OBSERVATIONS ON THE STRUCTURE OF THE SMALL INTESTINE IN FOETAL, NEO-NATAL AND SUCKLING PIGS

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[Plates 36 to 42]

CONTENTS

Light and electronmicroscopic observations of changes throughout the small intestine of foetal, and both suckled and unsuckled newborn pigs are reported. Foetal animals between 73 days gestation and term showed vacuolation in the terminal ileum. This was most extensive between 90 and 100 days when the terminal 30% of the small intestine contained vacuolated cells. The apical region of such cells contained a system of smooth tubes and vesicles, some of which showed evidence of a characteristic surface pattern. The vacuoles contained material of variable electron density and were sometimes seen apparently discharging their contents into the dilated intercellular spaces. Unsuckled newborn animals showed most of the features described above, but, in addition, the vacuolated cells contained large numbers of electron dense inclusions. In suckled animals from birth to 70 h of age there were considerable variations in cellular structure, which could be related to the position in the small intestine, the position on the villus and the age of the animal. The structural features described are discussed in relation to the transfer of colostrum immunoglobulins into the circulation. Keywords: swine, foetus, newborn, small intestine, structure.

Introduction

The plasma of unsuckled newborn pigs contains only traces of the immune globulins and immediate postnatal immunity is acquired by the absorption of intact antibodies from the sow's colostrum (Morris 1968). This absorption occurs in the small intestine, particularly in the jejunum (Matisson & Karlsson 1966), by an effectively non-selective mechanism: heterologous antibodies (Olsson 1959*a*, *b*; Payne & Marsh 1962*a*, *b*; Kaeberle & Segre 1964; Kim, Bradley & Watson 1966; Hardy 1969*b*), other large proteins (Lecce, Matrone & Morgan 1961; Lecce 1966*b*; Balconi & Lecce 1966), and even the inert macro-molecule, polyvinyl

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pyrrolidone (Lecce *et al.* 1961; Hardy 1965, 1969*a*; Lecce 1966*b*) are all absorbed. There is some breakdown of immune globulins both in the stomach (Hardy 1969*b*) and in the small intestine, partly by a trypsin-like protease (Nordbring & Olsson 1958; Hardy 1969*b*). Nevertheless, proteins which are absorbed retain their biological activity, even in the case of sensitive molecules such as iso-enzymes (Balconi & Lecce 1966; Lecce 1966*b*), and hormones (Asplund, Grummer & Phillips 1962). The material is taken into the epithelial cells by pinocytosis (Lecce 1966*a*, *b*), but the mechanisms by which it is transported across the cells and discharged are not known, although the latter processes are influenced by the medium in which the macromolecules are fed (Hardy 1969*a*).

Histological studies have shown that the absorbed material is taken up within the apical cytoplasm as small droplets, which then coalesce to form large globules (Comline, Pomeroy & Titchen 1953; Payne & Marsh 1962*a*, *b*). Material also appears in the lymphatics and blood vessels of the villus. Vacuoles without a stainable, condensed content have been reported in foetal piglets 2 to 3 weeks before birth and in neo-natal animals which have not suckled (Comline *et al.* 1953). In fully weaned pigs there are neither vacuoles nor globules, which suggests a connexion between the presence of such structures and the ability to absorb macromolecules.

All the studies on the fine structure of the intestine of the neo-natal pig which are known to the authors have concerned pieces of jejunum (Kenworthy, Stubbs & Syme 1967; Matisson & Karlsson 1965, 1966; Sibalin & Björkman 1966; Vodovar & Fléchon 1966; Staley, Jones & Marshall 1968), although observations in this laboratory have suggested that vacuolation in neo-natal, unsuckled piglets is best developed in the terminal third of the small intestine. There are no ultrastructural studies of foetal piglets known to the authors and little attention seems to have been paid to the structural changes which occur after the initial suckling period.

In this preliminary survey, a wide range of animals has been examined in order to discover the main changes which occur during the period of late intra-uterine and early neo-natal life. Emphasis has been placed on the terminal third of the small intestine in an attempt to discover more about the vacuolation which is found there.

Description of plate 36

Figures 1 to 5 and 10 to 15, plates 36 and 38, inclusive are light micrographs of tissue fixed in Susa and stained with iron haematoxylin and Alcian blue. Figures 6 to 9, plates 36 and 37 and 16 to 33, plates 39 to 42 inclusive are electron micrographs. With the exception of figure 18 which was double-fixed in glutaraldehyde and osmium tetroxide, the tissues were fixed in osmium tetroxide and stained with uranium and lead. The percentages, given immediately before the magnification, show the position at which the specimen was taken expressed as a percentage of the length of the small intestine measured from the pyloric incisura.

Figure 1. 93-day foetus. Duodenal region, without vacuoles or apical reticulum. 7%, ×1280.

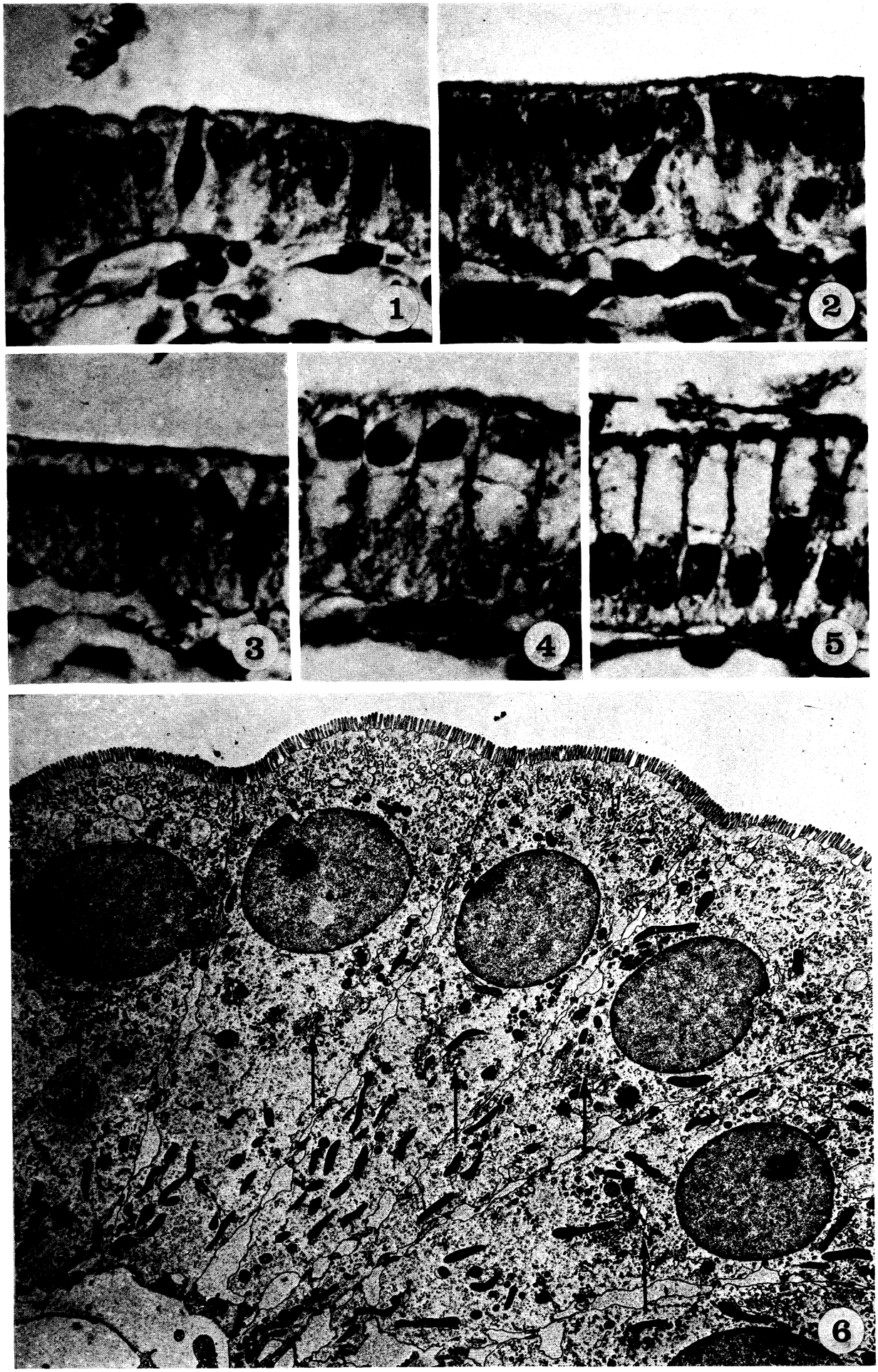
Figure 2. 93-day foetus. First traces of the apical reticulum. 21%, ×1280.

Figure 3. 93-day foetus. Presence of small apical vacuoles. 48%, ×1280.

Figure 4. 93-day foetus. Inversion of vacuole and nucleus. 70%, ×1280.

Figure 5. 93-day foetus. Large apical vacuoles. 85%, ×2800.

Figure 6. 101-day foetus. Survey, showing the general zonation of the cell organelles. The Golgi apparatus (arrows) is subnuclear. 55%, ×2330.



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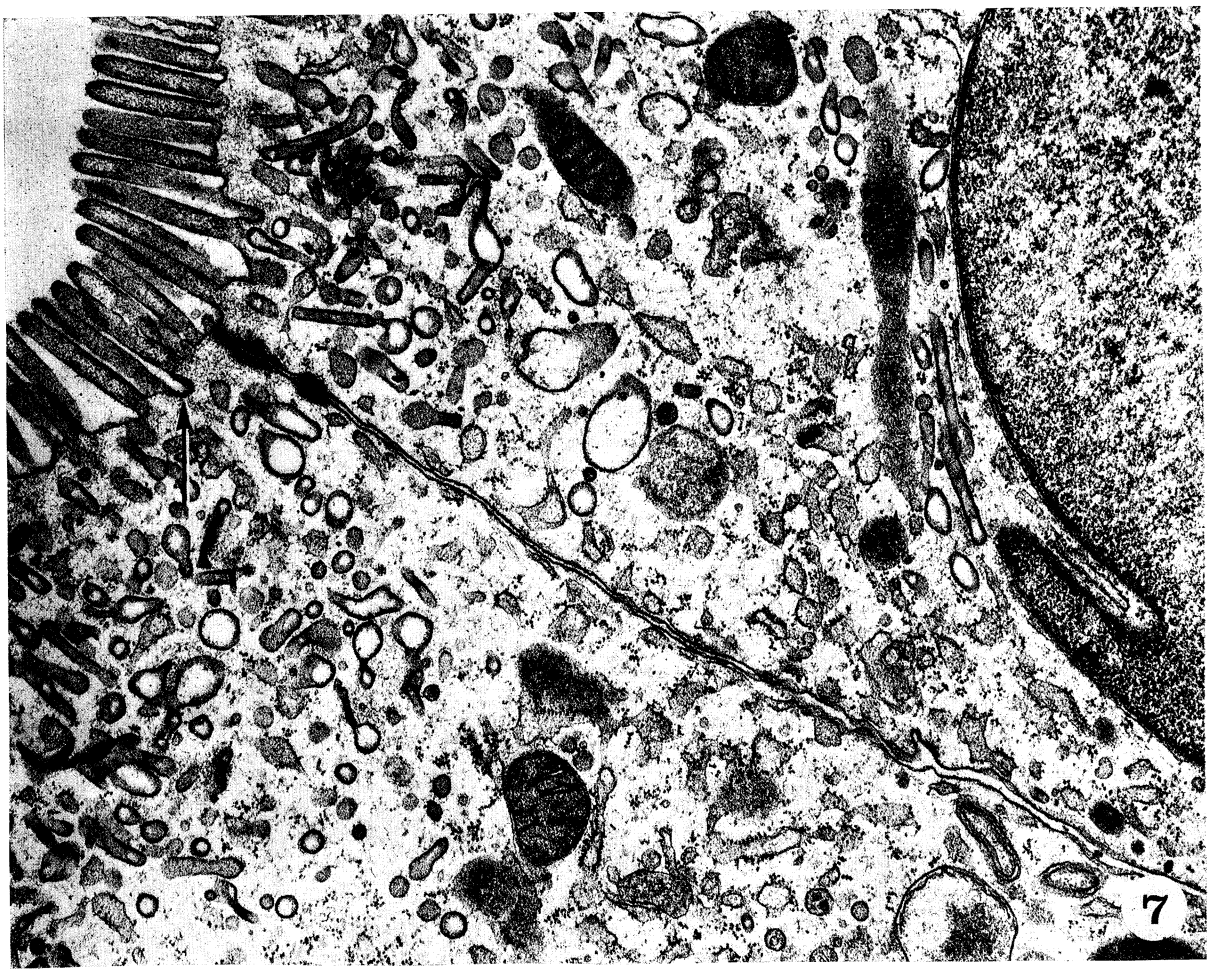

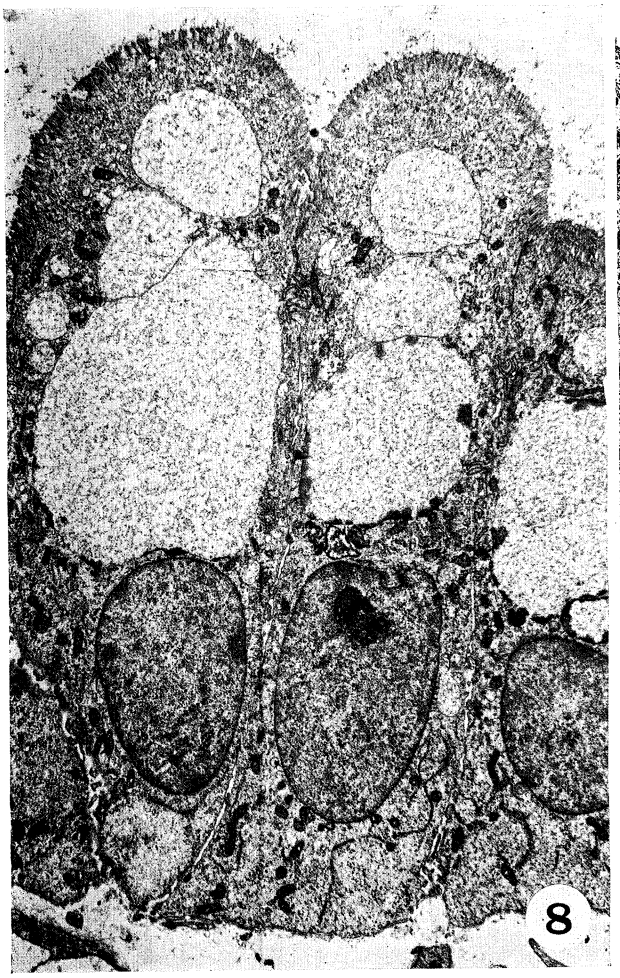

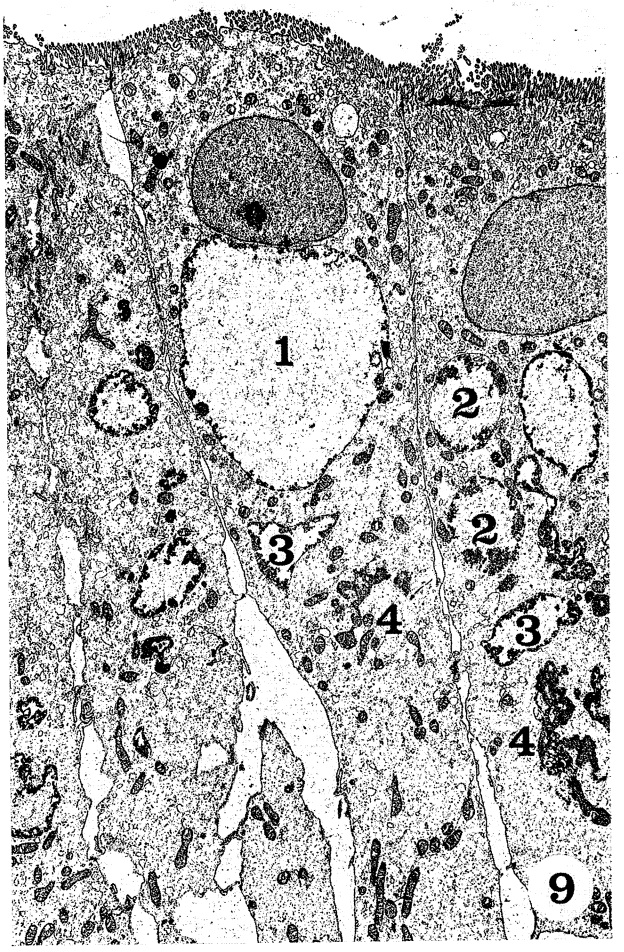

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Materials and methods

Twenty-seven Landrace and Large White pigs were used: details of the animals, and the locations of the specimens examined (given as a percentage of the length of the small intestine, measured from the pylorus) are given in table 1.

Table 1. Details of experimental animals

The positions from which pieces of intestine were taken are expressed as a percentage of the total length of the small intestine, measured from the pyloric incisura. F = foetal; UN = unsuckled neonate; SN = suckled neonate. Foetal ages are given in days from conception. Neo-natal ages are hours after birth.

			samples for	
			light	electron
no.	type	age	microscopy	microscopy
1	F	73	0 → 100	—
2	F	73	0 → 100	—
3	F	79	67; 80; 90	67; 80; 95
4	F	93	0 → 100	70; 88
5	F	101	59; 88	55; 80
6	F	106	0 → 100	64; 89
7	F	108	75; 91	87; 93
8	UN	2	40 → 100	—
9	UN	4	—	84; 91
10	UN	5	40 → 100	48
11	UN	8	78; 88; 97	80; 89
12	UN	28	0 → 100	85; 92
13	UN	43	87; 93; 97	—
14	SN	4	—	50; 87; 92
15	SN	7	—	50; 85; 92
16	SN	7	82; 90; 98	84; 91
17	SN	9	0 → 100	18; 25; 34; 44; 50 66; 82; 90; 98
18	SN	16	60 → 100	78; 90; 94
19	SN	19	85	86
20	SN	27	82; 90; 98	84; 91
21	SN	28	0 → 100	86
22	SN	28	0 → 100	53; 76; 99
23	SN	33	0 → 100	29; 46; 63; 81; 98
24	SN	43	87; 93; 97	—
25	SN	49	0 → 100	22; 41; 60; 79; 98
26	SN	57	0 → 100	35; 54; 63; 86; 99
27	SN	73	0 → 100	53; 98

Foetal animals were delivered by Caesarean section under halothane anaesthesia, and postnatal animals were given a lethal dose of Nembutal by intraperitoneal injection. Immediately after delivery, or after the inset of anaesthesia, the abdomen was opened and the entire gut

Description of plate 37

Figure 7. 101-day foetus. The apices of two epithelial cells, showing the junctional complex, the apical system, and several surface invaginations, one of which is 'coated' (arrow). 55%, ×2100.

Figure 8. 108-day foetus, showing large apical vacuoles which contain both precipitated material and conglomerations. 95%, ×2330.

Figure 9. 93-day foetus. Note the electron-dense conglomerations within the subnuclear vacuole, and the transitional forms (2) (3) between vacuoles (1) and electron-dense, irregular bodies (4). 70%, ×2330.

from pylorus to ileo-caecal valve was removed, unravelled and freed from mesentery. The specimens were cut and perfused with fixative within 5 to 10 min, and subsequently immersed in fixative.

For light microscopy, the tissues were fixed in Susa, embedded in paraffin wax, sectioned at 4 to 6 μm, and stained with iron haematoxylin and Alcian blue. Where long lengths of gut were processed, they were cut into 5 cm lengths immediately after fixation and subsequently either sectioned longitudinally and examined, or representative transverse sections from the centre of each piece were examined. The sections were photographed with a Zeiss photomicroscope.

For electron microscopy, the tissues were fixed either in 1 % osmium tetroxide or in 4 % glutaraldehyde followed by postfixation in 1 % osmium tetroxide. Both fixatives were used in a phosphate buffer at pH 7.4 and were cooled in melted ice. During fixation, the tubes of gut were cut into rectangular pieces, which were subsequently embedded in Araldite (Luft 1961), sectioned in the range 40 to 100 nm (Peachey 1958; Williams & Meek 1966), stained in alcoholic uranyl acetate (Stempak & Ward 1964) and lead citrate (Reynolds 1963), and examined on a Siemens Elmiskop 1.

Orientation

On any single villus there is a spectrum of cells at different stages of differentiation, ranging from the dividing cells near the base to the cells being shed at the apex. Unless otherwise stated, the descriptions which follow are of cells in the upper halves of the villi, but excluding the apex where cells are in the process of degenerating and being shed. In considering any epithelial cell, the surface nearest to the core of the villus is considered as basal or lower, and the surface furthest from the core of the villus as apical or upper.

Observations

1. *Foetal animals*

(*a*) *Light microscopy*

A gradual and progressive change in the structure of the epithelial cells occurred along the length of the small intestine from the pyloric incisura to the ileo-caecal valve. The duodenal cells contained homogeneous, uniformly stained cytoplasm (figure 1, plate 36), but at some

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Description of plate 38

Figure 10. Unsuckled neonate, 8 h old. Absence of vacuoles. 80 %, × 1280.

Figure 11. Suckled neonate, 9 h old. Subnuclear inclusions. 40 %, × 1280.

Figure 12. Suckled neonate, 9 h old. Supranuclear inclusions. 85 %, × 1280.

