Henry Ford Hospital Medical Journal

Volume 7 | Number 3 Article 4

9-1959

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OBSERVATIONS ON ULTRATHIN SECTIONS OF DOG LUNG BY METHODS OF ELECTRON MICROSCOPY

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The application of ultrathin sectioning techniques to biological tissue, and electron microscopy of the sections are well known, and new references are constantly being added to the subject. However, whenever a research group undertakes a problem involving the study by these methods of any type of tissue, it must be a first task to establish the 'normal' morphology for the techniques, instrumentation and methods of that group. It was our concern to establish for this laboratory, the cytological features of control dog lung, as a preliminary aspect of a larger project involving the eventual infiltration of the vascular system of lung with ultramicroscopic crystals of alpha iron†[1], both radioactive and non-radioactive. The findings reported here agree with those determined earlier by Low[2] and confirmed and expanded by others.[3,4]

While the work of others can be taken, and should be taken as criteria to be used for comparative and interpretative purposes, one must 'see' for oneself what the tissue looks like. This attitude must be taken first with regard to the normal material, and then with regard to the pathological or experimental matter. Further, the valuable information derivable from light microscopy of the same material must be utilized, especially for orientation of the observer between the well known and recognizable structures observed at the magnifications, resolution and contrasts (colors) inherent in the light microscopy, and the same quite different magnitudes and qualities of the same quantities in electron microscopy. For instance, there is a considerable difference in the size of field observable at X900 in a light microscope and at anywhere from X900 to X10,000 or much higher in an electron microscope.

There is often appreciable confusion if one attempts to step directly from a resolution of about 1500 angstrom units in light microscopy to 50 angstrom units or better in electron microscopy with all the wealth of unexplainable and unexplained detail such images are likely to demonstrate. Coupled with this is the 'new' information derivable from the fact that in electron microscopy there are none of the usual stains or colors so well-loved of light microscopists, but everywhere there are mere shades of black and white which are a result of more or less 'staining' by the molecules of the relatively high electron scattering fixative osmium tetroxide, (or other similar compound). The contrasts may be quite divergent from the appearances expected from light microscopy.

Any observation can be made only under some set of artificial conditions. Nothing can ever be seen *à la nature,* but only under the circumstances of the observation. Ingenious attempts are made to approach nature or to simulate it, but at best these attempts fail in one aspect or another. This is a true, (although often less strikingly so) of electron microscopy as of any other method of observation, and all interpretation must be made accordingly.

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†Unique crystals of iron, as well as of others of the transition elements, notably cobalt and copper, are provided for this work by Dr. Michael W. Freeman.

The tissue is fixed as rapidly as possible because autolysis of the ultramicroscopic structures will be understandably rapid. Fixation-staining must be carefully controlled so as to produce as few artifacts as possible, and several normal runs should be studied to make certain that results are reproducible and that the fixation is such as to delineate all the structures without obscuring some or producing others which do not actually exist. Embedding must also be controlled, first to preserve the structures, second to make the cutting of thin sections possible, and finally so as to be effected uniformly everywhere throughout the block without introduction of artifacts. The tissue and the block must be trimmed and observations made eventually far enough from the area trimmed that artifacts from the trimming process itself will not be recorded as characteristic of the tissue. In the cutting, the knives must be carefully chosen and many discarded before one that is suitable is found, and often one or two cuts are enough to ruin the blade, depending upon whether a knife edge of steel, glass, or diamond is used. During the cutting and preparation of the block the room temperature must be controlled and kept a little lower than is normally comfortable, about 68°F. Ideally room and microtome vibration should be non-existent. A thin section, (the order of 1/40 microns) is striven for, but often a section which is too thin can be as useless as a one which is too thick. Setting the microtome mechanically for some desirable thickness is usually not sufficiently satisfactory, since an unexpected temperature change, an undetected vibration, or other factors may feed a little less or a little more of the block to give an undesirable result.

Mounting the section on the electron microscope grid can lead to artifacts. Water is notably dirty, even when distilled. Sections tend to fold rather than to stay flat. The formvar or carbon supporting films may be dirty, too thick, separated or broken. And when the section is finally in the electron microscope, lack of resolution due to a myriad of reasons, too numerous to be discussed here, or effects of electrons upon the section to evaporate[5], break or contaminate it must be expected, and be reduced to a minimum.

MATERIALS AND METHODS

Lung tissue was obtained from six day old dogs, killed without anesthesia by a blow on the head. The lung tissue was removed immediately to cold, buffered 1 percent osmium tetroxide solution, and left in this fixative for 2 hours after being cut into cubic millimeter pieces. Karrer's technique of dehydration with acetone was used[6]. Embedding was effected in prepolymerized *n*-butyl methacrylate. Before final polymerization, the capsules which contained the tissue in the prepolymerized monomer were evacuated and flushed with dry nitrogen, according to Moore[7]. Polymerization was completed in the Canalco embedding chamber at 80°C.

Glass knives were used to section the material with a Porter-Blum ultramicrotome, and the sections were mounted upon Formvar-coated Athene Grids. A modified EMU-2B electron microscope was used for the microscopy.

