Electron Microscopic Study of the Phagocytosis Process in Lung*

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ABSTRACT

Diluted India ink was instilled into the nasal cavity of mice and the lungs of some animals were fixed with osmium tetroxide at various intervals after one instillation. The lungs of other animals were fixed after 4, 7, 9, 16, or 18 daily instillations.

The India ink was found to be phagocytized almost exclusively by the free alveolar macrophages. A few particles are occasionally seen within thin portions of alveolar epithelium, within the "small" alveolar epithelial cells, or within occasional leukocytes in the lumina of alveoli. The particles are ingested by an invagination process of the plasma membrane resulting in the formation of intracellular vesicles and vacuoles. Ultimately large amounts of India ink accumulate in the cell, occupying substantial portions of the cytoplasm.

The surfaces of phagocytizing macrophages show signs of intense motility. Their cytoplasm contains numerous particles, resembling Palade particles, and a large amount of rough surfaced endoplasmic reticulum. These structures are interpreted as indicative of protein synthesis. At the level of resolution achieved in this study the membranes of this reticulum appear as single dense "lines." On the other hand, the plasma membrane and the limiting membranes of vesicles and of vacuoles often exhibit the double-line structure typical of unit membranes (Robertson, 37).

The inclusion bodies appear to be the product of phagocytosis. It is believed that some of them derive from the vacuoles mentioned above, and that they correspond to similar structures seen in phase contrast cinemicrographs of culture cells. Their matrix represents phagocytized material. Certain structures within this matrix are considered as secondary and some of these structures possess an ordered form probably indicative of the presence of lipid.

The possible origin and the fate of alveolar macrophages are briefly discussed.

INTRODUCTION

The phagocytosis occurring in normal and experimentally altered lung has been investigated in the past (for review, see 38). Most of the older light microscopic studies tried to determine which cells in the lung have phagocytic properties, and were less concerned with the alterations in the cellular structure which occur during the phagocytosis process. Such a morphologic study of the phagocytosis process proper had to await the development of the modern electron microscope techniques which afford the high resolution necessary for such an investigation.

Such electron microscopic studies on "normal" and phagocytizing alveolar macrophages have recently been presented (25–27). It was shown that the alveolar macrophages readily phagocytize intranasally instilled India ink particles, and that the phagocytosis process is comparable to pinocytosis.

The present report is a follow-up of those earlier studies. The morphology of phagocytizing alveolar

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macrophages is analysed in detail, and the possible origin of these cells is discussed.

Material and Methods

India ink (American black India ink, Higgins) was instilled into the noses of young adult albino mice (Princeton strain) of both sexes. The India ink had previously been diluted 1:4 with sterile saline. The mice were anesthetized with 0.25 per cent surital sodium (Parke, Davis and Co., Detroit), and injected i.v. (0.25 ml.) or i.p. (0.40 ml.). A few mice were killed and their lungs fixed at varying intervals after a single intranasal instillation. Other mice were given India ink daily for a maximum period of 18 days. These animals were killed 2 hours to 1 day after the 4th, 7th, 9th, and 16th instillation, and 1 mouse was killed 9 days after the 18th instillation.

The lungs of the animals were fixed by infusion (23, 24) using 1 per cent osmium tetroxide in a veronal buffer containing saline (29). Small pieces of lung were then fixed *in vitro* over an additional period of 2 to 4 hours. They were dehydrated in ascending concentrations of acetone in water (total dehydration time about 1 hour) and impregnated in methacrylate (2 changes about 1 hour each). The whole procedure was carried out at about 4°C. The methacrylate was a mixture of 3 parts *n*-butyl and 1 part ethyl methacrylate containing 0.1 per cent benzoyl peroxide. The tissue pieces were finally embedded in this same mixture which had been prepolymerized to very high viscosity. Polymerisation (in number 5 gelatin capsules) was carried to completion in a vacuum oven at 80°C.

Thin sections were cut with a Porter-Blum microtome and glass knives. They were mounted on carbon films supported by 200 mesh copper grids. Some of these preparations were then stained with lead hydroxide (48) for 15 to 30 minutes. All preparations were studied in a Siemens and Halske Elmiskop 1 *b* electron microscope (40) at 60 or 80 kv., using the double condenser and 30 micron molybdenum (36) objective apertures.

A few thick (0.5 to 1 μ) sections were cut by the same method, spread by means of xylene, and mounted on glass slides. When dry, they were covered with prepolymerized methacrylate and a coverglass and were studied with a phase contrast microscope.

OBSERVATIONS

Nearly all mice appeared healthy even during prolonged daily administration of India ink. Only a few, which had received 18 daily instillations and which were kept beyond the 18th day, eventually showed signs of illness and of these some died. The lung of one severely ill animal was examined with the light microscope and was found to show evidence of pneumonia and lung abscesses. The lungs of sick or dead animals were not used for the electron microscope study.

Lungs of mice which appeared healthy looked macroscopically normal if only 1 or a few India ink instillations had been made. After as many as 18 daily instillations, however, the lungs showed macroscopic black patches which appeared slightly harder than the rest of the parenchyma. Small pieces from such regions tended to sink when immersed in the fixative, indicating some consolidation or atelectasis.

Extent of Phagocytosis within the Lung Parenchyma:

In sections examined with the phase contrast microscope the India ink appears only within free alveolar macrophages (*am*, Figs. 1, 2). Cells containing India ink can be found as soon as 20 minutes after 1 intranasal instillation, but they are more numerous after repeated instillations. In the latter case the cells often appear greatly enlarged and are densely packed with particles (*am*, Figs. 1, 2). In contrast to the alveolar macrophages (1), the "large" (32) and the "small" (32) alveolar epithelial cells do not contain India ink (*l* and *s*, Figs. 1, 2).

One can confirm with the electron microscope the impression that it is the *free* alveolar macrophages which take up the India ink in abundant quantities (*am*, Fig. 3). Rarely, however, a few India ink particles are also encountered within thin portions of the alveolar epithelium (i_1, Figs. 6, 7). Small finger-like protrusions (*ps*, Fig. 7) may occur where particles are in contact with the epithelial surface suggesting active motility of this surface. Furthermore, isolated particles can sometimes be seen within the "small" (32) alveolar epithelial cells (*i*, Fig. 30). In general, however, the alveolar epithelium is conspicuously free from phagocytized particles (Fig. 3).

After repeated administration of India ink certain cells lying within the thickened alveolar septa may also contain particles (*am*, Fig. 8). These cells tend to resemble the free alveolar macrophages in that they are rather large and contain a non-segmented nucleus and specific inclusion bodies. Probably they represent macrophages which were formerly free, but which are migrating to the lymph channels within the interstitial connective tissue.

Morphological Evidence of Phagocytosis:

India ink particles are frequently found in close contact with the plasma membrane, and it is assumed that such particles are about to be engulfed. They may be aggregated in small groups

(i_1, Fig. 11) or in large agglomerations (i_1, Figs. 9, 12, 13). The plasma membrane appears indented at the point of contact (i_1, Fig. 11), or it is invaginated with the formation of large inpocketings containing India ink (i_1, Figs. 9, 13). The smaller ones of these indentations and inpocketings are comparable to the "caveolae intracellulares" of Yamada (51). Often a large "flap" or "ruffle" of cytoplasm delimits such a pocket on one side (*ru*, Fig. 13). Similar "flaps" may also occur unrelated to India ink particles, either singly (ru_2, Fig. 17) or in succession (*ru*, Figs. 10, 14).

Rows of small vesicles and of larger flattened vacuoles frequently extend into the cytoplasm and appear to originate from such invaginated portions of the plasma membrane (*v*, Figs. 9, 10, 13, 14, 26). Several rows of vesicles may occur in parallel, corresponding to adjacent inpocketings of the cell surface (*v*, Fig. 12).

Intracellular Location of India Ink:

A few intracellular India ink particles are contained within small membrane-bounded vesicles which apparently derive from the vesicle chains mentioned above (i_2, Fig. 11; *i*, Fig. 26). Other particles lie within the lumina of variously shaped vacuoles (i_3, Fig. 13; *i*, Fig. 20). A third rather typical location of phagocytized India ink is within large (1 to 1.5 μ) spherical inclusion bodies (Fig. 16; *i*, Fig. 21; i_1, Fig. 22; i_2, Fig. 9). These bodies are bounded by a unit membrane (37) the image of which consists of 2 dense lines separated by a less dense zone (*m*, Fig. 9).

After repeated instillations of India ink many macrophages become filled with enormous masses of particles. Nevertheless, even these particles lie in distinct clusters (Fig. 3) marking the site of former inclusion bodies, the matrix of which has disappeared.

The Fine Structure of Phagocytizing Alveolar Macrophages:

These cells increase in size and apparently also in number as they engulf more and more India ink (Figs. 3, 5). They also tend to assume a more rounded form than do the "normal" macrophages, but sometimes they still exhibit long pseudopods (*ps*, Fig. 3). In general, they correspond to the "normal" alveolar macrophages which are always present within the alveoli (27). However, sometimes certain phagocytizing cells which lie free within alveoli are recognized as leukocytes because of their segmented nucleus, their smaller size, their generally denser cytoplasm, and the low number of inclusion bodies within them (*le*, Fig. 4). On one occasion such a leukocyte which contained India ink was seen to have been engulfed by an alveolar macrophage.

Plasma Membrane, Vesicles, and Vacuoles.—The plasma membrane of actively phagocytizing alveolar macrophages is sectioned normally only along limited lengths. It appears in such places as a dense line or at higher magnifications as a compound structure consisting of two dense lines with a separating less dense zone (pm_1, Fig. 14). Because of this "triple-layered" structure the plasma membrane corresponds to Robertson's "unit membrane" (37). The total width of the plasma membrane is about 70 A. Where the plasma membrane is obliquely or tangentially cut it shows as a dense ill defined zone of varying width (pm_2, Figs. 13, 14, 26).

In one instance a peculiarly patterned structure was seen at the surface of an alveolar macrophage. It consists of a dense zone on the outside of the plasma membrane (*pm*, Fig. 18), within which a pattern of variously arranged dense lines appears. This structure is interpreted as a nearly tangential section through a finely corrugated portion of the cell surface, with the lines representing oblique sections through the plasma membrane.

The vesicles mentioned earlier, which originate at invaginations of the plasma membrane (caveolae intracellulares (51)), are bounded by a unit membrane similar to the plasma membrane (*v*, Fig. 14). The same is true in the case of larger (0.2 to 0.5 μ) vacuoles (*va*, Figs. 14, 27 insert). The membranes of vesicles and vacuoles are smooth surfaced; that is, no Palade particles are attached to their outer surfaces.

The larger vacuoles occur in widely varying number and shape within different macrophages. They may be spheroidal, especially when located in the cell interior (*va*, Figs. 14, 27), or they appear flattened in those cases in which they lie close to the cell surface (*va*, Fig. 17). Sometimes a great number of small vesicles are arranged around a vacuole (27) (*v*, Fig. 27 and Fig. 27 insert). Some vacuoles appear nearly empty (*va*, Figs. 14, 17, 27), whereas others contain ill defined materials of varying density (*va*, Fig. 20).

Palade Particles and Rough Surfaced Endoplasmic Reticulum.—The phagocytizing alveolar macrophages show a considerable number of particles resembling Palades particles. These are variously arranged, but often they appear in

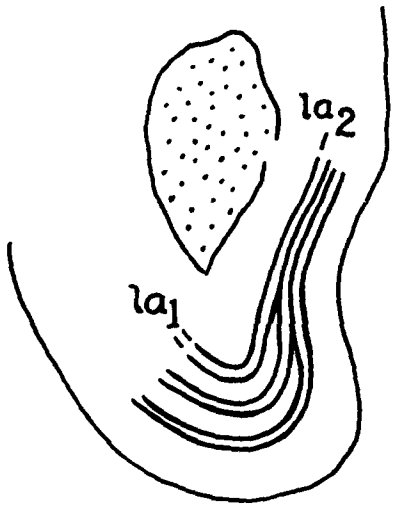
small clusters (*p*, Fig. 14). Others are lined up on the outer surfaces of membranes belonging to the endoplasmic reticulum. The diameter of single particles is about 150 A.