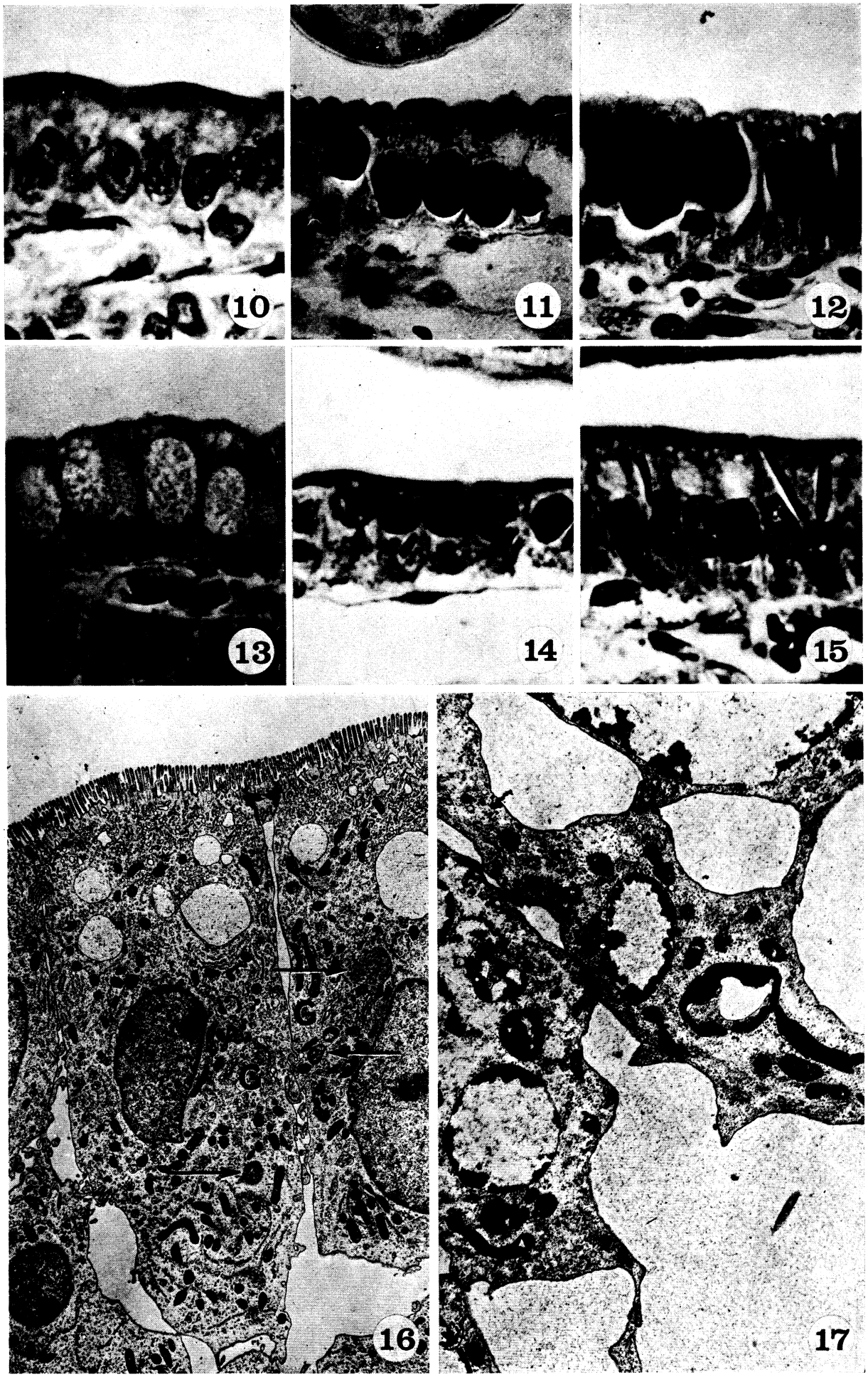
Figure 13. Suckled neonate, 9 h old. Supranuclear vacuoles containing flocculent material: near the base of a villus. 50 %, × 1280.

Figure 14. Suckled neonate, 57 h old. Subnuclear, spiculate inclusion bodies. 45 %, × 1280.

Figure 15. Suckled neonate, 57 h old. Supranuclear, spiculate inclusion bodies. 85 %, × 1280.

Figure 16. Unsuckled neonate, 28 h old. Note the well-developed apical system, the position of the Golgi apparatus (G), and the presence of vacuoles with an electron-dense content (arrows). 92 %, × 3870.

Figure 17. Unsuckled neonate, 28 h old. Basal region of an epithelial cell showing transitional forms between vacuoles containing dense conglomerations, and electron-dense bodies. 85 %, × 4530.



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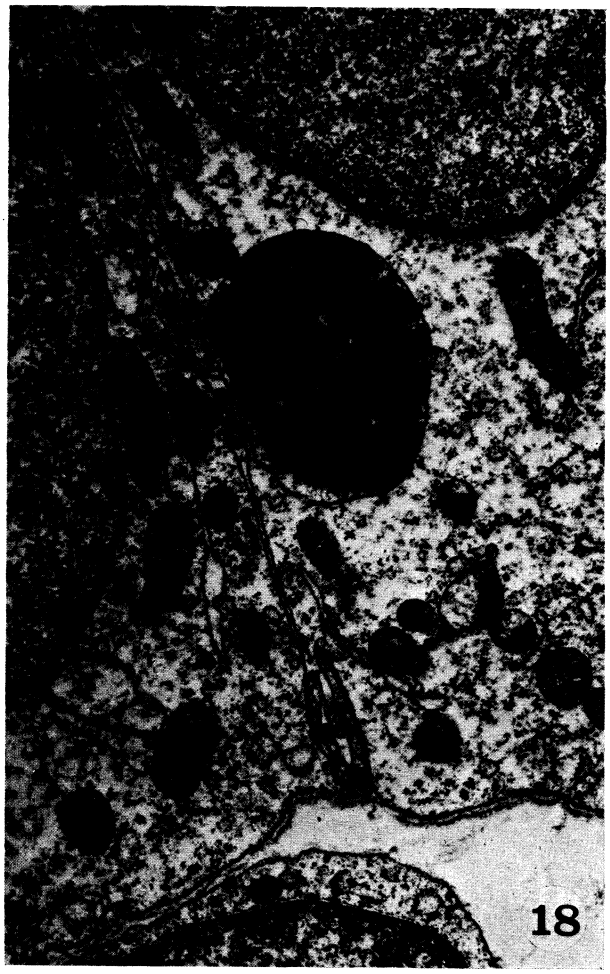

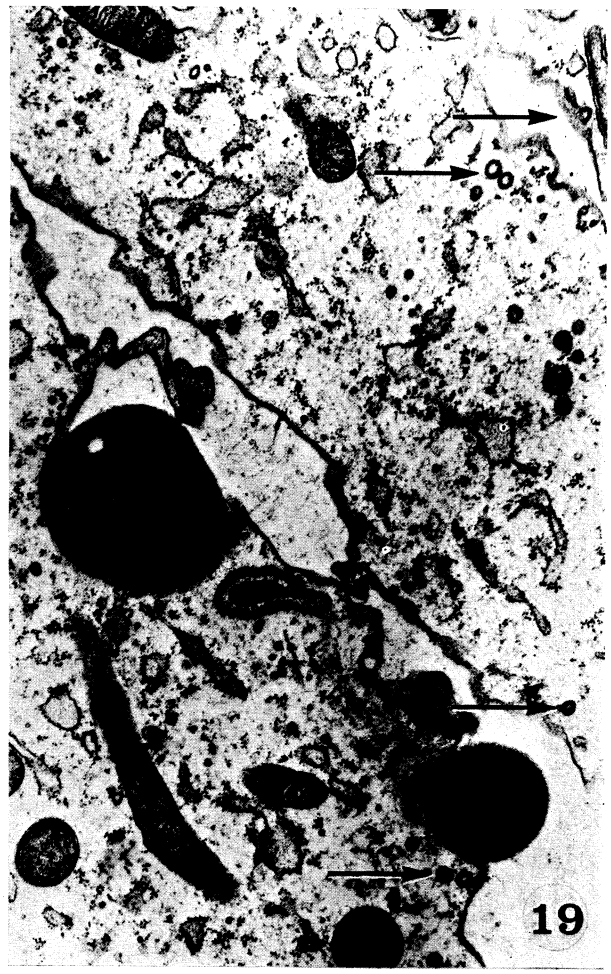

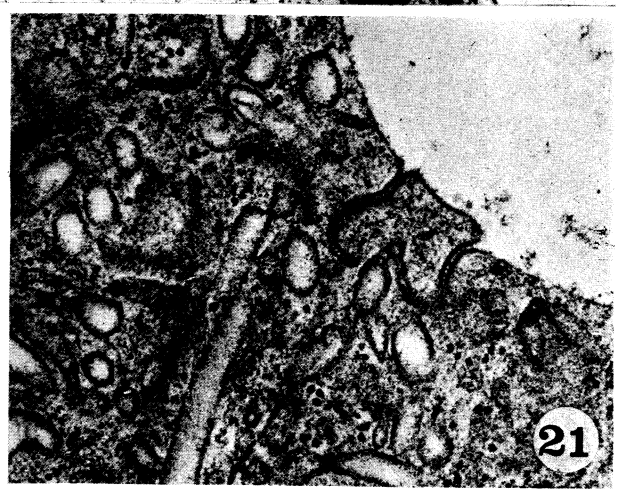

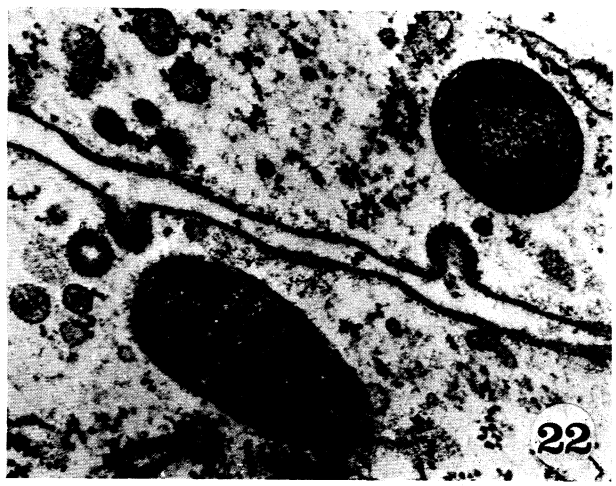

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point in the ileum an indistinct, unstained reticulum could be found in the apical cytoplasm (figure 2, plate 36). This reticulum became more prominent and more vesicular with increasing distance (figure 3, plate 36) until, in the terminal ileum (figure 5, plate 36), the cells usually contained one large unstained vacuole, up to 15 μm in diameter, which pushed the nucleus towards the base of the cell. The position at which obvious vacuolation started varied with age: in the youngest foetuses (73, 79 days), and in those just before term (106, 108 days), it was confined to the last 10 % of the small intestine, but at intermediate ages (93, 101 days) it was more extensive and occupied the terminal 30 %.

The reticulum and vacuoles usually occurred in the apical cytoplasm and progressively displaced the nucleus towards the base of the cell. In the 93-day and 101-day foetuses, however, the vacuoles in the most proximal part of the vacuolated ileum were sometimes below the nucleus (figure 4). This reversal of position usually occurred near the apex of a villus, the cells nearer the base having vacuoles in the cell apex. The occasional presence of cells with a vacuole lateral to the nucleus, or containing two vacuoles, one above and the other below the nucleus, suggested, that in this region of the ileum, vacuoles which had formed in the cell apex were able to migrate past the nucleus.

(*b*) *Electron microscopy*

Several features were common to all the cells examined (see table 1). In the apical cytoplasm, immediately below the microvilli, there was a narrow, homogeneous zone ('the terminal web': Sauer 1937), which was free of organelles except for occasional invaginations from between some of the microvilli (figures 6 and 7, plates 36 and 37). These invaginations appeared sometimes to connect with a system of smooth tubules and vesicles—the apical system—which lay between the terminal web and the nucleus (figures 7 and 20, plates 37 and 39). The commonest element of the apical system was a narrow elongated profile which sometimes branched and frequently extended to form a cisterna (figure 7). Circular and irregular profiles of various sizes were also present, and the general impression was of a branched and sinuous labyrinth of labile, tubular passages, which communicated occasionally with the apical surface and contained frequent dilatations.

Below the apical system, and sometimes separated from it by vacuoles, was the nucleus (figures 6 and 8, plates 36 and 37). The Golgi apparatus was usually situated below the nucleus (figure 6), but in some cells it occurred between the nucleus and the lateral cell boundary. In both cases it consisted of the usual, characteristic array of smooth cisternae and vesicles.

Description of plate 39

Figure 18. Unsuckled neonate, 28 h old. An electron-dense inclusion body adjacent to the lateral cell membrane. 85%, ×10670.

Figure 19. Unsuckled, neonate 28 h old. Electron-dense inclusion bodies in communication with the intercellular space. Note the presence of coated vesicles (arrows). 85%, ×7330.

Figure 20. Unsuckled neonate, 4 h old. An extensive invagination of the surface membrane with a modified lumenal surface. Note the rod-like modifications (arrows). 91%, ×41670.

Figure 21. Unsuckled neonate, 4 h old. Two coated vesicles in communication with an intercellular space. 84%, ×32000.

Figure 22. Unsuckled neonate, 4 h old. Two coated vesicles in communication with an intercellular space. 84%, ×32000.

Ribosomes and mitochondria were present throughout the cytoplasm (figure 6), although, in general, there were more mitochondria and rough endoplasmic cisternae in the basal part of the cell than in the apex.

The lateral membranes of adjacent cells were joined at the luminal border by junctional complexes (figures 6 and 7), which contained the three zones described by Farquhar & Palade (1963). Further down, they were linked by occasional *maculae adhaerentes*, although parts of the intercellular space were often dilated. Such dilatations usually contained traces of background material (figures 6 and 9, plates 36 and 37), although leucocytes and even erythrocytes were sometimes present. In areas where the intercellular space was not dilated and, in particular, just below the junctional complex, the adjacent cell membranes were often interdigitated (figure 6). A clearly defined basement membrane separated the epithelial cells from the mesenchyme of the villus.

The occurrence and distribution of vacuoles conformed to the pattern described with the light microscope, but it could now be seen that many vacuoles contained material ranging from a little, electron-dense precipitate to electron-dense conglomerations which were usually arranged along the inner surface of the membrane (figures 8 and 9). Where reversal had occurred (figure 9), the vacuoles which lay below the nucleus were usually more irregular in shape, often smaller, and appeared to contain more material. It was possible to find a series of profiles which suggested a gradual collapse and condensation of the vacuoles to form irregular, tubular, or globular bodies with an electron-dense content (figure 9). On rare occasions it was possible to find such bodies opening to the intercellular space and, apparently, discharging their contents into it. This process also occurred in unsuckled and in suckled neonates, and is described more fully later (figures 17 to 19, plates 38 and 39).

2. *Neo-natal, unsuckled animals*

(*a*) *Light microscopy*

The structure was almost identical with that described for late term foetuses. With increasing age, however, both the unstained reticulum in the apices of the cells and the vacuoles in the cells near the ileo-caecal valve become less prominent (figure 10, plate 38).

(*b*) *Electron microscopy*

Again, the structure (figures 16 and 20, plates 38 and 39) was almost identical with that described for late-term foetuses and only minor differences were observed. The Golgi apparatus was now usually lateral to the nucleus (figure 16), but the main difference was the large number of electron-dense, globular, tubular, or irregular structures present in the terminal third of the ileum (figure 17, plate 38). These structures were found both above and below the nucleus, and it was possible to trace a series of transitional forms between vacuoles with

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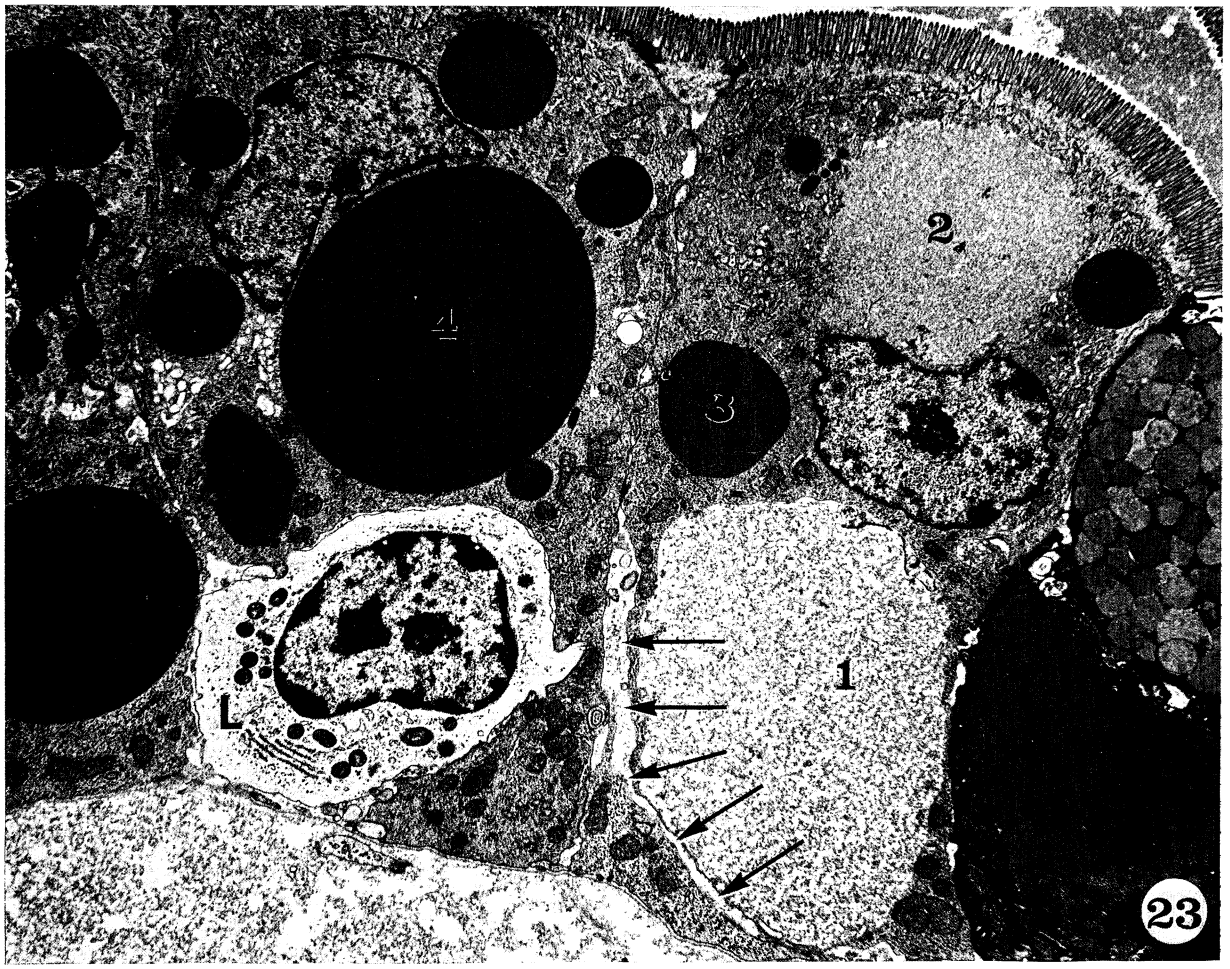
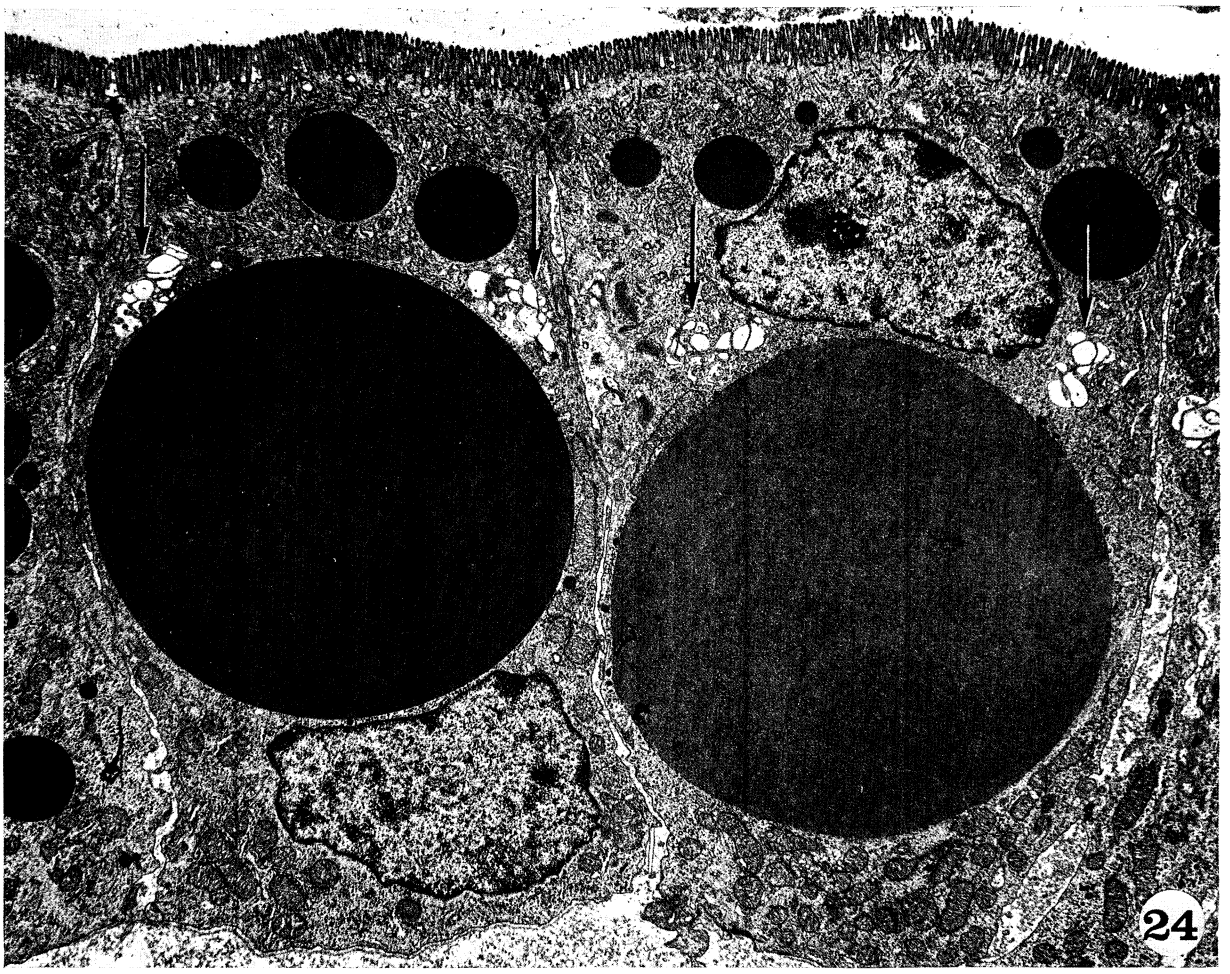
Description of plate 40

Figure 23. Suckled neonate, 9 h old. Survey showing vacuoles and inclusion bodies of graded electron-density (1–4), a leucocyte (L) lying between two cells, a deep invagination (arrows) running into a cell from the intercellular space, and the presence of a flocculent material between and below the cells. 34%, × 4670.