OBSERVATIONS

The electron micrographs are cross sections of the alveolar capillary tissue of the dog lung. Macroscopically, the areas represent the alveoli (Alv), or air spaces, the septum or air-blood "barriers", and the capillaries (Cap). Plate 1 shows a typical

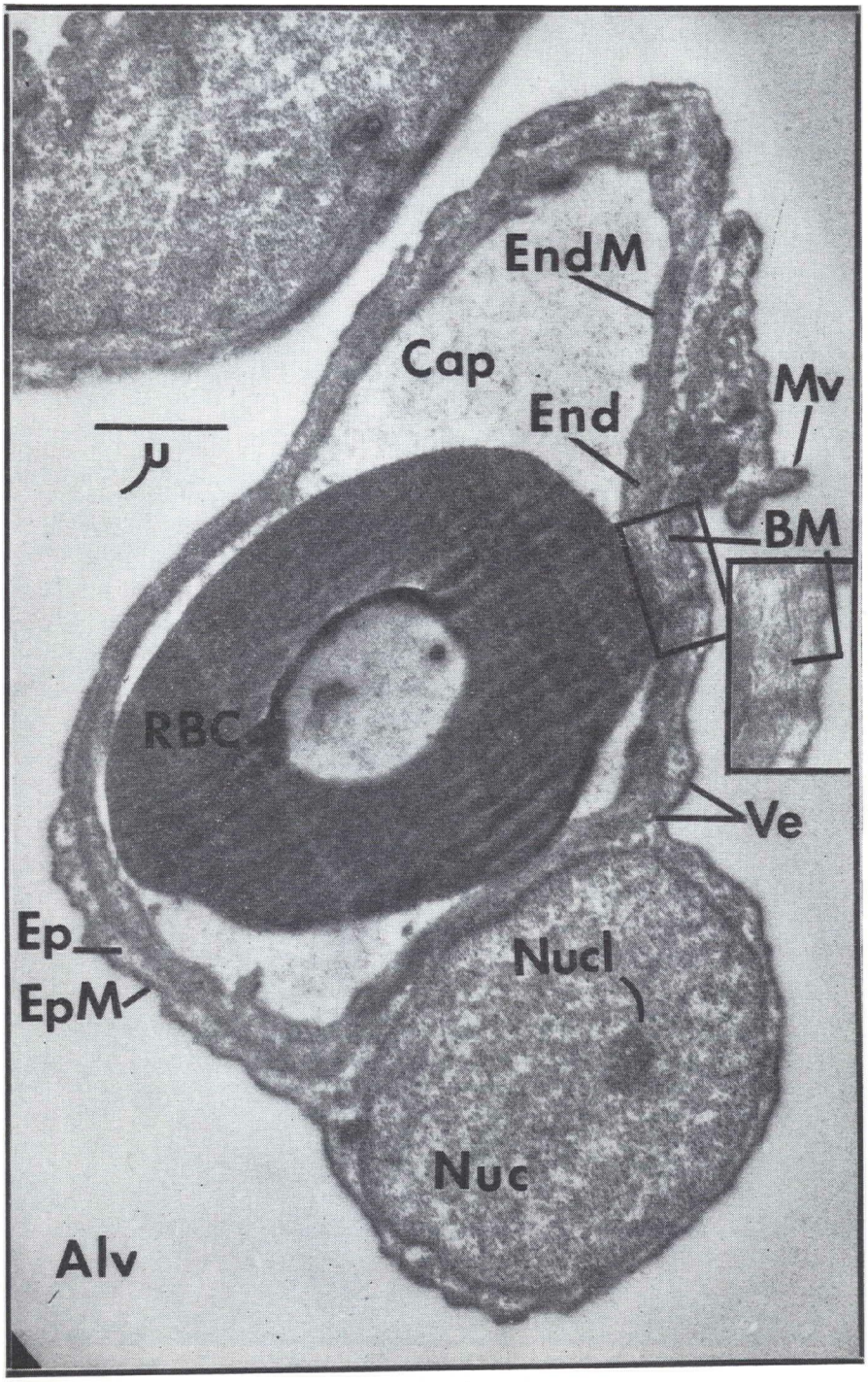

Plate 1
Puppy Lung, x19,000. Insert, x28,000.

