ULTRASTRUCTURE OF THE PULMONARY ALVEOLAR EPITHELIAL CELLS OF THE STRAIN A (HESTON) MOUSE

by

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INTRODUCTION

I. Statement of the Problem

There have been several previous electron microscopic studies of alveolar lining cells in mammalian lung. In the rat (6, 77), two types of alveolar lining cells were described. One type, designated the type A cell, is squamous with a very thin layer of cytoplasm spread out over the alveolar wall. The other type, called the type B cell, is cuboidal, located in niches in the alveolar wall, and is characterized by membranous bodies in the cytoplasm. Similar cells have also been described in mouse lung (30, 76).

The mouse lung adenoma, which occurs spontaneously in several strains of mice, especially the high incidence strain A (Heston) mouse, has been extensively studied (68, 69). Several investigators (21, 46) who have studied the histology of these mouse lung tumors considered that they arise from an alveolar epithelial cell. Two recent electron microscopic studies of mouse lung adenoma (33, 70) indicated that a strong morphological similarity exists between the adenoma cells and the type B alveolar epithelial cell. However, these two studies did not include illustrations of normal mouse lung for purposes of comparison, and this information is a necessary prerequisite to a

detailed study of the biology of the adenoma. Such a study of the adenoma is now in progress by the author.

The purpose of the present study is to describe the ultrastructural characteristics of the alveolar lining cells of fetal and adult lung of the strain A (Heston) mouse as a control for studies of pulmonary adenomas occurring in the same strain.

II. A Survey of the Literature relating to Lung Development and Morphology

Although the air bladder of certain fish is considered to be the organ from which the lung of land-dwelling animals developed, Goodrich (20) has cautioned that the origin of the air bladder is not definitely known. Indeed, Jones (28) considered the possibility that the swim bladder may have been a lung-like organ originally. If this were true, then the swim bladder of fish is a modification of an earlier organ developed primarily to hold swallowed air. In any case, the air bladder, as does the lung, develops as a diverticulum in direct open communication with the gut. The pneumatic duct, which connects the diverticulum to the gut, becomes obliterated in some fish (physoclistic), but remains open in others (physostomous). The opening from gut to air bladder primoridum is dorsal, unlike the lung, but may shift laterally during subsequent development. The bladder is completely absent in some modern fish which are specialized for bottom living or for rapid swimming. The

bladder, when present, varies in position, size, and shape from species to species. The physostomous fish can swallow air into the bladder where it is retained by the closing of the pneumatic duct whose walls have a sphincter-like musculature. In the physostomous and physoclistic fish, a portion of the bladder epithelium secretes a mixture of gases directly into the bladder. The gas mixture varies from fish to fish but usually has a high proportion of oxygen. The bladders have a part devoted to gas secretion and another part concerned with gas resorption. The secretory epithelium is in close approximation to a very extensive capillary bed. The entire bladder is surrounded by layers of a gas impermeable tissue consisting mainly of smooth muscle.

It has been suggested (22) that under nervous stimulation the epithelial cells secrete an acid substance which diffuses into the underlying capillaries causing, thereby, a liberation of carbon dioxide from blood bicarbonates. The carbon dioxide, in turn, brings about an above-normal dissociation of oxyhemoglobin in the blood. The released oxygen is then assumed to diffuse through the epithelium into the gas bladder. An alternative method of gas secretion has been suggested by Leiner (36) who found that carbonic anhydrase, an enzyme that catalyses the removal of carbon dioxide from carbonic acid, is present in large quantities in gas gland tissue. This enzyme could act to release carbon dioxide

from the blood. Fange (17) experimentally tested this suggestion by injecting an inhibitor of carbonic anhydrase into fish which had the gas removed from their bladder. He found that there was an abnormally low oxygen content in experimental fish bladders one to three days later as compared to control fish whose bladders were similarly emptied. He concluded from this finding that carbonic anhydrase may have an active role in gas secretion.

The resorbing portion of swim-bladder epithelium was thought by Tracy (72) to be derived from the pneumatic duct in the physoclistous fish. This specialized portion of the bladder is in the form of a shallow pouch, or "oval" as it is usually termed, which can be opened or closed by sphincter and dilator muscles. Tracy followed the change in shape, location, and condition of the pneumatic duct in various fish and deduced from his observations that the duct, cut off as it was from the gut, gradually became modified to function as the gas resorbing site in the higher forms of physoclistous fish. It is to be noted that underlying the resorbing epithelium is a large capillary network.

A recent electron microscopic study of the swim-bladder of the eel by Dorn (15) tends to confirm the concept of Tracy. Dorn noted that the pneumatic duct of the eel resembled the lung of such primitive forms as Protopterus, Amblystoma, and Xenopus. The duct in the eel has a thin, squamous epithelium overlying a connective tissue space

containing abundant capillaries. In the eel, both proximal and distal ends of the duct are narrow and the duct has a smooth musculature which suggests that it can be closed off from both the esophagus and the bladder. It would appear, then, that the pneumatic duct, rather than the gas bladder, may be closer to the line of lung development in the lower animal forms.

Goodrich (20) thought that in order to account for the blood supply to the duct and bladder it was most reasonable to consider that they were derived from a posterior pair of gill pouches. From similar reasoning, he suggested that the lung of land animals arose from a pair of "pouches" just ventral to the vestigial sixth pair of gill pouches. These "pouches"join very early in development to form a medial ventral depression representing the rudiment of the larynx and trachea. The prototype of the lung in land animals is thought (20) to be similar to the air bladder which Polypterus, a very primitive but still surviving fish, uses as a lung. This lung prototype is a simple bilobed sac protected by a glottis, opening onto the pharynx. The sac wall is very thin, highly vascular and is covered by ciliated and squamous epithelium, the latter over the blood capillaries.

In amphibia, except for the lungless salamanders, the lung is a relatively straight-forward modification of the

prototype described by Goodrich (20) above. In addition to the development of a definite larynx, trachea, and bronchi, the important change is a folding of the respiratory portion of the lung sac wall into few to numerous folds, depending upon species. These folds create chambers of varying size and thereby increase the respiratory surface.

In reptiles, the main changes from the amphibian lung are the formation of secondary bronchi and further folding of the lung into smaller and smaller cells, thereby increasing the respiratory surface even further.

The lung of birds represents a considerable departure from the previous pattern. In birds, the lung is divided essentially into two functional parts. One part, the respiratory portion, is concerned with gas exchange and the other part with air storage. The respiratory portion lies between the trachea and numerous air storage sacs. During breathing, air passes back and forth, at approximately uniform pressure, through small epithelial lined air tubules which are in intimate contact with blood capillaries. Gas exchange occurs during inspiration and expiration making the bird lung a very efficient respiratory organ.

Mammalian lung development follows the pattern seen in amphibia and reptiles, although it is not possible to trace the changes directly since the earlier forms are no longer extant. However, the direction of development is towards increased subdivision of the bronchial tree and smaller and

more numerous respiratory sacs or alveoli.

The development of the pig lung, which has been thoroughly described and well illustrated, provides a convenient model of mammalian lung development. Flint (18), in 1906, starting his study with a pig embryo 3.5 mm. long, found that the gut, just caudal to the last gill pouch, has an irregular, rhomboidal shape with dorsal, ventral, and lateral angles. The ventral angle deepens to form the pulmonary groove which terminates just above the future hepatic duct. This termination is the site of an asymmetrical projection: the unpaired anlage of the lungs. As the embryo grows, the pulmonary anlage widens more on the right than on the left. The right side evaginates to form the anlage of the right bronchi which grows laterally and caudally. The left side evaginates a little later to form the left bronchial anlage which grows at right angles to the pulmonary groove. By the time the embryo is 5 mm. long, derivatives of the pulmonary groove can be recognized as anlagen of the trachea and right and left bronchi. The primordial right stem bronchus then puts off a lateral bud: the anlage of a first order bronchus. This is followed by a lateral budding off from the left stem bronchus. Each first order bronchus eventually gives rise to second order bronchi and these in turn to third order bronchi, and so forth; in each case by a process of lateral budding. By the time the pig embryo is 5 cm. long, Flint was able to

describe a total of 32 first order bronchial anlagen, 105 second order bronchial anlagen, and 99 third order bronchial anlagen.

According to Clements (13), also working with the pig embryo, at the 5 cm. stage ill-defined septa divide the primitive lung lobe into primordial lobules. At the 10 cm. stage, the number of primordial lobules has increased about five-fold. The bronchial anlagen which branch through these lobules appear as epithelial lined tubules with dilated tips. At the 15 cm. stage, the smallest tubules are lined with cuboidal epithelium and cilia are present in the large bronchi. At 20 cm., the previously continuous epithelial lining of the tubules begins to appear discontinuous. Cuboidal and columnar cells are found in the tips, or end buds, of the epithelial tubules even after the epithelium has "disappeared" proximally. Mitoses are still frequent in the end buds. At 25 cm., each lobule has four or five orders of tubules. Epithelium-free spaces, suggestive of alveolar ducts are present. Most of the end buds are completely open and lined by only a few cuboidal cells. Capillaries are now more prominent around the duct-like spaces. At 29 cm., which is the length of the embryo at term, most of the end buds have disappeared. In any given section, two or more potential alveolar ducts arise from each terminal bronchiole. Large cells, with vacuolated cytoplasm, are prominent in the angles of the septal wall.

Capillaries are very evident and appear to be completely exposed to the primordial air space.

At the cellular level (1), the primitive lung sacs are epithelial tubes lined by a double layer of cells: the inner by columnar cells and the outer by small polygonal cells. The tubes are surrounded by a simple membrana propria of connective tissue derivation. As these tubes (i.e., the bronchi) grow, they acquire a coat of mesodermal cells which differentiate to become the cartilaginous cells of the trachea and bronchi. The epithelium is proximally thrown into folds which eventually develop into small glands containing mucous and serous secreting cells. Ciliated cells develop from the innermost of the double row of cells. Distally, the bronchi are lined by columnar epithelium becoming cuboidal in the smaller bronchioles.

All of these structures of entodermal origin grow within a mass of mediastinal mesenchymal cells which are simultaneously multiplying. As the two tissue systems enlarge within the coelom, they are covered with a lining layer of connective tissue and mesothelium which form the visceral pleura. The mesenchyme gives origin to cartilage, smooth muscle, connective tissue cells, and collagen and elastic fibers. Blood vessels, lymphatic vessels, and nerves grow into the mesenchyme.

In relation to the mesodermal portion of the lung, Ham and Baldwin (23) noted that the mesenchyme of the

developing lung is of two types. One is cellular and the other relatively non-cellular. These authors suggested that the invasive unit of the developing lung is not a simple branch of the entodermal tree but rather a complex consisting of a branch capped with cellular mesenchyme. It is this complex which they believed invades the relatively non-cellular mesenchyme. As a consequence of this dual nature of the growing lung unit, it was postulated that the capillaries eventually go on to line the alveoli, lying naked against air with no epithelial cover.

As an extreme view of the mesodermal nature of lung, Waddell (74) considered that the entire lung is derived from totipotent tissue and that bronchial and alveolar epithelium may have been derived by differentiation from mesoderm.

Other authors, for example Rose (57), admitted the entodermal origin of the bronchial epithelium, but thought that a continuous epithelium finally ended at the alveolar ducts where capillaries "broke through" to come directly in contact with air.

In 1952, Low (38), using the electron microscope, described a nucleated pulmonary epithelium lining the alveolar wall in the rat lung. This observation was subsequently extended to other laboratory mammals and man (39). Shortly thereafter, several investigators (3, 29, 32, 55, 61) confirmed and added to the description of lung ultrastructure. As the high-magnification re-investigation

of the lung proceeded, it became evident that either two types of cells were present on the alveolar wall or that there were two forms of a single cell type. All authors agreed that the capillaries were covered by squamous epithelial cells having an unusually attenuated cytoplasm spread out from a small cell body. This cell was almost identical in appearance to the capillary endothelial cell of the lung. However, as noted above, a second cell was seen in an epithelial position on the alveolar wall. This cell was cuboidal and had small cytoplasmic projections, or microvilli, on the luminal surface. The distinguishing feature of the cell was the numerous, membrane-filled, osmiophilic bodies within the cytoplasm. Schlipkoter (60), in 1954, was the first to describe the lamellar, membranous inclusions within the cytoplasm of the alveolar cells. These inclusions were the size of mitochondria, or larger, and consisted of vacuoles filled with osmiophilic lamelli. Later, other investigators (19, 30, 32, 55, 63), on noting the same structures, proceeded to give them somewhat varying descriptions and names. Schulz has pursued the nature of this alveolar cell lamellar inclusion further than any other microscopist and has described his findings in a series of papers (62 - 66) and finally in an atlas of electron micrographs of the lung (67). A description of some of the findings of Schulz will be given later, however it is convenient to note here that Schulz concluded from his studies

that the lamellar inclusions were remnants of altered or transformed mitochondria. This conclusion was based on the finding of numerous intermediate forms between normal appearing mitochondria and the lamellar inclusion bodies.

Karrer (29), in 1956, was the first to describe two different types of cells in the alveoli, both epithelial in character. However, Karrer thought that both cells had attenuated cytoplasm which spread over the alveolar surface. Policard and co-workers (55) had also recognized the two alveolar cell types and had called them small and large alveolar cells. Yasudo (77) had also described the two alveolar cells, calling them type A and type B, a convention that is followed in this paper. The type A cell of Yasudo is the squamous cell and the type B cell is the cuboidal cell with the lamellar cytoplasmic bodies. Campiche (6) gave a very good description of the two alveolar cells calling them type I and type II. From these several observations, it may be taken as established that there are two different alveolar cell types in the adult mammalian lung.

A study of fetal mouse lung by Woodside and Dalton (76) revealed that cells containing "inclusion bodies" were present in 18 and 19 day fetal lungs. While these authors did not consider that there were two different alveolar cells in the lung, their pictures of the "inclusion bodies" within the alveolar cells are identical in appearance to the lamellar

bodies in adult lung. It would appear likely, therefore, that two alveolar cell types are distinguishable in the lungs of the late mouse fetus.

The electron microscopic study of lung tissue from non-mammalian species is very limited. However, it is of significance that a cell can be found in amphibian lung (49, 67), bird lung (4, 73), and reptile lung (50) which appears similar to the type B alveolar cell of mammals. Moreover, Dorn (15) described two types of cells within the swim-bladder of the eel: a dark and a light cell. The dark cell contains a few bodies which resemble the lamellar bodies in the type B cell of mammalian lung; a similarity which the author recognized. In amphibia, one (67) or two cells (49) have been described lining the lung sacs. In both studies, lamellar bodies have been seen in the alveolar cells. In the bird, the cell which appears like the mammalian type B cell is limited to the smallest bronchi and atria where it forms a continuous cuboidal epithelium (4, 73). The small air tubules of the bird lung are lined by an extraordinarily thin layer of squamous cell cytoplasm (type A-like cell). In the reptile (50), both type A- and type B-like cells line the alveoli.

III. A Survey of the Literature relating to Alveolar Cell Function

The function of the type A alveolar cell is recognized by all investigators to be that of providing a thin, con-

tinuous cytoplasmic covering for the alveoli. In addition, several investigators (29, 55, 67), who have studied this cell at high magnification, have noted that there are numerous small vesicles scattered throughout the thin layer of cytoplasm. Moreover, many examples of tiny, cell membrane invaginations were seen on both the alveolar and capillary sides of the cell. From these observations, the above investigators suggested that the cell is probably engaged in fluid movement by the process of pinocytosis (cell drinking).

Very few investigators who have studied the morphology of the lung have attempted to suggest a function for the type B alveolar cell. Most light microscopists thought that all alveolar cells containing vacuoles or odd shaped cytoplasmic inclusions were macrophages. Low and Sampaio (40), in an electron microscopic study, attempted to determine if the alveolar epithelial cells were phagocytic. They introduced colloidal thorium dioxide (Thorotrast) into the lungs via the trachea. Thorium, which is opaque in the electron beam, could be well visualized within unequivocal alveolar macrophages but was not seen within either the type A or the type B alveolar epithelial cells. Earlier, Policard and co-workers (54) had noted that in experimental silicosis the alveolar cells did not show signs of active phagocytosis. Karrer (31) has also studied phagocytosis in the lung with the electron microscope. He instilled india ink in the

nasal cavity of mice daily for 4 to 18 days. Ink was found to be phagocytized, almost exclusively, by the alveolar macrophages. A small amount of the carbon in the india ink was observed in the "small" alveolar epithelial cells (i.e., the type A cells), and by connective tissue cells in the interalveolar septa following prolonged ink instillation. From the results of these several investigations, it would appear that the type B cell is not a phagocytic cell. It may be added that there was no evidence obtained from these, or any other studies, indicating that the type B cell is the precursor of the alveolar macrophage.

A secretory function for the type B alveolar cell was first suggested by Macklin (42). In a light microscopic study, Macklin noted the vacuolated cytoplasm of the type B cell, which he called a "granular pneumonocyte", and postulated that the cell secreted an acid mucopolysaccharide. It was thought that this substance formed a fluid film in the alveoli which aided in transporting dust particles out of the alveoli.

Hayek and co-workers (25), in an electron microscopic study of lung, described a layer material in a few places along the alveolar surface. These workers believed that this layer material was the mucopolysaccharide film suggested by Macklin's earlier observations. They accounted for the discontinuous nature of the film by pointing out that the fixative used in preserving tissues for electron

microscopic examination did not preserve polysaccharides. Chase (9) has also claimed to find some non-cellular material lining alveoli. However, Chase's study of the lung was made on tissue fixed by a freeze-dry technique, and the alveolar preservation which resulted from this method was too poor to permit certain identification of the tissue elements. Tyler and Pangborn (73) described a laminated, non-cellular membrane lining the tertiary bronchi and atria of chicken lung. This membrane consists of many layers of myelin-like material and may be up to 600 millimicrons thick in some places. The membrane is discontinuous and does not line the entire lumen. These authors suggested that this membrane material took its origin from osmiophilic inclusions found in cells lining the tertiary bronchi and atria.

The most recent suggestion for a secretory function for the type B alveolar cell was made by Clements (12), who postulated that a surface tension-reducing substance was produced by the cell. This suggestion of Clements is based upon physiological considerations originally presented by von Neergard (47), in 1929. Von Neergard, working with whole lung preparations, found that a greater pressure was needed to expand a degassed lung with air than with saline. The pressure needed to expand a lung with saline represented the elastic resistance of the lung. The difference between this pressure and that needed to inflate the lung with air was thought to be due to surface tension resistance produced

at the air-water alveolar surface interface. However, this difference in pressure was found to be less than expected if it were assumed that the alveolar surface was moistened by body fluids. Therefore, von Neergard concluded that the alveoli were moistened with some fluid having a lower surface tension than body fluids. Mead, Whittenberg, and Radford (43), in 1957, repeated the work of von Neergard and came to similar conclusions.

Pattle (51, 52) reported that pulmonary edema foam was very resistant to anti-foam agents and also that bubbles obtained by squeezing lungs are stable for long periods in air-saturated water. From these findings, he deduced that a surface tension-reducing material must exist in the alveoli capable of reducing the surface tension to almost zero.

Klaus and co-workers (34, 35) extracted lung and obtained a substance showing a surface tension-reducing property (i.e., a surfactant). It was thought by these workers that this surfactant had the correct properties to reduce the alveolar surface tension. Clements (12), in discussing this finding, pointed out that a surface active agent lining the alveolar walls would tend to "equalize the tension in the air spaces as they expand and contract" and would bring "about an even distribution of pressure between large and small alveoli"; moreover, it would act to decrease the overall pressure, thereby reducing "the muscular effort required for respiration." In addition, Clements postulated

that such a substance will function to assist "the osmotic forces acting across the surface of the lungs and so keeping the film of moisture on the surface from drawing fluid into the air spaces."

In 1960, I brought to the attention of Clements the morphological features of the type B alveolar cell. In the 1962 paper noted above (12), Clements offered the hypothesis that a surfactant may be synthesized within the mitochondria of the type B alveolar cells and passed into the tissue fluid coating the surface of the alveoli at the time when the transformed mitochondria were discharged from the cell. This idea was enlarged upon in a later paper by co-workers of Clements (35). However, an earlier study by this group (34) revealed that the surfactant material extracted from lung was a lipoprotein. If this were true, then it should be detectable with the electron microscope in osmium tetroxide fixed lung specimens. However, no electron microscopic study has shown an unequivocal non-cellular alveolar surface layer in mammalian lung.

