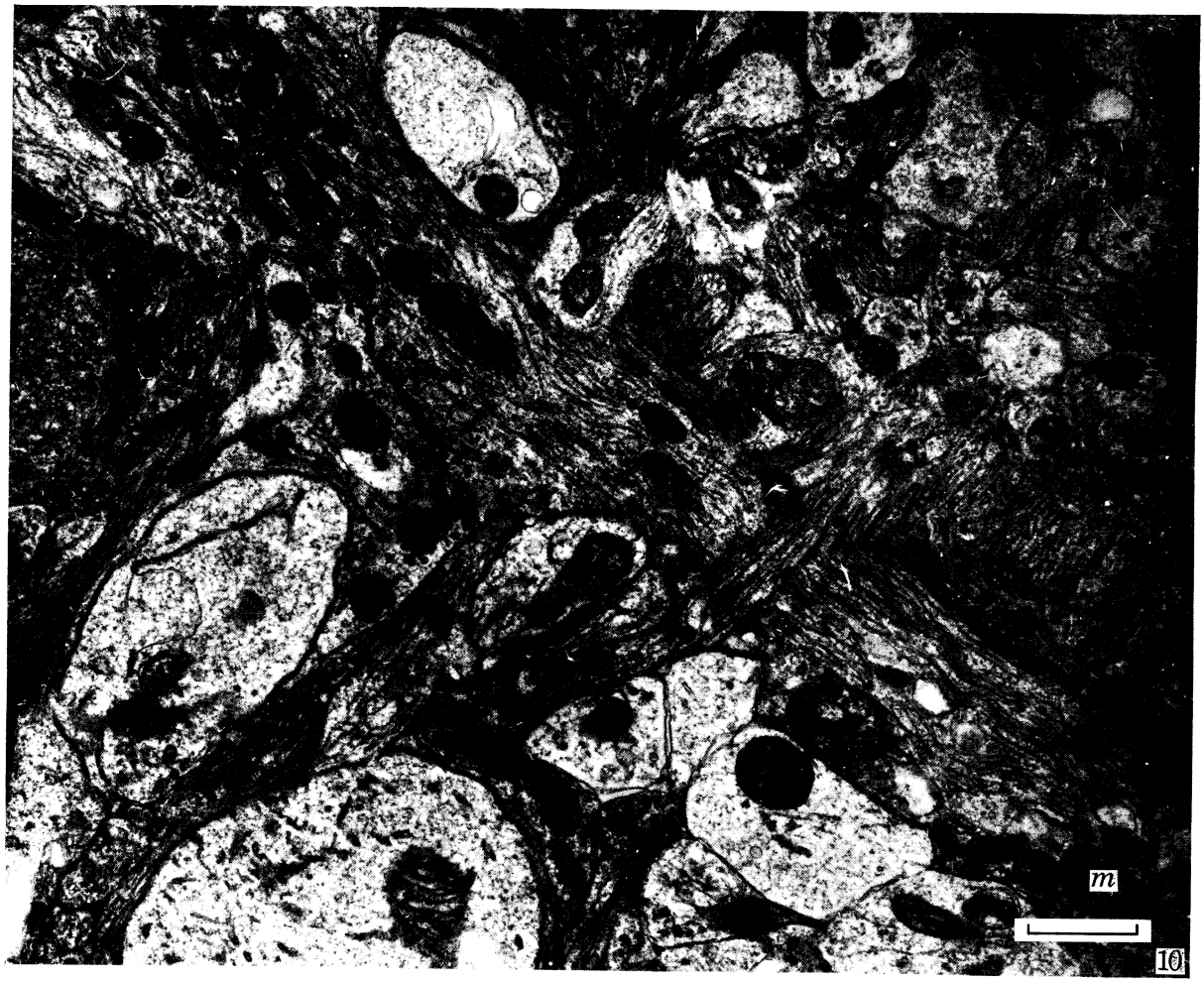
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Description of plate 66

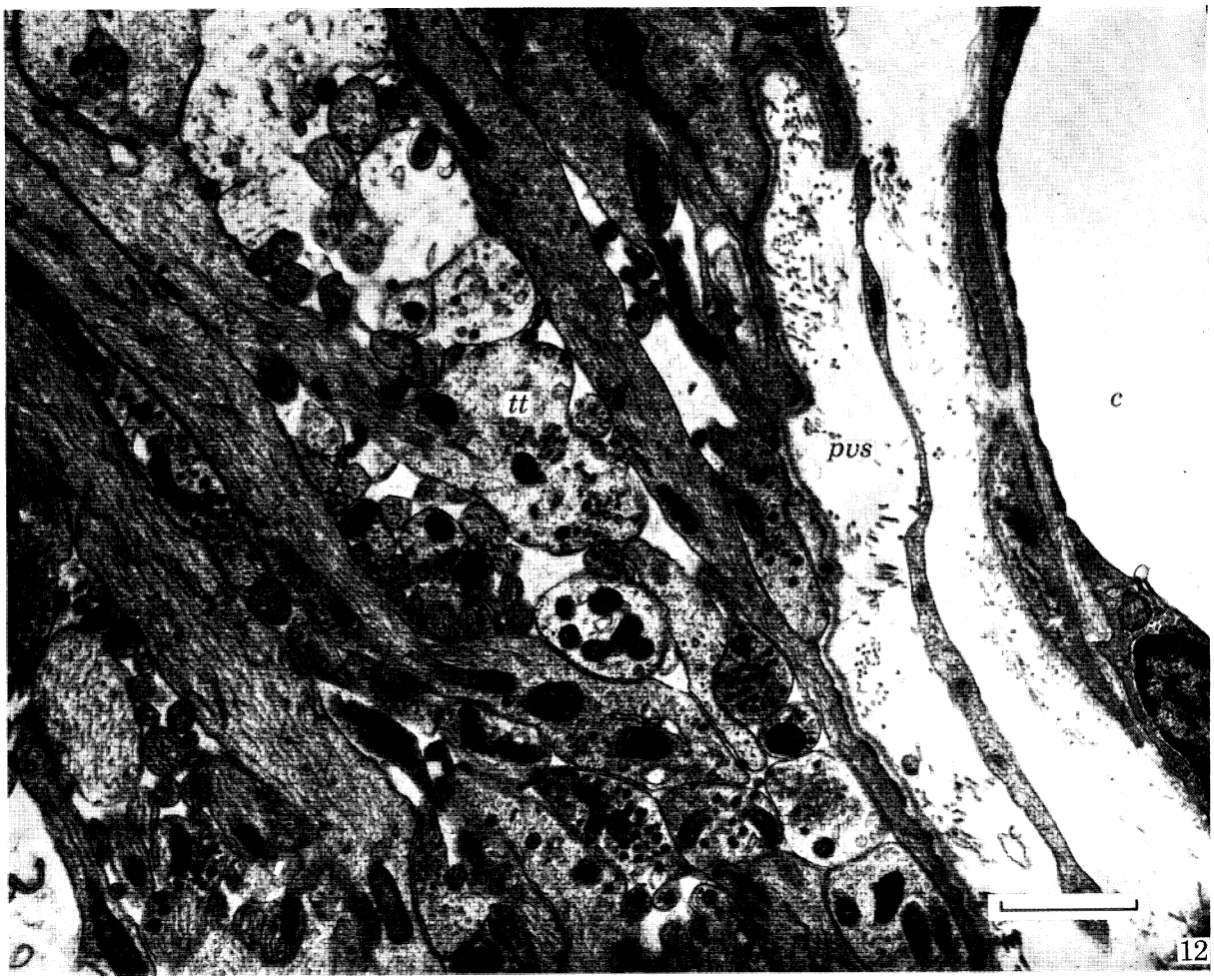
Figure 10. A portion of a Type C ependymal fibre in a region more distal to that depicted at figure 9, plate 65. The ependymal prolongations in this area have many lateral branches, some of which appear to fuse with neighbouring tanycytes.

Figure 11. In the more distal, preterminal, regions of the tanycyte fibres branching is more frequent; electron-dense granules (*g*) are more abundant here than in more proximal regions; mitochondria appear more spherical, irrespective of the plane of section. Scales = 1 μm.

Description of plate 67

Figure 12. A portion of the median eminence of a pre-ovulatory female, showing the spatial relationship between tanycyte terminals (*t.t.*), a perivascular space (*p.v.s.*) and a capillary (*c.*).

Figure 13. An area similar to that shown in figure 12, but more highly magnified. The tanycyte terminations contain mitochondria (*m.*), and electron-dense (*d.v.*) and electron-lucent (*l.v.*) vesicles. *p.v.s.*, perivascular space. Scales = 1 μm.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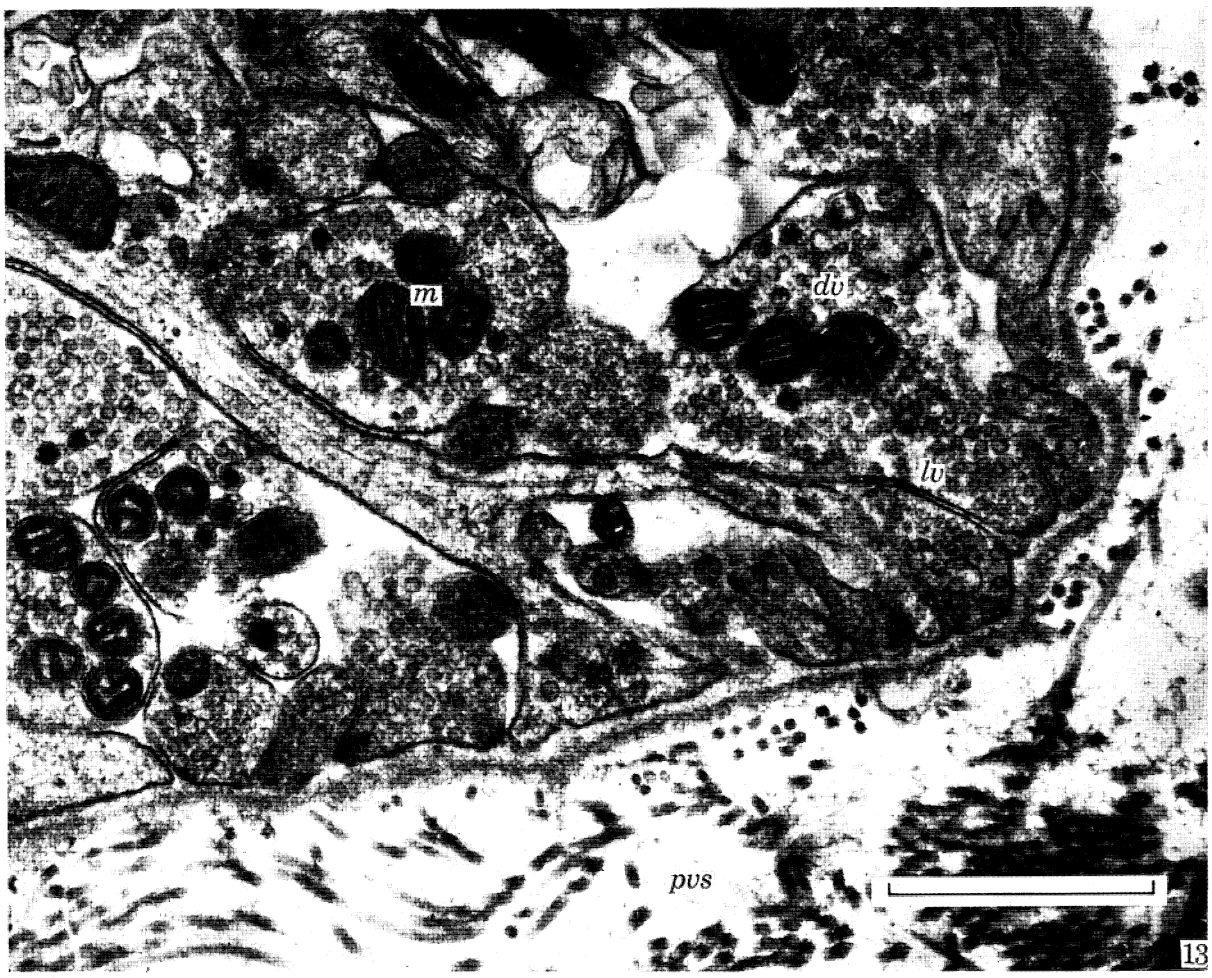
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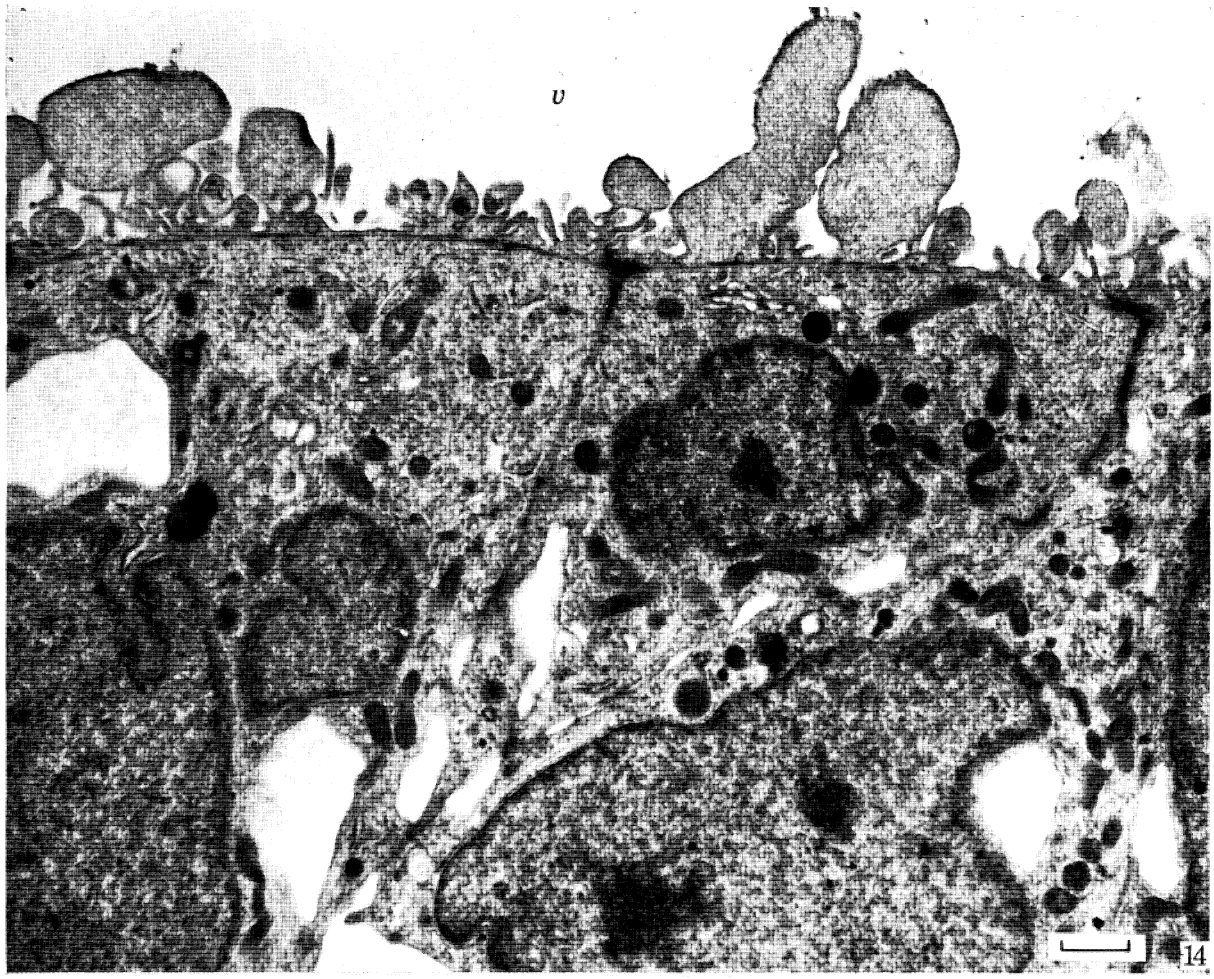
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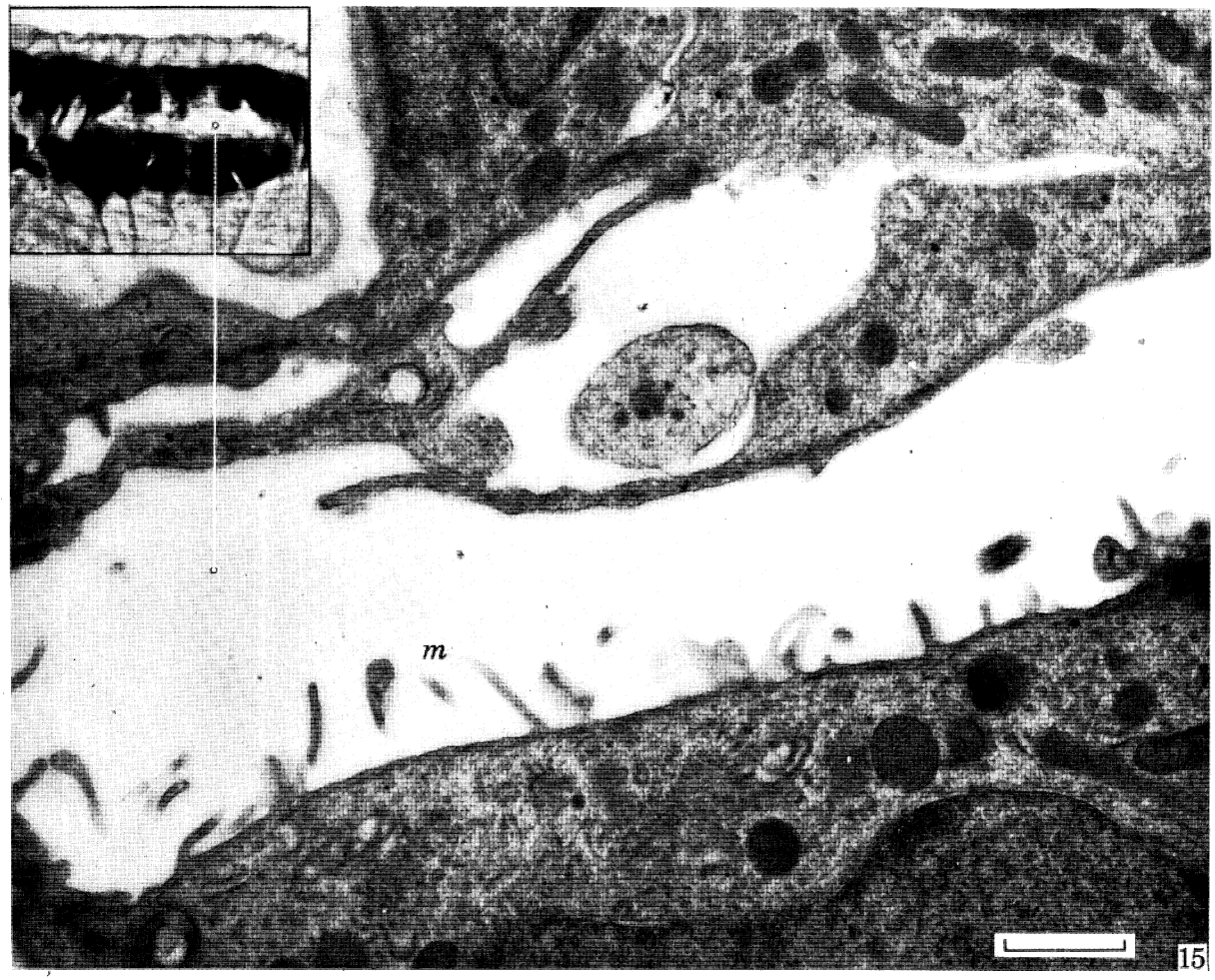
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Description of plate 68

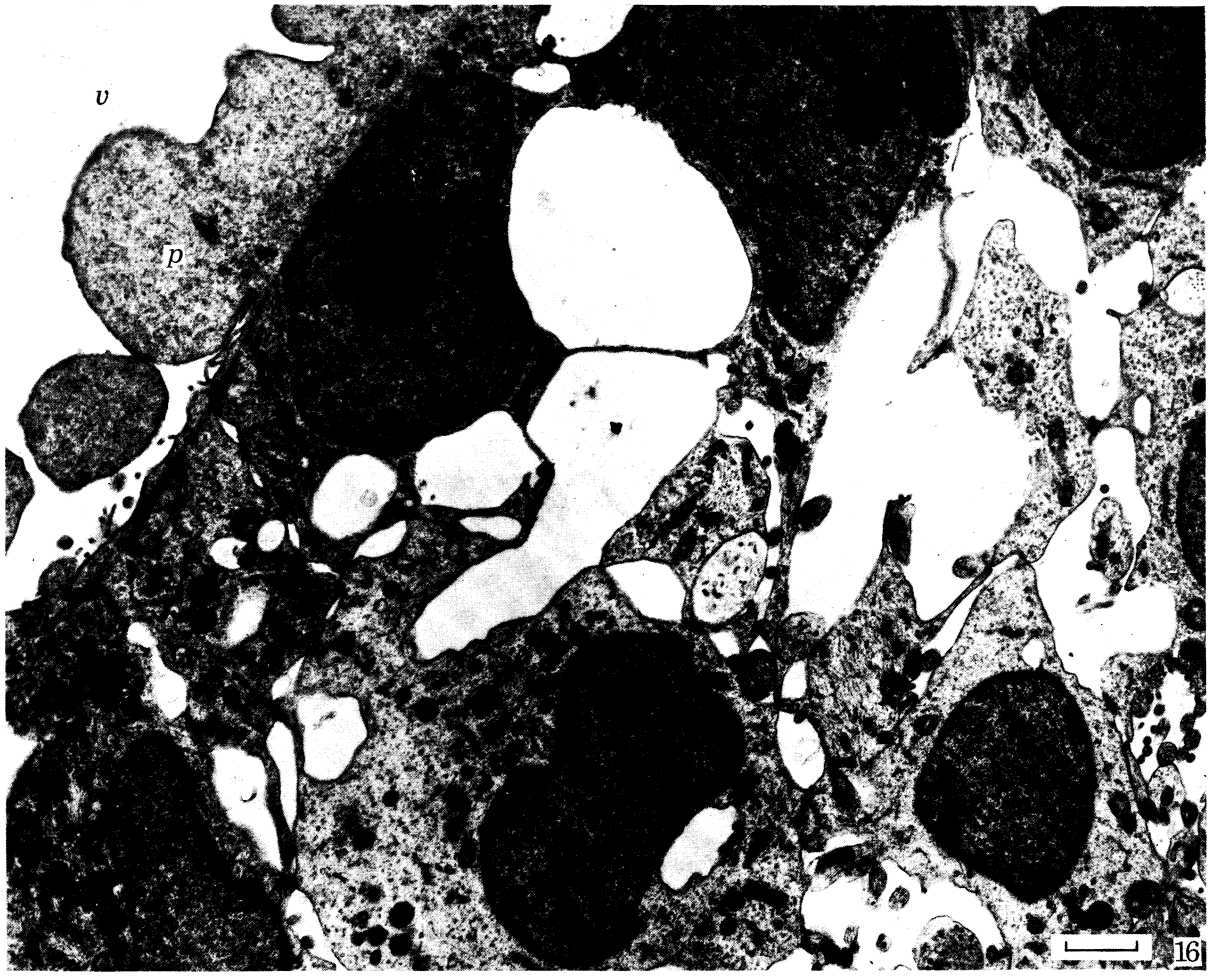
Figure 14. Type B tanycytes bordering the III ventricle (*v.*) in the male rhesus monkey.

Figure 15. As figure 14, but more highly magnified to show that in the male two distinct layers of tanycytes are separated by a space into which microvilli (*m.*) from the cells of the deeper layer extend. The small inset micrograph illustrates more clearly that the Type B region in the male has two separated layers of cells, in contrast to the same area in the female (see plates 65 and 69), and also depicts the positions of figures 14 and 15 relative to one another. Scales = 1 μm.

Description of plate 69

Figure 16. Type B tanycytes bordering the III ventricle (*v.*) in a female rhesus monkey, killed during the early pre-ovulatory period. *p.*, projection of ventricular surface membrane. (This figure should be compared with figures 7 to 9, plate 65.)

