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KEYWORDS: Myelin-like structures, ischemia, kidney

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MYELIN-LIKE STRUCTURES SEEN INTRACELLULARLY IN RENAL TUBULE CELLS SUBJECTED TO ISCHEMIA

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Abstract. Renal cortex was studied during experimentally induced ischemia. A transient increase in anerobic glycolysis occurred with concomitant swelling of both the Golgi apparatus and mitochondria. These intracytoplasmic organelles underwent marked changes in their intracellular positions. Infolding of cytoplasmic membrane at the basal side of proximal tubule cells increased in complexity and proceeded to enclose various intracytoplasmic microorganelles such as mitochondria and the Golgi apparatus. Piling up in layers was particularly marked around mitochondria. This piling up appeared as myelin-like structures on the free surface of, and within, proximal tubule cells, and followed disruption of the brush border at the free surface. Histological examination of thin sections showed that the fused portions of this brush border were actually brush border cytoplasmic membrane piled up in layers giving the appearance of myelin-like structures. After two hours of ischemia, parts of the membrane of these myelin-like structures were disrupted. Large vacuoles developed and these were thought to be related to the large vacuoles seen during cell degeneration.

Key words: Myelin-like structures, ischemia, kidney.

Renal blood flow comprises 25% of the cardiac output. Within the kidney, 90% of this blood flow goes to the cortex where it is used for aerobic oxidative metabolism. This consumes 6–7% of the total oxygen thus available (1–4). During deep anesthesia or shock, however, blood flow to other important organs is safeguarded at the expense of a fall in renal blood flow (5). Overall blood pressure fall is obviated but the kidney is plunged into ischemic conditions which cause damage to the kidney itself. If the ischemic conditions continue for long periods, this damage may remain well after the original situation has resolved and can be one cause of subsequent renal failure (4–6).

The authors investigated the changes which occur during renal ischemia after ligation of renal vessels in domestic rabbits over a period of 5 h from the onset of ischemia. The histochemical and histological changes in renal cortical tissue during this period were studied.

MATERIALS AND METHODS

Domestic rabbits (24 animals, body weight 2.0-2.5 kg) under pentobarbital anesthesia were incised in the lateral abdomen and renal peritoneum together with perirenal tissue was dissected away. The renal vessels were then ligated on both sides, creating ischemic conditions in the kidneys. The kidneys were returned to the retroperitoneal space and the wound closed. The kidney immediately after the establishment of ischemia was used as a control (6 rabbits). Ischemic kidneys for study were removed after 1 h (6 rabbits), 2.5 h (6 rabbits), and 5 h (6 rabbits) of ischemia.

Histochemistry. The kidney specimens obtained at each period were stained for acid phosphatase (Gomori method, 7), beta-glucuronidase (Hayashi method, 8) and lipid granules (Sudan III, 9). The stained sections were studied with a light microscope.

Histology. Kidney specimens were cut into rings 2-3 mm thick and fixed in 10% cold formalin. These rings were then dehydrated in a graded alcohol series, embedded in paraffin, sectioned finely to 4 μ thickness, stained with hematoxylin and eosin, and studied under the light microscope.

For transmission electron microscopy, the kidney cortical tissue was cut into 0.8 mm^3 blocks, fixed in iced 2.5% cacodylate buffered (pH 7.4) glutaraldehyde, then refixed with 1% cacodylate buffered (pH 7.4) osmium tetraoxide. The specimens were then dehydrated in a graded alcohol series, imbedded in Epon 812, and ultrathin sections prepared with a Type 1 Soval ultramicrotome. These were double-stained with lead and uranyl by Reynold's method (11) and studied using a Hitachi HU-11 transmission electron microscope at an acceleration voltage of 75 KV.

For scanning electron microscopy, the renal cortical tissue was finely sectioned ($2 \times 1 \times 1$ mm), double fixed in iced 2.5% cacodylate buffered (pH 7.4) glutaraldehyde, then refixed with 1% cacodylate buffered (pH 7.4) osmium tetraoxide. The fixed specimen was immersed first in glycerin, then in liquid freon, then split in liquid nitrogen. In that state, the tissue was dehydrated in a graded alcohol series, then dried in an Hitachi CPD-1 critical point drying apparatus (11). The cut surface was coated with gold in an Eiko IB-3 ion coating apparatus and studied with a U-3 Nihon Denshi scanning electron microscope at an acceleration voltage of 25 KV.

