The Ultrastructure of Mouse Lung: The Alveolar Macrophage*

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ABSTRACT

Free alveolar macrophages of normal mouse lung have been studied in the electron microscope. The tissue was obtained from several young adult white mice. One other animal was instilled intranasally with diluted India ink 1½ hours prior to the removal of the lung. Thin sections of the osmium-fixed, methacrylate-embedded tissue were examined either in an RCA EMU 2 electron microscope or in a Siemens and Halske Elmiskop I b. A few thick sections obtained from the same embeddings were stained for iron.

The normal alveolar macrophages, which are usually in contact with the alveolar epithelium, were found to contain a variety of inclusion bodies, along with the usual cytoplasmic components like mitochondria, endoplasmic reticulum, and Palade granules. Another typical component of the cytoplasm of these cells which appears as small ($\sim$ 6 mμ) very dense granules of composite fine structure is interpreted as ferritin. It is assumed that this ferritin is formed from red blood cells ingested by the alveolar macrophages.

The macrophages in the alveoli were found to phagocytize intranasally instilled India ink particles. Such cells, with engulfed India ink particles, were often of more rounded form and the particles were frequently seen lying inside membrane-bound vacuoles or vesicles of the cytoplasm. The membrane of a few vesicles containing India ink particles was seen as the invaginated portion of the cell plasma membrane, and in one instance these same vesicles were seemingly interconnected with a rough surfaced cisterna of the endoplasmic reticulum. The process of phagocytosis is recognized as related to the "normal" process of pinocytosis.

INTRODUCTION

For many years light microscopists have been studying the histological structure of lung tissue. Most of these investigations attempted to clarify the following questions:

1. Presence or absence of a continuous alveolar epithelium.
2. Nature and origin of the different cells seen in the alveolar septa.
3. Nature and origin of the free phagocytic cells inside the alveolar lumina.
4. Phagocytosis by the different cells of lung tissue.
5. The origin of cells seen under pathologic conditions and their relation to the different cells of normal lung tissue.

Most of the older light microscopic work has been reviewed by Bargmann (1).

Some of the above questions have been reinvestigated in more recent electron microscopic studies. Various authors agree that a continuous cellular lining of the alveoli does exist in the lungs of mammals (2–8), but their work does not correlate the various types of cells as seen in the electron microscope with the cell forms described by the light microscopists, nor does it solve the question of the origin of the alveolar epithelium. Other electron microscope studies have dealt with cells other than the alveolar epithelium (9–11), *e.g.*, capillary endothelium, blood cells, and thrombo-

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cytes inside lung capillaries, lung blood vessels, etc. And still other electron microscope investigations have been concerned with lung pathology (12–13).

The present report deals with the free phagocytic cell or alveolar macrophage. This cell which has been studied by numerous light microscopists (14) is known to have an important phagocytic function in normal lung and especially in pathologically altered lung. Some electron microscopic evidence of such phagocytosis will be given in the present report.

Material and Methods

Young adult (about 2 to 3 months) white mice (Princeton strain) of both sexes were used in these experiments. They had been bred in the laboratory in a clean animal room; however, no special precautions were taken to protect the animals from air-borne dust as is prevalent in the air of any large city. The mice were killed by breaking their necks, and their lungs were fixed by infusion through the trachea and through the heart (3, 4). 1 per cent veronal-buffered (15) osmium tetroxide in isotonic saline solution served as the fixative. Subsequent to the perfusion, small pieces of lung were fixed *in vitro* for at least 1 to 2 hours at 0°C. Some specimens were washed in distilled water at 0°C. for varying periods of time, but in the case of others, washing was omitted entirely. They were dehydrated in ice cold acetone of increasing concentration starting with about 20 per cent and allowing about 15 minutes in each change. The tissue was finally impregnated at 4°C. in a mixture of 3 to 4 parts *n*-butyl methacrylate and 1 part ethyl methacrylate containing 0.1 to 0.2 per cent benzoyl peroxide as catalyst. The small tissue pieces were then embedded (in No. 5 gelatin capsules) in the same methacrylate mixture which had been prepolymerized (16) to a very high viscosity. Polymerization was carried to completion in an oven set at 75 to 80°C. (16) over a period of 2 to 3 days. In later experiments a vacuum oven was used. Small holes were punched in the tops of the gelatin capsules which contained the tissue (17), and the oven was evacuated by means of a mechanical pump a few hours after the capsules had been placed in it. This procedure was followed in order to insure an even better polymerization of the methacrylate (17). A time lapse between placing specimens in the oven and the evacuation of this oven was necessary to prevent volatilization of the prepolymerized methacrylate matrix. In order to insure optimal cutting properties of all polymerized specimens, they were kept in a vacuum for several hours after the gelatin capsules had been removed (4).

In the preliminary phagocytosis experiment reported in this paper, the mouse was anesthetized with ether, and a 1:10 saline-diluted suspension of India ink (American black India ink, Higgins) was dropped into its nostrils (0.02 to 0.05 ml.). The animal was killed 1½ hours after the India ink instillation, and the lung processed as described above.

Sections, cut on a Porter-Blum microtome (18) with glass knives (19), were mounted on copper grids coated with carbon films (20) and were examined either in an RCA EMU 2 electron microscope (Figs. 2 to 5), or in a Siemens and Halske Elmiskop I b (Figs. 1, 6 to 18). In the latter the double condenser was used in combination with a 750 μ condenser I aperture and a 400 μ condenser II aperture.

In order to stain specifically intracellular inorganic iron, the Berlin blue reaction (using potassium ferrocyanide, 21) was performed on 3 micron thick sections from which the methacrylate had previously been removed by acetone. These sections were counterstained with eosin.

OBSERVATIONS

Alveolar Macrophages of Normal Lung:

General Appearance.—The alveolar macrophages are recognized as free cells in the alveoli. They were found in the lungs of all young adult mice that were examined. Their number was rather small so that not every thin section contained a macrophage. The same small number of such cells was found in the lung of one older (9 months) mouse. They are nearly always seen adjacent to an alveolar septum and they make contact with the epithelial lining of this septum by means of small pseudopods (*Pp*, Figs. 1 to 3). Their form is highly irregular, judging by their profiles which sometimes appear triangular (*Am*, Fig. 1) or polygonal. A few cells may also appear flat (Fig. 4). Some of them attenuate to form thin and elongated extensions of cytoplasm (Fig. 1). However, typical microvilli (22) such as are common with alveolar epithelial cells (4) are never observed.

These cells contain one nucleus of somewhat oblong shape (*N*, Fig. 4). No multiple or polymorphous nuclei are observed. This nucleus is bounded by a double membrane.

The cytoplasm of normal macrophages contains numerous structures which seem to vary from one cell to another in appearance as well as in number. These structures are: the mitochondria, various elements of the endoplasmic reticulum, some large vacuoles, various dense inclusion bodies, Palade granules, and very dense granules interpreted as ferritin.

The Mitochondria.—The mitochondrial profiles (*Mi*, Figs. 1, 3, and 4) are rather small (0.5 to 1 μ), round or oblong in shape, and are not numerous in "normal" alveolar macrophages. They are bounded

by two closely apposed membranes. The inner membranous structures (cristae, 23, 24) appear in some electron micrographs as closed profiles (*Mi*, Fig. 6), and are therefore believed to have the shape of tubules or flattened tubules (25–29), not of "double membranes" (30, 31). Some of these mitochondria contain a conspicuously large (50 mμ) intramitochondrial granule (*Mi*, Figs. 1 and 7).

The Endoplasmic Reticulum.—This canalicular system (32) is composed of variable subunits. Some of these are short flattened tubules or cisternae with dense Palade particles adhering to the outside of the limiting membrane (rough surfaced variety (32)) (*E*, Figs. 5 and 6). These rough surfaced profiles of the reticulum are nearly always short and inconspicuous. They are encountered but rarely in the cytoplasm of "normal" alveolar macrophages. Another portion of the endoplasmic reticulum appears in the form of small (40 to 60 mμ) round or oblong profiles of vesicles. They are often arranged in rows and presumably represent sections through tubules (*Ve*, Figs. 5 and 6). No Palade particles adhere to their limiting membranes (smooth surfaced variety (32)). At times a larger vacuole without recognizable contents can be identified as yet another portion of the reticulum because of its continuity with a rough surfaced profile (Va_1, Fig. 6).