The only other theory for a function of the type B cell was postulated by Meesen (44). According to Meesen, "the alveolar epithelium, under the influence of the partial pressures of oxygen and carbon dioxide from the air and blood, regulates the blood flow and the exchange of the gases of the alveoli." Based on this theory, Schulz, a co-worker of Meesen, conducted a series of experiments to determine

the effect of different conditions on the alveolar cells. In an early study (63), he exposed rats to a gas mixture of 3.5% carbon dioxide in air. He found that many, but not all, of the mitochondria of the alveolar cells in the carbon dioxide treated animals suffered a membrane damage of an extent and form not seen in normal lung. The damage was primarily to the inner membranes of the mitochondria and to the matrix material. The internal membranes appeared to be disconnected from the outer envelope membrane, and the mitochondrial matrix appeared empty. The altered mitochondria were left with dark, thick lamelli extending from side to side. From this appearance, Schulz described the mitochondrial alteration as a "lamellar transformation". In another experiment (63), rats were exposed to pure oxygen. It was found that almost all the type B cell mitochondria were vacuolated, the inner membranes almost entirely disappearing. In another early study (62), Schulz examined normal human lung and lung from a patient with mitral stenosis. In both cases, alveolar cells were seen with cytoplasmic bodies resembling the transformed mitochondria described above. In a later paper (65), Schulz reported that the lamellar transformation is found in normal as well as carbon dioxide treated animals, and is also present in embryonal rat lung. Of particular interest, Schulz observed lamellar material within the air spaces. This material was identical in appearance to the lamellar remnants of the transformed mitochondria. He found that this material was

most abundant in the new-born animal. In an experiment in which diamox, a carbonic anhydrase inhibitor, was administered to rats, Schulz (65) noted that two to four hours following administration there were few transformed mitochondria in the alveolar epithelium. In an experiment (65) in which rats were subjected to a severe lack of oxygen, the alveolar cell mitochondria became swollen but did not suffer a lamellar transformation. In a study of the dormouse during hibernation, Schulz (64) found that almost all alveolar cell mitochondria were transformed into the lamellar type of inclusion bodies. He attributed this change to the hypoxia and elevated carbon dioxide which is known to occur in this animal during hibernation.

While some of these findings tend to show a relation between high alveolar carbon dioxide content and increased lamellar transformed mitochondria, the postulated relation to the control of blood flow in alveolar capillaries was not experimentally tested. The authors imply that carbonic anhydrase is implicated in the latter relation. In this regard, Meesen (44) referred to information "from the older light microscopic findings of the effect of carbonic acid on the bronchi, on the size of the alveoli and capillaries, and on the alveolar epithelial cells." To add more weight to his hypothesis, Meesen (44) stated that, together with Knierem (reference not cited), he had found, by histochemical means, carbonic anhydrase in animal and human lung but not

in fetal rat lung. However, he used the method of Hausler (24) to detect carbonic anhydrase, and it has been shown by Fand and co-workers (16), and also by Pearse (53) that the method is not specific for carbonic anhydrase.

In summary, a definite function for the type B alveolar cell has not been proved.

MATERIAL AND METHODS

Strain A (Heston) mice (26) were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. Mice of both sexes were employed in this study. Fetal and neonatal mice were obtained as offspring from the above mice bred in the Pathology Department laboratories. Thirty fetal, 6 neonatal, and about 50 adult mice ranging in age from 3 to 14 months were used for this work. The age of the fetuses was measured from the day that a vaginal plug was found in the mated female. Fetuses were removed surgically from ether-anesthetized females. The 18 and 19 day old fetuses were carefully removed with membranes intact and placed into a vessel containing sufficient saline to cover them. The fetuses were dissected under liquid and the lungs removed without the animal breathing air. A small number of 19 day fetuses were delivered from their membranes and placed on moist cotton in a 37° C. incubator. These mice were allowed to breathe air for one to two hours and were then sacrificed and lung tissue taken for study.

In preparing tissues for electron microscopic examination, lungs were rapidly removed from ether-anesthetized mice and a small slice taken from the lung periphery. A small volume of fixing fluid was dropped onto the slice

completely covering it. The slice of lung was then cut into blocks about 1 cu. mm. in size, and the blocks transferred to a vial containing 1 ml. of fresh fixing fluid. Fixation was carried out for 1 - 2 hours either at room temperature or at refrigerator temperature. The fixative used for all tissue preservation was 1% aqueous osmium tetroxide, buffered with veronal-acetate to pH 7.4 according to the method of Caulfield (7). Sucrose was added to the fixative, in the amount of 45 mg. per ml. of fixative, just before use. Following fixation, the tissues were rapidly dehydrated with ethanol through a series of concentrations from 50% to 95% ethanol. Dehydration was completed through this series in from 15 to 90 minutes. Tissues were then left in 95% ethanol, at room temperature, until they were infiltrated with plastic. Prior to infiltration and embedment in epoxy plastics, the tissues were first dehydrated further with absolute ethanol and then with two changes of propylene oxide. Following this dehydration, the tissues were infiltrated for three hours with a one-to-one mixture of propylene oxide and epoxy embedding mixture. The epoxy embedding mixture was made up using EPON 812 (a Shell Chemical Co. epoxy resin), dodecenyl succinic anhydride and methyl nadic anhydride (curing agents), 2,4,6-tri (dimethylaminomethyl) phenol (accelerator). Two stock solutions of the epoxy plastics, A and B, were made up as recommended by Luft (41) and the proportion of 6 parts of A to 4 parts of B

was used in embedding most of the tissues. The tissue blocks were embedded in gelatin capsules and the plastic polymerized by heat as follows: 14 hours at 35° C., 10 hours at 45° C., and about 16 hours at 60° C.

Sections of the plastic embedded tissues were cut with glass knives using either the Porter-Blum or the Huxley microtome. Sections one micron thick were stained with methylene blue or with toluidine blue, and mounted on glass slides for study with the light microscope. Thin sections, about one-twentieth of a micron thick were mounted on formvar-covered copper mesh specimen screens and examined with the electron microscope. The contrast of sections examined with the electron microscope was increased by treating the thin sections with heavy metal "staining solutions". Uranyl acetate, lead acetate, lead hydroxide, or lead citrate was used for "staining" (37, 56, 75). The thin sections were examined and photographed in an RCA, model EMU 3, electron microscope. The electron micrograph negatives were photographically enlarged and printed on Kodabromide photographic paper.

RESULTS

I. General Description of Strain A (Heston) Mouse Lung

A. Adult lung. The general relations and appearances of alveolar and terminal bronchiolar cells are shown in Fig. 1, which is a highly diagrammatic representation. The alveoli are lined by two epithelial cells, type A and type B. The type A cell is squamous and covers the greatest part of the alveoli. Both it and the type B cell overlie a continuous basement membrane. The type B cell usually lies deep within the alveolar septum. Some examples can be found where the type B cell appears to extend completely through the alveolar septum to border on opposite alveolar spaces, as B_2 in Fig. 1. The alveolar macrophages, shown lying free in Fig. 1, in reality contact some part of the alveolar wall. In the alveolar septum, there is found endothelial-lined capillaries, connective tissue cells, and collagen and elastic fibers. Two types of cells line the terminal bronchiole: ciliated and non-ciliated cells.

A typical electron microscopic view of mouse alveolar structures is shown in Fig. 2. The perinuclear portion of a type A cell is shown in this figure. The cytoplasm immediately adjacent to the nuclear zone attenuates to become the major covering of the alveolus. The thin layer of attenuated type A cell cytoplasm may measure one-tenth micron or less

in some places in the mouse alveoli. The perinuclear zone of the type A cell contains many ribosomes but very little membranous endoplasmic reticulum. Only a few mitochondria are seen in the cell. The attenuated portion of the cell, which by far makes up the bulk of the cell, contains small vesicles and a very few mitochondria, Fig. 3. The cell membrane facing towards the air is irregular in contour but does not have microvilli (i.e., short evaginations of the cell surface).

The various positions which the type B alveolar cell occupies on the alveolar wall are shown in Figures 4 through 7. In Figure 4, the cell is seen in its most usual position indented into an angle formed by the alveolar septum. The cell is bordered at its base and sides by basement membrane. The junction between the type A and B cell may extend for as much as one micron, Fig. 4. At times, the type B cell may be seen on the alveolar wall occupying a position similar to the type A cell, Fig. 5. In this case, the cell is not indented into the septum. Occasionally, as in Fig. 6, the type B cell extends deep within the alveolar septum to contact the basement membrane of the type A cell on the opposite side of the septum. This position suggests a penetration of the septum by the type B cell, as does the position shown in Fig. 7, where a type B cell borders two air spaces.

The type B alveolar cell contains many mitochondria,

a variable amount of endoplasmic reticulum, and an inconspicuous Golgi apparatus. The endoplasmic reticulum is often markedly dilated. The luminal plasma membrane of the cell has numerous evaginations of microvilli.

The type B cells are easily recognized by the appearance of lamellar, osmiophilic bodies within their cytoplasm: the transformed mitochondria of Schulz (65). These lamellar bodies are found in several forms. In one form, thin membranes lie parallel to each other within an otherwise empty membrane-enclosed vacuole having the size and shape of a mitochondrium. In another form, the thin membranes are coalesced into a few thick membranes or bands. In still another form, membranes are whorled within large vacuoles to give a myelin-like appearance, Figs. 4 - 7.

Membranous material, similar to that seen in the type B cell, is also found in the air spaces, occurring in large quantities in late fetal and neonatal lung, Fig. 8.

The alveolar macrophage is a large, irregularly shaped cell found within the alveolus. The macrophage is separated from the alveolar basement membrane by a layer of epithelium, Figs. 9 and 10. There is usually abundant pleomorphic, phagocytized material within the cytoplasm of the cell. Broad pseudopods are often seen at the border of the macrophage, but microvilli do not occur. Membranous material, similar to that seen in the type B cell, is frequently found in vacuoles within the macrophages, Fig. 9.

The blood capillaries in mouse lung are very thin-walled and may extend completely across the narrow alveolar septum, separated from the adjoining air spaces only by a thin basement membrane and attenuated cytoplasm of the type A alveolar cell. It is noted that the type B alveolar cell does not overlie the capillaries directly.

The alveolar septum contains, in addition to the blood capillaries, connective tissue cells, Fig. 11, and a small amount of collagenous and elastic fibers. The connective tissue cell is a large cell with greatly extended, thin, cytoplasmic processes. This cell does not appear to have phagocytized material in its cytoplasm.

The cells lining the terminal bronchiole are of two types, ciliated and non-ciliated. Neither the ciliated nor the non-ciliated cell resembles the two alveolar cells or the alveolar macrophage. The ciliated cells of the terminal bronchiole are small, inconspicuous, and have very few cilia, Fig. 12. The non-ciliated cells outnumber the ciliated cells at least two-to-one. The non-ciliated cells, Fig. 13, contain abundant agranular endoplasmic reticulum, a small amount of granular endoplasmic reticulum, and numerous mitochondria. The mitochondria are atypical in that they are made up almost entirely of "matrix" material and have only very short internal membranes.

B. Neonatal lung. The alveolar architecture of the neonatal lung is very similar to that of the adult. The

main differences are a greater inter-alveolar septal thickness in the neonatal lung, immaturity of the non-ciliated bronchiolar cells, and the occurrence of large masses of membranous material in the air spaces, Fig. 8.

C. Fetal lung. The gestation period of the mouse averages 20 days. At the 19th day, the lung appears, in most areas, very similar to neonatal lung. The alveoli are open and the type A cells are in the squamous form, Fig. 14. The type B cell is recognizable at this fetal age by the presence of lamellar bodies in the cytoplasm, Fig. 15. At 19 days of fetal age, the type B cell contains relatively large amounts of a material having the morphological characteristics of glycogen. This material is found in most lung cells of the 17, 18, and 19 day fetus, but is not seen in adult lung. As in neonatal lung, masses of membranous material can be found in the alveoli of the 19 day fetus, Fig. 16. Ciliated and non-ciliated bronchiolar cells are readily distinguishable from the alveolar cells at 19 days, Fig. 17. Both cells are columnar, but only the ciliated cell is well differentiated.

The 18 day fetal lung is much less adult-like than the 19 day lung. At 18 days, while some of the alveoli are open, and the type A cells are squamous in such alveoli, other alveoli are closed and the cells are cuboidal. In the open alveoli, Fig. 18, the air spaces are relatively small and the alveolar septa quite thick. The type B cell

is recognizable at this stage of development due to the presence of lamellar bodies in the cytoplasm. Most of the alveolar cells are well filled with glycogen-like material, contain few mitochondria, and little endoplasmic reticulum. At this age, many of the alveoli are still closed, Fig. 19, but even here the type B cells contain lamellar bodies. Bronchiolar cells are similar to those at 19 days.

At 17 days of fetal age, the alveoli are closed and the alveolar cells are indistinguishable one from the other, Fig. 20. The alveolar cells form a solid cord of tissue. Glycogen-like material is not as abundant in the cells as at 18 days. The bronchiolar cells are more difficult to distinguish as to type. While the cells are columnar, very few of the ciliated cell precursors have cilia, although many of them show development of the basal bodies which give rise to the cilia.

At 16 days of fetal age, it is no longer possible to distinguish the bronchiolar cell types. The alveolar precursor cells are in solid cords and appear alike, Fig. 21. Glycogen-like material is absent at these earlier ages. Mitoses are frequent.

At 15 days of fetal age, alveolar precursor cells are difficult to find, and at 14 days of fetal age they cannot be found. Mitoses are very frequent at these ages.

D. Premature lung. The lungs of 19 day fetal mice, surgically delivered and allowed to breathe for one or two hours, appeared similar to both neonatal lung and 19 day, non-breathing, fetal lung, Fig. 22. Breathing at this age did not produce obvious alterations in either the type A or type B alveolar cells.

II. Description of Special Features of Strain A (Heston) Mouse Lung

A. Type B alveolar cell. The outstanding morphological characteristic of the type B cell is the cytoplasmic lamellar bodies. Yet, there is a great variation in the number of such structures and in their appearance, as has already been described. For example, the cell shown in Fig. 23 has only one such body in this section, while the cell shown in Fig. 24 has many. In some cells, Fig. 25, a mitochondrial origin is easily recognized. In other cells, Figs. 26 and 27, the origin is by no means evident. In addition to the lamellar bodies, multivesicular bodies are occasionally seen in the cytoplasm, Fig. 28. Such bodies are found in many different cells of the body, and are possibly a variety of lysosome. It is of interest to note that some of the mitochondria in the cell illustrated in Fig. 28 are encircled by endoplasmic reticulum, an atypical finding.

B. Non-ciliated bronchiolar cell. The non-ciliated bronchiolar cell merits additional attention since it appears to be a very active cell. This cell is unusually rich in

both mitochondria and agranular endoplasmic reticulum, Figs. 29 and 30. Moreover, the endoplasmic reticulum circumferentially surrounds many of the mitochondria. As noted above, such a relation between mitochondria and endoplasmic reticulum can occasionally be seen in the type B alveolar cell.

C. Pinocytosis. Lining cells, such as endothelial, epithelial, and mesothelial frequently show evidence of pinocytotic activity. This activity is indicated by the presence of small invaginations at the cell surface and microvesicles within the cytoplasm. The extent of pinocytotic activity can be judged by the number of microvesicles found in the cytoplasm adjacent to the cell membranes and also by the number of membrane invaginations. The pinocytotic activity of the endothelial and epithelial cells illustrated in Fig. 32 is moderate. Slightly more activity is seen in Fig. 33 which shows part of a connective tissue space between the endothelium and epithelium.

D. Search for a non-cellular alveolar lining layer. In order to determine if a non-cellular surface membrane lies on the air side of the alveolar epithelial cells, it is necessary to search for sections which show cell membranes cut at right angles to the plane in which they lie. However, the epithelial cell membranes are greatly irregular in contour since, in life, they are not in contact with an opposing surface. Tangential sections of undulating cell

membranes give the impression that the membranes have different thicknesses. Therefore, a search for a non-cellular alveolar lining layer, possibly thinner than cell membranes, is necessarily limited to areas where opposing cell surfaces come into close, but not complete contact. Such areas are shown in Figs. 33 and 34. It is not possible to detect, in these areas, the two thin lines expected if a surface membrane covered opposing cell membranes.

DISCUSSION

The alveoli of the normal adult strain A (Heston) mouse lung are populated by two epithelial cell types, A and B. The squamous type A alveolar cells line the alveoli with a thin layer of cytoplasm which serves to protect the alveolar septum and also provides a minimal barrier to gas diffusion. The cuboidal type B alveolar cells occur singly, interspersed between the type A cells. The two epithelial cell types appear approximately in equal numbers, but the type A cell covers the greatest part of the alveolar surface. The several positions which the type B cell occupies on the alveolar wall suggest that the cell has a capability for motion. However, the motion is apparently limited to the penetration of the alveolar wall from one air space to the adjacent one. The type B cell is characterized by the presence of lamellar bodies, the transformed mitochondria of Schulz (65). Both the type A and B alveolar cells are morphologically distinguishable from the alveolar macrophages, the connective tissue cells of the alveolar septum, and the ciliated and non-ciliated cells lining the terminal bronchiole. These findings are in general agreement with those of most other investigators who studied other strains of mice as well as other mammals. Certain aspects of the findings, as they deal with the function of the two alveolar

cells will be discussed later in greater detail.

Two distinct alveolar cell types are also distinguishable in neonatal and late fetal lung. In the mouse, with a gestation period of 20 days, the type B cell can be identified by the presence of lamellar bodies on the 18th and 19th day of fetal life. On the 19th day, the type A cell is recognized by its squamous appearance. Mice delivered surgically at the 19th day of fetal age appear capable of normal breathing, at least for two hours, which reflects the adult-like morphology of the lung at that age. At 18 days fetal age, about half of the alveoli are opened and the type A cells can be recognized in the alveoli by their squamous form. In the closed alveoli at this age, the type A cell precursor is cuboidal and can not positively be identified. It is not possible to distinguish differences between alveolar cell precursors at 17 days of fetal age or younger.

Many 17, 18, and 19 day fetal lung cells are rich in material considered to be glycogen, a cytoplasmic component not found in adult lung and also not found at earlier fetal ages. The absence of glycogen during early fetal development is believed to be related to the active multiplication of cells at this stage. The presence of glycogen in many lung cells from 17 to 19 days fetal age is thought to indicate that the cells are in a resting stage of development. That is, the cells at this age store glycogen in preparation for rapid differentiation into adult forms.

The cells lining the terminal bronchioles are also of two types, ciliated and non-ciliated. The non-ciliated cells are large, more numerous than the ciliated cells, and appear to be engaged in some type of secretory activity. In this regard, the extensive development of the agranular reticulum and the presence of numerous mitochondria give the non-ciliated cells an appearance somewhat similar to the striated duct cells of the salivary glands (71). It may be suggested that the non-ciliated cells produce a serous secretion which moistens the lower bronchioles and possibly also the alveoli.

From the extensive studies of Schulz (67) on mammalian lung, it can be assumed that most, if not all, mammals have two alveolar cell types. It is of equal interest that similar cell types can also be found in non-mammalian lungs.

In bird lung (4,5) the small air tubules are lined by squamous cells which are very similar to the type A cells of the mammal. Also, the atria of the bird lung (5, 73) are continuously lined by cells which appear very similar to the type B alveolar cell of the mammal in that they both contain osmiophilic, lamellar bodies in the cytoplasm.

The snake lung also contains two types of alveolar cells, squamous and cuboidal (5, 50). The appearance and spatial relationships of these two cell types in the snake are similar to that of the type A and B cells of the mammal.

The frog lung is different from mammalian or snake lung

in that the alveoli of the frog lung are lined by only one cell type (5, 49). However, it is noteworthy that this one cell combines the features of both the mammalian type A and type B cell since it is squamous yet contains lamellar bodies in the cytoplasm.

It may be concluded from the reported morphological findings on the cells of mammalian, bird, snake, and frog lung that at least two different functional needs are met by the alveolar lining cells.