Figure 24. Suckled neonate, 9 h old. Non-inverted and inverted inclusion bodies in adjacent cells. Note the position of the Golgi apparatus (arrows), and the presence of flocculent material between and below the cells. 44%, × 4800.





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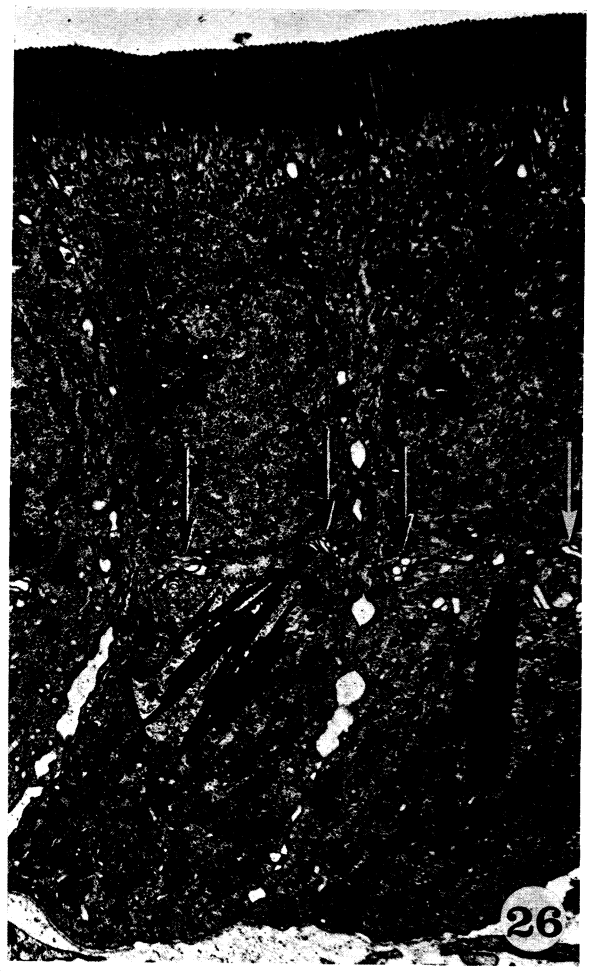

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a little, flocculent precipitate, and dense, uniformally stained globules or tubules. It was of particular interest that some of these structures lay very close to the lateral surfaces of the cell (figure 18, plate 39) and that occasionally they could be found in cup-shaped depressions of the lateral cell surface which were open to the intercellular space (figure 19, plate 39). Such observations suggested that the condensed globules could be discharged from the cell by reversed pinocytosis (exocytosis).

It is convenient to describe here three modifications of the apical system which were also present in foetal and in newborn, suckling animals as well as in unsuckled neonates. First, the lumenal surface of the membrane of some elements of the system was modified, and appeared in transverse sections as a series of heavily stained rods or granules, and in surface view as a pattern of dense lines (figure 20, plate 39). This modification was seen on parts of the cell surface, in particular on the membrane just above the *zona occludens* of the junctional complex, as well as on surface invaginations and in tubules and vesicles of the apical system. It could not, however, be clearly discerned on all elements of the system. Secondly, in specimens fixed with glutaraldehyde, tubular elements of the apical system could be seen to open abruptly into large vacuoles (figure 21, plate 39). Thirdly, a few surface invaginations (figure 7, plate 37), a few vesicles of the apical system, and many small vesicles (70 to 200 nm diam.) within the cell were surrounded by haloes of coarsely granular cytoplasm similar to the 'coated vesicles' described by Roth & Porter (1962, 1963). The small vesicles within the cell were usually located along the lateral and basal surfaces of the cell and in the Golgi zone. In some cases they could be seen in communication with the intercellular space (figure 22, plate 39).

3. *Suckled animals*

(*a*) *Light microscopy*

There were wide variations of structure in the animals examined, but one consistent feature was the presence of inclusion bodies in the majority of the ileal cells. Stages in their development were followed in adjacent cells of the younger animals. They first appeared in the apical region of the cell as vacuoles with a stainable content, which then migrated inwards and fused together to form one or a few large bodies, which occupied the greater part of the cell volume. During the formation of the inclusions, the height and area of the sectioned cells increased progressively. In the apical half of any villus, the majority of the inclusion bodies were densely and homogeneously stained (figure 11, plate 38), and were often displaced or torn from the cells as discrete bodies. The general appearance and irregular distribution of these areas suggested that such displacement was a preparation artefact. A few cells contained vacuoles with little or no stainable content and some cells contained vacuoles filled with

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DESCRIPTION OF PLATE 41

FIGURE 25. Suckled neonate, 9 h old. Very large supra-nuclear inclusion bodies. Arrows indicate the position of the Golgi apparatus. 90%, × 3470.

FIGURE 26. Suckled neonate, 28 h old. Spicules within regressing vacuolar inclusion bodies. Note the position of the Golgi apparatus (arrows). 53%, × 4400.

FIGURE 27. Suckled neonate, 28 h old. Inclusion bodies, showing various stages of regression. Some reversal has occurred, but two inclusion bodies remain above the nucleus. Note the well-developed apical system, and the position of the Golgi apparatus (arrows). 76%, × 6130.

flocculent, lightly stained material (figure 13, plate 38). This last type of vacuole was particularly common in the lower third of the villus (see below).

Some of the variations in structure could be related to three parameters: the position of the cells along the length of the small intestine; the position of the cells on the villus; and the age of the animal.

(i) *Position along the length of the small intestine.* The presence and position of inclusion bodies enabled the small intestine to be divided into four zones: their extent varied from animal to animal, and the figures given below are intended only as a general guide. The first zone occupied the initial 5 to 10 % of the small intestine measured from the pyloric incisura, and contained neither vacuoles nor inclusions. The second zone (5–10 % to 60–70 %) contained inclusion bodies which were usually located below the nucleus (figure 11, plate 38). The third zone (60–70 % to 70–80 %) contained inclusion bodies which varied in their positions: some were above the nucleus, some below, and some lateral to it. The final zone extended to the ileo-caecal valve (70–80 % to 100 %) and contained inclusion bodies which were generally located above the nucleus (figure 12, plate 38).

(ii) *Position on the villus.* It was sometimes possible to trace stages in the development of inclusions by examining the range of differentiating cells extending from the base to the apex of a villus. Intermediate stages were found which suggested that the situation in zone two (above) was the result of the inversion of a partially or fully formed inclusion body and the nucleus. The most striking change, however, was in the contents and staining properties of the inclusions. In the lower third of the villus, the majority of the inclusions were vacuoles containing varying amounts of a flocculent, lightly stained material. In the apical two-thirds, the majority of cells contained uniformly and densely stained globules. It was sometimes possible, on a single villus, to find a range of transitional forms, extending from vacuoles in cells near the base, to globules in cells near the apex.

(iii) *Increasing age.* There was a gradual change in the structure of the inclusions with increasing age. In the younger animals, the inclusion bodies were usually round or oval in form. In older animals, they became smaller and scalloped or irregular in shape. Finally, they were greatly reduced in size and either very irregular or spiculate in form (figures 14 and 15, plate 38). Their position, either above or below the nucleus, was maintained throughout these changes and corresponded with the zonation described earlier (figures 14 and 15).

Description of plate 42

Figure 28. Suckled neonate, 7 h old. Flocculent, precipitated material lying between the cells and below the basement membrane. 50 %, × 6400.

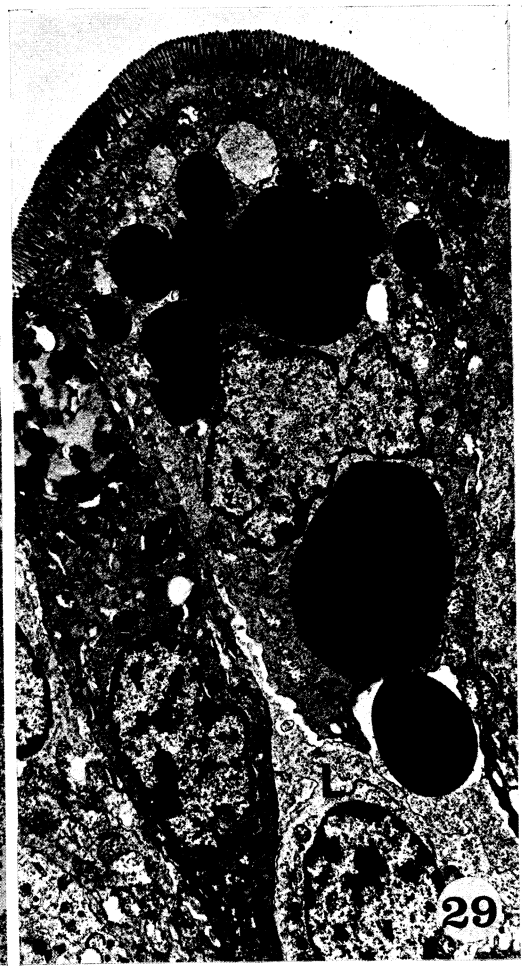
Figure 29. Suckled neonate, 9 h old, showing an inclusion body in communication with the intercellular space, and a leucocyte (L) lying between the cells. 18 %, × 4670.

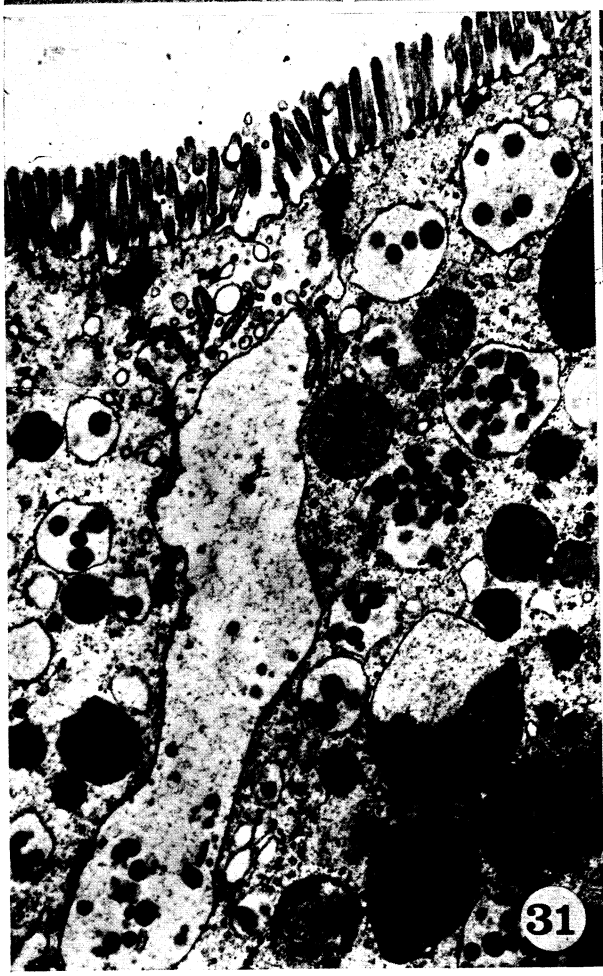
Figure 30. Suckled neonate, 19 h old. Fat droplets between two epithelial cells and below the basement membrane. 86 %, × 26 670.

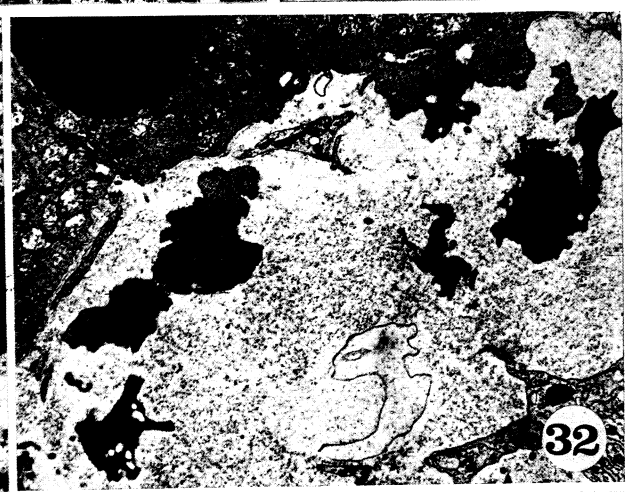
Figure 31. Suckled neonate, 16 h old. Fat droplets within the apical cytoplasm and between the cells. 90 %, × 20 000.

Figure 32. Suckled neonate, 9 h old. Membrane fragments and irregular pieces of cytoplasm lying below the basement membrane. 26 %, × 3730.

Figure 33. Suckled neonate, 9 h old. Apparent segregation of cytoplasm by the lateral fusion of smooth vesicles. 26 %, × 8270.







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(*b*) *Electron Microscopy*

Most features in the general architecture of the cells, and in the linkage and relationships between the cells, remained the same as in foetal and in newborn, unsuckled animals. Surface invaginations and an apical system, for example, were present in all the animals examined (figures 23 to 27, plates 40 and 41), although they were less developed in older animals and, in particular, the apical system contained fewer vesicular elements. The most conspicuous differences were a gradual increase in the electron density of the cytoplasmic matrix (compare figures 6, plate 36, and 23, plate 40), and the presence of inclusion bodies.

In young animals (up to 19 h) the inclusion bodies appeared as either vacuoles containing varying amounts of flocculent precipitate, or as electron-dense, uniform globules: by examining different parts of the ileum it was possible to find a range of forms intermediate between the two. Sometimes a cell contained several inclusions of various sizes (figure 23), but usually there was one large inclusion which occupied the greater part of the cell volume, with several much smaller bodies lying between it and the apical system (figure 24, plate 40). The largest inclusion bodies were found near the ileo-caecal valve (figure 25, plate 41).

The membrane of the inclusion bodies remained intact during the gradual changes with increasing age (figures 26 and 27, plate 41), and eventually the bodies were reduced to a single spicule, or to a cluster of spicules, closely invested by a membrane. Vacuoles containing flocculent precipitate also became irregular in shape and reduced in size, and eventually contained spicules (figure 26).

The position of the Golgi apparatus varied far more than in either foetal or newborn animals. The only constant relationship that could be established was in cells which contained one, large, globular inclusion body. In such cells, the Golgi apparatus was situated above, and usually close to, the apical hemisphere of the globule, a position it maintained even when the nucleus and globule were inverted (figures 24 to 27). As a result of this relationship, the Golgi apparatus was sometimes above and sometimes below the nucleus.

The lateral surfaces of the cells were often convoluted and sometimes deep clefts penetrated into the cytoplasm (figure 23). Particular attention was given to material in the intercellular spaces. Most common was a finely precipitated material which occurred both between the cells and in the interstitial space below the basement membrane (figures 23, 28, plates 40 and 42). It was found in all animals up to the age of 28 h, but was particularly abundant in the younger ones. The structure, staining properties and local variations in density of this material were strikingly similar to the flocculent material within the vacuoles.

Several electron-dense globules of up to 4 μm in diameter were found between the cells or below the basement membrane. They were morphologically similar to the small, globular inclusion bodies, which, on rare occasions, could be found partially surrounded by a cup of cytoplasm and apparently in the process of being discharged from the cells (figure 29, plate 42).

Much smaller, electron-opaque globules, 50 to 200 nm in diameter, were found in several animals. They tended to accumulate in the basal intercellular space just above the basement membrane (figure 30, plate 42), but were also present in the general interstitial space and enclosed by sacs of endoplasmic reticulum in the cell apex (figure 31, plate 42). These sacs were sometimes open to the intercellular space, and the overall pattern was nearly identical to the classical picture of fat absorption in adults. In the present material, however, absorption

seemed to be occurring along the entire length of the ileum. During the preparation of the specimens it was also noted that the lacteals along the entire length of the ileum were prominent and filled with milky chyle.

Irregular fragments of cytoplasm were present both between and below the cells. They were more commonly found below the cells (figure 32, plate 42) where they appeared to be formed from pseudopodial extensions of the basal surface which penetrated the basement membrane. There were also suggestions that areas of cytoplasm could be sequestered by the fusion of numerous enveloping vesicles of smooth endoplasmic reticulum (figure 33, plate 42), which could provide another source of origin of the cytoplasmic fragments described above.

White blood cells were observed frequently in the intercellular space (figures 23, 29, plates 40 and 42) where they penetrated as far as the junctional complex. Membrane fragments were also encountered both between and below the cells (figure 32).

Discussion

The ability to absorb intact macromolecules is present in premature animals born after 100 days gestation (Payne & Marsh 1962*a*), and in animals removed 3 to 5 days prematurely by Caesarean section (Kim, Bradley & Watson 1966). It also remains in unsuckled piglets until they die (70 h, Lecce & Morgan 1962; 72 h, Nordbring & Olsson 1958; 106 h, Payne & Marsh 1962*a*, *b*), although its efficiency falls off with time (Nordbring & Olsson 1958; Hardy 1969*a*). If the animals are allowed to suckle, however, absorption declines (or 'closes') rapidly. The usual pattern is a phase of vigorous absorption within the first 3 to 12 h of suckling (Nordbring & Olsson 1957; Speer, Brown, Quinn & Catron 1959; Miller *et al.* 1962; Payne & Marsh 1962*b*; Ullrey, Long & Miller 1966), which then falls to an insignificant level within 12 to 36 h (Miller *et al.* 1962; Lecce & Morgan 1962; Ullrey *et al.* 1966; Lecce 1966*b*, *c*), although traces of absorption may persist for up to 5 days (Kim *et al.* 1966).

There is good evidence from a number of species, including the pig, that endocytosis of macromolecules from the intestinal lumen into the tubules and vesicles of the apical system is the first step in the process of absorption (Clark 1959; Kraehenbuhl, Gloor & Blanc 1966, 1967; Kraehenbuhl & Campiche 1969). Our observations show, however, that there is only a partial correlation between the presence of an apical system and the ability to absorb macromolecules into the circulation: an apical system is present in foetuses for up to 4 weeks before birth, and in suckled neonates for an extended period after the transfer of maternal immunoglobulins into the circulation has effectively ceased. It is suggested, therefore, that the ability to endocytose is a property of the ileal epithelium which is present during a considerable period of development and which spans the normal time of birth. Such an ability does not imply that endocytosed material can be absorbed into the bloodstream, since this will only be achieved if other mechanisms such as intracellular transport and release are operative.

Lecce (1966*b*, *c*) has suggested that the mechanism of closure may be an apical event, possibly the exhaustion of surface membrane available for endocytosis, but two of our observations imply that this is not the case. First, a well-developed apical system is present for a considerable period after the normal time of closure, and secondly, some endocytosed material is retained within the cells as inclusion bodies, even though absorption in the pig is effectively non-selective. There is also evidence that the apical system may persist for a far longer period than that covered in our investigation: Kenworthy *et al.* (1967) have reported

a well-developed apical system in the jejunum of suckling piglets after 6 days and have found that traces of it are still present after 5 weeks. For these reasons, it is suggested that endocytosis is not a limiting factor, and that closure represents a failure either of intracellular processing or of release. A similar conclusion has been reached recently by Clarke & Hardy (1970) who showed that cellular uptake was not the limiting factor in the transfer of polyvinyl pyrrolidone across the wall of the neo-natal pig intestine. In these experiments, the polymer was found to be taken up by the cells for up to 14 days after birth.

In the present studies, some areas of the surface membrane, and some parts of the apical system, were structurally modified, and at least two sorts of modification could be found in all three categories of animals examined. The most frequent and noticeable modification involved the luminal surface of the membranes which, in transverse section, appeared as a series of granules or rodlets and, in surface view, as a pattern of dense lines. Similar structures have been reported in earlier studies of neo-natal piglets and described as 'bristles' (Sibalin & Björkman 1966), or as 'spinous processes' (Staley *et al.* 1968). They are probably analogous with structures found in similar locations in the ileal cells of suckling rats, which have been variously described as 'bristles' (Oberti 1961), 'spherical particles arranged in parallel rows' (Graney 1964), 'closely packed parallel ridges' (Porter, Kenyon & Badenhausen 1967), and 'regular plaques with a particle associated with each' (Wissig & Graney 1968). Their significance is unknown, but it has been suggested that they might represent either receptor sites or digestive enzymes (Staley *et al.* 1968; Wissig & Graney 1968).

The second modification was seen less frequently. It consisted of a fuzzy or particulate coating attached to the cytoplasmic side of the membrane, and was seen covering a few of the surface invaginations, some of the vesicles in the apical system, and some small vesicles (70 to 200 nm diam.) near the Golgi zone and along the lateral and basal surfaces of the cell, with which they were often joined. These structures closely resembled the 'coated vesicles' described by Roth & Porter (1962, 1963), who suggested that they might be specialized for the cellular uptake of protein. Further work has strengthened this hypothesis, and there is evidence that coated vesicles are involved in the uptake of albumen, ferritin, peroxidase and haemoglobin in the proximal convoluted tubule of the kidney (Ericsson 1965; Maunsbach 1966*a*, *b*; Graham & Karnovsky 1966), in the uptake of peroxidase in the cells of the vas deferens (Friend & Farquhar 1967), in the uptake of ferritin into neurones (Rosenbluth & Wissig 1964), in the uptake of yolk proteins into oocytes (Stay 1965), and in the cellular uptake of haemolymph in aphids (Bowers 1964).

Whatever the function of coated vesicles in the intestinal epithelium may be, it seems unlikely that they represent the exclusive channel for protein uptake: most of the surface invaginations and most elements of the apical system are not coated, and neither vacuoles nor inclusion bodies are surrounded by coated membranes. Friend & Farquhar (1967) have suggested that there may be several types of coated vesicle: in studies on the rat vas deferens they distinguished at least two categories—the 'large' (over 100 nm diam.) which were involved in the cellular uptake of protein, and the 'small' (75 nm diam.) which were concerned with the transport of enzymes, and possibly of other materials, from the Golgi zone. The vesicles which we have observed would include both of these categories.