Figure 17. As figure 16, but in a female rhesus monkey killed during menstruation. The inset photo-micrograph showing a comparable area shows that the bulbous juxta-ventricular projections (*p.*) become greatly reduced at the time of menstruation. *v.*, ventricle. Scales = 1 μm.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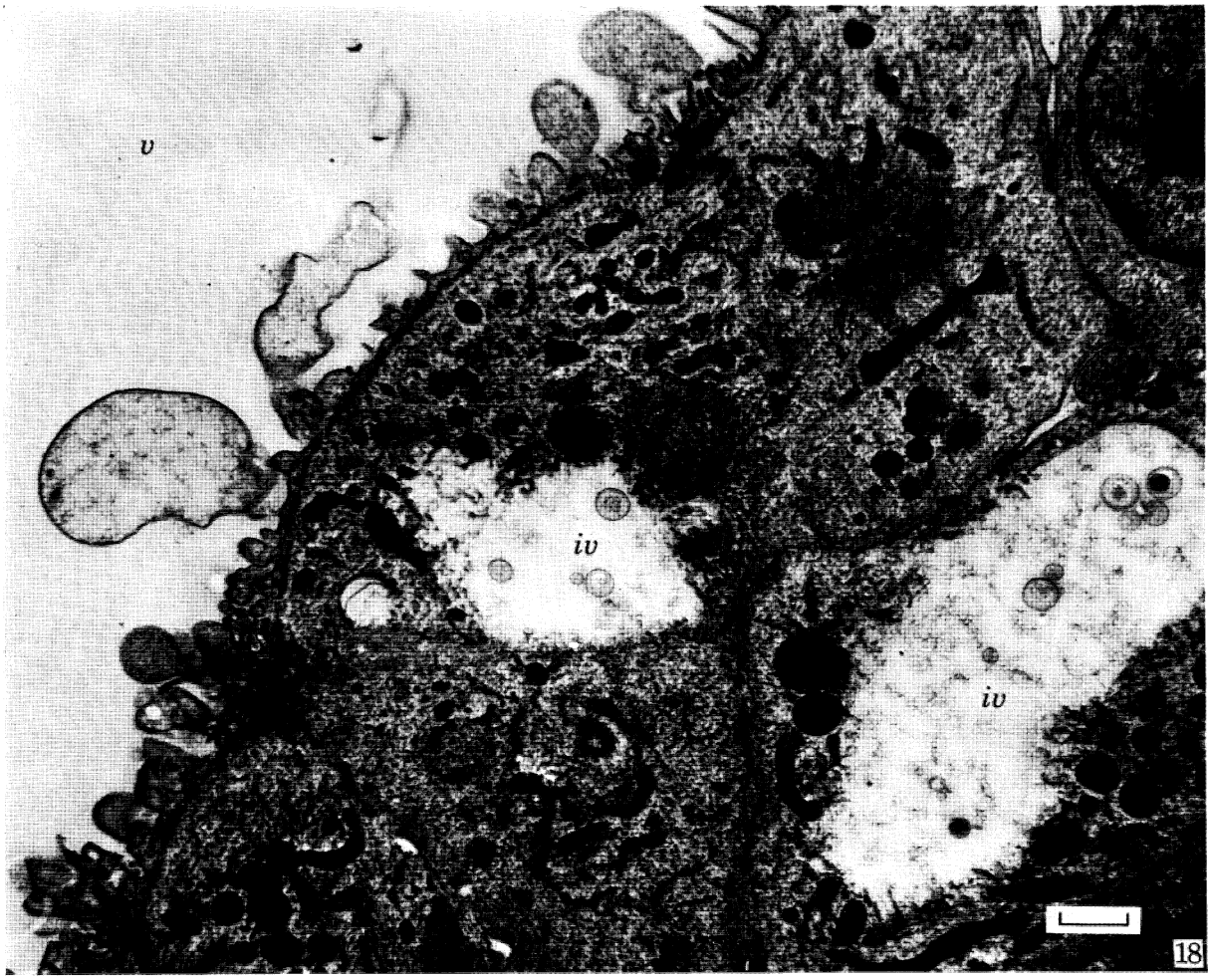
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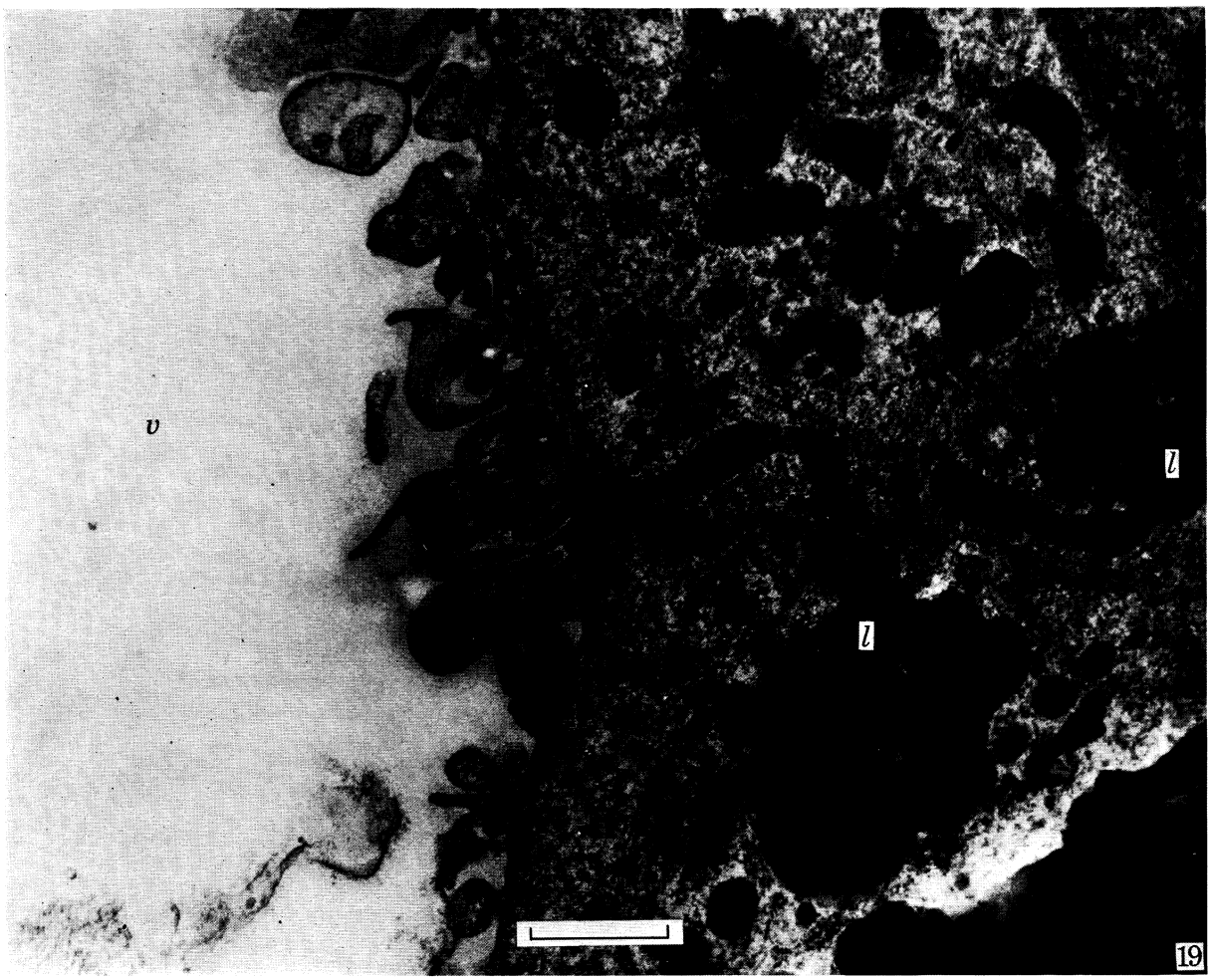
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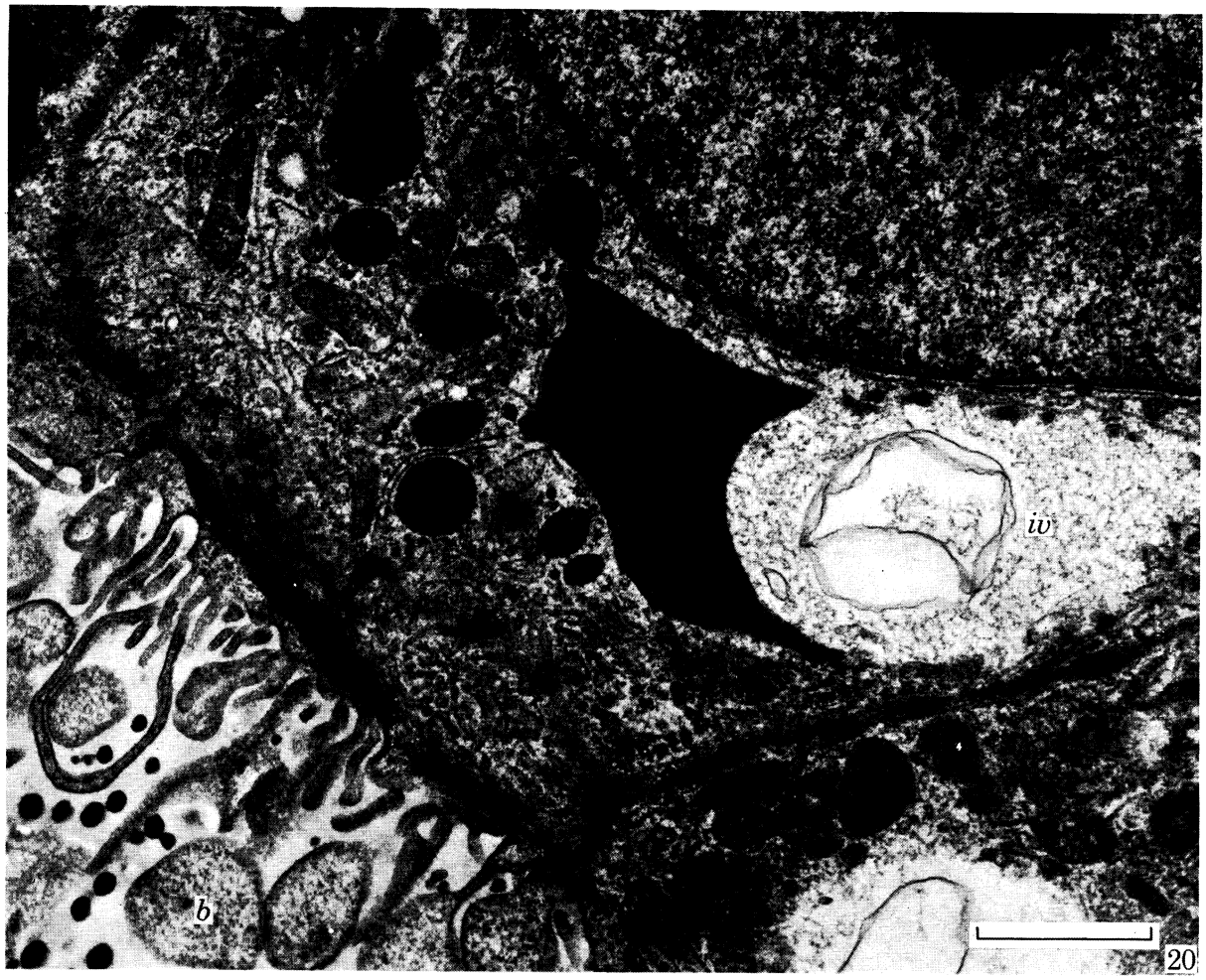
Description of plate 70

Figures 18, 19. Tanycytes bordering the III ventricle (*v.*) in animals killed during a late pre-ovulatory phase, closer to mid-cycle than that depicted in figure 16, plate 69. As the cycle proceeds lysosomes (*l.*), and other cytoplasmic inclusions become more abundant; intracellular vacuoles (*i.v.*) shrink and contain some electron-dense material. Scales = 1 μm.

Description of plate 71

Figures 20, 21. As the preceding figures 18 and 19 (plate 70), but from animals killed during an early post-ovulatory phase. During this phase of the menstrual cycle the bulbous projections (*b.*) extending into the III ventricle become notably larger. The cytoplasm of the tanycytes is densely filled with mitochondria and spherical electron-dense bodies; intracellular vacuoles (*i.v.*) are partially or completely filled with electron-dense material. Scales = 1 μm.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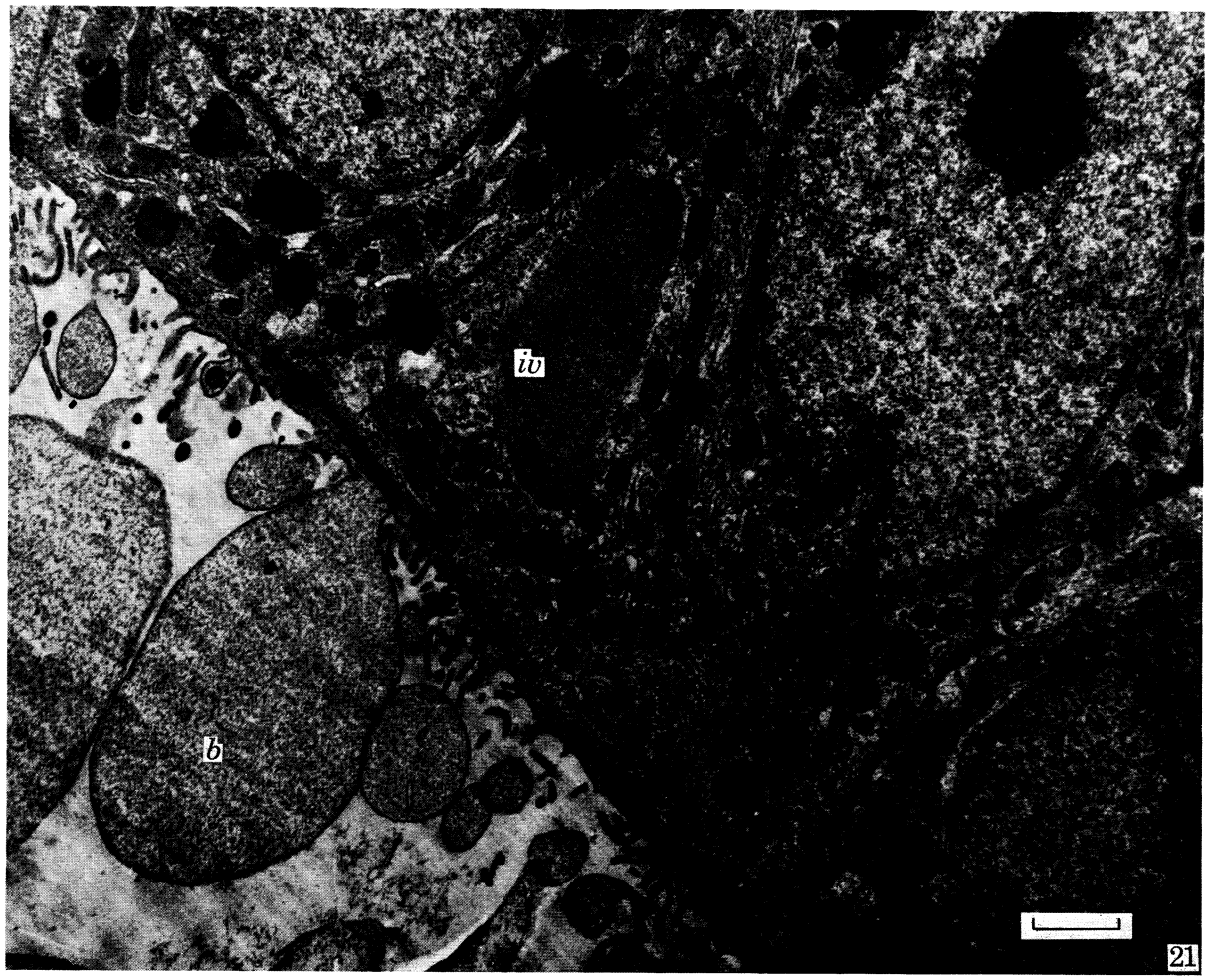
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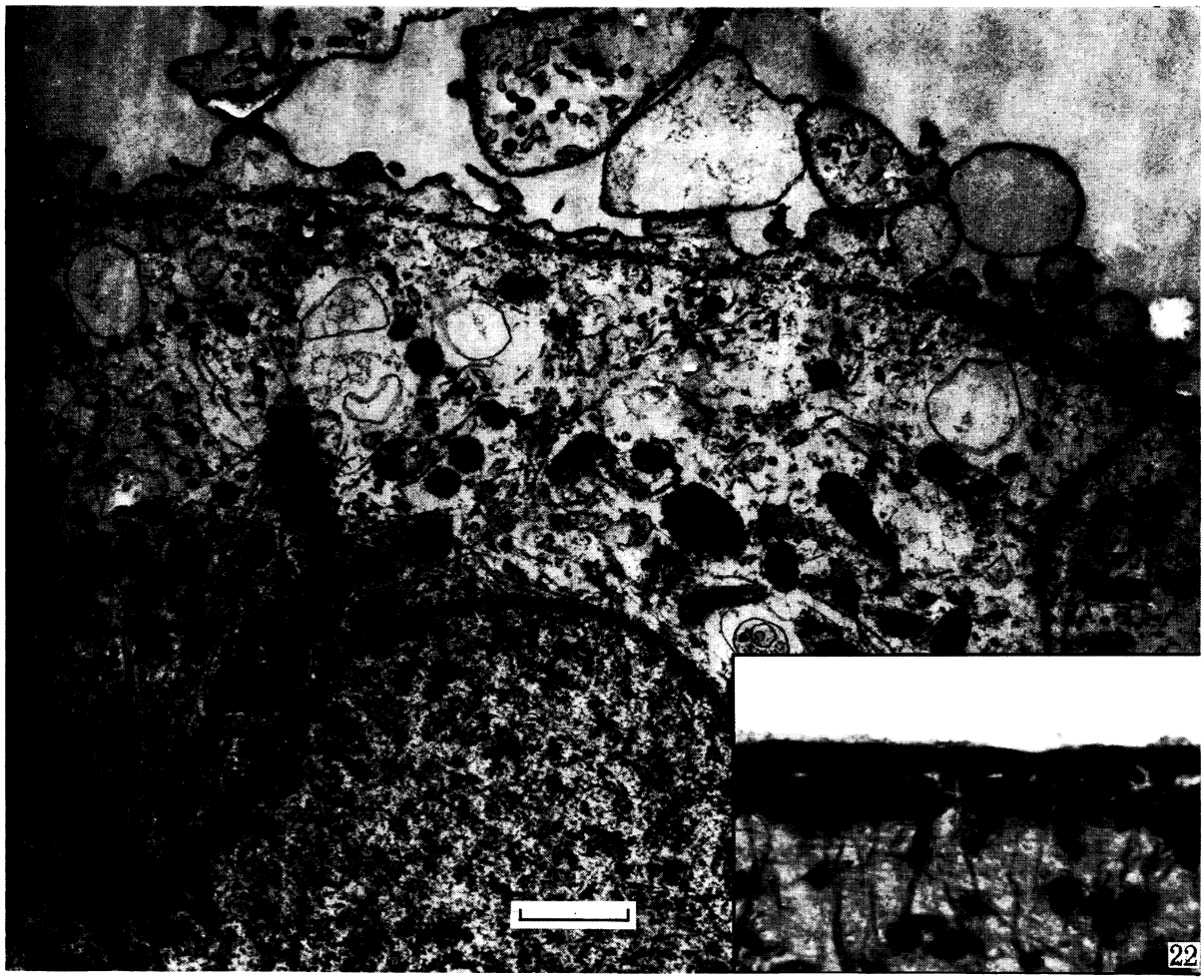

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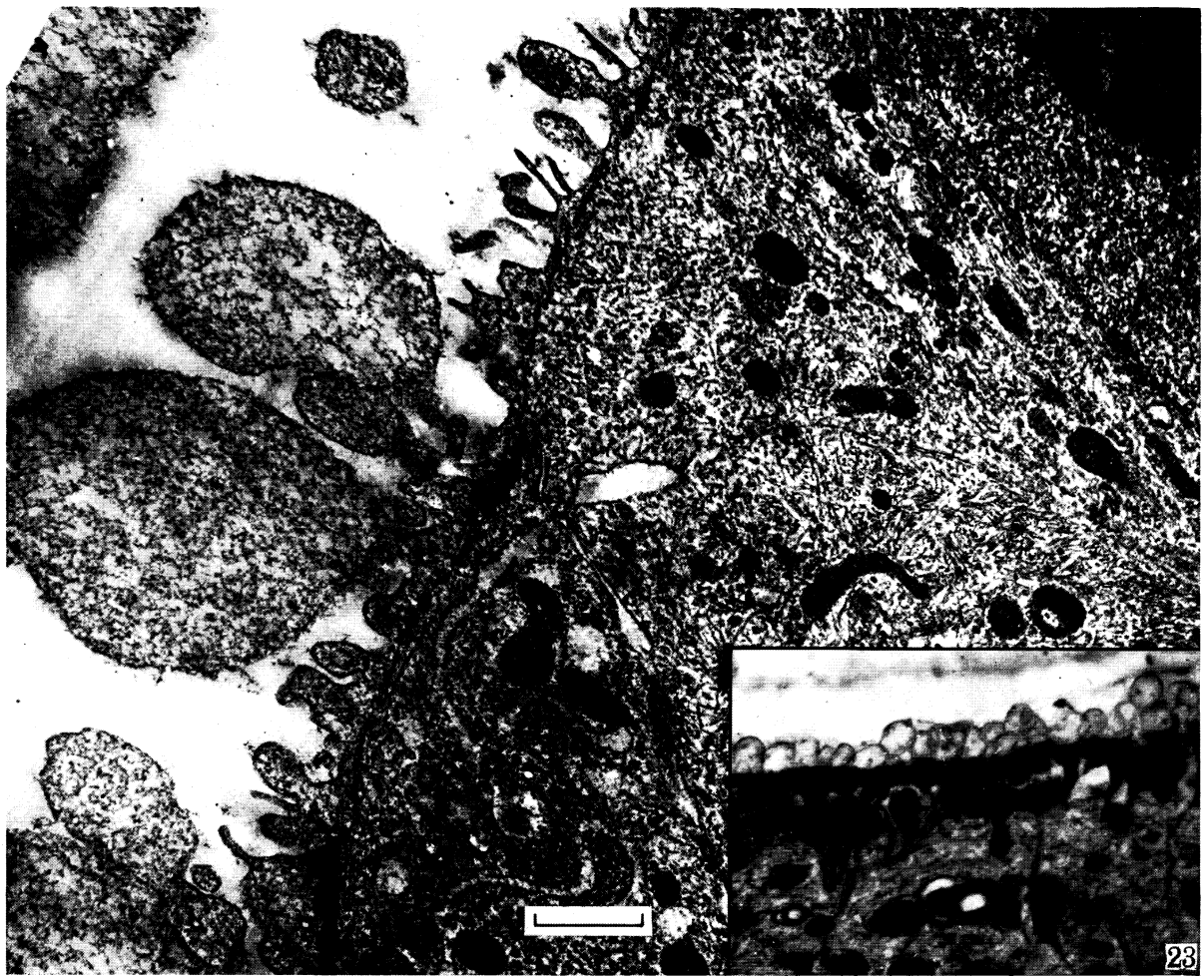

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any single continuous space, though at certain stages of the menstrual cycle intercellular spaces are present (plate 69). The bulbous projections are generally large but, as will be described later, vary during the course of the menstrual cycle.

The space separating the two layers of Type B cells in the male was examined under the electron microscope and it was found that the inner layer of cells bore microvilli projecting into the space (figure 15, plate 68).

4. Changes in relation to reproduction

(*a*) *The menstrual cycle*

Under the optical microscope a clear distinction between Type B ependyma and also C′ cells could be observed when animals killed during menstruation were compared to those killed at other times during the cycle. At menstruation (figure 17, plate 69) the juxta-ventricular border of Type B cells was reduced almost to the juvenile condition (see figure 25, plate 73), whereas at other times in the cycle the bulbous projections were very evident (plates 69 to 71). Type C′ cells were degranulated at menstruation compared to their condition at other times during the cycle.

Under the electron microscope it was possible to distinguish not only changes in the ventricular border of the Type B ependymal cells, but also alterations in their contents. In making comparisons great care was taken to select tissue from a comparable site in each animal examined in order to make sure that the differences seen were related to different stages in the sexual cycle rather than to possible variation in ependyma in different regions of the III ventricle. The sequence of changes may be summarized as follows:

(1) *Menstruation* (figure 17, plate 69)

Type B cells taken from animals killed while menstruating had microvilli, but bulbous projections of the ependymal ventricular border were rare or absent. The cytoplasm of the Type B cells was relatively electron-lucent and appeared to contain few electron-dense bodies. Lysosomes were rarely observed and mitochondria seemed few in number. Large intracellular and intercellular spaces were frequently observed.

(2) *Pre-ovulatory phase* (figure 16, plate 69 and plate 70)

The ventricular plasma membrane had few microvilli but the bulbous projections were very well developed. Indeed, optical microscope studies have shown that they sometimes attain their maximum size during this stage of the cycle. At menstruation they measure 4 μm but during mid-cycle they measure about 12 μm.

Description of plate 72

Figure 22. Tanycytes Type B from a female rhesus monkey which had been ovariectomized 2 months previously. The electron micrograph shows that the tanycytes of this animal bear some resemblance to those in a normal animal at menstruation (figure 17, plate 69); the inset shows that the tanycytes have become arranged in a double layer, with a slight space between, as in males (figure 15, plate 68); the bulbous projections are less pronounced than in either normal males or normal females.

Figure 23. As figure 22 but from an ovariectomized animal which had been given one dose of 5 mg oestradiol. These tanycytes resemble those of normal female animals killed at mid-cycle or during the pre-ovulatory period. Scales = 1 μm.

The cytoplasm was more electron-dense than at menstruation and contained more cytomembrane systems and a number of spherical electron-dense membrane-bound bodies measuring approximately 700 nm in diameter. In the early pre-ovulatory phase lysosomes were rare and mitochondria were not abundant. Intercellular and intracellular spaces were prominent.

(3) *Post-ovulatory phase* (plate 71)

In contrast to the pre-ovulatory condition the Type B ependymal cells of three animals killed in the post-ovulatory stage contained, in addition to numerous lysosomes and mitochondria, electron-dense bodies of many shapes and sizes. Some were spherical, as in the previous phase, and measured *ca.* 500 nm in diameter; others were irregular in form (figure 20, plate 71) and seemed to be contained in the large intracellular vacuoles previously noted. The bulbous projections of the juxta-ventricular surface were still very prominent. Among these and the microvilli electron-dense bodies were found in the ventricles (figure 21, plate 71); it was not possible to decide whether these electron-dense bodies were attached to the juxta-ventricular plasma membrane, but there were some indications of this.

(*b*) *Castration* (plates 72 and 73)

Following gonadectomy changes in Type B ependyma were observed in both sexes.

In the male the double-layered structure, separated by a space, was no longer evident, though intercellular spaces were seen (figure 26, plate 73). The bulbous projections were not greatly altered, but were slightly smaller in size.

In six ovariectomized females, striking changes ensued. The bulbous projections vanished almost entirely and a small space, reminiscent of that in the male, appeared between the two layers of ependyma (figure 22, plate 72, inset).

At the level of ultrastructure details resembling those of the post-ovulatory phase, just prior to menstruation, were seen, notably numerous mitochondria and lysosomes and other electron-dense bodies, including some clearly inside the juxta-ventricular bulbous projections (figure 22, plate 72).

(*c*) *Injection experiments*

A castrated male killed 72 h after a single injection of testosterone showed the space, which is normally absent from castrate males; bulbous projections were even smaller than in castrate males.

Description of plate 73

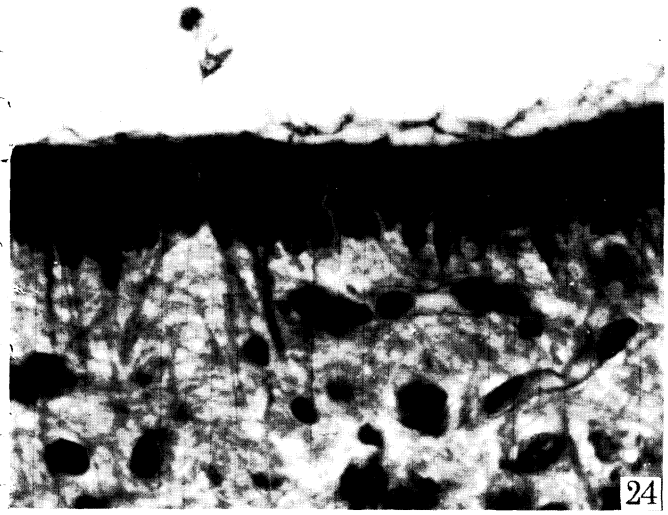
Figure 24. Type B tanycytes in a juvenile male rhesus monkey. This photomicrograph should be compared with figure 15 (inset), plate 68 which depicts the condition of these tanycytes in the adult male.