Because the present study is intended to serve as a basis for future research on induced lung tumors in the mouse, it is desirable to discuss the probable functions of the two alveolar cells, especially the type B cell which is thought to give rise to the tumor.

Although two cell types are found in the pulmonary alveoli of many different animals, only one functional need is apparent from morphological considerations; namely, that of covering the alveolar wall. This function would appear to be adequately satisfied by the type A cell of the mammal and the squamous alveolar cells of the other animal classes. However, a second function can be postulated from physiological considerations. Since it is necessary for optimal gas diffusion that the alveoli remain relatively free of fluid, it is reasonable to suggest that the type A alveolar lining cell may have the additional function of controlling the volume of alveolar surface fluid. The morphologic

evidence of apparent pinocytotic activity of the type A cell is consistent with this suggestion.

The morphological features of pinocytosis (cell drinking) have been described by many electron microscopists (27, 48, 59) in many cell types and are similar to the features described earlier in this paper. In some cells which border moist surfaces (e.g., endothelial and mesothelial cells), it is noted that cell membrane invaginations are present at opposite sides of the cell. Moore and Ruska (45) suggested that these features indicate that fluid is being moved completely across the cell. These authors offered the term "cytopempsis" (transmission by cell) to describe this activity. Cytopempsis differs from pinocytosis in that the former activity is concerned with fluid being moved across the cell, while the latter activity is concerned with fluid incorporated within the cell.

Since the capillary endothelium also shows the morphological features of cytopempsis, there may exist in the lung a mechanism for transfer of fluid from alveolar surface to blood capillary.

The type B alveolar cell of mammalian lung has no known function. Three suggestions as to its function can be found in the literature. The first, made by most light microscopists who observed the cell, was that the cell was a macrophage and that, therefore, its function was phagocytosis. The second suggestion was that the cell may secrete a

substance which would reduce alveolar surface tension. The third suggestion was that the cell is possibly involved in the regulation between ventilation and capillary blood flow at the alveolar level.

The concept that the type B alveolar cell is a fixed macrophage or a precursor of the alveolar macrophage finds no confirmation from the present study. The type B cell has the appearance of a mature cell. It seems unlikely that it would give rise to any other type of cell, except possibly under pathological conditions.

That the type B cell produces a surface tension-reducing agent, as suggested by Clements (12), can neither be confirmed nor denied by this study. It has been assumed by Clements that the agent is a lipoprotein. Such a substance, if it formed a lining layer in the alveoli, should be well preserved by the current techniques used in electron microscopy. However, the present study has failed to reveal a layer of electron-dense material covering the air side of the alveolar epithelial cells. On the other hand, if the surface tension-reducing material was a phospholipid (14) spread on the alveolar surface fluid as a monomolecular layer, and if the lipid part of the molecule was saturated, the osmium tetroxide used to fix the tissue would not preserve such a surface layer material.

That the type B cell may function to regulate the local flow of blood in alveolar capillaries, as suggested by

Meesen (44), can neither be confirmed nor denied by the observations of this study. While the observations of this study confirm the deduction of Schulz (65) that the lamellar bodies of the type B cell are membranous remnants of transformed mitochondria, no further direct information can be derived from such observations. A preliminary attempt (5) to repeat the work of Schulz (65) on the effect of carbon dioxide on the type B cell showed that there was a high but not abnormal number of transformed mitochondria in the type B cells of treated mice. It was noted, however, that treatment of mice with carbon dioxide caused a patchy atelectasis. Cedergren and co-workers (8) had observed that the type B cell, in areas of atelectasis, contained greater numbers of transformed mitochondria. It may be that the type B cell responds to atelectasis in this manner.

Since the three suggestions for type B cell function are unconfirmed by the present study, further consideration of what is known concerning this cell may help to suggest other possible functions.

The occurrence of mitochondrial transformation may be the best clue to the function of the type B cell. The most striking aspect of this transformation is that the mitochondrial matrix material disappears first leaving the internal membranes intact. Moreover, there is no evidence that the earliest alteration is one of swelling or rupture. In other described pathological or traumatic alterations of

mitochondria, there is swelling and early membrane disappearance (58).

The matrix of the mitochondria contains soluble enzymes (2). It may be speculated that these enzymes are released into the alveolar surface fluid where one or more of them fulfills some special function. It has already been pointed out that the type A cell is the primary functional cell of the alveolus, since it lines the greatest part of the alveolar wall and may also control the volume of fluid within the alveolus. The type A cell is specialized to carry out these functions while in a greatly attenuated state. It is observed that there are very few mitochondria in the attenuated portion of this cell. Since fluid transport by cytopemptic action may require a constant source of energy, it is possible that the type A cell needs protection from toxic substances that would impair its mitochondrial respiration. It is suggested, therefore, that the function of the type B cell is the production and release into the alveolar surface fluid of a substance, for example: cytochrome oxidase, which could bind with and detoxify respiratory poisons, such as carbon monoxide, present within the alveolus.

SUMMARY

The alveoli of the strain A (Heston) mouse are surfaced by two epithelial cell types, A and B. The type A alveolar cell is squamous and covers the alveolar surface with a very thin layer of cytoplasm. The cuboidal type B alveolar cell occurs singly, interspersed between the type A cells. The two cells are found in approximately equal numbers, but the type A cells cover the greatest part of the alveolar surface.

The perinuclear cytoplasm of the type A cell contains a few mitochondria, many ribosomes, but relatively little membranous endoplasmic reticulum. The attenuated, outspread portion of the cell contains a few scattered mitochondria, and a variable number of microvesicles. The plasma membrane has many small invaginations on both basal and luminal surfaces.

The type B alveolar cell most commonly occupies a position in an angle of the alveolar septum. It may be deeply indented into the alveolar wall. The basal and lateral sides of the cell are in contact with basement membrane. The cell membrane at the luminal side is characterized by numerous, short microvilli. Occasionally, the type B cell is seen to completely span the alveolar septum and face on two air spaces.

The type B cell contains a variable amount of endoplasmic reticulum, a small Golgi apparatus, and many mitochondria, similar to those in other cell types. An osmiophilic, lamellar body also occurs in large numbers in the cytoplasm of the type B cell. Intermediate forms between mitochondria and the dense, lamellar bodies are observed. Similar lamellar or membranous material is also found in large quantities in the alveoli of late fetal and neonatal mouse lungs. In adult mouse lung, membranous material resembling that of the lamellar bodies is found in vacuoles of alveolar macrophages. The morphological data suggest that the mitochondria of the type B cell are transformed into the lamellar bodies and that these are subsequently expelled into the alveolar air spaces.

The type A and B alveolar cells of the strain A (Heston) mouse are morphologically distinguishable from the bronchiolar lining cells, from the cells within the alveolar septum, and from the alveolar macrophages.

In the mouse (gestation period 20 days), type A and B alveolar cells are recognizable in 19 day old fetuses. At this age most alveoli are open, and the two cells are morphologically very similar to those seen in neonatal and adult lung. At 18 days of fetal age, some of the alveoli are open, and in such alveoli the two alveolar cell types are similar to those seen at 19 days. In closed alveoli at this age, the type B cell-precursor already contains transformed

mitochondria in the cytoplasm. At 17 days of fetal age, and earlier, the alveolar cell-precursors cannot be distinguished as to type.

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ABBREVIATIONS FOR FIGURES

A - type A alveolar cell

AGR - agranular endoplasmic reticulum

B - type B alveolar cell

BM - basement membrane

C - ciliated bronchiolar cell

CAP - capillary

CIL - cilia

CN - connective tissue cell

CTS - connective tissue space

EN - endothelial cell

EP - epithelial cell

ER - endoplasmic reticulum

GA - Golgi apparatus

GL - glycogen-like material

JN - junction between epithelial cells

LB - lamellar body

LIP - lipid

LY - lysosome

MAC - macrophage.

MIT - mitochondria

MV - microvilli

NUC - nucleus

PD - speudopod

PV - pinocytotic vesicle

RBC - red blood cell

TRM - transformed mitochondria

WBC - white blood cell

Figure 1

Half-tone schematic drawing of bronchiolar-alveolar pulmonary fine structure. The drawing shows a cross-section of the distal portion of a terminal bronchiole and adjacent alveoli. Alveolar ducts are not shown. The terminal bronchiole is lined by ciliated, C, and non-ciliated, NC, cells. The adjacent alveoli are lined by type A alveolar cells, A, and type B, B, alveolar cells. One of the type B cells, B_2, is shown lying between adjacent alveoli. The type A cell is squamous. The type B cell is cuboidal when it is on the alveolar wall, but may take various shapes when it lies deep within the wall. Both type A and B alveolar cells are in contact with basement membrane (heavy black line) at their basal surfaces. The inter-alveolar septa are made up of connective tissue spaces, CTS, connective tissue cells, CN, and blood capillaries, CAP. Endothelial cells, EN, with thin cytoplasm, line the capillaries. Alveolar macrophages, MAC, lie free within the alveoli but are in contact with alveolar epithelial cells.

Approximate magnification - 1400 X

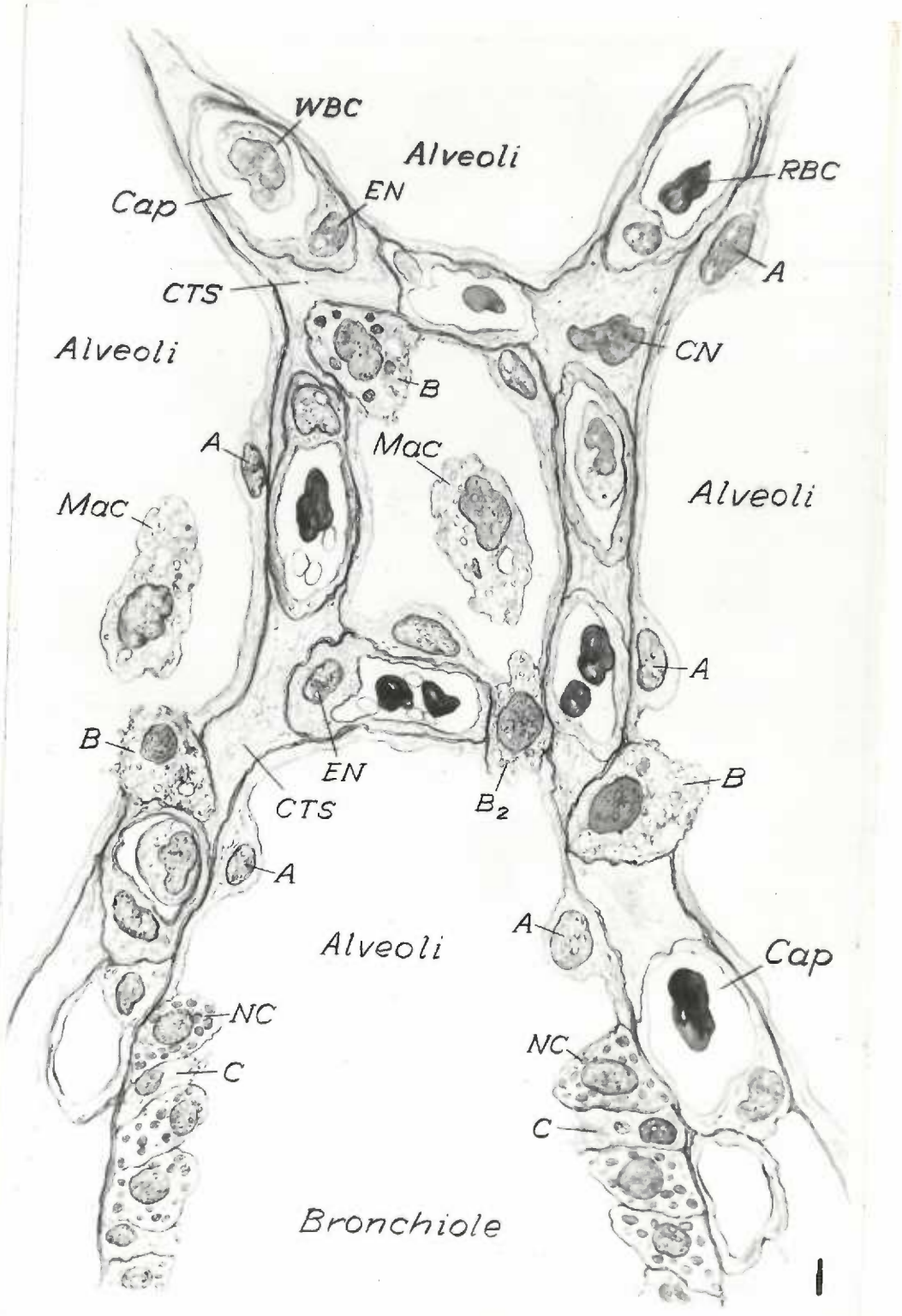

WBC
Alveoli
RBC
Cap
EN
A
CTS
Alveoli
CN
B
A
Mac
Mac
Alveoli
A
B
EN
CTS
B_2
B
A
Alveoli
Cap
NC
C
NC
C
Bronchiole

Figure 2

Normal mouse lung. This picture is of a portion of lung which has collapsed during the process of removal and fixation. As a result, the gross morphology is altered but the cellular constituents are typical of that seen in electron microscopic preparations of normal lung tissue. The perinuclear portion of a type A alveolar cell, A, fills the center of the picture. Immediately above the capillary is a narrow connective tissue space containing a thin cytoplasmic extension from the adjacent connective tissue cell, CN. Above the narrow connective tissue space is a capillary, CAP, containing several red blood cells. The basement membrane underlying both endothelial and epithelial cell cannot easily be distinguished from the ground substance of the connective tissue space in this area. Two other thin-walled capillaries are seen in the lower part of the picture. The shortness of the air-blood path is evident in this area.

Magnification - 15,300 X

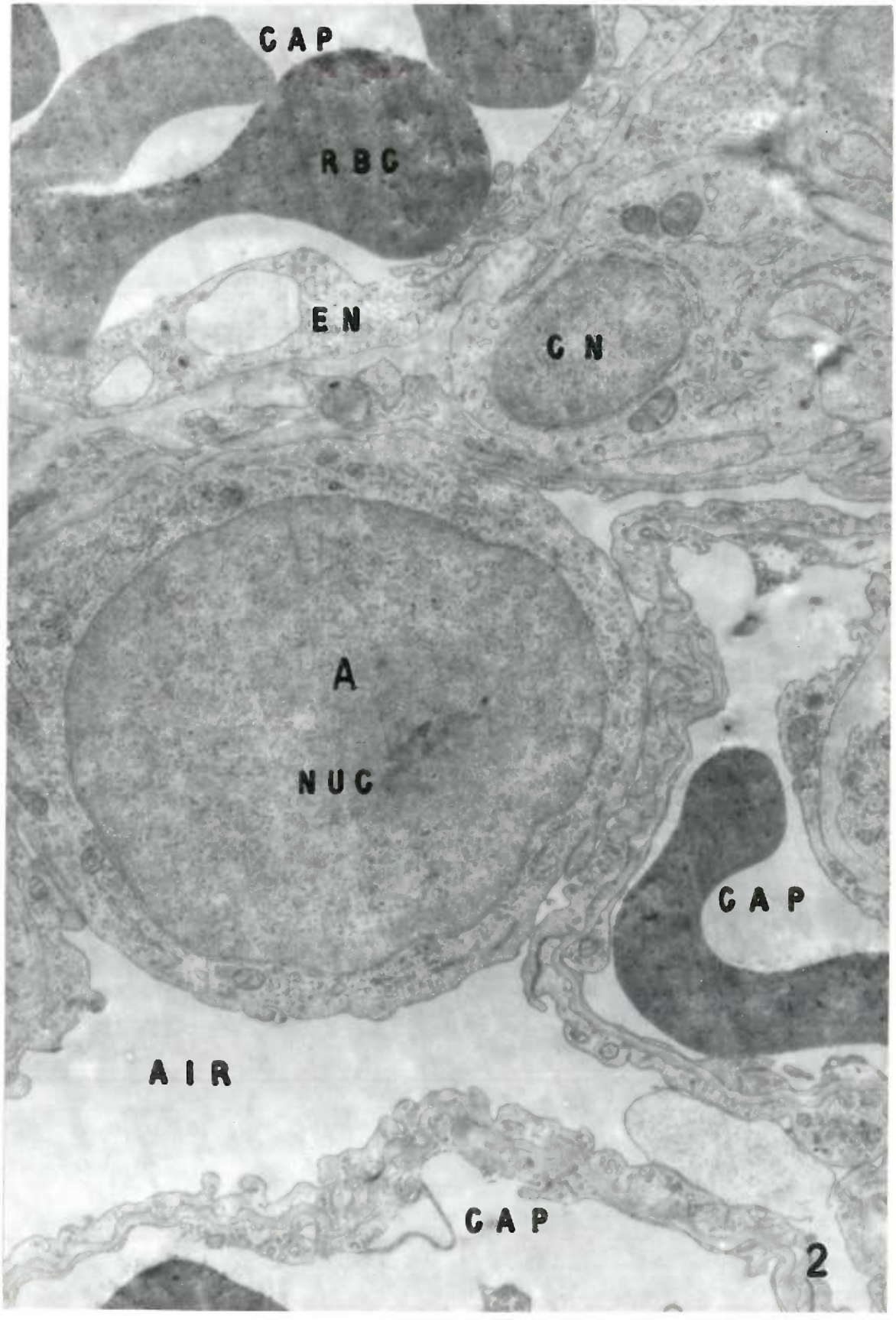

CAP
RBC
EN
CN
A
NUC
CAP
AIR
CAP
2

Figure 3

Type A alveolar cell. The type A cell shown is seen to have a narrow cytoplasmic bridge between the perinuclear and basal portions of the cell. The thin cytoplasmic layer around the nucleus, NUC, contains many small black particles, the ribosomes, and many small vesicles. The scarce endoplasmic reticulum, ER, a portion of a mitochondrium, MIT, and a lipid droplet, LIP, are indicated. The cell attenuates abruptly (arrows) at the basal region to spread out as a thin cytoplasmic layer over the alveolar surface. The basal part of the cell is in contact with the underlying basement membrane, BM.