The endoplasmic reticulum consists of elongated cisternae (*r*, Fig. 9) with adhering Palade particles (*p*, Fig. 9). Sometimes a cisterna can be found arranged parallel to a chain of vesicles (*r*, Fig. 12). Some rough surfaced units (r_1, Fig. 25) show small smooth surfaced dilatations (r_2, Fig. 25). The membranes of the reticulum measure about 50 A in thickness, that is, somewhat less than the membranes bounding the above discussed vesicles. At the resolution achieved in this study the membranes of the endoplasmic reticulum appear as a single dense "line" and do not display the "triple-layered" or "unit membrane" structure which the plasma membrane shows (Fig. 25). However, Robertson (37) has described a unit membrane structure for rough as well as smooth internal membranes of cells. It remains to be seen whether the rough surfaced membranes of the endoplasmic reticulum of the alveolar macrophages are truly different from the membranes described by Robertson. In contrast to the vesicles, the rough surfaced cisternae have not been seen to contain India ink or any other particulates.

Inclusion Bodies.—The same types of inclusion bodies which have been previously seen (27) are recognized in the present study. In the following, some additional structural details of these bodies will be described.

The simplest type of inclusion is bounded by a unit membrane (*m*, Fig. 24). Its matrix is homogeneous or slightly granular. These inclusions may contain India ink (ib_1, Figs. 21, 22).

Certain inclusions show concentric lamellae or membranes within their matrix. Some of these membranes seem to terminate with free edges, since in sections they appear as "lines" of finite length which do not enclose anything (*la*, Figs. 16, 22, 25). Other membranes, however, show no free edges, but form ellipsoidal closed systems separating 2 compartments (*la*, Fig. 15). When there are only a few of these membranes present within an inclusion body they are arranged at the periphery of the inclusion (*la*, Figs. 16, 22, 23, 25), whereas the more numerous membranes of other inclusion bodies appear to "progress" from the periphery towards the center, with only a small central portion of uniform matrix remaining (Fig. 15). These micrographs suggest that the formation of the membranes starts at the periphery



TEXT-FIG. 1. Arrangement of lamellae (membranes) in a lamellated inclusion body. Compare with Fig. 29.

of the inclusions (*la*, Figs. 16, 22, 23, 25) and progresses towards the center (Fig. 15). It also appears that the membranes first formed are those with free edges (*la*, Figs. 16, 22, 23), but that they are secondarily transformed into the ellipsoidal closed systems (Fig. 15). The membranes are about 60 A thick, and each one consists of 2 denser strata separated by a less dense zone (la_1, Fig. 29). The intervals between 2 lamellae measure about 80 A. In localized areas these intervals appear to be obliterated, resulting in a fusion of neighboring lamellae in the manner indicated by Text-fig. 1. The resulting regular periodicity (la_2, Fig. 29) measures about 75 A.

The matrix of other inclusions shows dense areas in the center (*c*, Fig. 23), which at high magnification show a pattern of parallel straight "lines" (*c*, Fig. 28). The "lines" cross each other in a rectangular lattice. They are about 60 A apart. This fine structure suggests the presence of a crystal-like order in the inclusion body.

The matrix of still other inclusions appears vacuolated and shows irregular dense masses (*ma*, Fig. 21), some of which show a concentric lamellation (*ma*, Fig. 22 and Fig. 22 insert). Small patches of dense masses may also occur within inclusions showing the peripheral concentric lamellation (ib_2, Fig. 23). These inclusion bodies may also contain India ink (ib_1, Fig. 27).

Finally, certain rather small (250 to 450 $m\mu$) inclusion bodies show a centrally dense matrix and a peripheral narrow "shell" of lesser density.

They are bounded by a unit membrane (*ib*$_4$, Fig. 23). They may contain small very dense granules (*ib*$_3$, Fig. 27) which are indistinguishable from similar granules inside mitochondria (*mi*, Fig. 27). Some of them also show thin lamellae (*ib*, Fig. 19). India ink has not been seen within these small bodies.

DISCUSSION

Possible Origin and Ultimate Fate of Alveolar Macrophages:

Four different origins of alveolar macrophages have been postulated on the basis of various light microscopic studies: from the alveolar epithelium (6, 7, 10, 17–19, 43, 44, 49, 50), from the (interstitial) connective tissue (21, 22, 47), from the capillary endothelium (13), or from leukocytes (14). More recent investigations seem to indicate that the free macrophages most probably are detached "alveolar cells" and are of connective tissue, not epithelial, origin (3, 4).

The present study indicates that some older light microscope observations on the phagocytosis by the alveolar epithelium (6, 18, 19, 43) were in error. Quite likely those free macrophages which cling to the alveolar wall (Fig. 5) were, in those studies, interpreted as phagocytizing alveolar epithelium. Very limited phagocytosis by the alveolar epithelium does occur (*i*, Fig. 30), but not to the degree described by those studies. It remains to be seen if under different experimental conditions the epithelium might not play a greater part in the phagocytotic process.

The present study does not permit a decision as to which is the true origin of the free cells. Cells detaching themselves from the alveolar epithelium have not been found. However, this is an inconclusive finding in view of the inadequate sampling inherent in electron microscopic techniques. The striking fact that the free alveolar macrophages are almost the only phagocytizing cells (28) does not necessarily prove that they do not originate from the alveolar epithelium, but it does lead to the conclusion that the physiological properties of free macrophages and sessile alveolar cells are radically different. Morphologically the free macrophages differ from the "large" (32) alveolar cells in that they contain no typical osmiophilic (vacuolated) inclusions, but instead show a variety of other inclusions as well as ferritin (usually absent within alveolar epithelial cells).

It is the writer's opinion that, in view of these contradictory findings, additional evidence is needed to determine with certainty the true origin of the alveolar macrophages. Such evidence may be obtained through experiments using infectious or toxic agents (for instance silica (31)) rather than the relatively inert India ink.

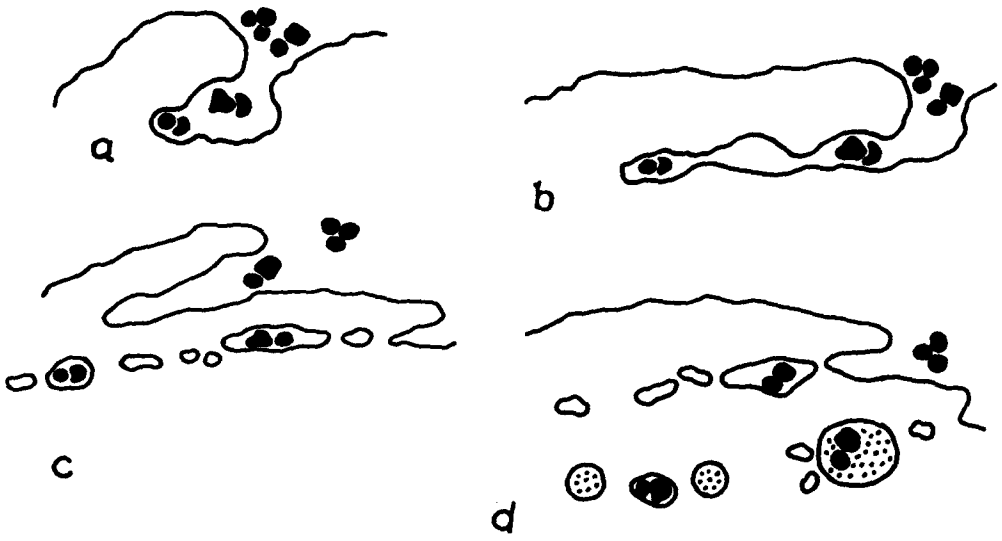
The presence of phagocytizing leukocytes (*le*, Fig. 4) within the alveoli does not indicate that all alveolar macrophages are of leukocytic origin. These leukocytes are very rarely observed and might have escaped from the blood stream as a response to slight local irritations by the instilled India ink. The fact that such leukocytes have been seen phagocytized by the macrophages suggests that they themselves may represent abnormal "foreign bodies" within the alveoli.

Ultimately the free phagocytes seem to enter alveolar septa (Fig. 8). Probably they will eventually reach the lymph channels, an assumption based on numerous histopathological observations.

Mechanism of Phagocytosis:

Phagocytosis as observed earlier (27) was seen as a process associated with plasma membrane infolding and the formation of vesicles (compare Figs. 11, 26). The present findings suggest that, as a variant of such a simple invagination process, large "flaps" or "ruffles" might trap "large" volumes of extracellular material as the "ruffles" backfold upon the cell (Text-fig. 2). The resulting inpocketings are subsequently subdivided into smaller vacuoles and vesicles as the backfolded "ruffle" fuses at different points with the cell surface (*ru*$_1$ and *ru*$_2$, Fig 12; *ru*$_1$, Fig. 17). This process has been accurately described in the case of macrophages from origins other than the lung (30), and somewhat similar phenomena have been observed in leukocytes phagocytizing bacteria (15, 16). The larger vacuoles (*va*, Figs. 17, 20, 27) correspond to the pinocytotic droplets observed in phase contrast cinemicrographs (39). The membranes limiting the vesicles and vacuoles derive from the plasma membrane, which is also borne out by the fact that they show the same "triple-layered" structure (*v* and *va*, Fig. 14 and Fig. 27, insert).

Phagocytosis and pinocytosis obviously are closely comparable processes, distinguished only on the basis of the ingested material: solid in the case of phagocytosis, fluid or semifluid in the case of pinocytosis. Thus, it is questionable whether the use of both terms serves a useful purpose.



TEXT-FIG. 2. Four progressive stages (*a*, *b*, *c*, *d*) of phagocytosis and of the formation of inclusion bodies. Compare with Figs. 9, 12–14, and 17.

Both processes are made possible by the very active, engulfing motion of the cell surface. There is ample morphologic evidence for such a surface activity. The undulations of the plasma membrane are demonstrated by the fact that the membrane appears sectioned under many different angles in the same micrograph (*pm*$_1$ and *pm*$_2$, Figs. 13, 14). The wrinkling in the membrane which precedes pinocytosis (5) can be seen (Fig. 18), and the "ruffles" of the cell surface in motion appear as single (*ru*, Fig 13) or multiple (*ru*, Figs. 10, 14, 26) "flaps." These electron microscopic findings are the exact parallel of phase contrast cinemicrographic observations which show a similar undulating surface activity in cultured cells. Once a vesicle or vacuole has formed and has separated from the plasma membrane, it moves freely within the cytoplasm as evidenced by motion pictures (39). Its bounding membrane undergoes the same shifts, which means that portions of the plasma membrane are constantly fed into the cytoplasm where they (and possibly enzymes connected therewith) might serve specific purposes. This "membrane flow" which has been theoretically postulated (2) was recently demonstrated quite beautifully for the case of amoebae "stained" with fluorescent globulin and examined by ultraviolet as well as by phase contrast microscopy (5). The phagocytized India ink within vesicles and vacuoles undergoes the same positional shifts, so that it is finally found distributed throughout the whole cell (Fig. 3).

Even within those cells which contain large masses of India ink the particles are still arranged in separate clusters (Fig. 3) corresponding to the original ink-containing vacuoles (*va*, Figs. 13, 20). Possibly some of the clusters may also have been formed by the fusion of several such vacuoles.

Palade Particles and Rough Surfaced Endoplasmic Reticulum:

The available electron micrographs do not reveal a "unit membrane" structure of the endoplasmic reticulum, although they do show this structure in the case of the plasma membrane and its derivatives (vesicles and vacuoles). This is in apparent contradiction to findings made by Robertson, who stated that the "unit membrane" structure is present also in the reticulum (37). The reason for this discrepancy is not well understood at the present time. Possibly the resolution obtained in the present study is not adequate to reveal the "compound membrane" structure of the reticulum, or this reticulum is not favorably sectioned. On the other hand, the possibility is clearly indicated that in alveolar macrophages a true difference of fine structure exists between the membranes of the reticulum and the plasma membrane.

The considerable development of rough surfaced reticulum and the numerous Palade particles (interpreted as containing ribonucleic acid) within phagocytizing macrophages is noteworthy (27)

and suggests active protein or enzyme synthesis by the cells. Increased enzyme synthesis can be expected since hydrolases are presumably necessary for the disposal of the phagocytized materials. Connections between pinocytotic vesicles and rough surfaced reticulum have not been found, but some micrographs do suggest that rough surfaced cisternae, arranged parallel to the vesicle chains (*r*, Fig. 12) may have been formed from such vesicles through a fusion process, a concept which had been previously formulated (11, 20).

