THE ULTRASTRUCTURE OF MOUSE LUNG

General Architecture of Capillary and Alveolar Walls*

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Plates 45 to 48

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In a previous report a few morphological features of normal mouse lung were discussed (11), and earlier electron microscopic studies on this subject by other authors (7, 12–15, 19, 24, 25) were reviewed. In that report, Low's finding of an alveolar epithelium (13, 15) was confirmed, but, in contrast to the observations of Kisch (12), discontinuities in this epithelial layer could not be verified. It was also shown (11) that the endothelium of lung capillaries is without pores, in contradistinction to kidney capillaries (2, 18, 22), and that it may become as thin as 100 A.

The present study extends the previously reported findings. In addition, it discusses some of the methods of tissue processing which were used, especially as regards their ability to provide good preservation of the tissue. Processing methods that would not be damaging to the tissue were considered essential for a study of lung, since only on the basis of undamaged specimens could the long debated question as to the continuity or discontinuity of endothelium and alveolar epithelium be solved. In damaged specimens the artefactitious disruptions in cells and membranes could simulate discontinuities in endothelium (19) or epithelium (12).

In the search for optimal processing methods a starting point was provided by a few reports in which the influence of different steps of processing upon the preservation of the tissue had been analyzed. Two of these reports had stressed the importance of the composition of the fixative and of the time lapse between death of the donor animal and fixation of the tissue (16, 21). In the present study, buffered (16) isotonic (21) osmium tetroxide was therefore used, and an effort was made to diminish postmortem changes (21) by a rapid initiation of fixation. This was obtained by infusion fixation. Another report dealing with embedding had presented evidence on the importance of a high polymerization temperature and of prepolymerization of the methacrylate for good tissue preservation (4). In the present study, a temperature of

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80°C. and prepolymerization to a very high viscosity were adopted for all but a few embeddings. In a further attempt to define optimal methods of tissue processing, the washing of the fixed tissue was varied in different experiments, by using either distilled water or balanced salt solution (9).

Besides proper fixation and embedding an optimal cutting property of embedded tissue was held desirable, since cutting artefacts that occur with poor embedding leave the tissue of the sections damaged to varying degrees. In the search for methods that would yield an improved cutting property of the tissue and thereby minimize cutting artefacts several modifications in the customary steps of tissue processing were adopted. These were prolonged acetone dehydration, ammonium sulfide treatment, prolonged dehydration and impregnation, the use of different amounts of catalyst, a polymerization time of several days, and vacuumization of embeddings (5). These modifications will be discussed later.

Material and Methods

Adult white mice (Princeton strain) were killed by breaking their necks, and the trachea was ligated below the larynx. ½ cc. of buffered, isotonic, cold (6°C.) 1 per cent osmium tetroxide solution (16, 21) was injected intratracheally below the ligature. This procedure usually required only 2 to 3 minutes. The left upper lobe of the lung was then perfused through the heart with approximately 2 cc. of cold (6°C.) fixative in the manner previously described (11). Small pieces of the left upper lobe were then fixed *in vitro* in cold osmium tetroxide solution, for an additional ½ to 1 hour. Some specimens were then washed in three changes of cold distilled water for a total period of 2 hours, others were washed in four changes of balanced salt solution (9) for a total period of 40 minutes. Dehydration was performed in one change of cold (6°C.) 70 per cent acetone for 1 to 2 hours, and in three changes of cold 100 per cent acetone. The tissue was agitated mechanically in each of the changes of 100 per cent acetone, using a small electric motor set up in a refrigerator (6°C.). The total period of time allowed for dehydration in the 100 per cent acetone was usually about 16 hours. The tissue was impregnated in a mixture of differing proportions of *n*-butyl methacrylate and methyl methacrylate containing benzoyl peroxide as catalyst; the quantity of catalyst used in different embeddings varied (0.2 or 0.5 per cent). Three changes of monomer were used, and the tissue was kept mechanically agitated in it during a total period of at least 16 hours. The tissue was embedded in batches of the same methacrylate mixture that had been used for impregnation. This had been prepolymerized to a very high viscosity. No. 5 gelatin capsules were used. Polymerization was carried to completion in an oven at 80°C. (4). The capsules were left in the oven for at least 72 hours.

Some tissue pieces were subjected to a different treatment. After having been fixed in osmium tetroxide and washed in balanced salt solution these pieces were immersed and agitated for 10 minutes in a cold (6°C.) ammonium sulfide solution. Such a solution was prepared immediately before use by adding 1 volume of ammonium sulfide, C.P. (ammonium hydrosulfide, light; Fisher Scientific Co.) to 99 volumes of balanced salt solution. Following this treatment the tissue was washed in two changes of balanced salt solution (without sulfide) for a total of 20 minutes. It was then dehydrated, impregnated, and embedded in the manner described above.

A few of the earlier embeddings were allowed to polymerize at a lower temperature (45°C.) instead of at 80°C. For some of these embeddings, prepolymerization had not been used.

After polymerization, most of the decapsulated embeddings were placed in the vacuum (5) of a shadow-casting machine for periods of several hours, with the hope of removing residual acetone as well as unpolymerized monomer (5). Sectioning was done by means of glass knives and a servall microtome (20). An RCA model EMU 2 electron microscope was used for microscopy.

OBSERVATIONS

The Effects of the Tissue Processing upon General Appearance, Preservation, and Cutting Properties of Lung Tissue

Effects of Fixation and of Washing.—Intratracheal fixation prevented the collapse of the lung upon opening of the mouse's chest. In addition, it perhaps contributed to good tissue preservation, although this point was not proved since no alternate methods of fixation were used. When washed in cold distilled water the lung tissue was found to be about as well preserved as tissue washed in balanced salt solution. Varying the length of time of washing (1 to 2 hours) did not seem to influence the quality of tissue preservation.