Magnification - 26,700 X

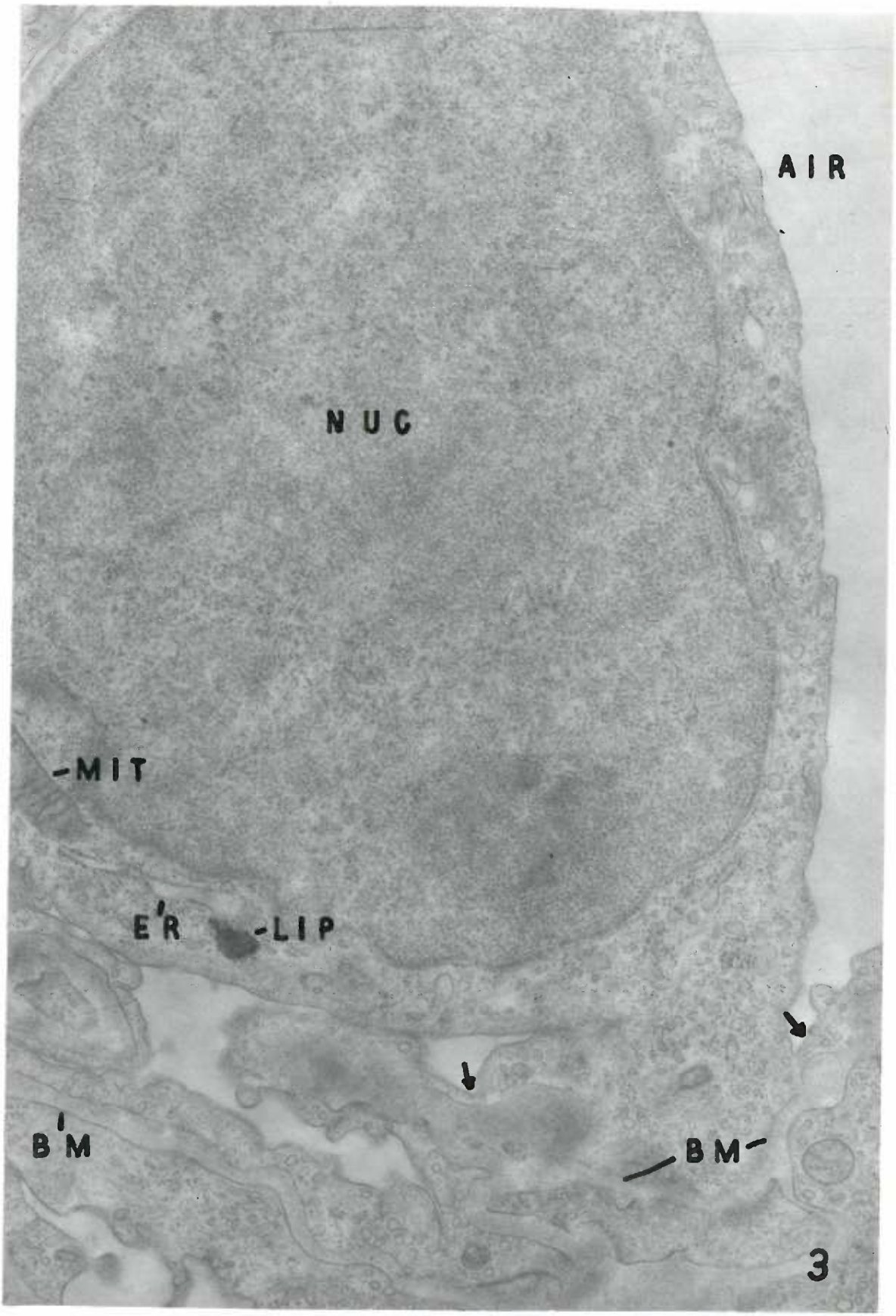

AIR
NUC
-MIT
E'R
-LIP
B'M
BM
3

Figure 4

Type B alveolar cell. This type B cell is in its most typical position in relation to the alveolar wall. The cell is continuously bordered at its base and sides by basement membrane, BM. At the air surface, the thin cytoplasmic layer of the type A cell abuts up against the sides of the type B cell in a relatively long junction, JN. The type B cell membrane bordering the air surface usually has many short evaginations, or microvilli, MV. The type A cell, while often having an irregular cell membrane, does not have microvilli. Within the cytoplasm of the type B cell, normal mitochondria, MIT, and lamellar bodies, LB, are evident.

Magnification - 15,300 X

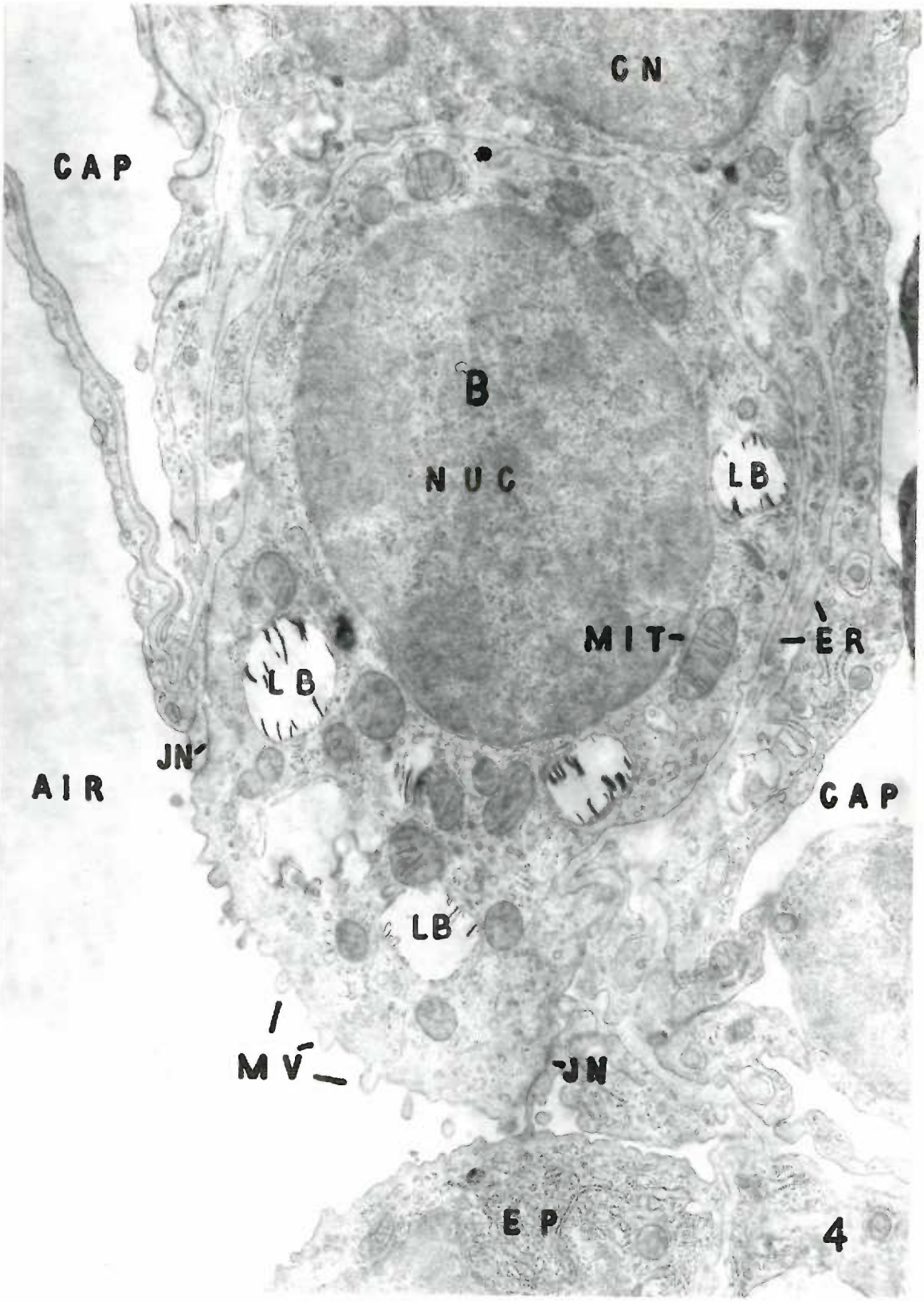

CN
CAP
B
NUC
LB
MIT
ER
LB
JN
AIR
CAP
LB
MV
JN
EP
4

Figure 5

Type B alveolar cell. This type B cell is indented into the alveolar wall. The cytoplasm contains many normal mitochondria and lamellar bodies. The cell membrane at the base of the cell shows several areas of extensive invagination.

Magnification - 13,200 X

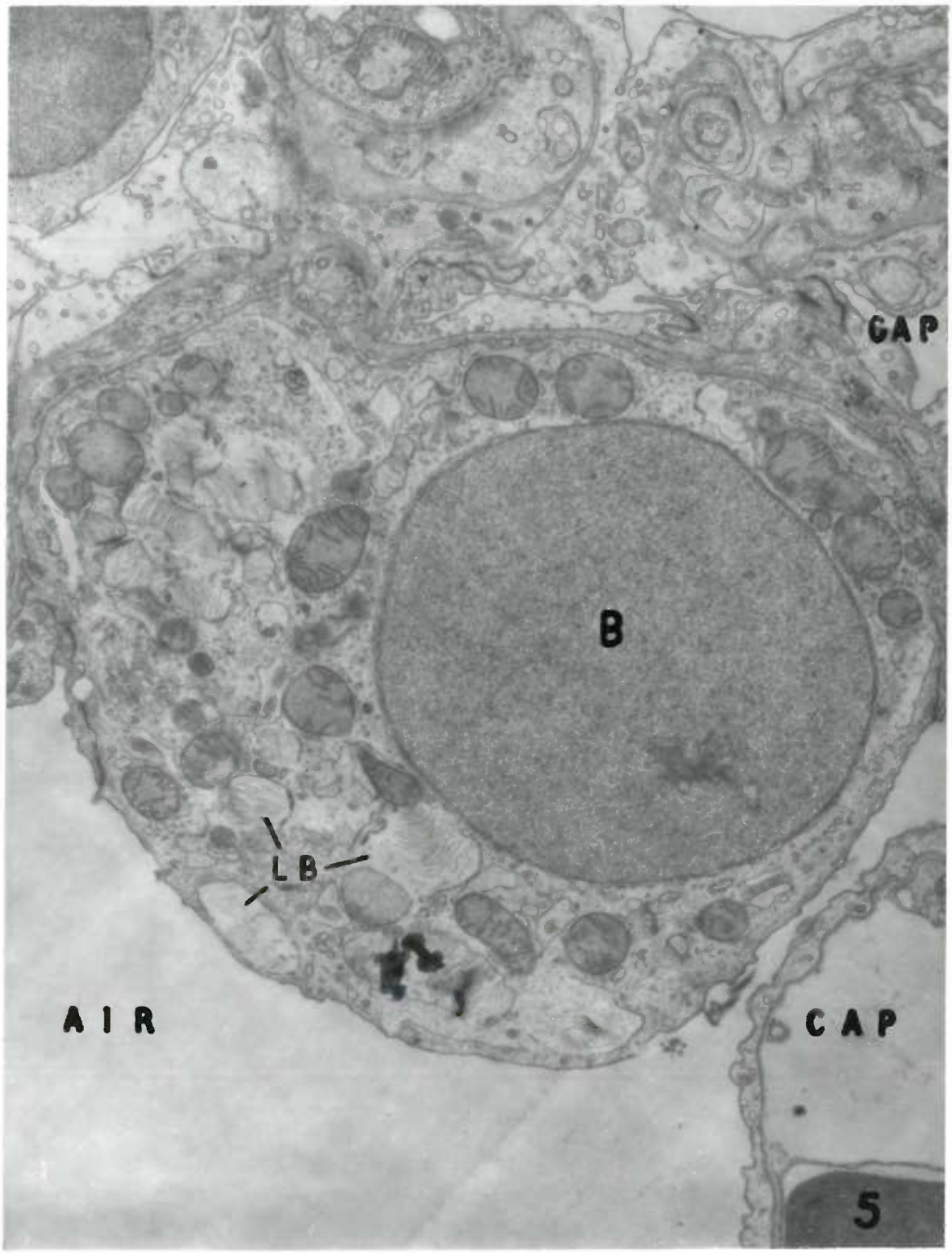

CAP
B
LB
AIR
CAP
5

Figure 6

Type B alveolar cell. This type B cell is in a less usual, but not atypical, position in the alveolar septum. The basal portion of the cell lies against the basement membrane of a type A cell which lines one alveolus, and the apical portion of the cell borders the air space of another alveolus.

Magnification - 15,300 X

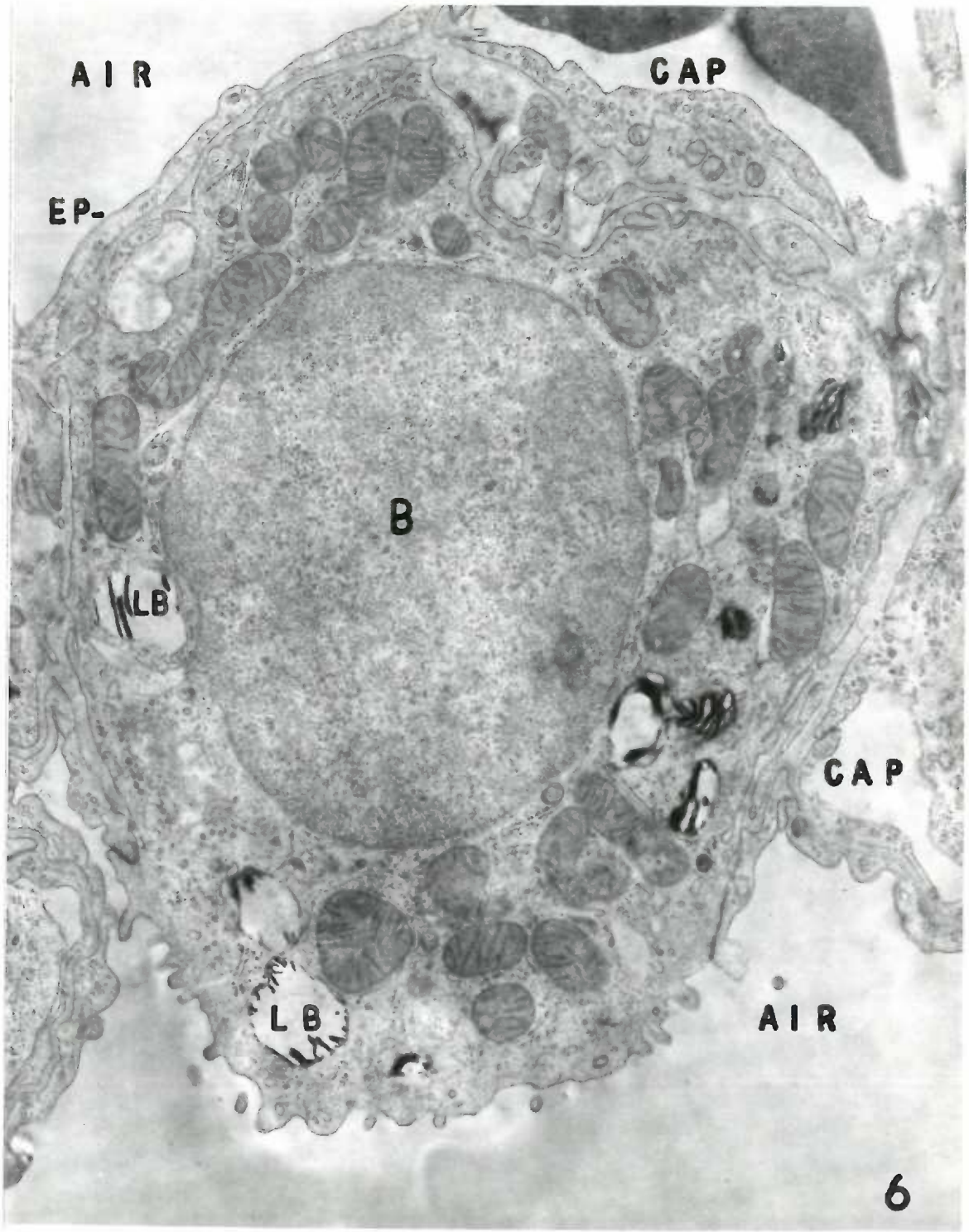

AIR
CAP
EP-
B
LB
CAP
LB
AIR
6

Figure 7

Type B alveolar cell. This elongated type B cell appears to extend from one alveolus to the adjacent one. Microvilli occur at the opposite ends of the cell where it faces the air spaces. The junction between this cell and the type A cells is long at the lower alveolus but short at the upper alveolus, suggesting that the cell may have penetrated from the lower to the upper alveolus.

Magnification - 15,300 X

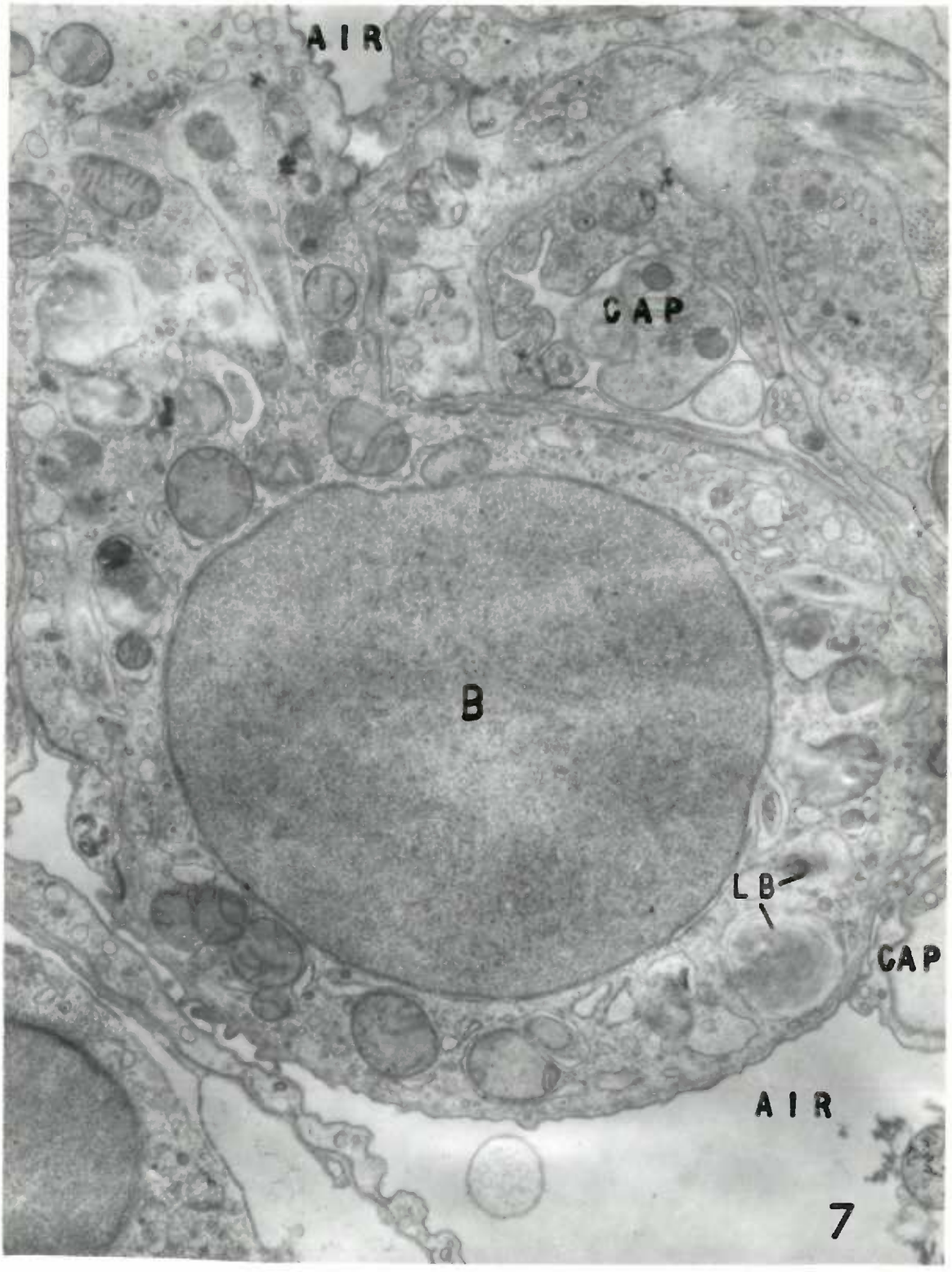

AIR
CAP
B
LB
CAP
AIR
7

Figure 8

Membranous material within an alveolus. This mass of membranous material was found within the alveolus of a two day old mouse.

