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CRYSTALLINE INCLUSIONS IN ENDOTHELIAL CELLS OF POST-CAPILLARY VENULES OF MOUSE LYMPH NODES

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INTRODUCTION

In the previous paper ('64), the writers reported that the endothelial cells of post-capillary venules in mouse lymph nodes contained a prominent Golgi complex and three types of inclusions ; a crystalline inclusion, a lysosome-like dense body and a multivesicular body. The writers were attracted by the crystalline inclusion, because the inclusion seemed to be specific to the venules.

This paper is a further study of crystalline inclusions with special reference to their orgin and fate.

MATERIALS AND METHODS

Mandibular, superficial axillary, subiliac and cranial mesenteric lymph nodes of 11 mice of dd strain and 5 of NIH were used. The names and locations of the lymph nodes were taken from descriptions by KAWASHIMA et al. ('64). Specimens were fixed with 1% osmic acid (PALADE or MILLONIG) and embedded in Epon 812 (LUFT) as a routine procedure. Sections were cut on a HITACHI-UM 3 ultramicrotome using glass knives and staned with uranyl acetate (WATSON) and/or lead citrate (RAYNOLD), then examined with a JEM CHD 4 electron microscope at magnifications varying from 4,000 to 8,000.

RESULTS

The post-capillary venules have a layer of high endothelial cells, whose cytoplasm contains various types of inclusions (figs. 1~3). Numerous small lymphocytes seem to migrate by passing through the venules, though it is difficult to be certain whether lymphocytes are moving from blood to node or from node to blood (fig. 1). In this paper, crystalline inclusions will be described principally.

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1. Structures of the crystalline inclusions

The inclusions with crystalline structures are surrounded by a limiting membrane, spherical in shape and approximately 0.5~2 μ in size. One inclusion usually has one crystal, but sometimes several crystals are seen. The shapes and sizes of the crystals are variable. The shapes are usually irregular polygonal, such as square, rhombic, trapezoid, pentagonal and hexagonal (figs. 6~11). The three dimensional forms of the crystals appear to be nearly cubid, because various cubic sections show all of the above mentioned shapes in photographs. The crystalline substance is homogenous and of moderate density, and at times includes a vesicle-like light area in the inner portion (figs. 7, 9 & 11). In the magnification used in this study, the molecular structures of the crystals are not clear. In addition to crystals, dense particles and lamellar structures are sometimes observed in the finely granular matrix of the crystalline inclusions.

The occurrence of the crystalline inclusions vary from mouse to mouse and from node to node.

2. The relationship of the crystalline inclusions to other inclusions

It is difficult to determine the origin and fate of the crystalline inclusions, but some observations were made which might explain their origin and fate.

A well-developed Golgi complex is characteristic of the endothelial cells and contains numerous vesicles, lamellae and vacuoles (figs. 4 & 5). The vesicles seem to derive from the rough-surfaced endoplasmic reticulum which is located around the Golgi complex (fig. 5). Near the Golgi complex, multivesicular bodies are frequently observed and seem to be formed from Golgi vacuoles and vesicles (figs. 1 & 4), as described in the previous paper. The matrix of the multivesicular bodies varies from a light, empty-like matrix to finely granular one. Moreover, transitional forms are found among them (figs. 1, 3 & 4).

The crystalline inclusions are also variable not only in the shape of the crystals but also in features of the matrix and limiting membrane (figs. 6~11). Transitional forms are observed among same crystalline inclusions, multivesicular bodies with a fine granular matrix and some lysosome-like dense bodies; the latter two kinds of bodies sometimes iuclude a substance which is difficult to distinguish from typical crystals in its density (figs. 1, 3 & 5).

The vesicles with a fine granular matrix are frequently observed in the basal portion of the endothelial cells and are sometimes irregular in shape (figs. 1~3 & 12). The limiting membrane of some crystalline inclusions is uneven and related to the vesicles with a fine granular matrix (fig. 9). Figure 12 clearly shows that the vesicles open to the intercellular space.

It is morphologically clear that the crystalline inclusions are closely related to the rough-surfaced endoplasmic reticulum, the Golgi complex, and other types of inclusions and vesicles with fine granular matrix.

DISCUSSION

The fine structures of the post-capillary venules of lymph nodes were reported by BURWELL ('62), CLARK ('62), MARCHESI & GOWANS ('64), MIKATA & NIKI ('66) and SUGIMURA et al. ('64), but details of the crystalline inclusions were not described.