Effects of High Temperature Embedding and of Prepolymerization.—Lung tissue was preserved best if an elevated embedding temperature (80°C.) was combined with the use of prepolymerized methacrylate. Preservation was judged to be good if the cells were uniformly dense, and if membranes were continuous (4). Tissue damage was most severe if a low embedding temperature (45°C.) was used in combination with methacrylate that had not been prepolymerized. The damage could be so severe that the tissue was not even recognizable as lung. The damage was manifested in a disrupted continuity of membranes (4), especially of the surface membranes of cells, and in a general rarefaction and discontinuity of all the cellular material.

Effects of Methods Designed to Improve the Cutting Properties of Embeddings.—Prolonged acetone dehydration, prolonged impregnation and polymerization, and vacuumization (5) of the embeddings all seemed to improve somewhat the cutting properties. No ill effects on tissue preservation were noted. There was no recognizable difference in the cutting properties of embeddings prepared with different amounts of catalyst (0.2 and 0.5 per cent). The sulfide treatment did not appreciably improve the cutting properties of the tissue, but it produced several changes in its appearance. Macroscopically, the previously sepia-brown tissue turned black when immersed in the sulfide solution. In the electron microscope, sulfide-treated tissue appeared well preserved, but showed an over-all increased contrast and, at high magnifications, an increased granularity. Black amorphous masses (*D*, Figs. 2, 2 *b*) were considered to be produced by the sulfide treatment, since they were not observed in untreated lung tissue. Another more specific effect of the sulfide treatment on some morphological structures will be mentioned in the description of the alveolar epithelium.

Morphological Findings

Alveolar Epithelium.—The alveolar epithelium consists of cells which in the region of their nucleus reach a height of about 4μ, and which attenuate peripherally to form a thin (average 50 to 100 mμ) uninterrupted layer of cellular material that lines the alveolar air spaces (*Ae*, Fig. 2).

Two morphological "types" of alveolar epithelial cells are recognized. In one "type," shown in a previous report (11), the portion of the cell that contains the nucleus bulges more or less into the alveolar lumen, surface microvilli (3) are missing, and mitochondria and inclusions are rare or absent. In the other "type," the nucleated portion of the cell is often located in a niche of the alveolus (*Aec*, Figs. 3 and 5), its free surface is covered with numerous microvilli (*Mv*, Figs. 3 and 5), and it contains many mitochondria and typical inclusion bodies. These mitochondria have a dense matrix and numerous inner double membranes or cristae (*M*, Fig. 6) which run in planes roughly perpendicular to the longitudinal axis of the mitochondria. From some mitochondrial profiles it can be deduced that the mitochondria are long and filamentous (Fig. 5). The inclusion bodies appear more or less vacuolated in tissues not treated with ammonium sulfide (*I*, Figs. 3 and 4), but are much darker and less vacuolated in sulfide-treated tissue, where they seem to consist of densely packed granules (*I*, Figs. 5 and 6). The largest inclusions measure about 500 mμ in diameter.

The thin portions of the alveolar epithelium are seen to contain small vacuoles, as well as some mitochondria (*M*, Fig. 2).

Capillary Endothelium.—The endothelium is made up of cells which reach their greatest thickness (about 2μ) in the region of the nucleus and attenuate peripherally to a thin (average about 150 mμ) sheet.

The nucleated thick portion of the endothelial cells usually lies adjacent to other capillary loops, or opposite the thick portion of an alveolar epithelial cell (*Cec*, Figs. 3 and 5). Other endothelial cells, which belong to capillary loops above or below the plane of the section, are seen to be surrounded on all sides by the basement membrane (*Cec*, Fig. 2). In sections the nucleus frequently appears oval (*N*, Figs. 3 and 5); it is bounded by a double nuclear membrane. It is made up of small granules which appear especially dense along the nuclear membrane in a layer of about 500 mμ in thickness. In the region of the nucleus a few mitochondria and some larger vacuoles (*V*, Fig. 5) may be present in the cytoplasm, and numerous small vacuoles are also seen.

The thin portions of the endothelium are continuous and do not show any pores or interruptions of any kind. Even in those areas where the endothelial sheet becomes extremely thin (10 mμ in Fig. 2 *a*) it is limited by two continuous dense plasma membranes. These very thin portions of the endothelium are usually found adjacent to an alveolus (Figs. 1 and 2). Mitochondria and

small vacuoles are usually found in the thin portions of the endothelium. Boundaries between the single endothelial cells have been recognized before (11); in sections they appear as two parallel dense membranes—the plasma membranes of the two adjacent cells—with an enclosed intermediate layer of lesser density (*Isp*, Figs. 1, 2 *b*).

Basement Membrane.—The generally thin (about 35 to 55 mμ) structureless layer (11) between epithelium and endothelium (*Bm*, Fig. 2) and between the endothelial layers of neighboring capillaries (*Bm*, Fig. 1; *Bm*, lower part of Fig. 2) is designated as basement membrane. In sufficiently well preserved tissue this layer appears a little denser than methacrylate alone (*Bm*, Figs. 2 and 3) and gives the impression of being homogeneous (*Bm*, Figs. 2 *a* and 2 *b*). There is continuity between the basement membrane and the translucent layer of boundaries between endothelial cells (*Isp*, Fig. 2 *b*).

The basement membrane thickens considerably in some areas, usually in the knee of a capillary loop (*Bm*, Fig. 1) or between different capillary cross-sections (*Bm*, at lower margin of Fig. 2). In such areas bundles of slender collagen fibrils may be seen (*F*, Fig. 1). The smallest measured diameter of a single fibril is about 25 mμ. In association with such fibril bundles knife marks and cutting artefacts are frequently found (Fig. 1).