Inclusion Bodies:

It is assumed that the inclusion bodies are products of phagocytosis, since they are limited by a unit membrane indistinguishable from the plasma membrane, and since they often contain India ink. If the membrane-bound vacuoles correspond to the pinocytotic droplets originating at the cell surface (39), then the dense inclusions, described in this study, probably represent the later stages in the development of such droplets, stages which appear dark in phase contrast (39). The dense matrix of these inclusions could be formed from the contents of the vacuoles by simple concentration, or by hydrolysis.

The present findings are in agreement with another study (41) in which it was suggested that the different morphologic types of inclusion bodies derive from each other through a series of step by step changes. Thus, the "initial" type of inclusion might be the one with a homogeneous matrix. This matrix may subsequently be converted into concentric lamellae (through a lamellation process starting peripherally), into a dense body exhibiting a crystal-like lattice (this process starting centrally), or into irregular dense masses. On the other hand, the polymorphism of certain inclusion bodies could also reflect the variable composition of the phagocytized substances.

The different morphological structures seen within inclusion bodies might be indicative of chemically distinct compounds. The question as to the nature of these compounds could be answered only if chemical data on isolated inclusion body fractions were available. In view of the absence of such data, all interpretations, in chemical terms, of the observed morphological structures must necessarily be speculative.

The lamellae or membranes present within certain inclusion bodies could represent lipid or lipoprotein, since lipids have been shown to exhibit lamellae of the same size order (12, 45, 46) and since lipoprotein structures like the myelin sheath also show a comparable lamellation. The lipid could either derive from lipid-containing material ingested along with the India ink, or it could possibly also be synthesized by the cell from the ingested material. The latter possibility is suggested by the fact that the lamellae seem to appear secondarily within the uniform matrix of inclusion bodies (*la*, Figs. 16, 22). The fact that the first lamellae appear at the periphery of the inclusion might indicate that the limiting unit membrane (*m*, Figs. 16, 22) plays an important role in this hypothetical lipid synthesis. Concentrically lamellated bodies similar to those described here have been observed in a variety of cells (8, 9, 33-35). Lamellations have also been seen within inclusion bodies which were shown to contain mucoprotein, but the possibility that the lamellated portions of those bodies represented lipid could not be excluded (42).

The dense irregular masses which also appear within certain inclusions remind one of neutral fat. The vacuoles which mostly occur along with the dense (fat-like) material (ib_2, Fig. 21; ib_3, Fig. 22) may be artifacts created by the extraction of fat during the acetone-dehydration of the tissue. On the other hand, the vacuoles could also be real and this would indicate the "disappearance" of the (homogeneous) matrix substance as this substance is being metabolized by the cell.

The homogeneous matrix substance (ib_1, Figs. 21, 22; *ib*, Fig. 24) might contain intermediates of lipid metabolism as well as protein. The significance of the crystal-like bodies (*c*, Figs. 23, 28) forming centrally within the matrix is not clear at the present time. Similar structures have been described within tracheal epithelial cells (34).

It is suggested that macrophages represent a suitable cell model with which to explore further the possible relation between phagocytosis, the synthesis of various compounds, and even the formation of mitochondria (37, 41). If known substances like proteins, lipids, or carbohydrates were "fed" to the phagocytizing cells, a combined morphological and chemical study of the phagocytes could yield valuable information on intracellular metabolism.

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EXPLANATION OF PLATES

Key to Abbreviations

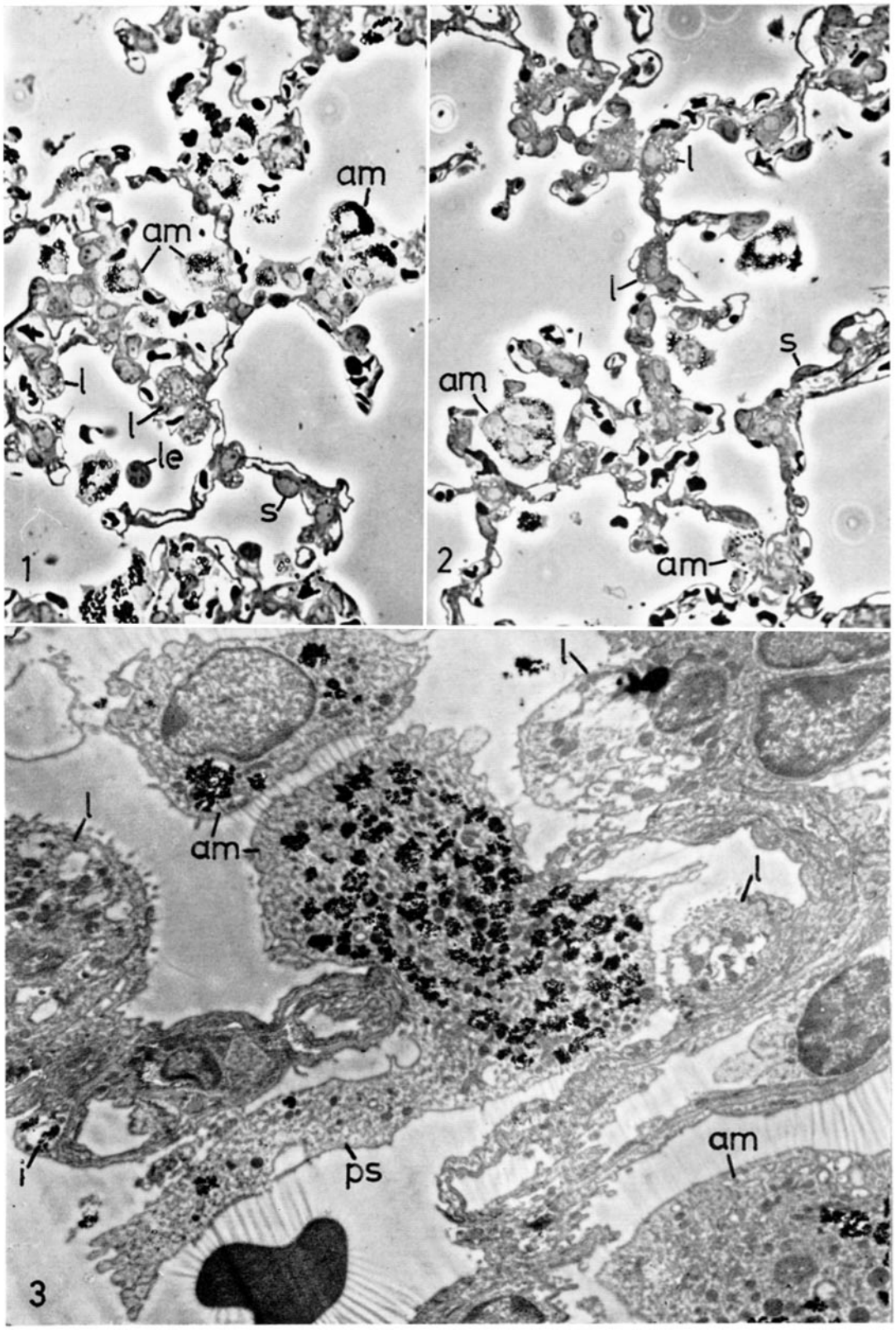
a, alveolar lumen
am, alveolar macrophage
b, basement membrane
c, crystalline body
ca, capillary
ce, centriole
e, alveolar epithelium
en, endothelium
f, ferritin
i, India ink
ib, inclusion body
l, "large" alveolar epithelial cell
la, lamella
le, leukocyte
m, membrane
ma, dense mass
mi, mitochondrion
n, nucleus
p, particle, resembling Palade particle
pm, plasma membrane
ps, pseudopod
r, endoplasmic reticulum
ru, "ruffle"
s, "small" alveolar epithelial cell
v, vesicle
va, vacuole

All micrographs represent sections of lungs of mice which received intranasal instillations of India ink for periods indicated in the explanation of each figure. They all are of sections of mouse lung fixed with buffered osmium tetroxide.

PLATE 186

FIGS. 1 and 2. After 18 daily instillations. Photomicrographs taken with phase contrast. Numerous alveolar macrophages contain large masses of phagocytized India ink (*am*). Some of these cells are touching the alveolar walls. A leukocyte located within an alveolus (*le*, Fig. 1) is smaller than the macrophages. The "large" alveolar epithelial cells (*l*) contain typical vacuoles or inclusions, but no detectable India ink. The "small" epithelial cells (*s*) contain no ink either. × 590.

FIG. 3. After 18 daily instillations. The 3 alveolar macrophages shown (*am*) contain varying amounts of India ink. The ink is arranged in clusters within the cell. The cell in the middle seems to migrate through an interalveolar pore and in so doing it sends out a long pseudopod (*ps*) which contains only few ink particles, in contrast to the rest of its cytoplasm. Three large alveolar epithelial cells are seen (*l*); they include no India ink. However, a few ink particles lie within a thin portion of the alveolar epithelium (*i*). × 4,400.



(Karrer: Phagocytosis process in lung)

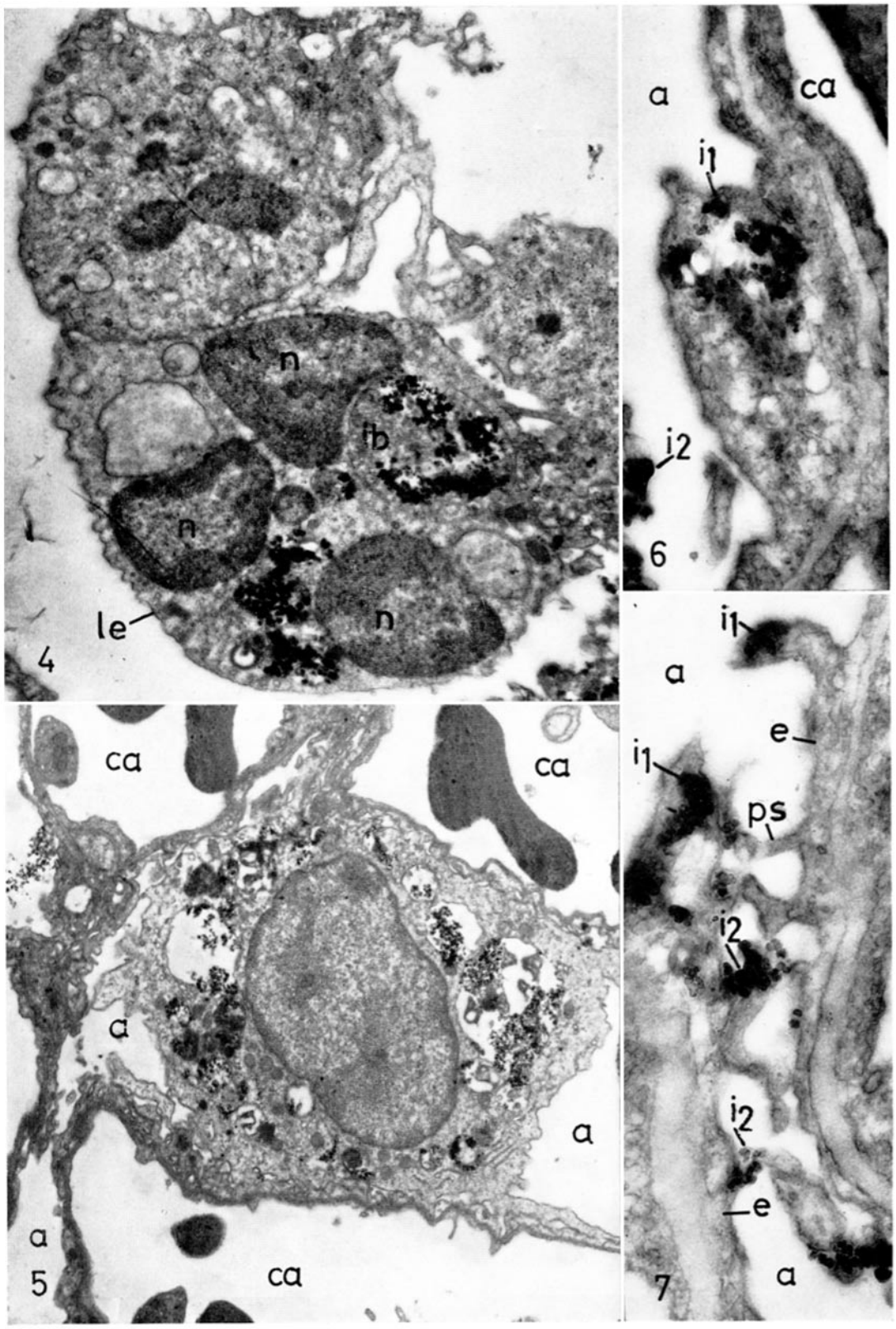
PLATE 187

FIG. 4. Fixed 24 hours after one instillation. Phagocytizing leukocyte within the alveolar lumen. The leukocyte (*le*) is characterized by its segmented nucleus (*n*). Some of the phagocytized India ink lies within a large membrane-bounded inclusion body (*ib*). The second cell (above) probably also is a leukocyte. × 15,000.

