NEW CYTOPLASMIC COMPONENTS IN ARTERIAL ENDOTHELIA

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ABSTRACT

A hitherto unknown rod-shaped cytoplasmic component which consists of a bundle of fine tubules, enveloped by a tightly fitted membrane, was regularly found in endothelial cells of small arteries in various organs in rat and man. It is about 0.1 μ thick, measures up to 3 μ in length, and contains several small tubules, ~150 A thick, embedded in a dense matrix, and disposed parallel to the long axis of the rod. In some of these cells, the cisternae of the endoplasmic reticulum are greatly distended by the accumulation of a dense, finely granular material. The nature and significance of these cytoplasmic components are yet unknown.

Surveying a large number of sections of rat and human lungs in the course of an electron microscopical morphometric study, we found that the endothelial cells of pulmonary arteries frequently contained two types of cytoplasmic components which, to our knowledge, have not yet been described. A subsequent extension of this survey to other organs revealed the presence of one of these components (a rod-shaped tubulated body) in practically all arterial, arteriolar, and endocardial endothelia investigated. Similar bodies were occasionally found in the endothelia of alveolar capillaries. The purpose of this note is to call attention to these structures, although, so far, we have no clue as to their functional significance.

MATERIALS AND METHODS

The lungs of rats, anesthetized by intraperitoneal injection of Nembutal, were fixed *in situ* by intratracheal instillation of 1 per cent OsO_4 buffered at pH 7.4 with 0.1 M potassium-phosphate. After a few minutes the lungs were removed, cut into blocks, and immersed in fixative at 4° for 2 hours. The small tissue blocks were dehydrated in ethanol and embedded in Epon 812. In some cases, the lungs were fixed in 0.1 M phosphate-buffered (pH 7.4) glutaraldehyde (1), postfixed in OsO_4, and treated in the same manner as the other blocks. The other rat organs studied, including thyroid, pancreas, intestine, and heart, were fixed in OsO_4 and embedded in Epon 812 by conventional methods. A limited number of observations were made on skin vessels in the tail fin of *Amblystoma punctatum* larvae prepared by the same methods. Sections were cut with glass or diamond knives, mounted on carbon-coated Formvar films, contrasted with lead by the second method of Karnovsky (2), and examined in a Siemens Elmiskop I.

The tissues used for histochemical studies were fixed in cacodylate-buffered (pH 7.4) formaldehyde or glutaraldehyde.

OBSERVATIONS

1. *Morphology of the Observed Cytoplasmic Components*

A. Rod-Shaped Tubulated Bodies

The cytoplasmic component, observed with great regularity in the endothelial cells of practically all small arteries investigated, is a small rod-shaped body of moderate density which usually occurs in groups, often concentrated in the perinuclear region of the cytoplasm (Figs. 1 and 2).

In this region, the cytoplasm also contains numerous mitochondria as well as smooth and rough surfaced vesicles and cisternae of the endoplasmic reticulum.

Cross-sections of these bodies are circular or elliptic; the long axis of some ellipses can be quite extended, often 6 to 8 times the small axis (Figs. 3 to 6). Some of these long sections appear to have a blunt end (Fig. 3). From these observations we deduce that this body is a long, cylindrical rod with sharply cut-off, rather than tapered, ends. In one relatively thick section such a rod was observed over its entire length; it measured 3.2 μ.

The rods are bounded by a tightly fitted, 60 to 80 A thick membrane, of unit membrane type, which often appears discontinuous possibly as a result of preparative artifacts (Figs. 3, 6, 7). The interior is occupied by small cylindrical tubules, 150 to 200 A in diameter (Figs. 2 and 4 to 7), embedded in a relatively dense matrix. The size of these tubules is apparently constant. Their wall is about 40 A thick (Fig. 6) and in some micrographs seems to be triple-layered. The content of their lumen is less dense than the surrounding matrix. In cross-section it sometimes includes a central dense dot, in three dimensions, probably a filament (Fig. 6). Without being arranged in a strict geometric lattice, the tubules are often regularly spaced, at a center-to-center distance of about 250 to 300 A, so that they are always separated from each other by a layer of matrix at least 100 A thick (Fig. 6). In transverse sections, we found that the rods contain 6 to 26 such tubules, the number being proportional to the cross-sectional area. Longitudinal sections showed that the tubules are straight and generally course parallel to the axis of the rod (Figs. 3 to 5). Occasionally they exhibited, however, a spiral or twisted course, or even appeared to form whorls (Fig. 7).

B. Dilated Cisternae of the Endoplasmic Reticulum

The second and more conspicuous type of inclusion observed consists of highly dilated cisternae of the endoplasmic reticulum filled with a relatively dense material. The low power electron micrograph of Fig. 9 shows a sector of the wall of a small pulmonary artery of a rat in a contracted or collapsed state. The endothelial cells protrude into the lumen; three of them are seen to contain large inclusions, which are identified by label *C*. The cross-section of these bodies, which have a thickness of the order of 1 to 2 μ, is elliptic; one is bent sharply in the same way the nuclei of endothelial cells are bent in contracted arteries.