Figure 25. Type B tanycytes in a juvenile female rhesus monkey. This photomicrograph should be compared with figures 4 (inset), plate 63 and 8, plate 65 which depict similar tanycytes, but in the mature female.

Figure 26. Type B tanycytes from a male monkey which had been castrated three months previously; this figure should be compared with figure 15 (inset), plate 68.

Figure 27. Type B tanycytes from a male monkey which had been castrated 2 months previously, and then given a single injection of 5 mg testosterone. Note that the normal double-layered condition of the tanycytes has been restored, but that the bulbous juxta-ventricular projections are even less noticeable than in the untreated castrate.

Figure 28. As figure 27 but after a single injection of 5 mg oestradiol. No clearly evident differences between the Type C tanycytes of this animal and the untreated male castrate were observed. Magn. × 820 for figures 24 to 28.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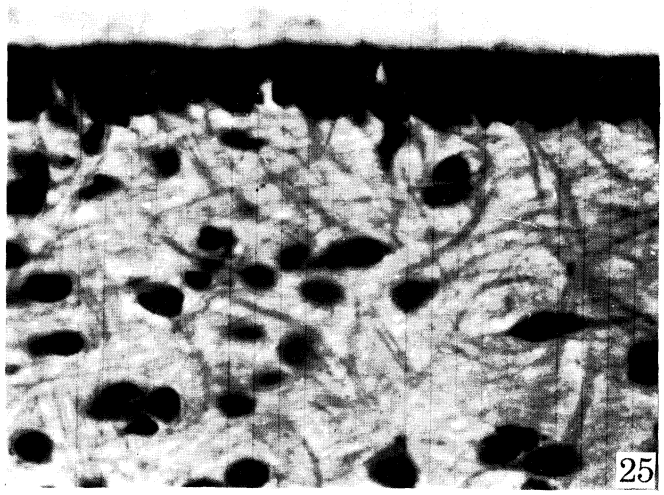
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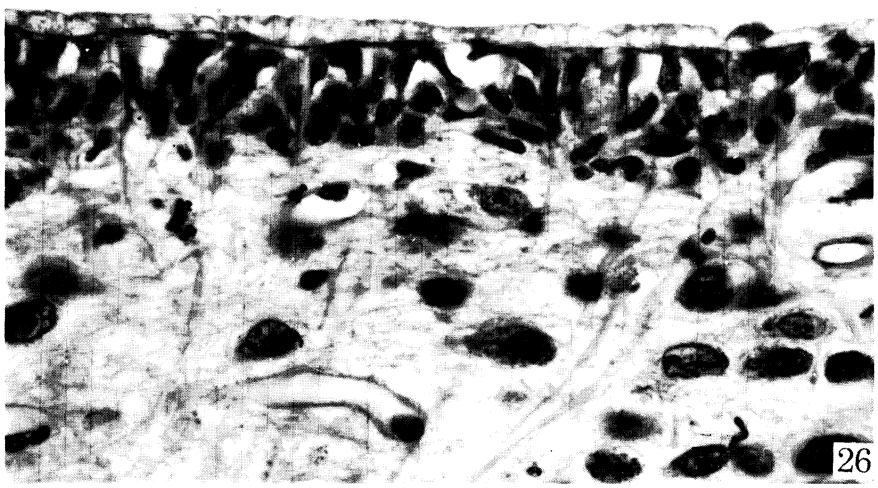
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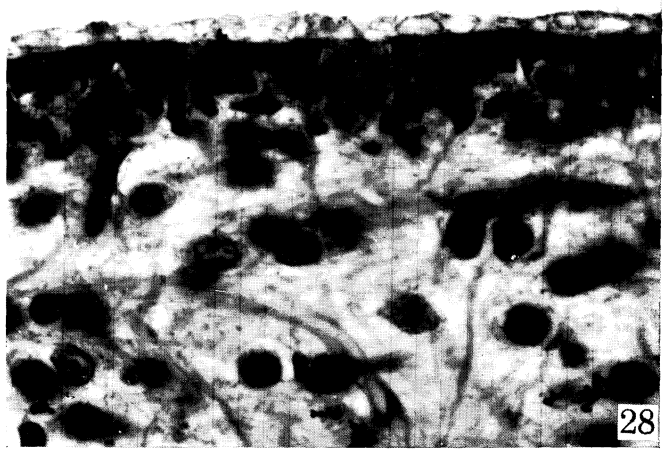
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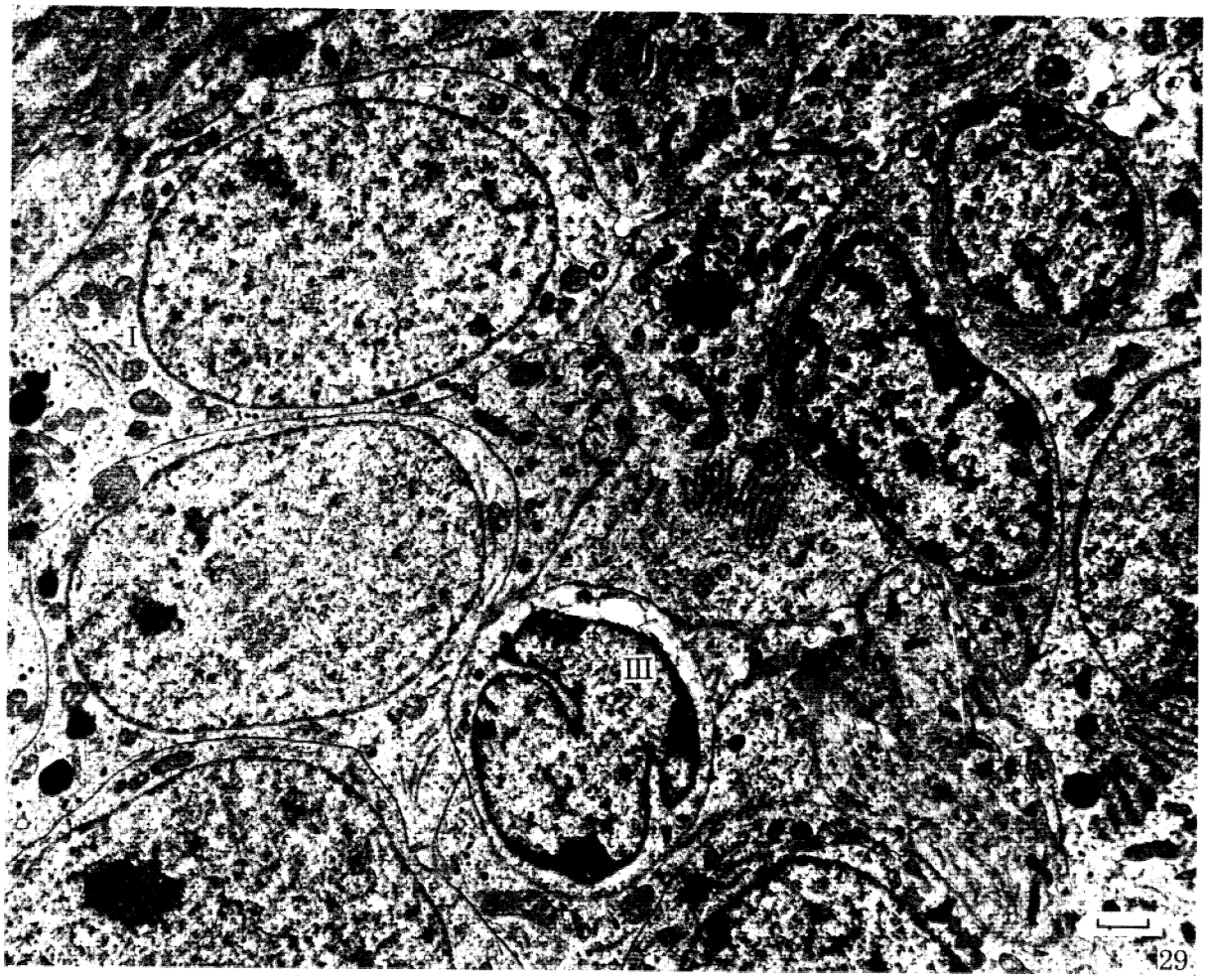
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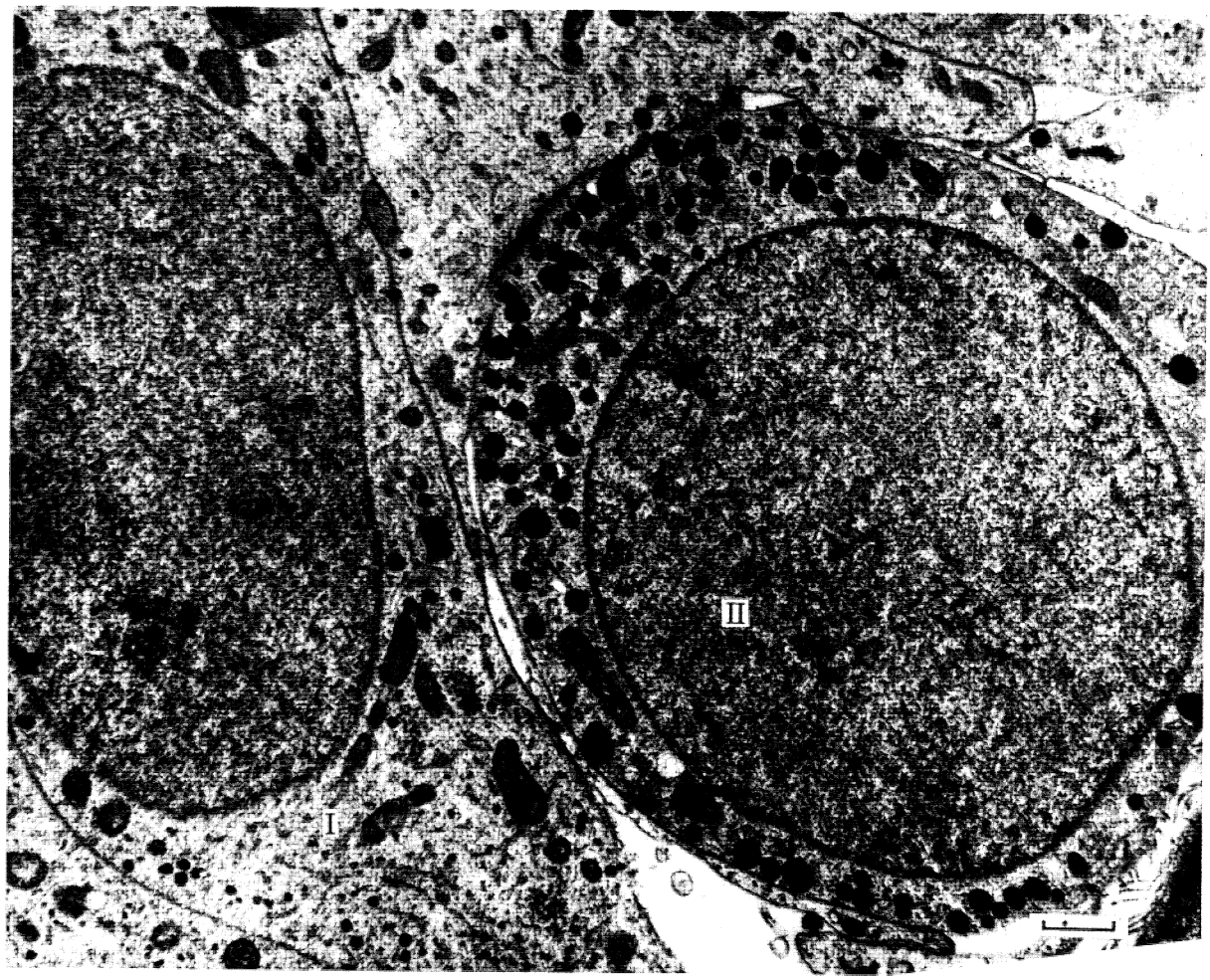
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Oestrogen administration, on the other hand, had no detectable effects on either the space or the bulbous projections of the Type B ependyma of a castrate male (figure 28, plate 73).

Injections of oestrogen to five ovariectomized females however restored the bulbous projections, which had almost disappeared after gonadectomy, to a size more like that of the mid-cycle condition (figure 23, plate 72, inset). The slight space which seemed to result from ovariectomy remained, but was less evident in the oestrogen-injected animals.

At the level of ultrastructure the bulbous projections, which formed as a result of oestrogen administration, were very granular but contained no evident electron-dense granules (figure 23, plate 72); in these respects they resembled the pre-ovulatory condition. The cytoplasm also resembled the normal pre-ovulatory state, though with less vacuolation.

PART II. THE PARS TUBERALIS

1. Introduction

Little is known about the structure and function of the pars tuberalis despite its close embryological relationship with the remainder of the adenohypophysis, and their intimate topographical relationship. Szentágothai *et al.* (1968) have pointed out that in some mammals blood supplying the adenohypophysis first irrigates the pars tuberalis.

In our preliminary studies on the hypothalamus of the rhesus monkey attention was drawn to the proximity of the terminations of the Type B ependymal processes to the cells of the pars tuberalis (Anand Kumar & Knowles 1967). Further studies were therefore undertaken to discover whether changes in the Type B tanycyte ependyma, in relation to reproduction, were paralleled by corresponding alterations in cells of the pars tuberalis.

2. The structure of the pars tuberalis

(*a*) *Structure*

Viewed by the optical microscope the pars tuberalis of the rhesus monkey showed strong basophilia. Using the performic acid-Alcian blue, PAS, Orange G method two types of cells could be distinguished—a predominant cell type stained by PAS and a less frequent cell type stained by Alcian blue. The general disposition of the cells, arranged as they are in cords around blood capillaries, resembled that which has been described for other species, and is similar to that seen in the pars distalis.

At the level of ultrastructure three types of cells could be distinguished (plate 74), by the size of the granules they contain as well as by some other features. Two of these, Types I and II, constituted the main body of the pars tuberalis; and probably represent those cells distinguished by light microscopy; their cytoplasmic constituents denoted synthetic activity; they were distinguished from one another by the size of their electron-dense granules.

Description of plate 74

Figure 29. A portion of the pars tuberalis in a female rhesus monkey in the pre-ovulatory phase of the menstrual cycle. Type I (I) and Type III cells (III) may be distinguished.

Figure 30. As figure 29, but showing Type I (I) and Type II (II) cells. Scales = 1 μm.

The more numerous cell type (I) contained the smaller granules, measuring *ca.* 170 nm in diameter; these granules were uniformly electron-dense and were membrane-bound. Other cytoplasmic contents included mitochondria, lysosomes and a very characteristic compact endoplasmic reticulum which consisted of a small number of parallel cisternae. These cells were in intimate contact with one another, and indeed frequently one cell appeared partially to envelop another. It seems likely that Type I cells represent the PAS-positive cells seen by light microscopy.

The other, less frequent, cell type (II) contained many electron-dense membrane-bound granules, measuring *ca.* 350 nm in diameter, evenly dispersed throughout the cell. The endoplasmic reticulum was likewise evenly dispersed and irregular in form (figure 30, plate 74). Mitochondria and occasional lysosomes were also present. This second type of pars tuberalis cell was less tightly packed than the former; intercellular spaces were common. Probably this cell type is that which stained by Alcian blue.

The third type of pars tuberalis cell (III) showed a deeply indented nucleus surrounded by little cytoplasm, in which a few granules were occasionally seen. These cells were generally separated from one another by narrow spaces into which their cell membranes protruded finger-like projections. The tinctorial affinities of this cell-type have not been determined.

(*b*) *Innervation* (figure 2)

Fibres, containing fibrillae and clusters of electron-lucent and some membrane-bound electron-dense vesicles, *ca.* 70 nm in diameter, were commonly found between the cells of the pars tuberalis. Metuzals (1956, 1959) has described a direct innervation of some pars tuberalis cells (Type I) by nerve fibres emerging from the median eminence, and its seems likely that many of the fibres forming neuroglandular junctions in the pars tuberalis of the rhesus have a similar origin and destination and represent a form of neurosecretion, possibly Type B or aminergic neurosecretion (Knowles & Bern 1966). Szenthágothai *et al.* (1968) have described fibres of this type, that terminate in the median eminence, arising from cells of the arcuate and adjacent hypothalamic nuclei. The origin of the nerve fibres innervating pars tuberalis cells was not determined in the present studies.

(*c*) *Tanycyte-gland junctions* (figure 2)

Occasionally a second type of contact between tanycyte terminals and pars tuberalis (Type I) cells was seen. This could be distinguished from the neurosecretory innervation by the following features:

(*a*) The fibrillae continued almost to the point of contact.

(*b*) The characteristic compact and densely staining mitochondria found elsewhere in the tanycyte terminals were present here also.

(*c*) The electron-dense granules were larger, *ca.* 100 nm in diameter.

(*d*) The relation between the tanycyte terminal and the pars tuberalis cell was usually intimate, and often the terminal was almost completely enveloped by the gland cell, but their cell membranes remained separated by a uniform space *ca.* 15 to 20 nm wide.

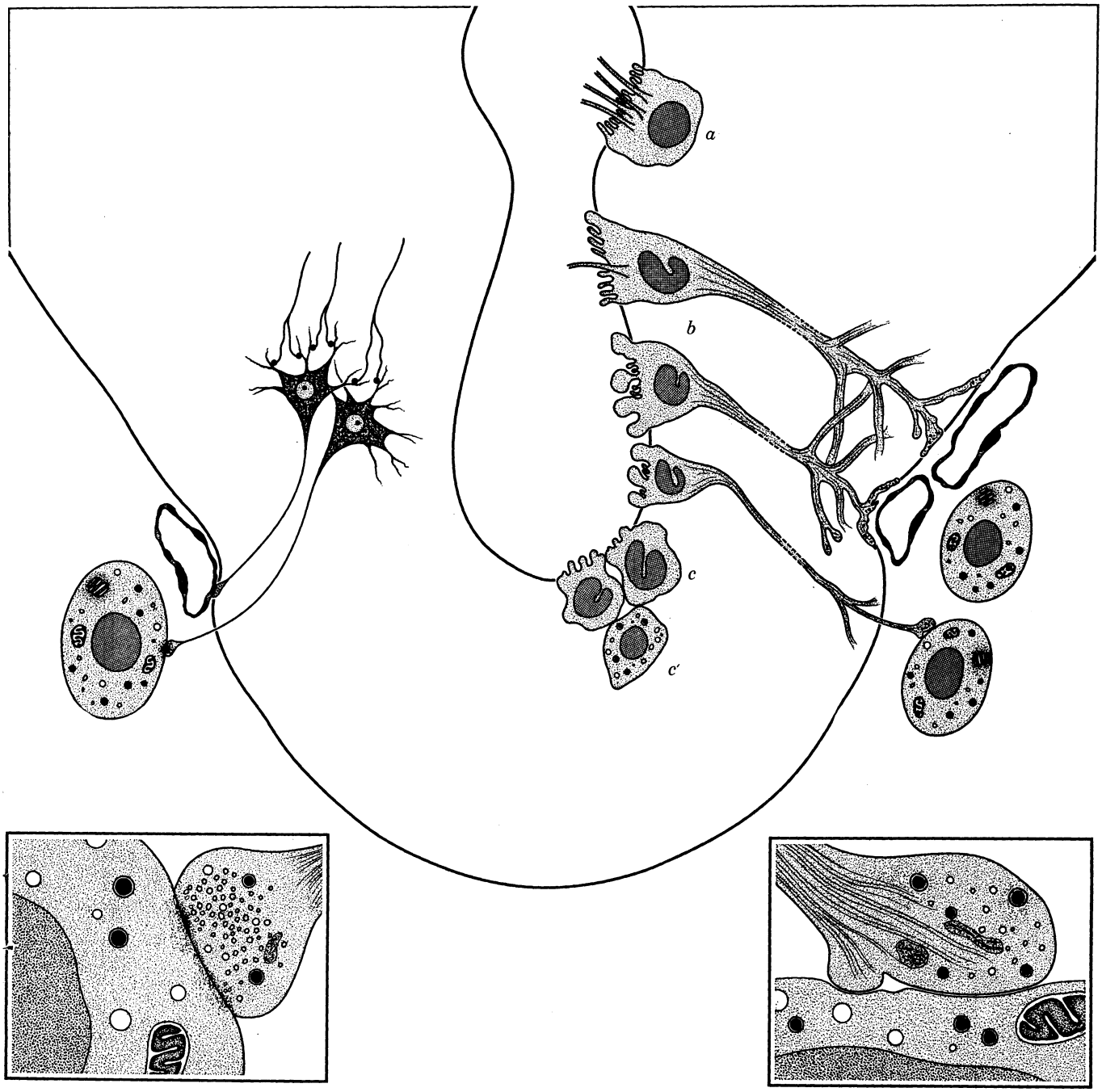

FIGURE 2. The relationship of neural and specialized ependymal elements to cells of the pars tuberalis, shown semi-diagrammatically. *Left*: two neurosecretory neurons which are related to the pituitary or its blood supply; one makes a direct synaptoid contact with a cell of the pars tuberalis—the other terminates in close proximity to a portal vessel. *Right*: various types of ependymal cells described in the text (this drawing should be compared with plates 63 to 65). Some of the Type B tanycyte cells (*b*) terminate in close relation to portal blood vessels—others appear to make direct contacts with cells of the pars tuberalis. The lower inset pictures (drawn from electron micrographs) contrast the type of junction made between neurosecretory fibres and pars tuberalis cells (left), or tanycyte ependymal cells and pars tuberalis cells (right).

3. Changes in relation to reproduction

(*a*) *The menstrual cycle*

At the time of menstruation the Type I cells of the pars tuberalis show indications of hypersecretion, namely a widening of the cisternae of the endoplasmic reticulum, vacuolation of the cytoplasm, distended mitochondria, lysosome activity and an abundance of moderately electron-dense granules (figure 32, plate 75).

During the pre-ovulatory and post-ovulatory phases a very different picture was seen (figure 31, plate 75). The endoplasmic reticulum was compact and inconspicuous, and there were few electron-dense granules and lysosomes. The mitochondria were electron-dense and almost spherical, and the general indications were that of a relatively quiescent cell.

There were therefore clear signs that Type I cells altered in relation to the menstrual cycle (figure 3). No such changes were detectable in either Type II or Type III cells.

(*b*) *Ovariectomy*

Following ovariectomy the cytoplasm of the Type I cells showed more pronounced vacuolation than in normal intact animals (figure 33, plate 76). There were indications of considerable lysosome activity.

(*c*) *Injection experiments*

In the ovariectomized animals given exogenous oestrogen the cytoplasmic vacuolation, characteristic of castrates, was no longer apparent (figure 34, plate 76). Instead the pars tuberalis Type I cells resembled those of intact animals killed at the pre-ovulatory stages of the cycle, with few lysosomes.

4. Discussion

The discovery that in the rhesus monkey, a cellular link between the III ventricle and blood vessels in the median eminence alters in relation to reproductive function, both under normal and under experimental conditions, extends our concept of the neuroendocrine control of pituitary activity. Previously a possible relationship between ependymal cells lining the III ventricle and pituitary activity has appeared in observations made by Löfgren (1960), Leveque *et al.* (1967) and Wittkowski (1967), and Hagedoorn (1965) has remarked that ependyma in the III ventricle of the skunk *Mephitis* alters in relation to the reproductive cycle. These authors however were not able to demonstrate that the changes they noted were meaningful in terms of pituitary function.

The present studies were based on the observations made by Knowles & Vollrath (1966), who, after demonstrating that certain ependyma lining the floor of the infundibular recess of the eel were innervated by neurosecretory fibres and appeared to alter in relation to pars nervosa activity, suggested that these ependyma might form a link in a feedback control of pars nervosa function by secreting into the c.s.f. The alternative possibility, that specialized ependyma might detect substances in the c.s.f. and subsequently have a neuroendocrine action was also postulated (Knowles 1967, 1969), and has now been tested in the present investigation which has shown that when the level of oestrogen production by the gonads is high certain tanycyte ependyma show a dilation of the plasma membrane, which might indicate absorption, and also signs of increased synthetic activity. It is relevant to note that Anand Kumar & Thomas (1968) have shown that tritiated oestradiol, injected intramuscularly into the rhesus monkey may

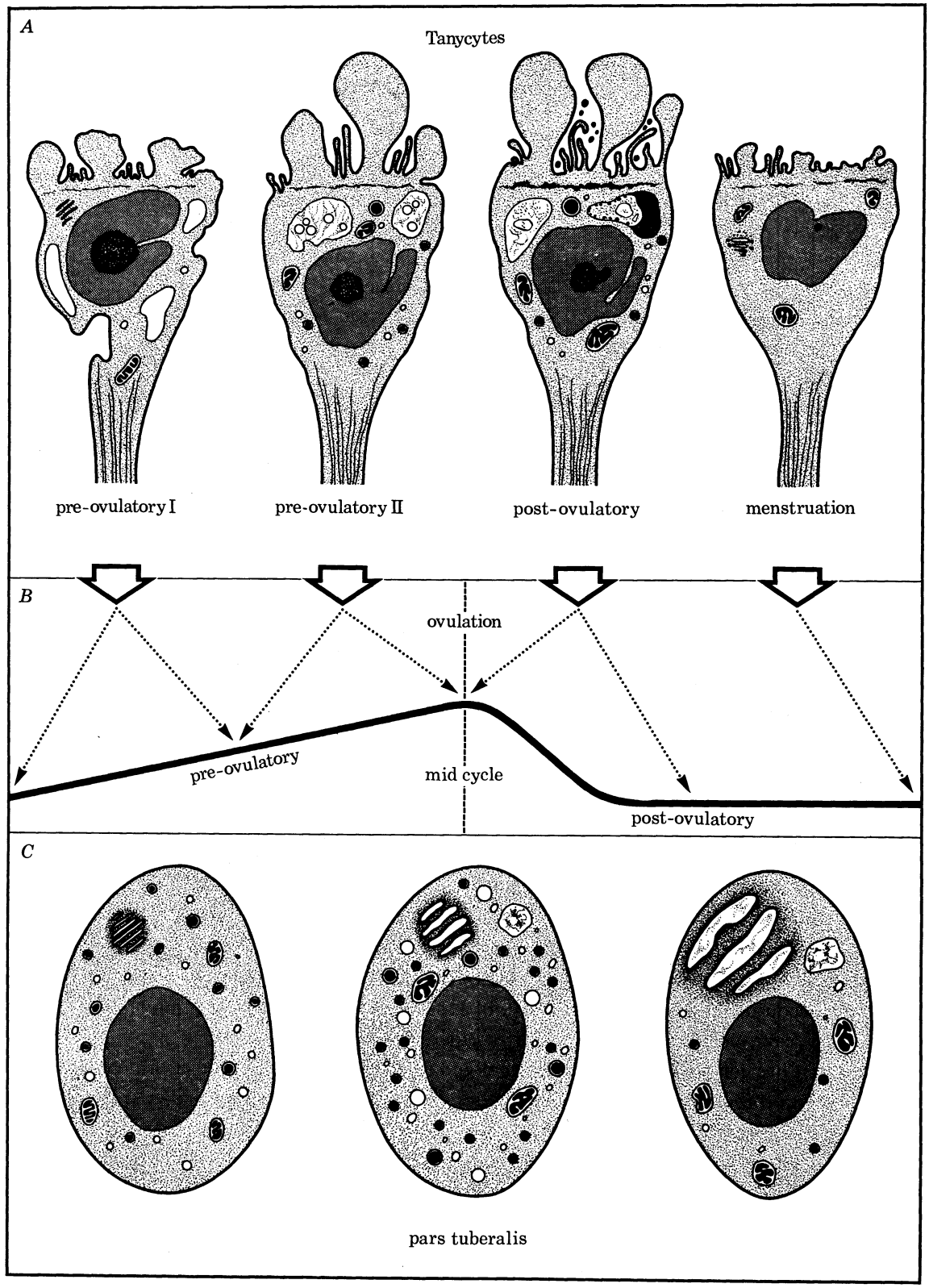

FIGURE 3. Changes in Type B tanycyte cells and pars tuberalis cells in relation to different stages of the menstrual cycle of the rhesus monkey. Simplified drawings of electron micrographs (*A* and *C*) are shown in relation to a curve (*B*), based on evidence from many sources, depicting the probable variation in the amounts of circulating oestrogen before and after ovulation.

be detected in the c.s.f. some hours later. It is therefore reasonable to postulate that a rise in oestrogen circulating in the blood would be followed by an increased oestrogen concentration in the c.s.f. and that changes in the tanycytes may denote a response to this rise in c.s.f. oestrogen level.