The Large Vacuoles.—Most macrophages contain a small number of larger (200 to 800 mμ) vacuoles that have no immediately apparent connection with units of the endoplasmic reticulum (*Va*, Figs. 1 to 5). They usually occur singly rather than in groups and are frequently found lying close to the cell surface (*Va*, Figs. 1, 2, 4, and 5). These vacuoles are bounded by a thin dense membrane. Their clear interior usually has the same density as the methacrylate-embedding matrix. In some cases, however, they appear to contain a material of low density (Va_1, Fig. 3).

The Inclusion Bodies.—All alveolar macrophages of normal lung contain a variable number of inclusion bodies of highly varying size, shape, and density (*In*, Figs. 1 to 13). They are usually of oblong shape and measure 0.3 to 1.5 μ in diameter. Several different types can be recognized on the basis of their density and fine structure. One type of inclusion contains small, very dense masses inside a matrix of medium density, and is bounded by a single or double membrane (*In*, Figs. 2, 3, 13; In_1, Fig. 4). Another somewhat similar type of inclusion contains small, very dense masses in greater number than the aforementioned type; it, however, is not bounded by a well defined membrane (*In*, Fig. 6). A third type shows a laminated fine structure of its matrix (In_2, Fig. 5; In_1, Fig. 11; Fig. 12). Less dense layers approximately 6 mμ wide, which are arranged concentrically, alternate with denser membranes, 4 to 6 mμ thick. These denser membranes in their turn are not single units but appear as triple layered structures (Fig. 12). A fourth and rather large (up to 1.5 μ) type of inclusion is bounded by a membrane. It is of medium density, but may consist of denser material at its periphery. This denser material either forms a continuous peripheral shell (*In*, Fig. 7; In_1, Fig. 10) or consists of globular masses (In_1, Fig. 8).

The Palade Granules.—These particles (33), which measure about 15 mμ in diameter, occur either randomly distributed (*G*, Fig. 2), or they are seen in conjunction with elongated profiles of the endoplasmic reticulum (*G*, Figs. 5 and 6). They are not numerous in the normal alveolar macrophages.

The Dense Granules (Ferritin).—In all normal alveolar macrophages a large number of extremely dense granules can be found distributed at random (*Fg*, Figs. 2, 5 to 8). They are only rarely found inside mitochondria (Fg_1, Fig. 6). Similar granules are sometimes arranged in clusters which lie either inside inclusion bodies (Fc, Figs. 8 and 9) or which are apparently independent from such bodies (*Fc*, Figs. 10 and 11). These clusters are always small (~200 mμ). With adequate resolution these granules are recognized as composite structures even in electron micrographs of low magnification (*Fg*, Figs. 6 and 7). At higher magnification, they are seen to consist of several dense subunits (*Fg*, Figs. 11, 13, and 14). In these electron micrographs the average diameter of the whole granule measures about 6 mμ; the subunits inside these granules, which in some cases form a tetrad (*Fg*, Fig. 11; Fg_1, Fig. 14) or a pentad (Fg_1, Fig. 13), measure about 1 to 2.5 mμ in diameter, and their centers are about 22 to 32 A apart. A light halo which surrounds the granules in some electron micrographs (Figs. 2, 8, and 9) does not appear on highly magnified and exactly focused pictures (Figs. 11 and 13). Accordingly, it is interpreted as a fringe phenomenon due to underfocusing rather than as a real structure of the granule itself. Very rarely, a few similar granules are encountered also inside the alveolar epithelial lining (Fg_2, Fig. 2). Because of their similarity to purified ferritin (34)

these gran les are interpreted as the iron hydroxide cores of ferritin molecules.

The presence of inorganic iron in alveolar macrophages is also recognized in thick sections of lung stained with potassium ferrocyanide (Berlin blue reaction). The free alveolar macrophages are readily recognized in such sections. Their cytoplasm is seen to contain blue masses of varying size, which thus indicate the presence of inorganic iron.

Alveolar Macrophages after India Ink Inhalation:

One and ½ hours after intranasal instillation of 1:10 diluted India ink the number of macrophages is not conspicuously different from that in normal lung. They are still not found in every thin section examined (estimated area of a few hundredths of 1 square millimeter). However, some of these macrophages appear different in their general shape compared with those of untreated lungs. They are more rounded and tend less to cling to the alveolar walls (Fig. 15). Invariably these macrophages contain huge numbers of India ink (carbon) particles, and clumps of similar particles are seen free in the alveolar air spaces (I_2, Fig. 15). Cells other than macrophages, notably those belonging to the alveolar epithelium, also appear altered to some degree but do not contain India ink. These changes in cells other than macrophages will be the subject of further studies.

The macrophages that contain India ink show mitochondria roughly similar in number and shape to those seen in normal macrophages. They also contain ferritin granules either in random distribution (Figs. 16 and 18) or in clusters (*Fc*, Fig. 17). The endoplasmic reticulum, however, appears somewhat altered (as compared with normal alveolar macrophages), since the rough surfaced profiles are more numerous and are often arranged in parallel rows (*E*, Fig. 18). Palade granules, compared with those in normal macrophages, appear more numerous within the cytoplasm between the rough surfaced (*E*, Fig. 18) profiles.

The India ink particles nearly always lie either inside large vacuoles (*Va*, Fig. 17) or inside smaller vesicles (*Ve*, Fig. 18) which are bounded by a dense membrane. The membrane which bounds the small vesicles can be seen as continuous with the cell plasma membrane in some cases (*Ve*, Fig. 16), and with the membrane of the rough surfaced endoplasmic reticulum in other cases (*Ve*, Fig. 18).

DISCUSSION

Alveolar Macrophages of Normal Lung:

Throughout this report the term alveolar macrophage is used to designate the free cells in alveoli of normal lung. This is done for the sake of convenience. It does not imply that these cells are necessarily considered the equivalent of connective tissue macrophages. These free cells are believed to cleanse the alveolar air spaces physiologically as well as in pathologic conditions by engulfing inhaled dust, cell debris, bacteria, etc. Thus, the numerous inclusion bodies as seen in the electron microscope (*In*, Figs. 1 to 4, 7 to 13) are interpreted as remnants of such phagocytized materials.

These inclusion bodies are of rather varying appearance, probably because they originate from different ingested materials. Their exact origin cannot be determined on the basis of their morphology. Nevertheless, certain large ($\sim$1.5 μ) inclusions (*In*, Fig. 7; In_1, Fig. 10) resemble cells sufficiently to be considered the remnants of ingested cells or cell debris. The accumulations of ferritin granules inside or close by such inclusions (*Fc*, Figs. 8 to 10) suggest that these cells might have been erythrocytes from which the ferritin was formed during their breakdown inside the alveolar macrophage. The evidence available at the present time does not permit one to conclude where and how alveolar macrophages have ingested the erythrocytes. Nor is it possible to decide how large a proportion of the normally observed inclusion bodies are remnants of extraneous dust inhaled by the animals. Such dust is prevalent in the air of large cities, and its presence in the air of the animal room used must be suspected.

The small dense granules that are found in the cytoplasm of all alveolar macrophages are interpreted as the iron-containing core of ferritin molecules, because of their resemblance to the iron-containing core in molecules of purified ferritin (34). This core measured up to 55 A in diameter and consisted of several iron-containing micelles about 27 A in diameter which sometimes were seen arranged in tetrads (34). Approximately the same dimensions and fine structure characterize the granules found inside alveolar macrophages. The protein hull of ferritin molecules is of too low a density to be detectable in tissue sections. Even in purified ferritin its outline was recognized only after shadow casting (34, 35).

Light halos around ferritin granules (*Fg*, Figs. 2, 8, and 9) are interpreted as underfocus fringe phenomena rather than as the protein hull (apoferritin) of the ferritin molecules since such halos are absent in accurately focused electron micrographs (Fig. 11). The iron nature of these granules is further supported by findings of similar granules, presumably ferritin, inside sectioned red blood cells (36–41) and hemosiderin bodies (42, 43). It is assumed that the ferritin of the alveolar macrophages is formed from the hemoglobin iron of phagocytized erythrocytes. The presence of inorganic iron compounds inside alveolar macrophages is known from previous (14, 44) as well as from our own light microscope studies in which specific iron staining had been used.