Alveolar Macrophages.—This designation is applied to free cells in the alveolar lumina which are not a part of the alveolar epithelium. Such cells are to be discussed in future publications. A small portion of such a cell is seen (*Am*, Fig. 1) in close contact with the free surface of the alveolar epithelium by means of pseudopodium-like short stalks.

DISCUSSION

The Effects of the Tissue Processing upon General Appearance, Preservation, and Cutting Properties of Lung Tissue

As has already been mentioned in the introduction, modified methods of tissue processing that would not damage tissue appreciably were considered essential for a successful study of the fine structure of lung. Such modifications have been described earlier in the paper. The following discussion will explain the reasons why they were selected, what effects were noted, and what the author's present opinion is as regards their usefulness.

Fixation and Washing.—The initiation of fixation by intratracheal infusion was found necessary to prevent lung collapse. Lung collapse was judged undesirable not only because it changes the spacial relationship of cells as present *in vivo*, but also because it could affect the single cells to a high enough degree to produce an alteration in their morphology. An additional reason why the method was judged superior to *in vitro* fixation was the fact that intratracheal infusion would initiate fixation quickly throughout the lung, and would thus minimize the possibility of postmortem changes (21) in as

yet unfixed cells. The subsequent vascular perfusion with fixative was done with the hope of obtaining an even better and faster fixation of all parts of the lung; its additional value has not been proved, however. The tissue was fixed *in vitro* for an additional ½ to 1 hour, since after the infusion of fixative the blood of larger pulmonary vessels was sometimes seen to be still red, indicating that perhaps not all parts of the lung were as yet adequately fixed. The length of this additional *in vitro* fixation (½ to 1 hour) did not seem to have any influence on the preservation of the tissue, which is in disagreement with the reported findings of Low (14).

Some embeddings were washed in distilled water with the hope of removing water-soluble salts along with osmium tetroxide, since it had been suggested that such salts may precipitate during dehydration and become visible in the form of granules or similar structures (5). Other embeddings were washed in balanced salt solution because physiological pH, composition, and tonicity were considered less apt to cause trauma with osmium-fixed tissue than distilled water. Balanced salt solution would not, however, remove soluble salts as effectively as water. Although no obvious difference was noted in the fine structure of tissues washed by the two methods, the balanced salt solution was generally preferred for the theoretical reasons mentioned.

Fixation and washing were done in the cold (6°C.) in order to reduce postmortem changes in the tissue (21) and precipitation of reduced osmium (21).

High Temperature Embedding and Prepolymerization.—The reasons why high temperature and prepolymerization are advantageous have been given by Borysko (4). The adoption of an elevated polymerization temperature in combination with prepolymerization of the methacrylate (4) was found essential for an over-all good tissue preservation; that is, a virtually undamaged appearance of the tissue in all embeddings and at all levels sectioned. Damage in lung tissue embedded at 45°C. in methacrylate which had not been prepolymerized closely resembled the changes that had been attributed to prolonged fixation (Low (14)). Such damage might be localized and relatively minor, or widespread and severe.

Methods Designed to Improve the Cutting Properties of Embeddings.—Certain cutting artefacts consisting of alternating thick and rarefied zones which traverse the sectioned tissue have been studied in chromium-shadowed sections of lung. Using this method it was realized that these cutting artefacts were often more severe inside the tissue, *e.g.* in an alveolar septum, than in the methacrylate surrounding the tissue, *e.g.* in an alveolar lumen. It was concluded that the embedded tissue had cutting properties inferior to those of the methacrylate surrounding it. It was suspected that one possible cause for the poorer cutting properties of the tissue was the rubbery consistency, *i.e.* the higher elasticity, of the methacrylate inside as compared with that

outside the tissue (5). It was already known that the polymerized methacrylate was rubbery if it contained small amounts of water, ethanol, acetone, or non-polymerized monomer (5), or if it had been exposed to the interfering action of oxygen (23) during the polymerization. The hypothetical rubbery consistency of the methacrylate inside the tissue could thus be caused by residual water, dehydrating agent, and non-polymerized monomer, or by oxygen liberated from residual osmium tetroxide (5). Tissue processing was therefore done in such a way that a nearly complete removal of water, acetone, and non-polymerized monomer could be expected, and some preparations were treated with sulfide in order to inactivate the residual osmium tetroxide.

Since uncollapsed lung resembles a sponge which during the processing is filled consecutively with large amounts of osmium fixative, water (or balanced salt solution), and dehydrating agent, it was believed that the complete removal of these large amounts of reagents would be difficult. Prolonged washing could probably have removed the osmium more efficiently, but for fear of its damaging the tissue the washing time was kept relatively short (1 to 2 hours). Instead, the osmium was inactivated, in some embeddings, by precipitation with sulfide, as discussed below. Prolonged dehydration and impregnation were believed to be advantageous for a more efficient removal of the water (or balanced salt solution) and of the acetone. Acetone was used for dehydration in preference to ethanol for the following reason. Acetone is known to be soluble in the polymer, whereas ethanol is not (5). If a trace of acetone were to remain inside the tissue even after prolonged impregnation, it would, during and after the embedding process, diffuse out of the tissue into the surrounding polymer in which it is soluble (5). Traces of ethanol on the contrary, being insoluble in the polymer, would remain trapped inside the tissue (5) and cause the methacrylate in the tissue to be rubbery. For the complete removal of the last traces of acetone the vacuumization of the polymerized, decapsulated embeddings had been proposed by Borysko (5). A prolonged polymerization time of at least 72 hours (at 80°C.) was adopted in order to insure a more complete polymerization and to decrease the amount of monomer remaining in the polymerized embedding; the last traces of such residual monomer would likewise be removed by the vacuumization (5).