RESULTS

Changes with time in proximal tubule cells. Light microscopy after 2.5 h of ischemia showed no marked differences from the normal. After 2.5 h of ischemia, parts of the tubule cell basement membrane and cytoplasm had begun to separate. By 5 h, pyknosis was evident in the nucleus and separation of basement membrane and cytoplasm had become marked. The tubule lumen had narrowed. Even after 5 h of ischemia, the myelin-like structures were not visible with light microscopy.

At normal time, proximal tubule cells, at their basement side, had complex infolding following the line of the basement membrane. Intracellularly, numerous large long mitochondria (Mt) extended almost at right angles from the basal side to the free surface. Cristae ran parallel within the mitochondrial matrix (photos 1, 2). At the free surface, the brush was ordered, smooth, and thick (photos 3, 4).

At 30–60 min of ischemia, the complicated infolding from the basal side showed signs of roughness, mitochondria and Golgi apparatus had begun to swell and the electron density of the mitochondrial matrix had decreased. The cristae alignment was disorganized and, within the mitochondria, small flocculant densities had appeared. Intracellularly on the basement membrane side, myelin-like structures existed as multilayers of concentric circles centered around mitochondria (photos 5, 7).

Scanning electron microscopy of the free surface of the tubule cells showed that, at normal time, the brush border was neatly arranged. At 30–60 min of ischemia, this was disorganized and showed entanglement of the villi. With the transmission electron microscope, these appeared as myelin-like structures (photos 6, 8).

At 2.5 h of ischemia, the infolding at the basal side was rough, and many vacuoles (Ve, photo 9) were present at the basement membrane side of the cell. The myelin-like structures were larger than at 1 h of ischemia. Vacuolization was occurring at their centers, and parts of the myelin-like membrane were disrupted. Mitochondria were swollen and the matrix electron density was further decreased. The flocculant densities were larger and cristae alignment was more disorganized (photos 9, 10).

Scanning electron microscopy of the cell free surface showed extreme disorganization of the brush border and increased entanglement of the villi. The brush border surface appeared very rough (photos 10, 12).

At 5 h of ischemia, the infolding from the basal side had disappeared, the myelin-like structures were disintegrating and becoming vacuolated. Large vacuoles were scattered intracellularly (photos 13, 14), there was separation of the basement membrane and cytoplasm (photo 15), and the tubule lumen was narrowed. Mitochondria had swollen even further and the electron density of their matrices was even further decreased. Large flocculant densities were present. The brush border on the free surface was extremely fused, adherent, and its surface was very rough (photo 16).

Changes with time in histochemistry of proximal tubule cells. During the 5 h period of study, no changes were seen in acid phosphatase, beta-glucuronidase or lipid granules.

DISCUSSION

The authors have been studying the changes which occur in the kidney after the development of a state of ischemia. The nature of changes in renal cortical tissue, and the progress of such changes, have been studied histologically. The morphological changes which occur with return of the blood flow to a kidney once plunged into ischemia, and the transition from reversible to irreversible change, have been studied over a period of 5 h from the onset of ischemia. The results have been reported previously (6, 13).

Experimentally, 30–60 min after the creation of ischemic conditions in the kidney, myelin-like structures became evident electron-microscopically both within proximal tubule cells and on their free surfaces. There have been other reports of myelin-like structures on the free surface of tubule cells early in ischemia (14–16), but these do not explain either what the myelin-like structures are, or why they are produced intracellularly.

To clarify these points, the authors' previous study (6) of tissue pH, LDH activity, and changes in lactate and pyruvate was supplemented in the present study by histological staining for acid phosphatase, beta-glucuronidase, and lipid granules. Specimens were studied by light microscopy, transmission electron microscopy, and scanning electron microscopy.

The object of the present experiment was to study as accurately as possible the changes due only to loss of renal blood flow. To achieve this, the kidney was completely dissected out from the surrounding capsular tissue. Blood flow from within the kidney after occlusion of lateral flow was confirmed before ligating the renal artery, veins, and ureter. The kidney was then returned to the retro-abdominal space and the wound closed.