It is not known how endocytosed macromolecules are released from the epithelial cells during the period of transcellular absorption, but we have regularly found a flocculent, precipitated material both between and below the cells of the ileal epithelium in suckled

animals up to the age of 28 h. It was particularly prominent in younger animals, but was not present in foetuses, in unsuckled neonates, or in suckled neonates over 28 h. Both its texture and its staining properties resembled the flocculent material present in some of the cellular vacuoles. These observations suggest that vacuoles may be able to discharge their contents into the intercellular space, an assumption which would also explain the irregularities and invaginations which were frequently present along the lateral surfaces of the cells.

This possibility receives some support from the fact that small inclusion bodies are sometimes discharged from the cells by exocytosis in foetal and in both unsuckled and suckled neo-natal animals. Since inclusion bodies are apparently derived from vacuoles, it seems reasonable to assume that vacuoles may be treated in a similar way. However, there was no evidence that large inclusion bodies, regressing inclusion bodies or spicules could be exocytosed. This may be because these categories of inclusion body are found only in cells which have 'closed', but the problem requires further investigation.

The range of structures found in the epithelial cells suggests that the homogeneous inclusion bodies are derived from vacuoles containing light, flocculent material, by a process of concentration and condensation. Such a process may account for the decline in vacuolation and for the increased number of inclusion bodies which are found in unsuckled animals after birth. It is perhaps also significant that the Golgi apparatus, an organelle which is implicated in the cellular partition of water (Grundmann 1966), is usually closely applied to mature inclusion bodies no matter whether they are above or below the nucleus.

Simple concentration cannot, however, explain the phase of regression during which the inclusion bodies are reduced to spicules. These structures can occur singly or in clusters, but in either case they are bounded by a membrane. They are visible with the light microscope and probably correspond to the lamellar bodies described by Clarke & Hardy (1970). It seems likely that a digestive phase is involved in their development, but the histo-chemistry of the process and the possible involvement of lysosomes remain to be investigated.

Fat absorption in the adult normally occurs in the early part of the small intestine (Strauss 1968), and experiments *in vitro* (Johnston 1959) suggest that this reflects the differing intrinsic properties of the intestinal cells. In the neo-natal suckled animals which we have examined, however, fat absorption is apparently occurring along the entire length of the small intestine, in a manner which is identical morphologically with that described for adult animals (Cardell, Badenhausen & Porter 1967; Strauss 1968). Previous workers have suggested that fat absorption might be atypical in young animals (Matisson & Karlsson 1966), but there has been no previous indication that it occurs over the entire length of the ileum. The presence of fat absorption, particularly if it is atypical, raises questions about the interpretation of our results, since large fat droplets appear very similar to inclusion bodies in the electron microscope. Indeed, in particular cases, it is not possible to decide whether a small globular body is a fat droplet or an inclusion body. However, many stages of fat absorption can be identified as such, and it is clear that the majority of the inclusion bodies described in this paper, especially the larger ones, are not fat droplets: they are present and stainable in wax sections; they are present in foetal animals and in unsuckled animals, where fat absorption is unlikely to be occurring; they are related to vacuoles through a series of transitional forms; and they undergo a very characteristic phase of regression. We feel, therefore, that the presence of fat absorption does not alter or invalidate our interpretations.

The obvious zonation of the small intestine in suckled animals raises several questions. The

first 5 to 10 % (measured from the pyloric incisura) contained neither vacuoles nor inclusions, which suggests that the duodenal cells are not involved in the uptake of ingested material. A similar conclusion was reached by Clarke & Hardy (1970), but there is at least one report (Kaeberle & Segre 1964) that orally administered albumins can be taken up by the duodenal cells. The significance of the differences between zones 2 and 4 remains enigmatic. It is apparently not related to any marked variation in the ability to take up macromolecules (Clarke & Hardy 1970): the only correlation which can be suggested is that inversion is least likely to occur in those cells which have absorbed a noticeable quantity of meconium *in utero* However, the most proximal part of the vacuolated area in foetal animals shows some evidence of inversion, and it seems probable, therefore, that the general zonation of the small intestine is established *in utero* and that the correlation is fortuitous.

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References

Asplund, J. M., Grummer, R. H. & Phillips, P. H. 1962 Absorption of colostral gamma-globulins and insulin by the new-born pig. *J. Anim. Sci.* **21**, 412–413.

Balconi, I. R. & Lecce, J. G. 1966 Intestinal absorption of homologous lactic dehydrogenase iso-enzymes by the neonatal pig. *J. Nutr.* **88**, 233–238.

Bowers, B. 1964 Coated vesicles in the pericardial cells of the aphid (*Myzus persicae*). *Protoplasma* **59**, 351–367.

Cardell, R. R. Jr., Badenhausen, S. & Porter, K. R. 1967 Intestinal triglyceride absorption in the rat: an electron microscopical study. *J. Cell Biol.* **34**, 123–155.

Clark, S. L. 1959 The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice. *J. biophys. biochem. Cytol.* **5**, 41–48.

Clarke, R. M. & Hardy, R. N. 1970 Histological changes in the small intestine of the young pig and their relation to macromolecular uptake. *J. Anat.* (in the Press).

Comline, R. S., Pomeroy, R. W. & Titchen, D. A. 1953 Histological changes in the intestine during colostrum absorption. *J. Physiol.*, **122**, 6*P*.

Ericsson, J. L. E. 1965 Transport and digestion of haemoglobin in the proximal tubule. II. Electron microscopy. *Lab. Invest.* **14**, 16–39.

Farquhar, M. G. & Palade, G. E. 1963 Junctional complexes in various epithelia. *J. Cell Biol.* **17**, 375–412.

Friend, D. S. & Farquhar, M. G. 1967 Functions of the coated vesicles during protein absorption in the rat vas deferens. *J. Cell Biol.* **35**, 357–376.

Graham, R. C. & Karnovsky, M. J. 1966 The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. Ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**, 291–302.

Graney, D. O. 1964 Ultrastructure of the apical plasma membrane of intestinal lining cells. *Anat. Rec.* **148**, 373–374.

Grundmann, E. 1966 In *General cytology*, pp. 256, 276. London: Arnold.

Hardy, R. N. 1965 Intestinal absorption of macromolecules in the new-born pig. *J. Physiol.* **176**, 19–20*P*.

Hardy, R. N. 1969*a* The absorption of polyvinyl pyrrolidone by the new-born pig intestine. *J. Physiol.* **204**, 633–651.

Hardy, R. N. 1969*b* The breakdown of [^{131}I]γ-globulin in the digestive tract of the new-born pig. *J. Physiol.* **205**, 435–451.

Johnston, J. M. 1959 Site of fatty acid absorption. *Proc. Soc. exp. Biol. Med.* **100**, 669–670.

Kaeberle, M. L. & Segre, D. 1964 Intestinal absorption of homologous and heterologous serum globulins by the new-born pig. *Am. J. vet. Res.* **25**, 1096–1102.

Kenworthy, R., Stubbs, J. M. & Syme, G. 1967 Ultrastructure of small-intestinal epithelium in weaned and unweaned pigs and pigs with post-weaning diarrhoea. *J. Path. Bact.* **93**, 493–498.

Kim, Y. B., Bradley, S. G. & Watson, D. W. 1966 Antibody synthesis in germ-free colostrum-deprived miniature piglets. In *Swine in biochemical research* (Ed. L. K. Bustad & R. O. McClellan, pp. 273–284. Richland, Washington: Battelle.

Kraehenbuhl, J.-P., Gloor, E. & Blanc, B. 1966 Morphologie comparée de la muqueuse intestinale de deux espèces animales aux possibilités d'absorption protéique néonatale différentes. *Z. Zellforsch. mikrosk. Anat.* **70**, 209–219.

Kraehenbuhl, J.-P., Gloor, E. & Blanc, B. 1967 Résorption intestinale de la ferritine chez deux espèces animales aux possibilités d'absorption protéique néonatale différentes. *Z. Zellforsch. mikrosk. Anat.* **76**, 170–186.

Kraehenbuhl, J.-P. & Campiche, M. A. 1969 Early stages of intestinal absorption of specific antibodies in the new-born. An ultrastructural, cytochemical and immunological study in the pig, rat and rabbit. *J. Cell Biol.* **42**, 345–365.

Lecce, J. G. 1966*a* Glucose milliequivalents eaten by the neonatal pig and cessation of intestinal absorption of large molecules (closure). *J. Nutr.* **90**, 240–244.

Lecce, J. G. 1966*b* Absorption of macromolecules by neonatal intestine. *Biol. Neonat.* **9**, 50–61.

Lecce, J. G. 1966*c* *In vitro* absorption of γ-globulin by neonatal intestinal epithelium of the pig. *J. Physiol.* **184**, 594–604.

Lecce, J. G., Matrone, G. & Morgan, D. O. 1961 Porcine neonatal nutrition: absorption of unaltered non-porcine proteins and polyvinyl pyrrolidone from the gut of piglets and the subsequent effect on the maturation of the serum protein profile. *J. Nutr.* **73**, 158–166.

Lecce, J. G. & Morgan, D. O. 1962 Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in neonatal pig and lamb. *J. Nutr.* **78**, 263–268.

Luft, J. H. 1961 Improvements in epoxy resin embedding methods. *J. biophys. biochem. Cytol.* **9**, 409–414.

Matisson, A. G. M. & Karlsson, B. W. 1965 Observations on the structure of intestinal epithelium cells in new-born piglets. *J. Ultrastruct. Res.* **12**, 243.

Matisson, A. G. M. & Karlsson, B. W. 1966 Electron microscopic and immunological studies on the small intestine of newborn piglets. *Ark. Zool.* **18**, 575–589.

Maunsbach, A. B. 1966*a* Absorption of ^{125}I-labelled homologous albumen by rat kidney proximal tubule cells. A study of microperfused proximal tubules by electron microscopic autoradiography and histochemistry. *J. Ultrastruct. Res.* **15**, 197–241.

Maunsbach, A. B. 1966*b* Absorption of ferritin by rat kidney tubule cells. Electron microscopic observations of the initial uptake phase in cells of microperfused proximal tubules. *J. Ultrastruct. Res.* **16**, 1–12.

Miller, E. R., Harmon, B. G., Ullrey, D. E., Schmidt, D. A., Lueke, R. W. & Hoefer, J. A. 1962 Antibody absorption, retention and production by the baby pig. *J. Anim. Sci.* **21**, 309–314.

Morris, I. G. 1968 Gamma globulin absorption in the new-born. In *Handbook of physiology*, Section 6, vol. III, pp. 1491–1512. Washington: American Physiological Society.

Norbring, F. & Olsson, B. 1957 Electrophoretic and immunological studies on the sera of young pigs. I. Influence of ingestion of colostrum on protein pattern and antibody titre in sera from suckling pigs and the changes throughout lactation. *Acta Soc. Med. upsal.* **62**, 193–212.

Norbring, F. & Olsson, B. 1958 Electrophoretic and immunological studies on the sera of young pigs. II. The effect of feeding bovine trypsin inhibitor with porcine colostrum on the absorption of antibodies and immune globulins. *Acta Soc. Med. upsal.* **63**, 25–40.

Oberti, C. 1961 Electron microscope studies of the intestinal epithelium. II. Pinocytosis. *Biologica* **31**, 77–88.

Olsson, B. 1959*a* Studies on the formation and absorption of antibodies and immune globulins in piglets. II. The intestinal absorption of antibodies and immune globulins by new-born piglets after the administration of bovine colostrum. *Nord. VetMed.* **11**, 355–390.

Olsson, B. 1959*b* Studies on the formation and absorption of antibodies and immune globulins in piglets. III. The intestinal absorption of heterologous antibodies and serum proteins in new-born piglets. *Nord. VetMed.* **11**, 441–460.

Payne, L. C. & Marsh, C. L. 1962*a* Absorption of gamma globulin by small intestine. *Fedn. Proc.* **21**, 909–912.

Payne, L. C. & Marsh, C. L. 1962*b* Gamma globulin absorption in the baby pig: the nonselective absorption of heterologous globulins and factors influencing absorption time. *J. Nutr.* **76**, 151–158.

Peachey, L. D. 1958 Thin sections: a study of section thickness and physical distortion produced during microtomy. *J. biophys. biochem. Cytol.* **41**, 233–242.

Porter, K. R., Kenyon, K. & Badenhausen, S. 1967 Specializations of the unit membrane. *Protoplasma* **63**, 262–274.

Reynolds, E. S. 1963 The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–213.

Rosenbluth, J. & Wissig, S. L. 1964 The distribution of exogenous ferritin in toad spinal ganglia and the mechanism of its uptake by neurons. *J. Cell Biol.* **23**, 307–325.

Roth, T. F. & Porter, K. R. 1962 Specialized sites on the cell surface for protein uptake. In S. S. Bresse (ed.). *5th International Congress for Electron Microscopy*, vol. 2, p. LL-4. London: Academic Press.

Roth, T. F. & Porter, K. R. 1963 Membrane differentiation for protein uptake. *Fedn. Proc.* **22**, 178.

Sauer, F. C. 1937 Some factors in the morphogenesis of the vertebrate embryonic epithelia. *J. Morph.* **61**, 563–580.

Sibalin, M. & Björkman, N. 1966 On the fine structure and absorptive function of the porcine jejunal villi during the early suckling period. *Expl Cell Res.* **44**, 165–174.

Speer, V. C., Brown, H., Quinn, L. Y. & Catron, D. V. 1959 The cessation of antibody absorption in the young pig. *J. Immun.* **83**, 632–634.

Staley, T. E., Jones, E. W. & Marshall, A. E. 1968 The jejunal absorptive cell of the newborn pig: an electron microscopic study. *Anat. Rec.* **161**, 497–515.

Stay, B. 1965 Protein uptake in the oocytes of the cecropia moth. *J. Cell Biol.* **26**, 49–62.

Stempak, J. G. & Ward, R. T. 1964 An improved staining method for electron microscopy. *J. Cell Biol.* **22**, 697–701.

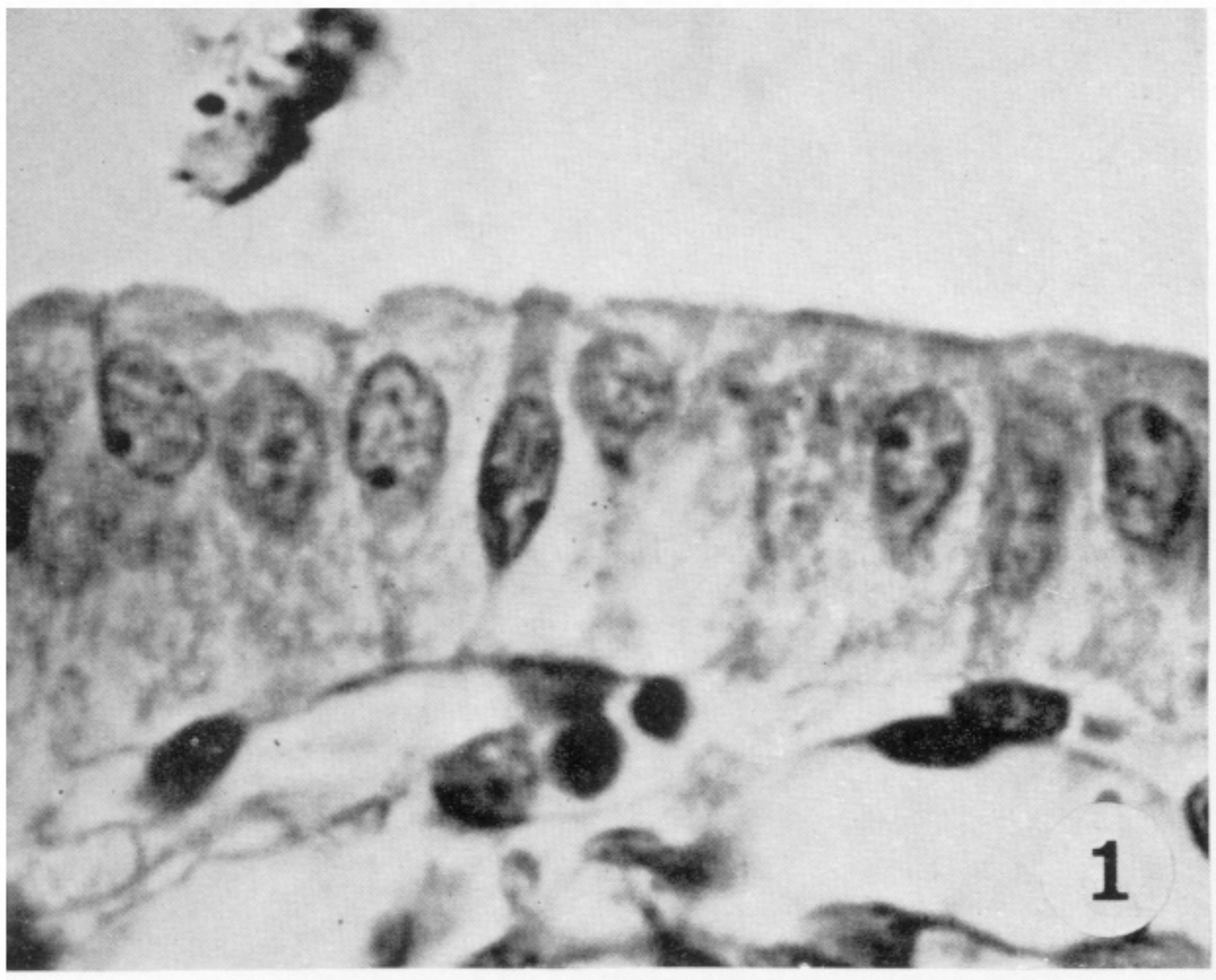
Strauss, E. W. 1968 Morphological aspects of triglyceride absorption. In *Handbook of physiology*, Section 6, vol. III, pp. 1377–1406. Washington: American Physiological Society.

Ullrey, D. E., Long, C. H. & Miller, E. R. 1966 Absorption of intact protein from the intestinal lumen of the neo-natal pig. In *Swine in biomedical research* (ed. L. K. Bustad & R. O. McClellan), pp. 249–262. Richland, Washington: Battelle.

Vodover, N. & Fléchon, J.-E. 1966 La cellule épithéliale absorbante de l'intestin grêle du porc ultrastructure. *Ann. Biol. anim., Biochem. Biophys.* **6**(i), 13–32.

Williams, N. A. & Meek, G. A. 1966 Studies on thickness variation in ultra-thin sections for electron microscopy. *Jl R. microsc. Soc.* **85**, 337–352.