Many kinds of crystalline inclusions are found in various organelles of various types of cells ; in the nucleus, mitochondria, the endoplasmic reticulum, the Golgi complex, in the secretion granules, and free in the cytoplasmic matrix (FAWCETT '66). Out of those the yolk platelets of frogs (KARASAKI '66) and the specific granules of eosinophil leukocytes (see fig. 13) are somewhat similar to the crystalline inclusions found in the post-capillary venules, but differe from them in detail. That is to say, the crystalline inclusions described in this report seem to be a new type.

Some lysosomes of reticular cells resemble the crystalline inelusions in size and possession of an outer membrane. In normal mice, the endothellial cells of post-capillary venules did not show any detectable activity of acid phosphatase in light microscopy (SUGIMURA et al. '63). On the other hand, MIKATA & NIKI ('66) histochemically observed activity of acid phosphatase in the venules of the infected mice. However, according to NOVIKOFF ('61) lysosomes are ferritin containing particles without crystalline bodies. It is suggested that the crystalline inclusions may differ from lysosomes, but further histochemical studies at the electron microscopic level are necessary.

The well-developed Golgi complex and the considerably numerous rough-surfaced endoplasmic reticulum are characteristic of the endothelial cells. The Golgi complex is closely related to the rough-surfaced endoplasmic reticulum. This feature is closely related to that of protein-secreting glandular cells. It is worthy of note that the endothelial cells frequently contain multivesicular bodies and lysosome-like dense bodies. In some photographs, two kinds of these bodies include a crystalloid substance.

The origin of the crystalline inclusion is difficult to determine, but these findings suggest the following ; a raw substance of the crystals is synthesized in the rough-surfaced endoplasmic reticlum and then transferred to the Golgi complex. The Golgi vesicles and vacuoles form multivesicular bodies in which the substance is condensed. Consequently, the matrix of the multivesicular bodies become finely granular and the bodies appear to trasform to lysosome-like dense bodies. In the multivesicular and lysosome-like dense bodies, the raw substance is crystallized.

Occurrences of the crystalline inclusions vary from mouse to mouse and from node to node. Accordingly, the crystals may appear only under some unknown conditions.

Concerning the fate of the crystalline inclusions, certain observations come the writers' notice ; it was noted that the vesicles with a fine granular matrix, which is similar to that of the crystalline inclusions, are often observed in the basal portion of the endothelial cells. The fine granular vesicles are closely related

with the outer membrane of the crystalline inclusions and a few vesicles open into intercellular space. This feature suggests secretion by a reverse pinocytotic mechanism.

From these observations, it is suggested that the endothelial cells of the post-capillary venules synthesize an unknown protein containing substance which is released into the intercellular and basal surfaces of the cells. The crystalline inclusions appear to be a storage form of a substance synthesized in the post-capillary venules.

In sections, lymphocytes migrating through the venules are most frequently found in the basal side of the endothelial cells as described by MARCHESI & GOWANS ('64); this means that lymphocytes may stay in this place for a longer time. It is worthy of note that the fine granular vesicles and the release of their granules are most frequently observed in the basal side of the endothelium. That is, the endothelial cells appear to produce any substance which would be useful in lymphocytic functions, probably in their immunological ones, but further evidence is necessary.

SUMMARY

The crystalline inclusions in the post-capillary venules of mouse lymph nodes were observed by use of electron microscopy with special reference to their origin and fate.

The inclusions are surrounded by a limiting membrane, spherical in shape and approximately $0.5 \sim 2\,\mu$ in size. One inclusion usually has one crystal, but sometimes several crystals. The three dimensional forms of the crystals appear to be nearly cubic. It is morphologically clear that the crystalline inclusions are closely related to the rough-surfaced endoplasmic reticulum, the Golgi complex, multivesicular bodies, lysosome-like dense bodies and vesicles with a fine granular matrix.

It is suggested that the endothelial cells synthesize a substance which is condensed in the Golgi complex and multivesicular bodies, and then the condensed fine granular substance is released into the basal and intercellular spaces of the endothelium. The crystalline inclusions are presumed to be a storage form of a substance synthesized by the endothelial cells.

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EXPLANATION OF PLATES

All scales printed in the figures are shown at 1 μ.