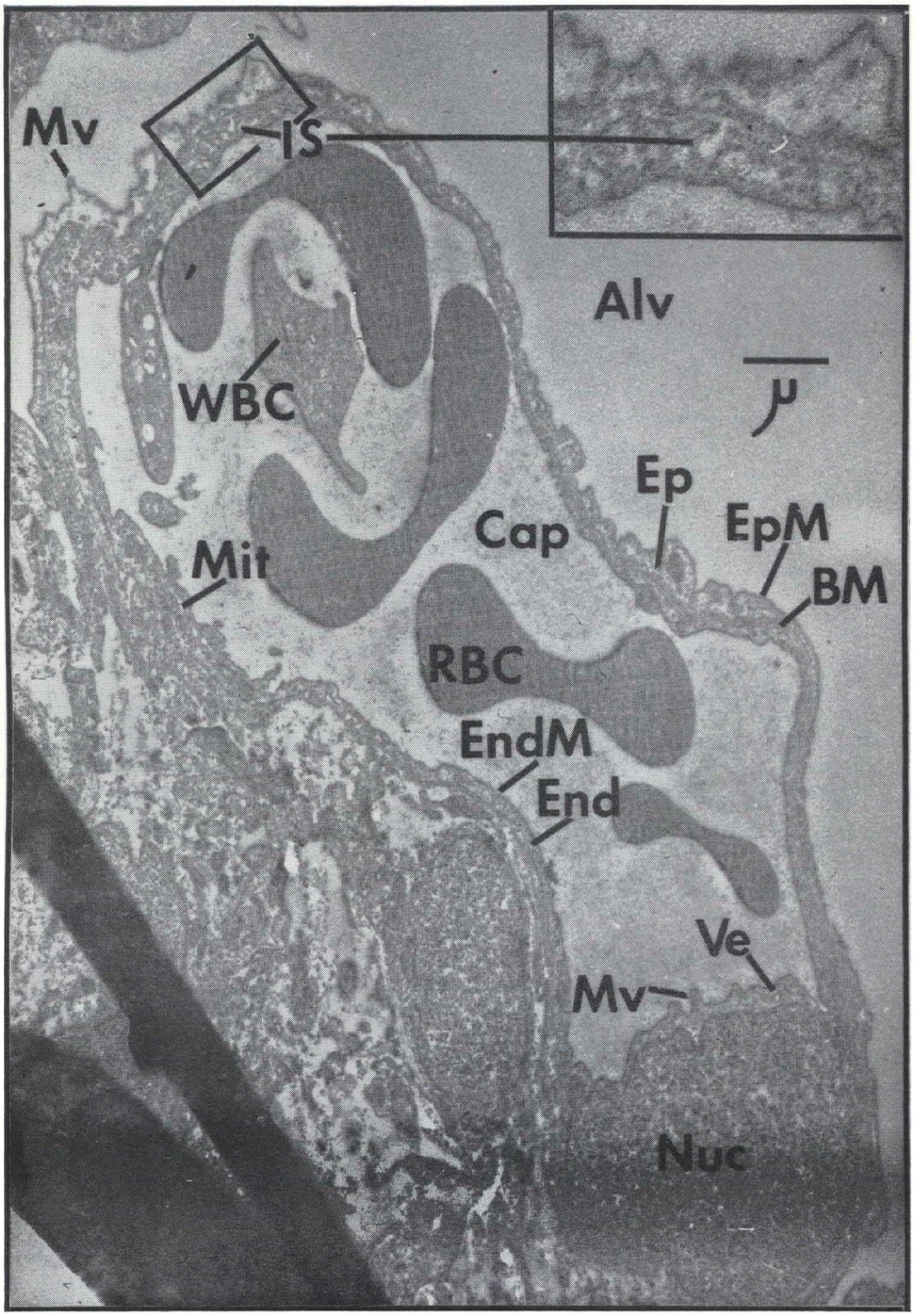

Plate 2

Puppy Lung, x12,000. Insert, x33,000.

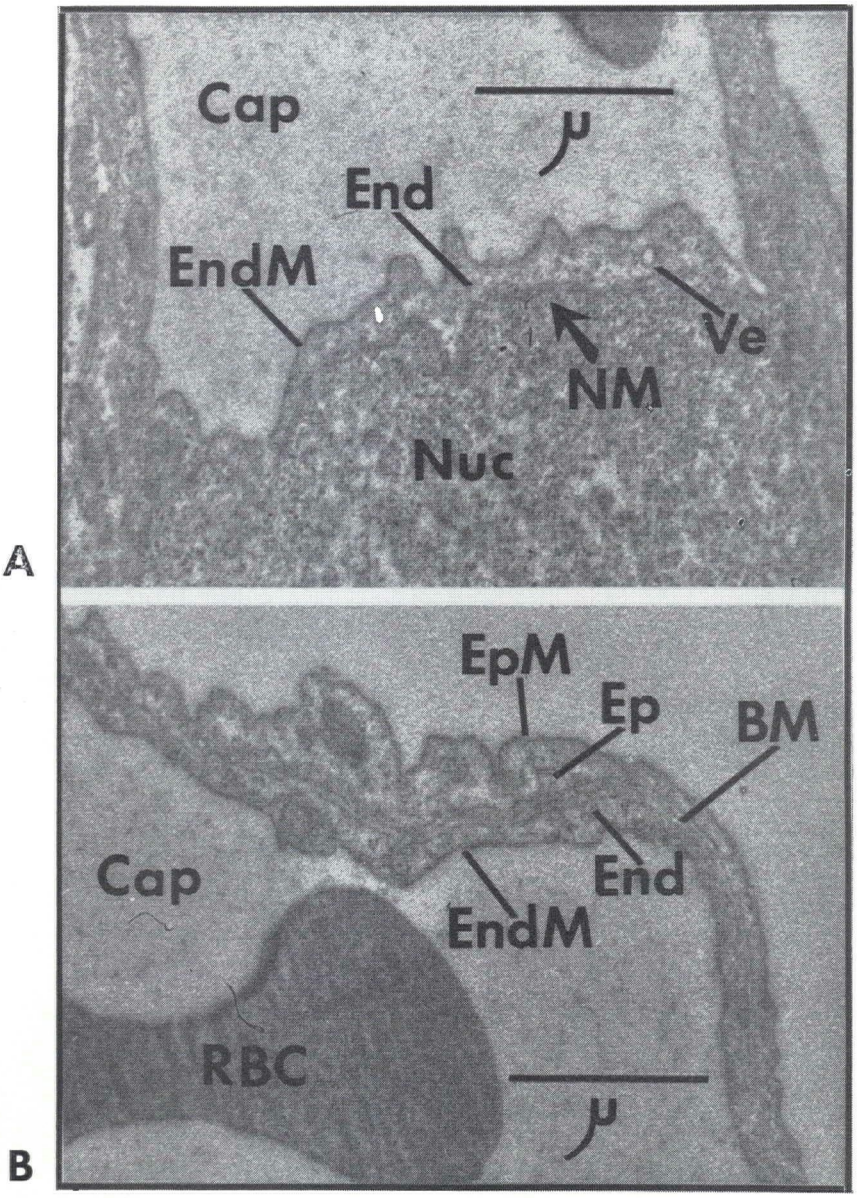

Plate 3

Enlarged Areas of Plate 2, x26,000.

example of a cross section through part of one alveolus and an adjacent capillary. The insert at higher magnifications demonstrates the finer structure of the basement membrane (BM), which in this area demonstrates a *triple* membrane nature. The narrow translucent layer between two thin dense membranes is referred to as the basement membrane. The inner one of these limiting dense areas is presumed to represent the surface membrane of the capillary endothelial cells: the outer one

to represent the inner surface of the alveolar epithelial cells. The third one which is observed in the present investigation is not immediately explainable and has not been reported earlier to our knowledge. Although often detected, plates 1, 3B, 7A, it is not observed continuously in our micrographs but becomes alternately distinct and indistinct along the septum, probably due to variations in the direction of the cut relative to it, or because of local differences in thickness of the section.