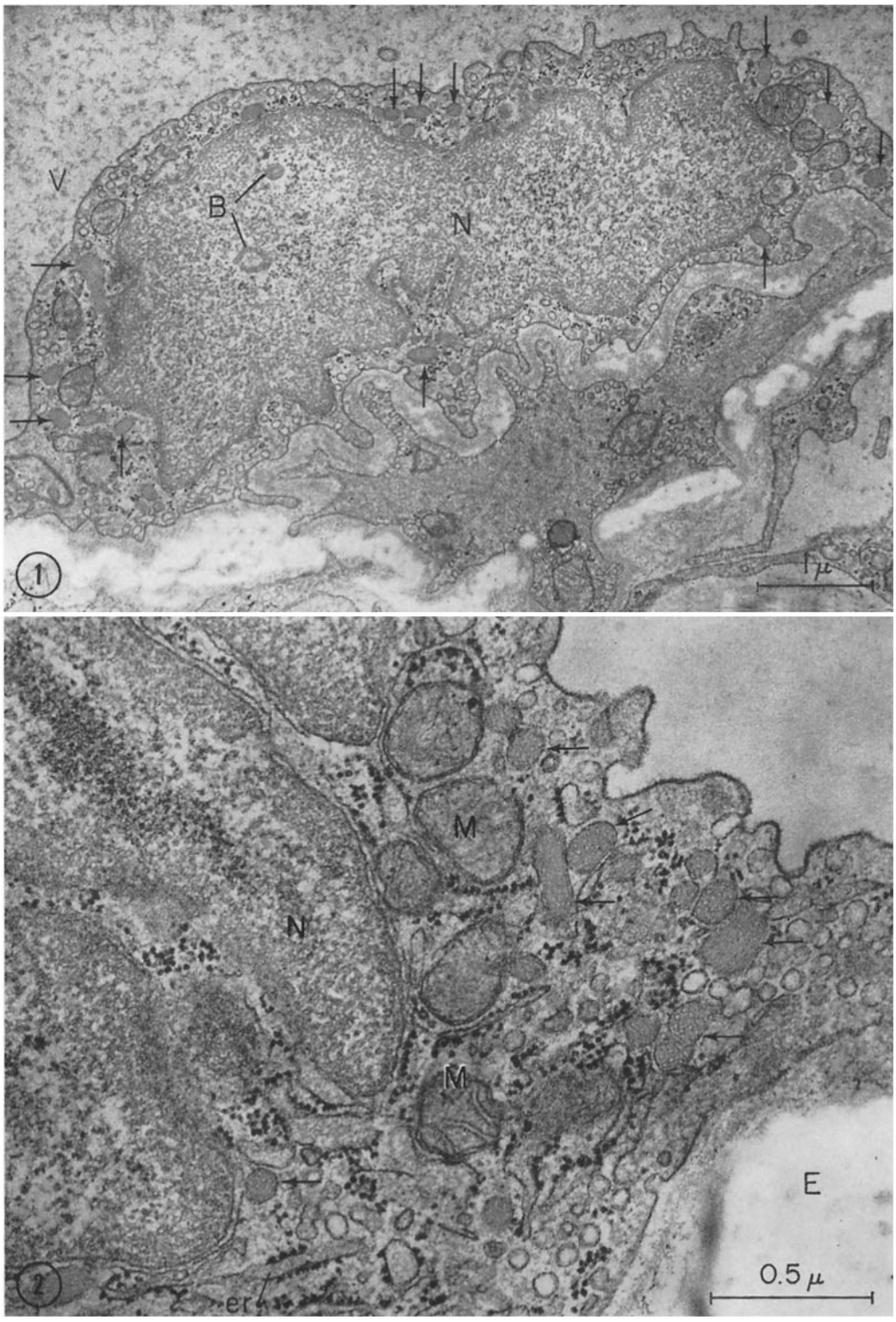
Another example of such inclusions is shown at a higher magnification in Fig. 10. The vessel in Fig. 10 is dilated so that the endothelium lies flat on the stretched internal elastic membrane. All the cross-sections of distended cisternae so far observed are elliptic. We can, therefore, conclude that in three dimensions these cisternae have the shape of flat discs. In dilated vessels, their largest cross-section lies parallel to the surface; in contracted arterioles, it remains parallel to the cell

Key to Abbreviations

C, dilated cisternae of endoplasmic reticulum.
E, internal elastic membrane of artery.
M, mitochondria.
N, nucleus.
R, rod-shaped bodies.
r, ribosomes.
T, tubules in rod-shaped bodies.
u, unit membranes.
V, vessel lumen.

FIGURE 1 Endothelial cell of a small pulmonary artery [rat]. Some of the numerous rod-shaped bodies, concentrated around the nucleus (*N*), are pointed out by arrows, *B*, nuclear inclusion. × 18,000.