Plate I

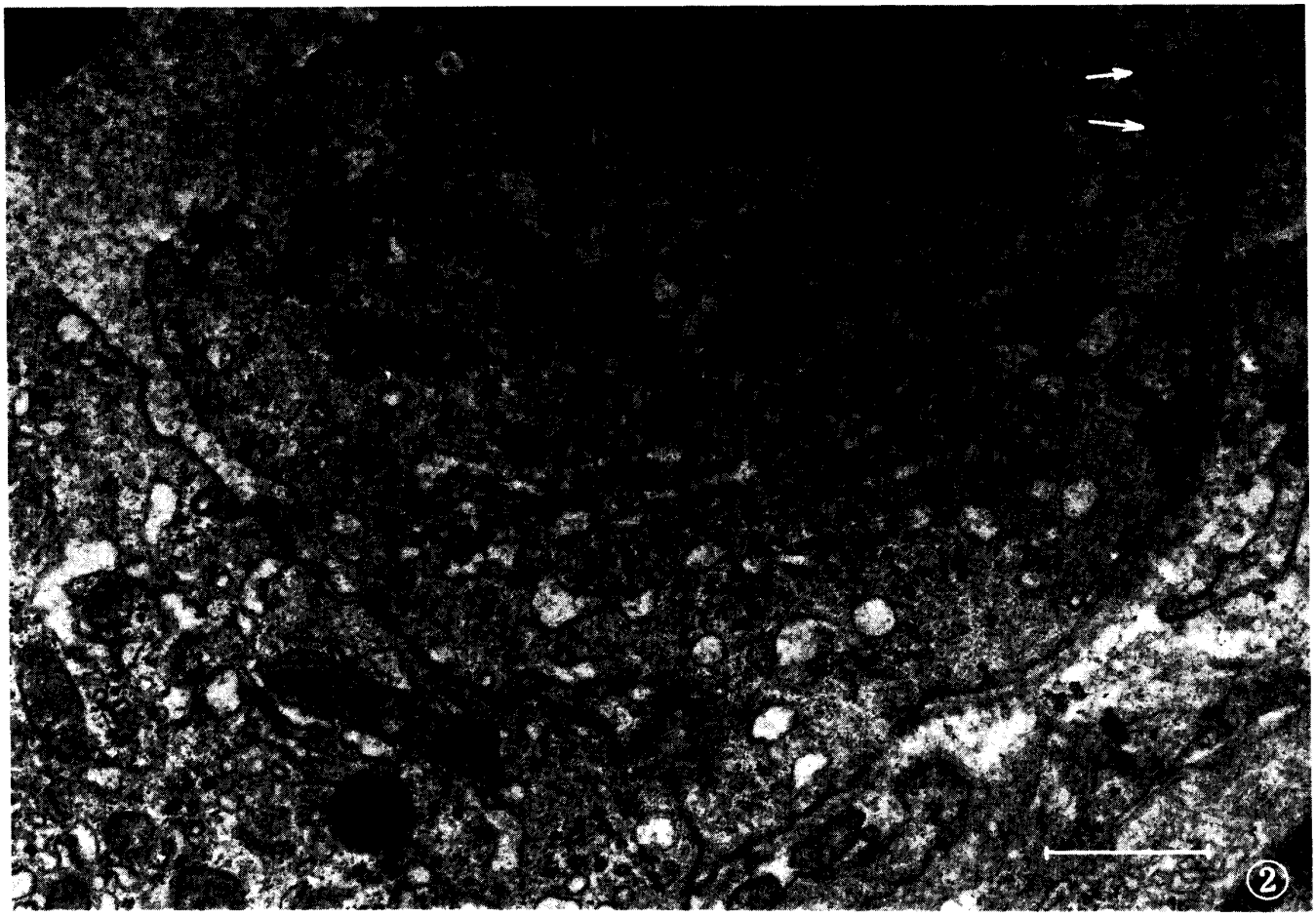
Fig. 1 In this figure, an endothelial (E) and a migrating lymphocyte (L) are shown. The endothelial cell contains a well-developed Golgi complex (G) and many inclusions; multivescular bodies with a light matrix (1), fine granular matrix (2), and crystalloid substance (3), inclusion with a rhombic crystal (4) and a degenerated inclusion (5),. Several dense irregular vesicles (6) aggregate near the basal surface of the endothelial cell and one of them appears to open into the intercellular space where a migrating lymphocyte is situated. It is suggested that various types of inclusions may be transformed from (1) to (6). Lead staining × 16,200