It was found that the combination of the above modifications in the tissue processing yielded embeddings of improved cutting properties. An exact evaluation of the relative importance of each of the steps was not made, but it was felt that the substitution of acetone for ethanol (5), prolonged impregnation and polymerization times, and subsequent vacuumization of embeddings (5) were all valuable, whereas the value of a prolonged dehydration was more questionable. The substitution of acetone for ethanol did not result in an

altered appearance of the tissue, with the possible exception of some contrast loss under acetone dehydration. Such a loss of contrast could be expected since acetone, in contradistinction to ethanol, does not reduce osmium tetroxide. Thus in acetone-dehydrated lung the inclusions of alveolar cells were observed to be considerably less dark than in ethanol-dehydrated lung.

The sulfide treatment was introduced in order to inactivate possible residues of osmium tetroxide, which could perhaps interfere with proper polymerization because of their oxidizing action (5). Since, in general, ethanol was not used for dehydration for the reasons given above, its reducing action in respect to osmium tetroxide was also lacking. Ammonium sulfide was chosen instead, since hydrogen sulfide (as present in ammonium sulfide solution) is known to precipitate osmium oxysulfides from osmium tetroxide solution at an acid (10) or neutral (6) pH. Contrary to expectations, this treatment did not alter appreciably the cutting properties of the tissue, but it did change its appearance, as described earlier in this report. The increase in over-all contrast and the fine grain visible at high magnification, which seemed to be produced by sulfide treatment, are analogous to changes observed in kidney tissue processed at an elevated temperature instead of in the refrigerator (21). In the latter case these changes have been ascribed to precipitated osmium (21). In sulfide-treated tissue they may be attributable to precipitated osmium oxysulfides. The black amorphous masses seen in sulfide-treated tissue (*D*, Figs. 2 and 2 *b*) may likewise represent osmium oxysulfides, which were precipitated in those areas where residual fixative had not been completely washed out. A third morphological change produced by the sulfide treatment, namely the black and compact appearance of the inclusion bodies inside alveolar cells, may be explained as follows: The possibility that these inclusions consisted originally of some lipoidal material was suggested by their dark appearance even in tissue not treated with sulfide. Sulfide would precipitate the osmium previously present in the region in the form of osmium oxysulfides. If the tissue were not treated with sulfide, part of the lipides would be removed during dehydration (1), especially if dehydration were prolonged, and vacuolization (*I*, Figs. 3 and 4) of the inclusions might result. Complete vacuolization of these inclusions has previously been described by others (19). On the contrary, the precipitated osmium oxysulfides, which are insoluble in acetone, would contribute to the dark and compact appearance of the inclusions as seen after sulfide treatment.

It appears that sulfide treatment of the tissue may not be desirable in instances in which high magnification and resolution are sought because it introduces a fine over-all grain that tends to obscure fine detail. The treatment may on the other hand be advantageous if increase in contrast is desired.

Its selective effect on certain structures, like the inclusions of alveolar cells, may possibly be utilized for the "staining" of such structures.

Morphological Findings

Alveolar Epithelium.—Two morphological types of alveolar cells have been recognized but the significance of this observation is not known at the present time. The numerous microvilli (3) at the free surface of one type (*Aec*, Figs. 3 and 5) may indicate an active motion of the cell surface, as is illustrated by cinemicrographs of tissue cultures (8), which suggests that these cells are detaching themselves from the alveolar epithelium (although they are still seen as a part of this epithelium, Figs. 3 and 5). If this is true these cells would represent the initial phase of a future free alveolar macrophage. The other type of alveolar epithelial cell (11) may be less motile, and the smaller number of mitochondria and the complete absence of inclusions in this type could be taken as an indication that it has a different metabolic activity, from the type of cell shown by Figs. 3 and 5, *Aec*.

Alveolar cells similar to the ones shown here (*Aec*, Figs. 3 and 5) have been recognized by others (12, 19, 24). Kisch (12) described these cells as "specific cells," and although he did not recognize that they belonged to the epithelium, he did describe and discuss their inclusion bodies, which in his material appeared very dark (presumably owing to ethanol dehydration) and partially vacuolated. Policard and others (19) saw only vacuoles inside these cells, having the same size and distribution as the inclusions (*I*, Figs. 3 and 5). In Schlipköter's micrographs (24) the inclusions appeared dark and lamellated.

It has been found impossible up to now to establish a relation between these inclusions and the mitochondrial profiles, although both occur side by side in the same alveolar cells and are comparable in size. It is not believed that the vacuolated inclusions, as observed in acetone-dehydrated lung, not sulfide-treated, (*I*, Figs. 3 and 4), have anything to do with the Golgi apparatus, in spite of a rather similar appearance, since they may also appear more compact than is indicated by Figs. 3 and 4, and since they are distributed throughout the whole cell. It is also unlikely that these inclusions are a postmortem change (21), a reaction product of a cell deprived of oxygen supply or subjected to some other change of environment after the death of the animal, because of the very short time interval (usually about 3 minutes) between the death of the animal and the intratracheal infusion of fixative.

Capillary Endothelium.—It is interesting to note that the endothelium gets thinnest where adjacent to alveolar air spaces (Figs. 1 and 2); *i.e.*, where the gas exchange is taking place. The extreme thinness of about 10 mμ that it may reach locally is also remarkable; this is less than the minimal thickness measured for the alveolar epithelium. The numerous small vacuoles within

capillary endothelia have previously been interpreted as evidence of pinocytosis by the endothelial cells (17), and suggestive evidence has been presented (11) that pinocytosis may be taking place in endothelium and epithelium.