FIGURE 2 Perinuclear portion of an endothelial cell from a small pulmonary artery [rat]. Several rod-shaped bodies (arrows) are sectioned in different directions. On transverse sections they are seen to contain small membrane-bounded tubules, which are embedded in a denser matrix. Compare the structure of these rods with that of mitochondria (*M*). Some cisternae of the endoplasmic reticulum (*er*) appear slightly distended by a finely granular material. × 50,000.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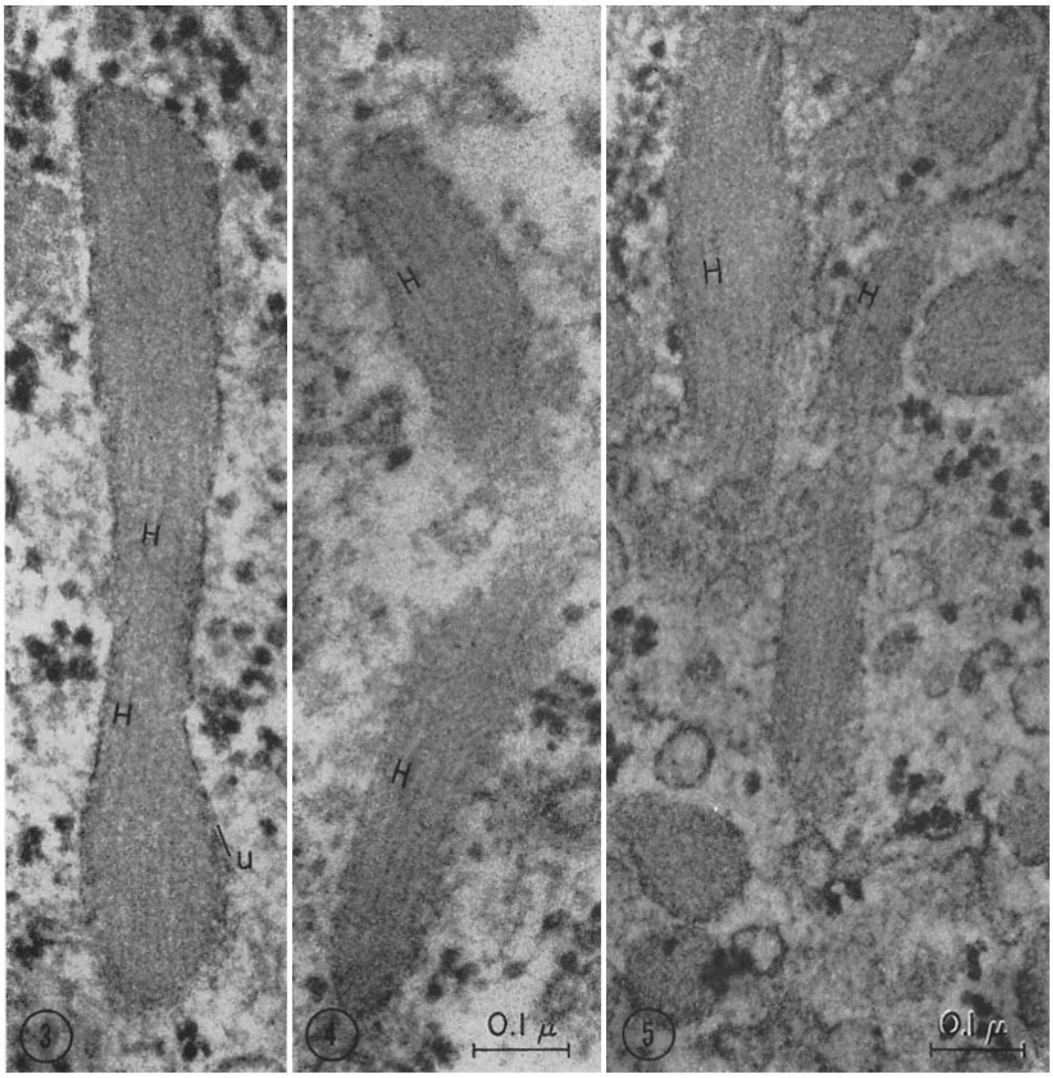
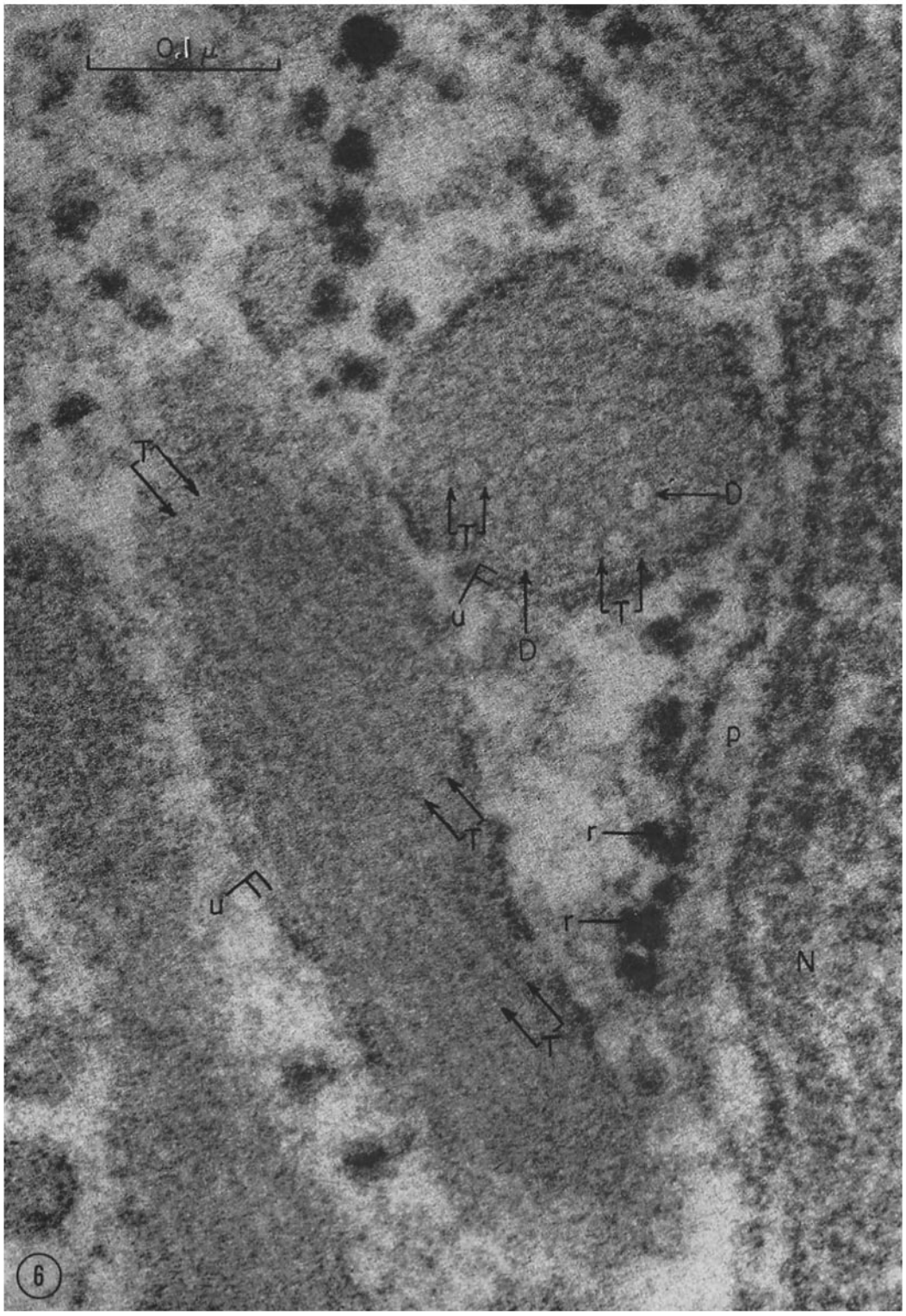