FIG. 5. Fixed 1½ hours after one instillation. Phagocytizing alveolar macrophage. The macrophage lies within the alveolar lumen (*a*) and touches the alveolar wall along nearly its entire circumference. It is surrounded by capillaries (*ca*) of alveolar septa. It contains masses of India ink which are mostly lying within the lumina of vacuoles. × 7,800.

FIG. 6. Fixed 3½ hours after one instillation. Phagocytized India ink is lying within the thin portion of the alveolar epithelium (i_1). Other particles (i_2) lie free within the alveolus (*a*). A capillary is seen at upper right (*ca*) × 35,000.

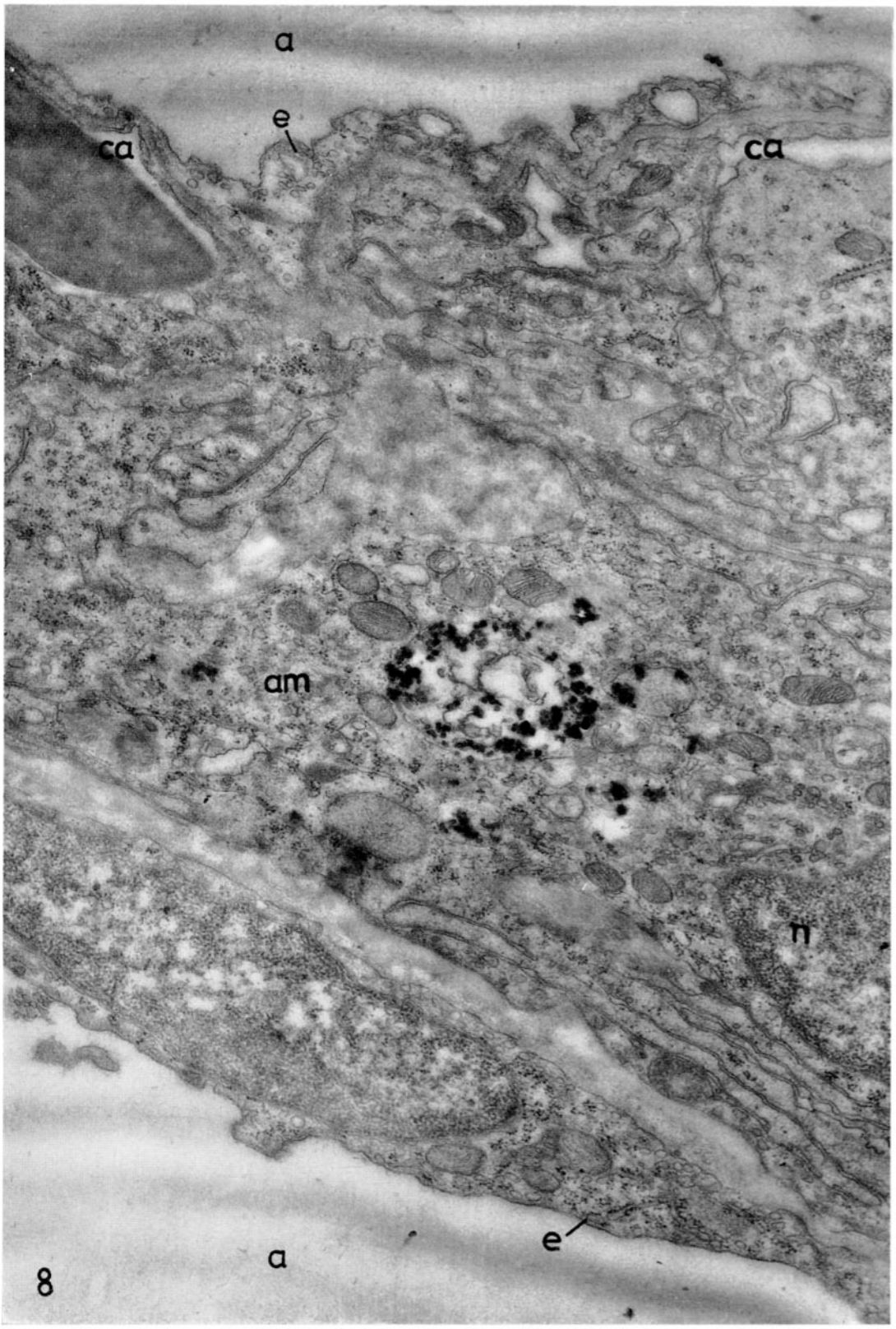
FIG. 7. Fixed 3½ hours after one instillation. Some India ink particles (i_1) have been phagocytized by the thin portions of the alveolar epithelium (*e*). Others still seem to be located extracellularly although they are in close contact with the surface of the alveolar epithelium (i_2). The epithelium surface shows a few pseudopods (*ps*) projecting into the alveolar lumen (*a*). × 45,000.



(Karrer: Phagocytosis process in lung)

PLATE 188

FIG. 8. Fixed 3½ hours after one instillation. The septum between 2 alveoli (*a*) is shown, with alveolar epithelium (*e*) and capillaries (*ca*). Within the septum lies a large cell which resembles an alveolar macrophage (*am*). A portion of its nucleus is seen at right (*n*). This cell contains phagocytized India ink particles which lie within inclusion bodies or vacuoles. × 25,000.

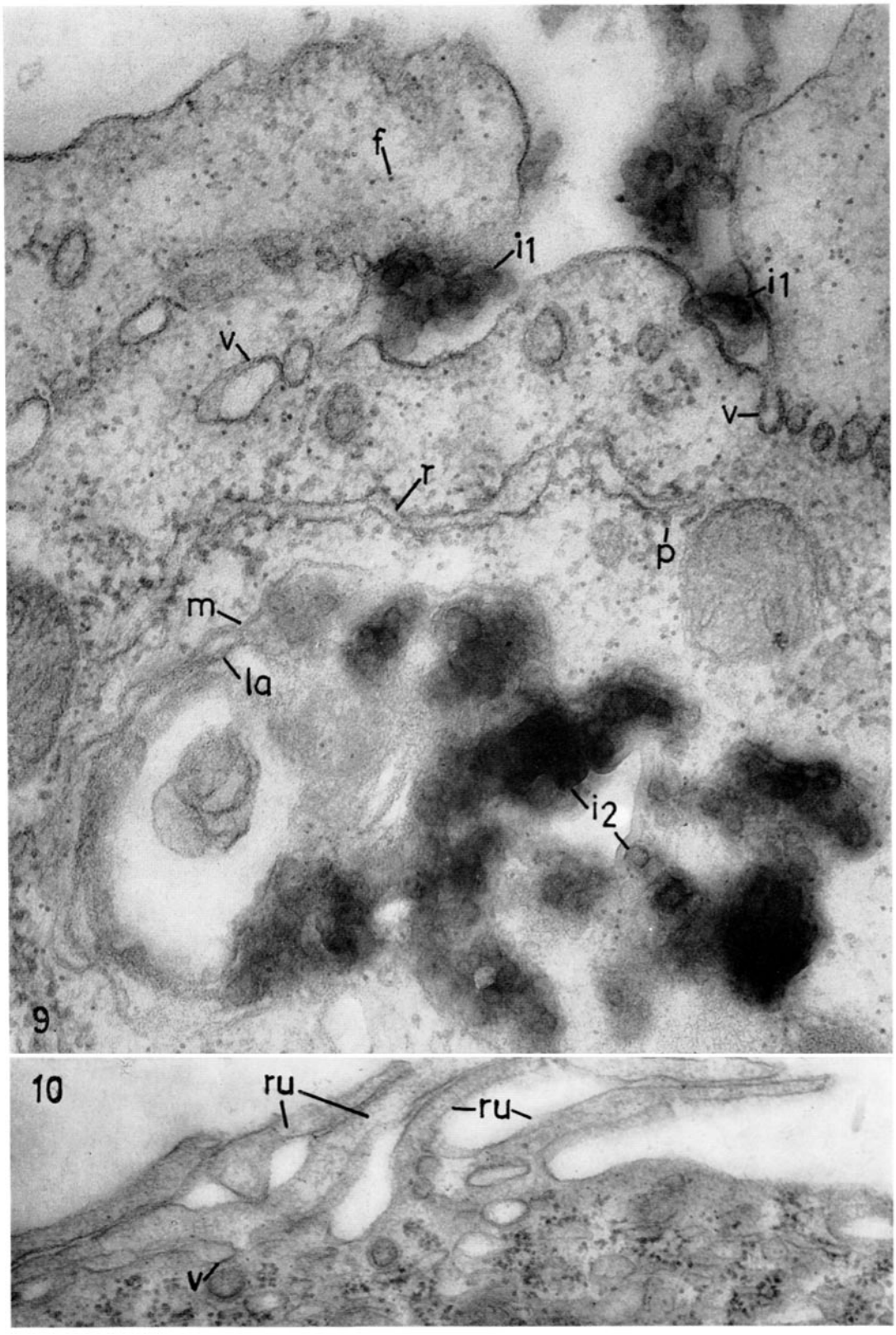


(Karrer: Phagocytosis process in lung)

PLATE 189

FIG. 9. Fixed 3½ hours after one instillation. Two clumps of extracellular ink (i_1) are apparently being trapped within an inpocketing (caveola (51)) of the cell surface. Chains of small vesicles (v) extend from the inpocketing. A cisterna of the endoplasmic reticulum (r) with adhering particles (p) runs roughly parallel with the cell surface. The phagocytized India ink (i_2) lies within an inclusion body, which is bounded by a "triple-layered" membrane (m), and which contains evidence of concentric lamella formation (la). Ferritin molecules appear as small black dots (f) in the cytoplasm. × 105,000.

FIG. 10. Fixed after 7 daily instillations. An alveolar macrophage shows several "ruffles" (ru) which backfold onto the surface. As one result of this process, small droplets (v) are probably trapped between ruffles and cell surface. × 45,000.