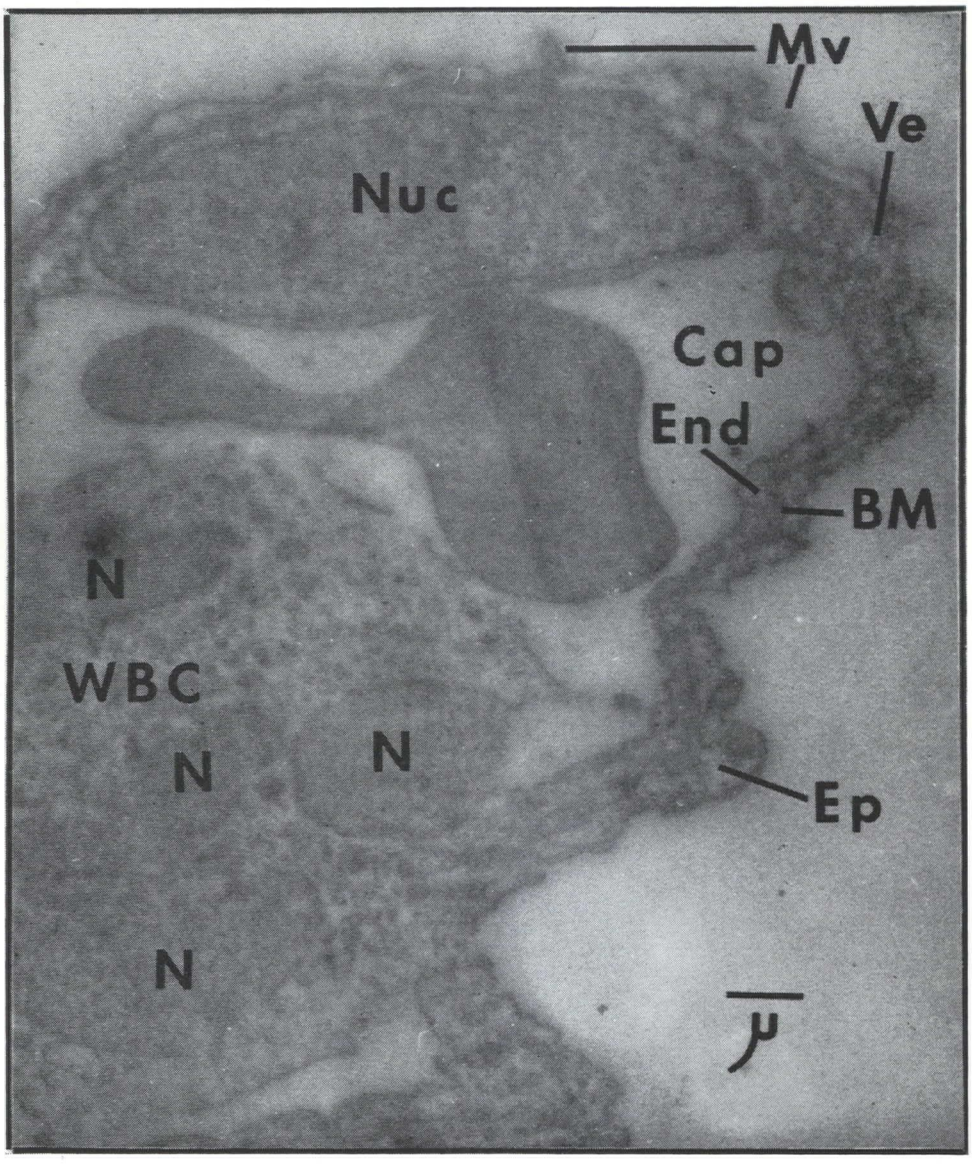

Plate 4
Puppy Lung, x10,000.