FIGURES 3 to 5 Oblique sections of rod-shaped bodies from pulmonary artery endothelium of rats, showing parallel arrangement of internal tubules (*H*) and tightly fitted enveloping membrane (*u*). × 127,000.

surface, but the orientation of the whole cisterna is determined by the shape of the corresponding endothelial cell. If the cell is compressed along the circumference of the vessel wall, a large cisterna can be folded up, indicating that its content must have a gel-like consistency.

Figs. 10 to 13 show that these cisternae are enveloped by a membrane which carries ribosomes

FIGURE 6 Transverse and oblique sections of rod-shaped bodies located near nucleus (*N*) in endothelial cell of small pulmonary artery of a rat. Arrow pairs point to clearly visible outlines of internal tubules (*T*) in transverse or longitudinal sections. The tubule wall appears as a membrane of ~40 A thickness. The lighter lumen often contains a dark central spot (*D*) visible in cross-sections. The tubules (*T*) of the obliquely sectioned rod appear to course in a steep spiral. The enveloping membrane (*u*) is seen to be of unit membrane type. *p*, perinuclear cisterna with ribosomes (*r*). × 290,000.


0.1 μ
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u
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6

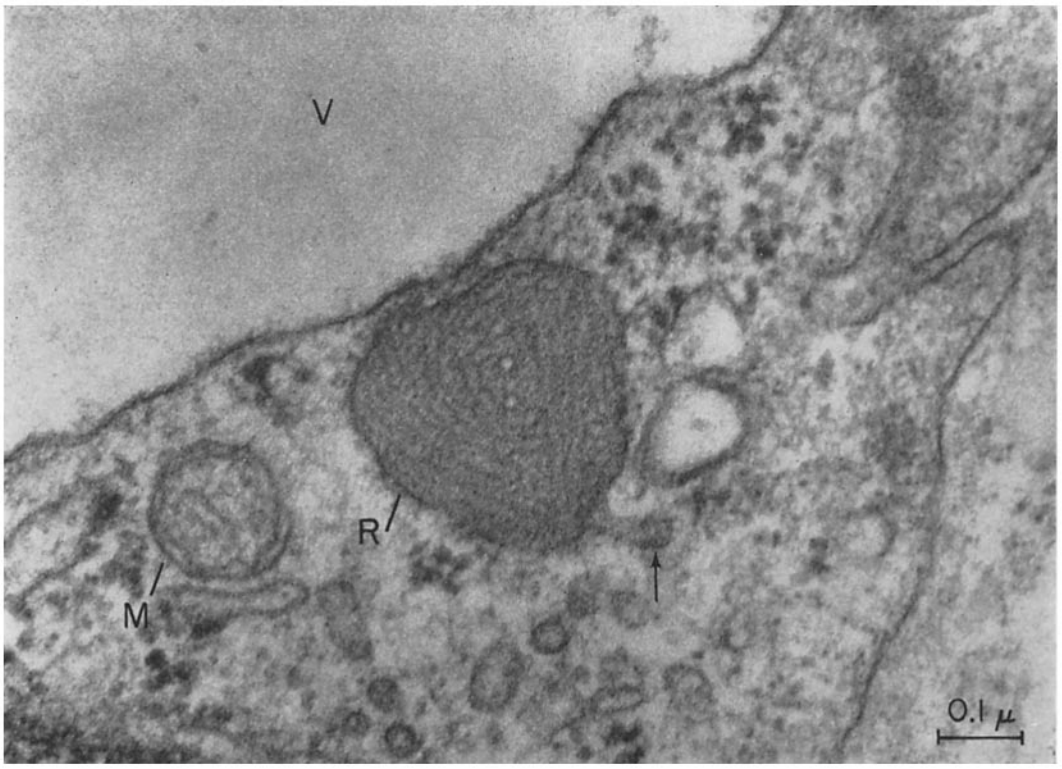


FIGURE 7 Transverse section of a rod-shaped body in an endothelial cell of the atrial endocardium (rat). The tubules, disposed in a whorl, appear in cross-section in the center of the body and in oblique section at its periphery. The arrow points to a smooth surfaced vesicle whose membrane is apparently in continuity with the limiting membrane of the rod-shaped body. × 115,000.