Alveolar Macrophages after India Ink Inhalation:

Phagocytosis by Alveolar Macrophages.—The phagocytosis experiment reported in the present study is but a portion of a more extensive investigation which is concerned with the function and the origin of the alveolar epithelium. The preliminary findings presented in this report do not add to our knowledge (for reviews see 45, 46) as to the possible origins of the alveolar macrophages. However, these findings do demonstrate the phagocytic activity of these cells, and they also provide additional insight into the mechanism of the phagocytic process.

India ink was chosen for these experiments because the high density of its carbon particles renders these particles easily detectable in electron micrographs of tissue sections. India ink was preferred to oil (which also would be readily detectable in the electron micrographs) because it was assumed to be less irritating to the lung, in spite of its content of antibacterial preservative. The additional irritating effect of the ether anaesthesia was considered irrelevant to the outcome of the experiment reported in this paper, especially since no significant changes in alveolar macrophages could be found in the lung of a normal etherized control mouse. Intranasal instillation of India ink was adopted because it is known that small particulates, *e.g.* viruses, will reach the lungs if administered by this route, a fact which has also been determined by mathematical reasoning (47). Moreover, this instillation was considered less traumatizing than the intratracheal route adopted by others (48).

This instillation experiment demonstrates the highly active phagocytosis of which alveolar macrophages are capable. One and ½ hours after the instillation, practically every macrophage found in thin tissue sections had engulfed numerous India ink particles (Fig. 15). This finding is in line with similar light optical studies, which have demonstrated phagocytosis by the same cells in as little as 10 minutes after an intratracheal instillation of *Staphylococci* (49). Nevertheless, clumps of India ink particles which lie free in the alveolar air spaces (I_2, Figs. 15 and 16) suggest that the population of alveolar macrophages is unable to engulf, within one and ½ hours, the entire mass of a rather heavy inoculum of foreign material. Phagocytosis by cells other than free alveolar macrophages was not observed in the one experiment described in this report. However, there is evidence from further experiments that attached portions of the alveolar epithelium may participate to a limited extent in the phagocytosis of carbon particles.

Mechanism of Phagocytosis.—The ingestion of India ink particles by the alveolar macrophages seems to occur in the following manner. At first, clumps of carbon particles (I_2, Fig. 16) are likely to get in contact with plump pseudopodia. Then invaginations of the plasma membrane are formed (*Ve*, Fig. 16), which appear as chains of small vesicles (*Ve*, Fig. 18). Since such vesicles are larger than the ingested carbon particles (*I*, Fig. 18), it is probable that they also contain fluid ingested together with the particles. The whole morphological appearance of this phagocytic process strongly resembles pinocytosis as seen on a light microscopic scale in tissue culture cells (50). It is thus suggested that the process of phagocytosis of solid particles as observed in alveolar macrophages is essentially comparable to the normal pinocytosis found in many different cell forms. Such a view is supported by the electron microscopic studies of other authors (51–53). This concept is further corroborated by the fact that most engulfed India ink particles seem to lie inside membrane-bound cavities or vacuoles of the cytoplasm (*Va*, Figs. 17 and 18). These vacuoles, which are similar to those observed inside normal alveolar macrophages (*Va*, Figs. 1, 3 to 5), could be products of pinocytosis, especially since such a relationship between inclusion droplets ("vacuoles") and pinocytosis has been established by cinemicrographic studies of tissue culture cells (54).

The fact that the membrane of a rough surfaced cisterna of the endoplasmic reticulum (E_1, Fig. 18) appears to be interconnected with the membranes of the aforementioned vesicles (*Ve*, Fig. 18) can be interpreted in two ways. Either this cisterna is being formed by the same process that also produces the strings of vesicles, or the cisterna has been present prior to the formation of vesicles, which only subsequently establish continuity with it. At the present time there is not sufficient evidence to prove or to disprove either of these two possibilities. If the rough surfaced cisternae were formed by the same infolding process of the cell plasma membrane that also produces the strings of vesicles (*Ve*, Fig. 18), one would expect to find some ingested India ink particles not only inside these vesicles, but also between the two membranes of the rough surfaced cisternae. Such a finding has not been made up to the present. On the other hand, the observation that rough surfaced cisternae occur in increased numbers inside those alveolar macrophages which are actively phagocytizing (*E*, Fig. 18), suggests an interrelation of this increased occurrence with the phagocytic process. Such an interrelation has been postulated for tissue culture cells, the cisternae of which have been considered as pinched-off portions of the cell plasma membrane (54).

It is hoped that additional studies on the phagocytosis process will eventually clarify the role played by the rough surfaced cisternae in this process.

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Note Added to Proof:—The following report, which describes the morphology of alveolar macrophages, has come to our attention since the completion of this manuscript: Schulz, H., *Beitr. path. Anat.*, 1958, **119**, 71.

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Explanation of Plates

Key to Abbreviations

Abm, Alveolar basement membrane.
Ae, Alveolar epithelium.
Am, Alveolar macrophage.
Aw, Alveolar wall.
Ce, Capillary endothelium.
Cl, Capillary lumen.
D, Dense material inside inclusion bodies.
E, Endoplasmic reticulum.
Fc, Cluster of ferritin granules.
Fg, Ferritin granule.
G, Palade granule.
I, India ink.
In, Inclusion body.
M, Membrane.
Mi, Mitochondrion.
N, Nucleus.
P, Plasma membrane.
Pp, Pseudopod.
Rbc, Red blood cell.
Va, Vacuole.
Ve, Vesicle.

Plate 346

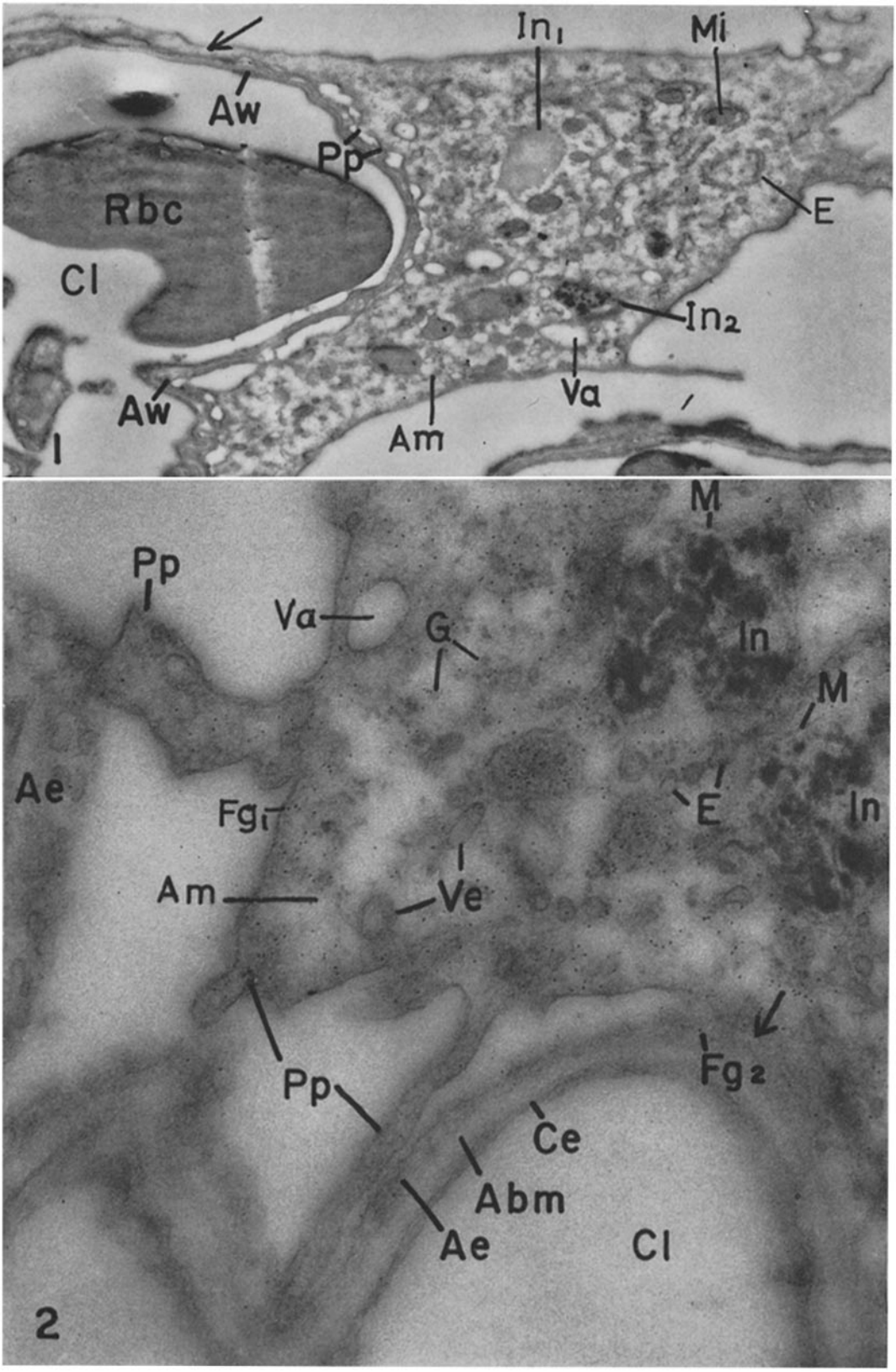
Fig. 1. Alveolar macrophage of normal lung. The alveolar macrophage (*Am*) closely contacts an alveolar wall (*Aw*) by means of numerous short pseudopods (*Pp*). The macrophage has an irregular outline. It attenuates at its periphery; two attenuated portions of its cytoplasm nearly surround a small capillary loop (*Cl*). Such an attenuated portion of the cytoplasm measures only 140 mμ in thickness (arrow), but it is nevertheless distinct from the alveolar wall. The nucleus of the macrophage is not included in this section. The cytoplasm contains several large vacuoles (*Va*) and numerous smaller vesicles. Some of these smaller units are seen arranged in rows (endoplasmic reticulum, *E*). Several small mitochondria (*Mi*) contain rather large and conspicuous intramitochondrial granules (black dots). One large inclusion body (In_1) has a homogeneous appearance and is of medium density. Other inclusions (In_2) contain irregular small masses of a very high density.