It could be argued that changes in the tanycytes in response to oestrogen level might have no special significance as the metabolism-stimulating potency of oestrogen is well established. If this indeed were the explanation, however, one might look for alterations in all the ependymal cells of the III ventricle in relation to the presence or absence of oestrogen in the body fluids, but in fact it was the Type B tanycytes, and not neighbouring ependymal cells, which showed changes in relation to gonadectomy and injections of oestrogen. It is, moreover, noteworthy that not only do the alterations in the tanycytes in relation to the normal reproductive cycle indicate that they are related to reproductive activity, but the experimental techniques so far employed have fulfilled the generally accepted criteria for determining endocrine relationships, i.e. extirpation of the source of hormones (the gonads) followed by replacement therapy (by gonadal hormones) in relation to the supposed target tissues (the tanycytes).

It is also of considerable interest that the Type B tanycytes differ in appearance in the two sexes, and that preliminary experiments have shown that they react differently in males and females to an experimentally induced absence or presence of gonadal hormones. Taken together these facts argue in favour of a role of Type B tanycytes in pituitary-gonad feedback control, maybe as receptors sensitive to the level of oestrogen in the c.s.f.

A possibility that Type B ependymal cells may secrete into the c.s.f. cannot be discounted, but the abundance of inclusions at their distal terminals, and the proximity of those to the blood-vessels of the median eminence and cells of the pars tuberalis denote rather a distal secretion, in the direction of pituitary tissues. Secretion in both directions, into the III ventricle and also the pituitary vessels should also be considered as a possibility during this preliminary stage of study of the Type B tanycytes, in which evidence of structure pointing to their implication in pituitary feedback control is considerable, but their precise role in terms of function is not yet clear. The balance of evidence, however, favours a concept that the Type B tanycytes absorb from the c.s.f. and secrete into the blood-vessels of the median eminence.

Also the precise nature of the part played by the pars tuberalis in the reproductive cycles is not yet evident, though its alterations in structure during these cycles indicate that it may be concerned in the control of reproductive function. A possibility that some pars tuberalis cells may produce hormones with direct action on the gonads cannot be excluded and this should be taken into account when assessing the results of experiments involving the section of the pituitary stalk or the severance of the pituitary portal blood-vessels.

Hitherto it has generally been assumed that gonadal hormones might affect pituitary activity either by (*a*) a direct action on pituitary tissues or (*b*) an action on hypothalamic centres and subsequent neuronal or neurosecretory action on the pituitary. The present studies on the

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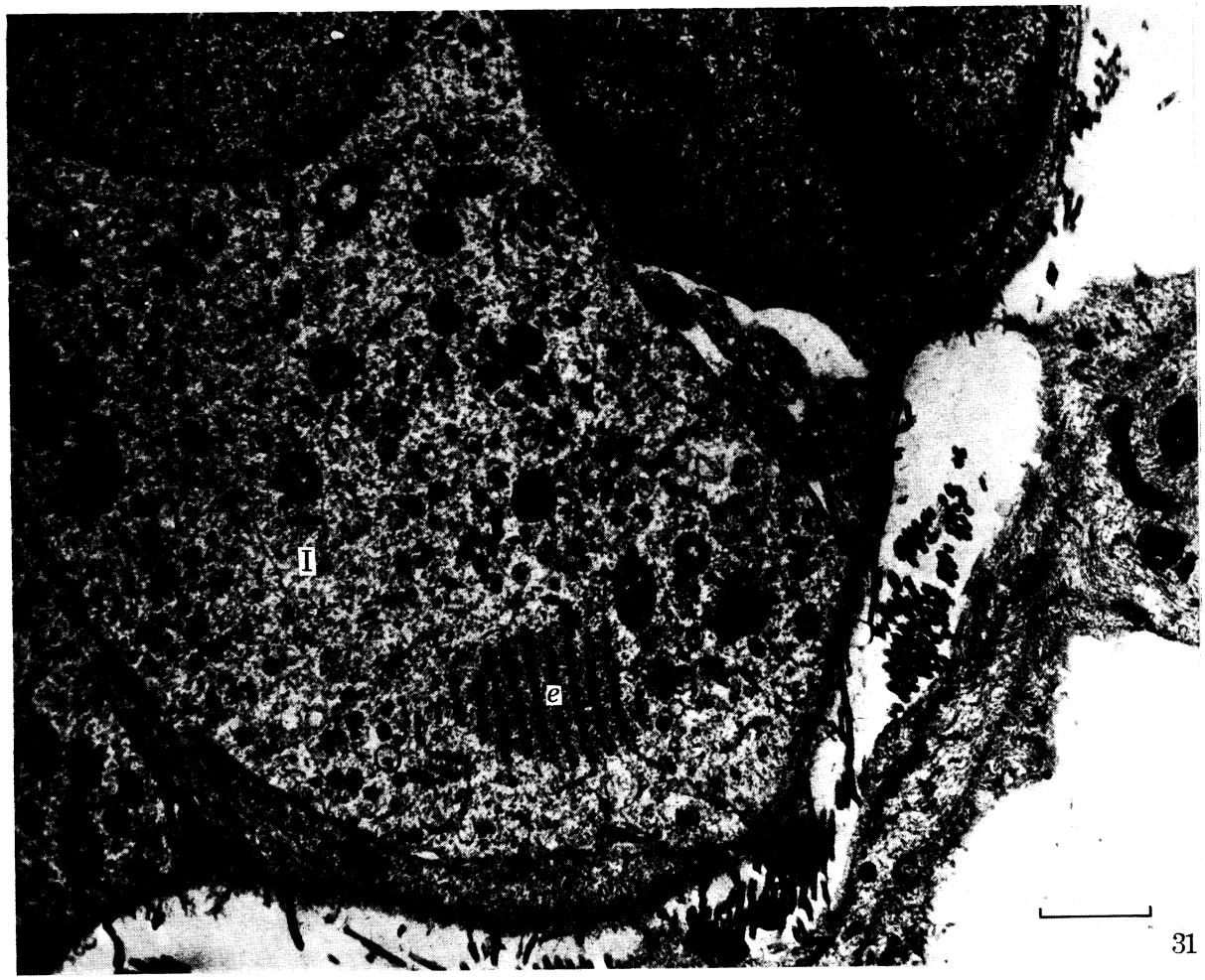
Description of plate 75

Figure 31. Cells of the pars tuberalis in a female rhesus monkey killed during the pre-ovulatory phase of the menstrual cycle. I: Type I cell; *e*, compact endoplasmic reticulum.

Figure 32. As figure 29, but from a female rhesus monkey killed during menstruation. Vacuoles (*v.*), a distended endoplasmic reticulum (*e.*) and lysosomes (*l.*) characterize this phase of the cycle. Scales = 1 μm.



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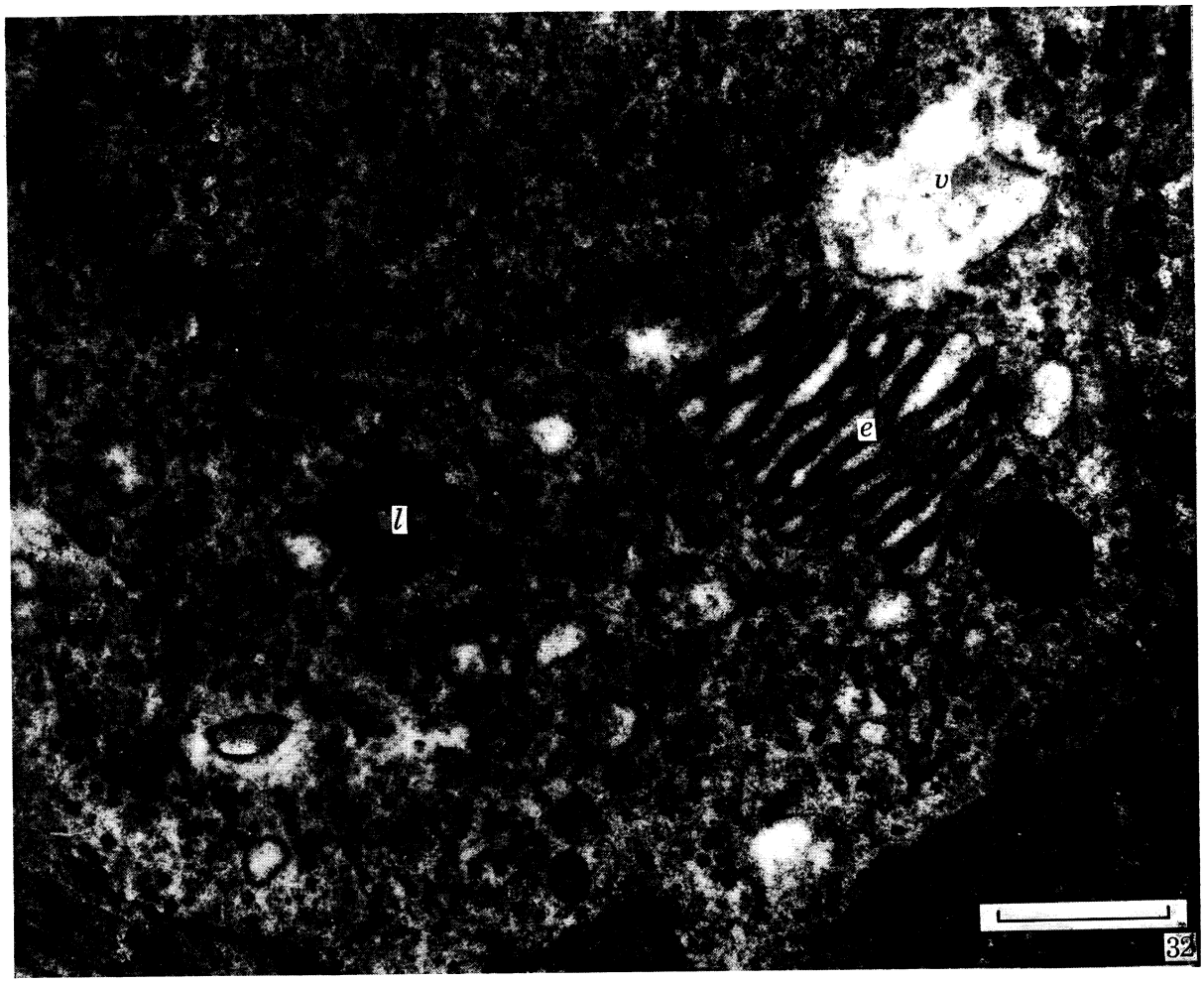

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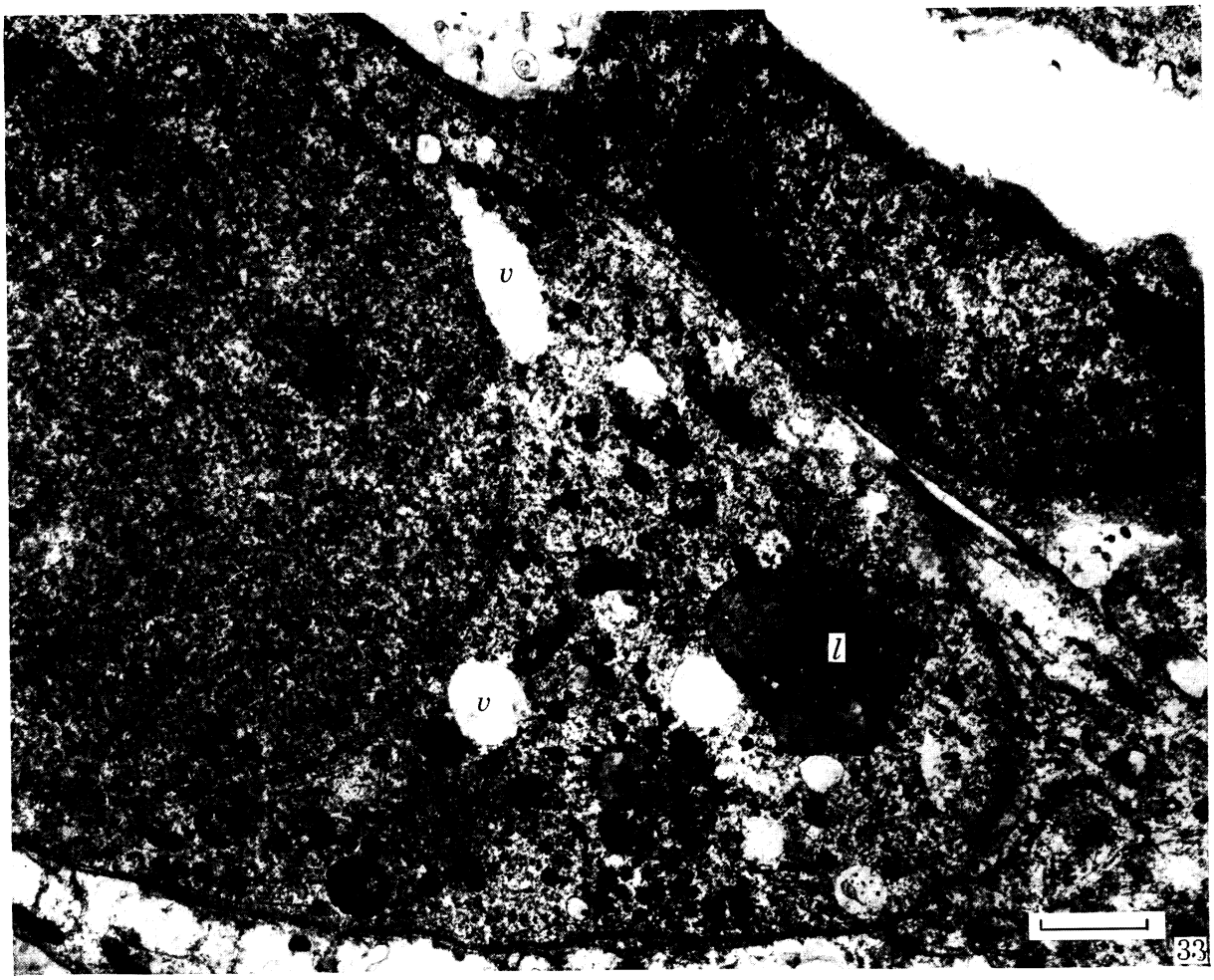
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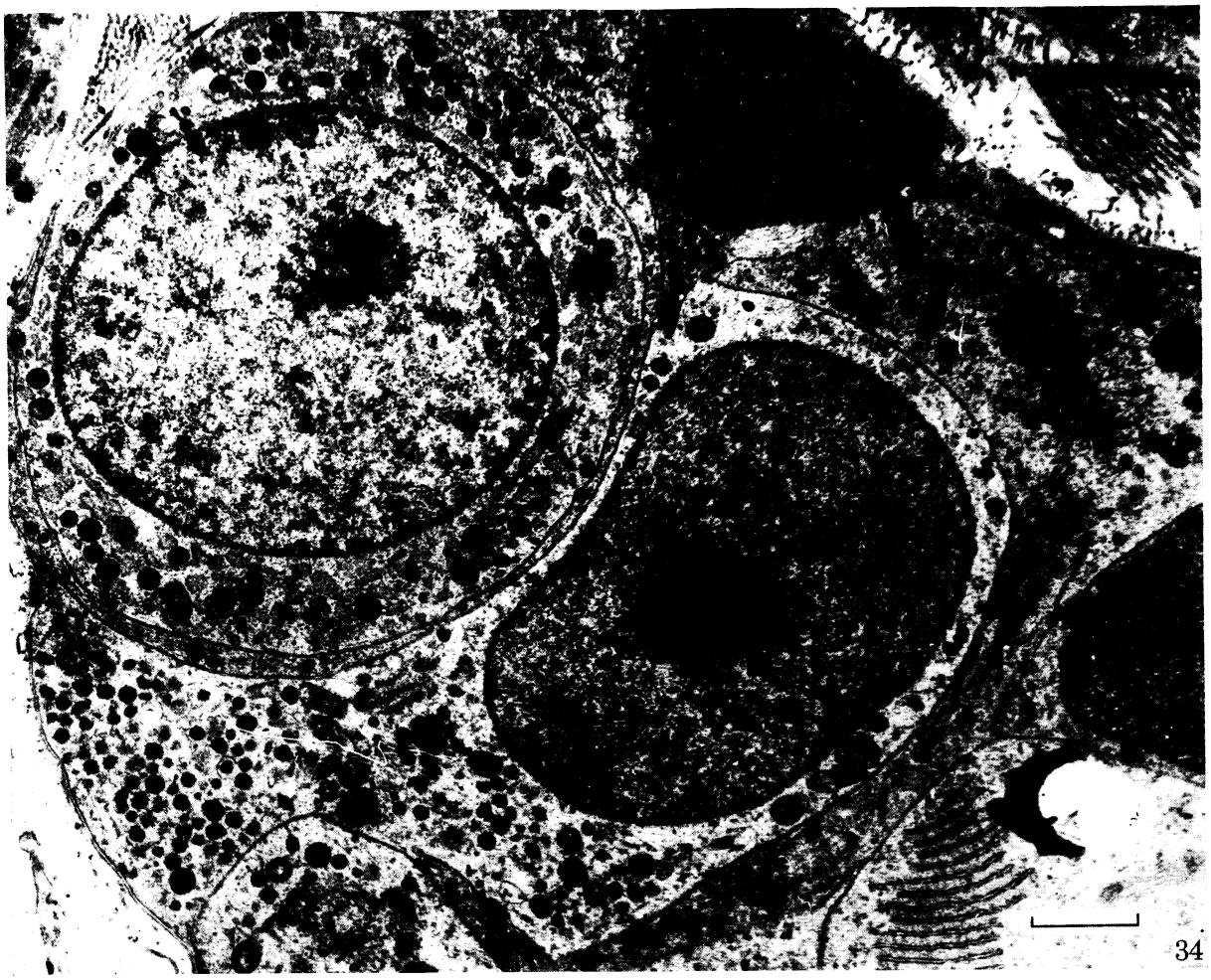
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rhesus monkey have indicated a third possibility, namely that modified ependymal elements might detect oestrogen in the c.s.f. and subsequently secrete some substance affecting pituitary function. The proposal that this ependymal pathway may exist is, however, not intended to diminish the importance of the well-documented evidence for the role of neurosecretory neurons originating in hypothalamic nuclei, whether peptidergic or aminergic (Knowles 1965; Bargmann, Linder & Andres 1967), in the control of pituitary function.

In their experiments in which they transplanted anterior pituitary tissue into different regions of the hypothalamus Halász *et al.* (1962) noted that gonadotropic function was maintained in transplants made in a circumscribed area, and remarked that this area contained, in addition to neurosecretory fibres, some ependymal tanycyte processes. These authors suggested that one should not exclude the possibility that tanycyte ependyma might play some part in pituitary control, but that their evidence favoured the arcuate and other hypothalamic nuclei as centres for the control of pituitary function. Conversely, the present studies on the rhesus monkey point to a possible role for tanycyte ependyma in the control of pars distalis function but do not exclude the importance of those monoamine-containing neurosecretory systems which also alter in relation to reproductive activity (Lichtensteiger 1967).

Experimental evidence has indicated that pituitary GTH function is affected by many factors in the external environment, e.g. illumination, smell, nutrition, tactile stimuli (Bissonette 1938; Critchlow 1963; Bruce 1959; Leathem 1962; Greulich 1934), and also by the level of circulating hormones in the blood (Burrows 1949). It is interesting to note that in the present studies on the rhesus monkey the modified ependymal elements described seem to be sensitive to changes in the internal milieu, and that no neuronal contacts, which might directly relate the tanycytes to exteroceptors, were seen. In this respect the tanycytes appear to differ from some neurosecretory systems related to pituitary function.

A distinction between neurosecretory elements and ependymal elements may be less fundamental than might, at first sight, be supposed. A number of structural, functional and cytogenetic resemblances have already been shown (Knowles 1967), including the fact that the perikarya of some neurosecretory systems in lower vertebrates project into the III ventricle and are bathed by the c.s.f. It may be argued indeed whether the tanycytes described in the present studies have more in common with some neurosecretory systems than with 'normal' ependymal cells and whether in fact they should be designated as ependymal cells, or be given some less specific title. Certainly they seem to belong to the group of specialized circumventricular 'organs' and tissues which, though known for many decades have only in recent years attracted the attention which they may merit (see Sterba 1966). Already the term tanycyte ependyma has been used to describe specialized cells with long processes in the lateral walls of the III ventricle (Schachenmayer 1967; Leonhardt 1966; Wittkowski 1967) and the term 'glandular ependyma' to describe certain cells in the floor of the III ventricle (Leveque, Stutinsky, Porte & Stoekel 1966). Therefore, to avoid confusion, the term ependyma has been retained in the present studies to describe the elements studied; it seems likely that the Type B

Description of plate 76

Figure 33. Cells of the pars tuberalis of a female rhesus monkey ovariectomized two months previously. *l*, lysosome; *v*, vacuole.

Figure 34. As figure 33, but from an ovariectomized female given one injection of 5 mg oestradiol. Note that the vacuoles have disappeared, and lysosomes are few or absent. Scales = 1 μm.

cells which alter in relation to reproduction may be homologous with at least some of the tanycyte ependyma described by other workers; the ependyma of the C area may represent that tissue described by Leveque *et al.* (1966) as the prechiasmatic gland.

The main interest of the present investigation lies, however, not in distinctions of terminology but in the demonstration that a cellular link between the III ventricle and the pituitary differs in the sexes and alters in relation to reproductive activity. The nature and precise function of the tanycytes, and also the part which the pars tuberalis may play, are far from clear. Nevertheless, the present evidence suggests that they and the cerebrospinal fluid may be implicated in some way in gonad-pituitary feedback control. Thus the suggestion that the cerebrospinal fluid may play an integral part in the endocrine system receives support, and possible new horizons in neuroendocrinology are indicated.