Magnification - 20,700 X

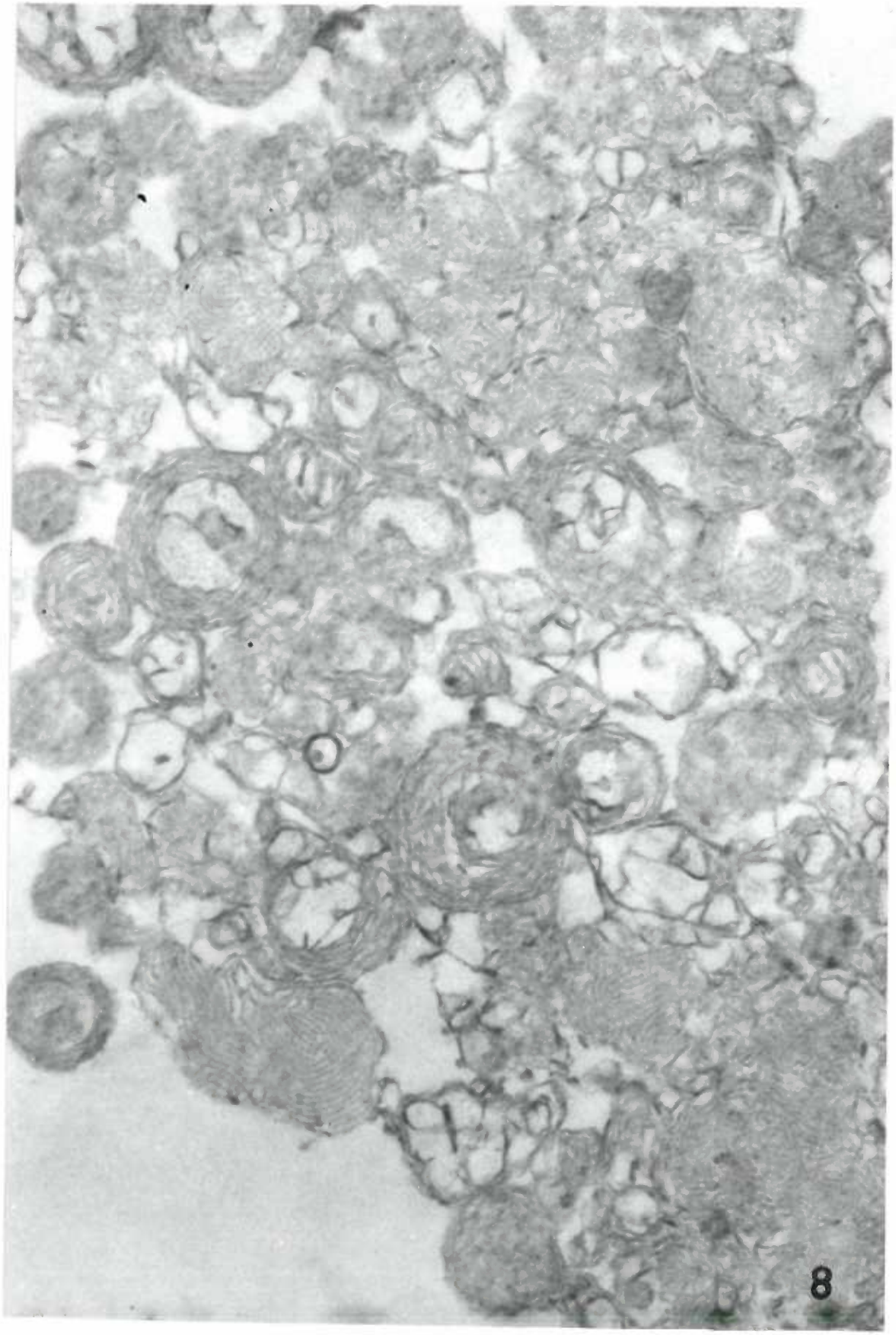

8

Figure 9

Alveolar macrophage. The alveolar macrophage is easily distinguished from the type B cell since the macrophage does not contact alveolar basement membrane. In addition, the macrophage does not have numerous short microvilli, but instead usually has broad cytoplasmic extensions or pseudopods, PD. The cytoplasm contains small mitochondria, and abundant pleomorphic, phagocytized material, including membranous material similar to that seen in the previous figure.

Magnification - 13,200 X

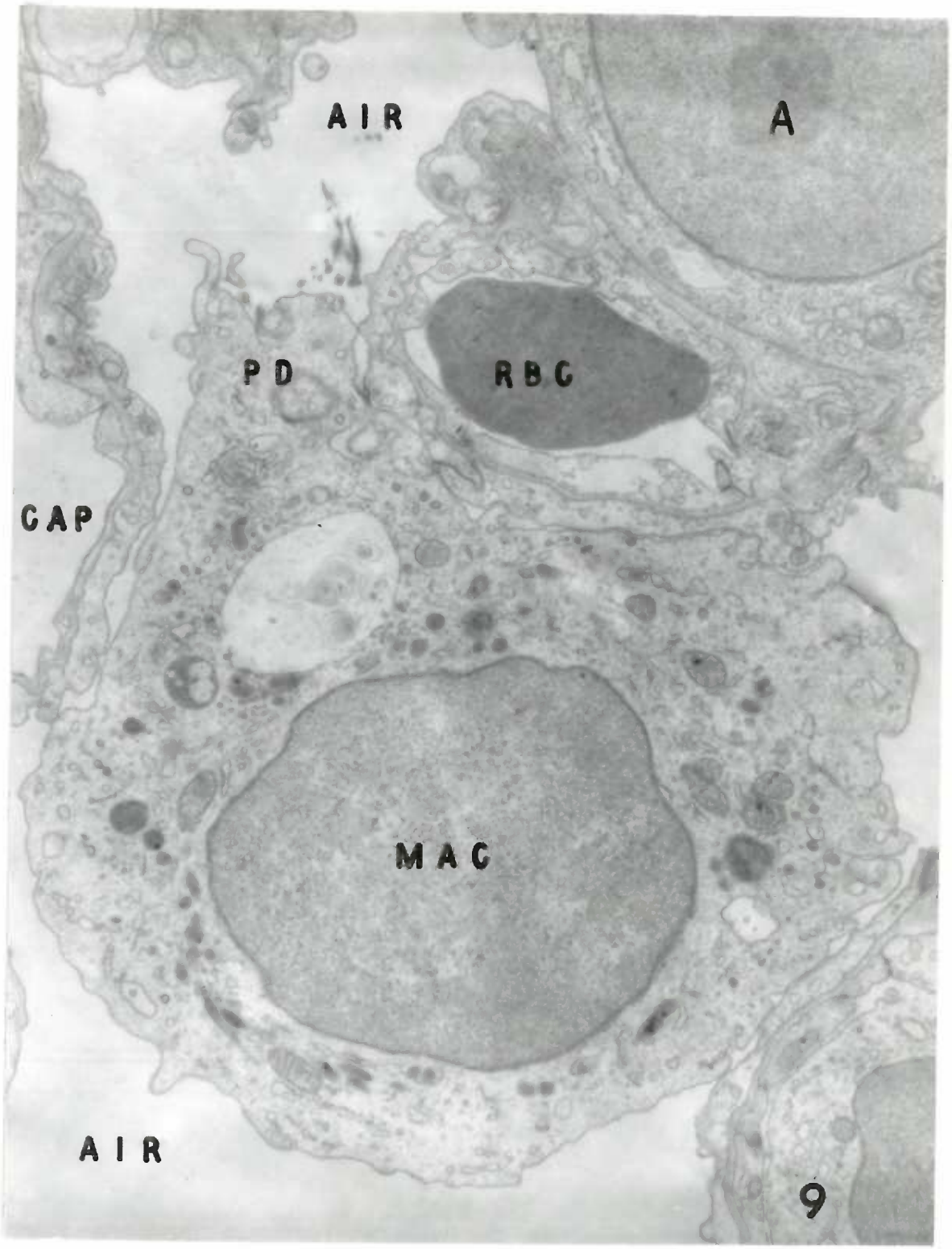

AIR
A
PD
RBC
CAP
MAC
AIR
9

Figure 10

Alveolar macrophage. This cell is more closely applied to the alveolar wall than the macrophage shown in the preceding figure, but is everywhere separated from the basement membrane by a layer of epithelial cytoplasm. The cell contains many different phagocytized objects. Some of the bodies seen in the cytoplasm may be lysosomes, LY, however these bodies are not consistently observed in these cells.

Magnification - 13,700 X

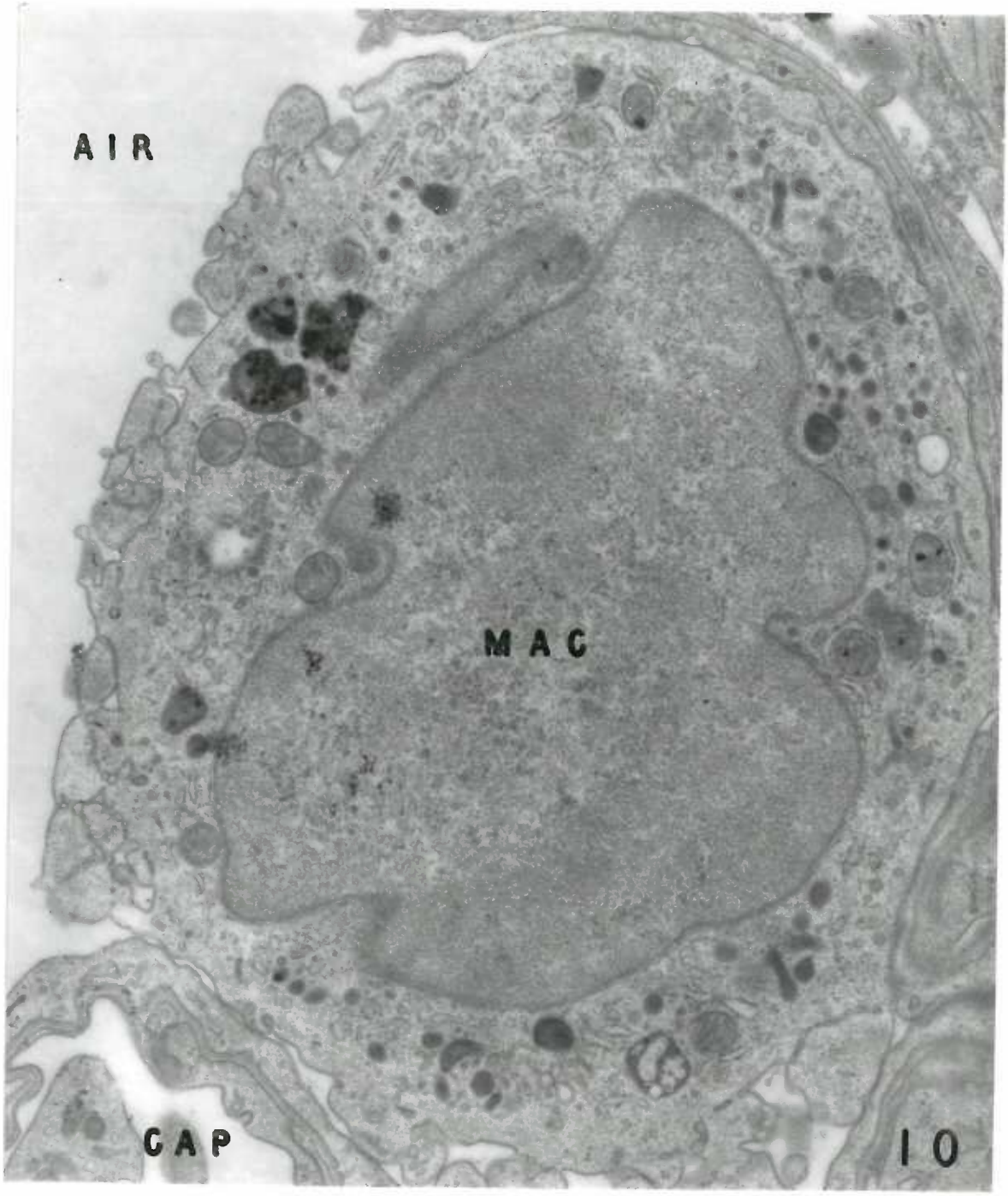

AIR
MAC
CAP
10

Figure 11

Connective tissue cell. The perinuclear portion of a connective tissue cell within the alveolar septum is illustrated. This cell has a small amount of cytoplasm around the nucleus, but has many cytoplasmic extensions proceeding in all directions from the cell body. The cytoplasm contains large amounts of ribosomes and endoplasmic reticulum. The cell lies in a connective tissue space.

Magnification - 13,400 X

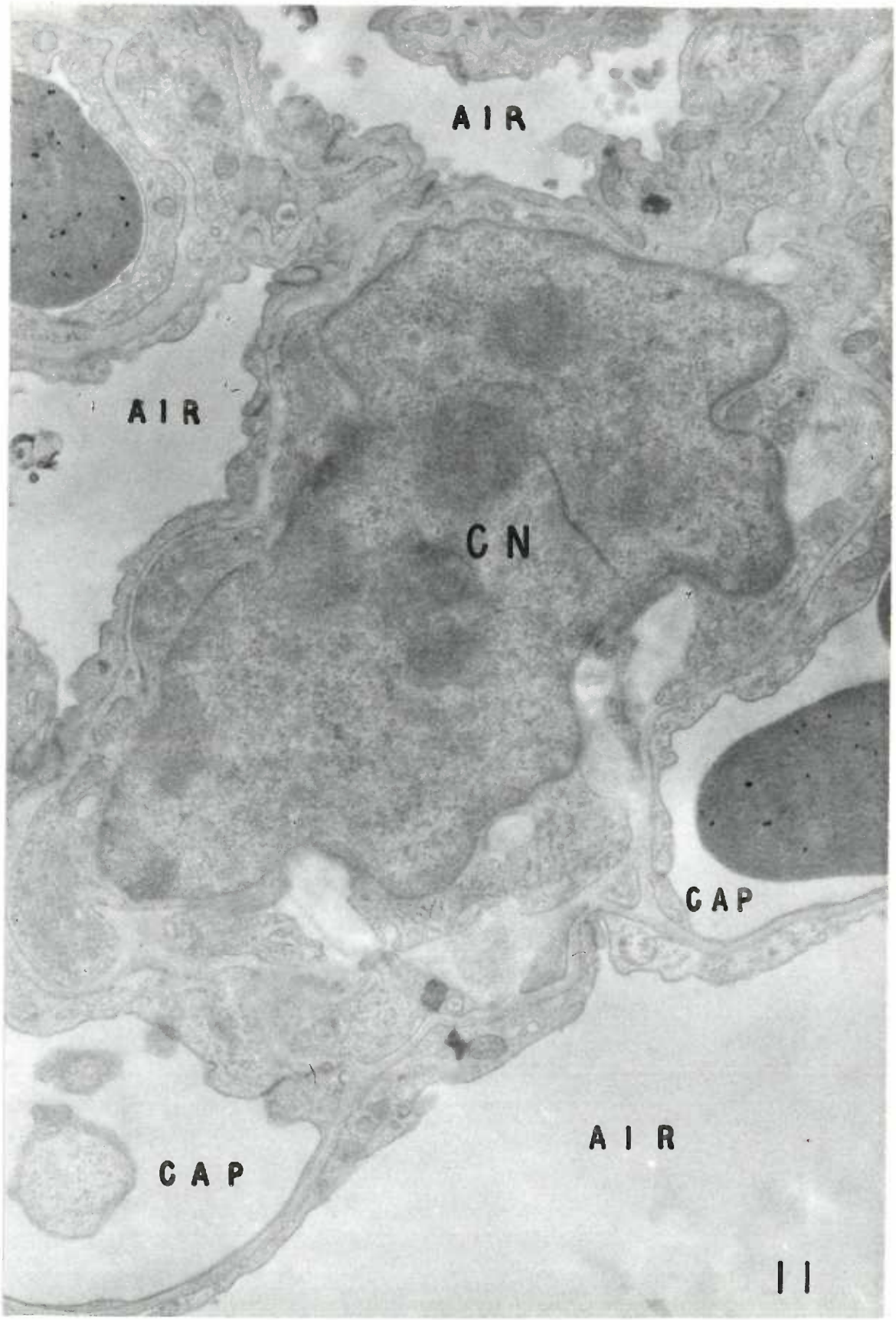

AIR
AIR
CN
CAP
CAP
AIR
11

Figure 12

Ciliated terminal bronchiole cell. The ciliated cell of the terminal bronchiole is a narrow columnar cell with only a few short cilia, CIL. The cytoplasm contains moderate numbers of mitochondria, a small Golgi apparatus, GA, and a very small amount of endoplasmic reticulum. Ciliary basal bodies, BB, can be seen at the apex of the cell.

Magnification - 18,000 X

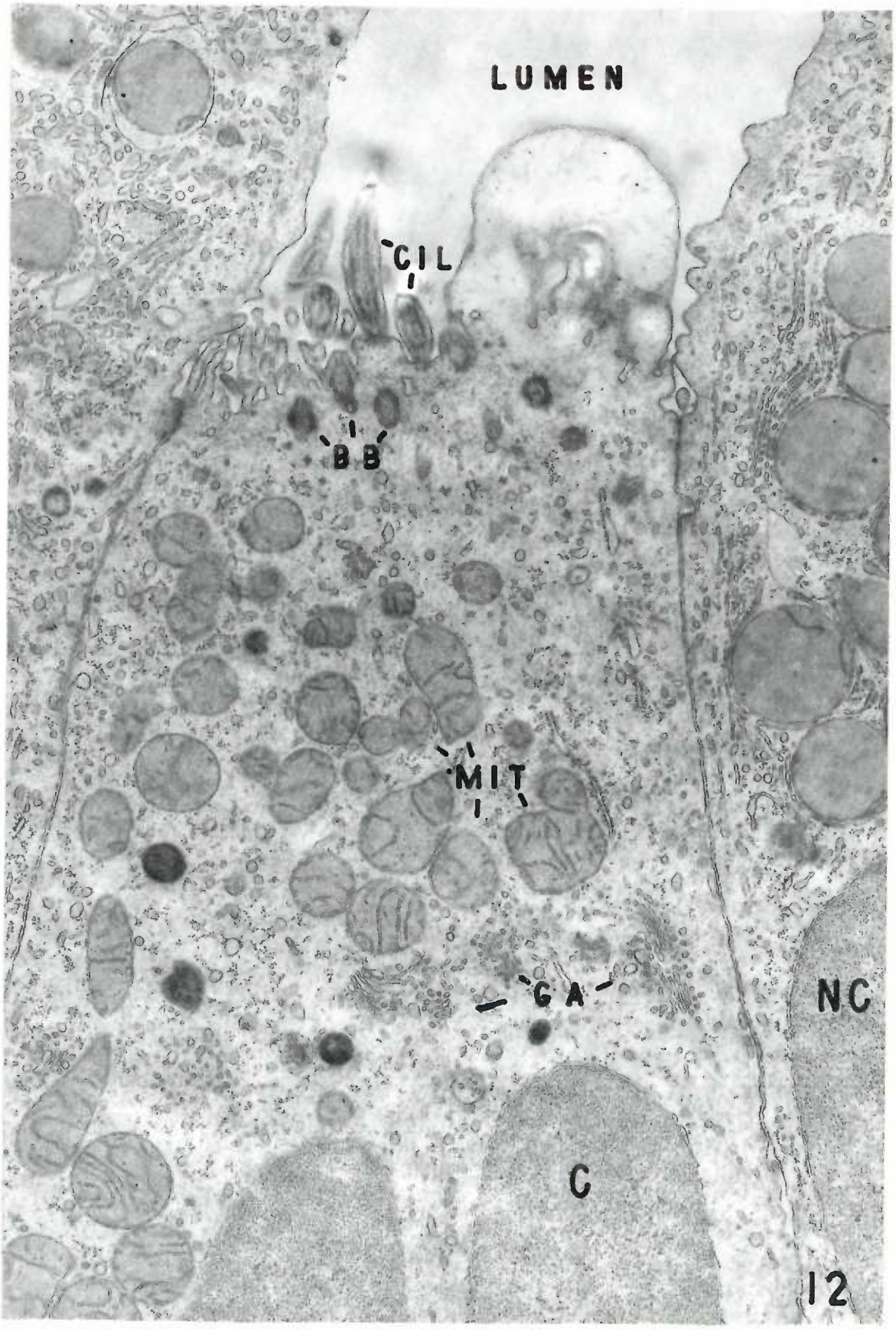

LUMEN
CIL
BB
MIT
GA
NC
C
12

Figure 13

Non-ciliated terminal bronchiole cell. About two-thirds of the cells lining the distal end of the terminal bronchiole are non-ciliated. The function of these cells is not known. The cytoplasm contains many spherical mitochondria characterized by an almost complete absence of internal membranes. A very large amount of agranular endoplasmic reticulum extends from the nuclear zone to the apex of the cell. Part of this reticulum is observed surrounding the periphery of the mitochondria.