This model of ischemic acute renal failure (ligation of the renal artery in domesticated rabbits) is said to resemble ischemic acute renal failure in man both histologically and functionally (17). In general, it begins with turbid swelling, hyaline degeneration, and pyknosis. At its peak, flattening of epithelial cells, necrotic desquamation, enlargement of vacuoles, and hyaline casts occur. The tubulorrhexis described by Oliver *et al.* (18), however, is not seen. Biochemical studies of these conditions indicated that the cell is maximally active for one hour after ischemia but that, thereafter, cell function begins to deteriorate (6).

From 30–60 min after the onset of ischemia, no change from the normal was evident with light microscopy. With the electron microscope, however, infolding from the basal side appeared coarse, mitochondria were swollen with decreased matrix electron density and disorganized alignment of their cristae. Small flocculant densities corresponding to those of Glaumann *et al.* (14, 15) were also present. Myelin-like structures were seen at the basal side of the cells. The alignment of the brush border on the free surface was swollen and disorganized,

and was fused in places. The surface had started to lose its smooth appearance. With the transmission electron microscope, this disorganization and fusion of the brush border was seen to center around cell projections and, as Suzuki *et al.* (17) suggested, the cytoplasmic membrane which made up the brush border is probably what makes up the myelin-like structures.

At 30–60 min of ischemia, the cell was considered as being in its maximally promoted functional state, with biochemical parameters such as anerobic glycolysis showing great activity (6). Histologically, also, the picture of promoted function was observed: swelling of mitochondria and Golgi apparatus had developed, and many of these organelles were fused together. Rapid changes in their intracellular positions resulted in the development of extended, convoluted infoldings from the basement membrane side. These infoldings were continuous with the membrane which enveloped the various intracellular organelles (double arrow, photo 5). Alkaline phosphatase activity was not studied histochemically, but photos 5, 6 and 8 clearly show that the myelin-like material appearing within, and at the free surface of, cells in response to ischemia was cytoplasmic membrane. With mitochondria at the center, this cytoplasmic membrane infolding in parts of the cell was what made up the myelin-like structures (double arrow, photos 9, 10, 11).

Morita *et al.* (16) reported that fragility of lysosome membrane, followed by autolysis, developed after one hour of ischemia. However, in the present experiments, the myelin-like structures appeared within one hour of ischemia; moreover, histologically, acid phosphatase, beta-glucuronidase and lipid granules were not demonstrable even 5 h after the onset of ischemia. Instead of activation of autolytic enzymes such as lysosome being responsible, therefore, the myelin-like structures were seen to consist of layers of cytoplasmic membrane.

By 2.5 h of ischemia, all cell metabolism has ceased biochemically (6, 14, 15, 19). At 2.5 h, mitochondria and Golgi apparatus were more swollen and numerous vacuoles were apparent intracellularly. The infoldings from the basement membrane side were almost completely absent and the myelin-like structures were larger. Parts of the cytoplasmic membrane of these myelin-like structures had ruptured. Mitochondria were lost from the central part and vacuolization was in progress. The electron density of the mitochondrial matrix was decreased, cristae alignment was disorganized, and large flocculant densities had become evident (photos 9, 10). Brush border alignment on the free surface was disorganized. There was fusion and increased entanglement so that the cell surface appeared coarse. In many tubule cells, separation of cell basement membrane from the cytoplasm had begun (photos 11, 12), and it was histologically evident that, by 2.5 h, the changes due to ischemia had become irreversible.

This point of time has also been reported in other histological studies (13, 20–23).

At 5 h of ischemia, the infoldings from the basal side were lost and numerous vacuoles were scattered intracellularly. Mitochondria and Golgi apparatus were extremely swollen and the electron density of mitochondrial matrices was even further decreased. Large flocculant densities, and mitochondria with parts of their external membrane disrupted, were present. The myelin-like structures were seen as large vacuoles at the center of disrupted cytoplasmic membrane (photos 13, 14).

Brush border on the free surfaces was fused, entangled, and bunched up. Its surface was rough. The basement membrane of the cell was completely separated from the cell cytoplasm (photos 15, 16).

It was concluded, therefore, that the myelin-like structures which appeared both within, and on the free surface of, proximal tubule cells after 30–60 min of ischemia consisted of layers and layers of cytoplasmic membrane centered on mitochondria. This membrane had previously been part of the brush border, or had surrounded intracellular organelles after developing into complex infoldings from the basal side of the cell as part of a temporary increase in cell function due to tissue anoxia. The intracellular myelin-like structures became disrupted with increase in time of anoxia, and lost their central mitochondria. Spaces developed which progressed to become large intracellular vacuolae.