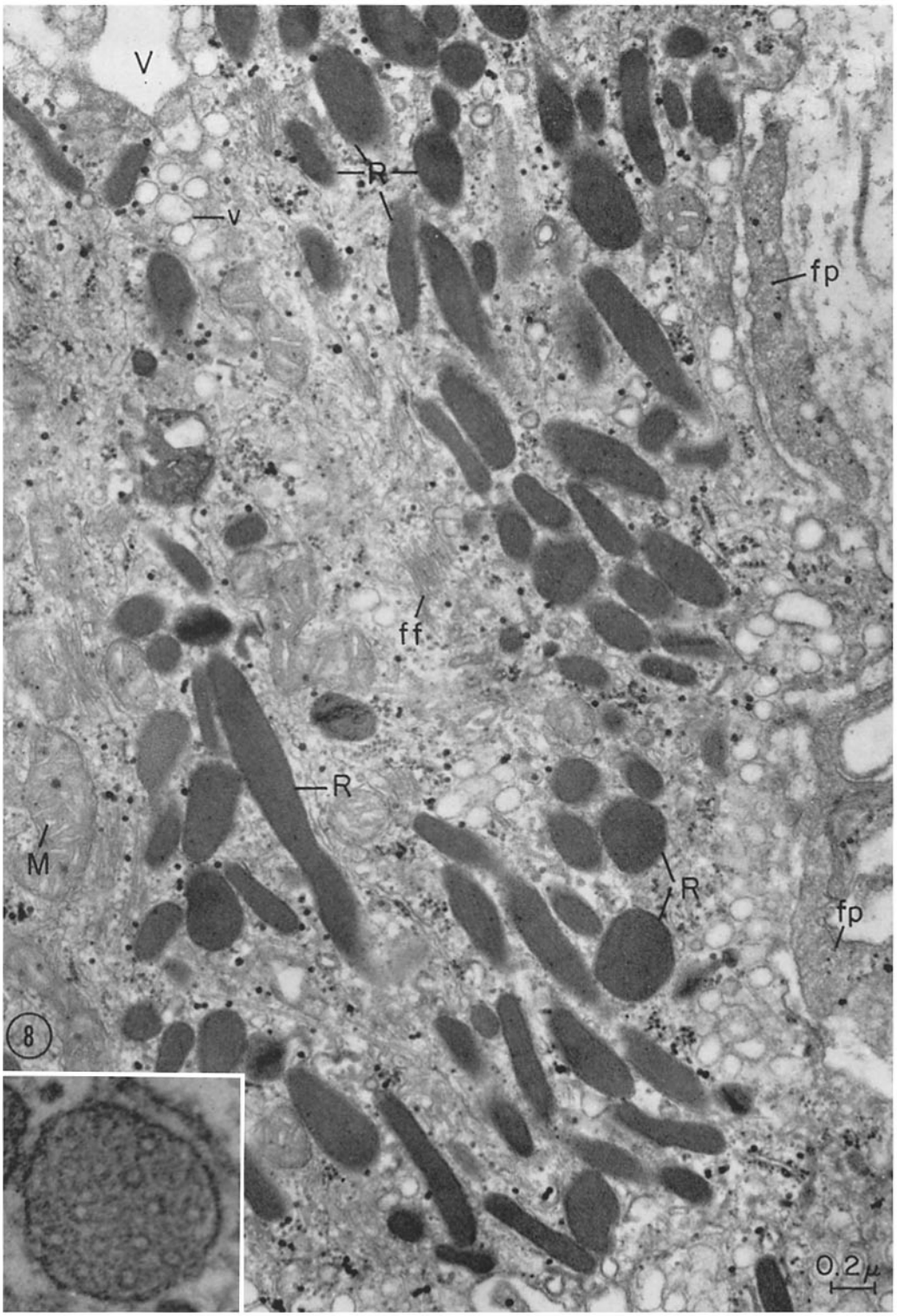
on its outer surface. Hence, these discoid bodies can be identified as segments of the rough surfaced endoplasmic reticulum which became dilated by the accumulation of a finely granular, or floccular material. At some points, the enveloping membrane is in continuity with the membrane of the rest of the endoplasmic reticulum. This feature is more frequently exhibited by the least distended cisternae (Fig. 11).

2. *Distribution of These Components*

Both cytoplasmic components were found with great regularity in endothelial cells of small branches of the pulmonary artery in rats. Much less frequently, rod-shaped bodies were also found in the endothelium of alveolar capillaries. Dilated cisternae were never encountered in these endothelia. The endothelium of arterioles of the systemic circulation also regularly contained

FIGURE 8 Small vessel in the tail fin of an *Amblystoma punctatum* larva (close to metamorphosis). A large population of rod-shaped bodies, a few mitochondria, a number of plasmalemmal vesicles (*v*) and a few bundles of fine fibrils (*ff*) appear in the cytoplasm of an obliquely sectioned endothelial cell. Pericyte foot processes can be seen at *fp*. Specimen fixed in glutaraldehyde and postfixed in OsO_4.