Basement Membrane.—The term basement membrane as used in the present paper and in a previous (11) publication designates the layer of low electron density between alveolar epithelium and capillary endothelium, and between the endothelial layers of neighboring capillaries. If used in this manner it designates, at the electron microscopic level, what has been called the alveolar and capillary basement membrane in light microscopy. The present report extends the previous one (11) in that Fig. 1 shows cross-sections of fibrils situated within the thickened basement membrane, a confirmation of previous reports (12, 13, 19). These structures are presumed to represent collagen fibrils. Elastic fibres as described by Low (13) have not been recognized.

The alveolar basement membrane, *i.e.* the layer between alveolar epithelium and capillary endothelium, is analogous to a layer between the epithelium of kidney glomerula and the endothelium of glomerular capillaries (22). In spite of this topographic analogy, the fine structure of the two membranes is dissimilar. For whereas the alveolar basement membrane appears of rather uniform density at higher magnifications, the membrane in kidney glomerula consists of a middle denser and two outer less dense layers (22).

Blood-Air Barrier.—The extreme over-all thinness of the blood-air barrier (11) is confirmed in the present report. In high magnification micrographs the minimal thickness seen has been 75 mμ, whereas minimal measurements for endothelium, basement membrane, and epithelium (measurements taken at different points) have been found to be approximately 10 mμ, 22 mμ, and 25 mμ respectively. All three layers of the blood-air barrier are uninterrupted, in contradistinction to findings in kidney glomerula (2, 22). Thus the gas exchange in the lung has to proceed across two intact layers of living cytoplasm in addition to the basement membrane, whereas in kidney glomerula the glomerular filtrate is assumed to pass through pores of endo- and epithelium (2, 22) and thus would have to traverse only one uninterrupted layer, the glomerular basement membrane.

SUMMARY

The general architecture of capillary and alveolar walls of the mouse lung was studied by means of the electron microscope. In order to minimize tissue damage and to improve the cutting properties of embeddings, several modifications in the tissue processing methods were adopted. These modifications were: fixation by infusion, a prolonged time of dehydration, of impregnation, and of polymerization, the use of acetone for dehydration, ammonium sulfide

treatment of the fixed and washed tissue, and an elevated (80°C.) polymerization temperature combined with the use of prepolymerized methacrylate. The generally favorable effects of these modified methods upon preservation and cutting properties of embedded tissue are discussed.

Both capillary endothelium and alveolar epithelium were found continuous and without pores. The endothelium was seen to be thinnest in those portions that were adjacent to alveolar air spaces. Two morphological "types" of alveolar epithelial cells were found. One protruded into the alveolar lumen with its thick portion containing the nucleus. The other was often located in a niche of the alveolar wall, and contained peculiar dark inclusions amidst numerous mitochondria. Both were attenuated at their periphery to form the thin epithelial layer. The layer between endothelium and epithelium was designated as basement membrane. It was seen to be generally thin and structureless, but was found thickened in some areas where it also contained collagen fibrils.

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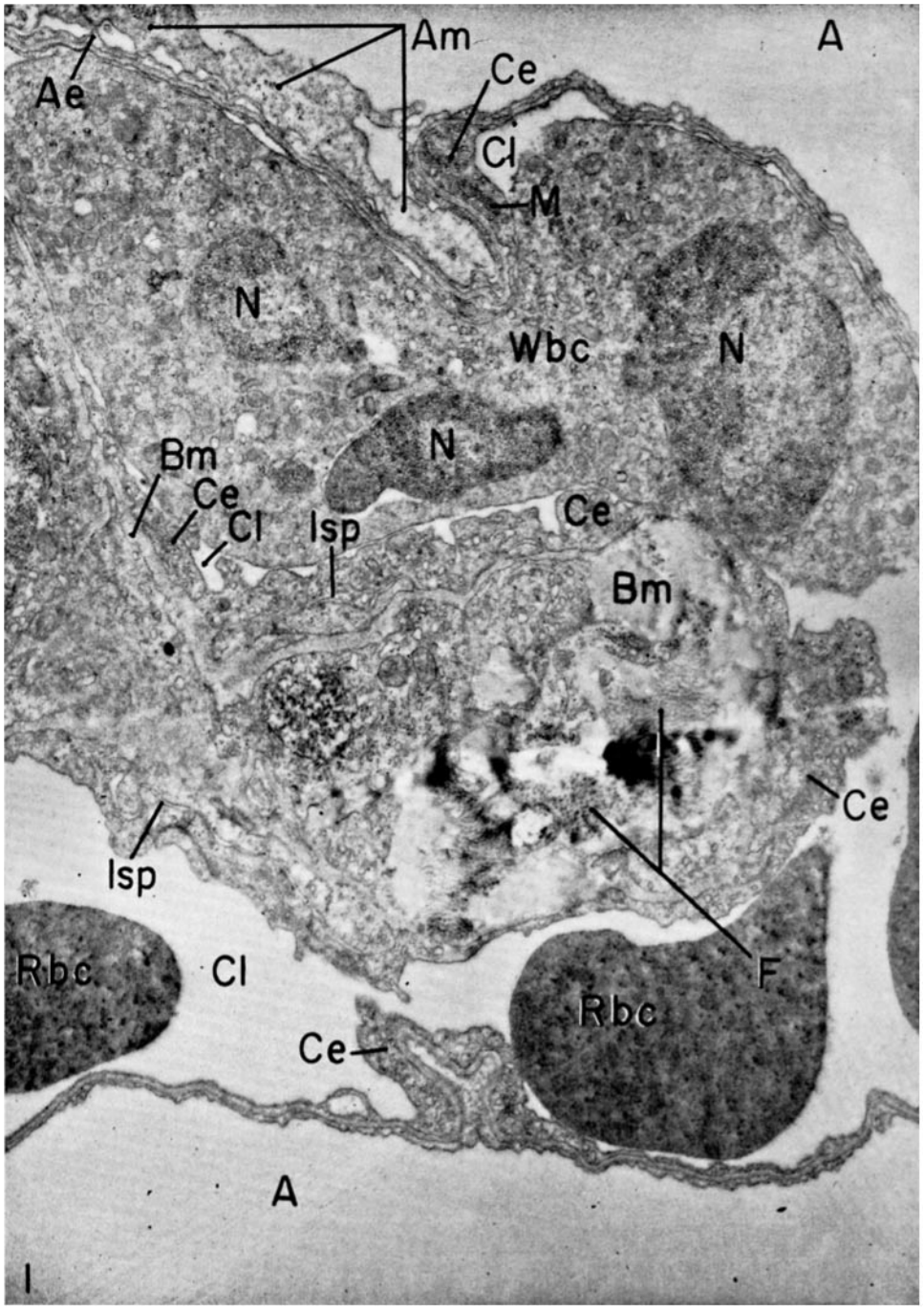
EXPLANATION OF PLATES