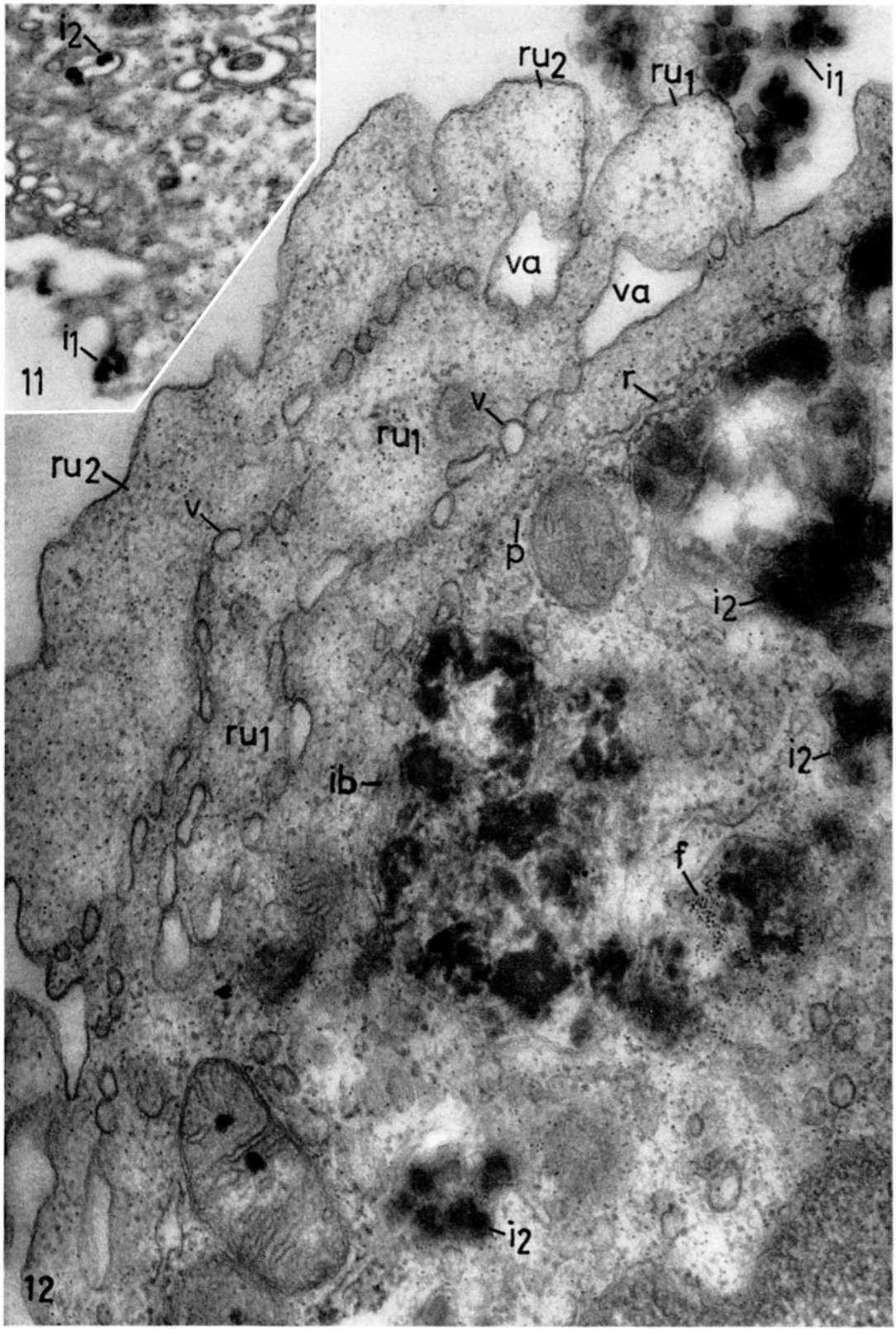


(Karrer: Phagocytosis process in lung)

PLATE 190

FIG. 11. Fixed 1½ hours after one instillation. Portion of an alveolar macrophage. A small group of India ink particles lies within an indentation (caveola (51)) of the cell surface (i_1). Other particles have been phagocytized and are seen within the lumen of a vesicle (i_2). × 40,000.

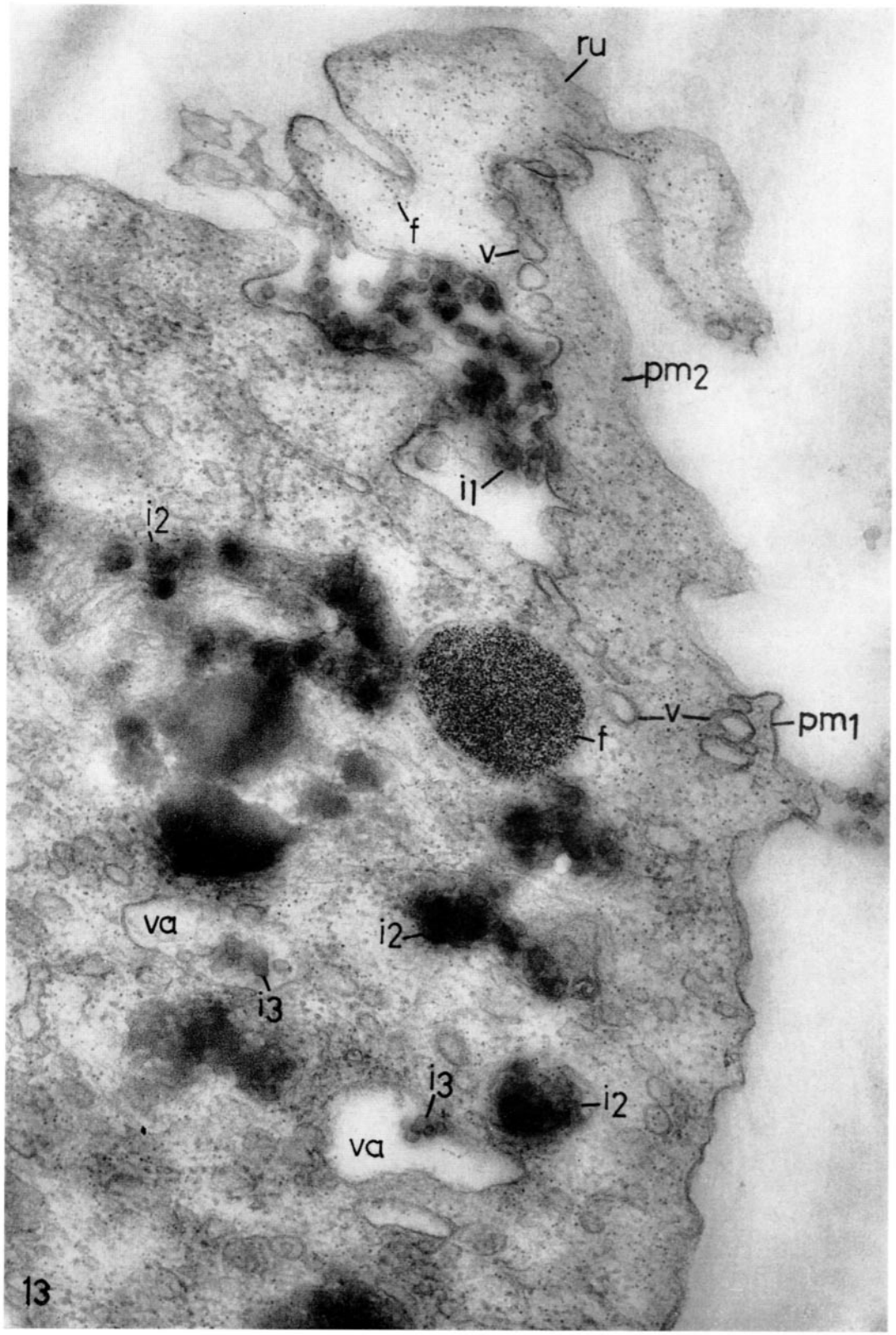
FIG. 12. Fixed 3½ hours after one instillation. Two "ruffles" or folds of the superficial cytoplasm are recognized (ru_1 and ru_2). They represent backfoldings (compare Fig. 10) which probably were formed in succession. Two chains of vesicles (*v*) and vacuoles (*va*) have been formed by this process. A cisterna of the endoplasmic reticulum (*r*) with adhering particles (*p*) runs parallel to these chains. A clump of extracellular India ink is partly engulfed at (i_1), other such clumps lie intracellularly (i_2). An inclusion body (*ib*) contains irregular dense masses. Ferritin molecules appear as small black dots (*f*) in the cytoplasm. × 70,000.



(Karrer: Phagocytosis process in lung)

PLATE 191

FIG. 13. Fixed 3½ hours after one instillation. A "ruffle" of superficial cytoplasm (*ru*) is backfolding upon the cell surface, thereby trapping a large clump of extracellular India ink (i_1). Similar clumps lie within the cell (i_2); they seem surrounded by membranes. Small groups of India ink particles (i_3) are also found within vacuoles (*va*). Small vesicles appear to form from infoldings of the plasma membrane (*v*). The plasma membrane is sectioned in many different planes, either normally (pm_1) or obliquely (pm_2). Intracellular ferritin appears as small dense dots, in some instances massed together in form of an inclusion body (*f*). × 65,000.

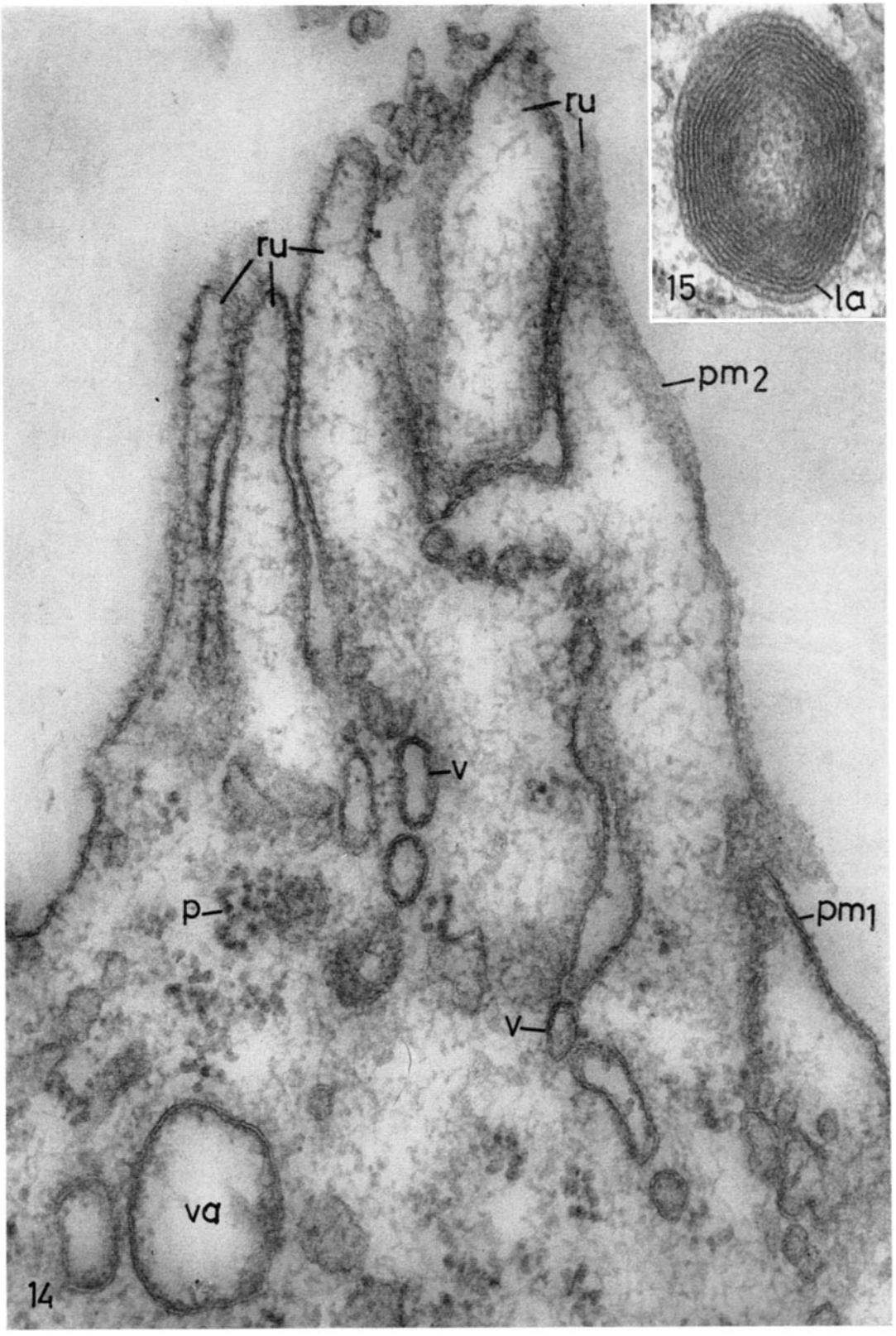


(Karrer: Phagocytosis process in lung)

PLATE 192

FIG. 14. Fixed after 9 daily instillations. Numerous "ruffles" of superficial cytoplasm (*ru*) are arranged in parallel. Between them the plasma membrane is deeply infolded. A number of small vesicles (*v*) seem to form from these infoldings. The vesicles as well as a large vacuole (*va*) are bounded by unit membranes, consisting of two dense strata with less dense interspace. The normally sectioned plasma membrane has the same fine structure (pm_1). Obliquely sectioned portions of the plasma membrane appear as ill defined zones of varying width (pm_2). A number of particles resembling Palade particles (*p*) occur free in the cytoplasm. × 120,000.