E
C
2
3
5
4

Plate II

Fig. 2 The cytoplasm of the endothelial cells contains numerous rough-surfaced endoplasmic reticulum and polysomes. Several vesicles with a fine granular matrix (arrow) are found in the basal pedicles of the endothelial cells. Double staining × 17,500

Fig. 3 In this figure, the basal portion of the endothelial cells is shown. In their cytoplasm, multivesicular bodies with a light matrix (MB 1) and a fine granular matrix (MB 2), and two lysosome-like dense bodies (DB) are observed. Note the crystalloid substance in the matrix of the larger dense body (arrow). Double staining × 17,500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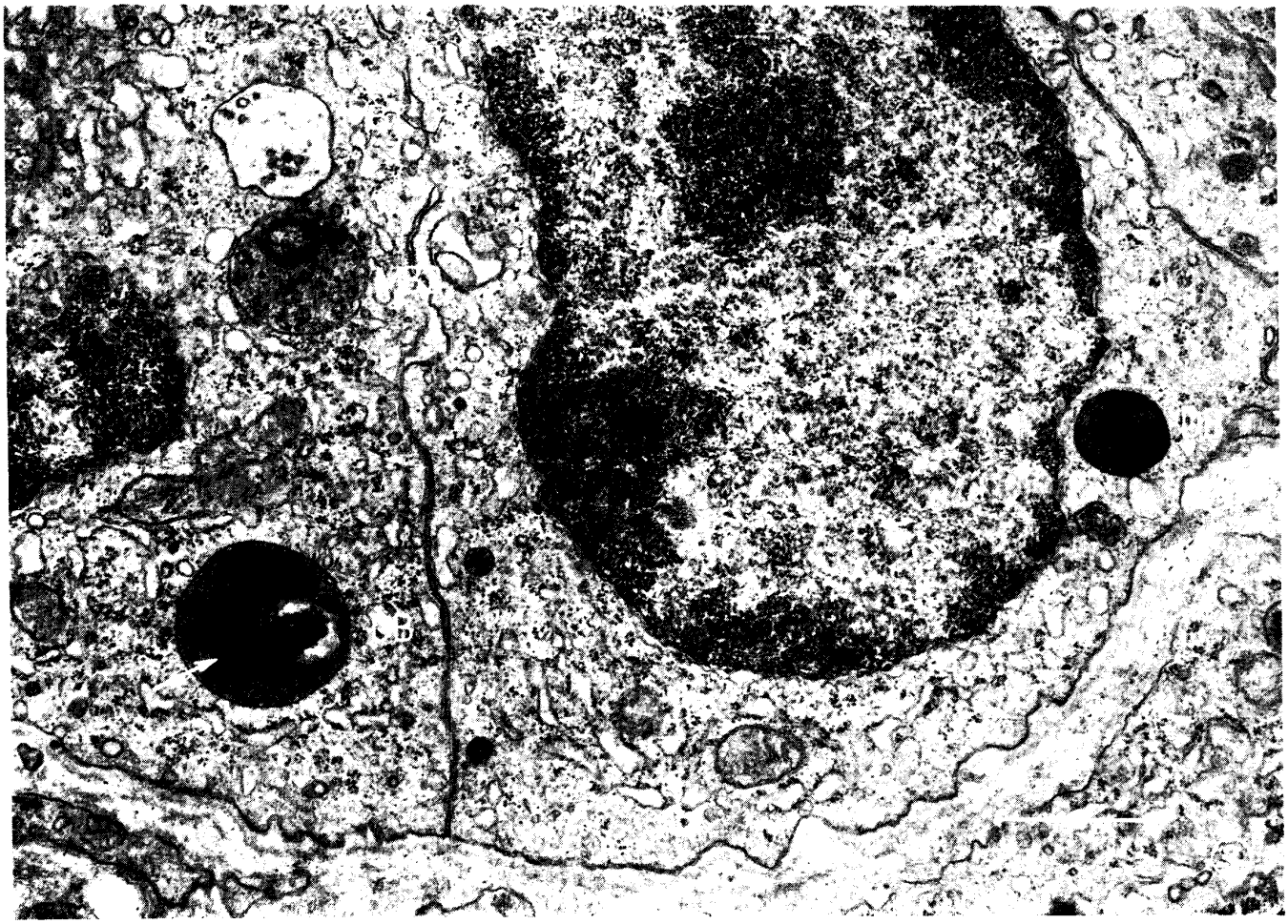