The characteristic fine structure of these rod-shaped bodies is shown in the inset in a similar specimen fixed in 1 per cent OsO_4 in 0.1 M phosphate buffer (pH 7.6). × 33,000; inset: × 160,000.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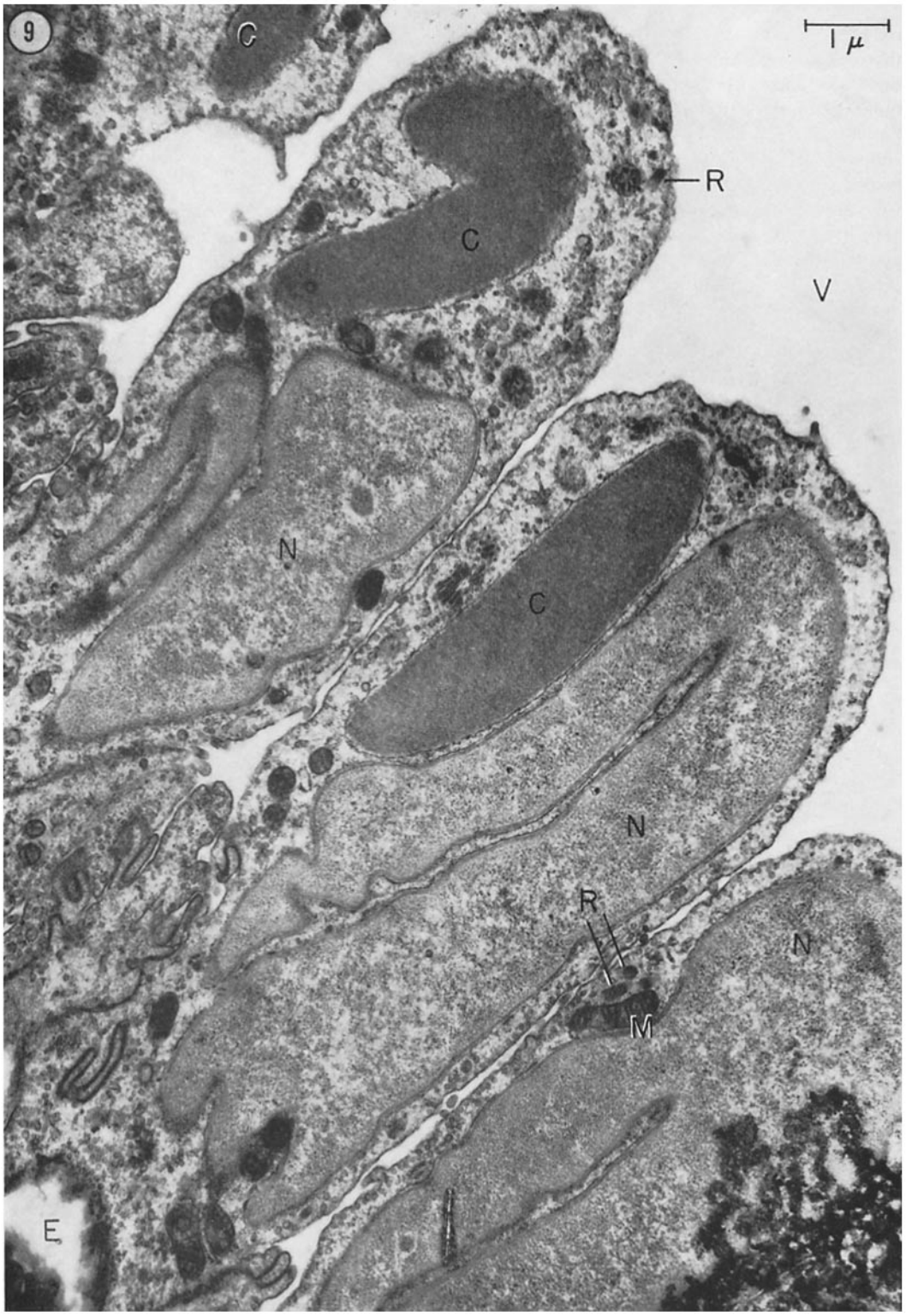
9
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E

rod-shaped bodies. Such vessels were surveyed in thyroid, pancreas, intestine, and myocardium sections. The bodies were also seen in relatively large numbers in the atrial endocardium of the rat. Similar bodies probably occur also in the aortic endothelium, since "dense granules" with a suggestion of "compartmentalized internal structure" were observed in this location in the rat by Pease and Paule (3). Dilated cisternae of the endoplasmic reticulum were occasionally observed in these instances, though not so greatly enlarged as in pulmonary arteries (Fig. 11).

Preliminary observations indicate that tubulated bodies occur in the endothelium of alveolar capillaries and splenic sinuses in man, and micrographs published by Hatt *et al.* (4) suggest that such bodies also occur in the endothelium of arterioles and capillaries of the human lung. Large concentrations of similarly structured bodies, measuring up to 0.4 μ in diameter, were found in the endothelium of skin vessels in *Amblystoma* (Fig. 8).

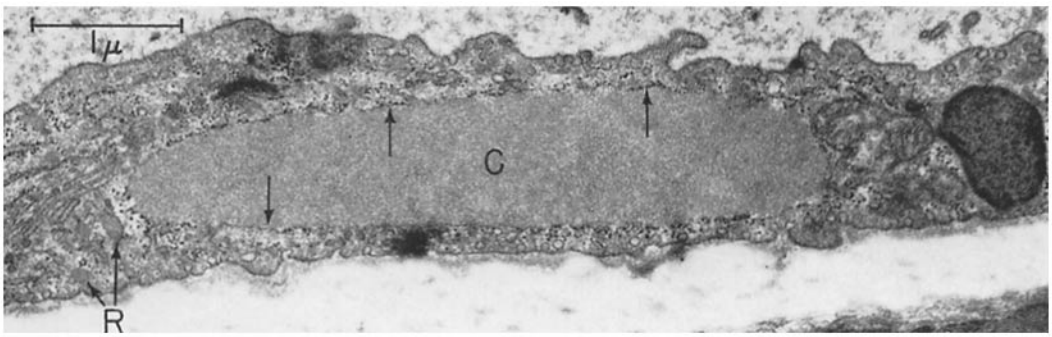

FIGURE 10 Endothelium of a dilated pulmonary artery (rat) containing a large discoid body (*C*) enveloped by a ribosome-studded membrane (unlabeled arrows). The last feature indicates that the body is a dilated cisterna of the endoplasmic reticulum. Rod-shaped bodies (*R*) are found in the neighborhood. × 23,000.