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LEGENDS TO PHOTOS

Abbreviations in Photographs 1-16:

Bb	brush border	Bm	basement membrane
Er	erythrocyte	Mt	mitochondria
N	nucleus	Ve	vacuole

The scale indicates one micron.

1, 2. Transmission electron micrographs of proximal tubule cells showing complex infoldings following the line of the basement membrane (Bm) at normal time. Almost at right angles to the basal side of the cell, there are numerous large, long mitochondria (Mt). Cristae are aligned in parallel within the mitochondrial matrix. A brush border (Bb) is present on the free surface.

3, 4. Scanning electron micrographs of proximal tubule cells at normal time. Infolding is more complex. The brush border on the free surface is smooth, well-ordered, and dense.

5, 6. Transmission electron micrographs of proximal tubule cells after 1 h of ischemia. The basal infolding is somewhat coarse, Mt are swollen, and small flocculant densities (arrow, photo 5) are now present in their matrices. The cytoplasmic membrane is piled up in layers centered on mitochondria and appears as myelin-like structures (double arrow, photo 5). Brush border alignment is disorganized and parts of it appear as myelin-like structures (arrow, photo 6).

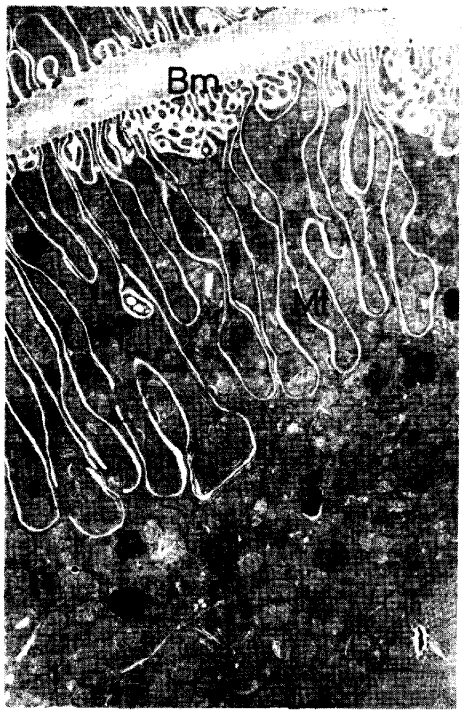
7, 8. Scanning electron micrographs of proximal tubule cells after 1 h of ischemia. The myelin-like structures of photo 5 are seen to be three-dimensional layers of cytoplasmic membrane (photo 7). Brush border alignment is disorganized and the villi are fused in places (photo 8). When sectioned, this is probably what appears as the myelin-like structures.

9, 10. Transmission electron micrographs of proximal tubule cells after 2.5 h of ischemia. The basal infolding is more coarse and the myelin-like structures are larger. Parts of the cytoplasmic membrane making up these myelin-like structures are ruptured and vacuoles have begun to develop. Large flocculant densities (arrows, photo 9, 10) are present in the mitochondrial matrix. Brush border alignment is extremely disorganized and appears as myelin-like structures (photo 9).

11, 12. Scanning electron micrographs of proximal tubule cells after 2.5 h of ischemia. Basal infolding and the cytoplasmic membrane enfolding the mitochondria have formed distinct layers centered on mitochondria (photo 11). The brush border alignment is disorganized and fusion of the villi seen here probably gives the picture of myelin-like structures seen in sectioned specimens (photo 12).

13, 14. Transmission electron micrographs of proximal tubule cells after 5 h of ischemia. Basal infolding has been lost and the myelin-like structures are disrupted and vacuolated (arrow, photo 14). Scattered large spaces have developed intracellularly. Mitochondria are more swollen and their matrices have decreased electron density. Large flocculant densities are present and, in parts, mitochondrial disruption has occurred. The brush border alignment is extremely disorganized and appears as myelin-like structures.