The alveolar wall (*Aw*) is made up of three layers: the alveolar epithelium, the alveolar basement membrane, and the capillary endothelium. Because of the low magnification of this electron micrograph, these three layers are not easily distinguished. In the capillary lumen (*Cl*) a red blood cell (*Rbc*) is seen. × 15,000.

Fig. 2. Alveolar macrophage of normal lung. The macrophage (*Am*) is in close contact with the thin alveolar epithelium (*Ae*) by means of short pseudopods (*Pp*). On the right, the boundary between macrophage and alveolar epithelium is ill-defined (arrow). Presumably this is caused by oblique sectioning through the alveolar wall at this point.

The cytoplasm of the macrophage contains profiles of vacuoles (*Va*) and vesicles (*Ve*). Some of these are recognized as portions of the endoplasmic reticulum (*E*) because they appear interconnected. The cytoplasm also contains numerous small (~15 mμ) granules of medium density (*G*). In addition, specific granules of much smaller size (less than 10 mμ) and of a very high density are scattered throughout the cytoplasm of the macrophage (Fg_1), and a few similar granules are seen here and there within the thin alveolar epithelium as well (Fg_2). Two large inclusion bodies within the cytoplasm of the macrophage (*In*) contain irregular clumps of very dense material. Faint traces of a thin membrane seem to delineate these inclusions (*M*).

The alveolar wall with which the macrophage is in contact is seen as a three layered structure. The different layers are: The alveolar epithelium (*Ae*), the alveolar basement membrane (*Abm*), and the capillary endothelium (*Ce*). The latter lines the lumen of a capillary (*Cl*). × 65,000.



(Karrer: Ultrastructure of mouse lung)

PLATE 347

FIG. 3. Alveolar macrophage of normal lung. The short pseudopodia (*Pp*) by means of which the macrophage is in contact with the thin alveolar epithelium (*Ae*) are clearly recognizable. In spite of this close contact, these pseudopodia are distinct from the alveolar epithelium. The alveolar basement membrane (*Abm*) is seen to support the alveolar epithelium, and the endothelium of capillaries is also recognized in places (*Ce*).

The cytoplasm of the macrophage contains several inclusion bodies of varying size and shape (*In*) which are usually surrounded by traces of a thin membrane. Their interior consists of a rather uniformly dense mass in which numerous very dense small grains and granules are embedded. A great number of vacuoles and vesicles of varying size are also encountered in the cytoplasm of the macrophage (*Va, Ve*). Ill defined material of a low density is seen within the lumen of few such vacuoles (Va_1). × 12,000.

FIG. 4. Alveolar macrophage of normal lung. This macrophage (*Am*) is in continuous contact with an alveolar wall, rather than by means of isolated short pseudopods. The gap between the plasma membranes of the macrophage and of the alveolar epithelium (*Ae*) is everywhere distinct. The alveolar epithelial lining is supported by the thin alveolar basement membrane (*Abm*), below which the endothelium of a capillary (*Ce*) can be seen. Within the lumen of the capillary (*Cl*) a red blood cell (*Rbc*) is recognized.

Portion of the nucleus of the macrophage is included in the section (*N*). The cytoplasm contains a few inclusion bodies (In_1) similar to those in Fig. 3, which are surrounded by traces of membranes. One such membrane gives the impression of being a double structure (*M*). A similar inclusion body (In_2) is seen inside the capillary endothelium (*Ce*). The cytoplasm of the macrophage also contains some large vacuoles (*Va*) without recognizable contents and a variety of small vesicular profiles. × 30,000.