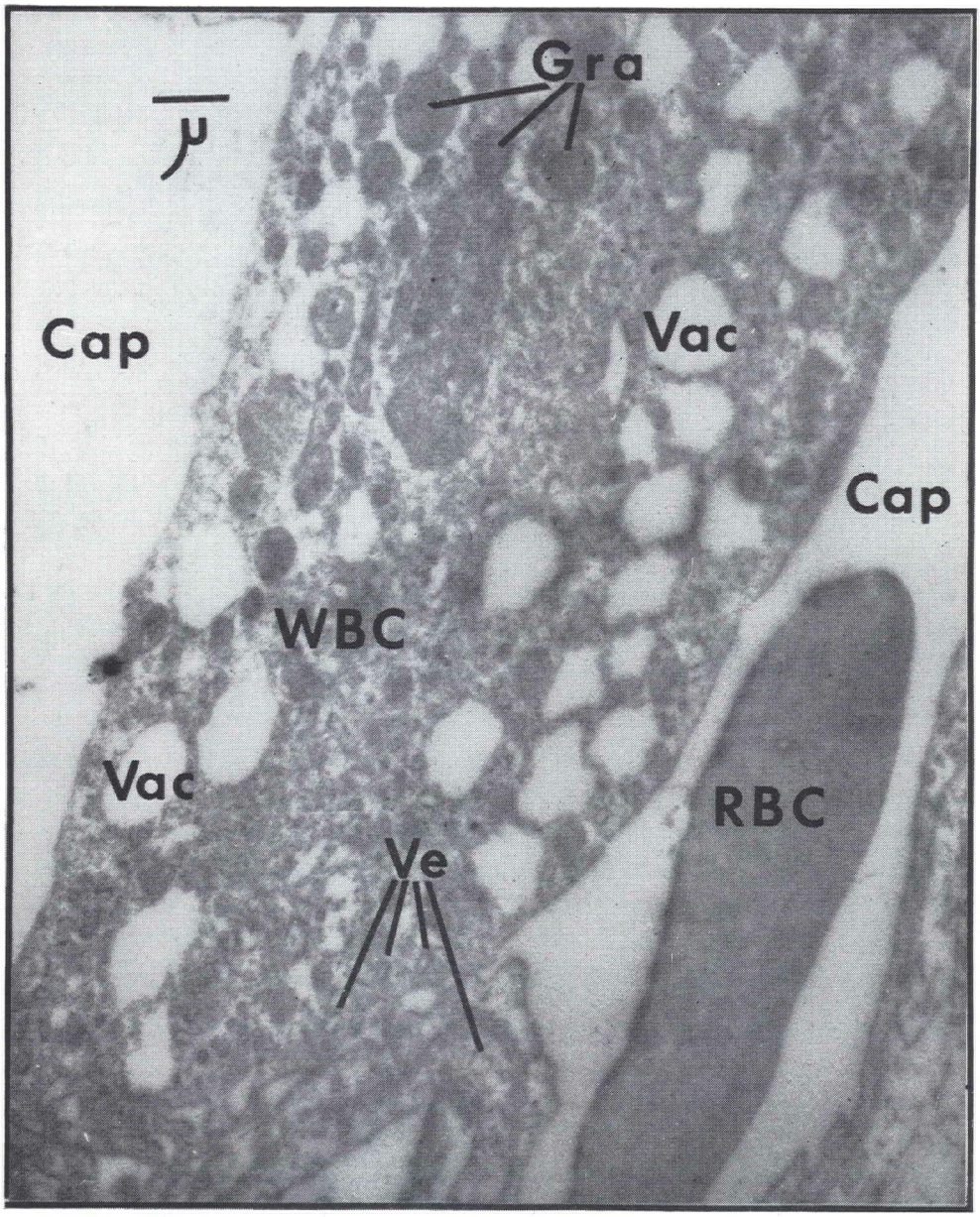

Plate 5

A Leucocyte in a Capillary of Puppy Lung, x10,000.

Immediately facing the alveolus and lining it, is a very thin continuous epithelium (Ep), which has a membrane (EpM) about 100 to 350 A.U* thick, represented in the micrographs as a dark line. The epithelium is composed of a single layer of

*1 angstrom unit (A.U.)=10-8 cms.