Key to Abbreviations

A, alveolus.
Ae, alveolar epithelium.
Aec, alveolar epithelial cell.
Am, alveolar macrophage.
Bm, basement membrane.
C, carbon particle.
Ce, capillary endothelium.
Cec, capillary endothelial cell.
Cl, capillary lumen.
D, deposit.
F, fibril.
I, inclusion.
Isp, intercellular space.
M, mitochondrion.
Mv, microvillus.
N, nucleus.
P, pinocytosis.
Rbc, red blood cell.
V, vacuole.
Wbc, white blood cell.

PLATE 45

FIG. 1. Electron micrograph of a U-shaped capillary loop containing red and white blood cells (*Rbc*, *Wbc*) and surrounded by alveolar air spaces (*A*). A small part of an alveolar macrophage (*Am*) is seen in direct contact with the alveolar epithelium (*Ae*). Intercellular spaces (*Isp*) between overlapping endothelial cells are recognizable. The endothelium (*Ce*) is thinnest where adjacent to alveolar air space (*A*) and contains mitochondria (*M*). The basement membrane (*Bm*) is thickened at the knee of the capillary loop, and the toughness of fibrils (*F*) is probably responsible for the horizontal knife mark. The black ill defined areas in this region represent tissue material that has been torn out from the adjacent translucent spaces during the sectioning; they are a cutting artefact. × 15,000.



(Karrer: Ultrastructure of mouse lung)