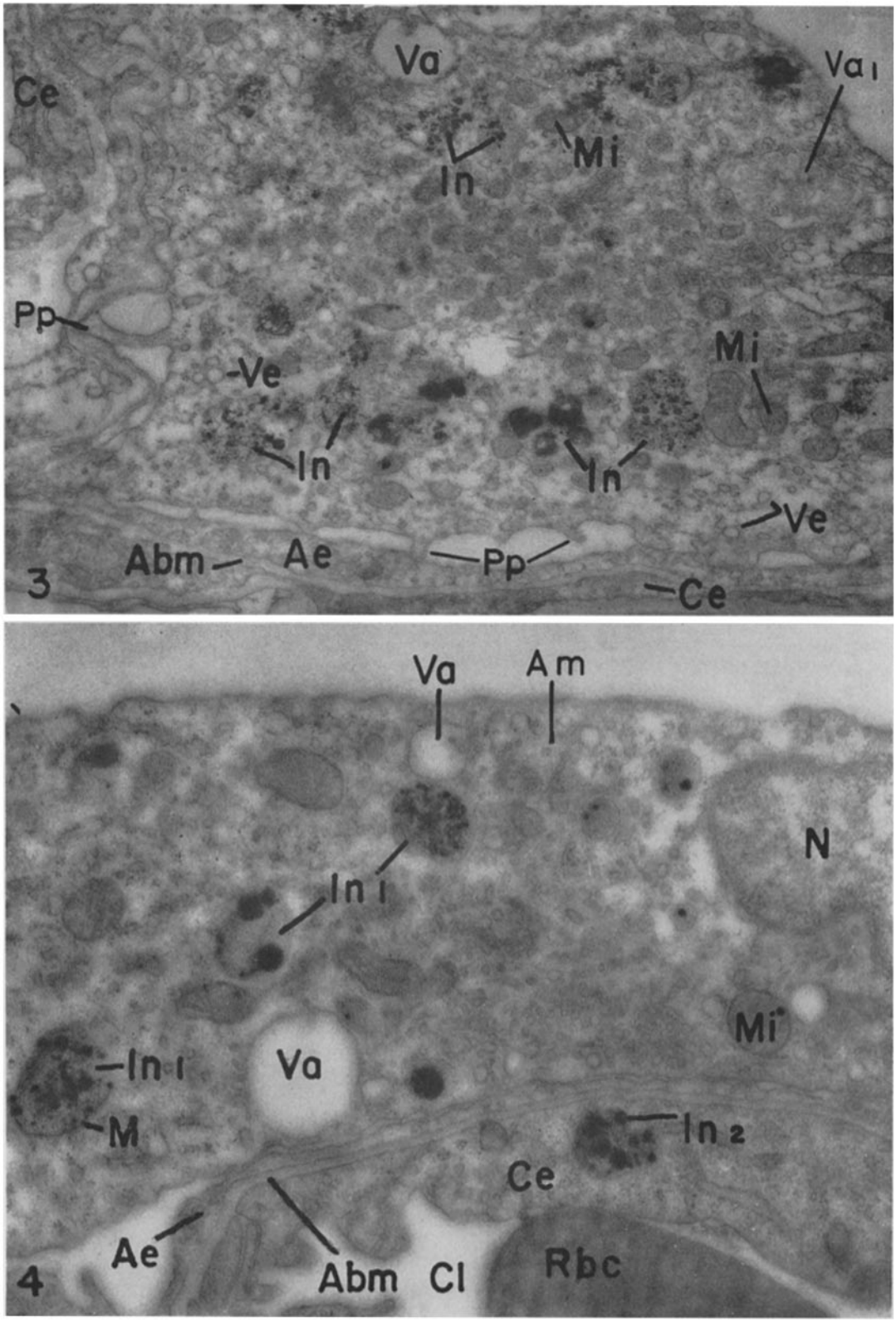


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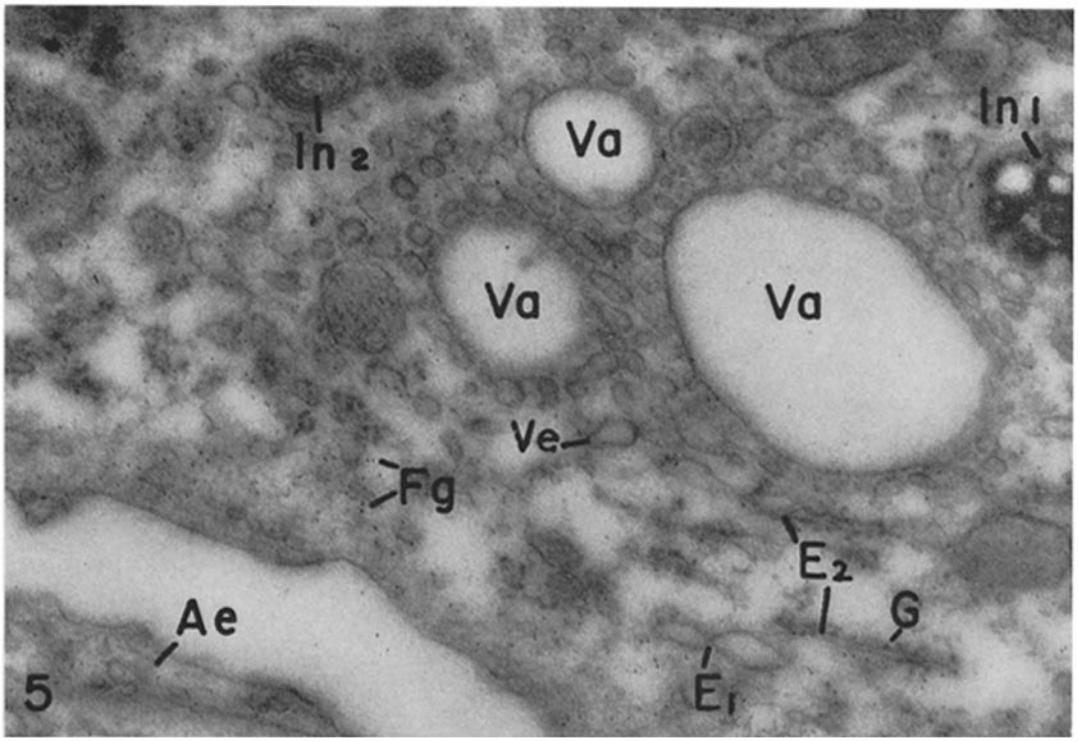
FIG. 5. Alveolar macrophage of normal lung. A portion of the macrophage cytoplasm and of the adjacent alveolar epithelium (*Ae*) are shown. Three large vacuoles (*Va*) are bounded by thin membranes. They do not contain any recognizable material. These vacuoles are surrounded by numerous small vesicles (*Ve*). In places these vesicles are seen to be interconnected and are thus believed to be portions of the endoplasmic reticulum (E_1). Other elongated units of this same reticulum (E_2) have small dense granules (*G*) attached to their membranes. A small inclusion body within the macrophage cytoplasm contains some very dense material (In_1). Another small inclusion shows alternating dense and less dense concentric layers (In_2). Throughout the cytoplasm, very small granules of high density are seen (*Fg*). × 55,000.

FIG. 6. Alveolar macrophage of normal lung. Two large vacuoles (Va_1, Va_2) and several small vesicles (*Ve*) are presumed to represent portions of the endoplasmic reticulum. This reticulum is also apparent in the form of elongated cisternae (*E*, E_1). The membranes of some such cisternae are smooth, whereas those of others have small particles adhere to their outside (*G*). One such unit (E_1) is seen interconnected with one of the large vacuoles (Va_1). Two inclusion bodies (*In*) contain dense masses of varying size (*D*). A considerable number of small (~5 to 6 mμ) very dense granules (*Fg*) are scattered throughout the cytoplasm. A few such granules (Fg_1) are seen within a mitochondrion (*Mi*), others seem to overlap with vesicular profiles (*Ve*). There are not any granules within the lumina of either vacuoles (Va_1, Va_2) or endoplasmic reticulum (*E*, E_1). × 120,000.