FIG. 15. Fixed after 7 daily instillations. Lamellated inclusion body within a macrophage. Concentric lamellae (*la*) fill the body from its periphery towards the center. A central portion of the inclusion appears still finely granular or homogeneous (compare with Fig. 24). × 75,000.



(Karrer: Phagocytosis process in lung)

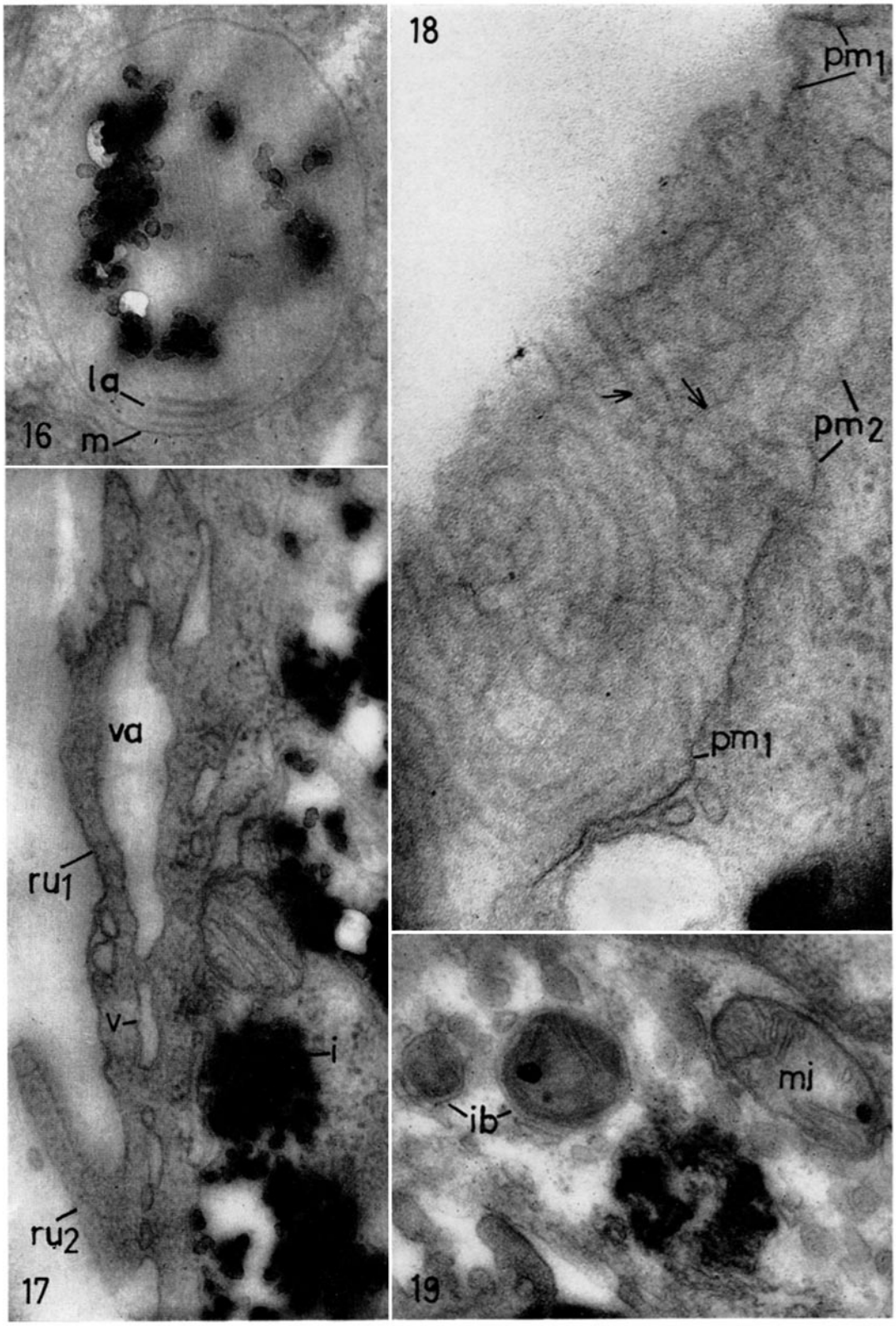
PLATE 193

FIG. 16. Fixed after 9 daily instillations. A large membrane-bounded inclusion body is filled with a homogeneous matrix, within which clumps of India ink particles are embedded. Three parallel lamellae or membranes have formed peripherally within this matrix (*la*). They are concentric, run parallel to the bounding membrane (*m*), and appear to have free edges. × 70,000.

FIG. 17. Fixed after 9 daily instillations. A "ruffle" of cytoplasm (ru_1) has backfolded onto the cell, trapping a vacuole (*va*) and vesicles (*v*) within the cytoplasm. Another "ruffle" (ru_2) is forming. Large clumps of India ink (*i*) lie within the cell; most of them seem to be in spaces bounded by membranes. × 60,000.

FIG. 18. Fixed after 9 daily instillations. Superficial cell portion showing the plasma membrane and a tangentially sectioned area. Where the plasma membrane is cut normally it appears as a dense "line" (pm_1); it fades and becomes indistinct where it is cut obliquely (pm_2). This obliquely cut portion (pm_2) is indistinguishable from an array of "lines" which form an irregular pattern within a dense zone adjacent to the plasma membrane. These "lines" (arrows) are, therefore, recognized as oblique sections through the plasma membrane, the honeycomb pattern indicating a fine corrugation of this membrane and of the cell surface. × 110,000.

FIG. 19. Fixed 20 minutes after one instillation. Within a macrophage, 2 small inclusion bodies (*ib*) lie close to a mitochondrion (*mi*). They are bounded by a membrane. Their matrix is dense in the center and a narrow peripheral "shell" of lower density separates the central dense portion from the limiting membrane. The inclusion on the right in addition shows a very dense small granule, comparable to the granule within the mitochondrion (*mi*), and a few indistinct lamellae. × 45,000.

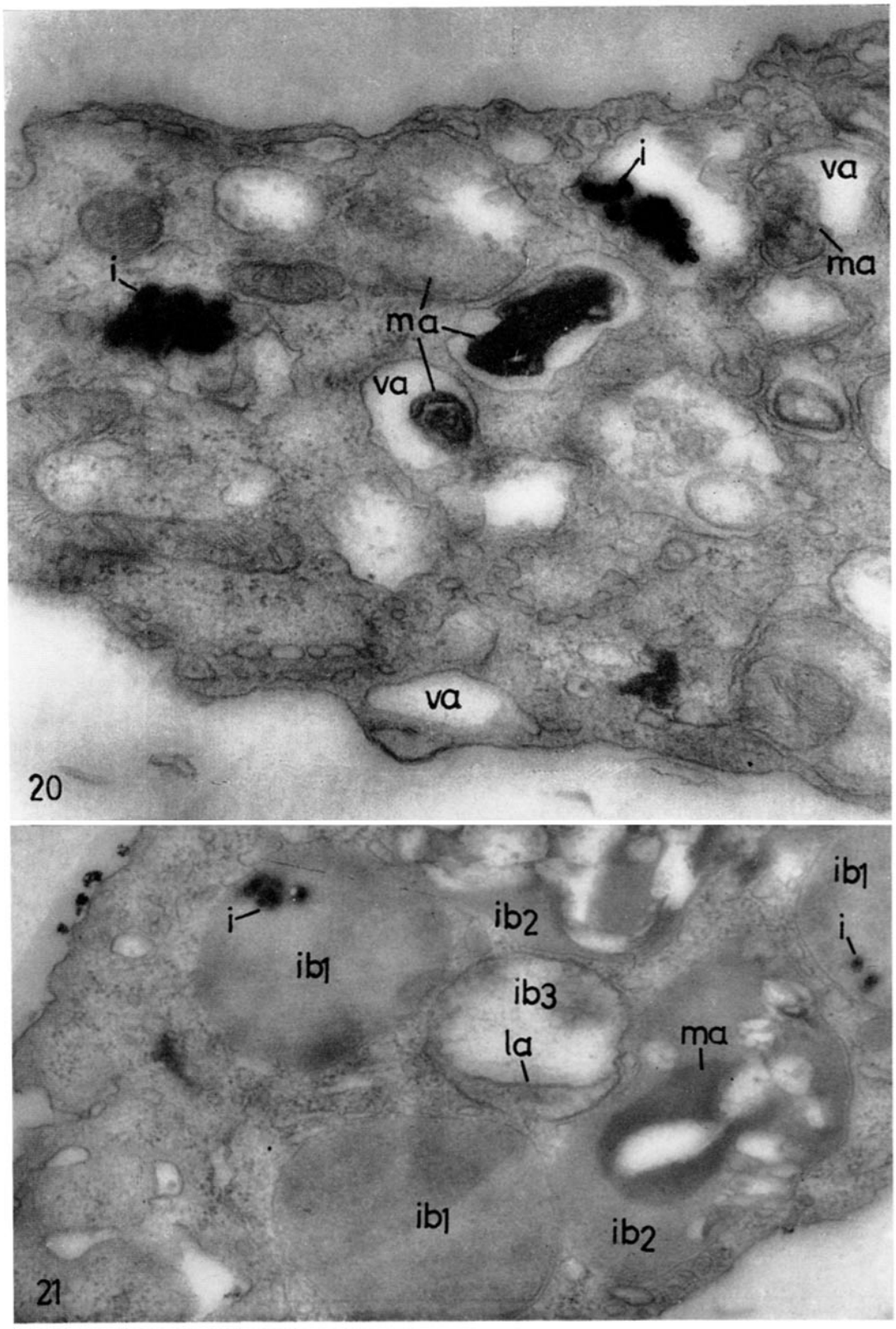


(Karrer: Phagocytosis process in lung)

PLATE 194

FIG. 20. Fixed after 9 daily instillations. Portion of a macrophage showing numerous vacuoles (*va*). Some of these contain masses (*ma*) of widely varying density. No structural detail can be resolved in some of these masses, whereas others appear as formed granules. Other vacuoles contain distinct ink particles (*i*). × 45,000.

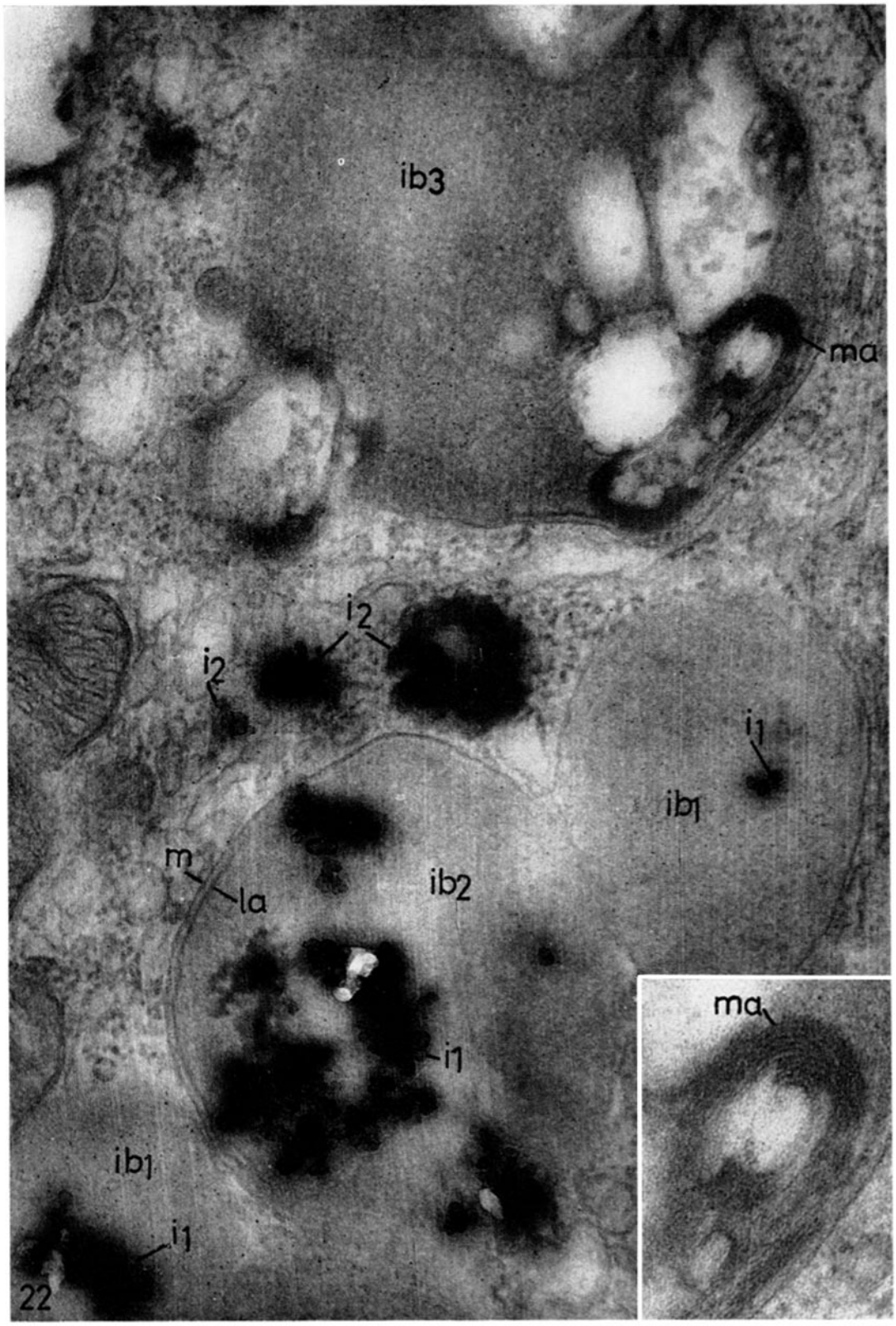
FIG. 21. Fixed after 9 daily instillations. Portion of a macrophage containing numerous large inclusion bodies. All of them are bounded by membranes. The simplest type contains a homogeneous matrix (ib_1). In others (ib_2) this matrix shows vacuolization and dark masses (*ma*). One inclusion (ib_3) contains a lamella or membrane consisting of 2 dense lines (*la*). Ink particles are recognized within 2 inclusions (*i*). × 40,000.