3. *Histochemical Studies*

A number of general histochemical reactions were carried out on rat lung tissue fixed with cacodylate-buffered (pH 7.4) formaldehyde or glutaraldehyde (1, 11). In the light microscope, cytoplasmic inclusions which correspond in size, location, and shape to the distended cisternae could be recognized in the endothelial cells of pulmonary arteries. With mercury-bromphenol blue (5) they stained blue, indicating that the floccular material is, or contains, protein. Chloramine-T, ninhydrin-Schiff, and PAS reactions did not yield conclusive results. With Sudan black B, dissolved in alcohol, acetone, or propylene glycol (5, 6), a fine perinuclear granularity was observed in some endothelial cells. This might be due to rod-shaped bodies, but definite conclusions cannot be drawn from these observations, since the dimensions of most of the rods (diam. $\cong$0.1 μ) are actually below the limit of resolution of the light microscope. In the electron microscope, it was observed that the rods "stain" intensely with phosphotungstic acid; they contain, therefore, a high concentration of basic groups.

Strong alkaline phosphatase activity has been reported in the endothelium of a variety of vessels, such as rabbit pulmonary artery (5), and small branches of systemic arteries (7). Wachstein (8) has reported a particularly strong 5′-nucleotidase activity in the endothelium of capillaries in the renal medulla. Hence, the question arose whether the cytoplasmic components described are sites of such enzymatic activities. Our studies, carried out on frozen and cryostat sections of rat lung fixed in

FIGURE 9 Endothelial cells bulging into the lumen (*V*) of a rat pulmonary artery fixed in a contracted state. The nuclei (*N*) appear folded. Three cells contain large discoid bodies within distended cisternae of the endoplasmic reticulum (*C*); one of them is sharply bent. The cisternal membrane which binds the discoid bodies is clearly visible. × 13,000.

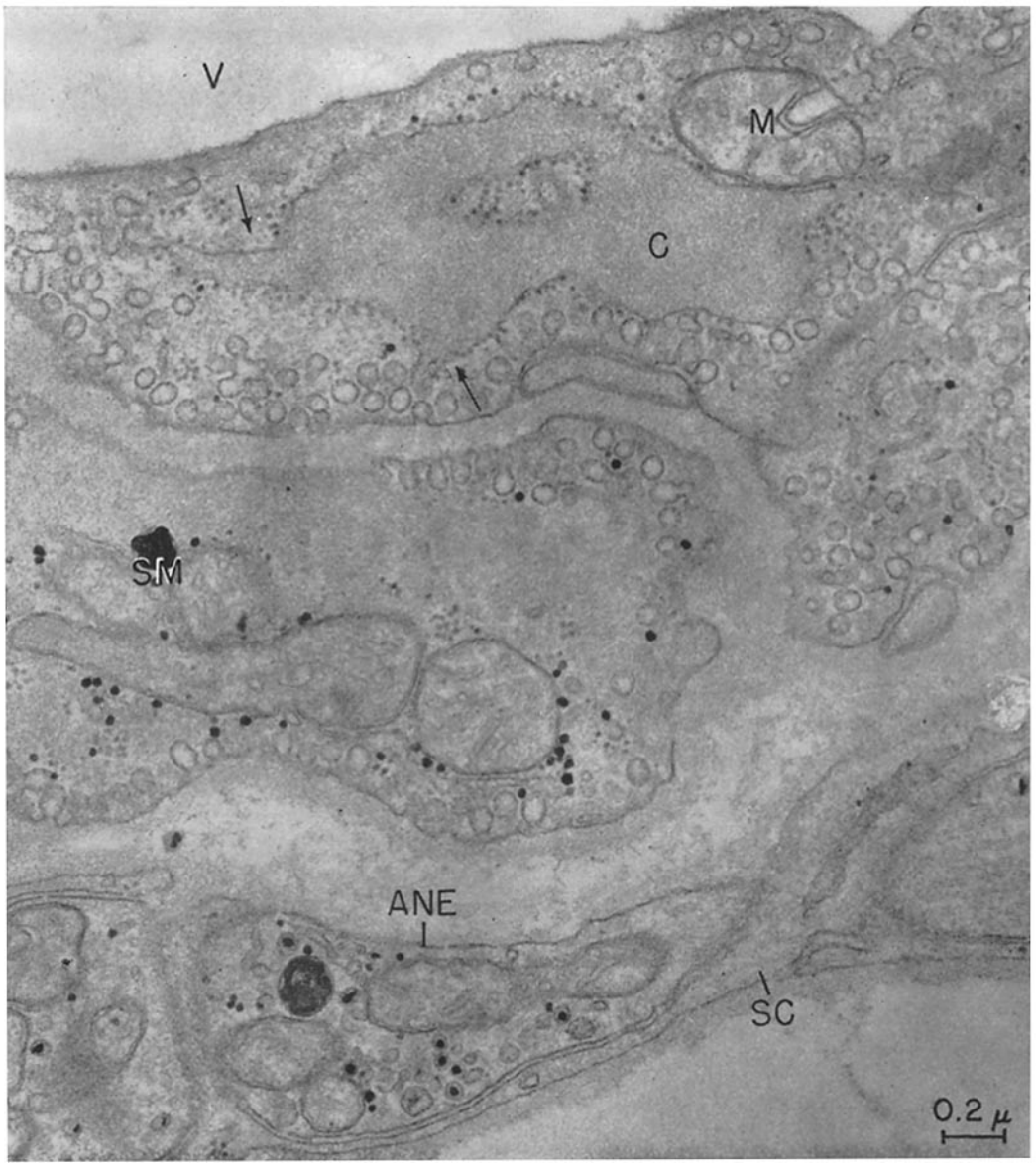

FIGURE 11 Arteriole in the ventricular myocardium of a rat. The endothelial cell to the left contains a distended rough surfaced cisterna which appears to be in continuity with other elements of the endoplasmic reticulum (arrows). Adrenergic nerve endings (*ANE*) partly covered by a Schwann cell (*SC*) can be seen close to a smooth muscle cell (*SM*). × 44,000.