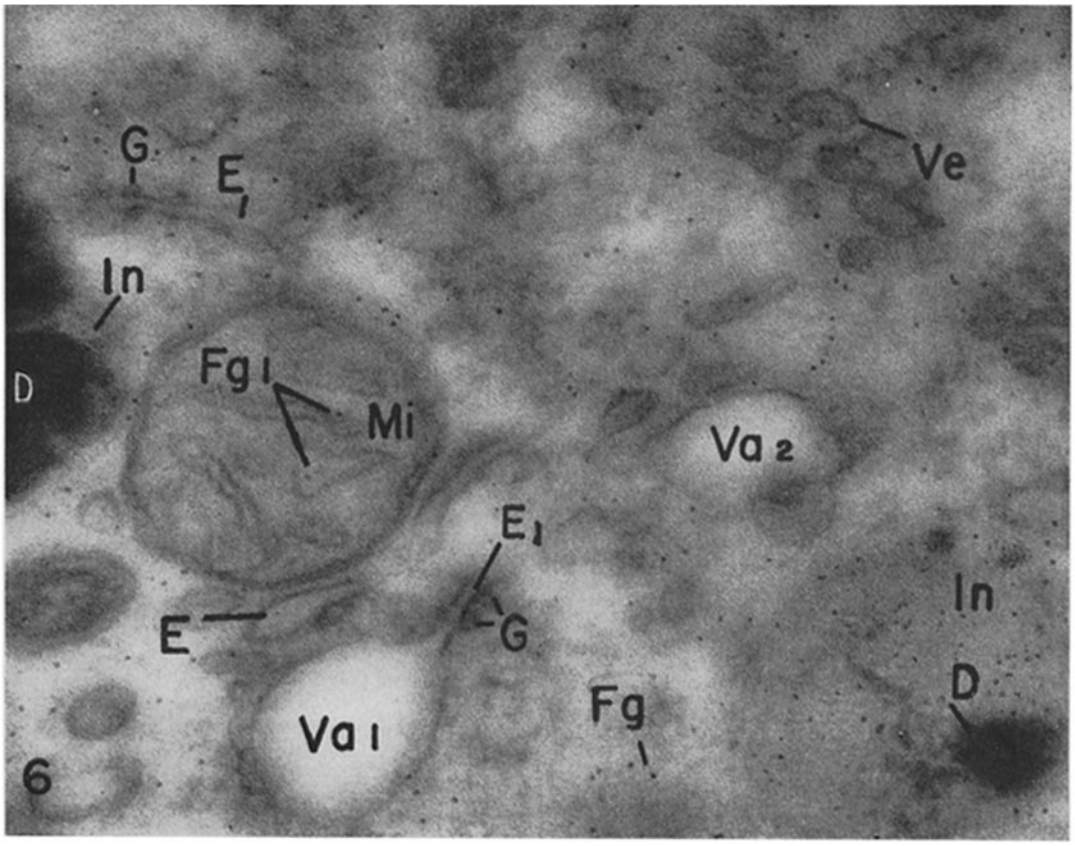


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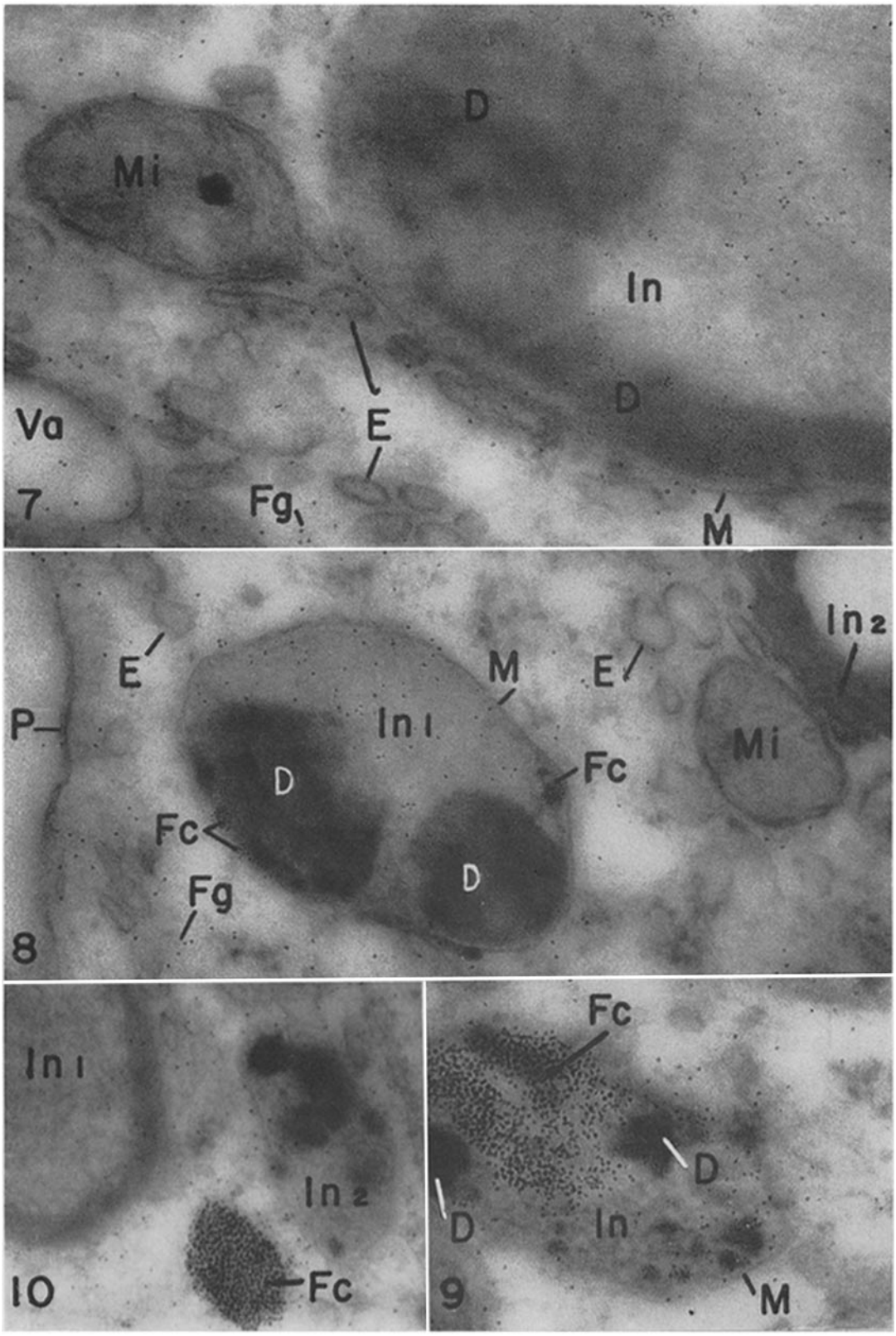
FIG. 7. Portion of a large inclusion body (*In*) is seen. It is delineated by a thin membrane (*M*). Its peripheral portion is highly dense (*D*). Small very dense granules (*Fg*) are scattered through the cytoplasm, and they are also found inside the inclusion (*In*) and the large vacuole (*Va*), but they are not seen inside a mitochondrion (*Mi*). Very small clusters of such granules are recognized within the dense portion of the inclusion body. × 110,000.

FIG. 8. An inclusion body (In_1) somewhat smaller than the one in Fig. 7 lies in proximity to the double plasma membrane (*P*) of the macrophage. It is surrounded by a thin membrane (*M*) which in places appears to be double. It is made up of material of rather low density within which lie two highly dense masses (*D*). A part of the dense portion of another inclusion body (In_2) is also seen. Small, very dense granules (*Fg*) are scattered through the cytoplasm, but they are not seen inside a mitochondrion (*Mi*) or within the units of the endoplasmic reticulum (*E*). Similar particles lie within the one inclusion (In_1). Three clusters of densely packed particles are located peripherally within the same inclusion (*Fc*). × 95,000.

FIG. 9. A small inclusion body (*In*) is delineated by remnants of a membrane (*M*). Its interior is made up of an irregularly dense material with some very dense masses (*D*). Within this inclusion body, a large cluster (*Fc*) of small, very dense particles is seen. This cluster occupies nearly one-half of the cross section of the inclusion. Similar small particles are seen scattered through the rest of the inclusion and through the cytoplasm of the cell. × 105,000.

FIG. 10. The portion of a large inclusion body (In_1) which is included in the electron micrograph consists of a dense peripheral zone with less dense center. A second, smaller inclusion (In_2) contains several highly dense masses within a less dense matrix. Between the two, a cluster of numerous small dense granules is recognized (*Fc*). Similar granules are scattered in the cytoplasm, and a few are seen inside the inclusion bodies. × 95,000.



(Karrer: Ultrastructure of mouse lung)

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FIG. 11. Alveolar macrophage of normal lung. A cluster of small, very dense granules is seen (*Fc*), and similar granules are scattered through the cytoplasm. The average diameter of these granules, which are assumed to be the iron hydroxide of ferritin molecules, is about 6 mμ. The composite structure of these granules is clearly recognized in all places. At least two of them (*Fg*) appear as tetrads, consisting of four smaller particles or iron-containing micelles (diameter about 1 to 2.5 mμ) arranged as corners of a square. Portions of three dense inclusion bodies are also apparent (In_1, In_2, In_3). One of these (In_1) shows a concentric layering. The ferritin molecules seem somewhat concentrated within two such inclusions (In_2, In_3). In the background outlines of cytoplasmic vesicles (*Ve*) appear under very low contrast. × 310,000.

FIG. 12. Alveolar macrophage of normal lung. A small inclusion body is made up of concentric dense and less dense layers, and thus resembles one of the inclusions shown in Fig. 11 (In_1). The less dense layers are about 6 mμ in width, the denser ones about 4 to 6 mμ. The latter appear in their turn as a triple layered structure, consisting of two denser units enclosing a less dense one. × 170,000.