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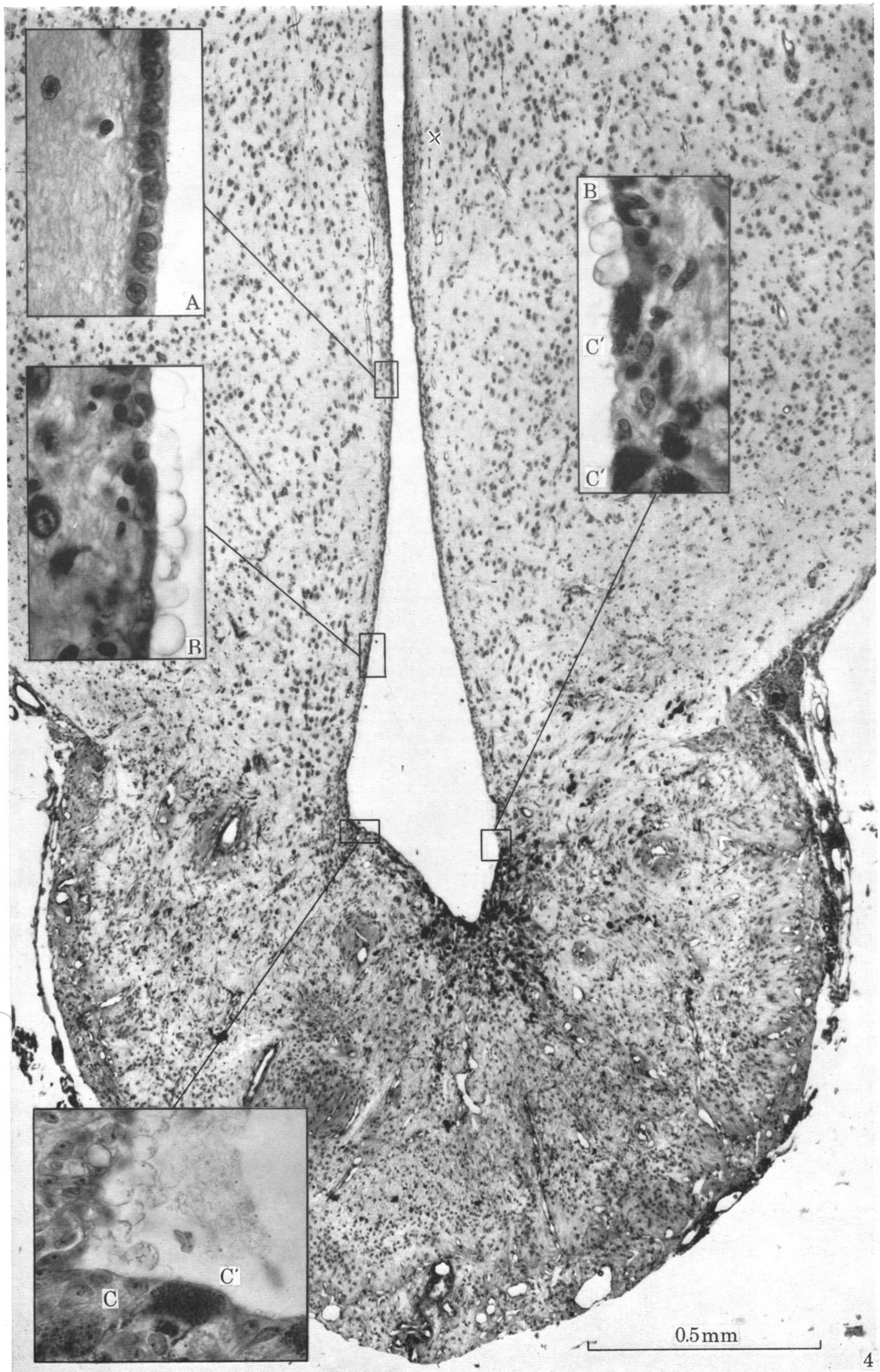
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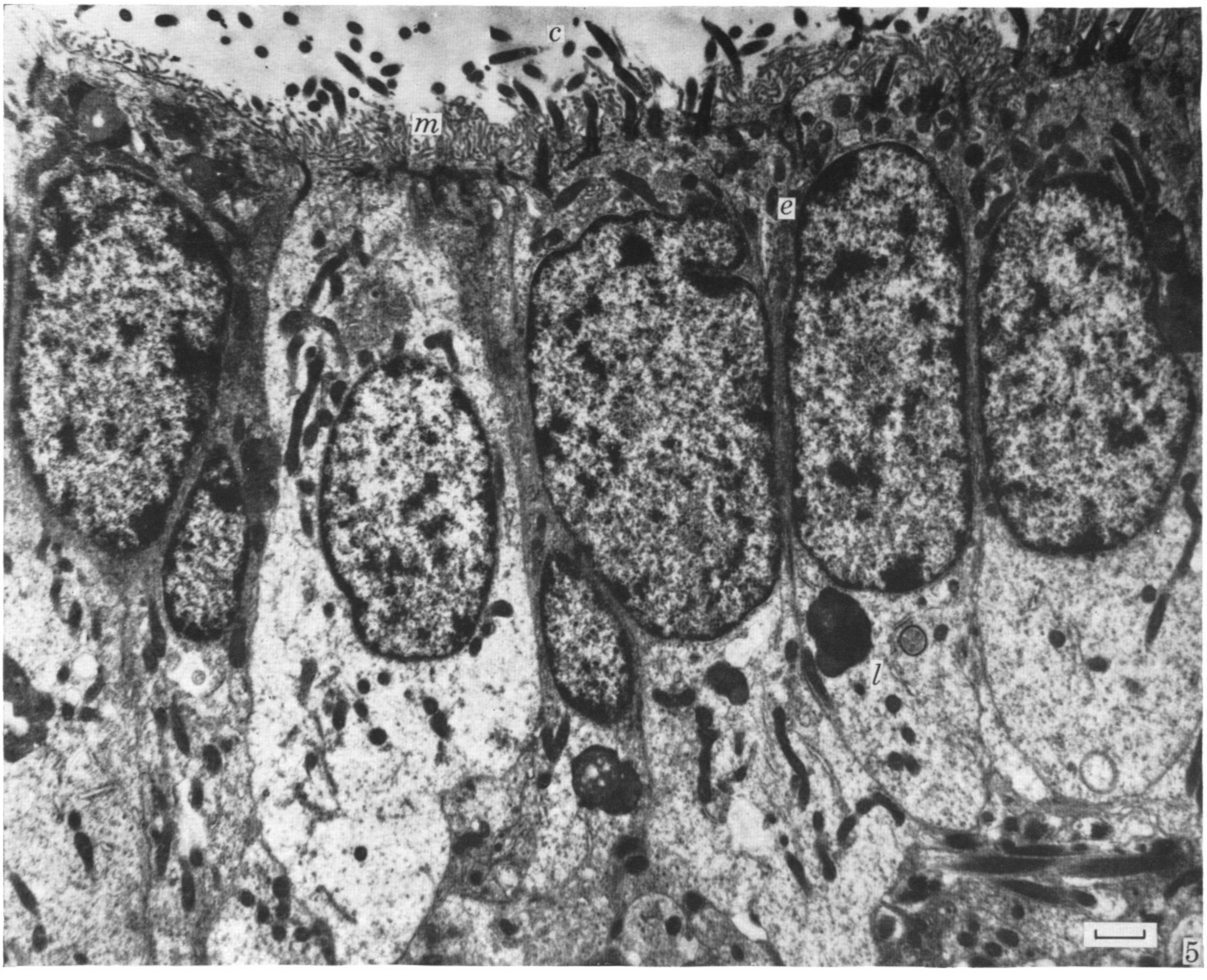

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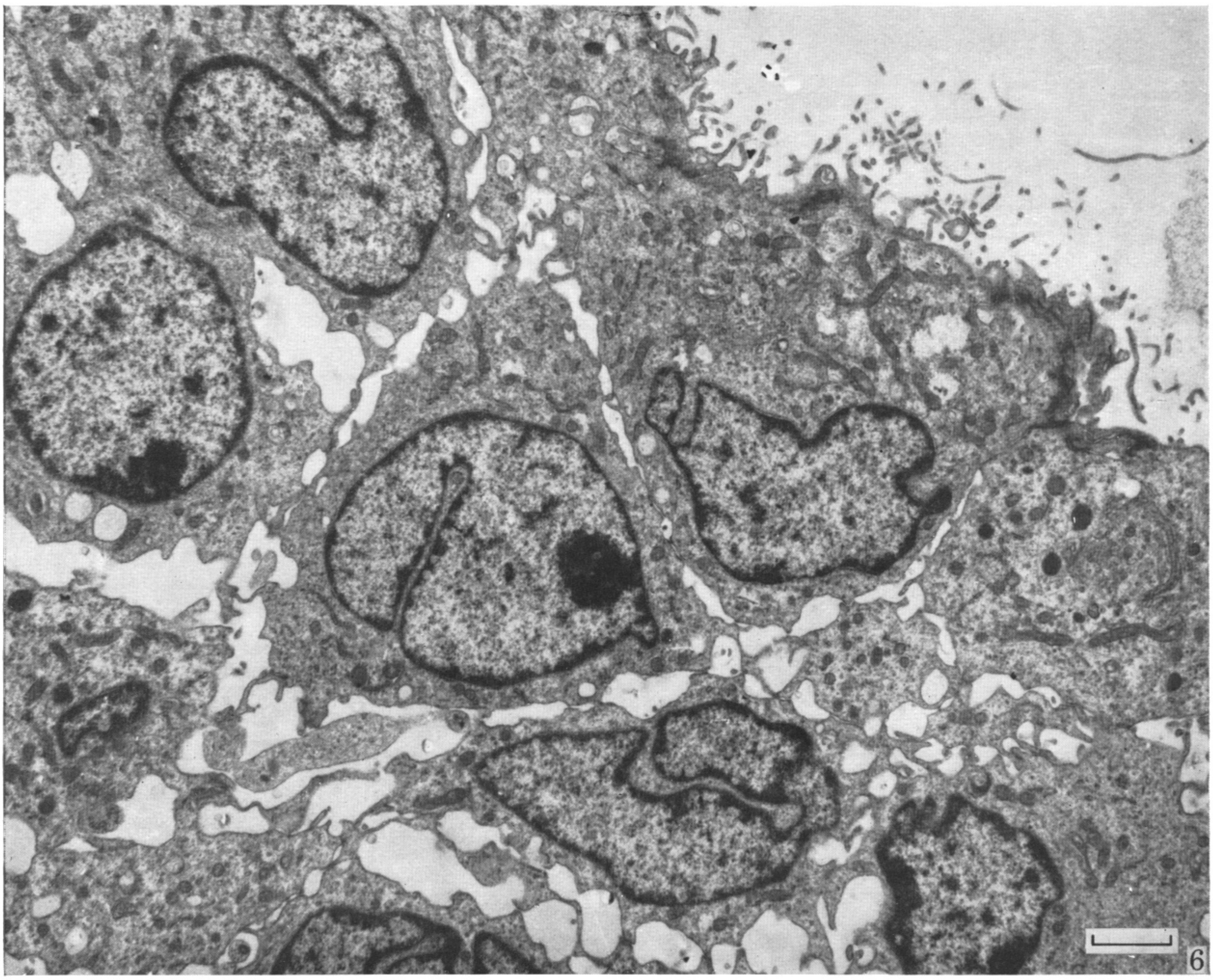

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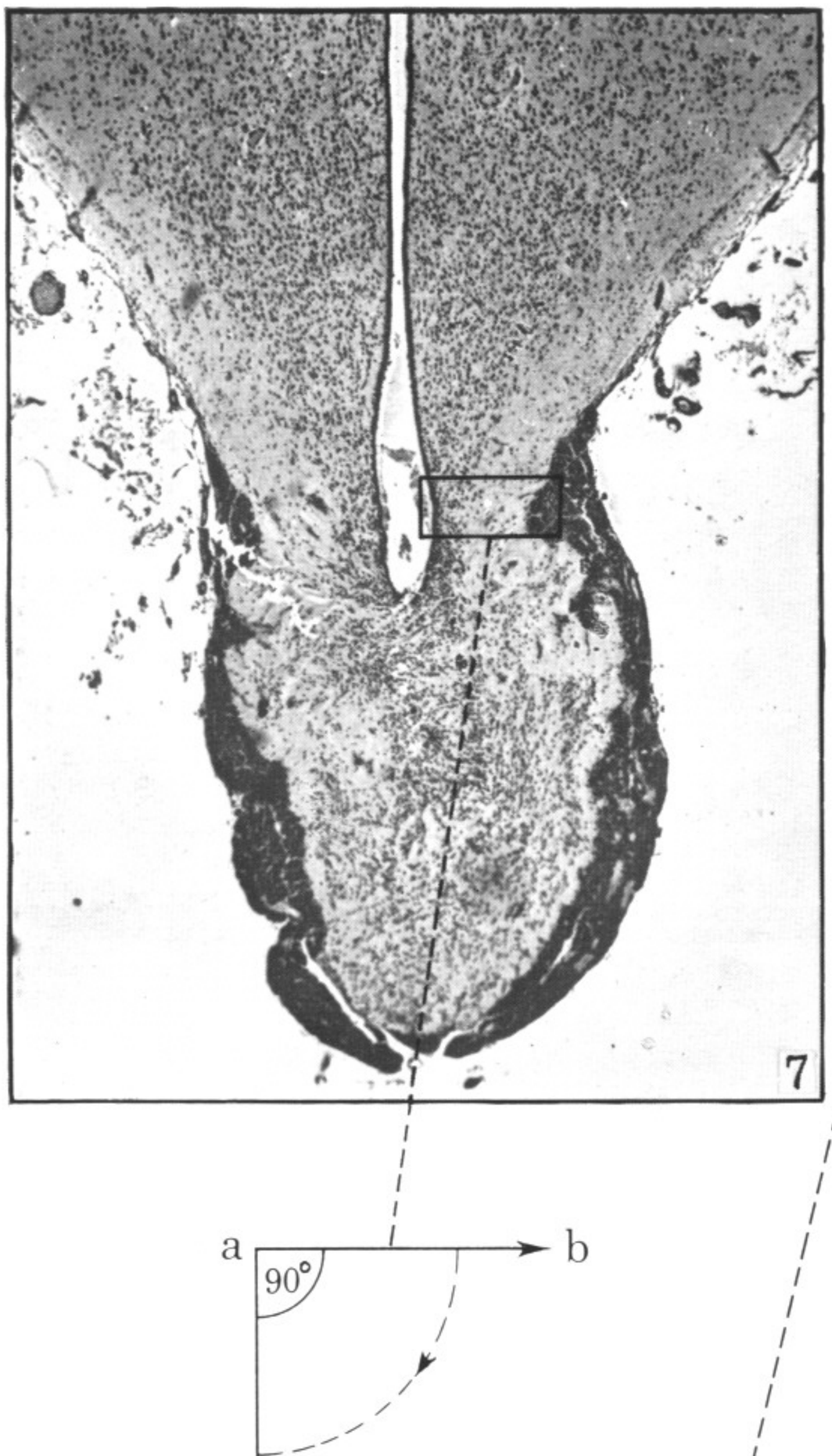

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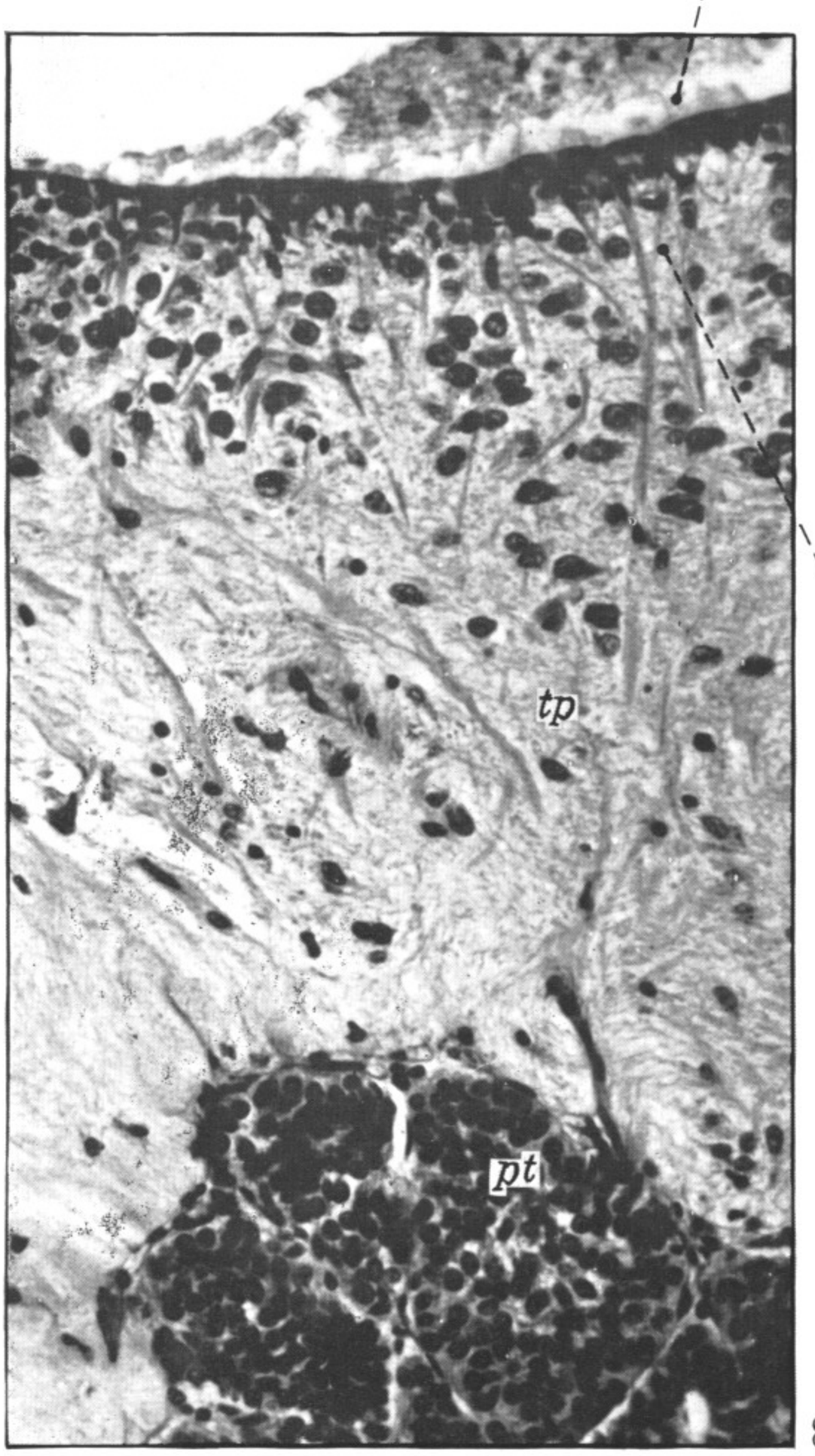

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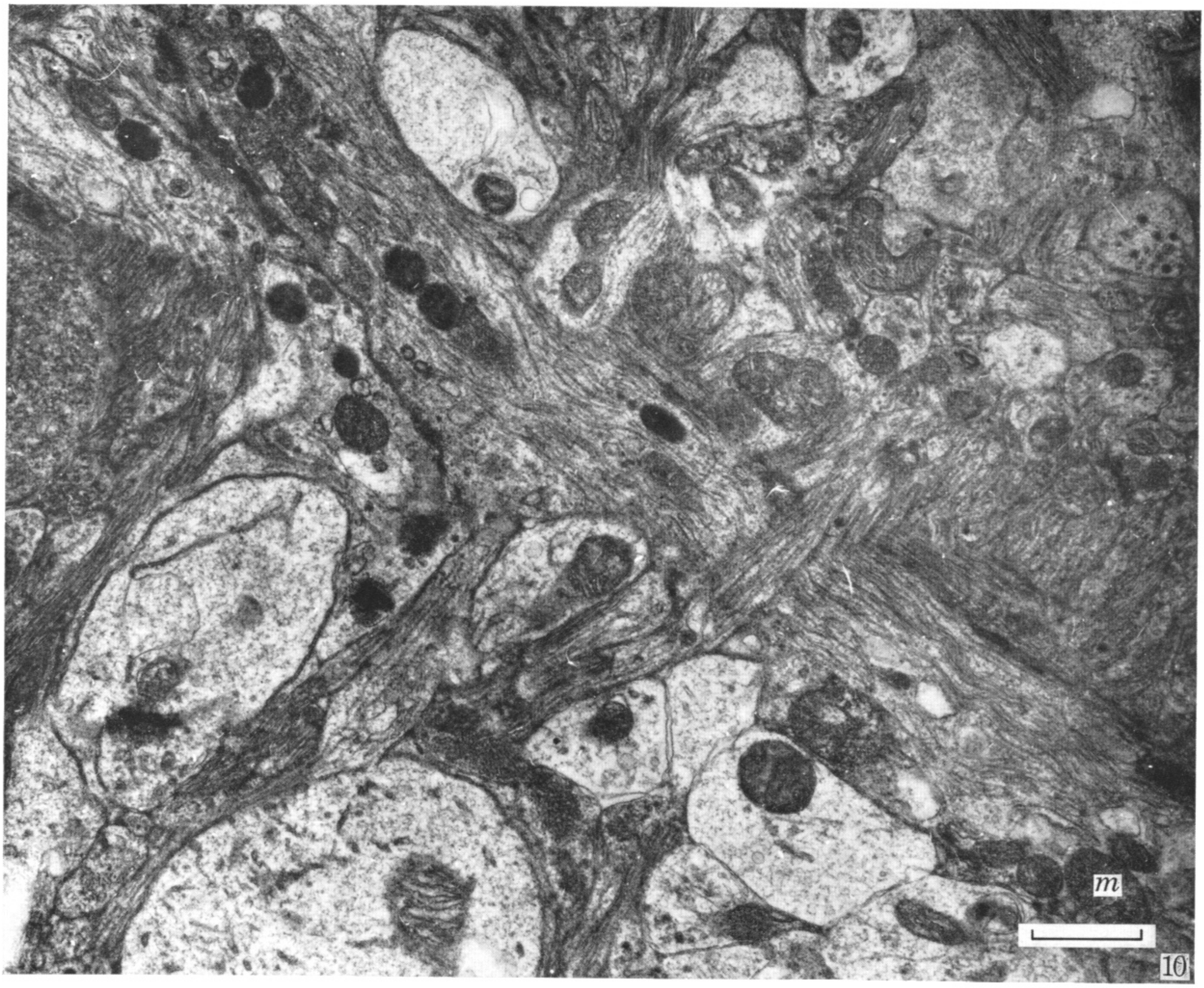

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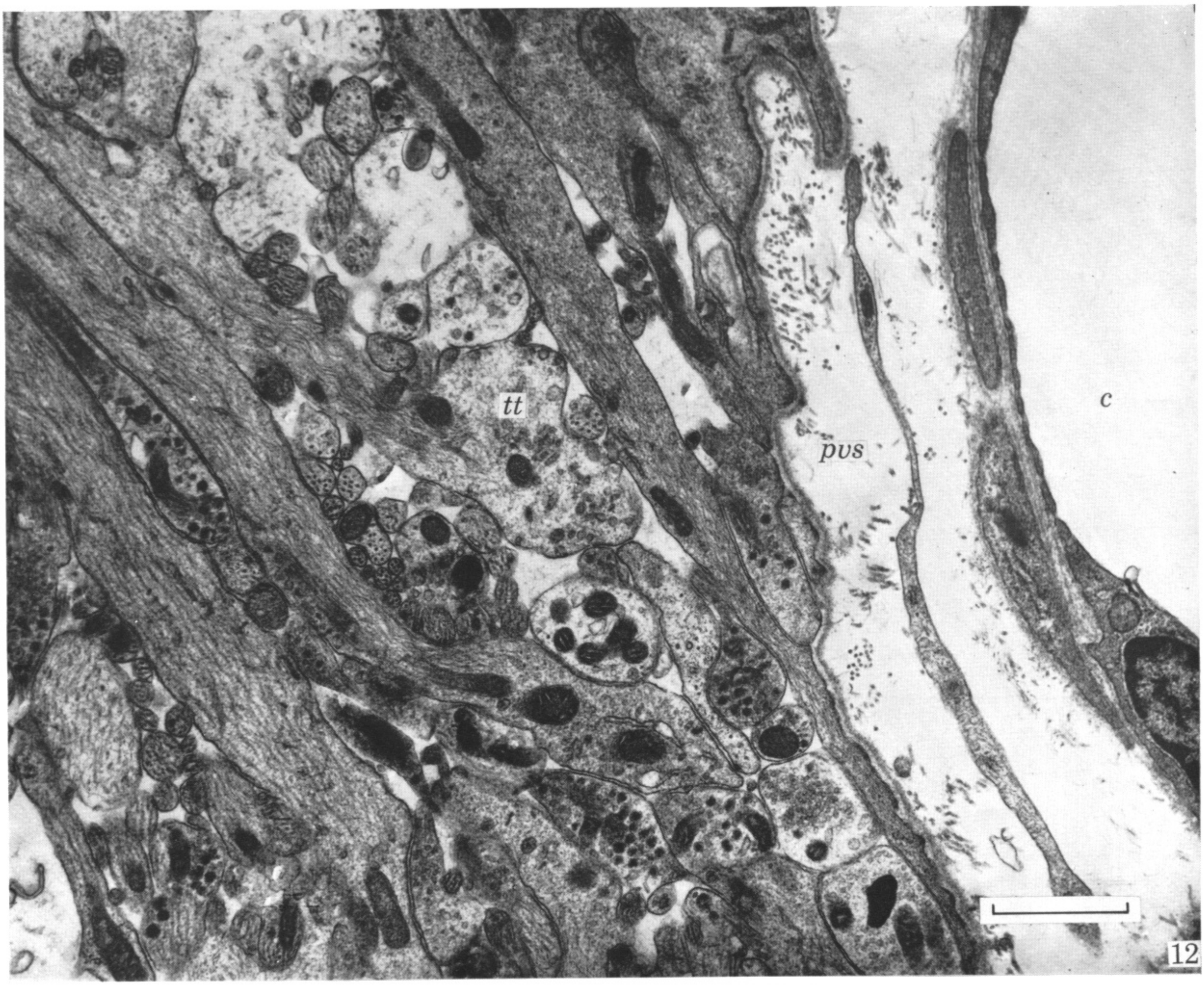
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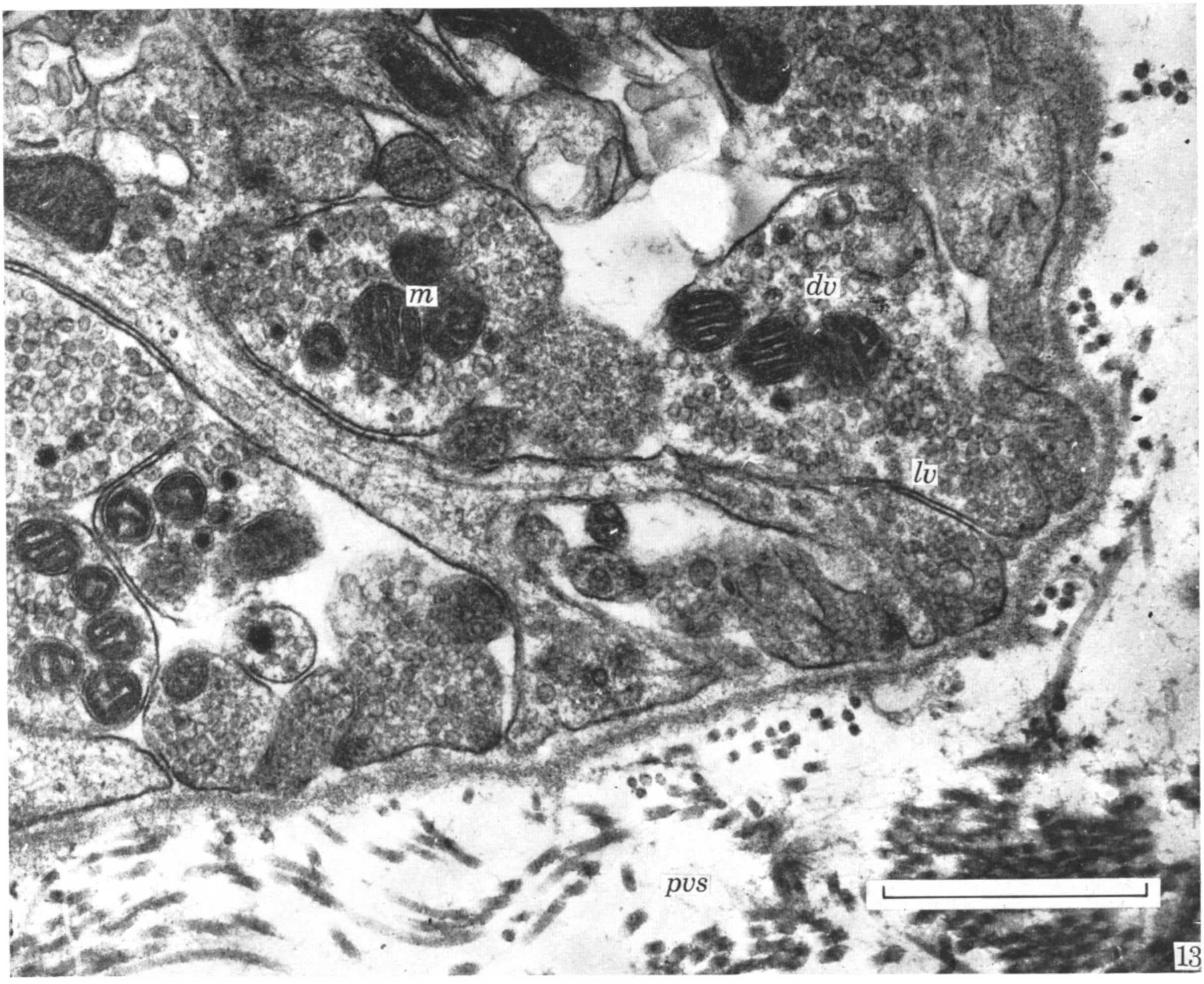
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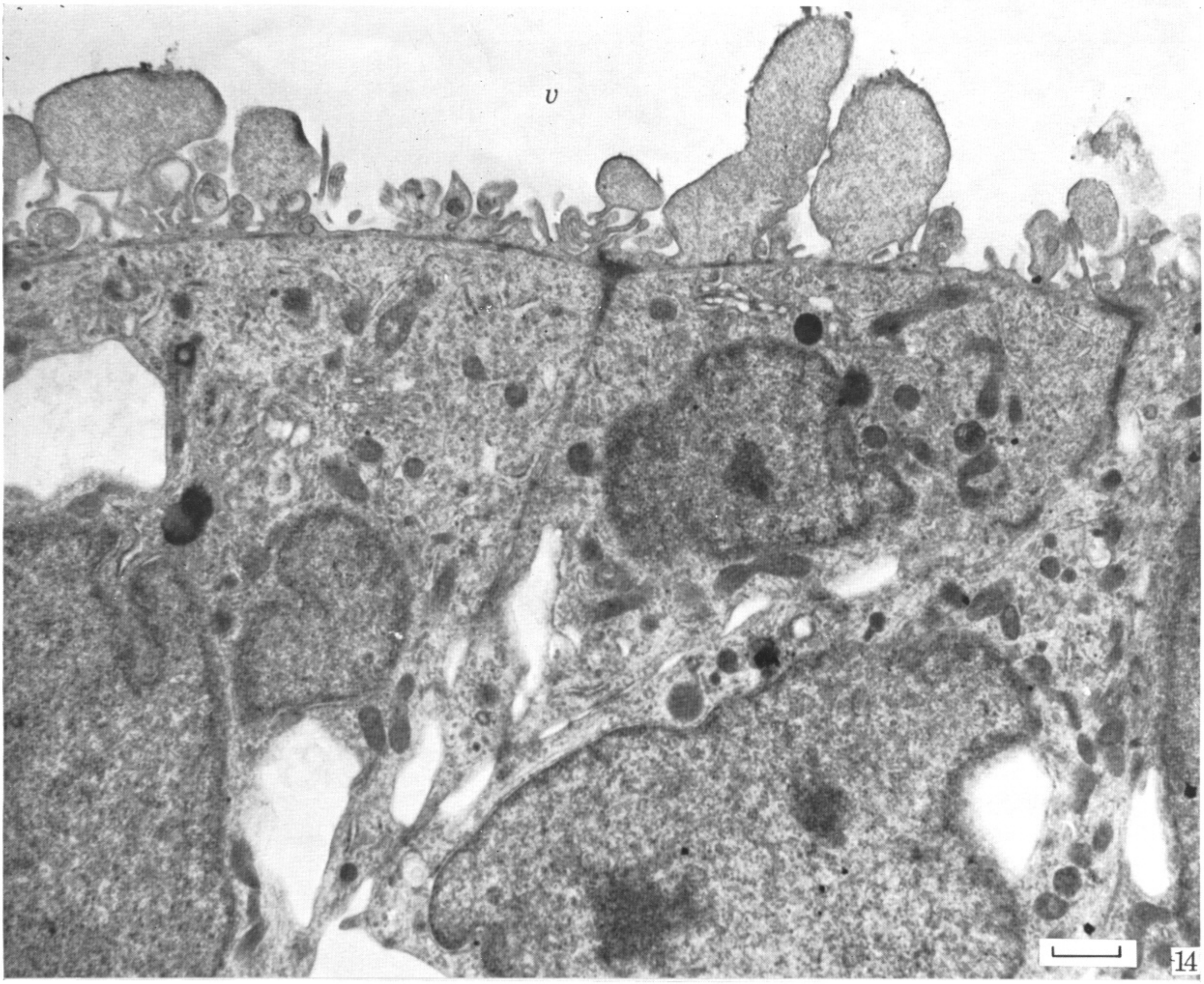