15, 16. Scanning electron micrographs of proximal tubule cells after 5 h of ischemia. Basement membrane and cell cytoplasm have parted and the tubule lumen is narrowed (photo 15). The brush border villi are in mutual contact and fused (photo 16).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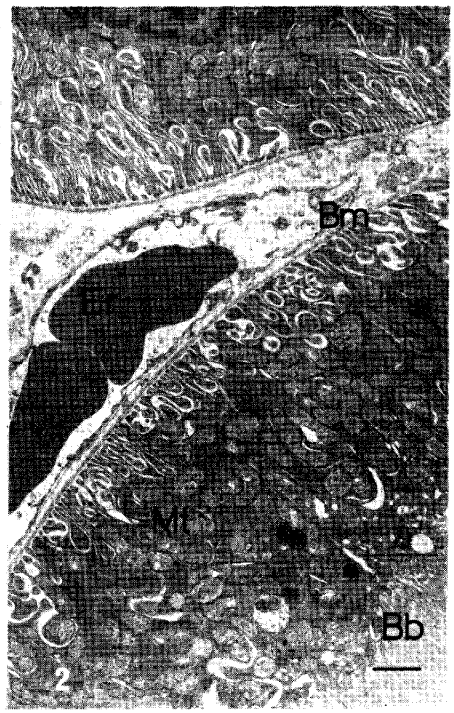
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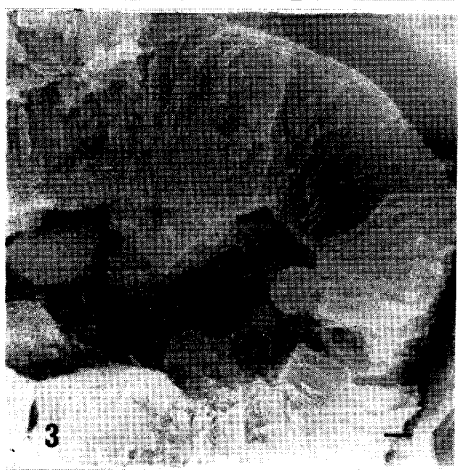
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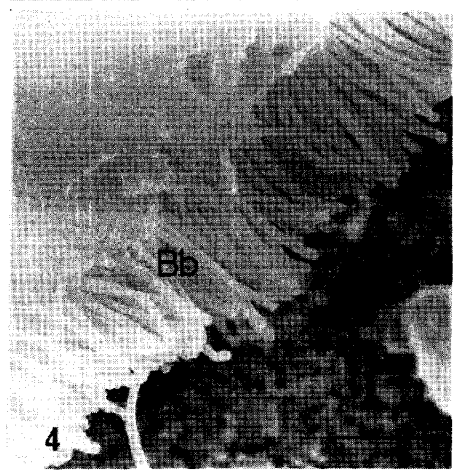
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