Magnification - 14,700 X

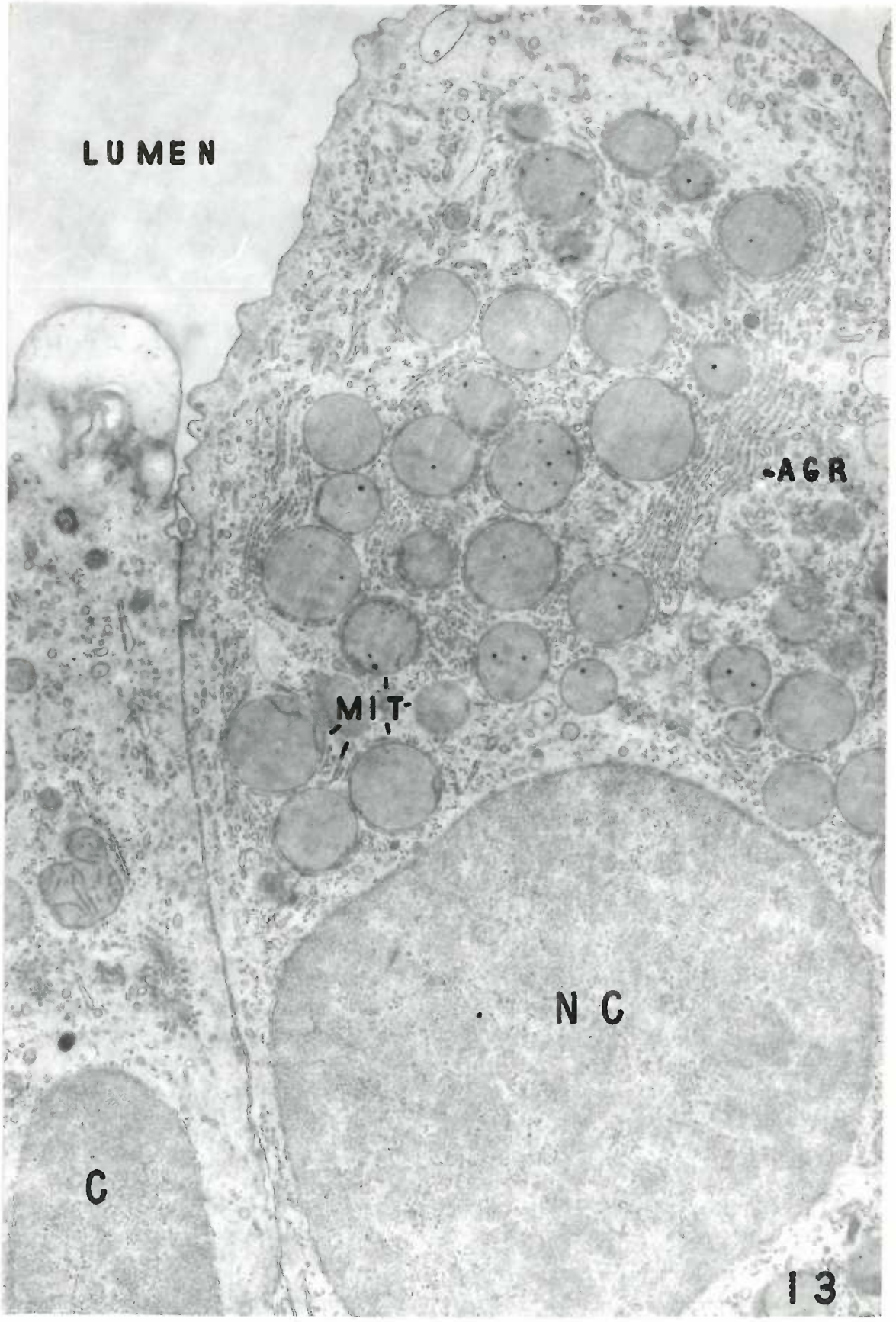
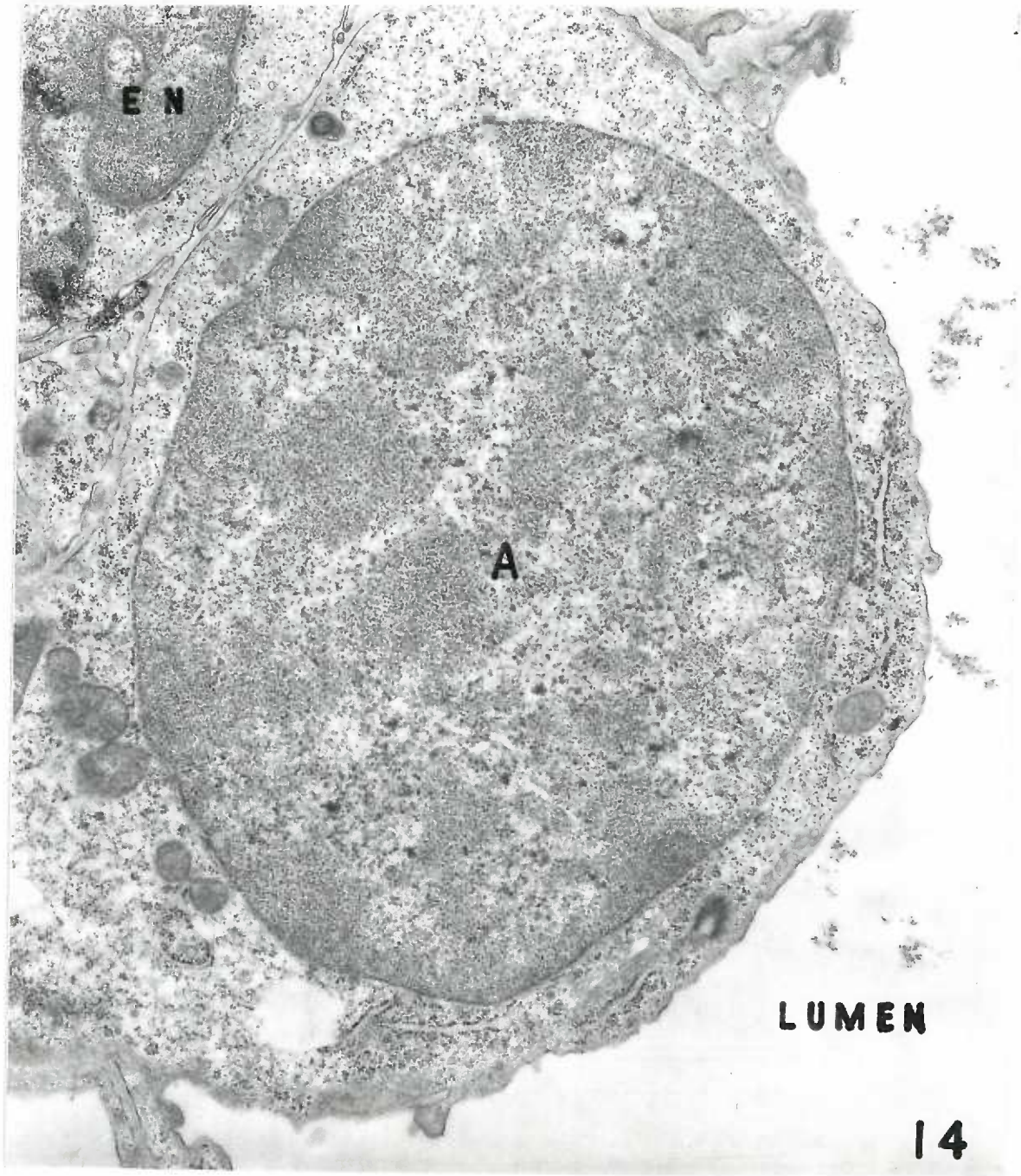

LUMEN
AGR
MIT
NC
C
13

Figure 14

Type A alveolar cell of a 19 day old fetus. At 19 days of fetal age, the alveoli of the mouse lung are open. The type A cell shown has assumed a squamous form, but the basal part of the cell is still partially indented into the septal wall. At this fetal age the perinuclear cytoplasm of the type A cell contains abundant ribosomes, but very few microvesicles.

Magnification - 18,000 X



14

Figure 15

Type B alveolar cell of a 19 day old fetus. The type B cell of the 19 day old fetus is very similar in appearance to the same cell type in the adult. Numerous mitochondria and lamellar bodies are observed at this age. Microvilli occur at the apical cell border. This cell appears to be caught at the stage of emitting the membranous remnants of a lamellar body (arrow) from the cytoplasm.

Magnification - 18,000 X

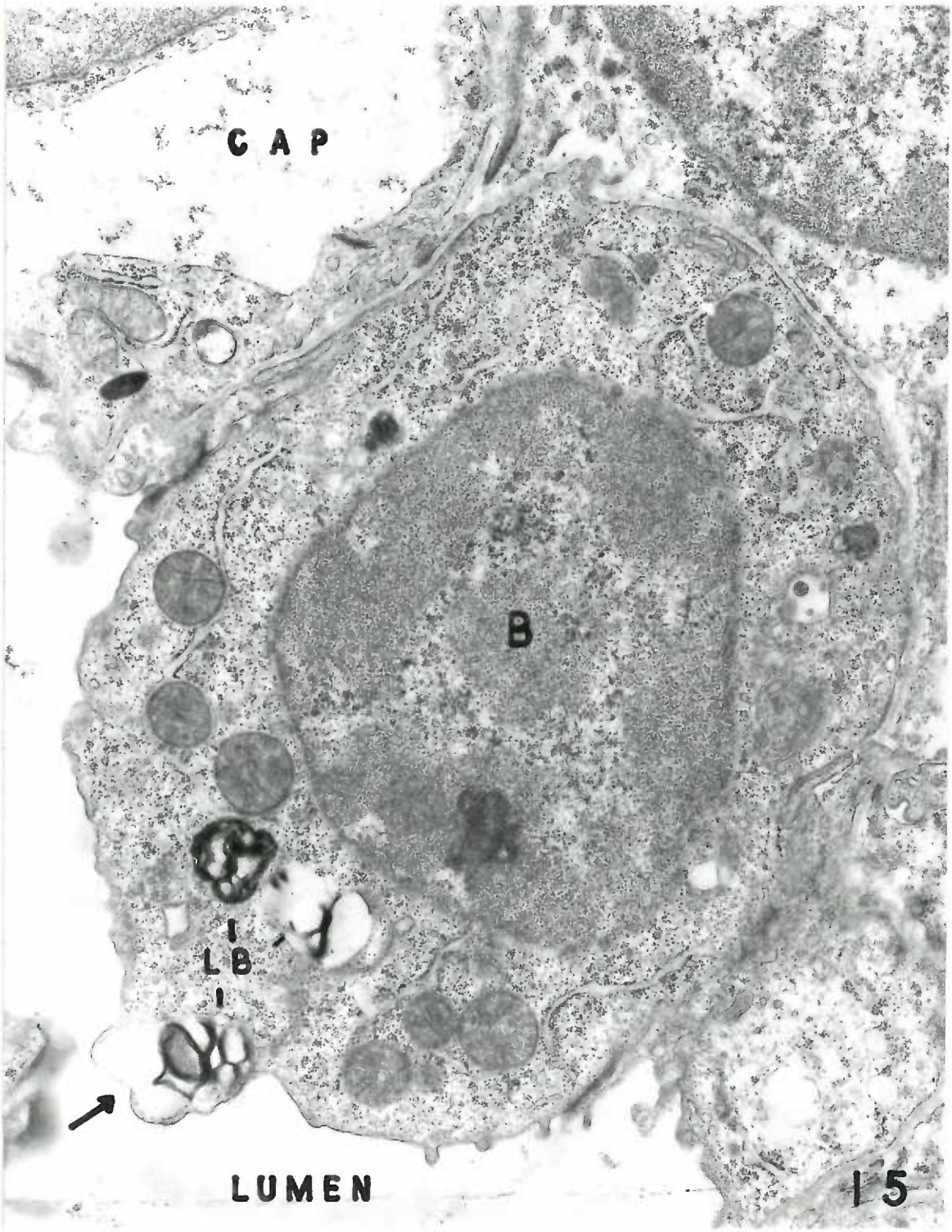
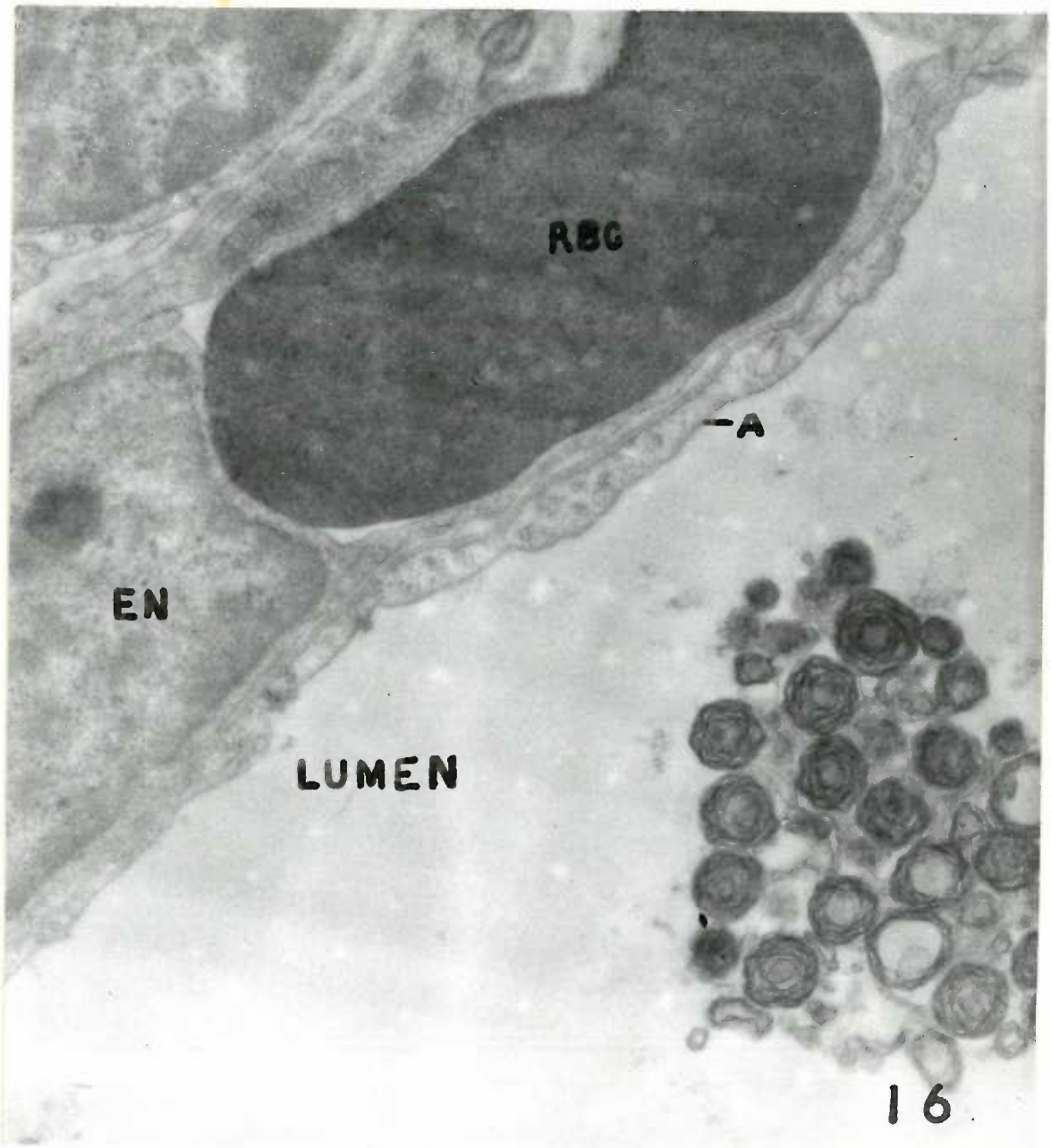

CAP
B
LB
LUMEN
15

Figure 16

Membranous material in the alveolus of a 19 day old fetus. As in the neonatal lung, masses of membranous material, in the form of circular whorls, are often observed in the alveoli of 19 day fetuses.

Magnification - 11,500 X



16

Figure 17

Terminal bronchiole cells of a 19 day fetal lung. Ciliated and non-ciliated cells are shown. The ciliated cell is well developed but the non-ciliated cell is still immature as evidenced by the lack of mitochondria, agranular endoplasmic reticulum, and other organelles of the mature cell. Moreover, the non-ciliated cell still retains a large pool of glycogen-like material, GL, typical of the lung cells in the later stages of fetal development.

Magnification - 10,700 X

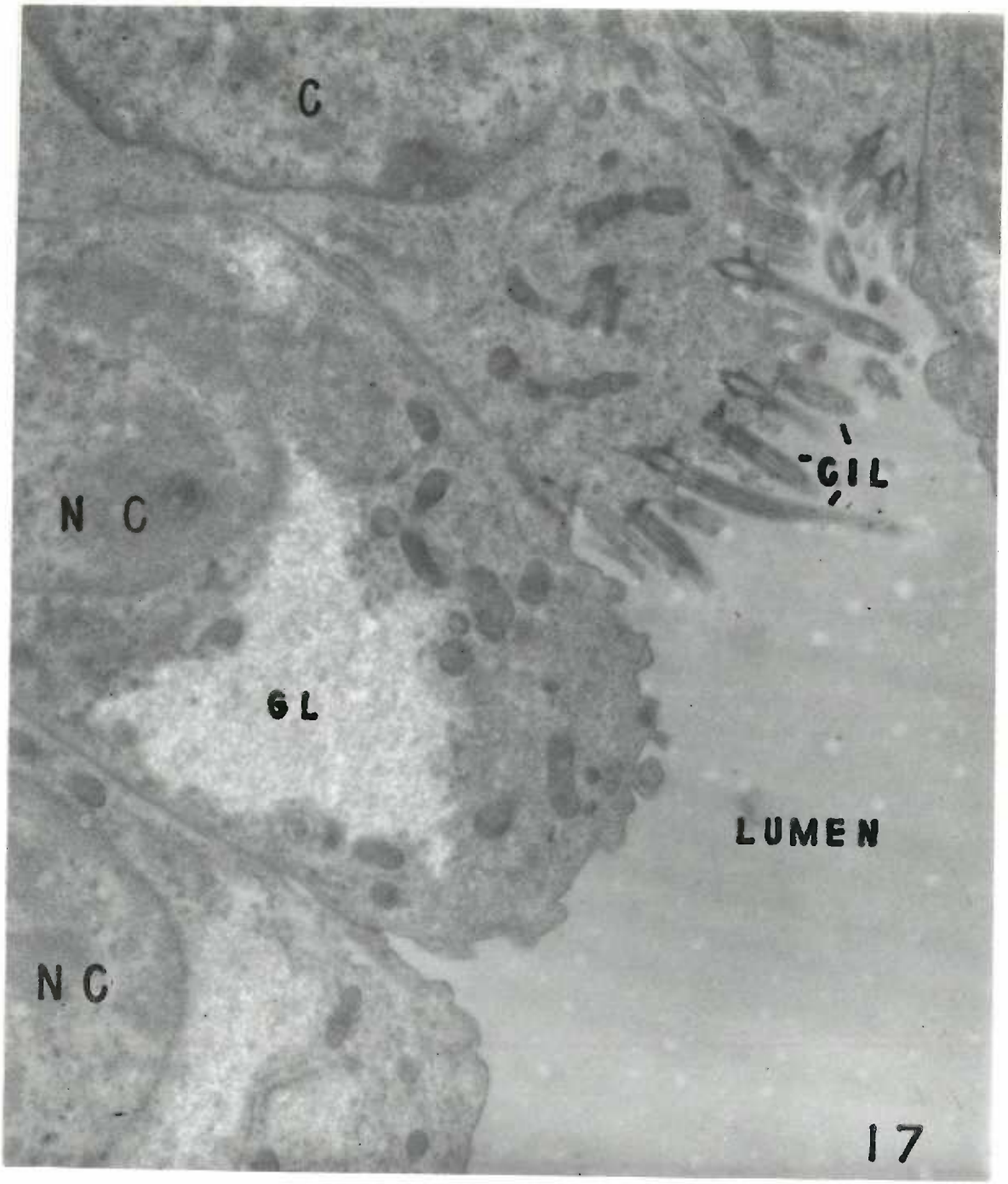

C
CIL
N C
GL
LUMEN
N C
17

Figure 18

Alveolus of an 18 day old fetus. At 18 days of fetal age, both open and closed alveoli can be found in the same lung. This picture shows a portion of an open alveolus. The septal wall is still very thick at this age. The type B alveolar cell is recognizable by the presence of lamellar bodies in the ctyoplasm.