Wissig, S. L. & Graney, D. O. 1968 Membrane modifications in the apical endocytic complex of ileal epithelial cells. *J. Cell Biol.* **39**, 564–579.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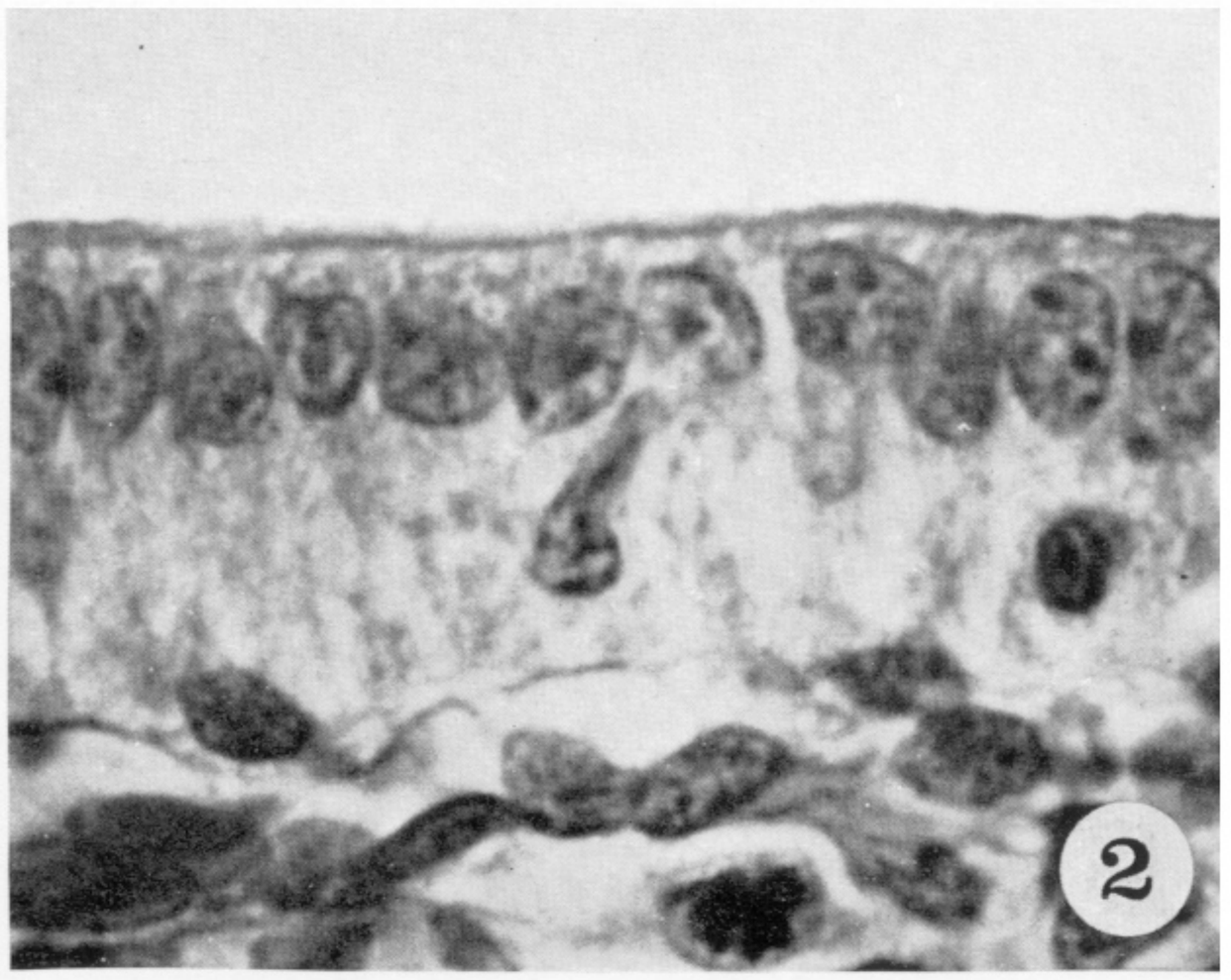
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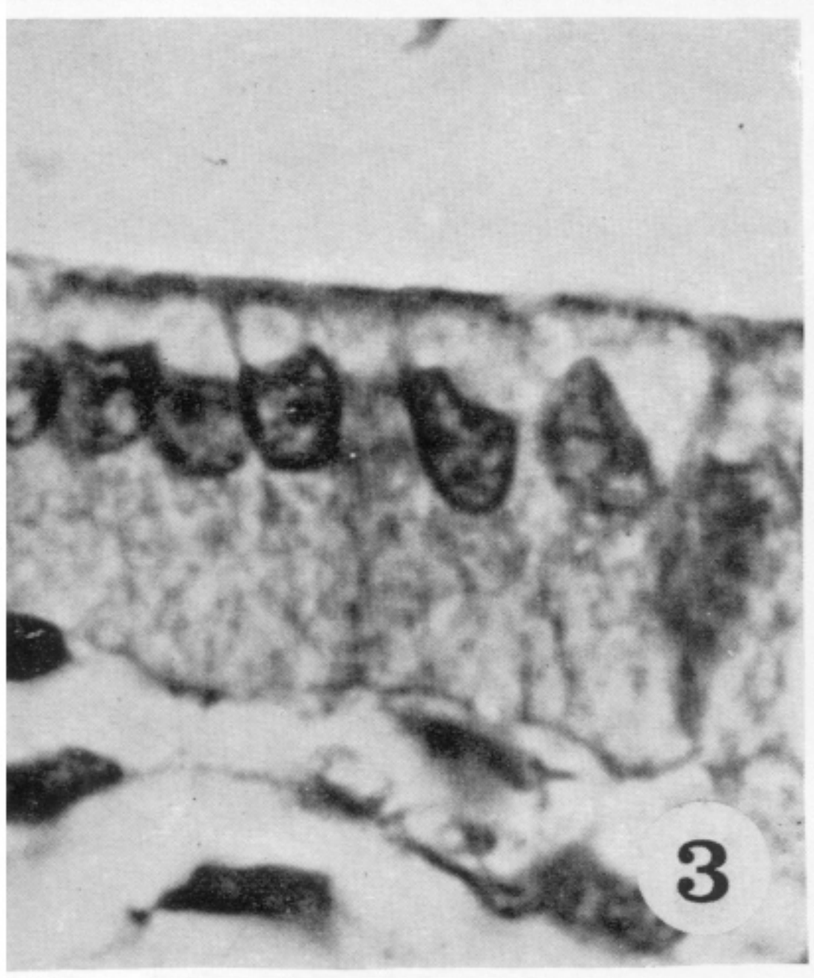
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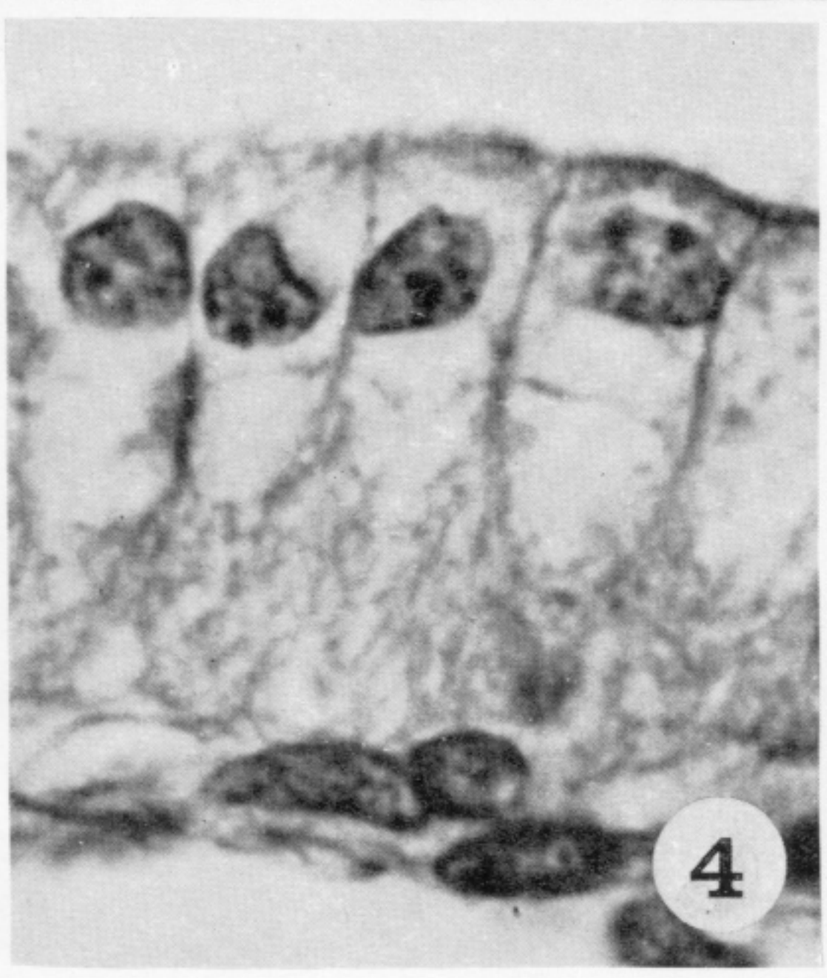
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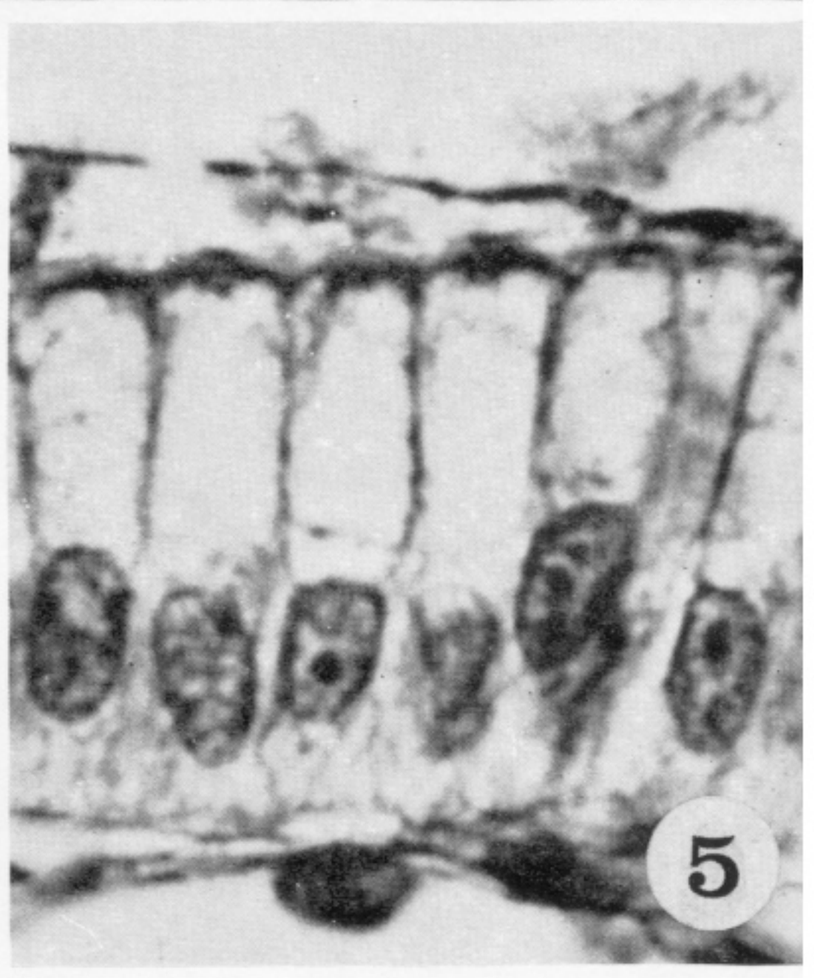
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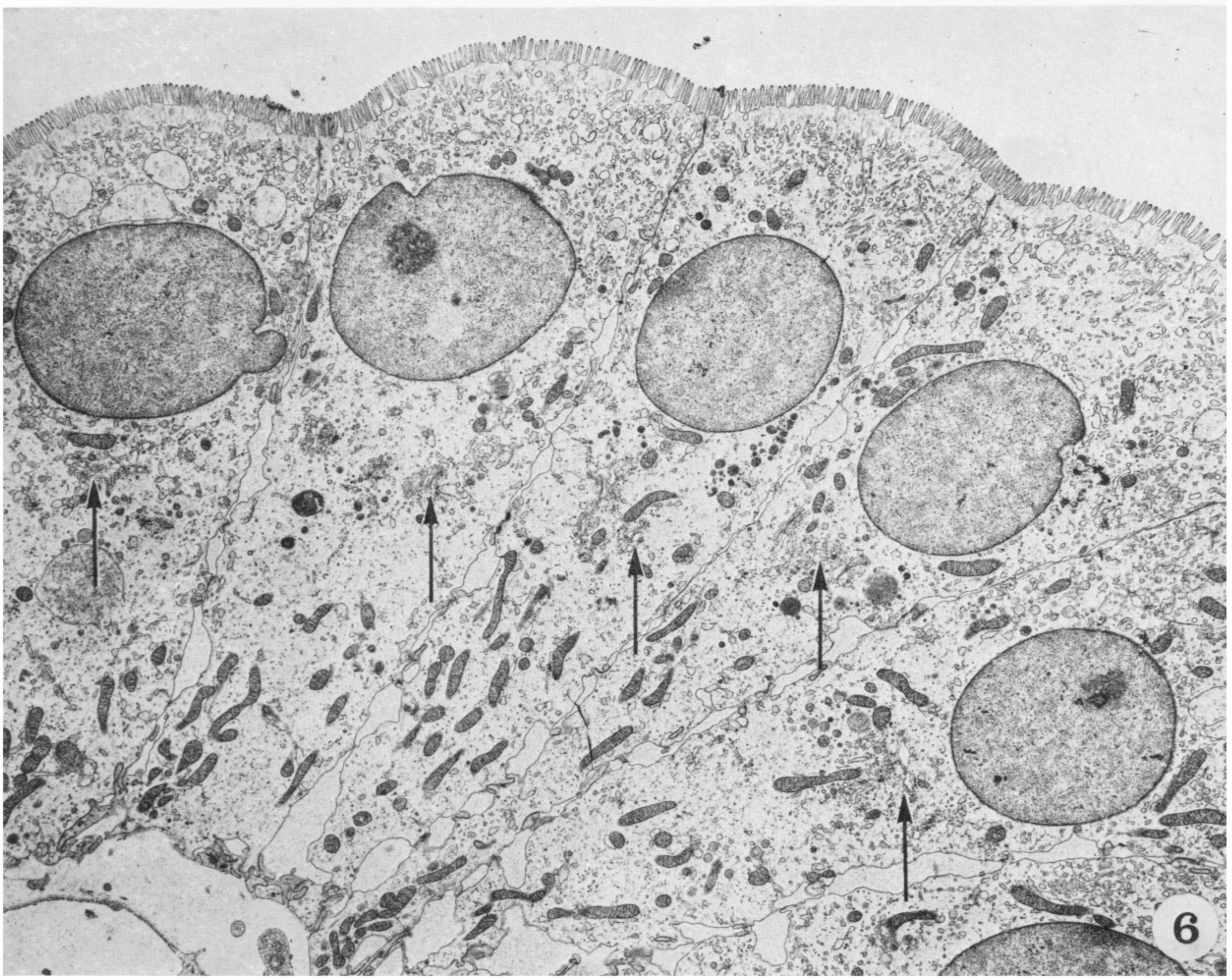
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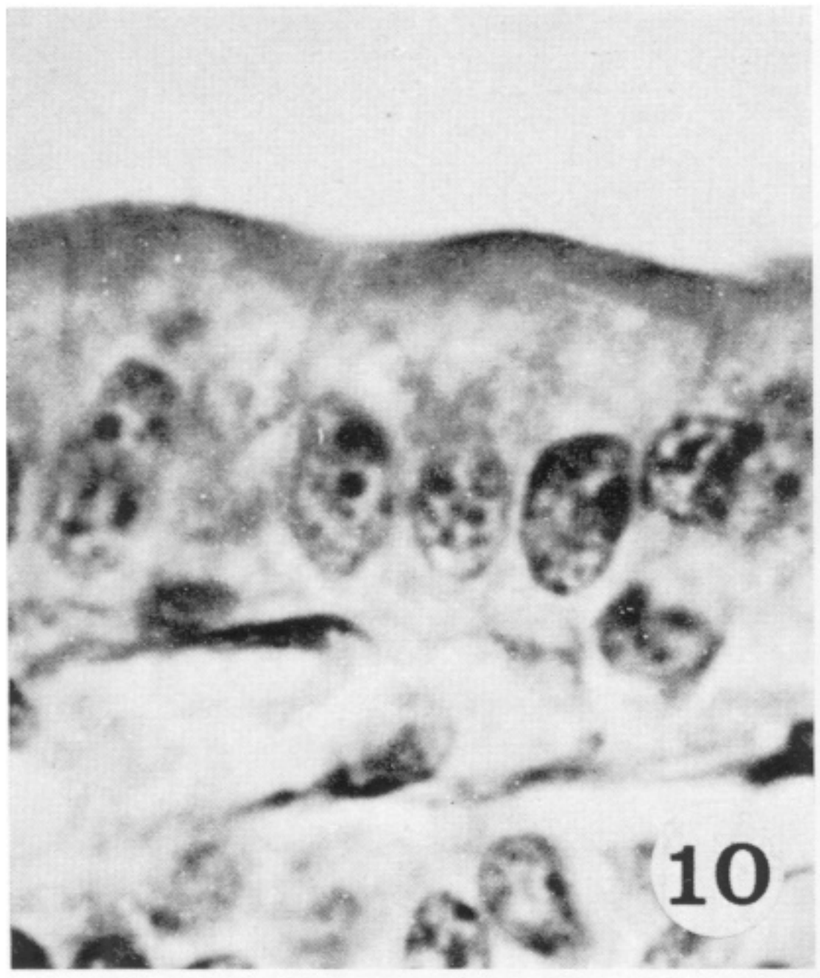

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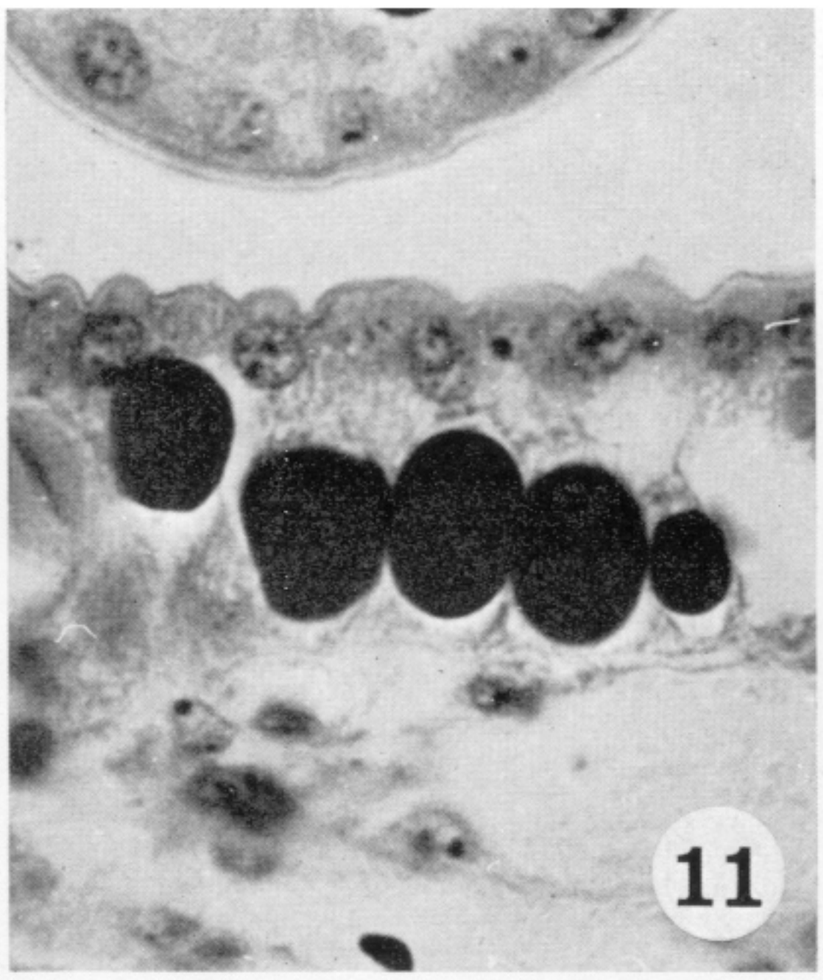

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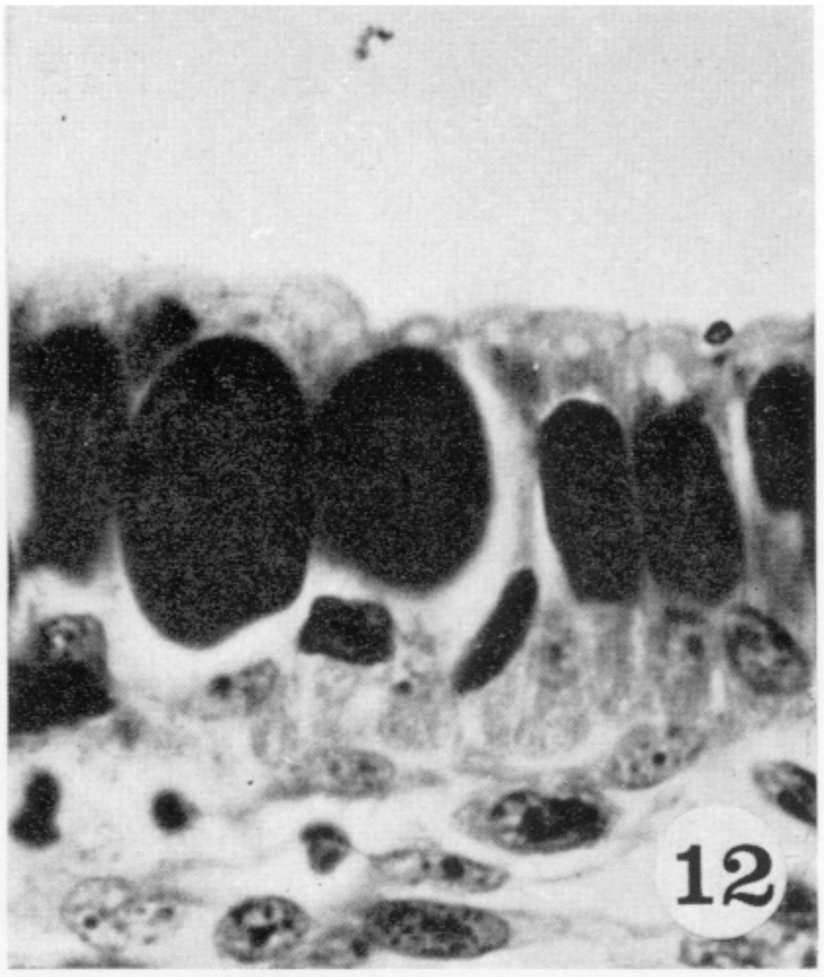

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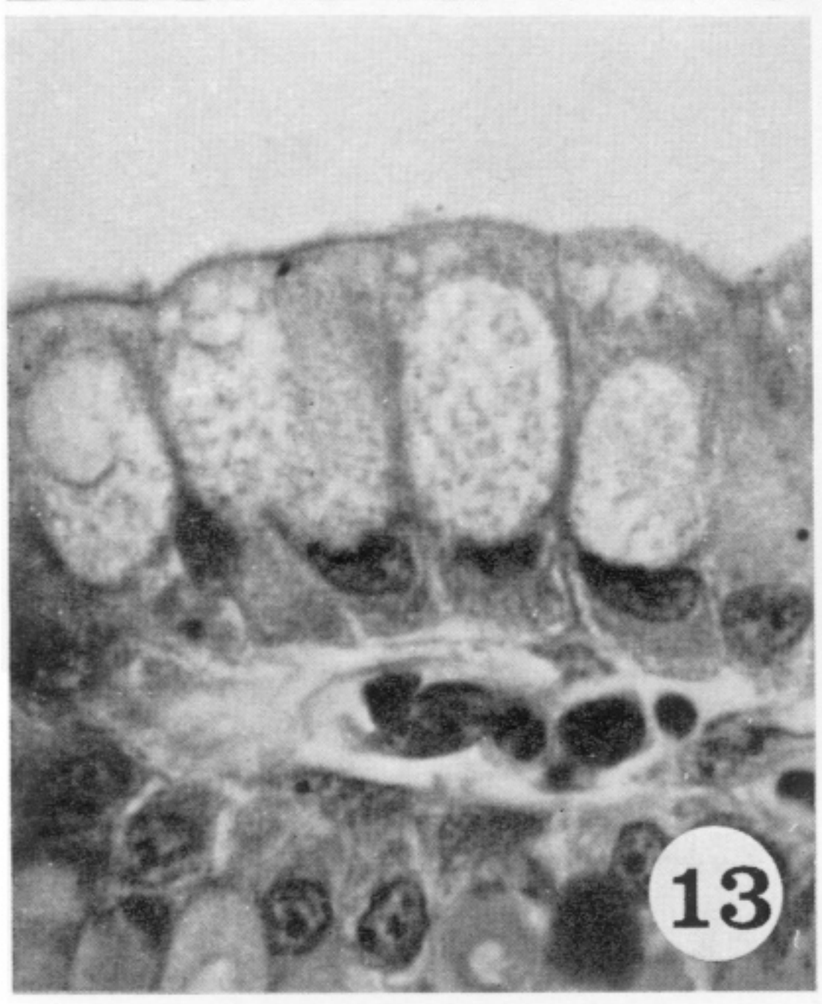