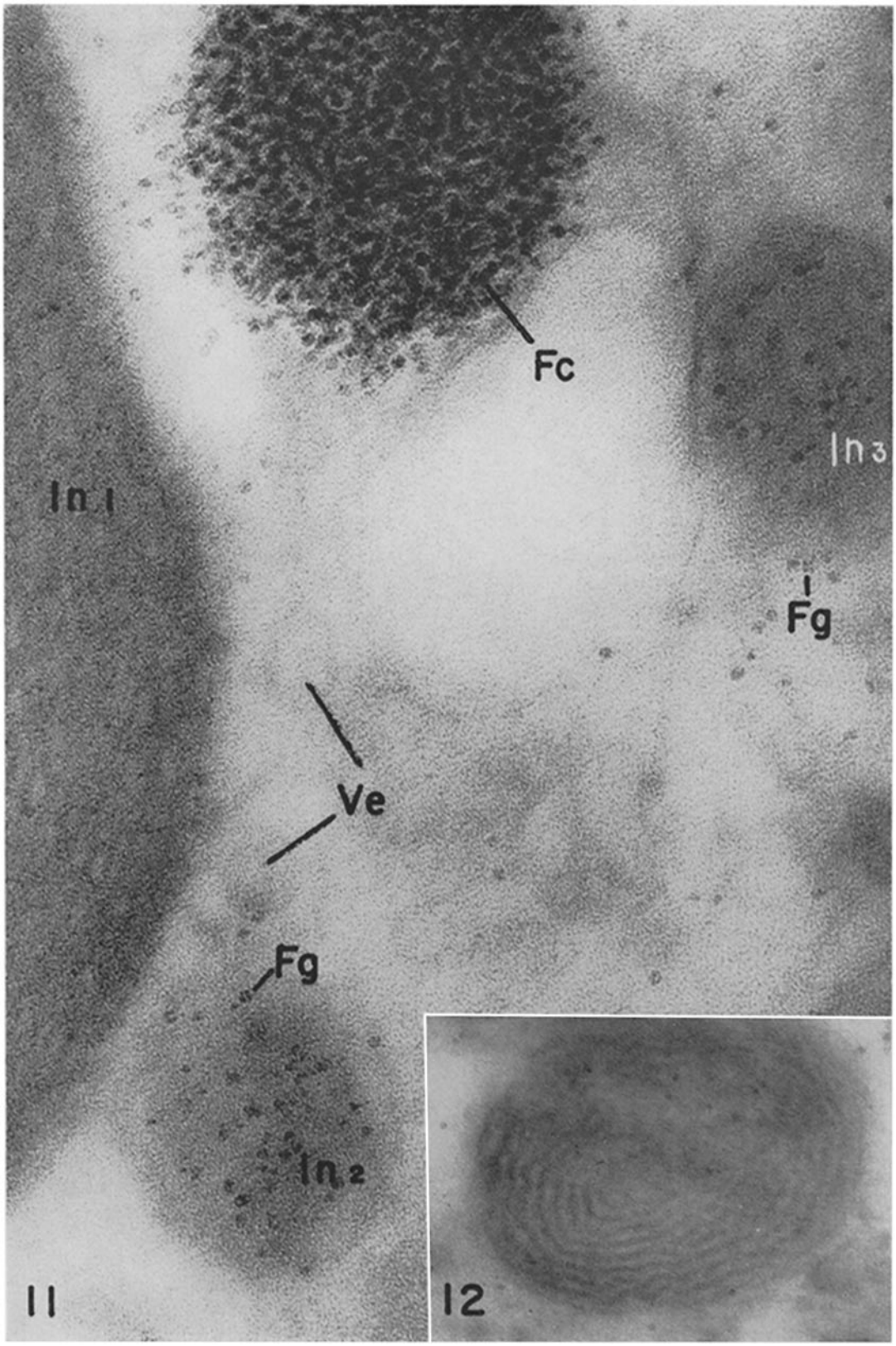

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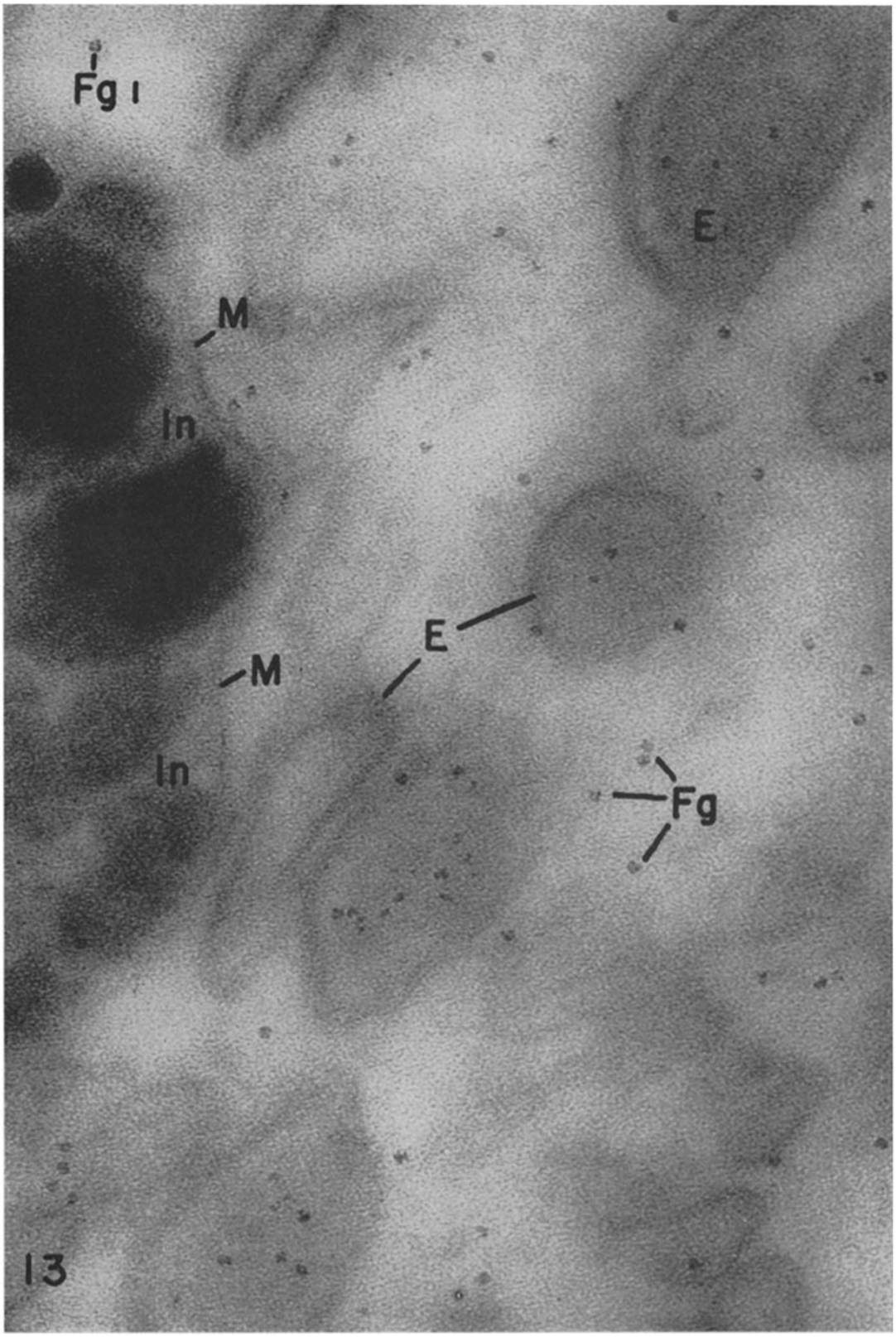
FIG. 13. Alveolar macrophage of normal lung. Small dense granules (*Fg*), presumed to represent the iron hydroxide core of ferritin molecules, are seen scattered through the cytoplasm. They also overlap with units of the endoplasmic reticulum (*E*). Their composite structure is apparent. A pentad, made up of five irregularly spaced smaller particles, is recognized (Fg_1). An inclusion body (*In*) is bounded by a thin membrane (*M*). × 305,000.



(Karrer: Ultrastructure of mouse lung)

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FIG. 14. Alveolar macrophage of normal lung. Small dense granules (*Fg*), presumed to represent the iron hydroxide core of ferritin molecules, show their composite structure. The contrast is somewhat enhanced due to underfocusing in this electron micrograph. One clear tetrad is shown (Fg_1), together with several other distorted or unfavorably oriented tetrads. × 350,000.