buffered glutaraldehyde (1), showed that most of the unspecific alkaline phosphatase activity (Ca-Co method at pH 9) resides in the adventitia of pulmonary arteries and in systemic capillaries associated with the striated muscle layer of pulmonary veins (9, 10); some activity is also present in alveolar capillaries, but practically none was found in the endothelium of pulmonary arteries. Although endothelial and adventitial cells showed 5′-nucleotidase activity, electron microscopical preparations indicate that the latter was not associated with tubulated bodies.

DISCUSSION

The rod-shaped body described in this article has been observed with regularity in numerous vascular endothelia of the rat, man, and *Amblystoma*. This indicates that it must be a structure of some functional significance which for the moment remains obscure. The observed restriction to vascular endothelium suggests, however, that these bodies are connected with vascular or blood physiology. It is hoped that further electron microscopical cytochemical studies may shed some light on the nature of the rods and their components. According to our observations, they occur chiefly in endothelial cells, especially in arterial endothelia and in endocardium, but their presence in other cells cannot yet be excluded with absolute certainty.

The disc-shaped, dilated cisternae of the endoplasmic reticulum filled with a dense proteinaceous material were observed with less regularity, so far only in vascular endothelia in rat tissues. In view of their coexistence in endothelial cells, it would be interesting to know whether these dilated cisternae stand in some functional relation to the rod-shaped bodies. But our observations do not reveal any striking structural relations between the two structures. The rods did not appear to be more closely associated with dilated cisternae than they were, for example, with other elements of the endoplasmic reticulum (compare Figs. 2 and 6), or with other cell organs (mitochondria, Golgi complex) (Fig. 2).

Some tubulated rods may appear superficially similar to certain forms of mitochondria (12, 13) on account of their bounding membrane and internal structure. On closer examination it is clear, however, that the two structures are different: the tubulated bodies are smaller, are provided with a

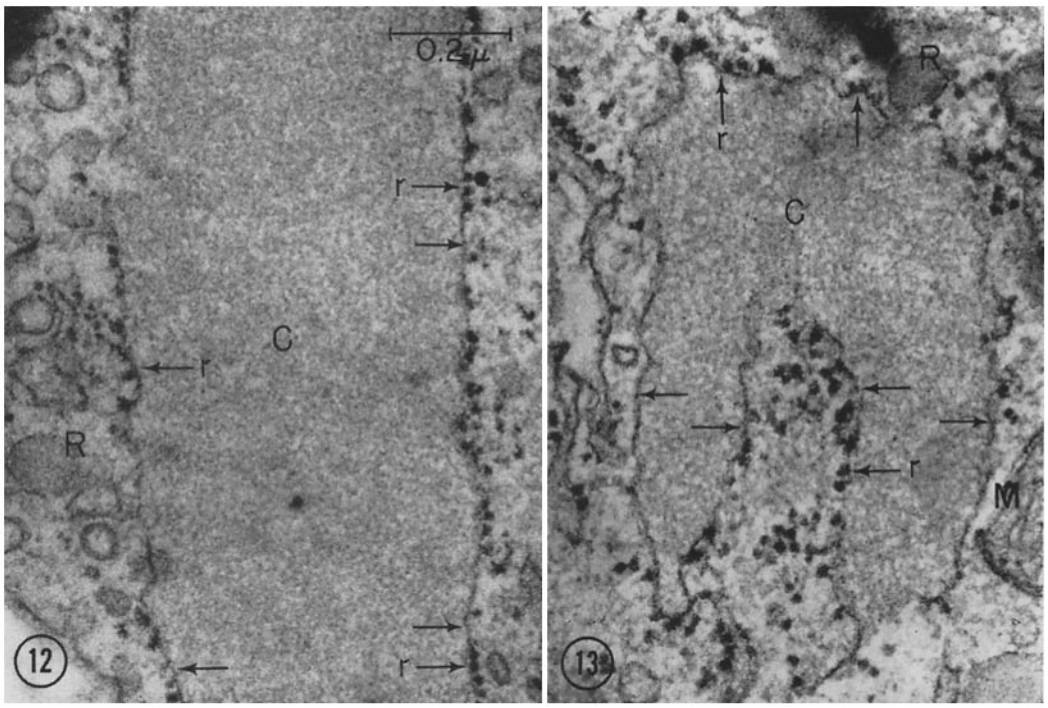

FIGURE 12 Portion of discoid body (*C*) showing the fine granular material and the enveloping membrane (unlabeled arrows) partially studded with ribosomes (arrows marked *r*). × 80,000.

FIGURE 13 A cisterna of the endoplasmic reticulum in an endothelial cell of a small pulmonary artery of a rat is somewhat distended by the accumulation of a fine granular material similar to that found in discoid bodies (compare with Figs. 10 and 12). The membrane (unlabeled arrows) is partially studded with ribosomes (arrows marked *r*). × 80,000.

single membrane, and as such their inner tubules do not represent equivalents of cristae.

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