Plate III

Fig. 4 Note the multivesicular bodies with a light matrix (MB1) and those with a granular matrix (MB2), and the dense body with a granular matrix (DB) near the Golgi complex (G). Lead staining × 14,000

Fig. 5 Near the well-developed Golgi complex (G), two dense bodies (DB) with crystalloid substance are observed. Golgi vesicles appear to be derived from the rough-surfaced endoplasmic reticulum (arrow). Lead staining × 28,000

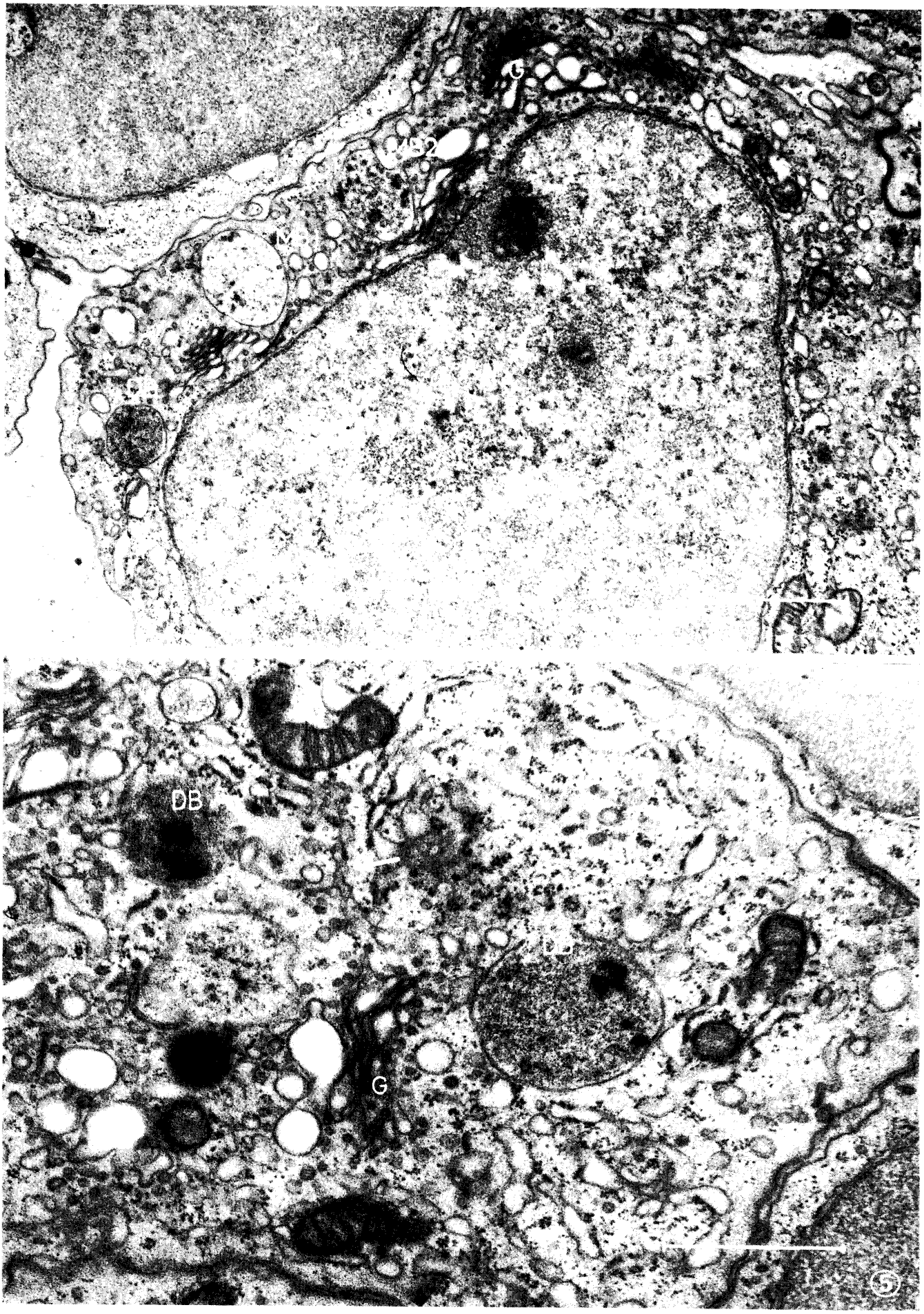

DB
G

Plate IV

In this plate, various shapes of crystals are shown. All figures are magnified to 28,000 times and stained by lead citrate.