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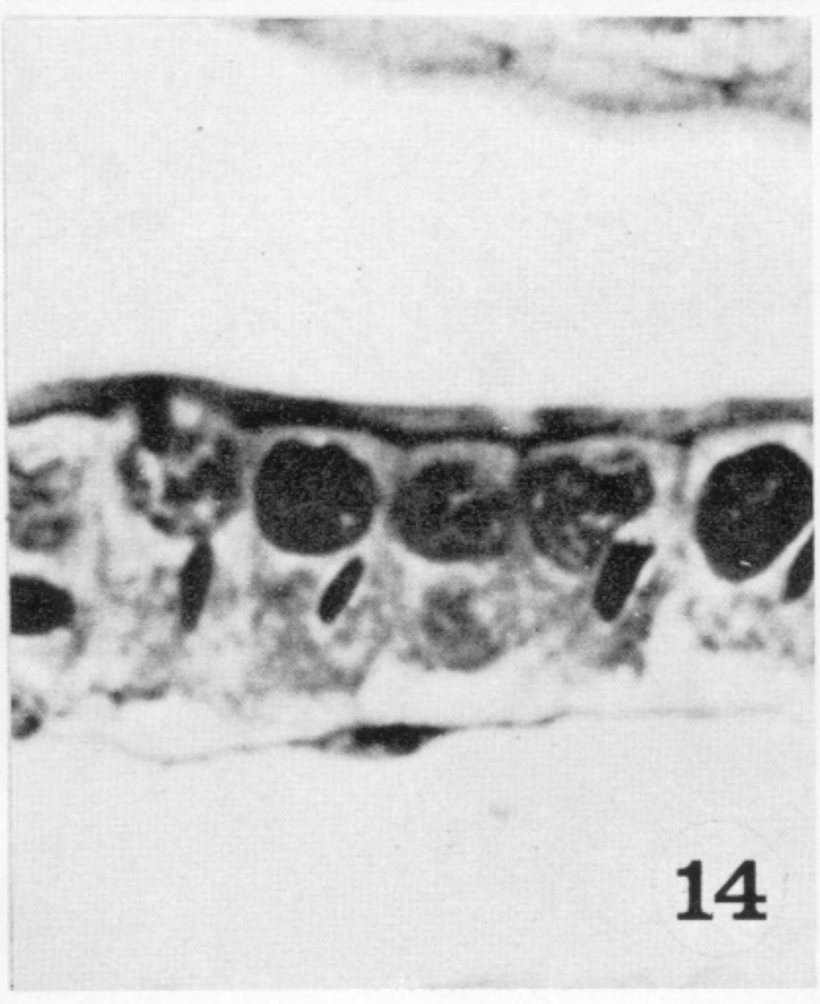

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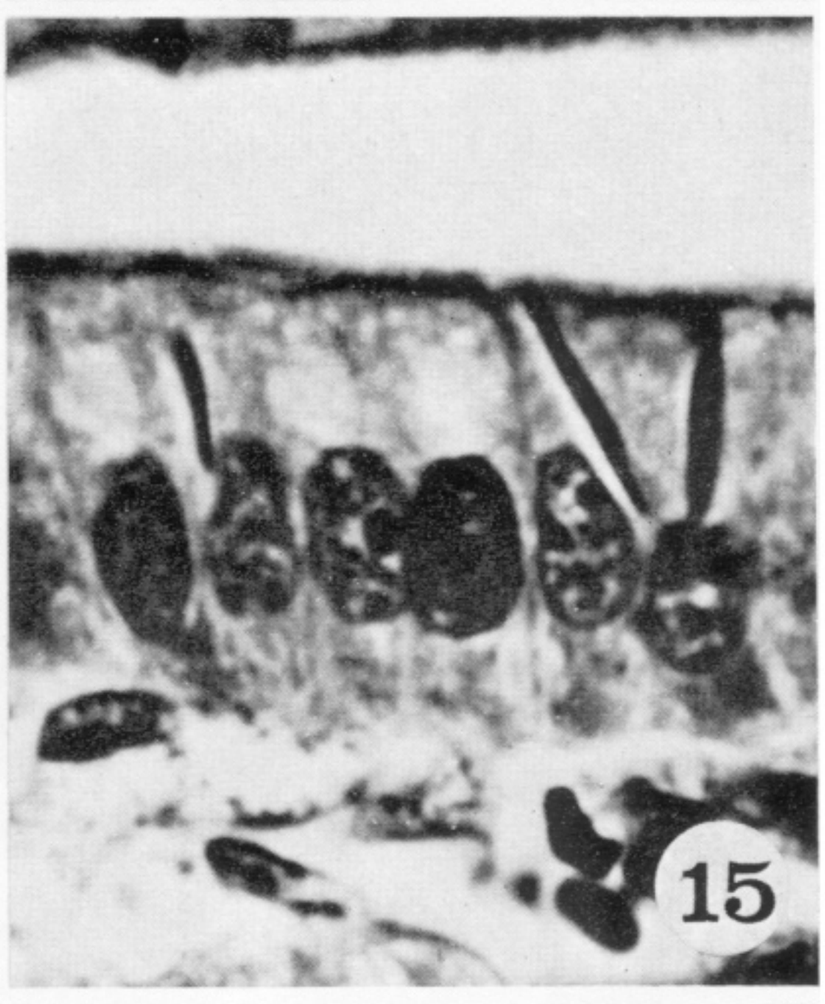

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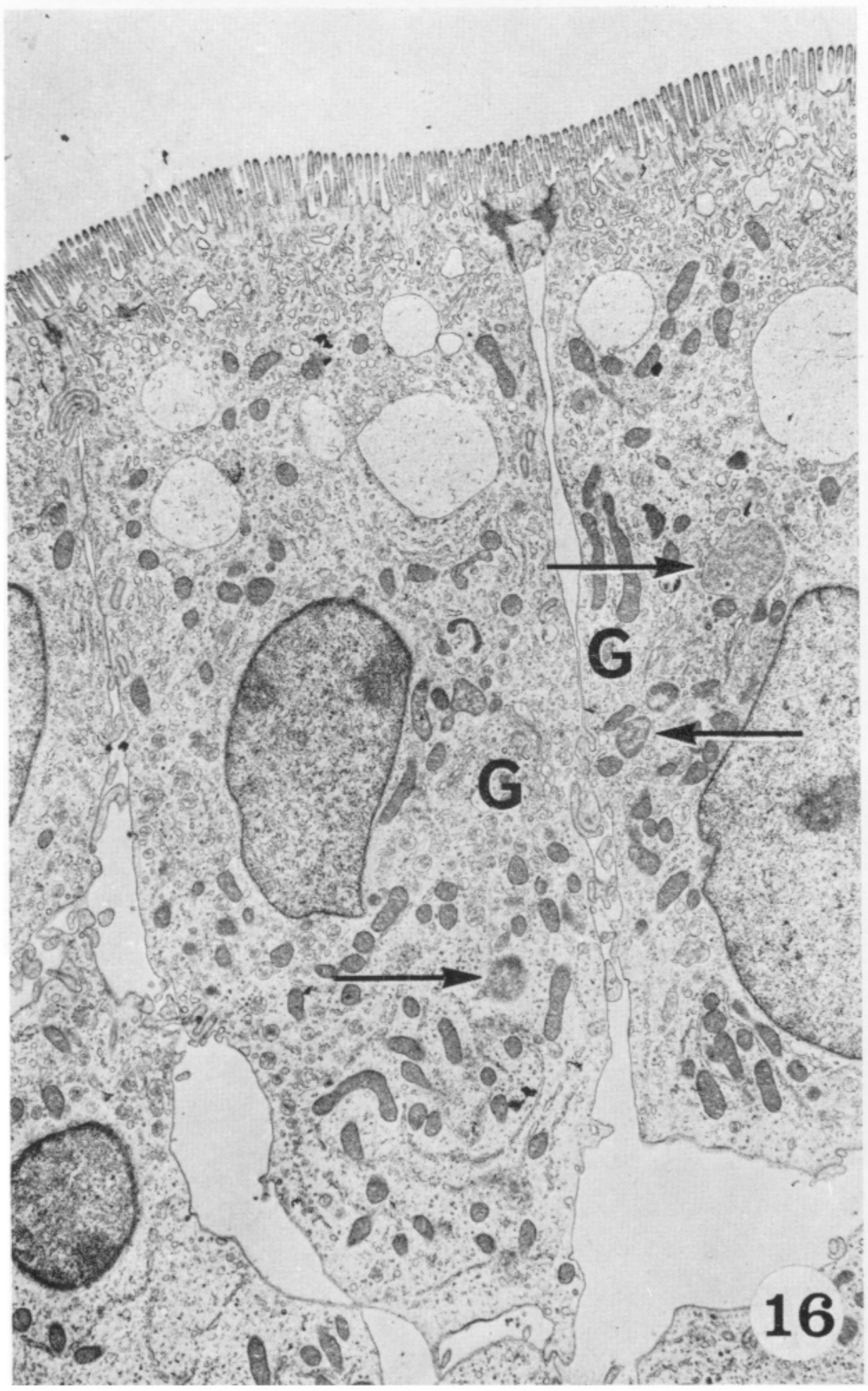

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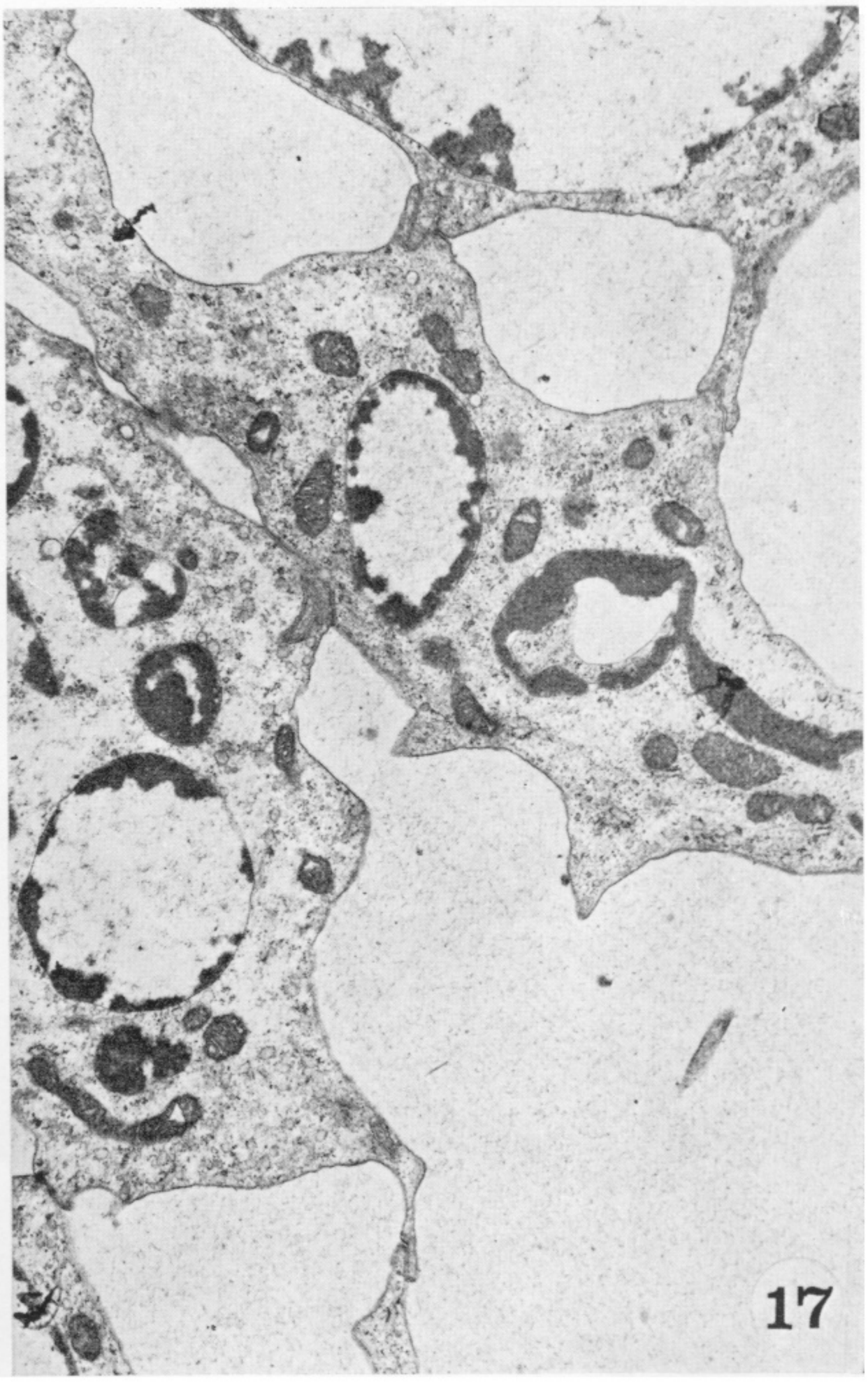

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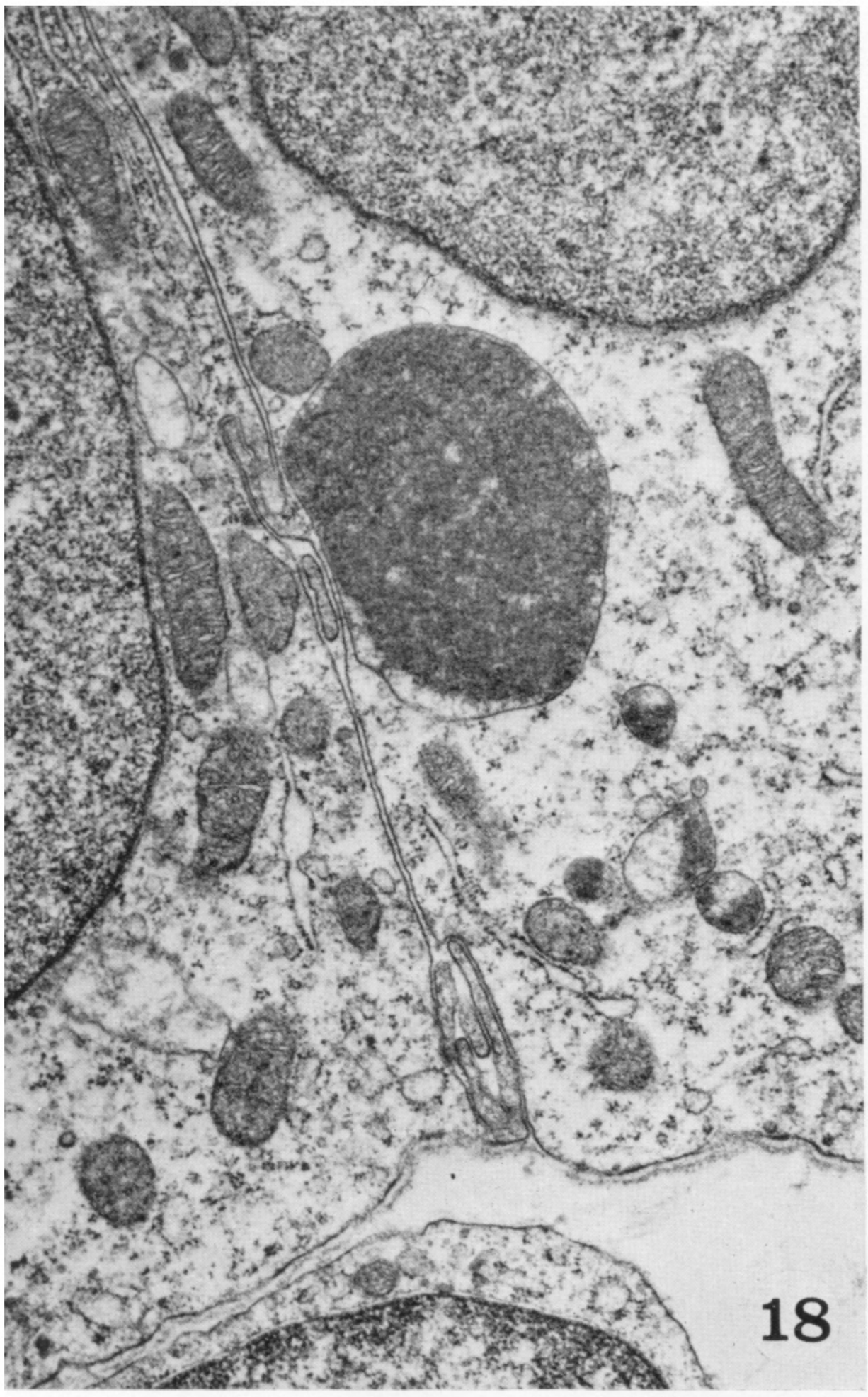

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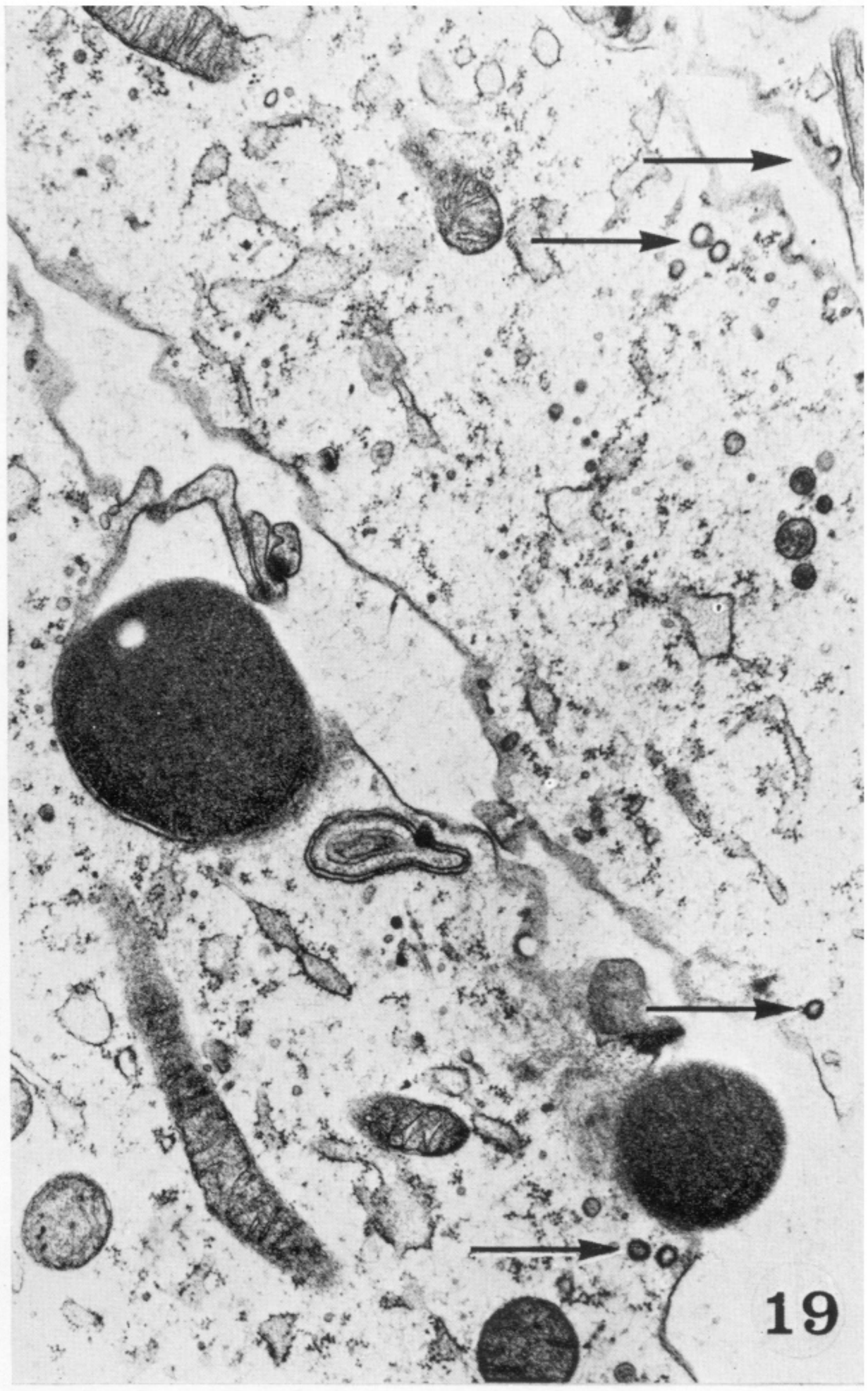

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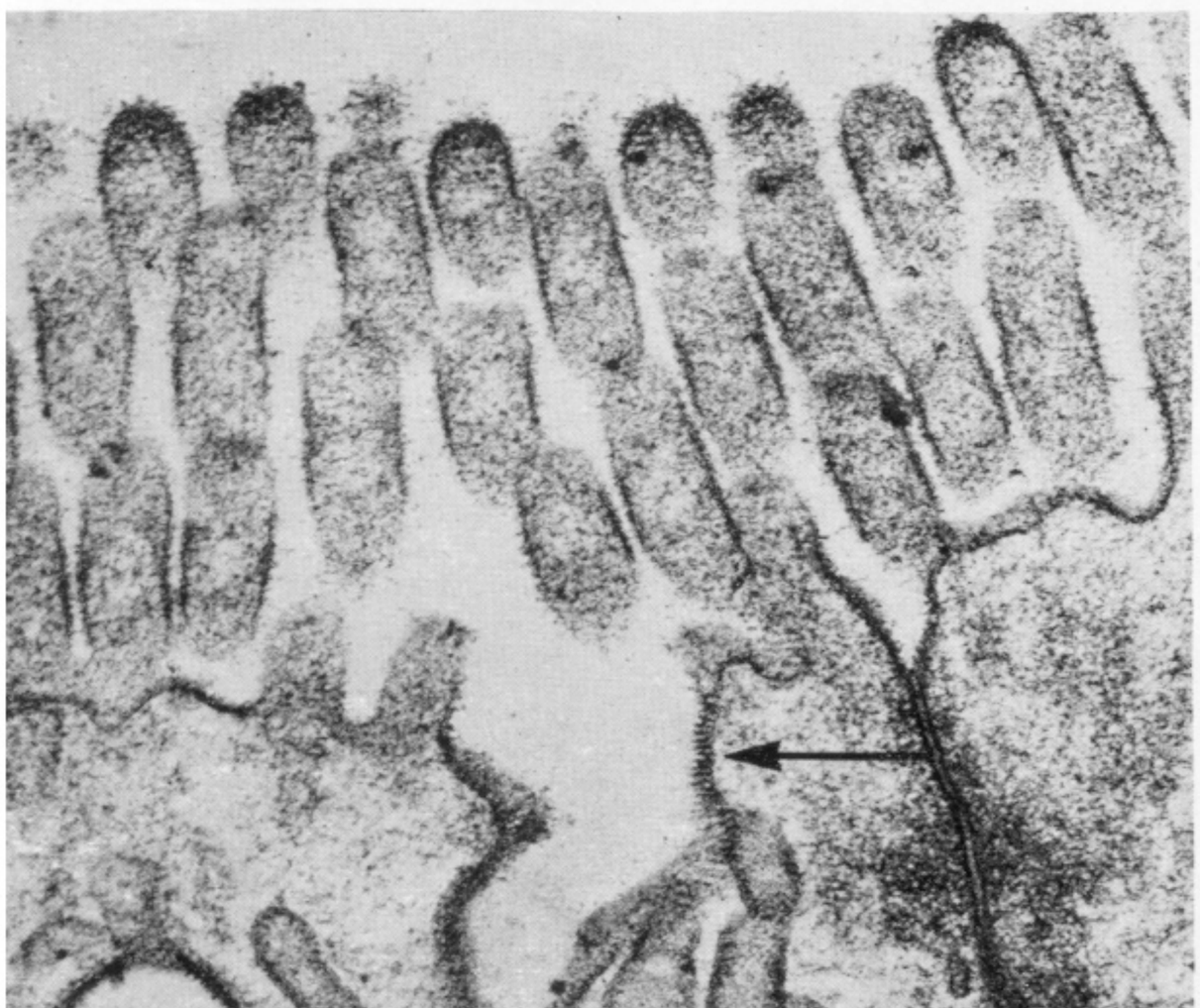