Magnification - 6300 X

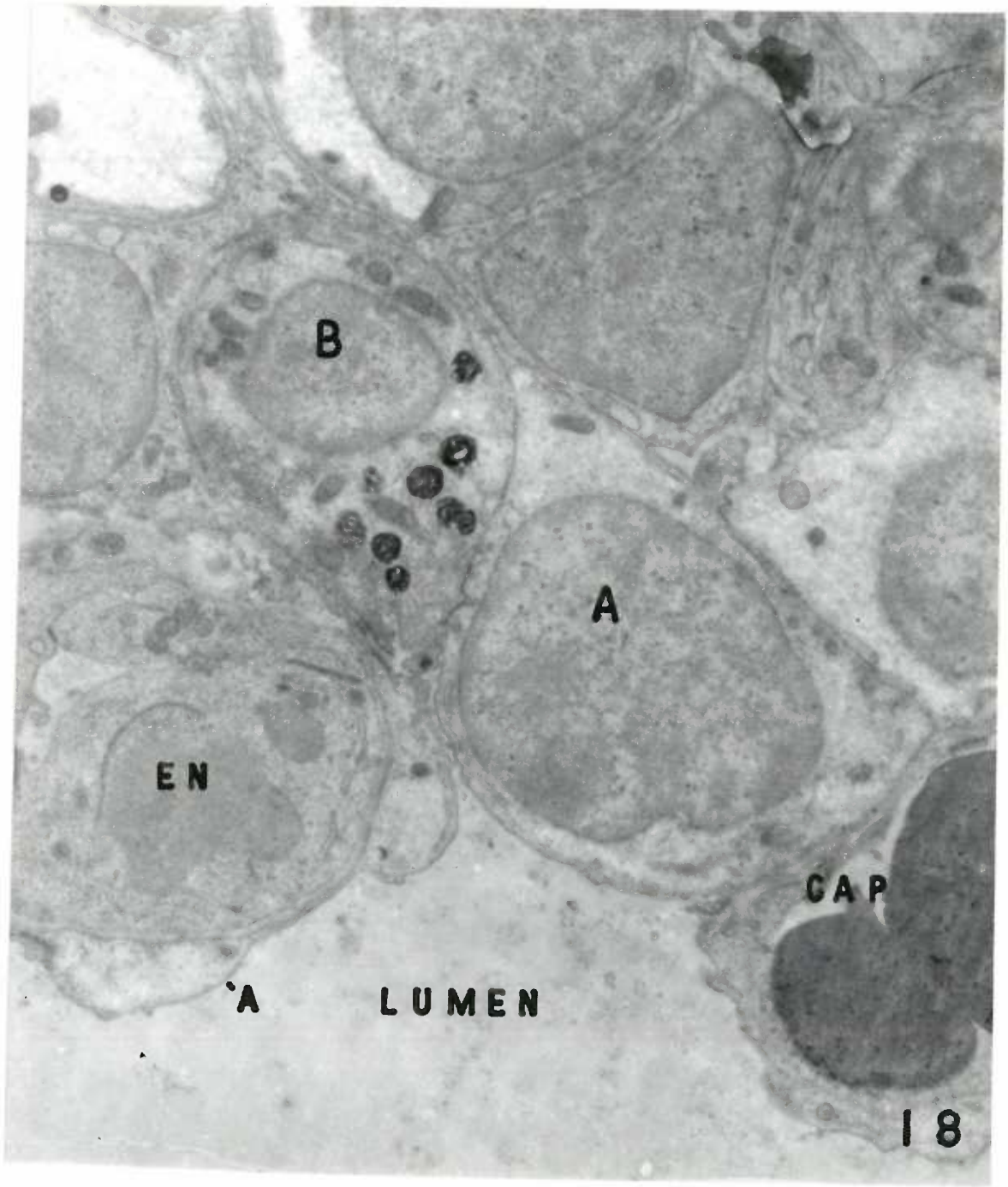

B
A
EN
CAP
LUMEN
18

Figure 19

Alveolar precursor cells in an 18 day old fetus. This closed alveolus contains cells which are rich in glycogen-like material. About half of the cells lining this alveolus are precursors of the type B cell as is evident from the presence of lamellar bodies.

Magnification – 10,700 X

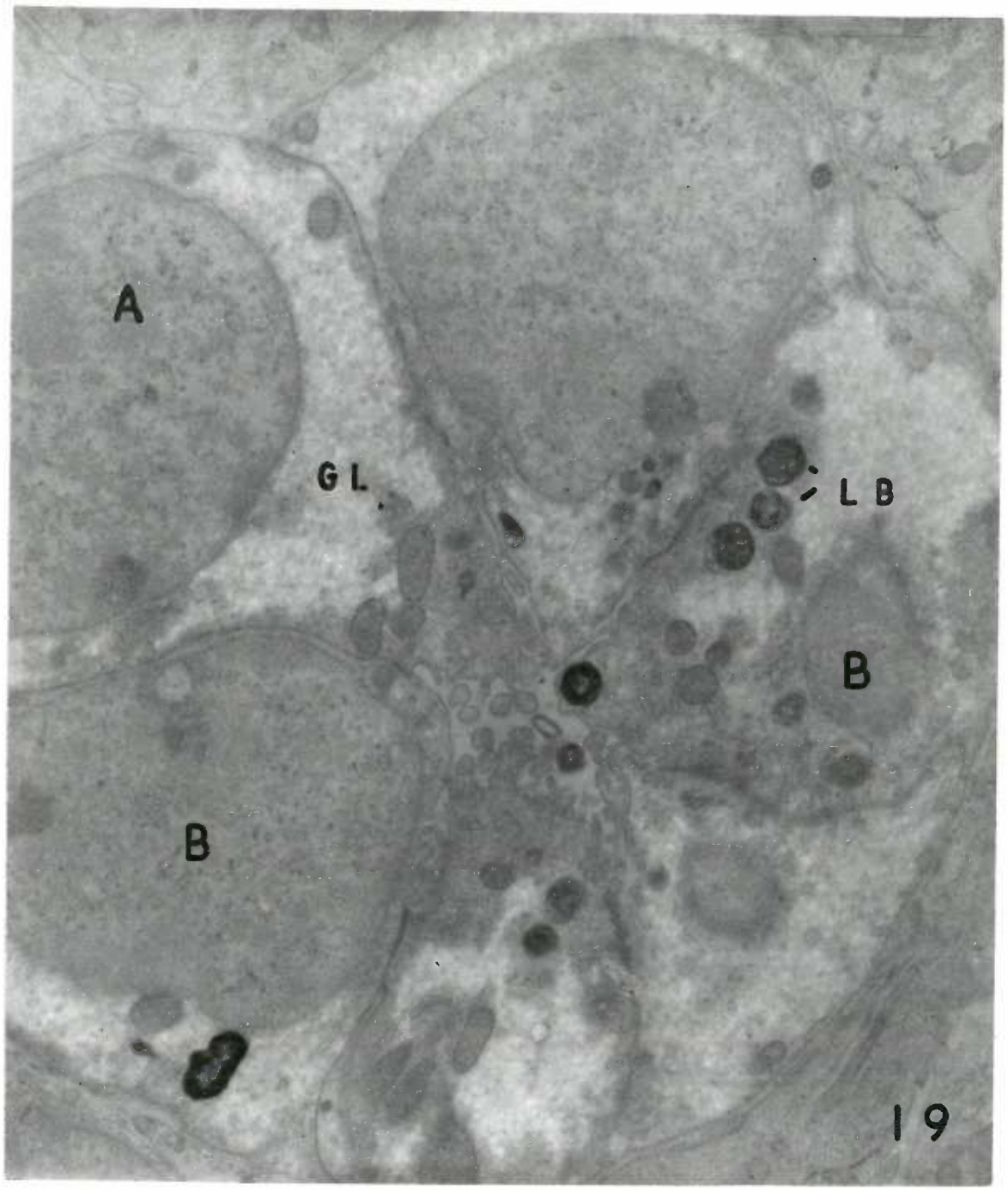

A
GL
LB
B
B
19

Figure 20

Alveolar precursor cells of a 17 day old fetus. At this age, the alveolar precursor cells are indistinguishable as to type. The cells form a solid cord. Glycogen-like material is not as abundant in the cytoplasm as at 18 days.

Magnification - 9400 X

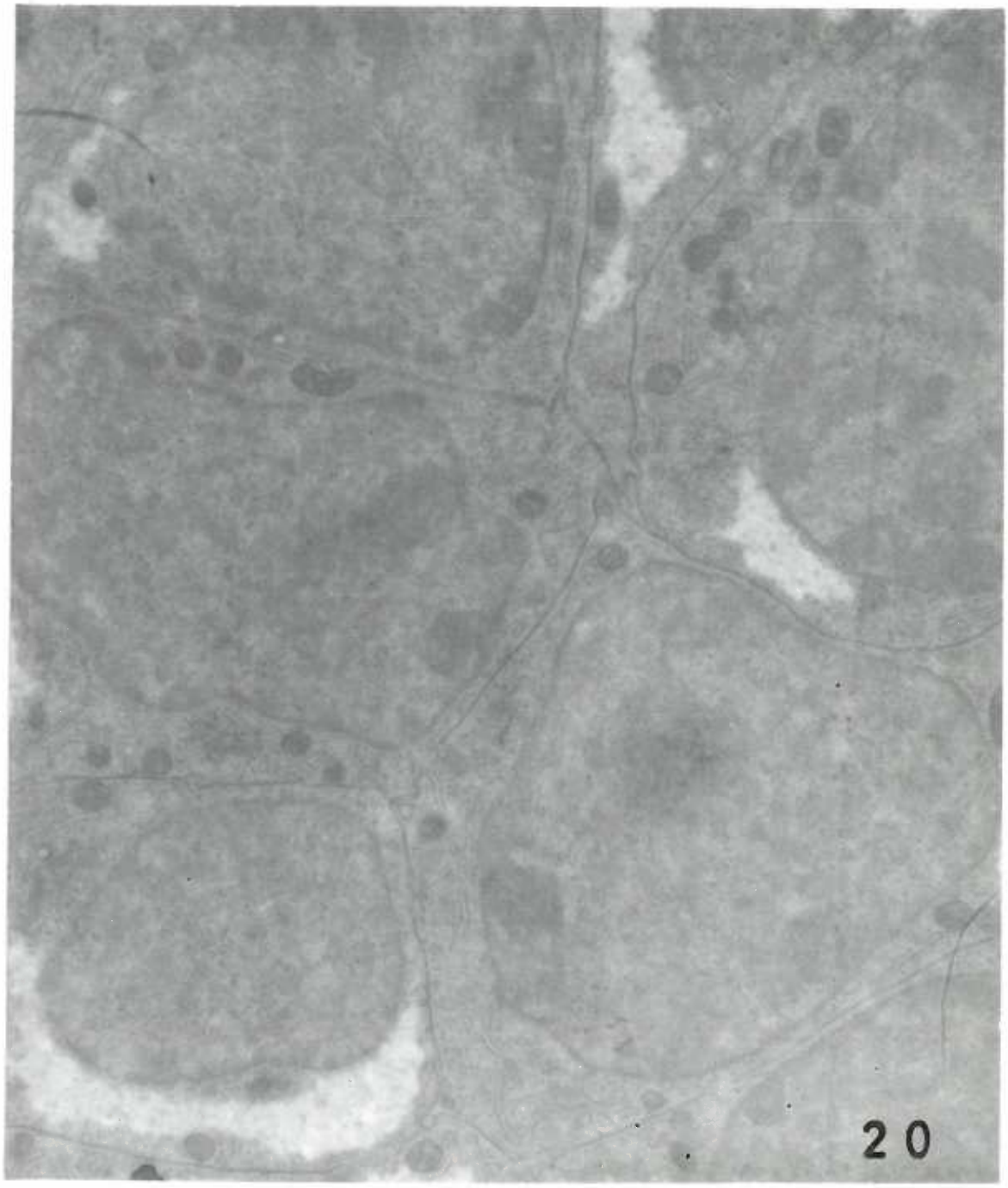

20

Figure 21

Alveolar precursor cells of a 16 day old fetus. As at 17 days of fetal age, the cells are arranged in solid cords. Glycogen-like material is almost entirely absent.

Magnification - 10,700 X

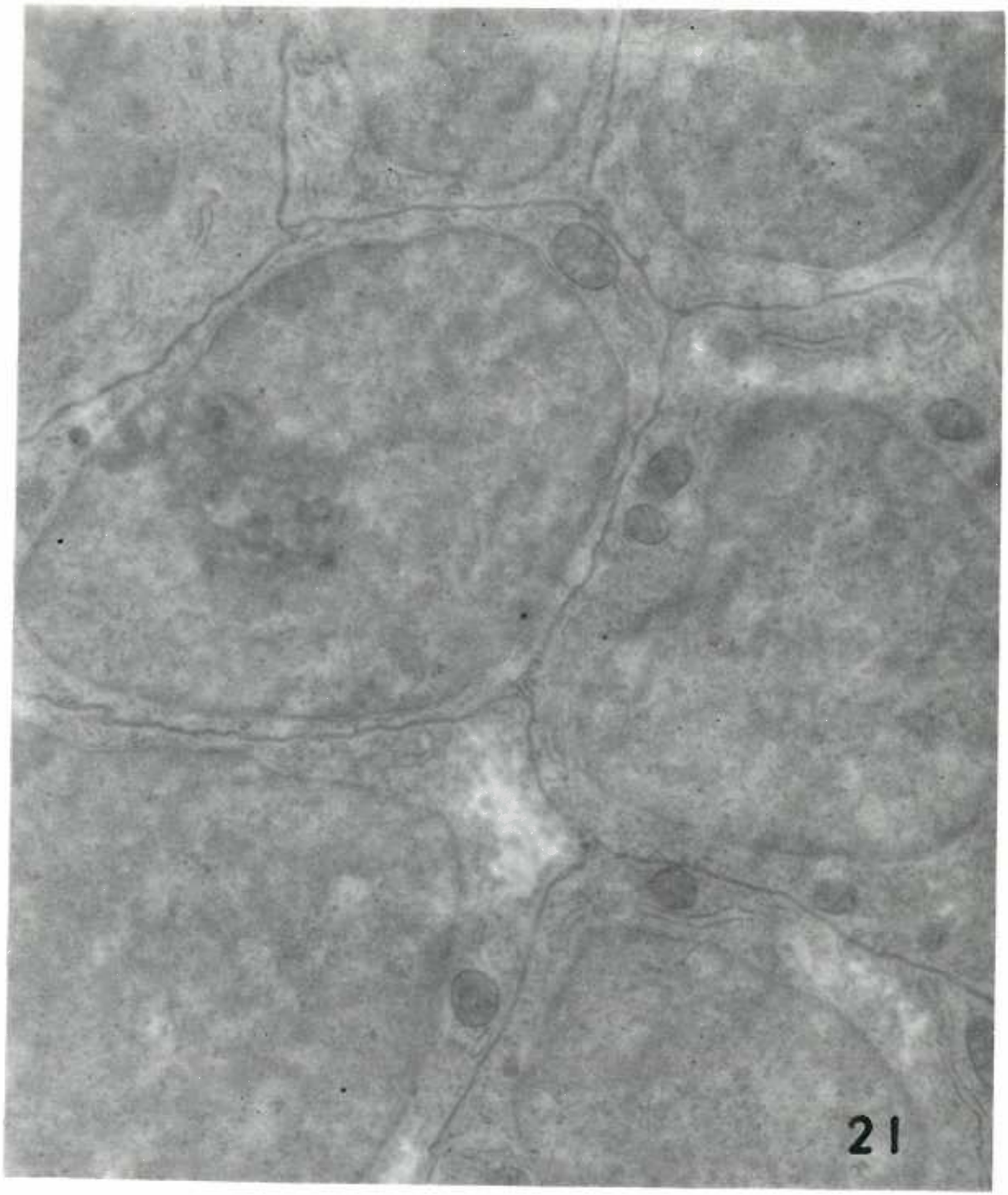

21

Figure 22

Alveolus of a 19 day old fetus which breathed for 2 hours. Expansion of a 19 day fetal lung by breathing for 2 hours does not significantly alter the morphology of the alveolar cells as compared to non-breathing 19 day lung. The type A cell is attenuated and the type B cell contains many lamellar bodies.

Magnification - 17,200 X

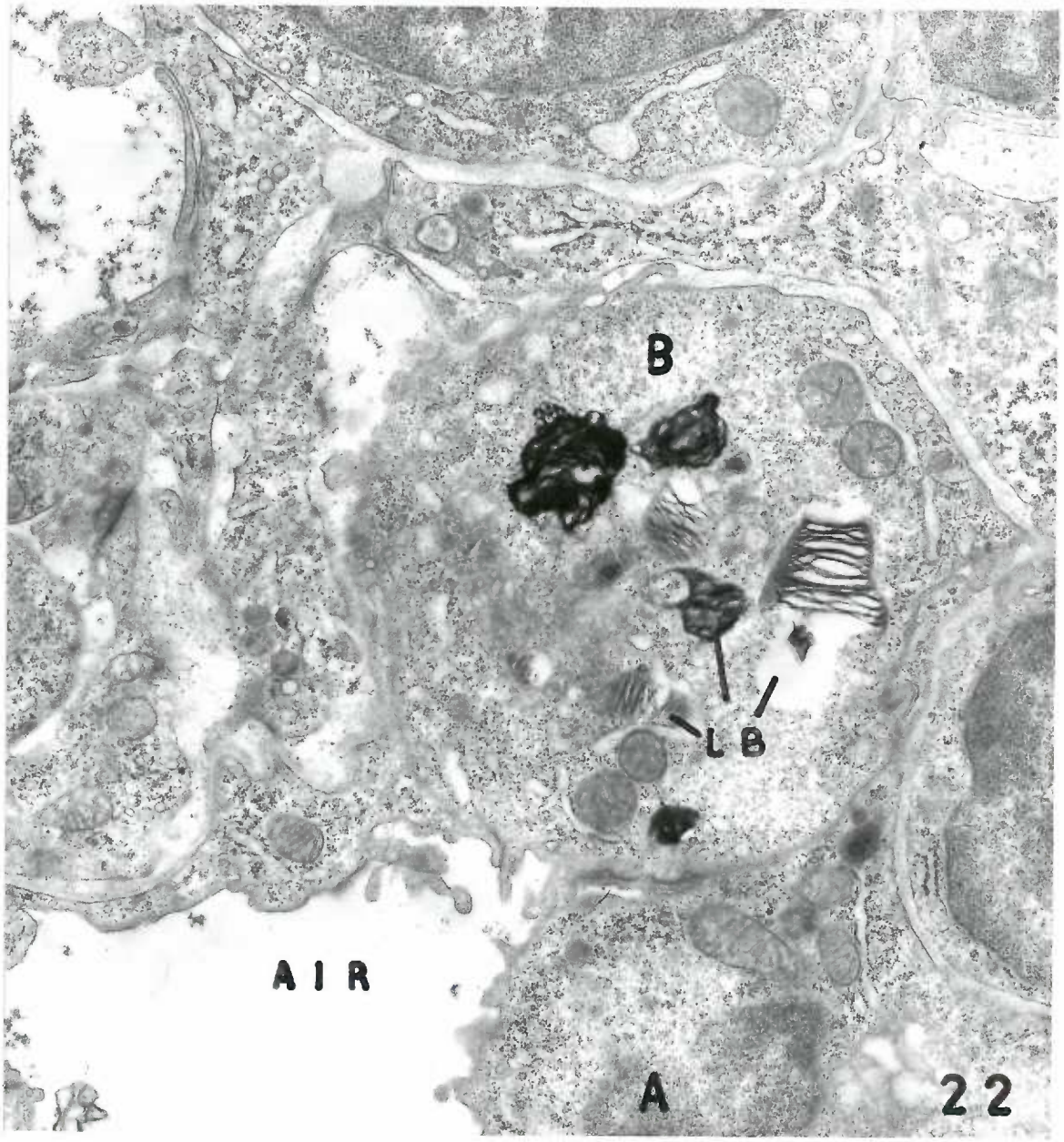

B
LB
AIR
A
22

Figure 23

Type B alveolar cell. This is the first of six pictures showing normal adult type B alveolar cells. The lamellar bodies in this series of pictures have been labelled as transformed mitochondria, TRM, in order to point to the numerous intermediate forms that exist between presumed early and late stages of mitochondrial alteration. The type B cell illustrated here has few mitochondria and only one transformed mitochondria in this section. The cytoplasm is rich in ribosomes but has relatively little endoplasmic reticulum.