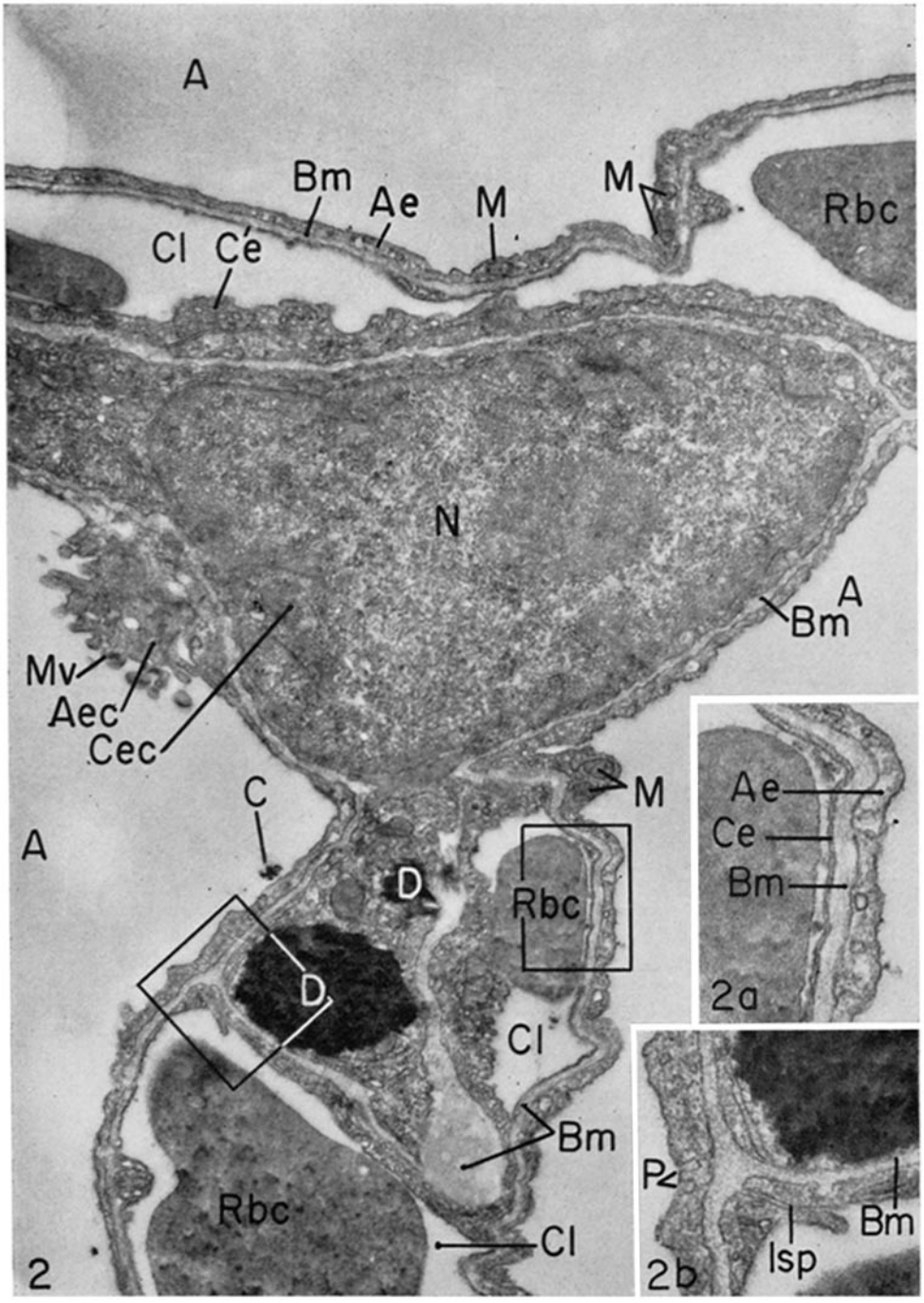
PLATE 46

FIG. 2. Electron micrograph showing a T-shaped septum between alveolar air spaces (*A*), which contains capillaries (*Cl*). The endothelial cell (*Cec*) of which the nucleus (*N*) is visible is thought to be bordering on a capillary lumen situated below or above the plane of the section. The basement membrane (*Bm*) shows one thickened area, between two capillary lumina (*Cl*, lower part of the figure) that probably belong to a single capillary loop. The alveolar epithelium contains mitochondria (*M*) in its thin parts. The microvilli (*Mv*) at the surface of a thicker area of the epithelium (*Aec*) suggest that this is the thick nucleated part of an epithelial cell, although the nucleus does not appear in this section.

The tissue shown in this electron micrograph had been treated with ammonium sulfide. The black deposits (*D*) are considered to be deposits of osmium oxysulfides. *C* is a carbon particle. × 22,000.

FIG. 2 *a*. The extreme thinness of the endothelium (*Ce*) is apparent. × 48,000.

FIG. 2 *b*. The intercellular space (*Isp*) between two endothelial cells is in continuity with the basement membrane (*Bm*). There is possible evidence for pinocytosis (*P*) by the epithelium. × 48,000.

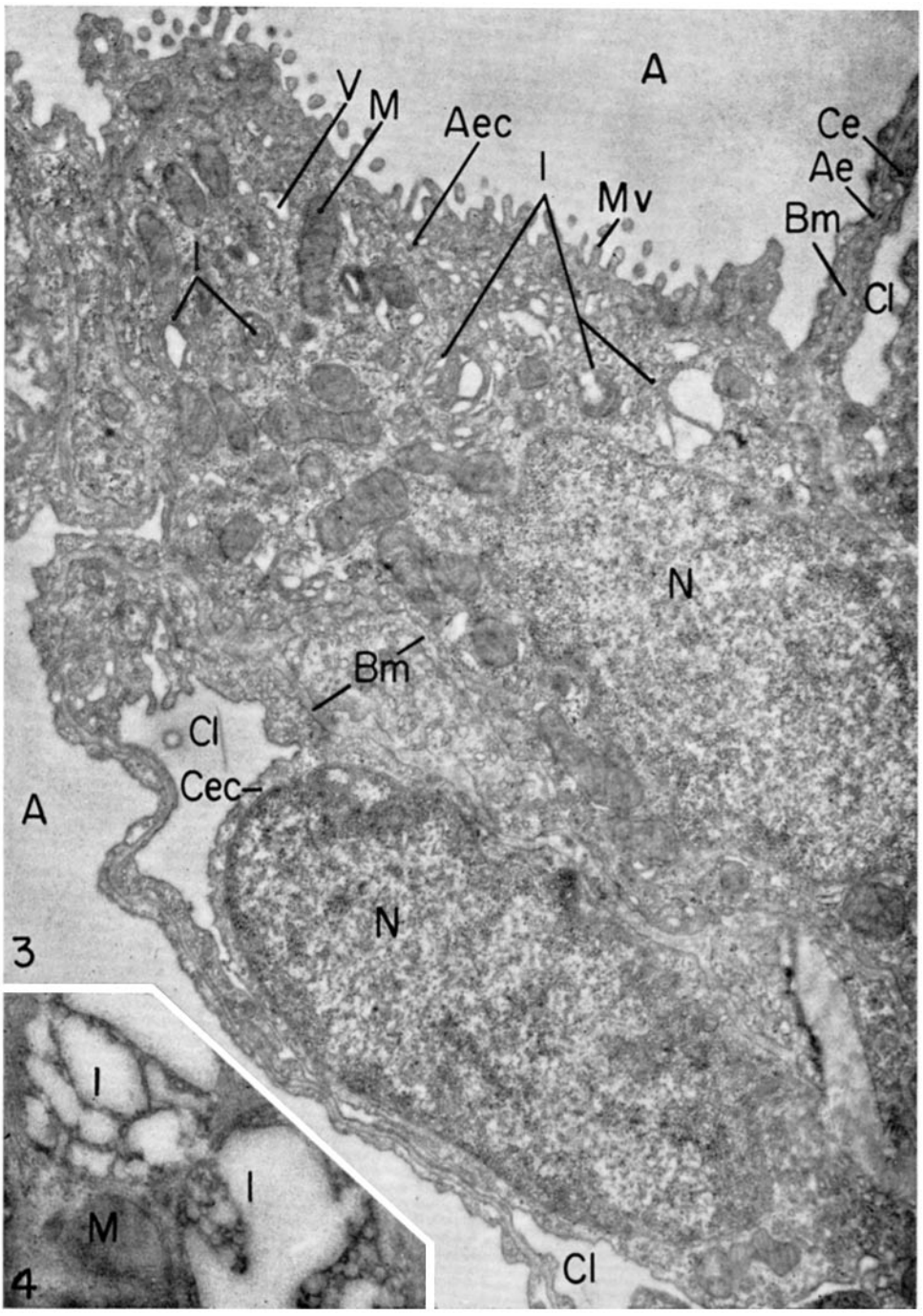


(Karrer: Ultrastructure of mouse lung)

PLATE 47

FIG. 3. Electron micrograph of an alveolar epithelial cell (*Aec*) and adjacent capillary endothelial cell (*Cec*). To be compared with Fig. 5. The epithelial cell seems continuous with the thin part of the alveolar epithelium (*Ae*). A few inclusions (*I*) contain dark lamellated material; they are located amidst numerous mitochondria (*M*). Numerous larger (*V*) and small vacuoles are scattered in the cytoplasm of the cell; small vacuoles are also evident inside the endothelial cell. × 14,500.