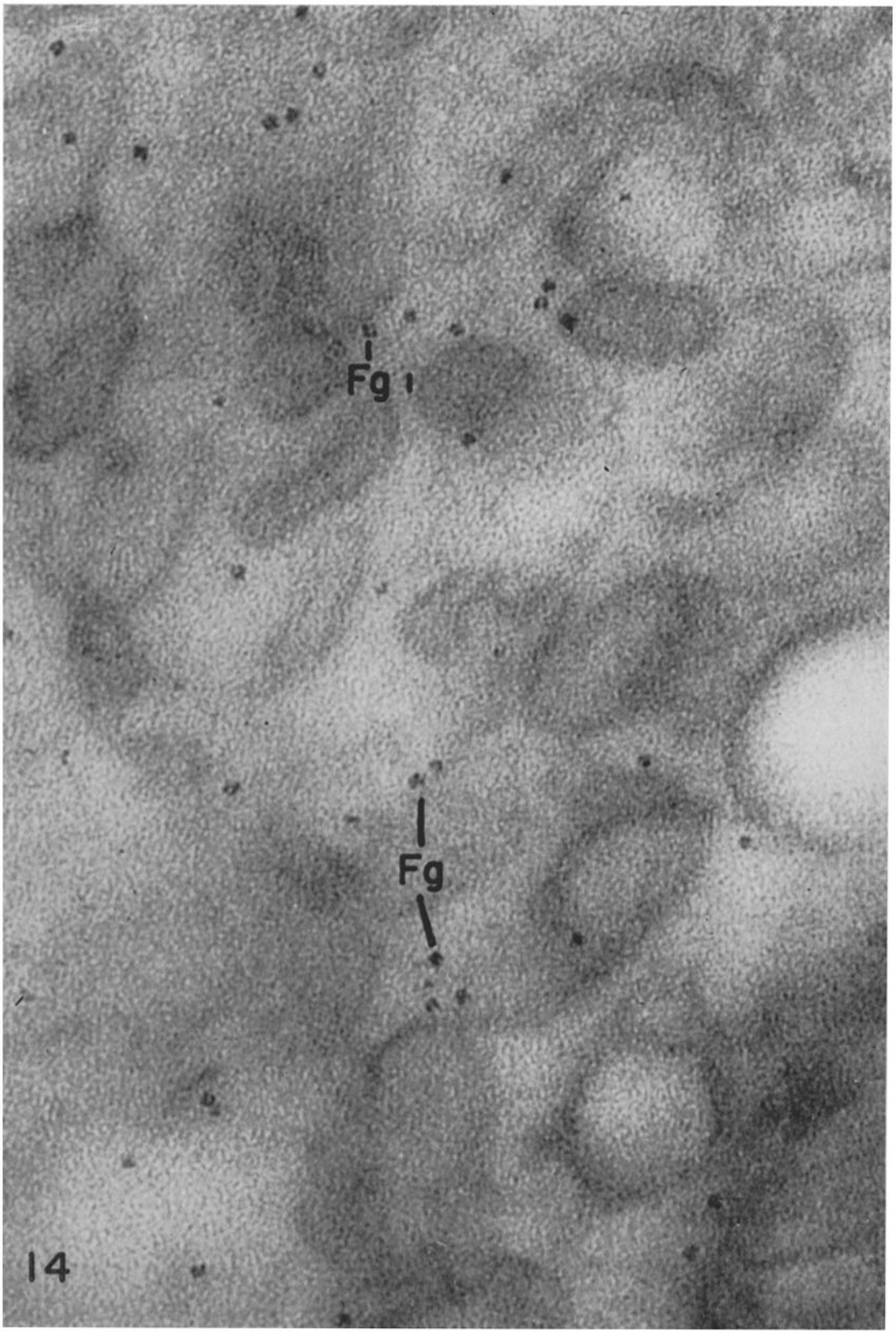

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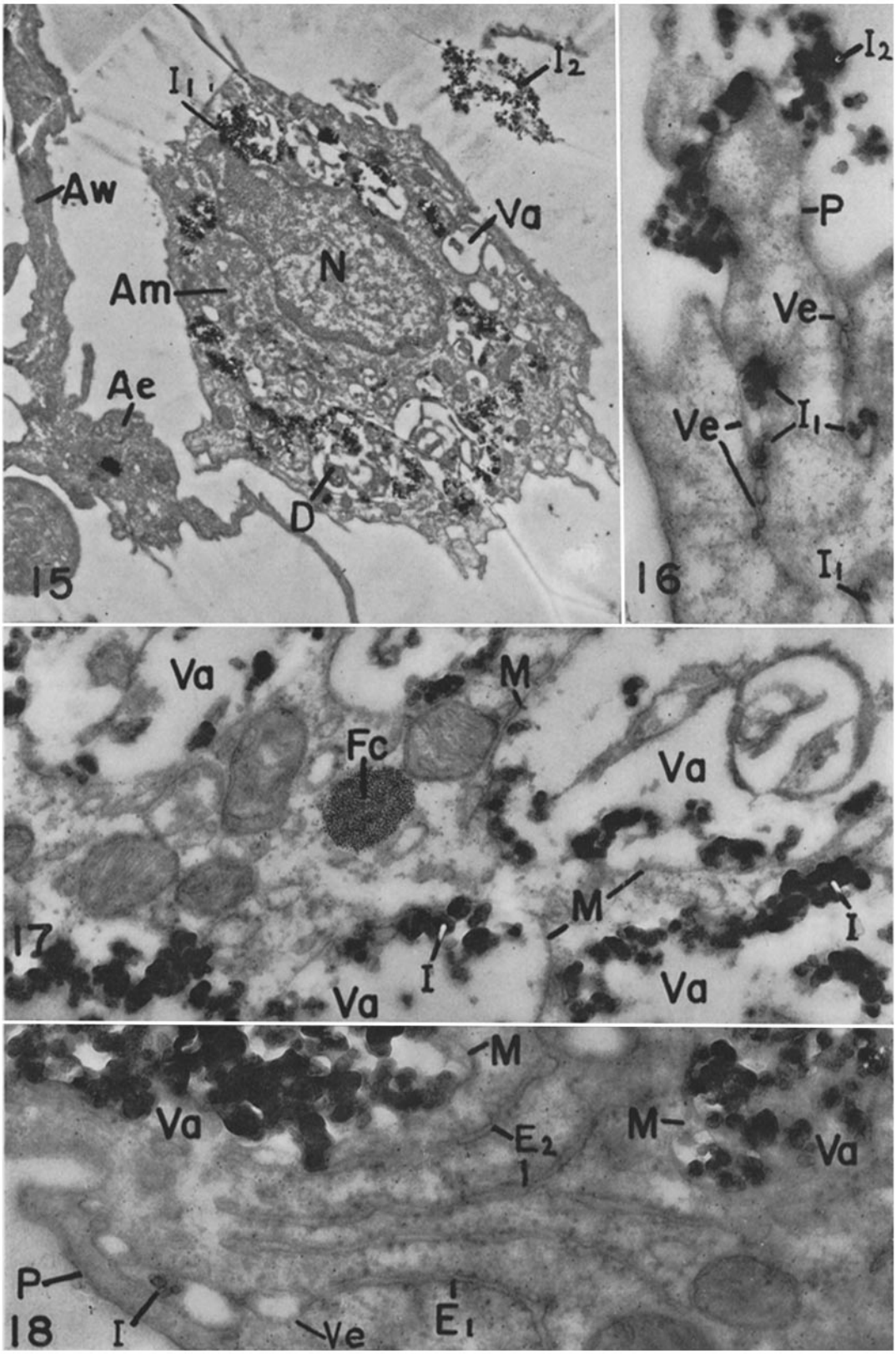
FIG. 15. Alveolar macrophage 1½ hours after intranasal India ink injection. The macrophage (*Am*) is seen close by, but not immediately adjacent to, the alveolar wall (*Aw*). Several large irregularly shaped vacuoles (*Va*) are seen arranged around the nucleus (*N*). Some of these vacuoles contain irregularly shaped dense material (*D*). Within all these vacuoles, groups of very dense carbon (India ink) particles are recognized (I_1). Similar particles are seen free in the alveolar lumen (I_2). They are not seen inside the alveolar epithelium (*Ae*). × 7,500.

FIG. 16. Alveolar macrophage 1½ hours after intranasal India ink injection. The macrophage is bounded by a dense plasma membrane (*P*). This plasma membrane invaginates in two places; it thus forms two strings of apparently interconnected vesicles (*Ve*) within the lumen of which small groups of India ink particles (I_1) can be recognized. Similar particles lie adjacent to the plasma membrane, still outside the cell (I_2). Within the cytoplasm, the ferritin molecules can be distinguished as very small dense dots. × 50,000.

FIG. 17. Alveolar macrophage 1½ hours after intranasal India ink injection. The electron micrograph depicts a central portion of the cell. A cluster of very dense small particles (*Fc*) is assumed to represent ferritin molecules. Similar dense particles are scattered throughout the cytoplasm. Portions of several large vacuoles (*Va*, compare Fig. 15) are bounded by membranes (*M*). These vacuoles contain some ill defined material resembling cellular debris and numerous India ink particles (*I*). These India ink particles are not usually seen within the cytoplasm proper beyond the membranes which bound the vacuoles. × 50,000.

FIG. 18. Alveolar macrophage 1½ hours after intranasal India ink injection. The macrophage is bounded by a dense plasma membrane (*P*). A string of small vesicles (*Ve*) is seen as an invagination of this plasma membrane. Within one of these vesicles, a few India ink particles are recognizable (*I*). The string of vesicles appears interconnected with a rough surfaced unit (exhibiting adhering particles) of the endoplasmic reticulum (E_1). Several additional rough surfaced cisternae of the endoplasmic reticulum (E_2) are arranged roughly parallel with the one first mentioned (E_1). Portions of two vacuoles (*Va*, compare Figs. 15 and 17) are bounded by thin membranes (*M*), and contain masses of India ink particles. Very small dense dots, presumed to represent ferritin molecules, are scattered through the whole cytoplasm. × 60,000.



(Karrer: Ultrastructure of mouse lung)