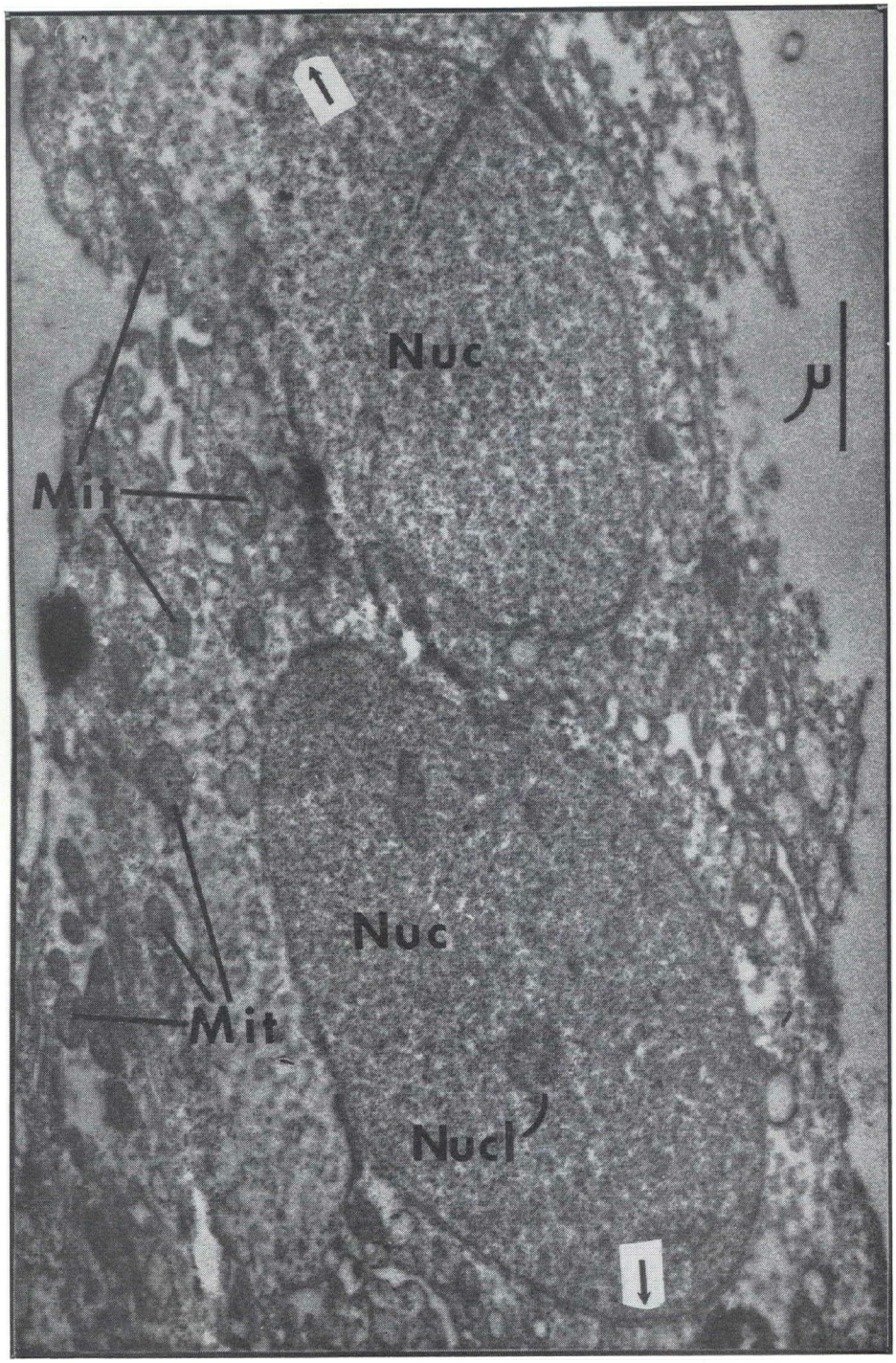

Plate 6
Puppy Lung, x20,500.

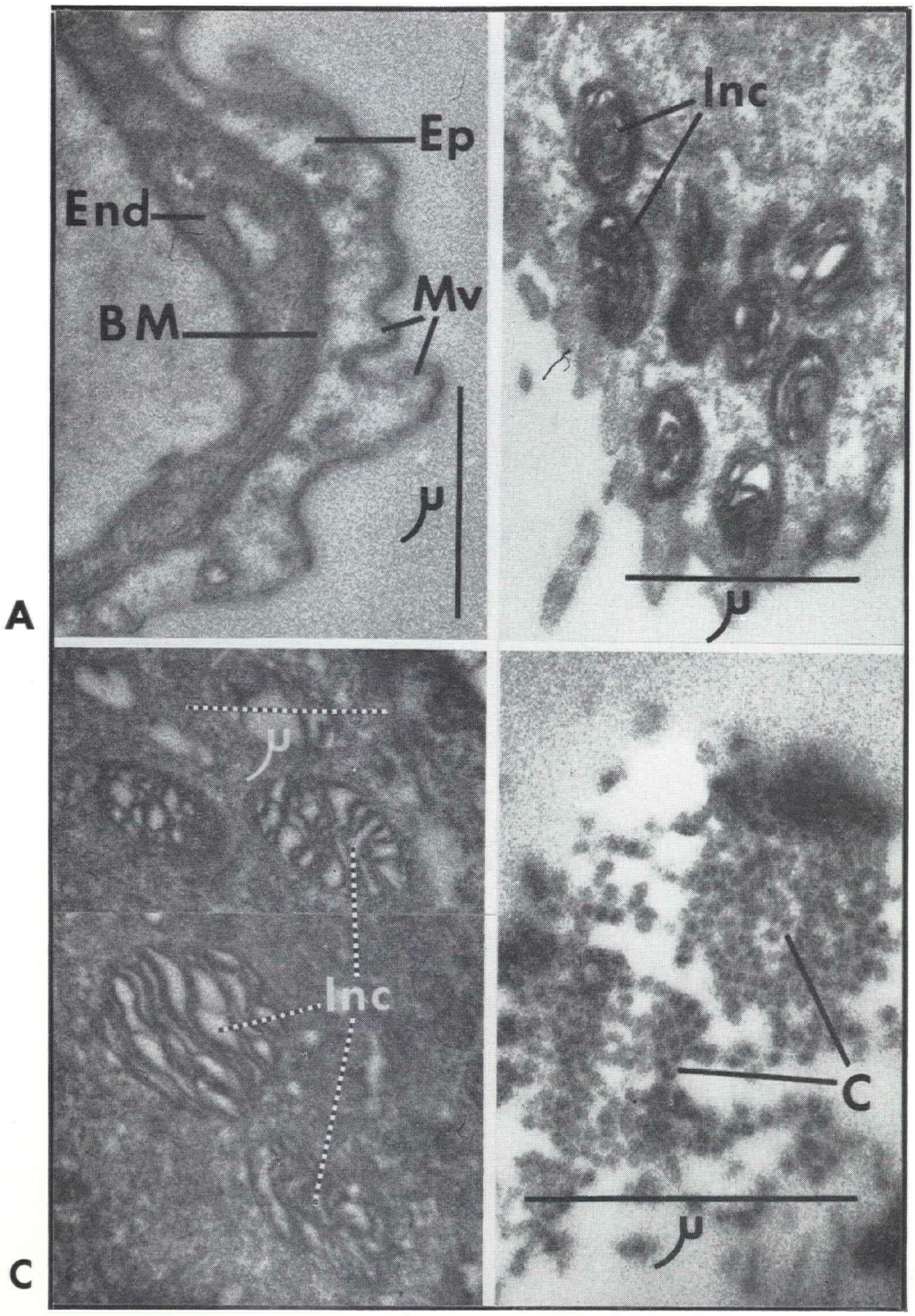

Plate 7

(A) Septum of Puppy Lung, x34,000 (B) Inclusions in Epithelial Cell of Puppy Lung, x34,000
(C) Inclusions in Epithelial Cell of Puppy Lung, x29,000.
(D) Cross Sections of Collagen Fibers in Puppy Lung, x49,000.