(Karrer: Phagocytosis process in lung)

PLATE 195

FIG. 22. Fixed after 9 daily instillations. Inclusion bodies within a macrophage. All inclusions are bounded by membranes. Their matrix generally is homogeneous (ib_1). In another (ib_2) a lamella (membrane) has formed at the periphery (*la*), parallel with the bounding membrane (*m*). This lamella appears to terminate with free edges. Vacuoles and dense masses (*ma*) appear within a third type of inclusion (ib_3 and insert). These dense masses show a concentric lamellation (*ma*, insert). Ink particles are found within inclusions (i_1) as well as without (i_2). × 70,000. Insert 140,000.

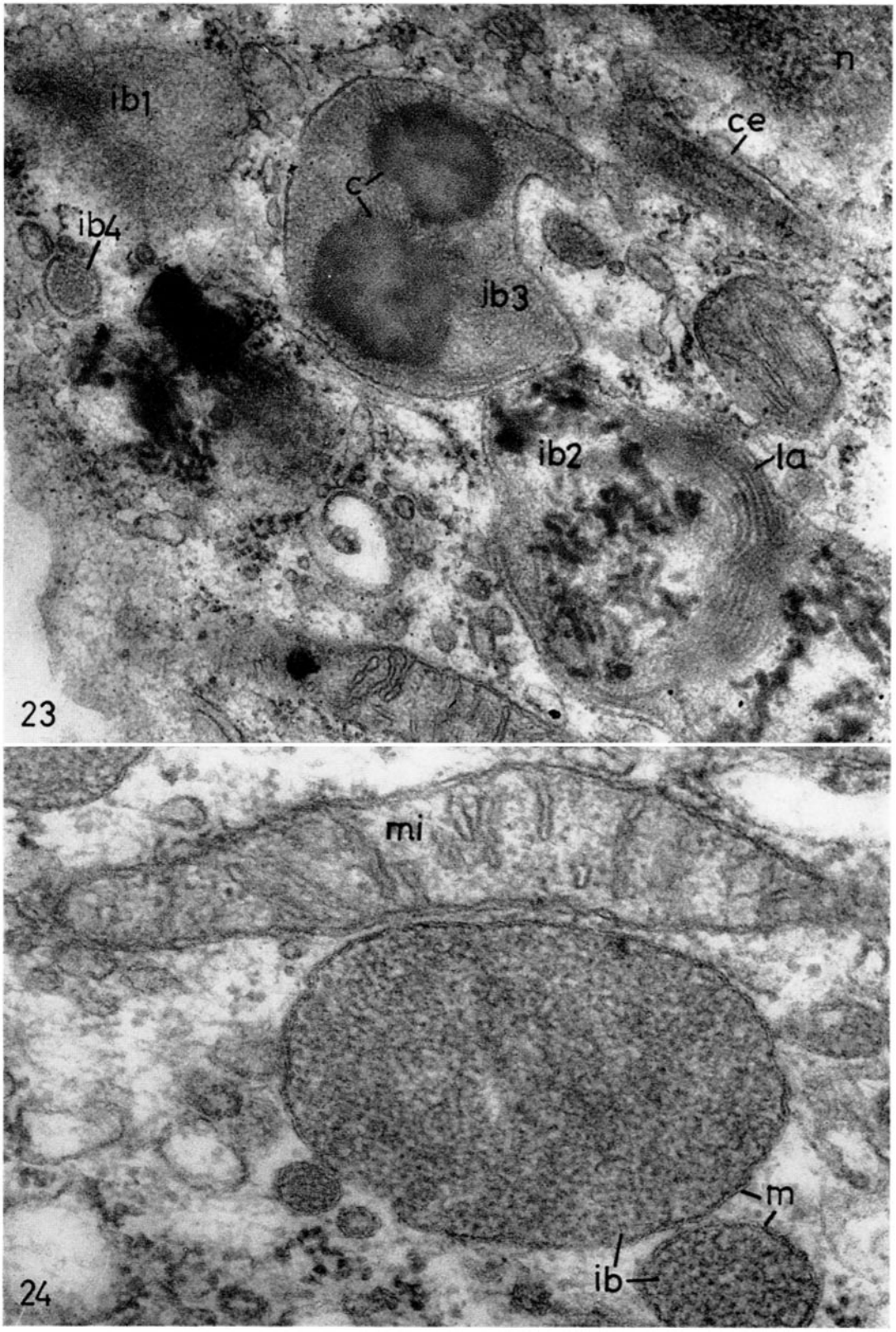


(Karrer: Phagocytosis process in lung)

PLATE 196

FIG. 23. Fixed after 7 daily instillations. Detail of cytoplasmic components. Portion of the nucleus is seen at upper right (*n*). Close to it a longitudinally sectioned centriole is seen (*ce*). One inclusion body (ib_1) shows a homogeneous matrix. A second one (ib_2) contains peripheral concentric lamellae and central dense masses of irregular shape. A third inclusion (ib_3) shows two dense bodies (*c*) within its otherwise homogeneous matrix. These correspond to the crystal-like body shown in Fig. 28. A fourth type of inclusion body is much smaller (ib_4). It contains a dense matrix in its center, which is surrounded by a less dense "shell." All inclusions are bounded by unit membranes. × 60,000.

FIG. 24. Fixed after 9 daily instillations. Inclusion bodies of the type with homogeneous matrix are shown (*ib*) (compare with Figs. 21 and 22). Here the matrix appears slightly granular, an effect possibly produced by the staining with lead hydroxide. The bounding membranes (*m*) are "triple-layered," that is, they consist of 2 dense strata with intermediate less dense layer. They, therefore, have the same structure as Robertson's unit membranes (37). A mitochondrion (*mi*) lies next to the inclusions. × 110,000.

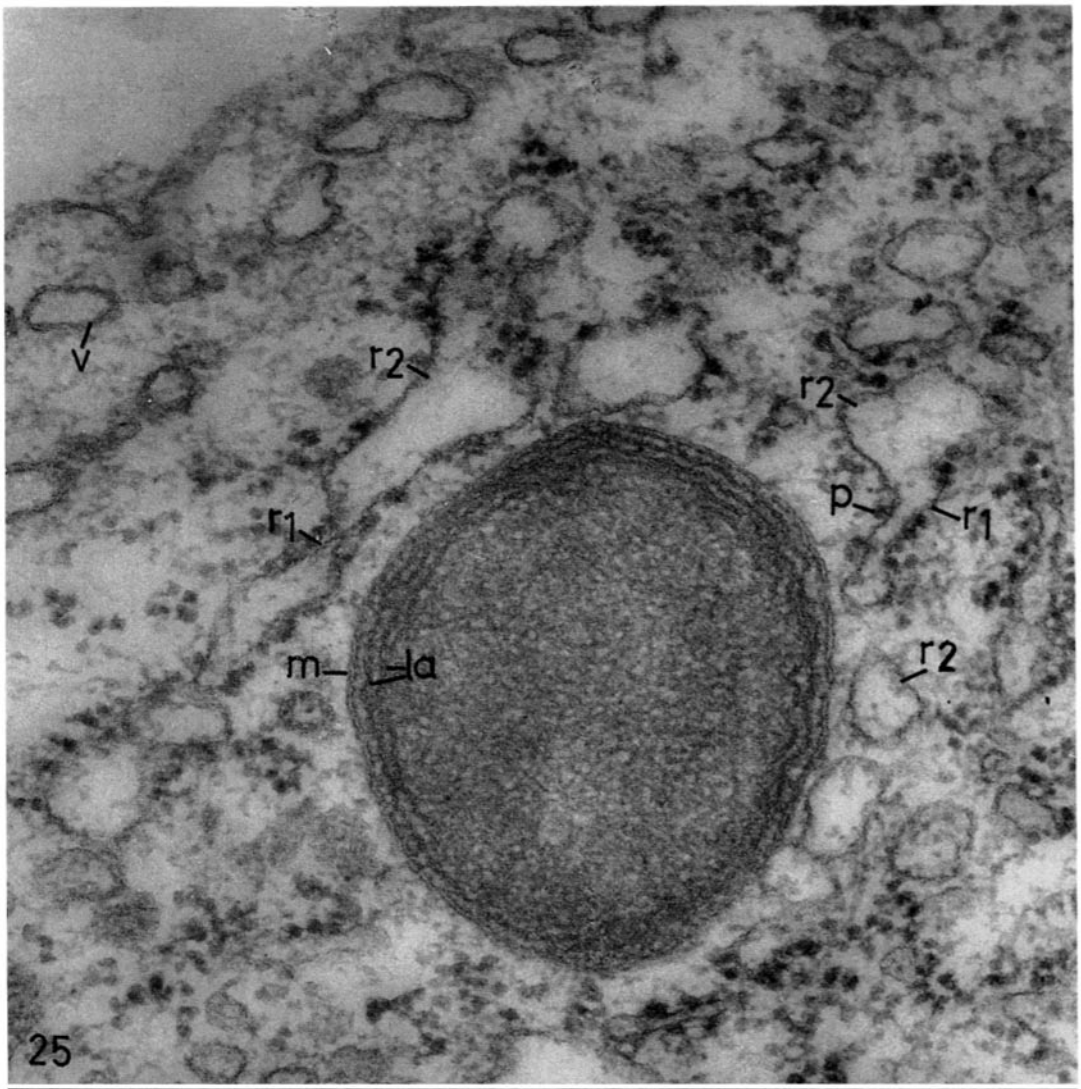


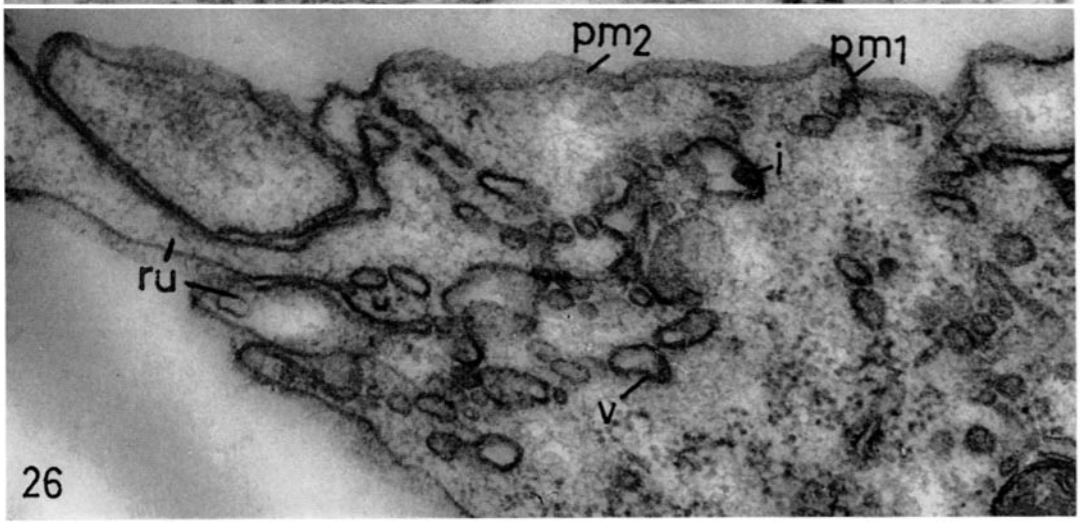
(Karrer: Phagocytosis process in lung)

PLATE 197

FIG. 25. Fixed after 9 daily instillations. A spherical inclusion body is bounded by a unit membrane (*m*). The central portion of the inclusion shows a homogeneous matrix (compare with Figs. 21, 22, 24). Peripherally, however, two to three concentric lamellae or membranes appear within this matrix (*la*). They seem "triple-layered," but not so distinctly as the bounding membrane. At upper left, a chain of pinocytotic vesicles (*v*) is seen beneath the plasma membrane; their bounding membranes are also "triple-layered." The rough surfaced endoplasmic reticulum (r_1) interconnects with smooth surfaced small dilatations (r_2); the bounding membranes of these units are not distinctly "triple-layered." They also seem to be thinner than the membranes bounding the vesicles (*v*). Dense particles resembling Palade particles (*p*) adhere to the membranes of the reticulum and also occur free within the cytoplasm. × 105,000.