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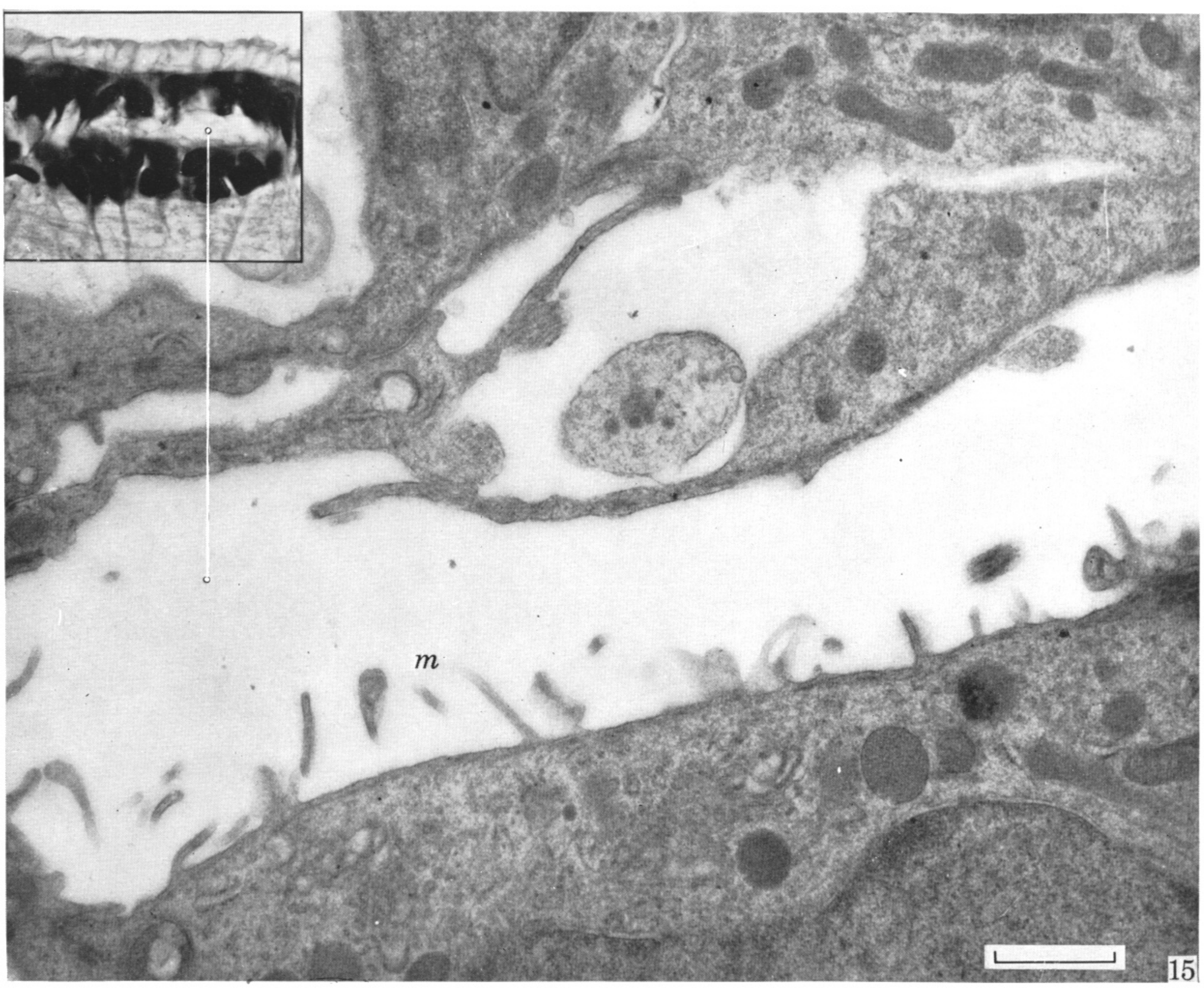

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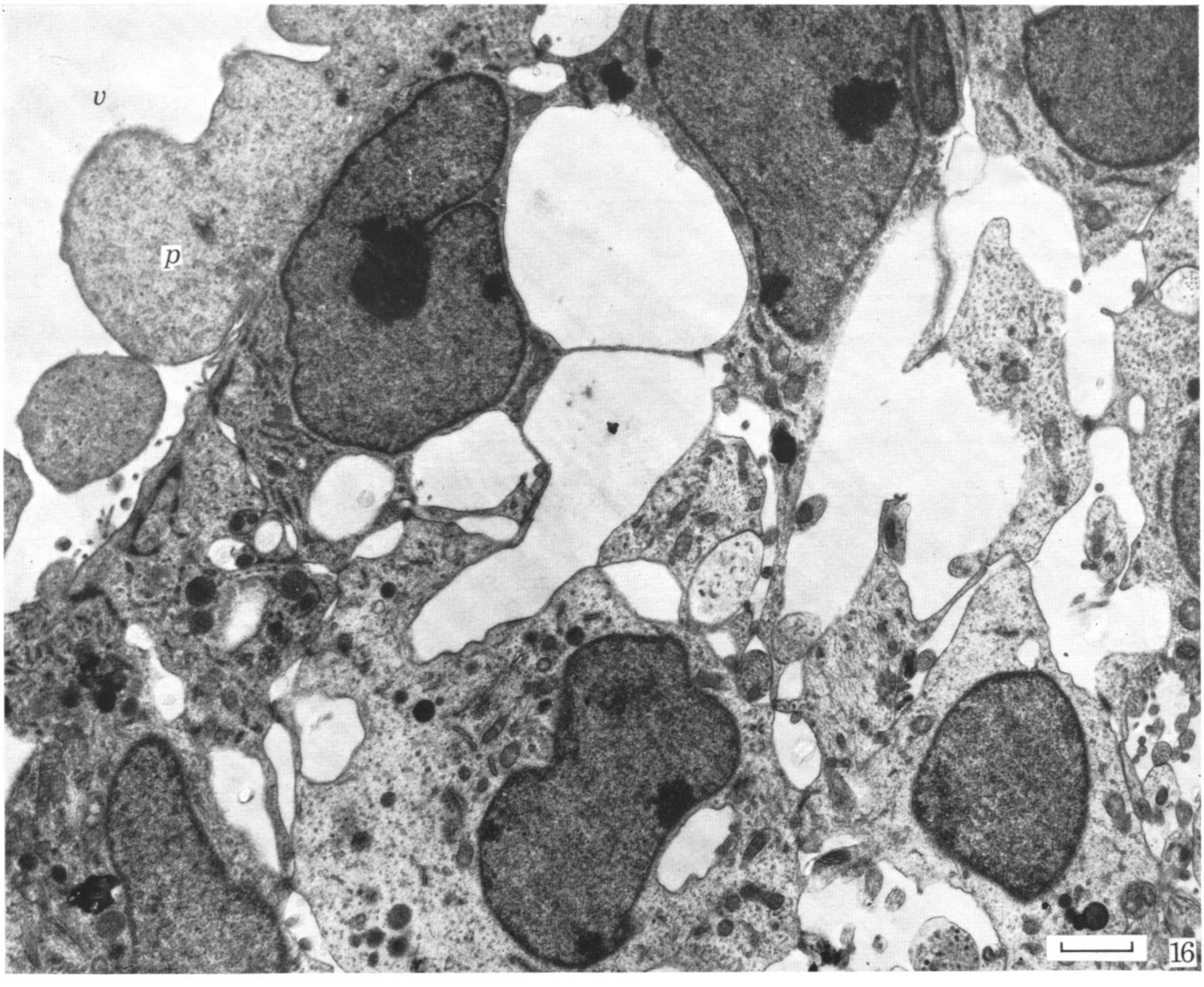

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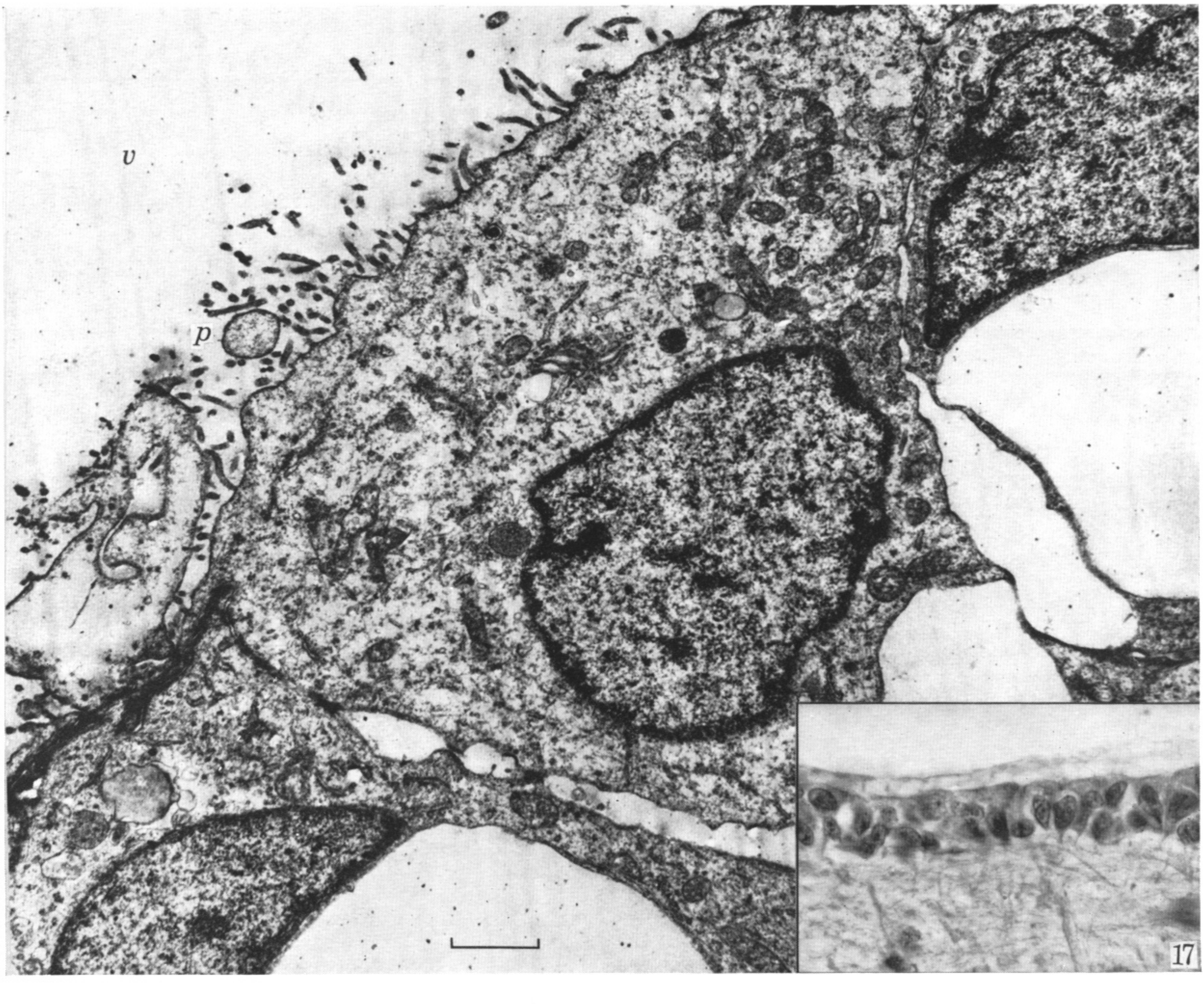

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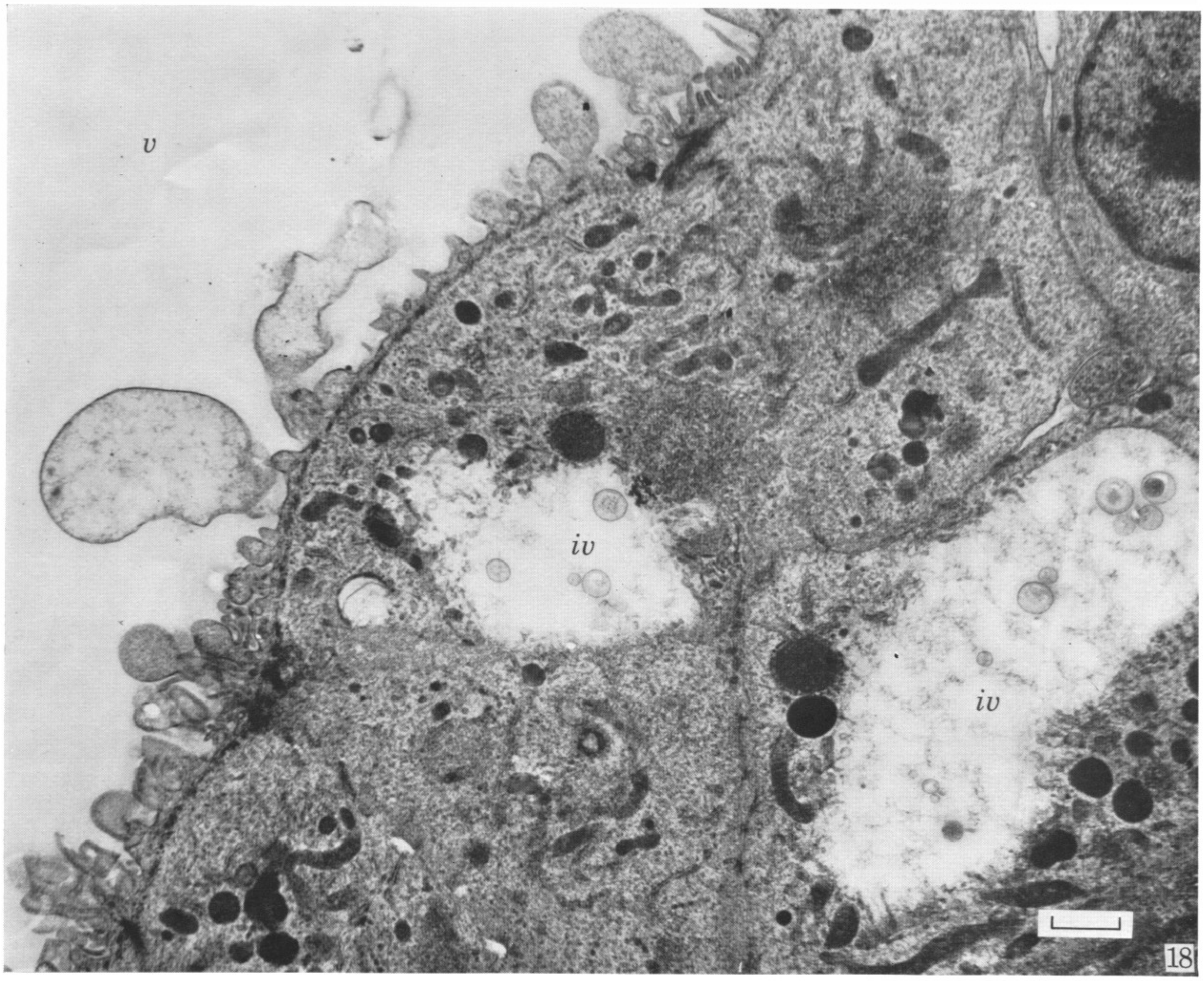
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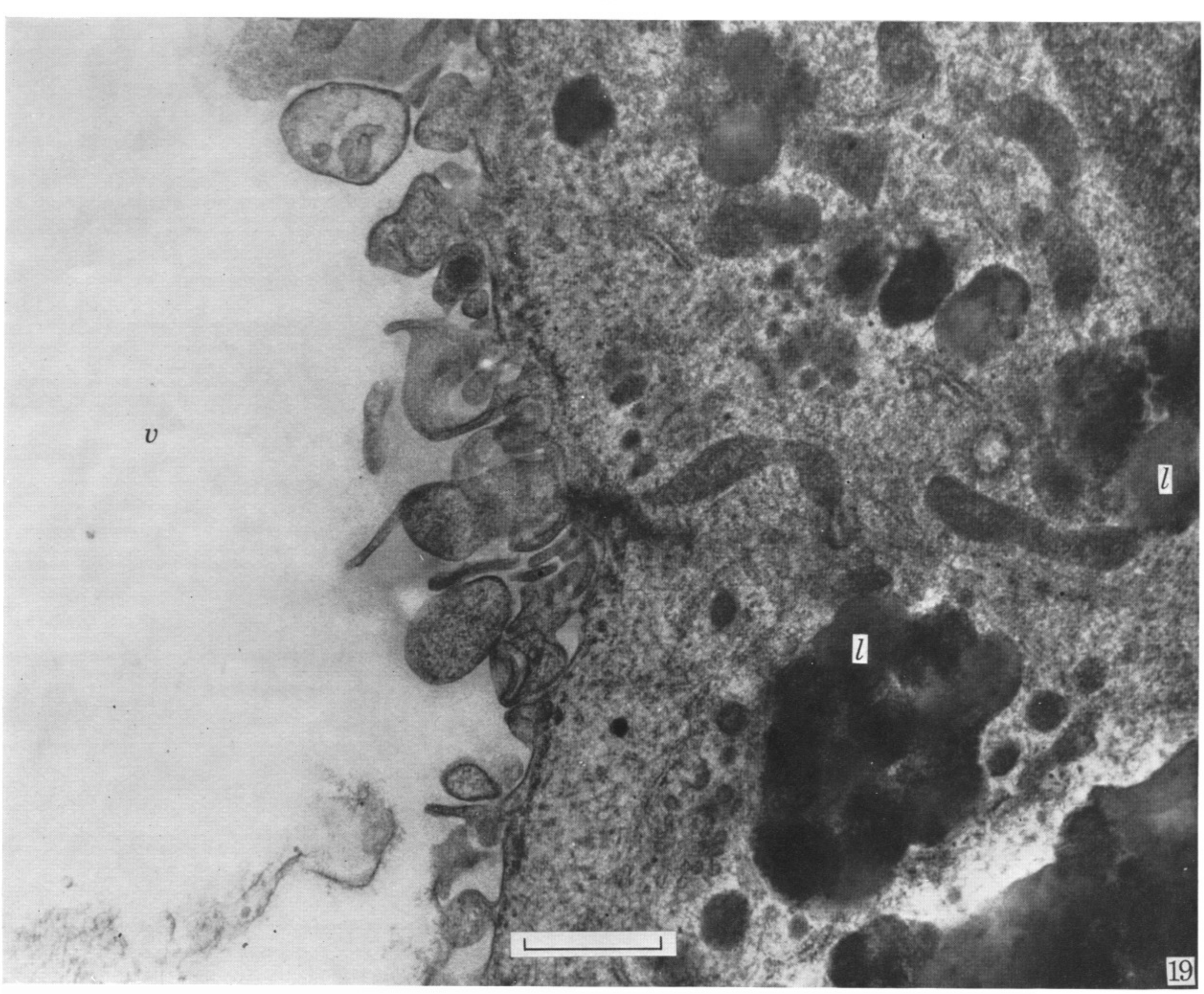
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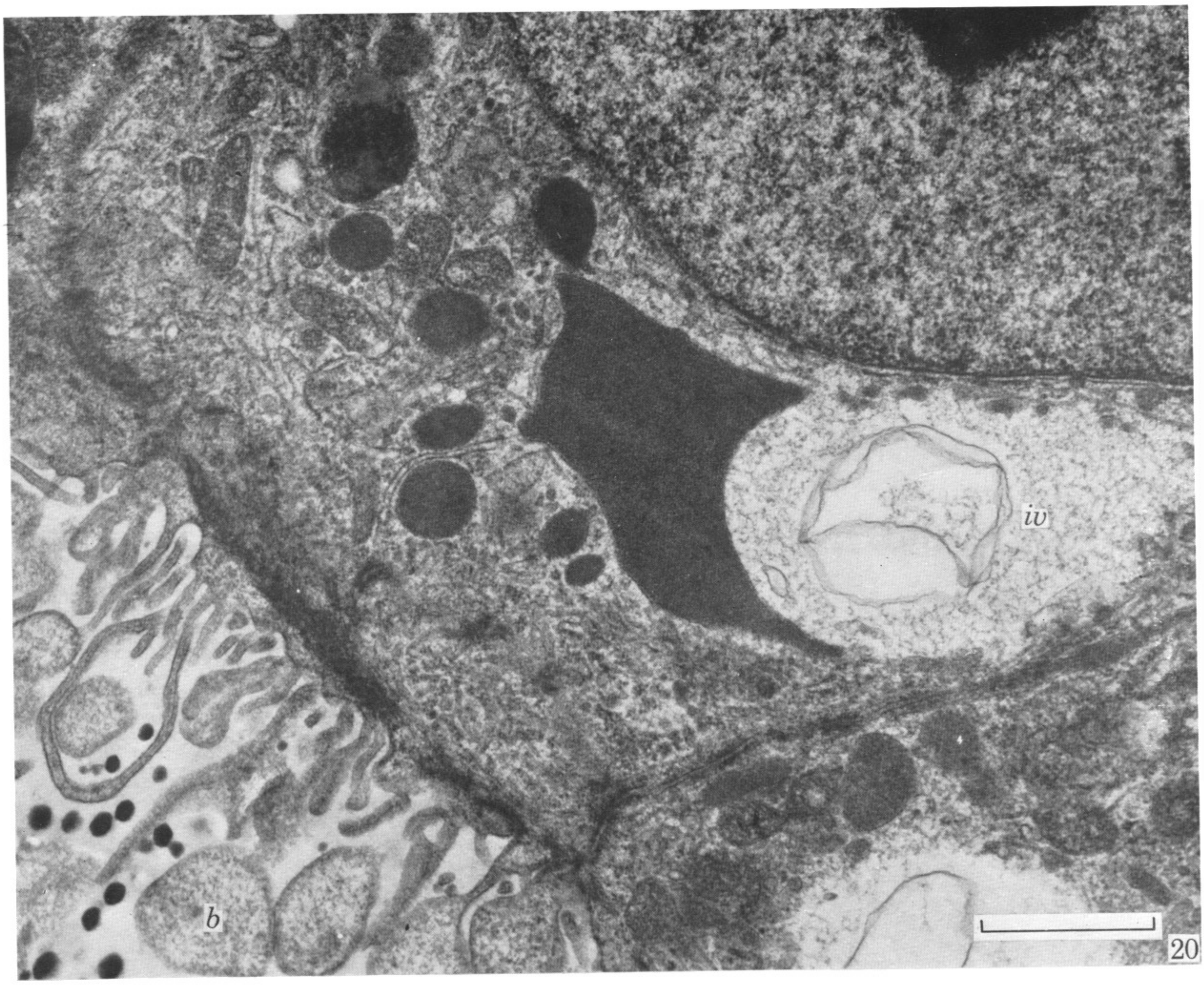