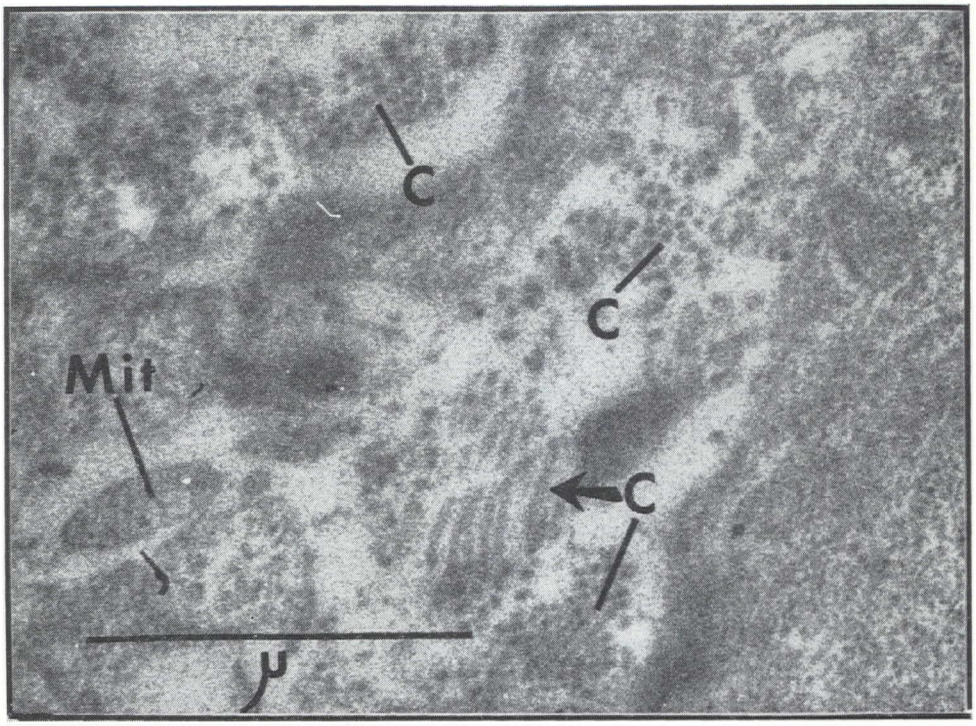

Plate 8

Section of Puppy Lung Showing Occurrences of Collagen, x51,000.

highly attenuated cells and is continuous without interruption in all the fields we have observed. In most cases the width of this epithelial lining is well below the limit of resolution of the light microscope, which is one reason why many investigators at one time doubted its existence[8,9].

We have found that it consists of cells which can attenuate to a thickness as small as about 0.05 μ, or in the region of an epithelial nucleus reach a thickness of as much as 3 μ or more, plate 1. In many places the plasma membrane of the various cell types is seen to project into the capillary or alveolar lumen in short columns or microvilli (Mv), plate 1 and others. Only infrequently was any evidence for the intercellular space (IS) detected in any of our micrographs of the septum, plate 2.

The epithelium lies directly upon the basement membrane. The basement membrane in turn is in direct contact with the capillary endothelium (End). This endothelium is likewise composed of a single layer of highly attenuated cells which surround the capillary lumen. Wherever it widens about an endothelial nucleus (Nuc), the endothelium can be as broad as 3.5 μ or more, plate 2. The endothelial nucleus in plate 3A is bounded by a double nuclear membrane (NM) (at the arrow). Plate 3B shows another enlarged segment of septum wherein the triple structure of the