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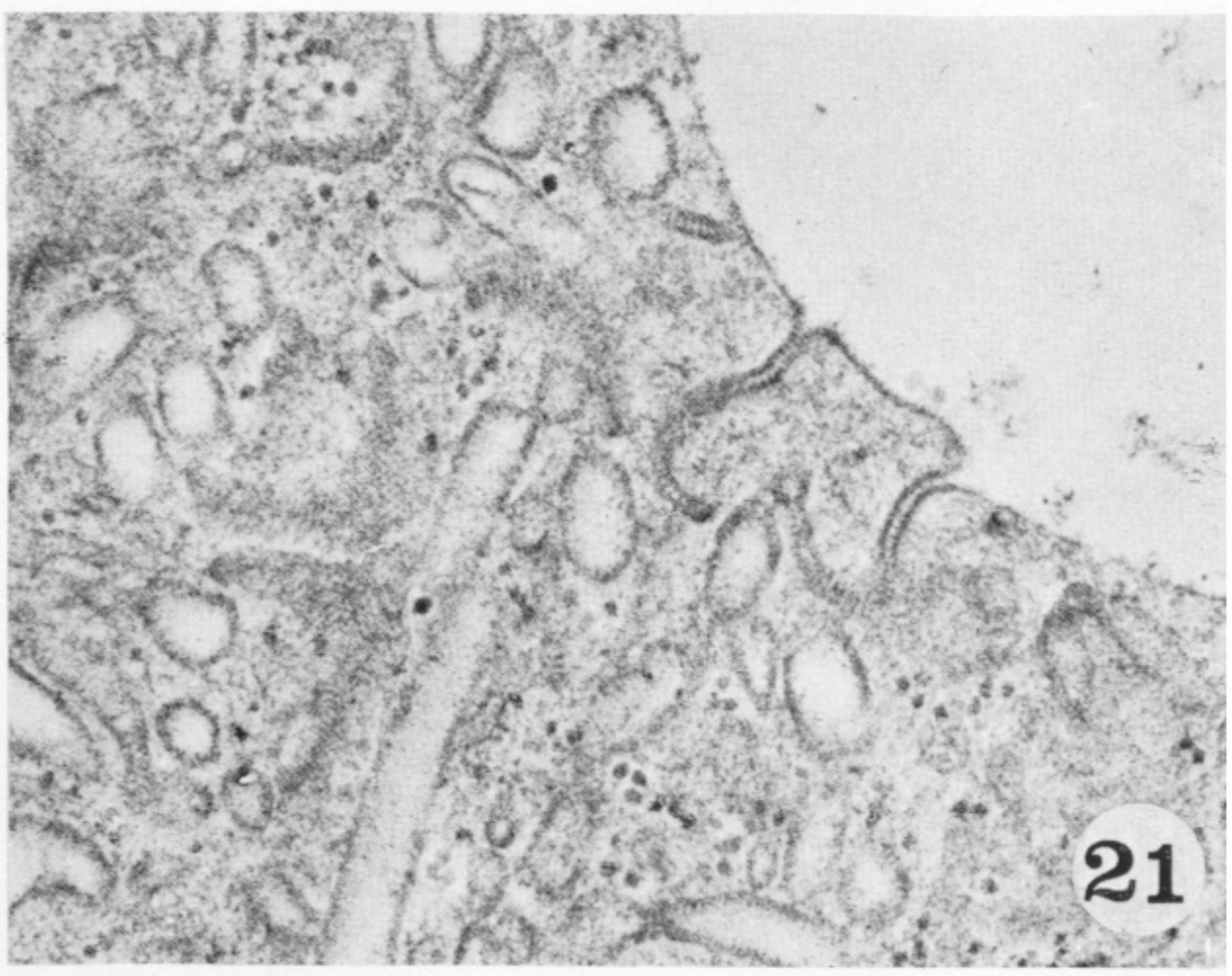

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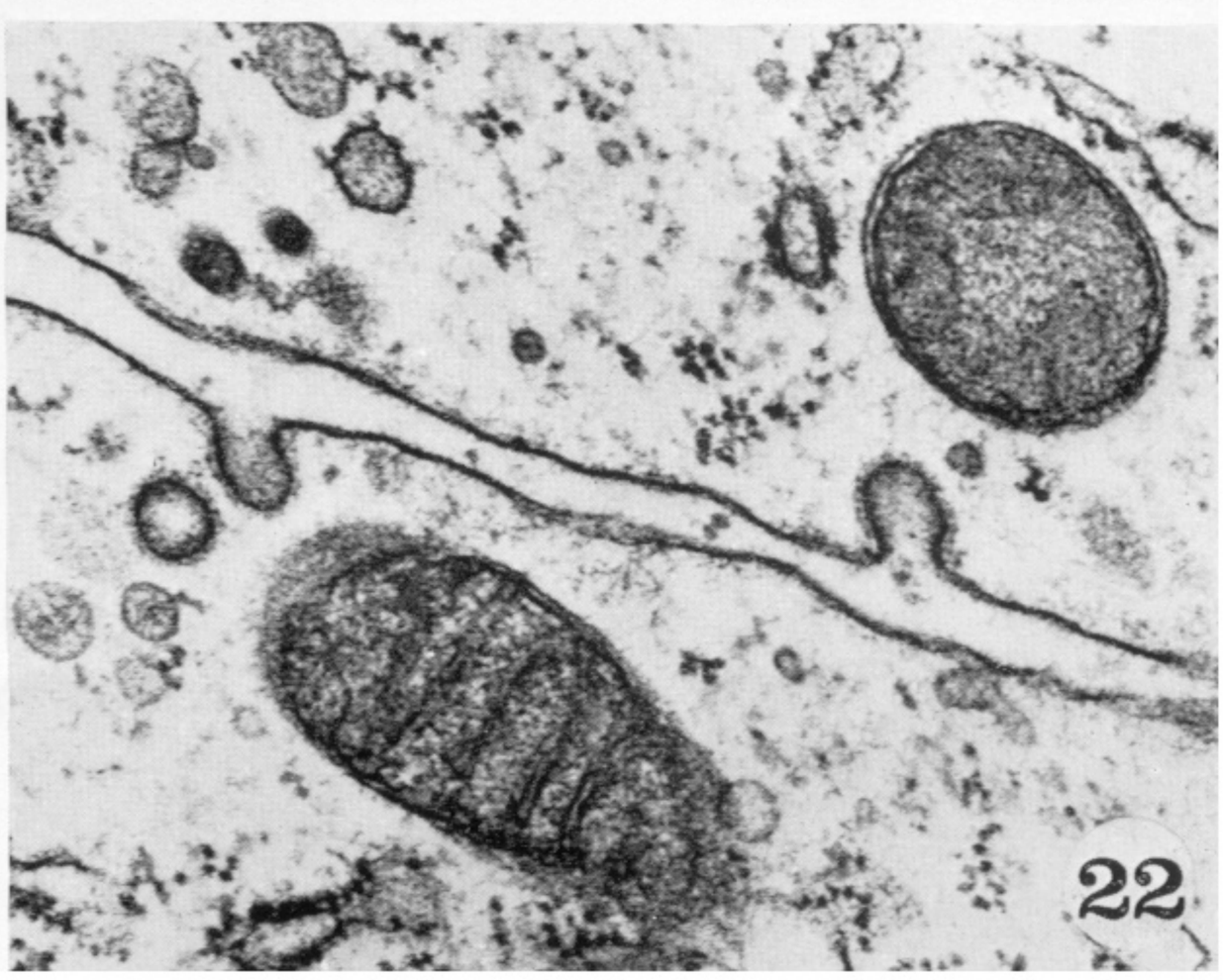

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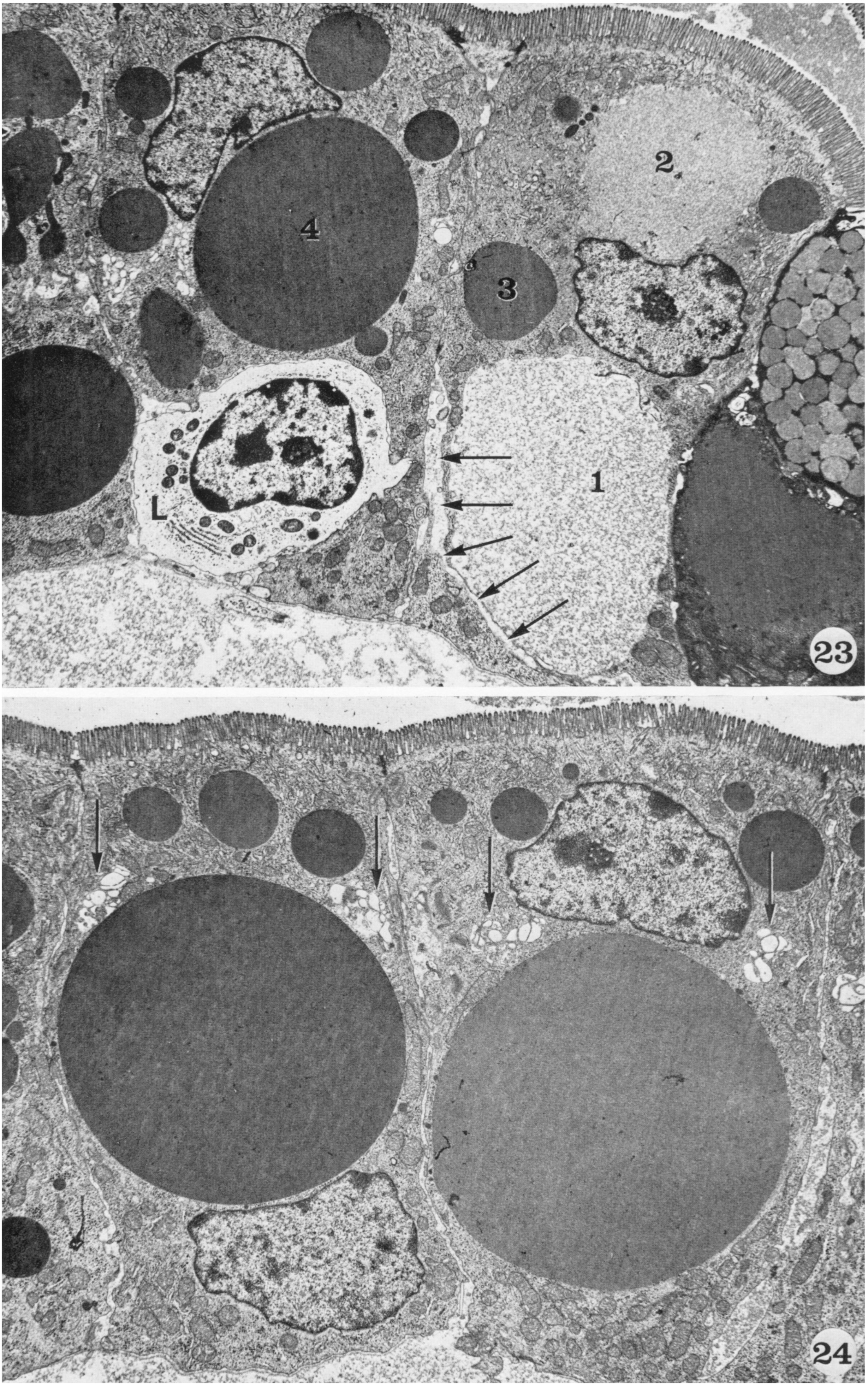
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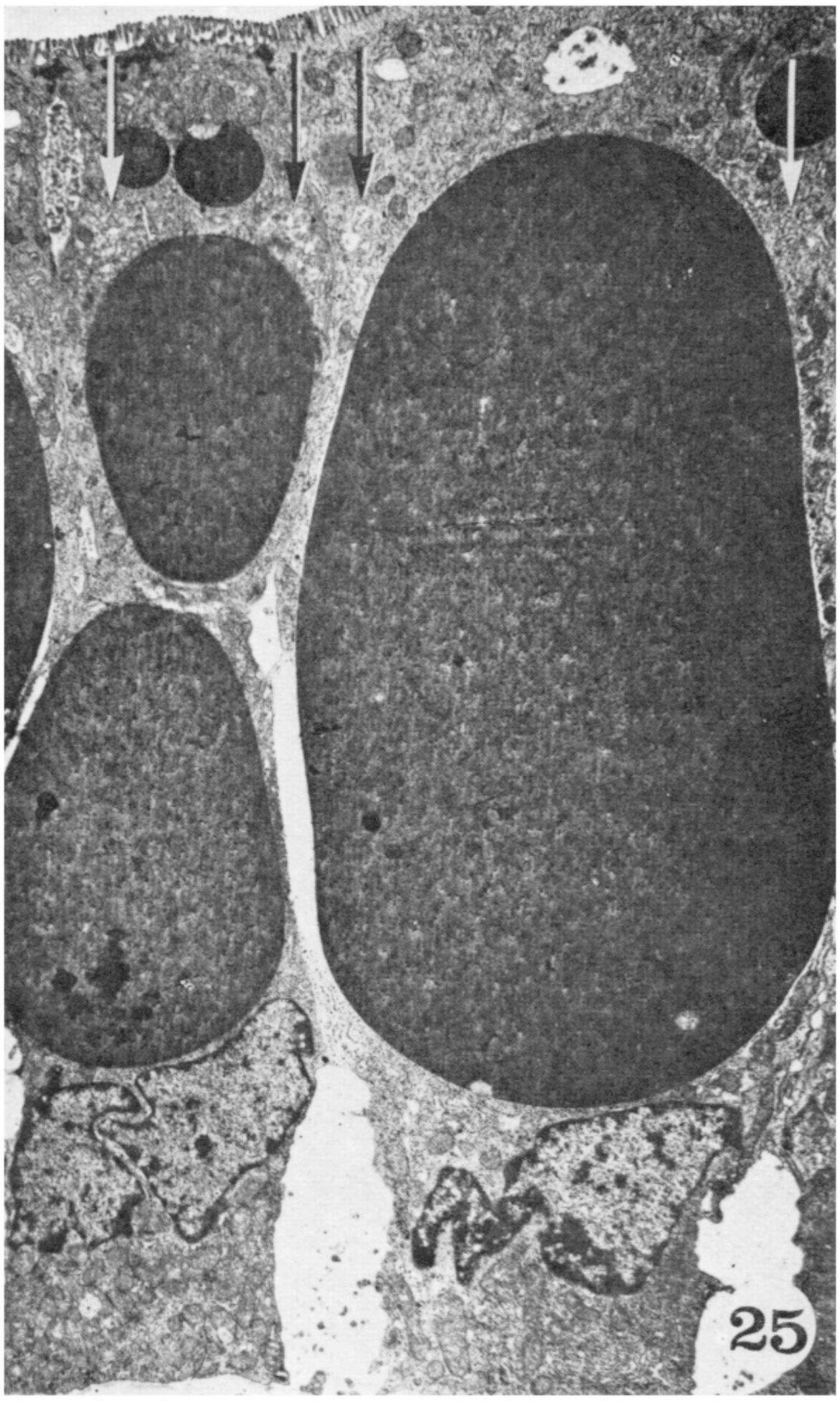

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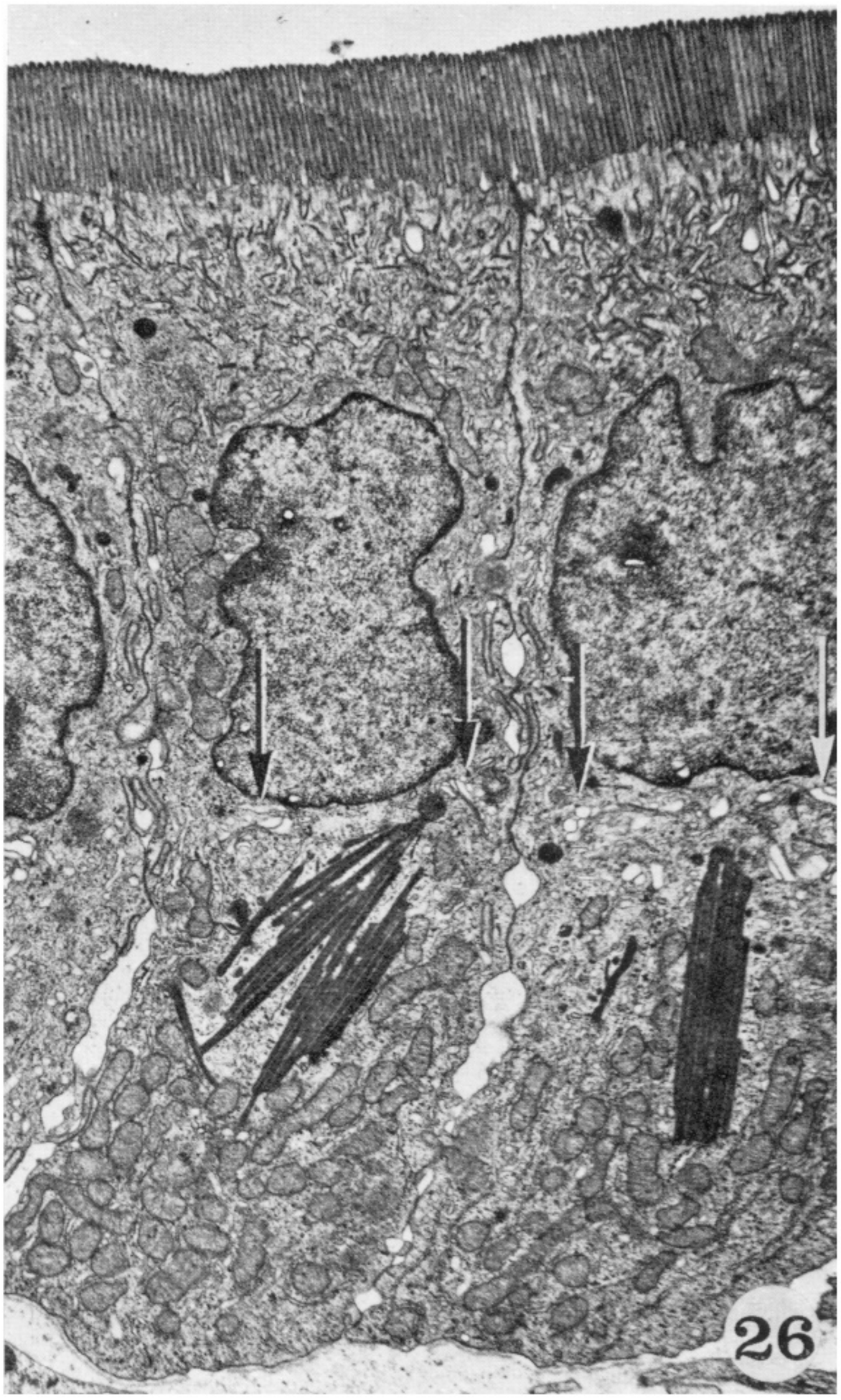

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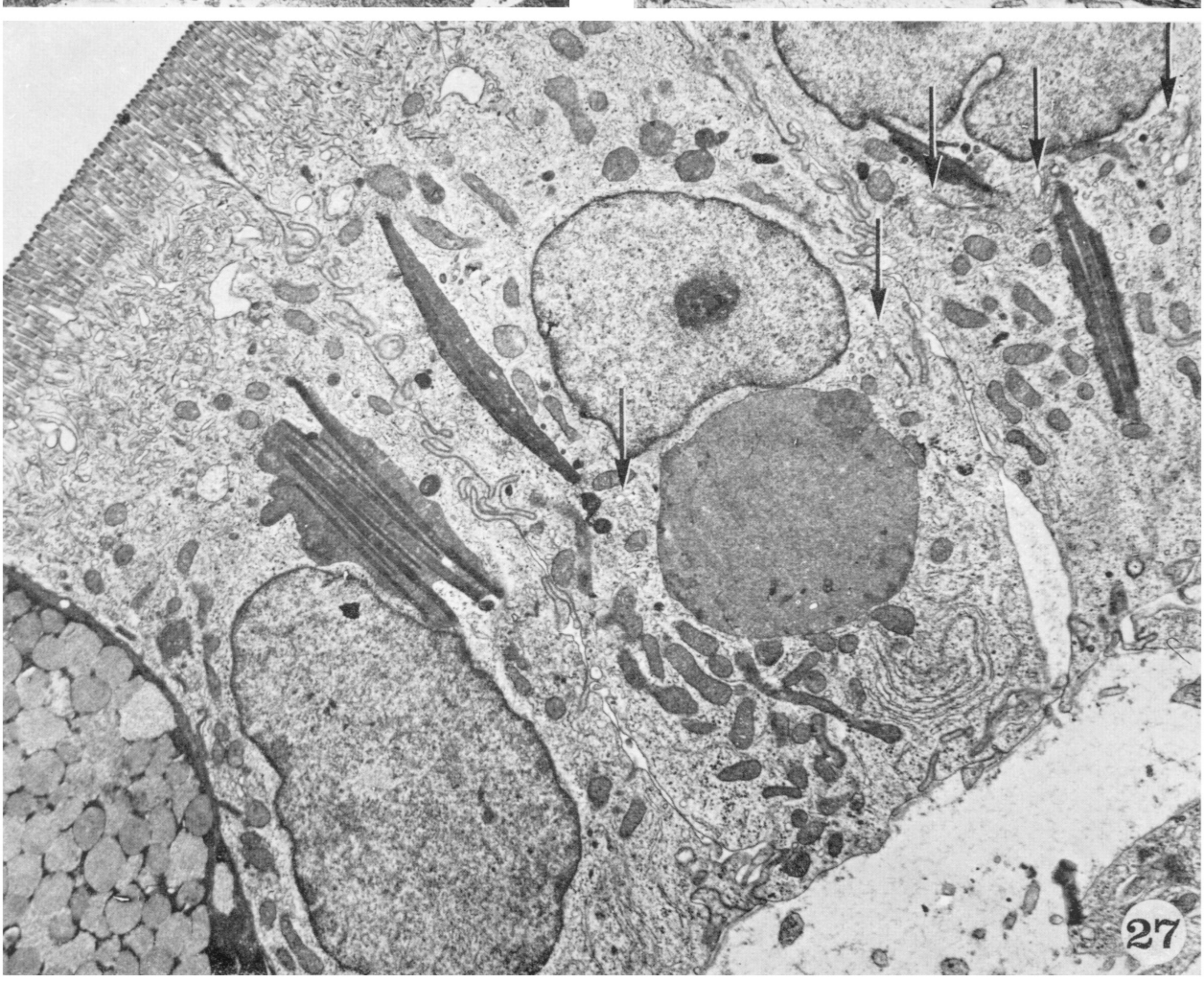