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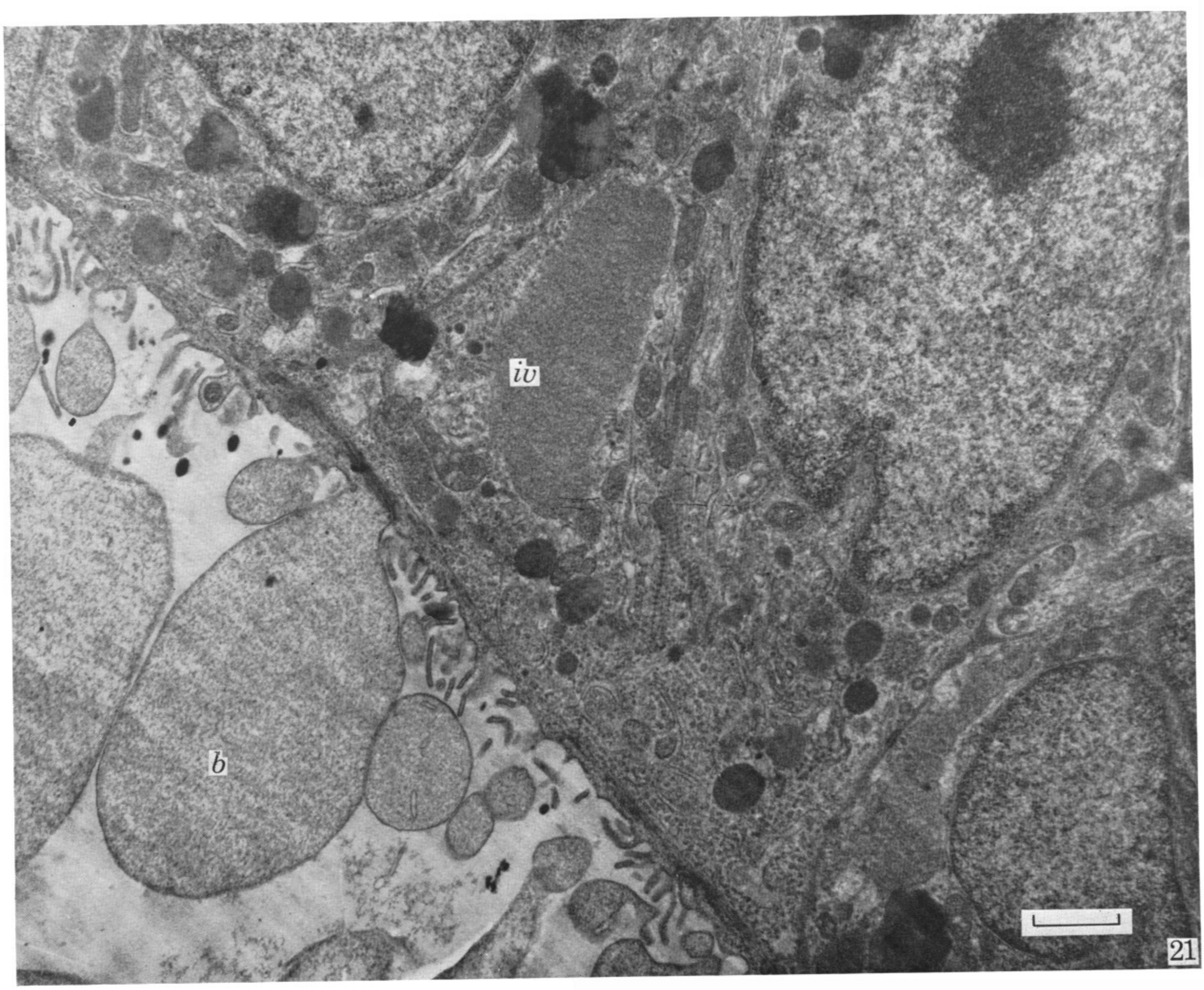

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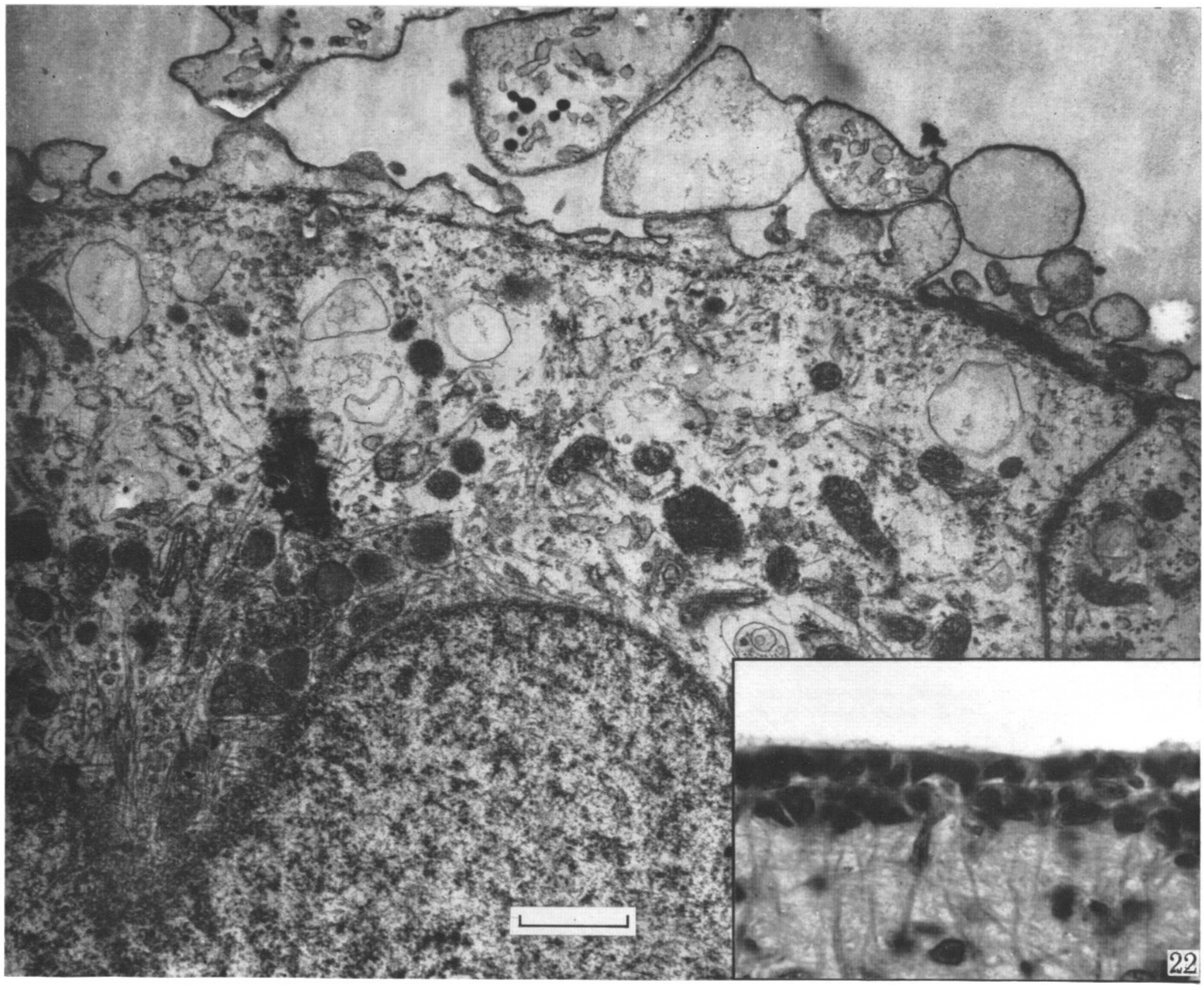
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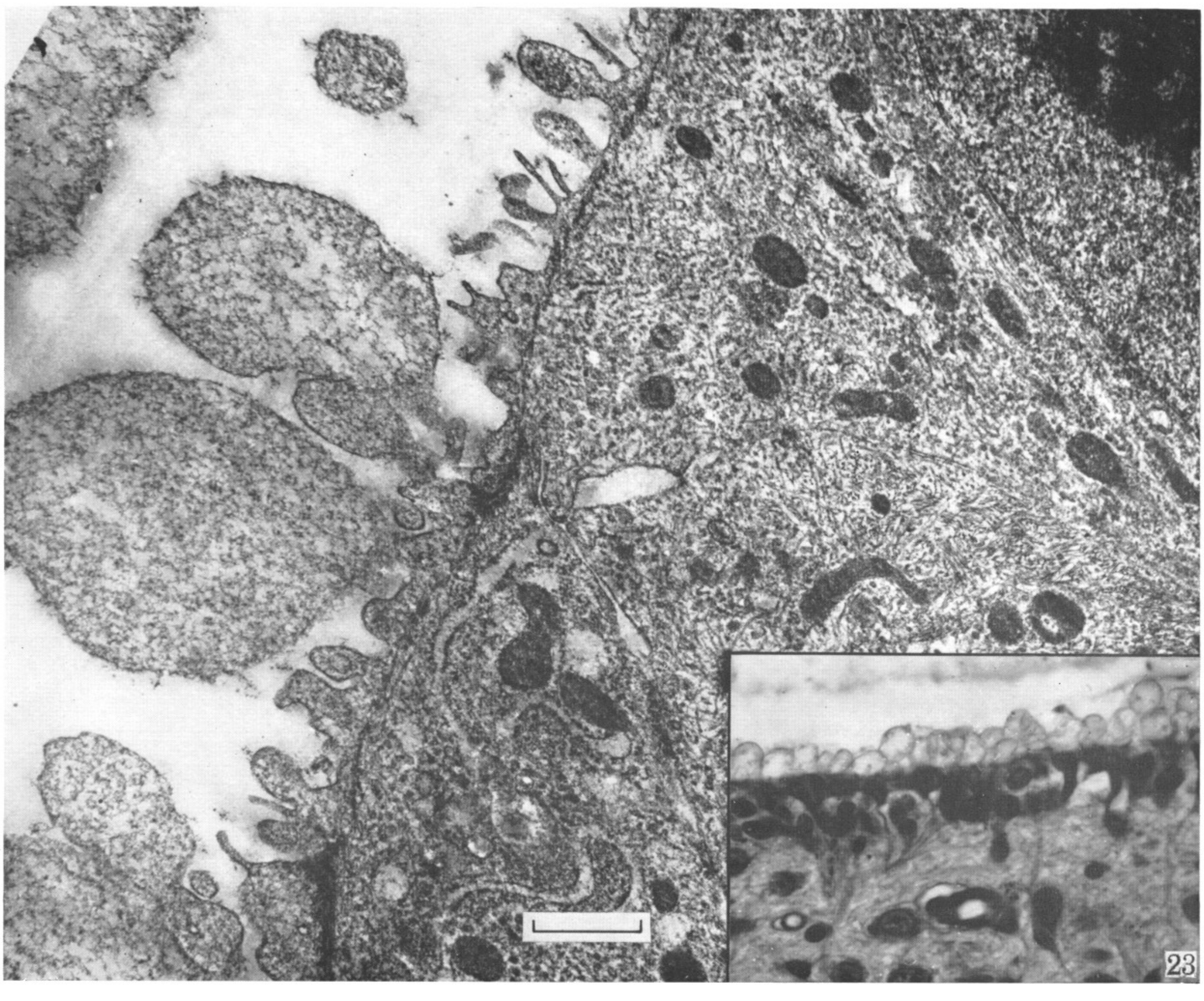
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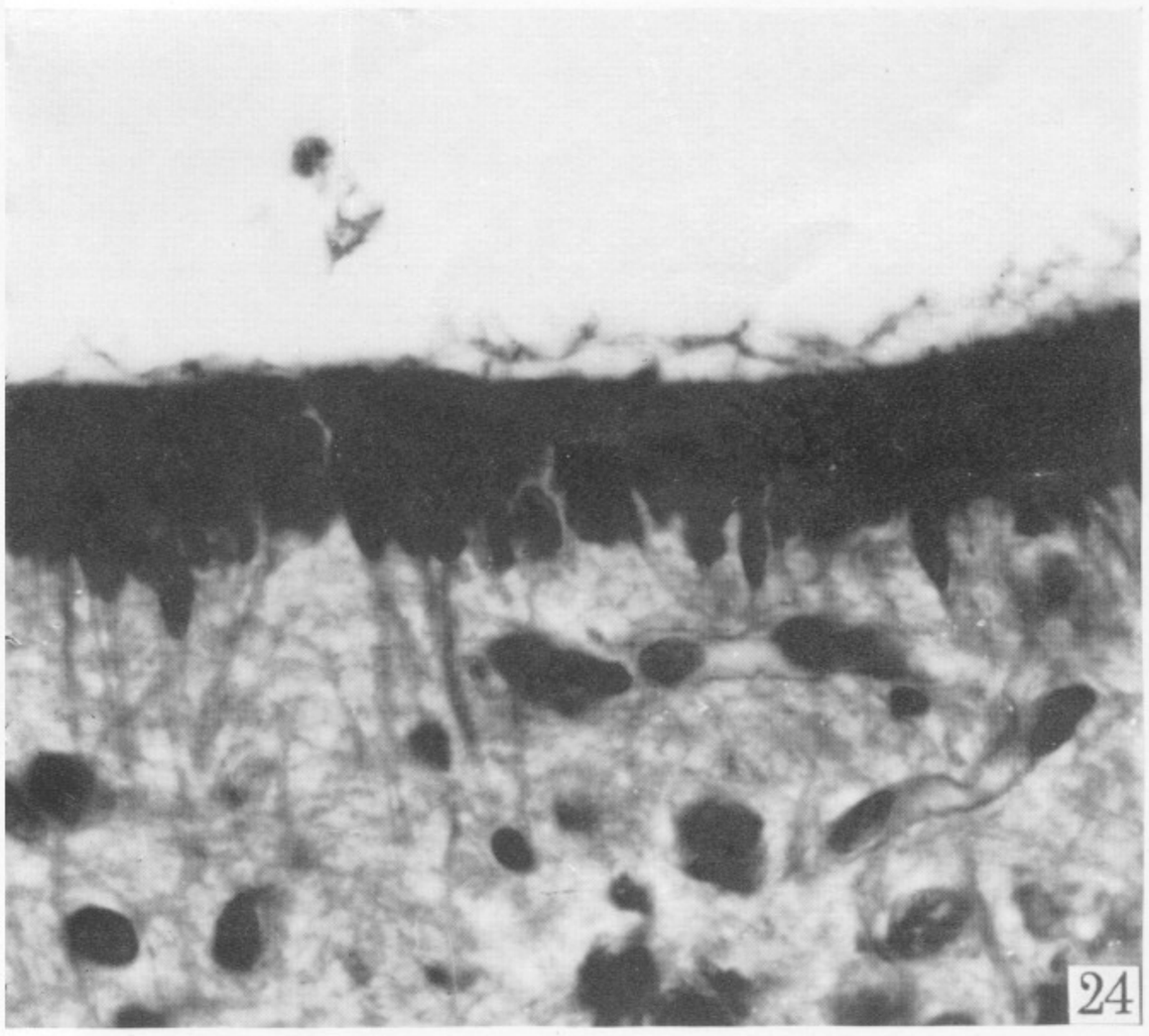
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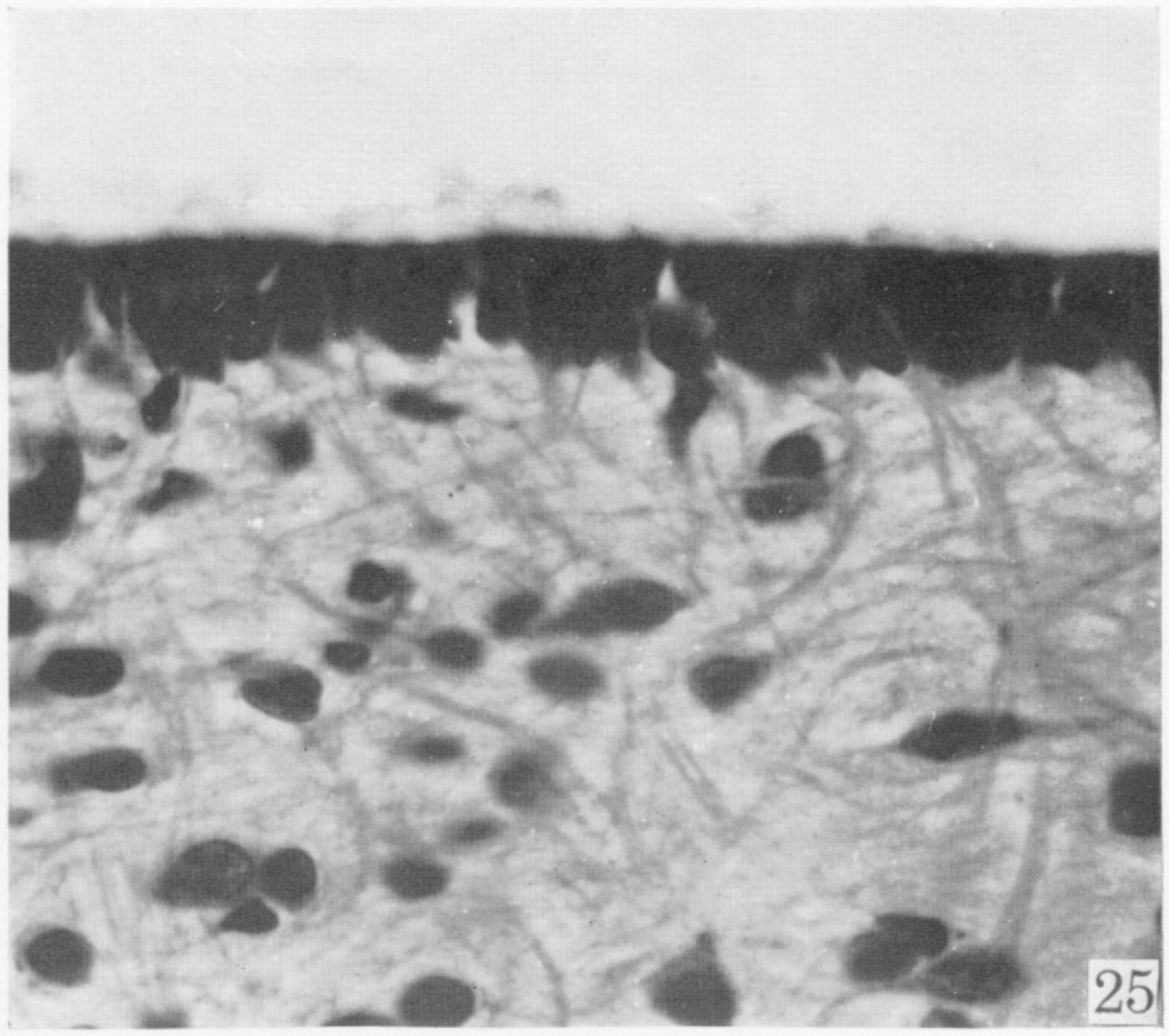
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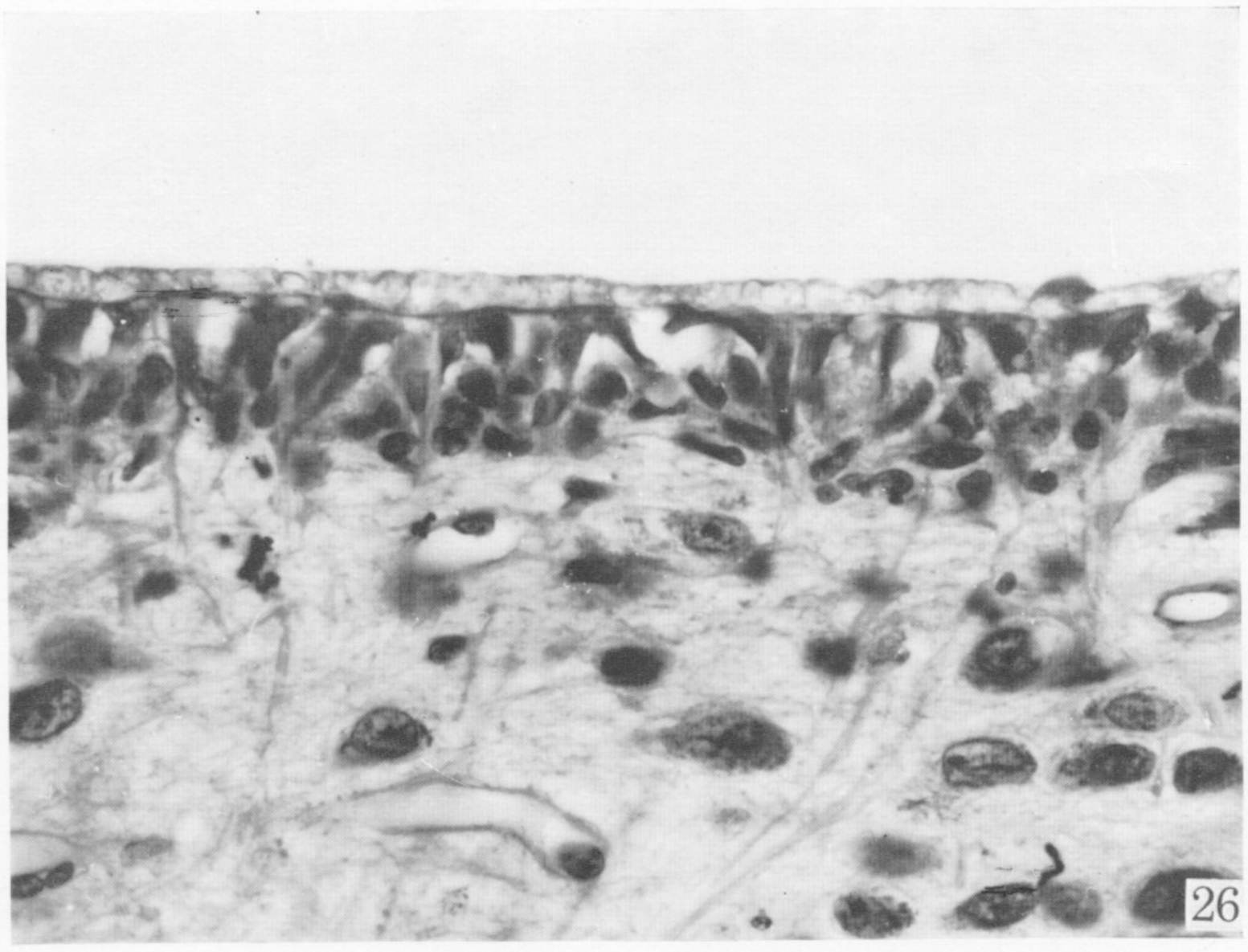
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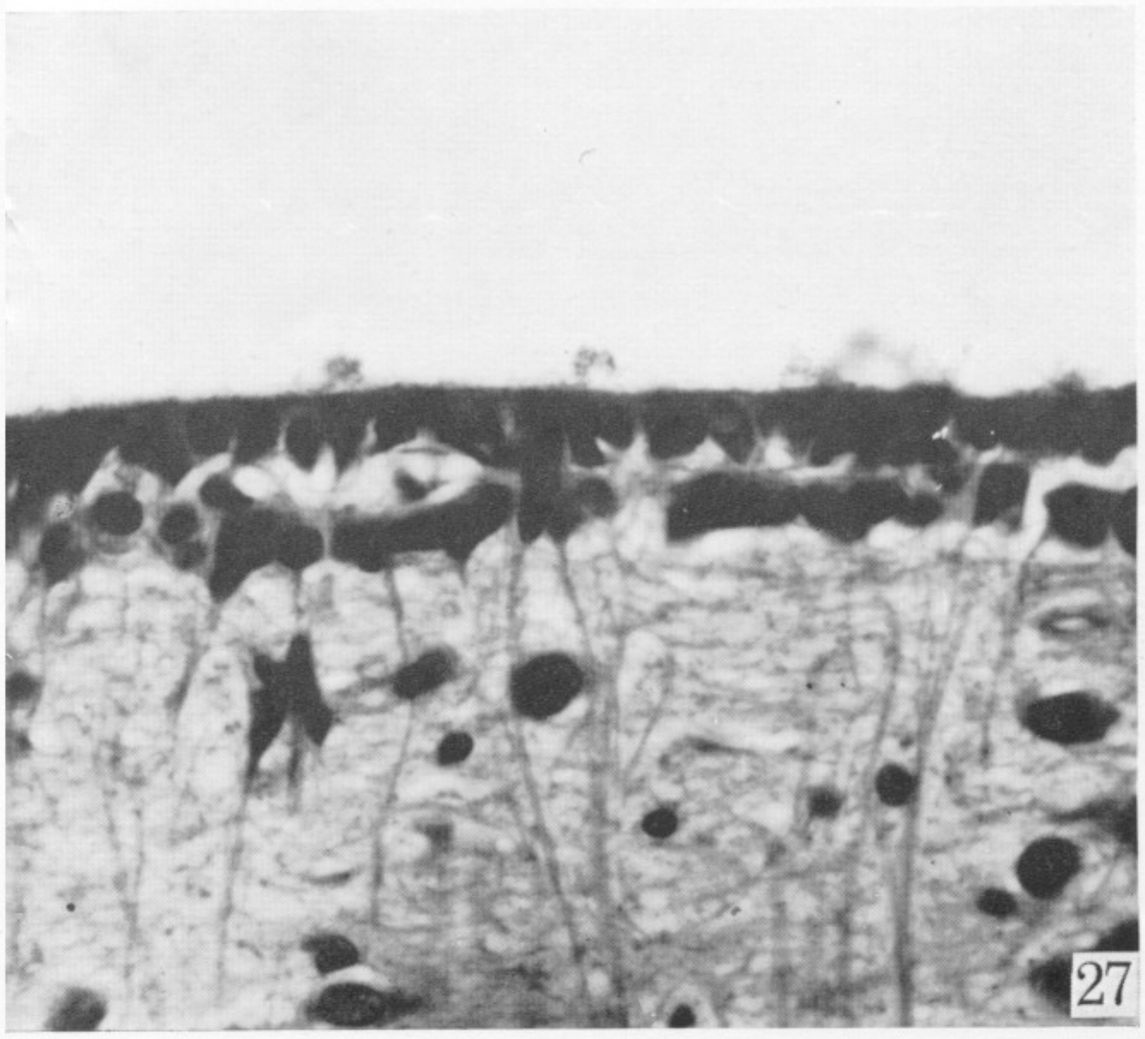
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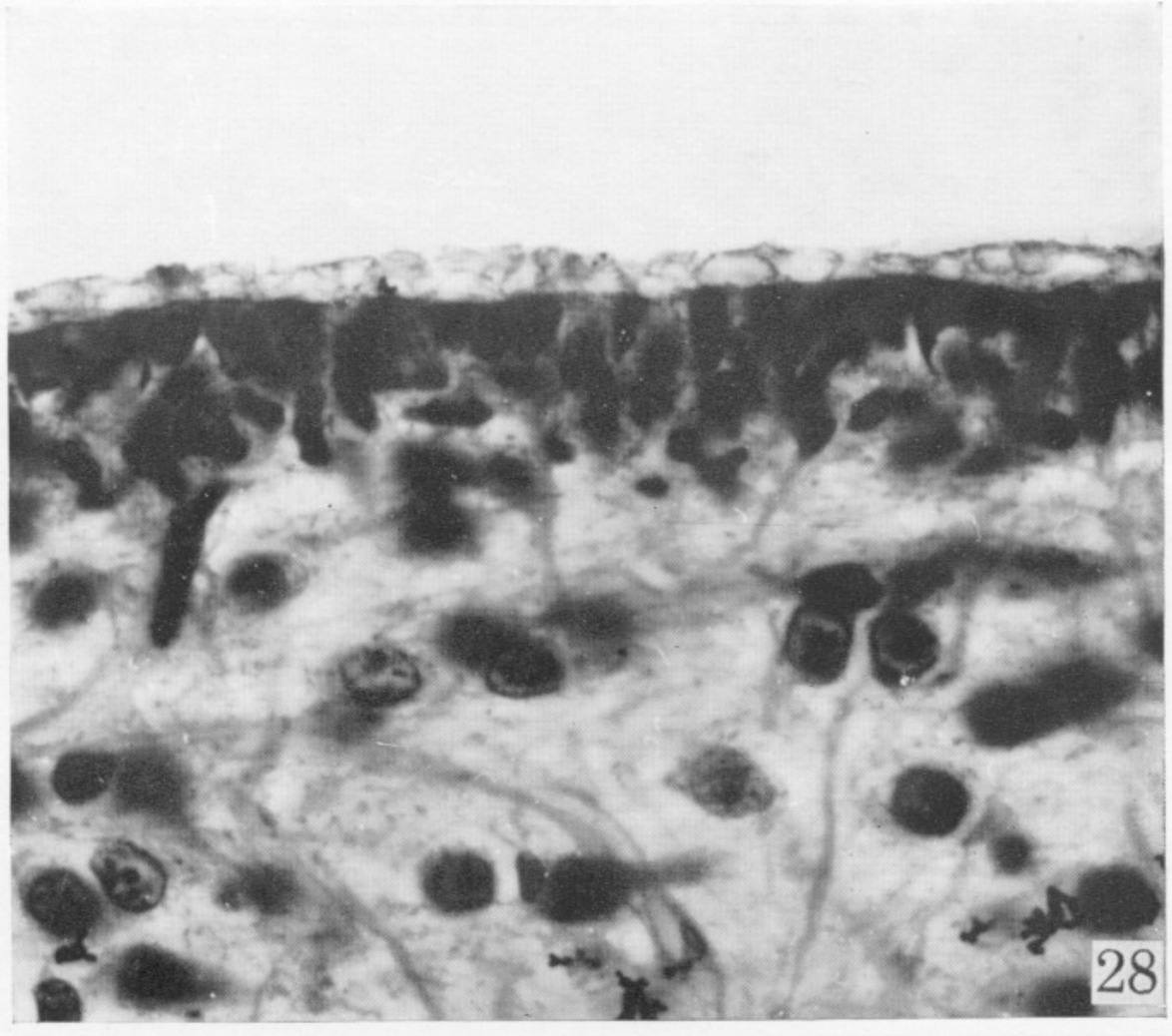
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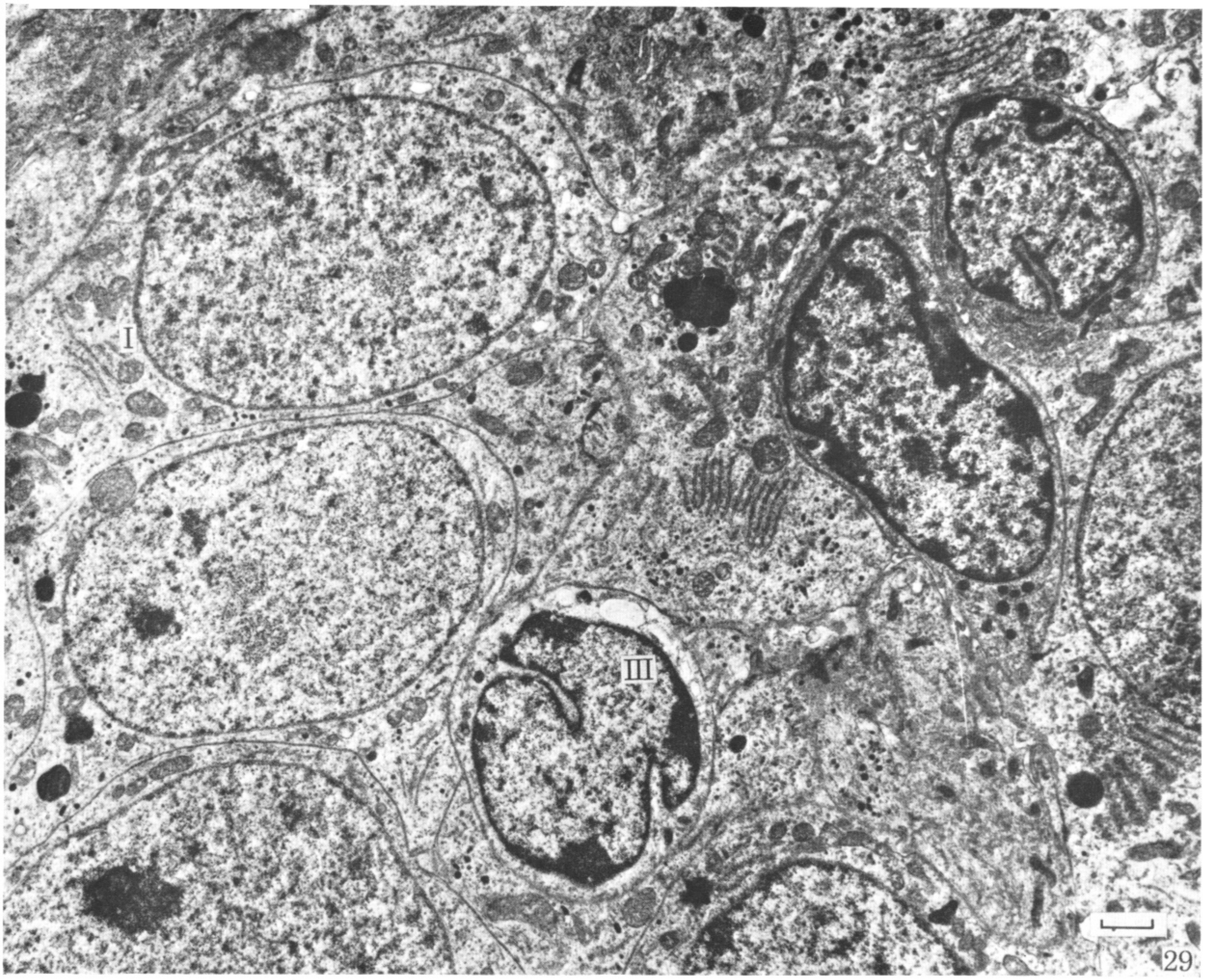
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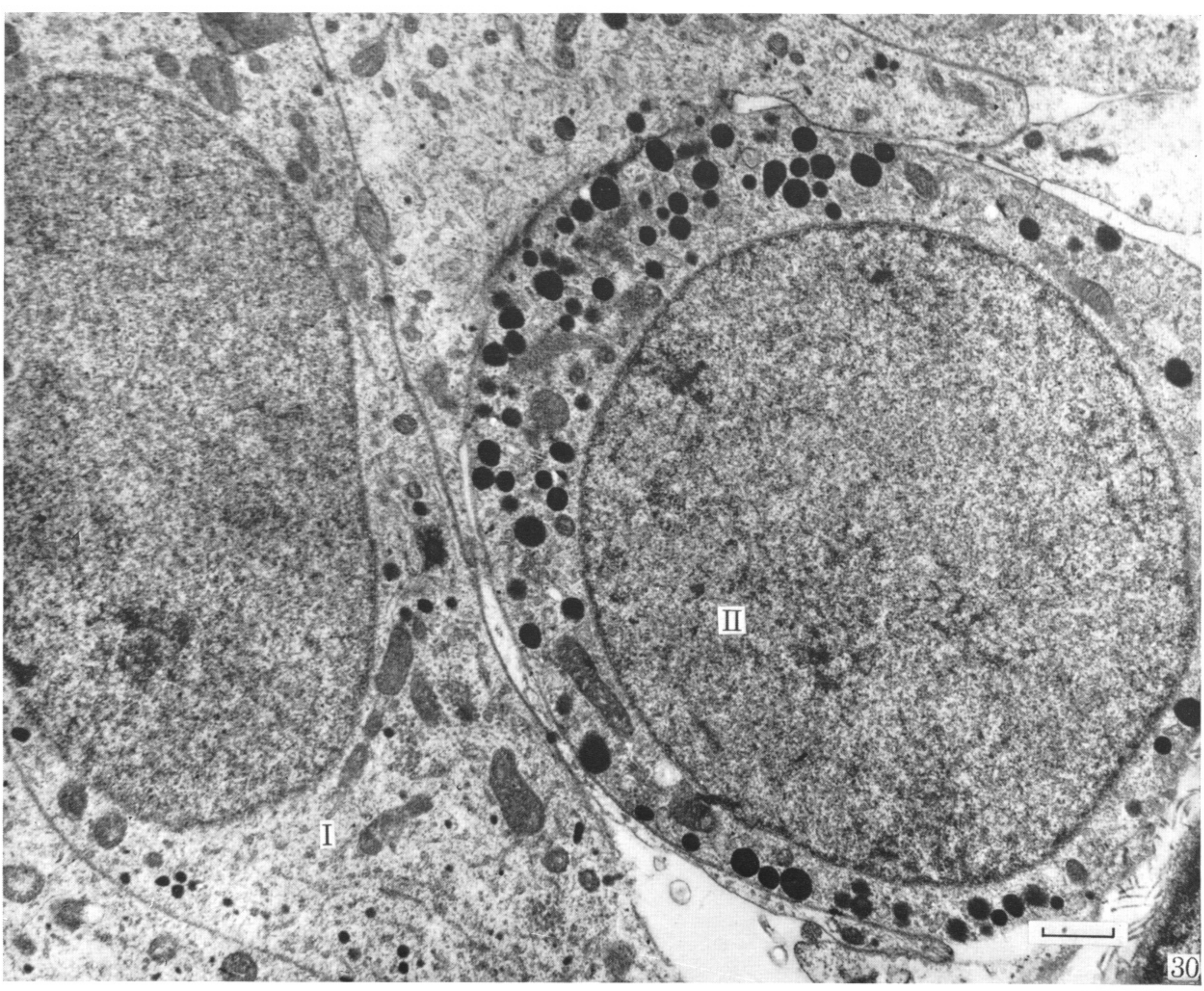
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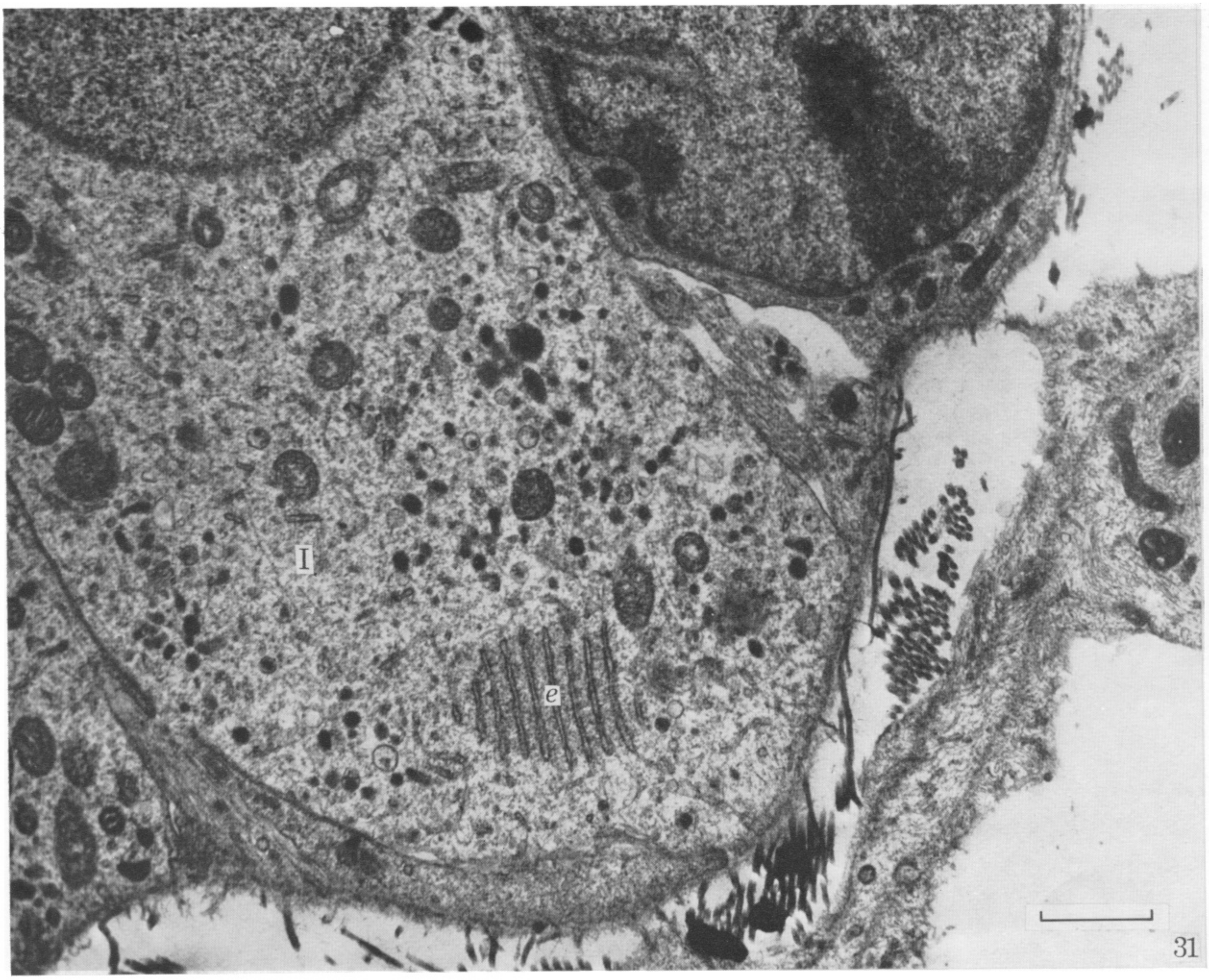