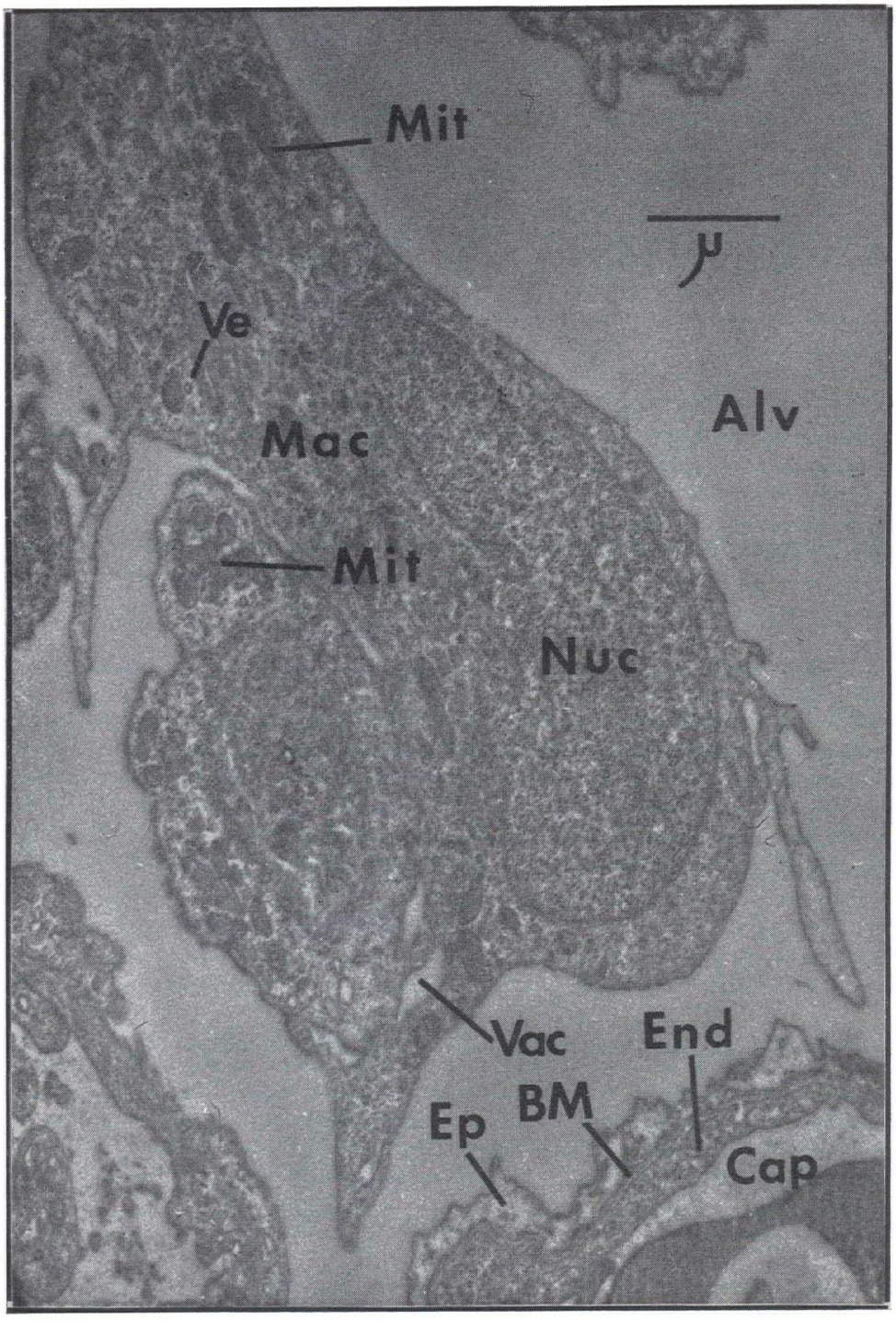

Plate 9

A Macrophage in the Alveolar Space of Puppy Lung, x19,000.

basement membrane is easily observed. The endothelium and epithelium membranes (EndM) (EpM) are also seen.

Within the capillaries, plates 1 and 2, both red and white blood cells (RBC) (WBC) are seen. The red blood cells are smooth in outline and homogeneous. Sometimes the white blood cells, which are more irregular in outline, are cut in such a manner as to demonstrate several segments of a multi-lobed nucleus (N), plate 4. Plate 4 also demonstrates several examples of microvilli (Mv) as well as an endothelial nucleus.

Small rounded profiles, which consist of a thin dark border, or membrane, surrounding a light interior, are observed scattered throughout the cytoplasm of the epithelium and endothelium. These are presumed to be some sort of vesicles (Ve), and are virtually a constant feature of most types of cells, such as the leucocyte in plate 5. Large dense granules (Gra) and large but less dense vacuoles (Vac), have been observed in some leucocytes, plate 5, as well as in certain cells which are probably phagocytic. Also found in the cytoplasm are denser bodies of the same general size, many of which are mitochondria (Mit), plate 6. The ground structure of the cytoplasm of all the cells observed except the red cells, is finely granular. Undoubtedly, some of these granules are the ribonucleic acid-rich granules described by Palade[10]. Again doubled walled nuclei are observed in plate 6 (arrows), and one of the nuclei shows evidence for what may be a nucleolus (Nucl).

Plate 7A shows another example at high magnification of a segment of septum with structure in the basement membrane. There are also several microvilli. Many inclusions (Inc) are also found in the sections. These are composed of closely parallel concentric lamellae, plates 7B and 7C. The inclusions shown in both plates are located in the epithelial cells. Sometimes, as in 7C the structure within these inclusion bodies resembles the cristae of mitochondria, except that the membranes are thicker and denser. This is particularly true in both 7B and 7C where the lamellae in the inclusion bodies are seen to be paired to consist of two dark lines (membranes) separated by a single light line. In different inclusions the lamellae are arranged with a variety of densities, but it may be that their separation is a preparation artifact. There are also evidences in the sections for the endoplasmic reticulum, a closed membrane system described by Porter[11] and Palade and Porter[12].

Although not usually as easily visible with osmium tetroxide fixation alone, as with a combination of staining, what are thought to be collagen fibers were often observed both longitudinally and in cross section, plates 7D and 8. In 7D they are observed in cross section to have a dark outline, with a less dense interior and to have an average diameter of about 350 A.U. The fibers may lie parallel in bundles, or separately without orientation. Plate 8 shows a group of six short fibers (at the arrow), lying parallel to each other in partial longitudinal section, as well as others in cross section.

Plate 9 shows what appears to be an alveolar macrophage. This cell contains a nucleus and several groups of mitochondria as well as large vacuoles and smaller vesicles. It also possesses what would appear to be well developed microvilli.

ABBREVIATIONS

Alv, Alveolus
BM, Basement Membrane
Cap, Capillary
End, Endothelium of Capillary
EndM, Endothelium Membrane
Ep, Epithelium of Alveolus
EpM, Epithelium Membrane
Gra, Granules
Inc, Inclusion
IS, Intercellular Space
Mac, Macrophage
Mit, Mitochondria
Mv, Microvillus
N, Nuclei Segment of Leucocyte
NM, Nuclear Membrane
Nuc, Nucleus
Nucl, Nucleolus
RBC, Red Blood Cell
Ve, Vesicle
Vac, Vacuole
WBC, White Blood Cell
C, Collagen

SUMMARY

Preliminary observations on the fine structure of control puppy lung studied with the electron microscope agree in general with the findings of other investigators who have used this technique with this tissue. A highly attenuated, continuous cellular epithelial and endothelial lining is found to separate the blood capillaries from the air spaces. The basement membrane, which appears to contain a central membrane, previously unreported, is described. A few blood cells are also shown. Cells believed to belong to the macrophage system are discussed. Large, dense, laminated inclusions are found scattered throughout the cytoplasm, and there are occurrences of collagen.

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Acknowledgment is made to Mr. J. D. Montgomery for his advise and criticism in the preparation of this manuscript.