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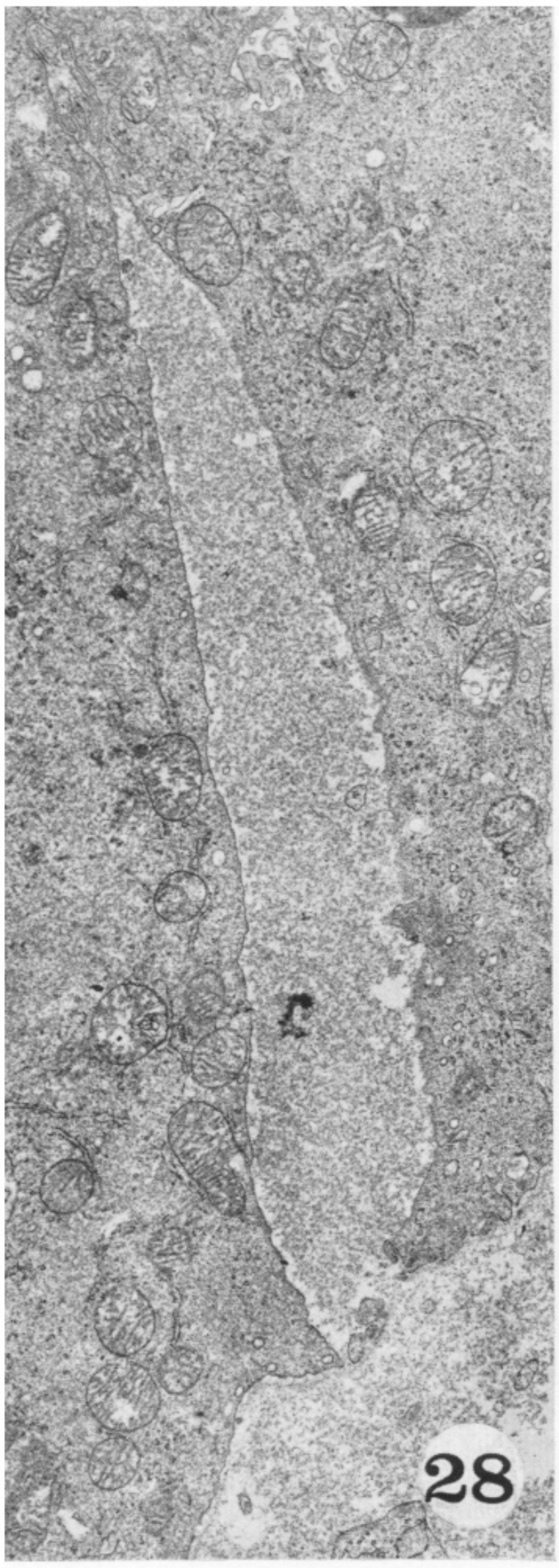
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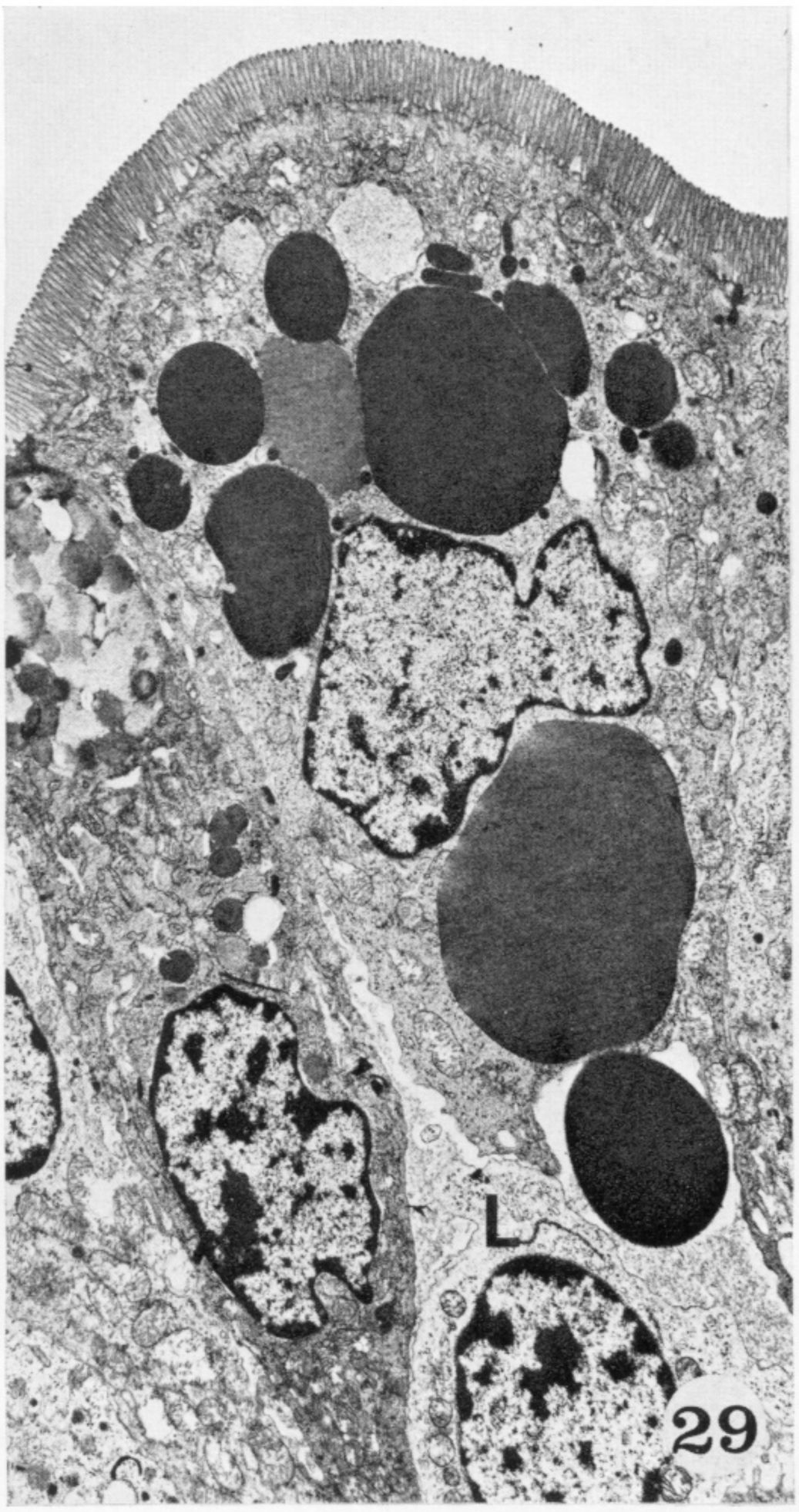

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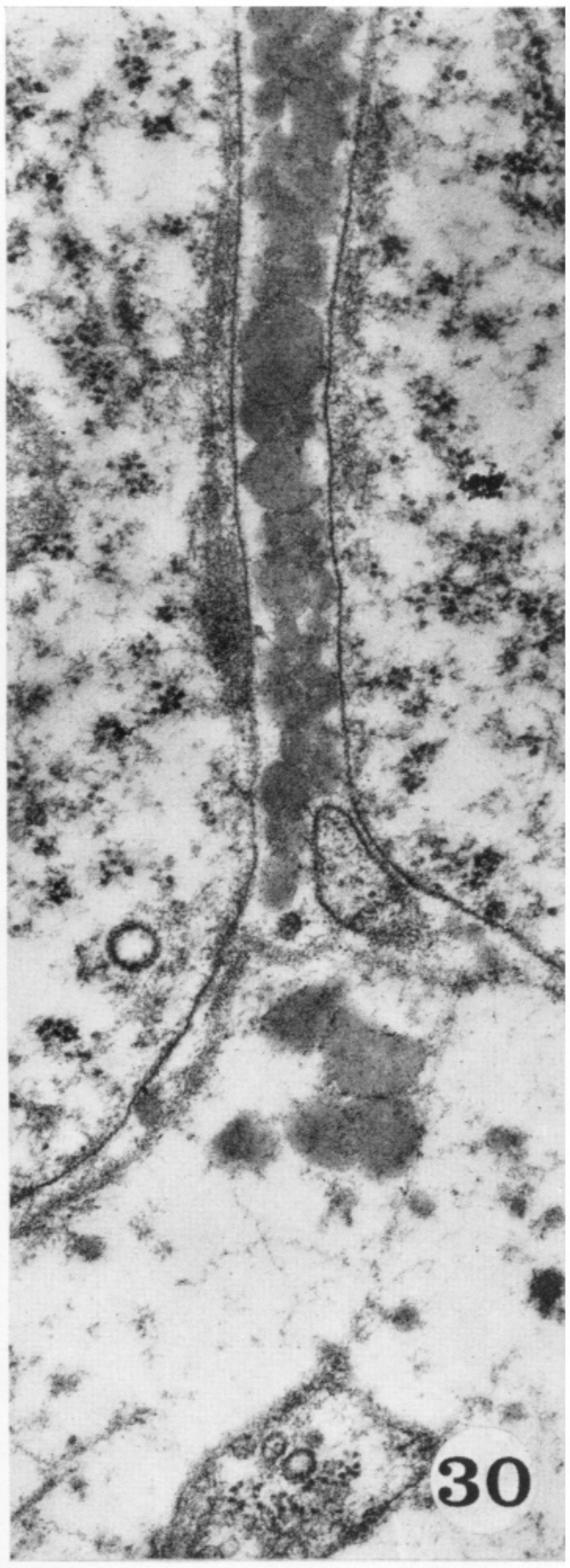
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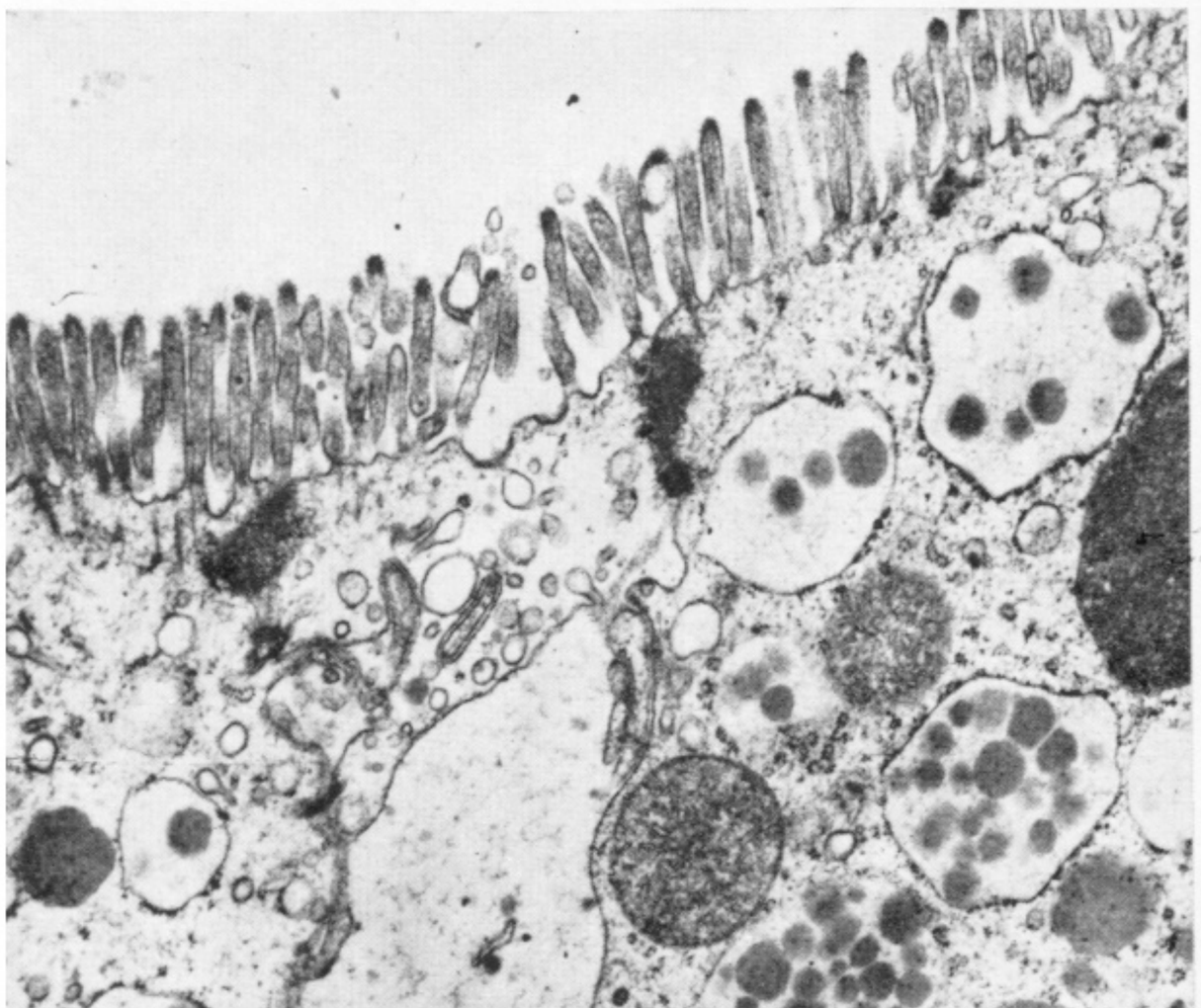
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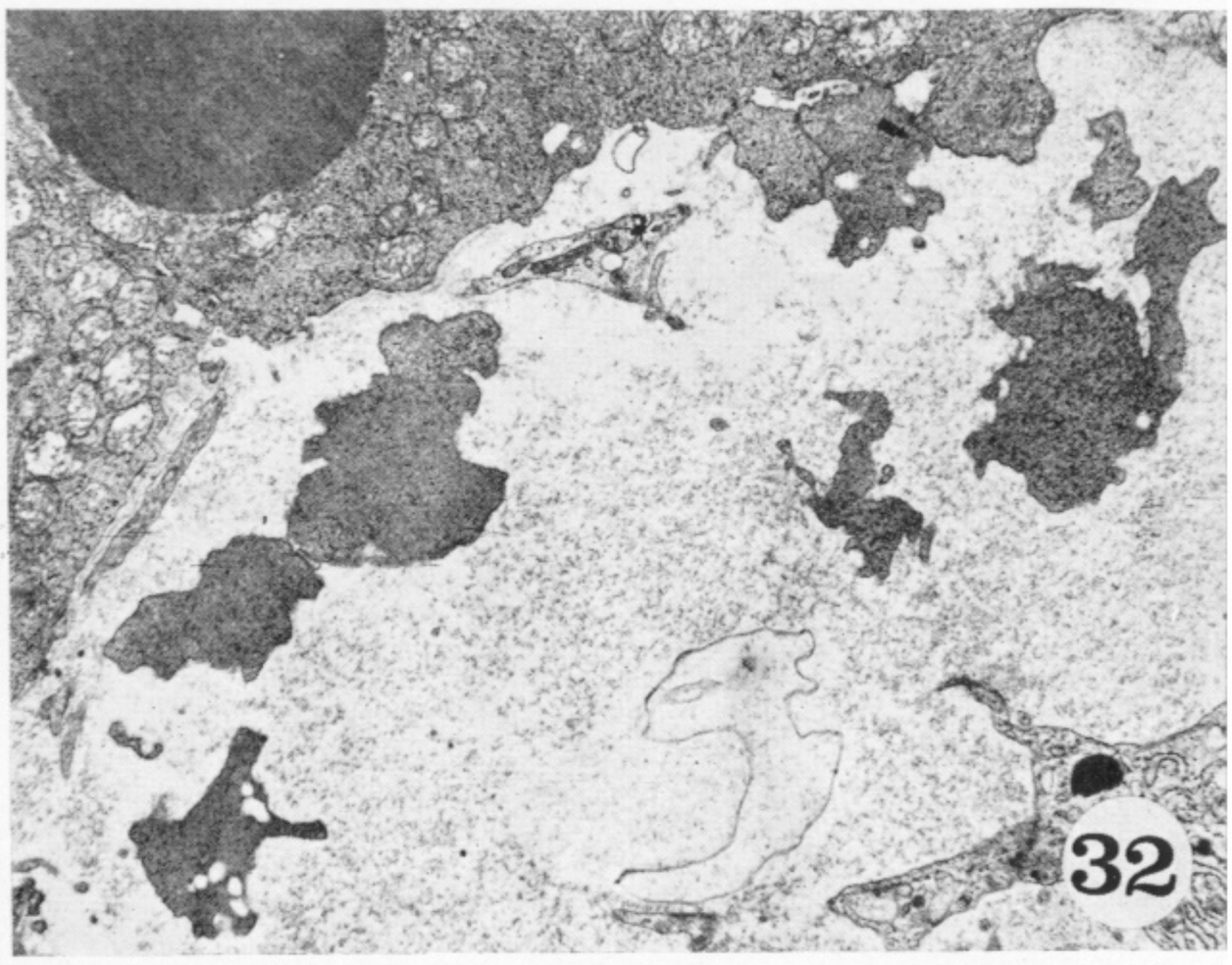
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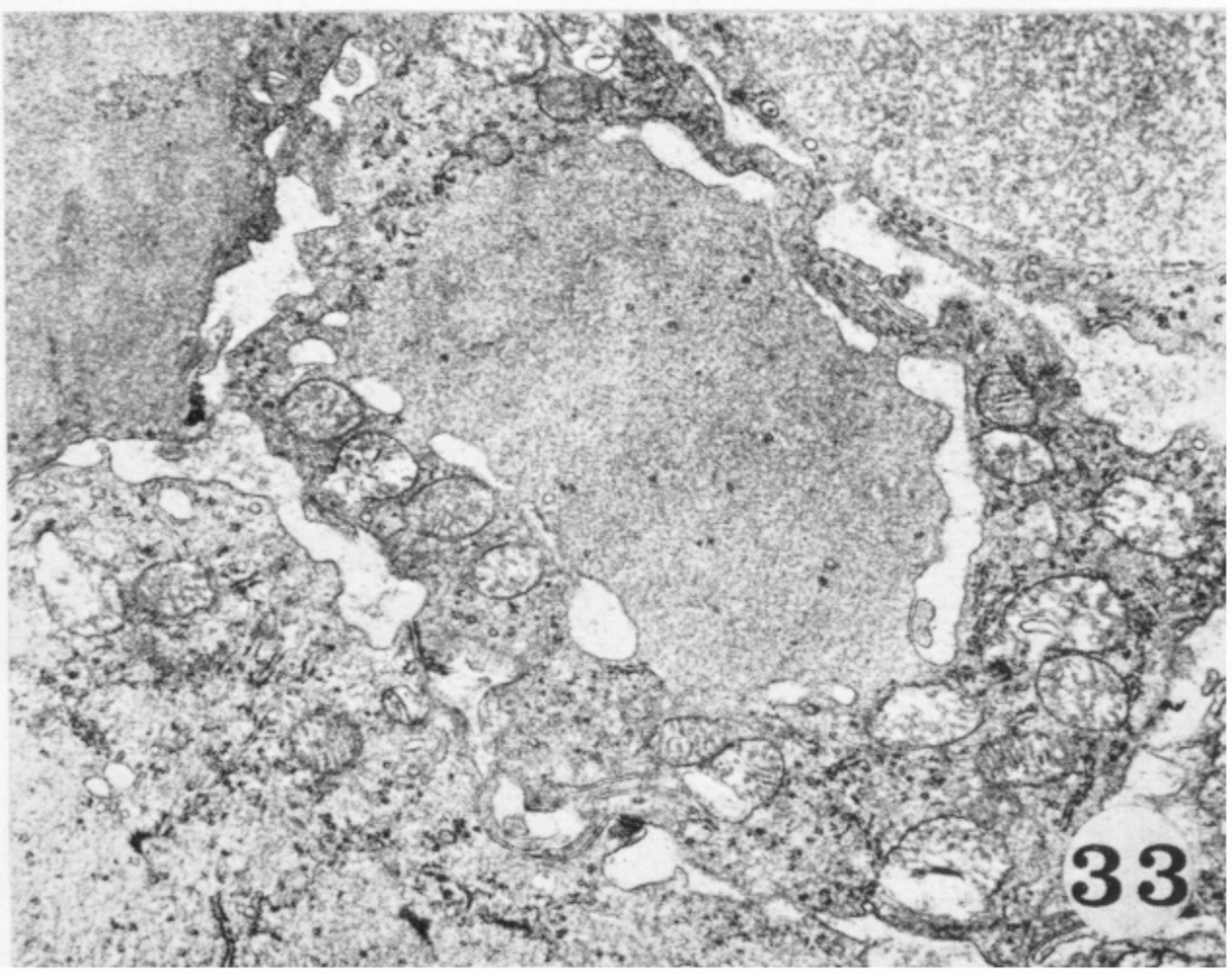
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