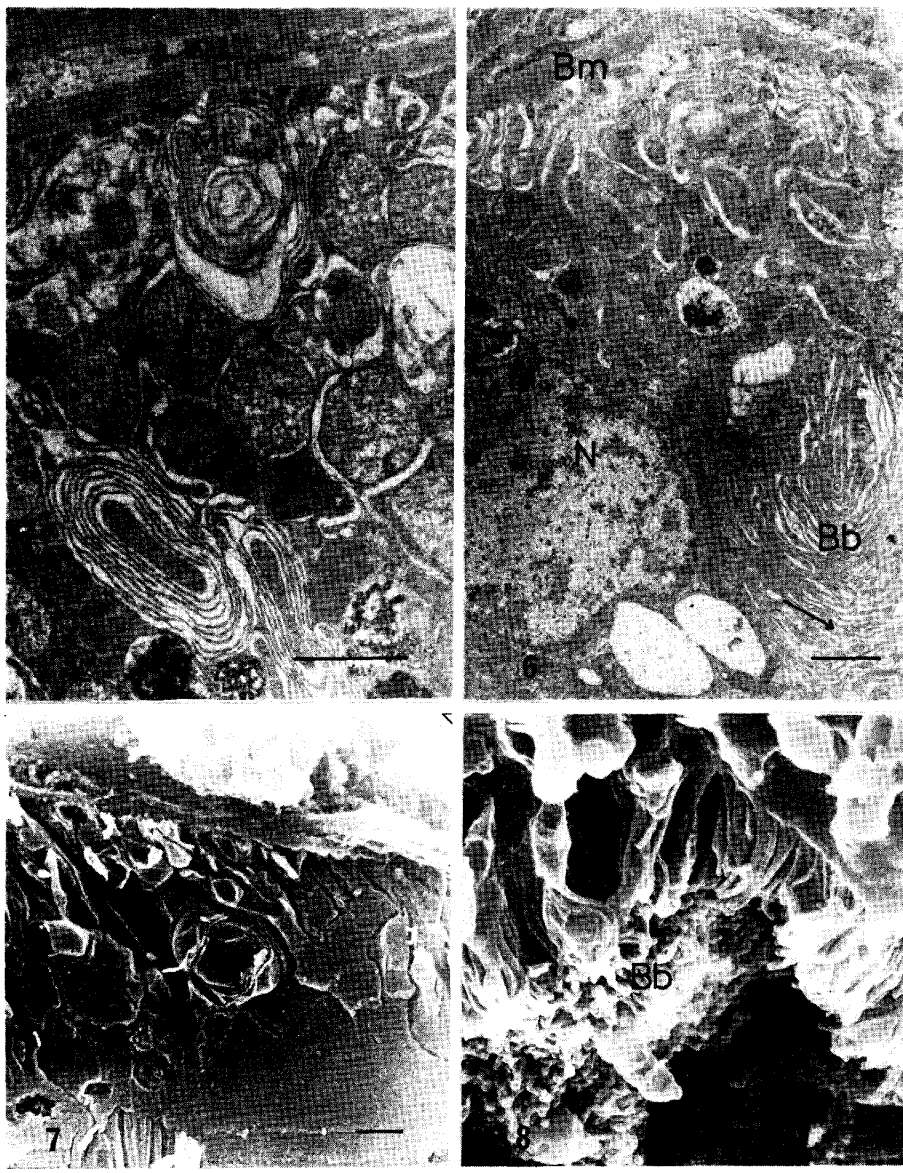
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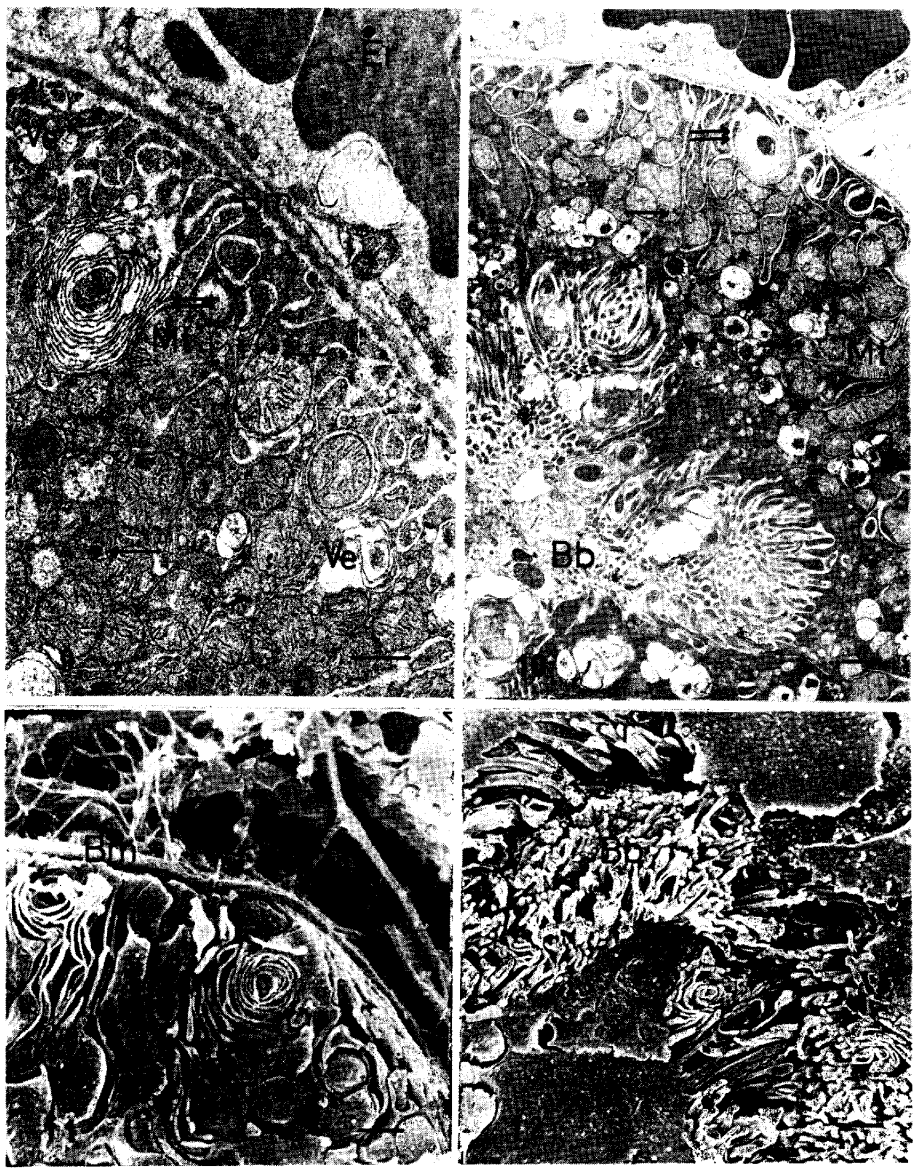
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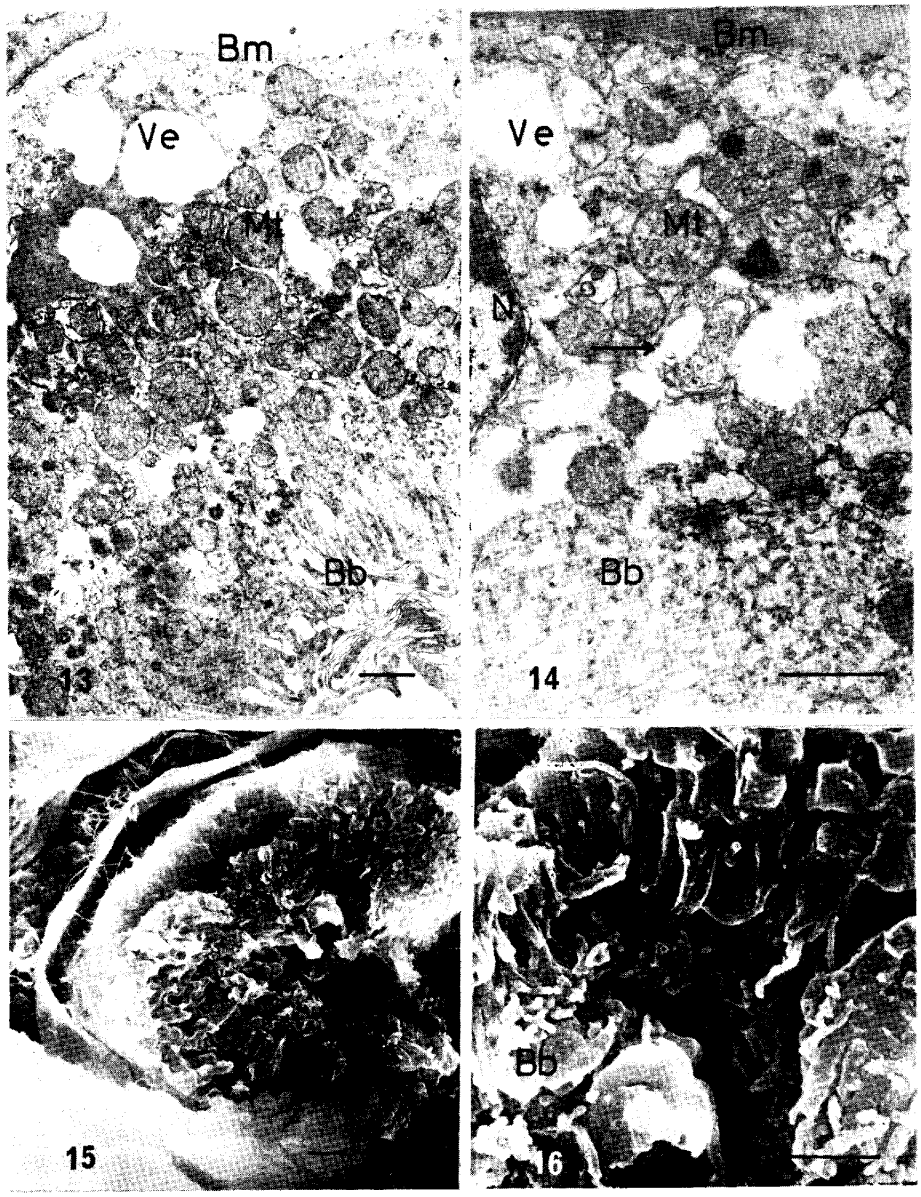
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