Fig. 6 This inclusion is surrounded by a limiting membrane. Crystal is square in shape.

Fig. 7 Note the rhombic crystal in this inclusion. Light vesicle-like areas are observed in the crystal.

Fig. 8 This inclusion contains four crystals. The central one is hexagonal and the lower one is pentagonal in shape.

Fig. 9 This crystal is a trapezoid shape. Note vesicles with the granular matrix (arrows) which are connected with the uneven limiting membrane of the inclusion.

Fig. 10 Several deformed crystals are observed in this inclusion which is surrounded by a uneven membrane. Dense particles and lamellar structures are found in the granular matrix. Near the inclusion, irregular vesicles with a granular matrix are found (arrows).

Fig. 11 Several deformed crystals are found in this inclusion. Note numerous light vesicle-like areas in crystals.

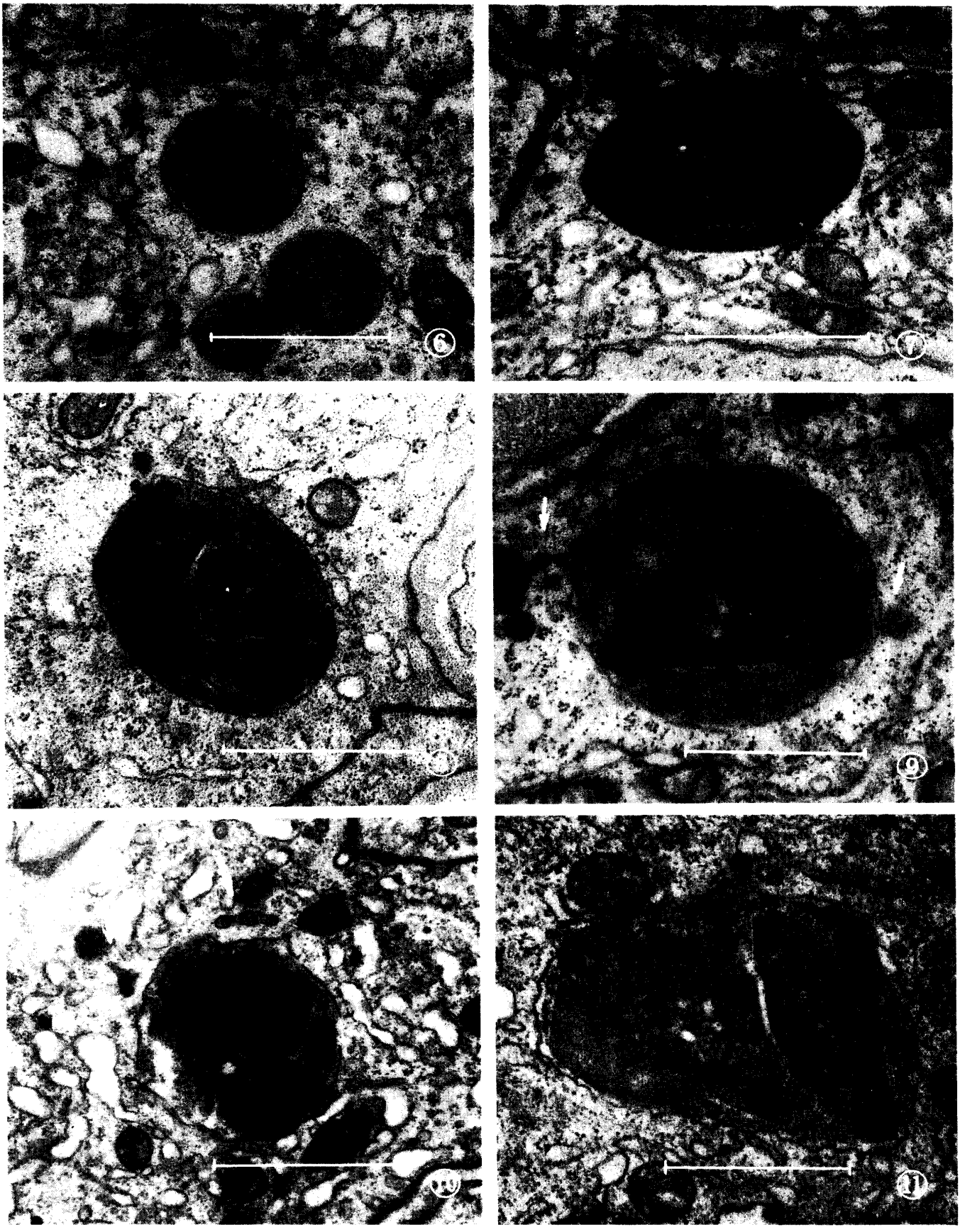
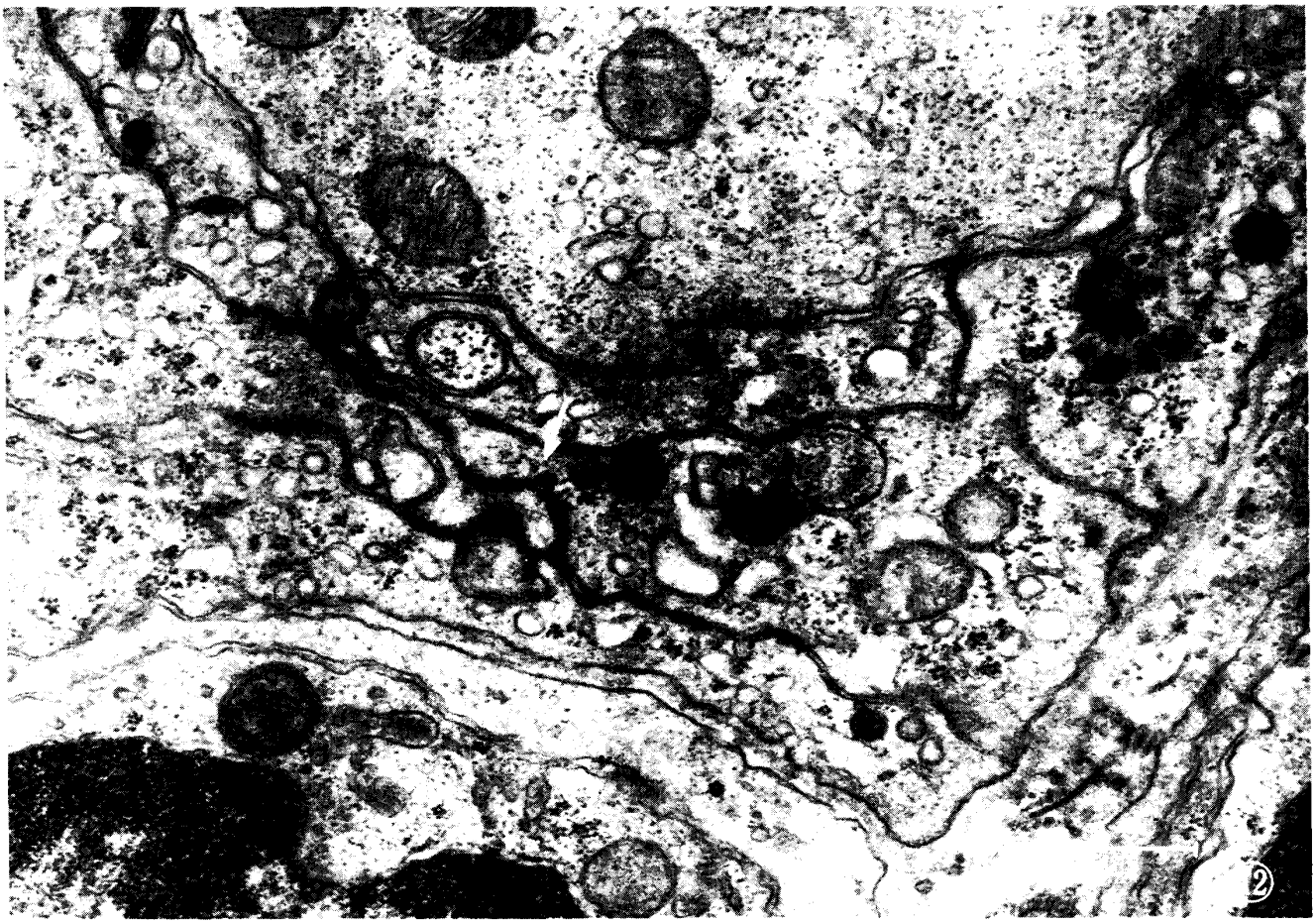
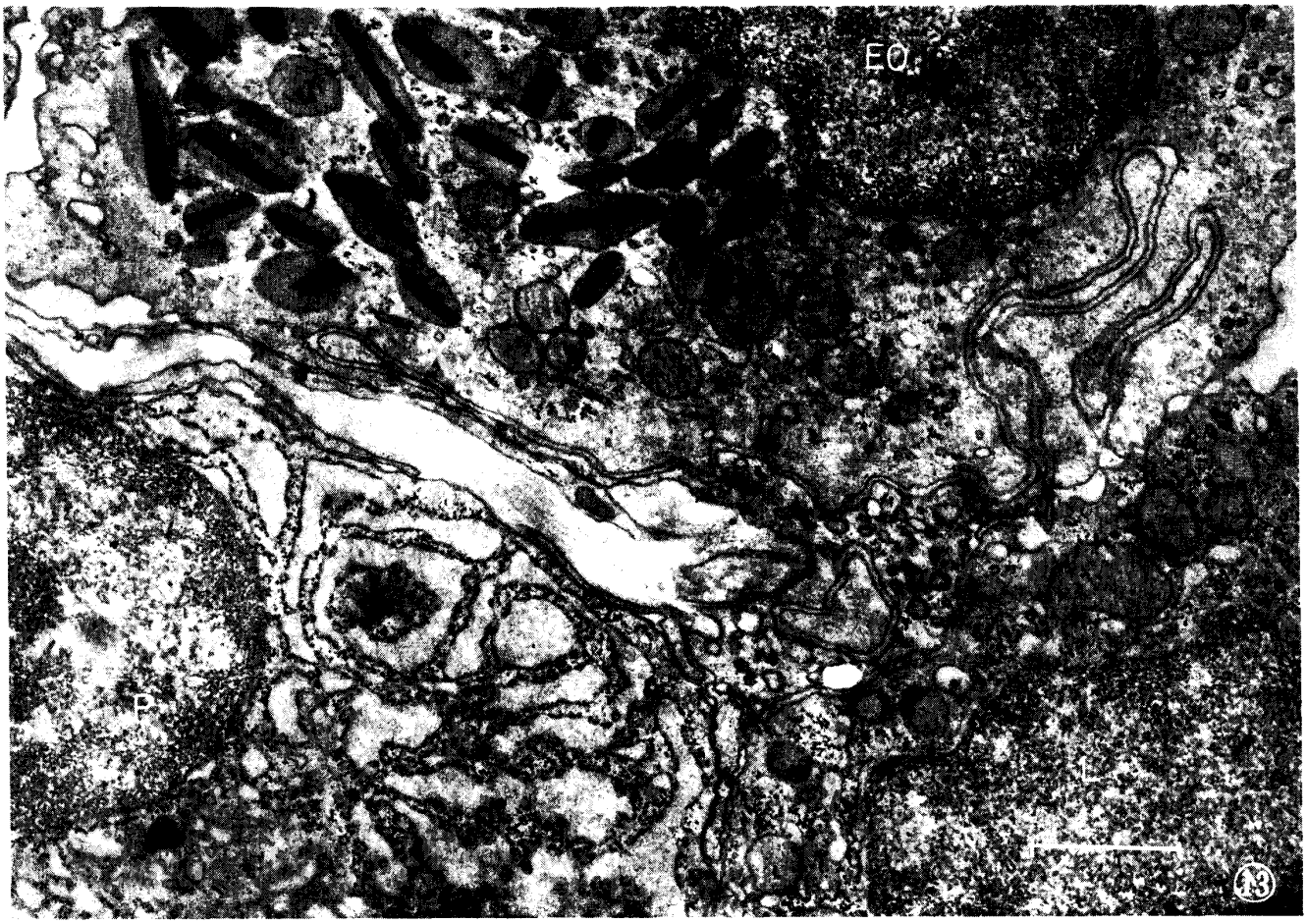


Plate V

Fig. 12 Terminal bar-like junctions and an interlocking of the pedicles of the endothelial cells are observed. There are numerous irregular vesicles with a granular matrix in the pedicles and a vesicle opens into intercellular space (arrow). Double staining × 28,000

Fig. 13 In this figure, a lymphocyte (L), plasma cell (P) and eosinophil leukocyte (EO) in medulla of the lymph node are shown. Specific granules of eosinophil leukocytes are somewhat similar to the crystalline inclusions, but differ in detail. Lead staining × 17,500




EO.