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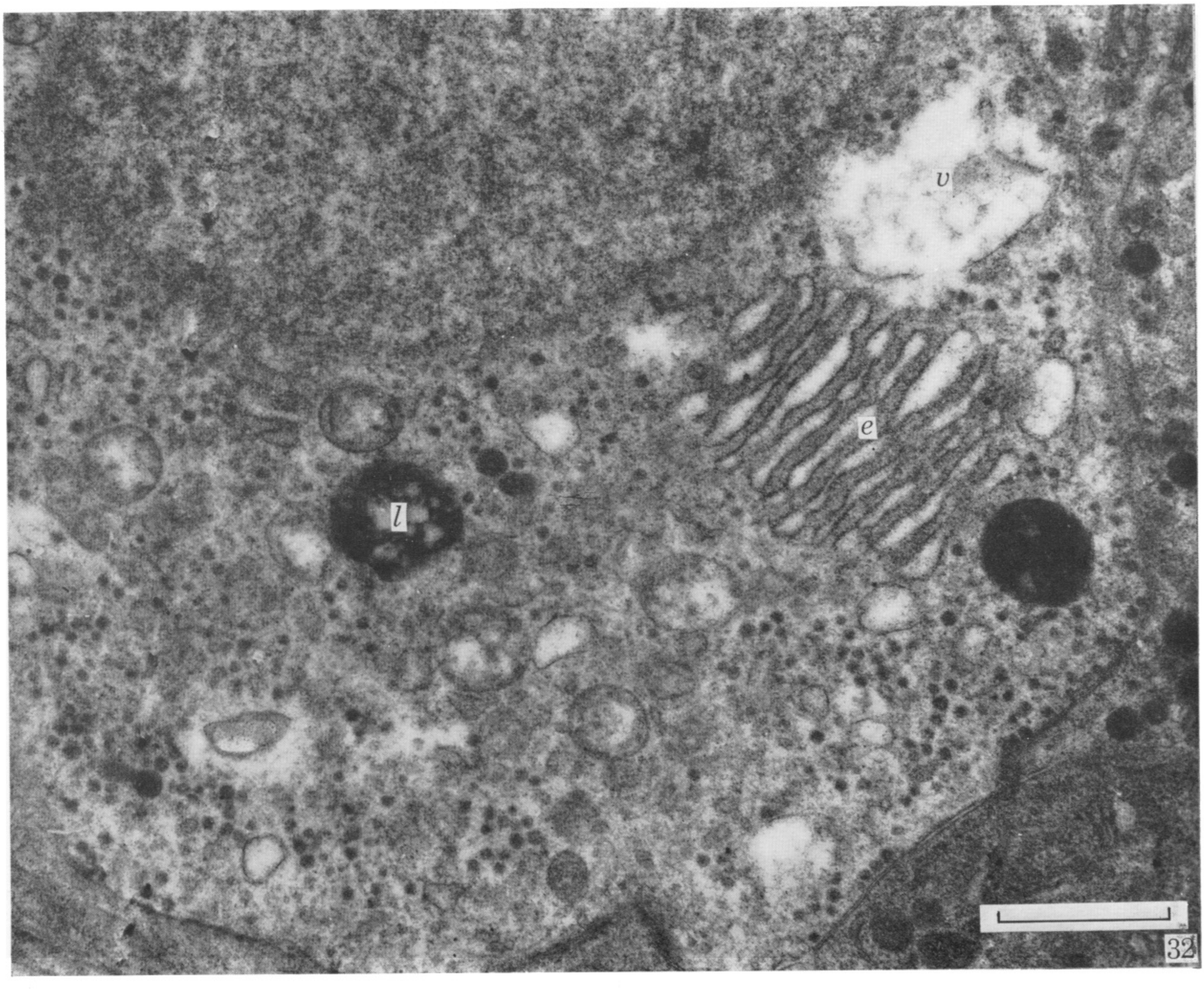

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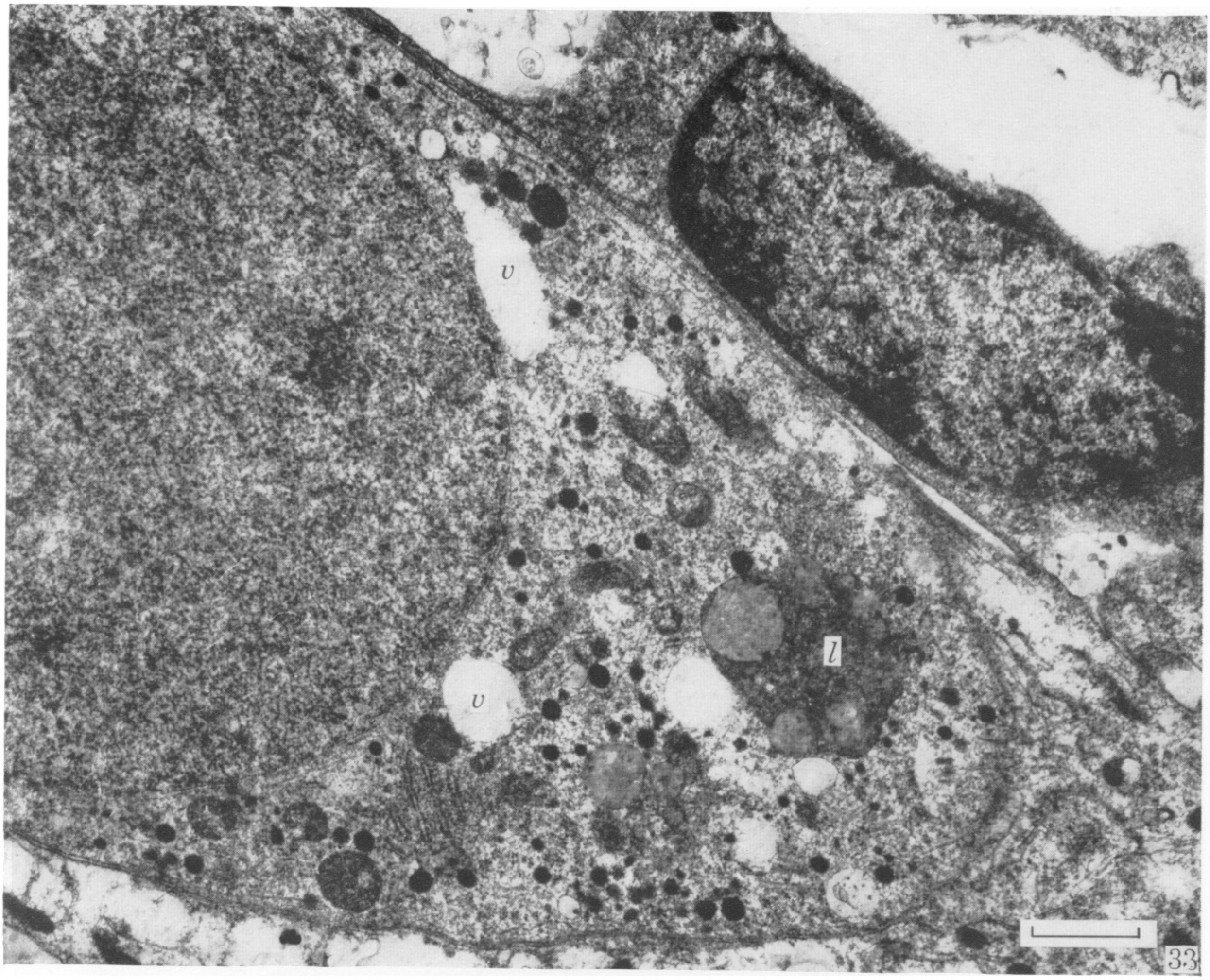

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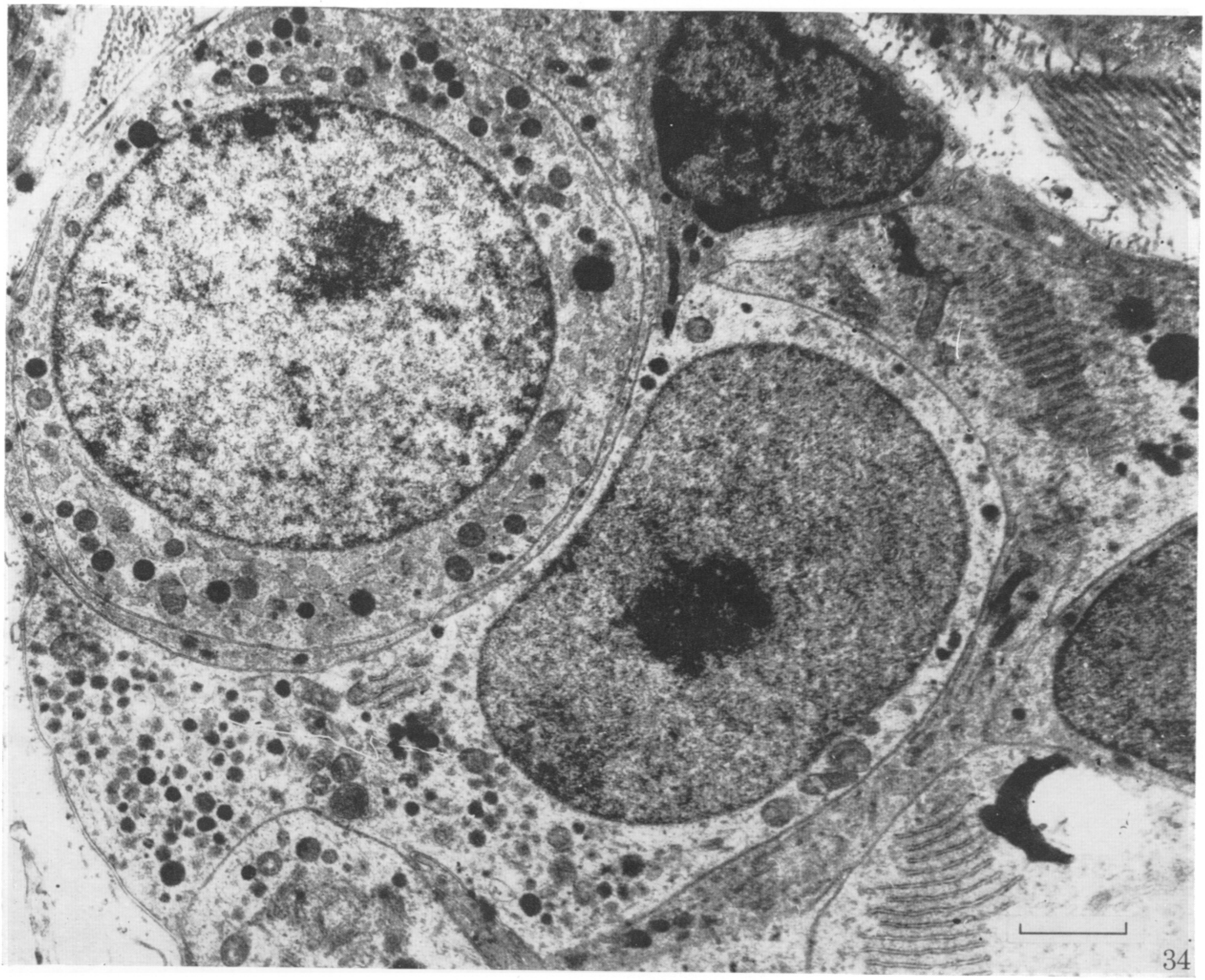

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