FIG. 4. Detail of inclusions of alveolar epithelial cells, from a section through a cell different from the one shown in Fig. 3. To be compared with Fig. 6. The dark material shows a net-like arrangement. The inclusions are in the proximity of a mitochondrion (*M*). × 34,000.

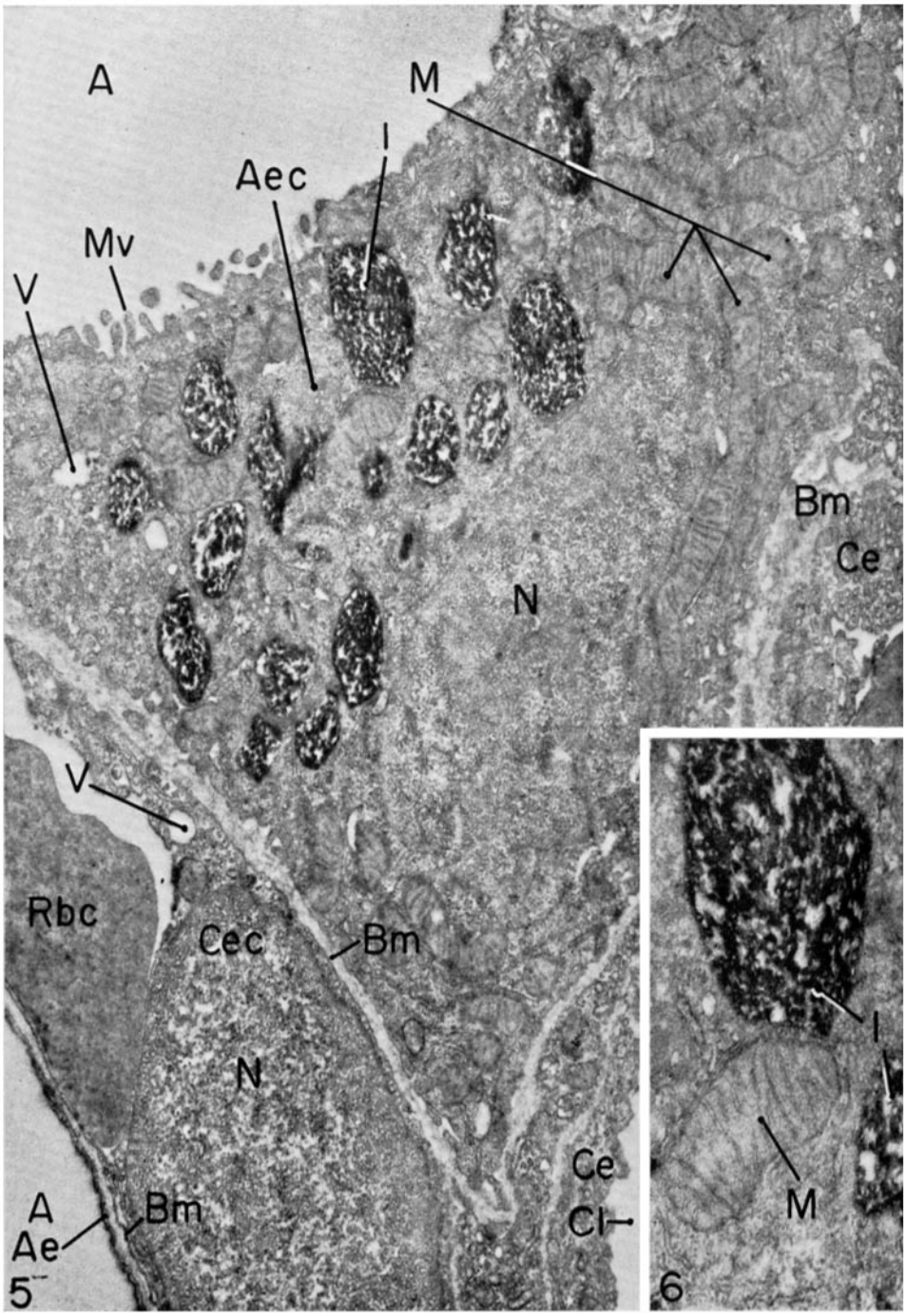


(Karrer: Ultrastructure of mouse lung)

PLATE 48

FIG. 5. Electron micrograph of an alveolar epithelial cell (*Aec*) and adjacent capillary endothelial cell (*Cec*). To be compared with Fig. 3. The tissue shown in this section was treated with ammonium sulfide. The inclusions (*I*) are considerably darker than in Fig. 3, and are less vacuolated. Mitochondria (*M*) are numerous, and apparently quite long. × 20,000.

FIG. 6. Detail of inclusions of alveolar epithelial cell, of tissue treated with ammonium sulfide. The section is different from the one shown in Fig. 5, but the same cell is shown. The dark material of the inclusion seems to consist of strands of small particles. × 44,000.



(Karrer: Ultrastructure of mouse lung)