FIG. 26. Fixed after 9 daily instillations. Several "ruffles" of the cell curface (*ru*) enclose deep plasma membrane infoldings between themselves. Small vesicles (*v*) take their origin from such infoldings. One vesicle contains an ink particle (*i*). The obliquely sectioned plasma membrane appears as a dense zone of varying width (pm_2), whereas normally sectioned portions of the membrane appear as dense "lines" (pm_1). × 50,000.

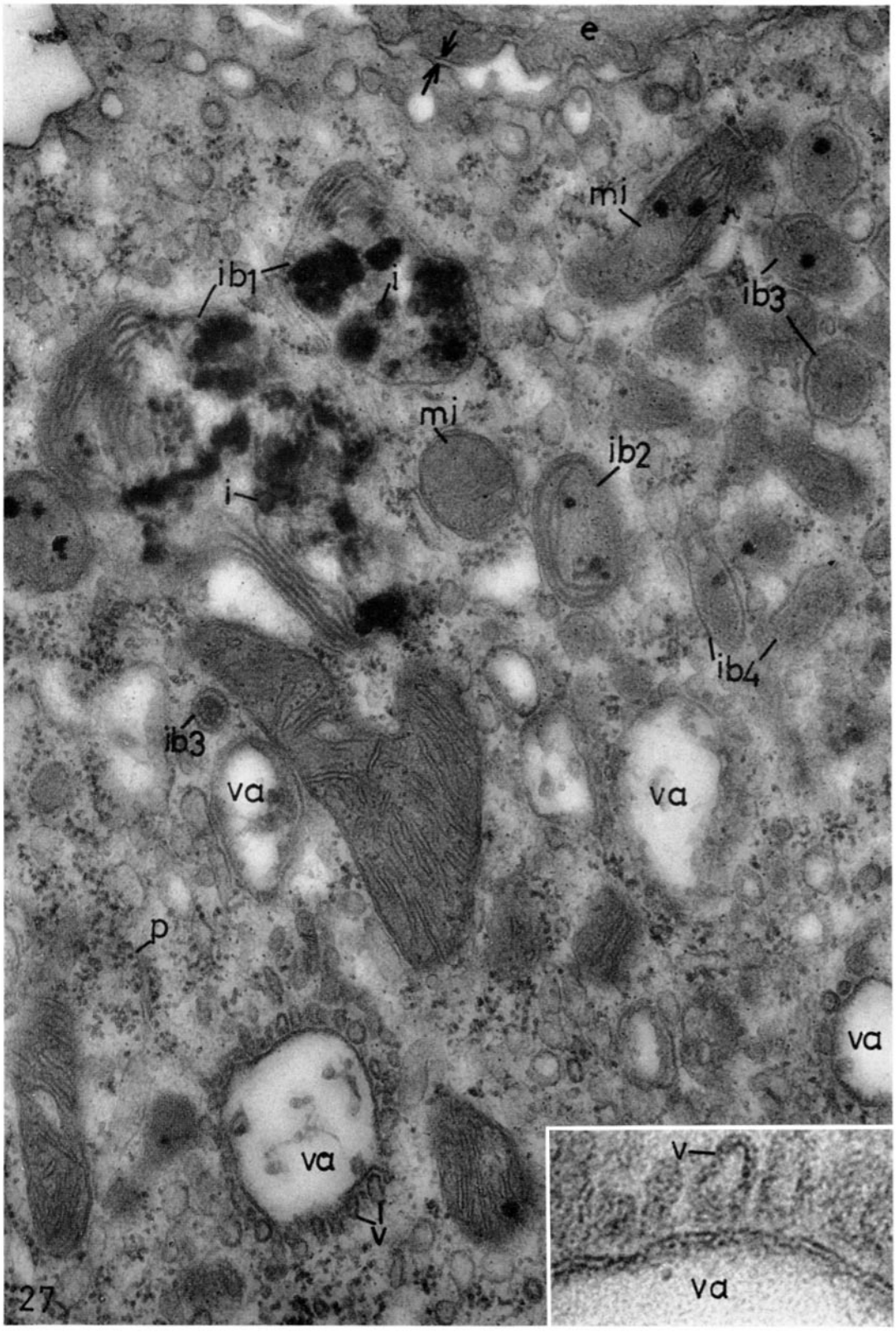




(Karrer: Phagocytosis process in lung)

PLATE 198

FIG. 27. Fixed after 7 daily instillations. Portion of an alveolar macrophage which rests upon the alveolar epithelium (*e*). Only a narrow intercellular space separates the free macrophage from the epithelium (arrows). Various vacuoles and inclusion bodies lie within the cytoplasm. The vacuoles (*va*) are bounded by unit membranes (insert). Small vesicles contained by similar membranes (insert) cluster around some of them (*v*). Two large inclusion bodies (ib_1) contain concentric lamellae, dense masses, and a few ink particles (*i*). Another, smaller inclusion (ib_2) contains a few lamellae and small dense granules. A third group of inclusions (ib_3) shows a centrally dense matrix which is surrounded by a less dense "shell." They also contain dense granules indistinguishable from the granules within mitochondria (*mi*). If suitably sectioned, these inclusions appear to be elongated (ib_4). Numerous particles resembling Palade particles (*p*) are scattered throughout the cytoplasm. × 60,000. Insert × 235,000.



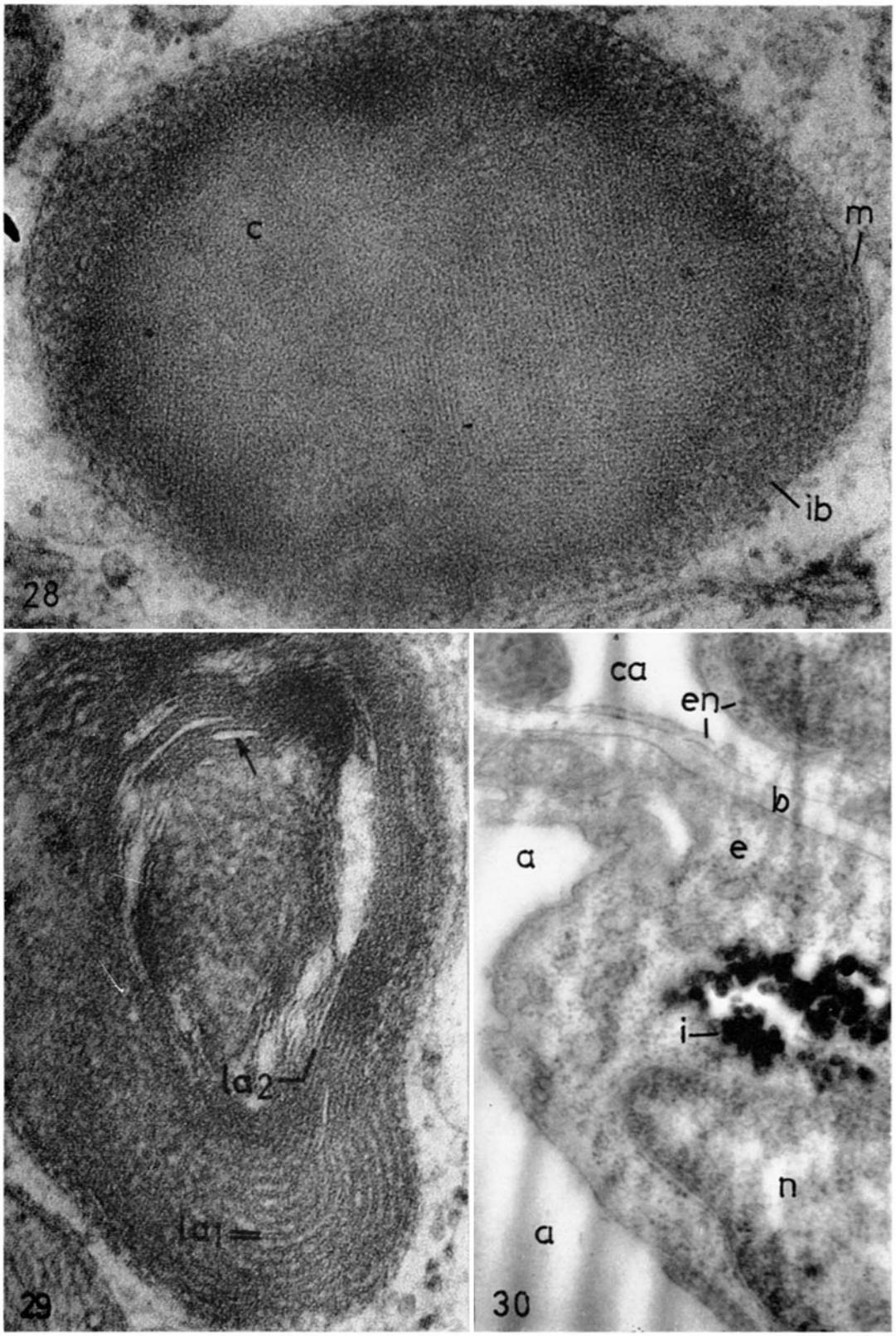
(Karrer: Phagocytosis process in lung)

PLATE 199

FIG. 28. Fixed after 7 daily instillations. Detail of an inclusion body. A unit membrane bounds the inclusion (*m*). The matrix appears homogeneous peripherally (*ib*), but in its center it consists of a large body showing a crystalline structure (*c*). Very thin parallel lines appear within portions of this body. Arrays of such lines cross each other at angles approximating 90 degrees. The periodicity created by these lines measures about 60 A. × 155,000.

FIG. 29. Fixed after 9 daily instillations. Detail of a lamellated inclusion body. The peripheral concentric lamellation surrounds a central portion of matrix which still appears uniform. The lamellae are transversely sectioned only within limited areas. In the other areas they appear blurred or indistinct. In one area the periodicity created by the lamellae measures about 120 to 140 A; there each lamella consist of 2 dense strata with separating less dense layer (la_1). It appears that in other areas a fusion occurs between neighboring lamellae (la_2) in a manner indicated in Text-fig. 1: there the periodicity is of the order of 75 A. Within these latter areas, local clefts may separate some of the fused lamellae (arrow). The whole lamellar arrangement closely resembles that within myelin. × 145,000.

FIG. 30. Fixed after 18 daily instillations. The micrograph shows a portion of a "small" alveolar epithelial cell (*e*) with its nucleus (*n*), the alveolar basement membrane (*b*), and portions of an endothelial cell (*en*) and of a capillary (*ca*). The alveolar epithelium lines the alveolus (*a*). A small clump of phagocytized ink lies within the epithelial cell (*i*). × 40,000.



(Karrer: Phagocytosis process in lung)