Magnification - 15,800 X

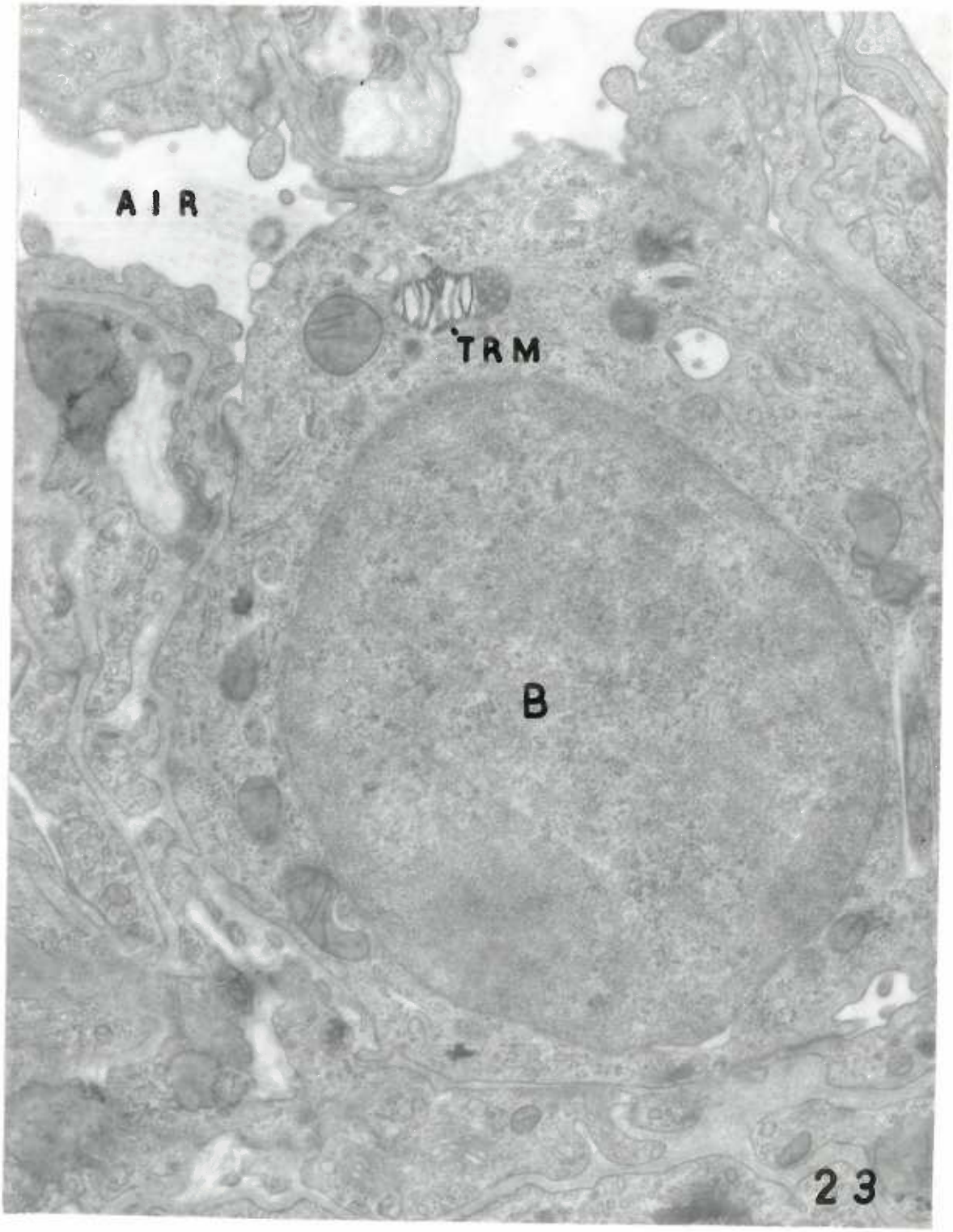

AIR
TRM
B
23

Figure 24

Type B alveolar cell. Several transformed mitochondria are present in the cytoplasm. This cell also has a number of long mitochondria which in section give the unusual forms noted by the arrows.

Magnification - 13,800 X

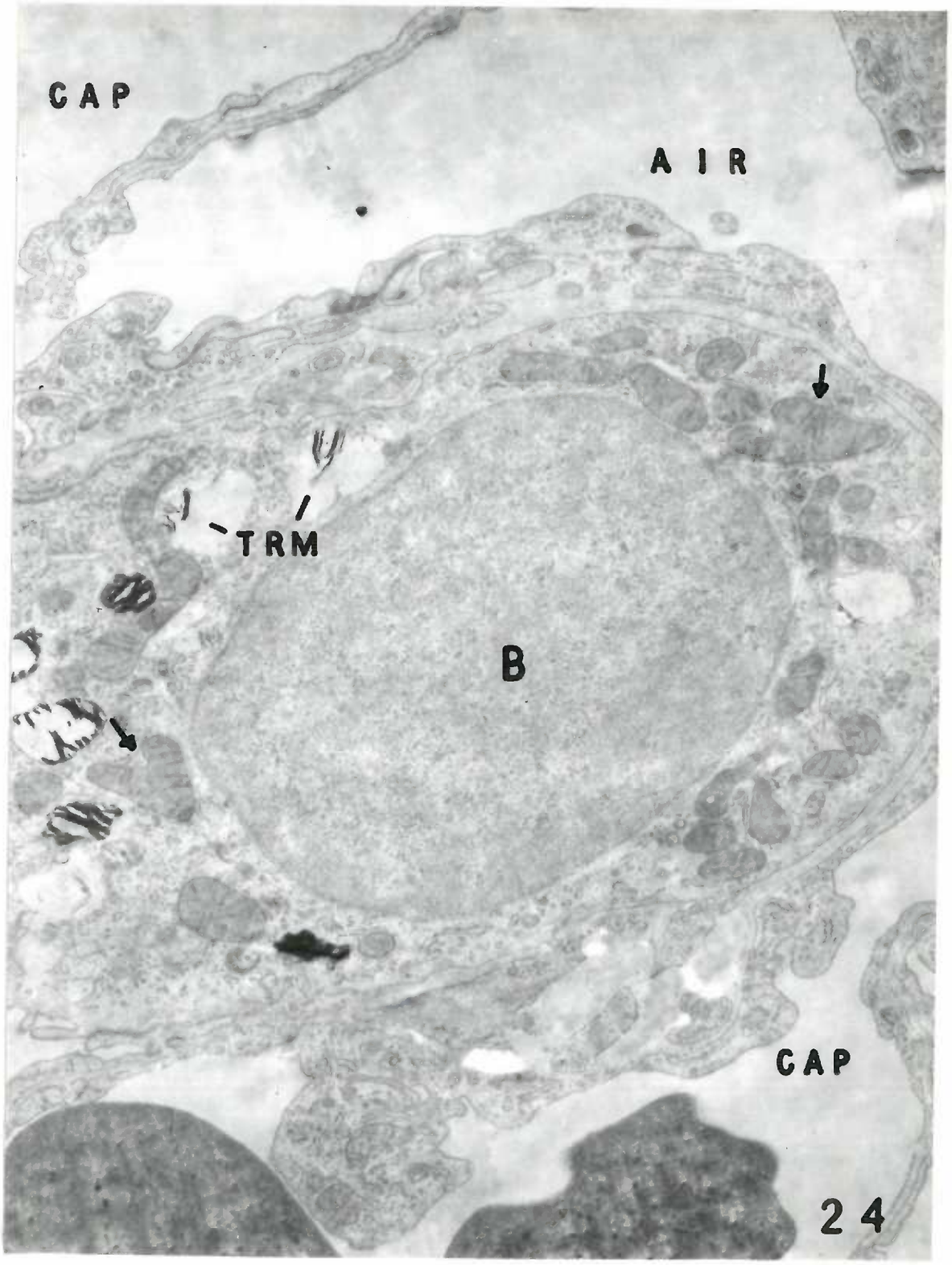

CAP
AIR
TRM
B
CAP
24

Figure 25

Type B alveolar cell. At higher magnification, the early stage of mitochondrial transformation is evident in this picture. The internal membranes of the transforming mitochondria are still partly intact and continue to extend from side to side. The upper transforming mitochondrium shows beginning clumping of the membranes.

Magnification - 42,200 X

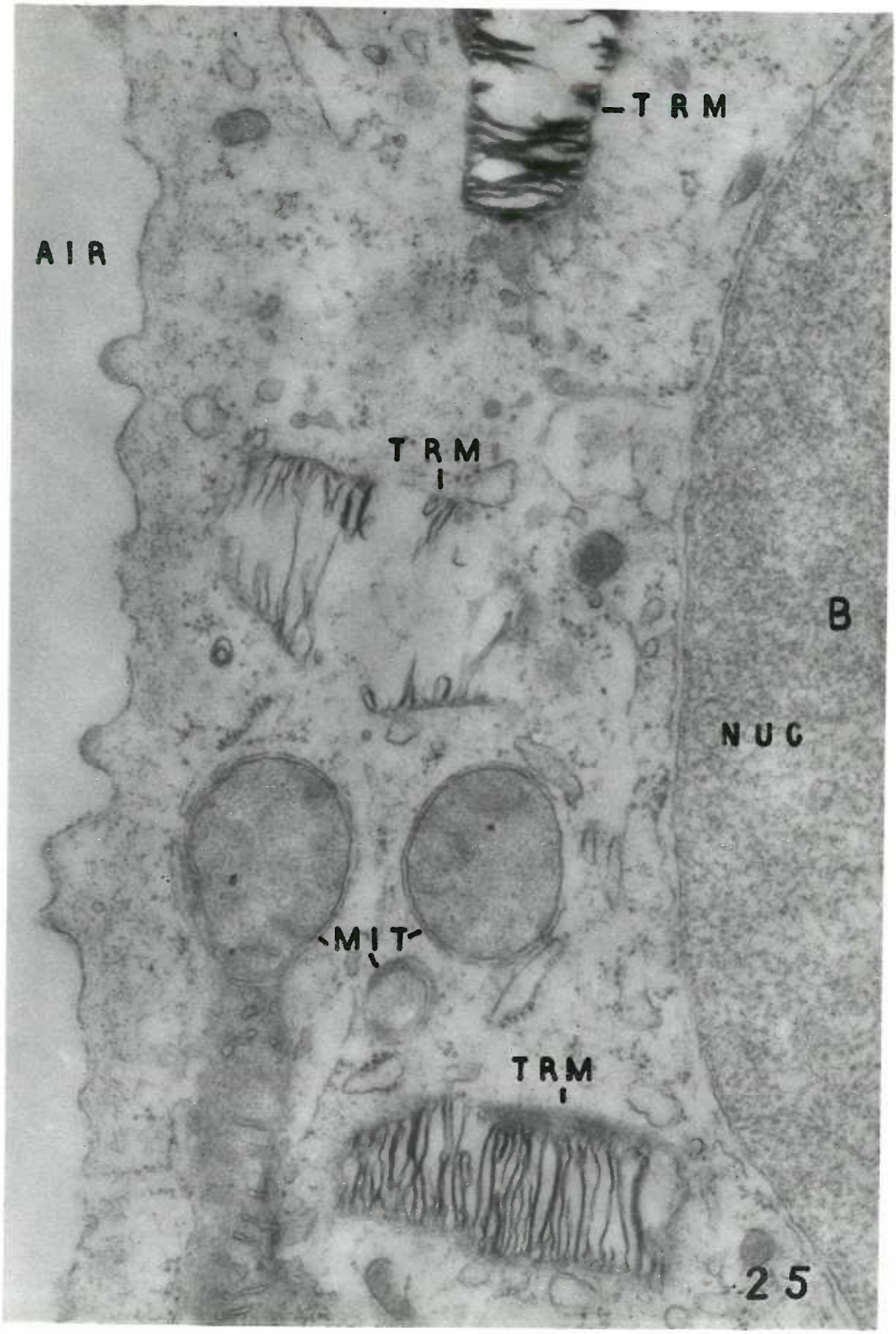

TRM
AIR
TRM
B
NUC
MIT
TRM
25

Figure 26

Type B alveolar cell. In this cell, the membranous remnants of the transforming mitochondria appear as coalesced dense material. These altered mitochondria are assumed to represent a later stage of alteration than those seen in the previous figure.

Magnification - 42,200 X

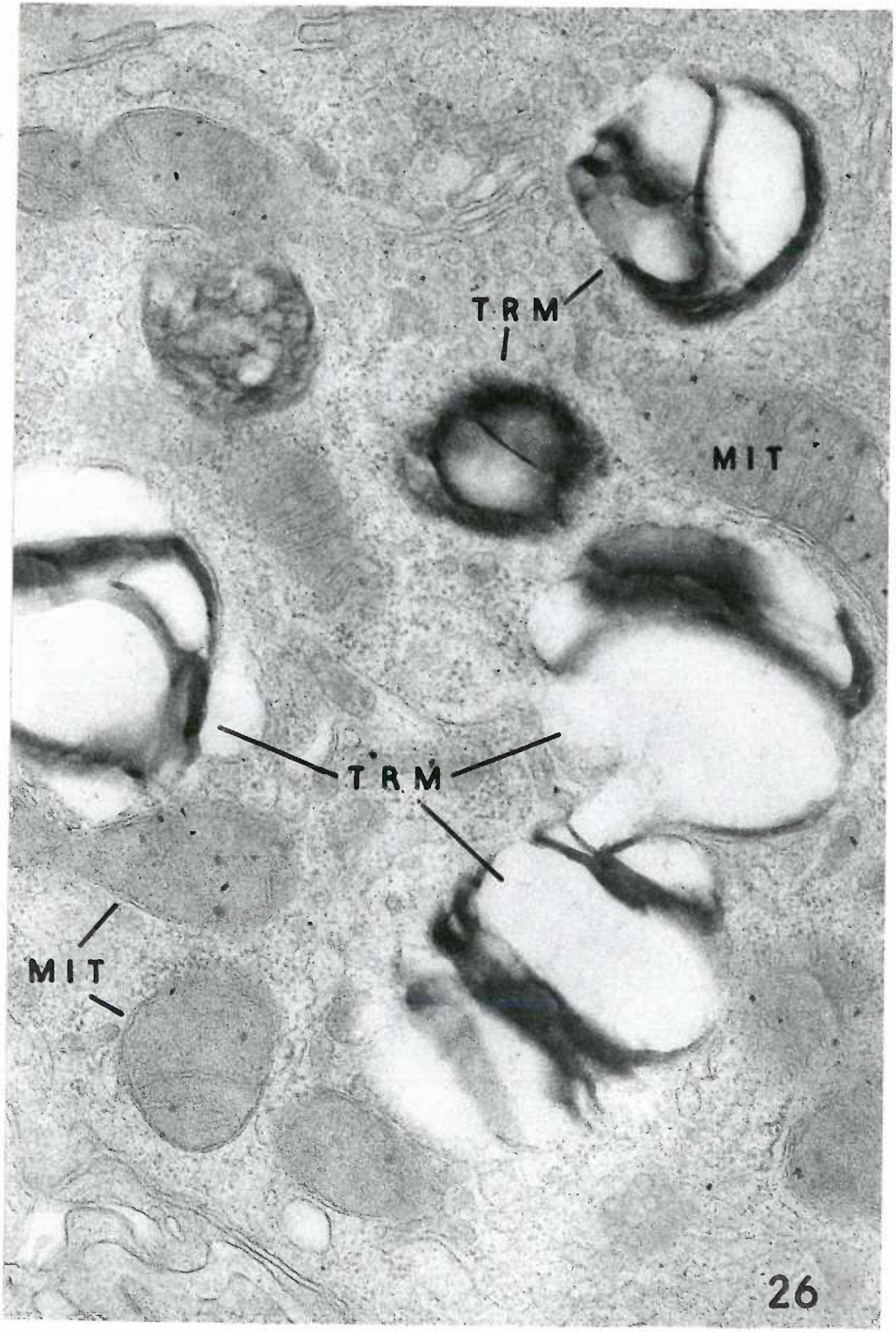

TRM
MIT
TRM
MIT
26

Figure 27

Type B alveolar cell. This picture, as the preceding one, shows various late forms of assumed mitochondrial transformation.

Magnification - 42,200 X

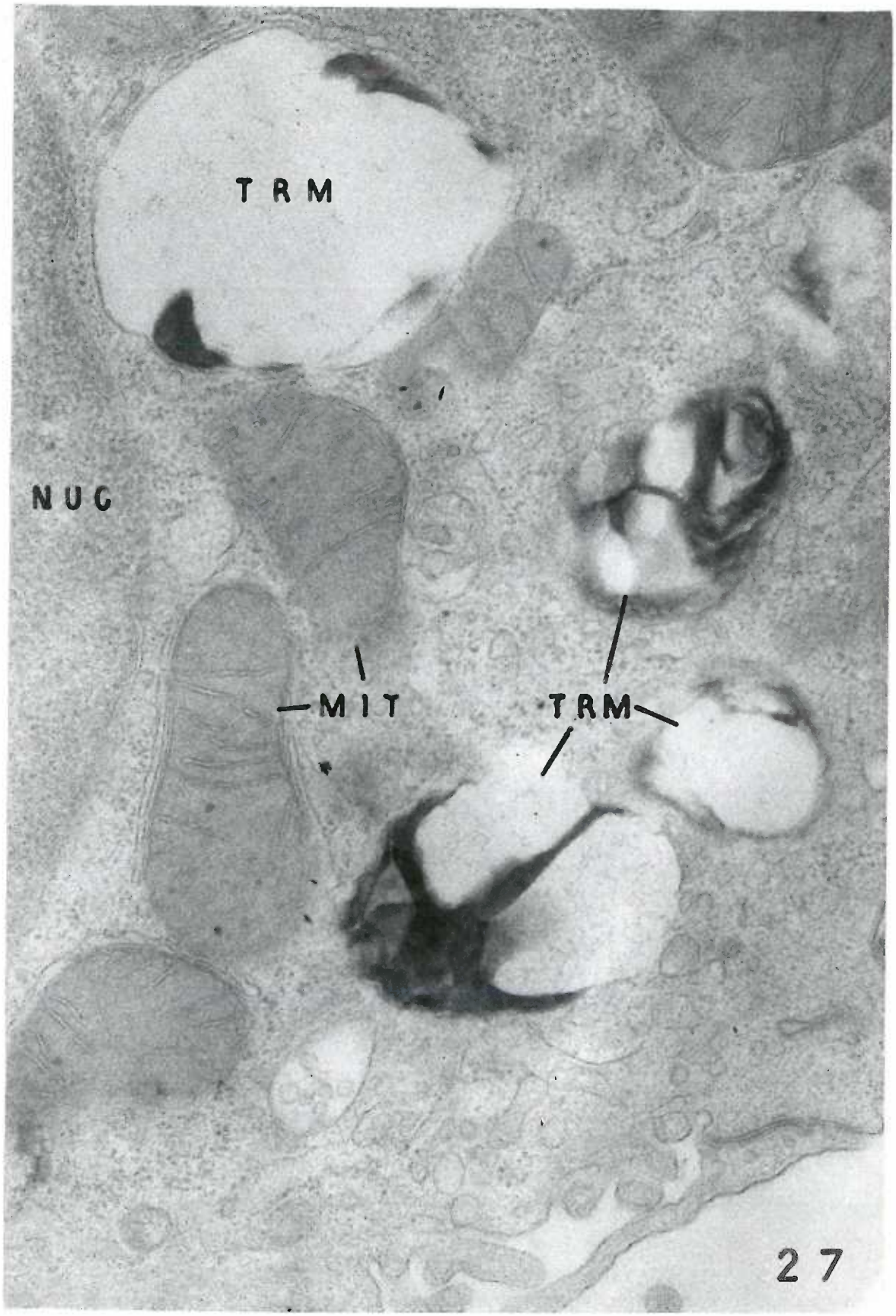

TRM
NUC
MIT
TRM
27

Figure 28

Type B alveolar cell. The basal portion of this type B cell contains two transformed mitochondria and a membrane-enclosed body filled with small vesicles (arrow). This latter body, seen also in cells from other parts of the organism, has been termed a multivesicular body. Its function is not known. The mitochondria in this illustration are surrounded by a circumferential layer of endoplasmic reticulum.

Magnification - 52,300 X

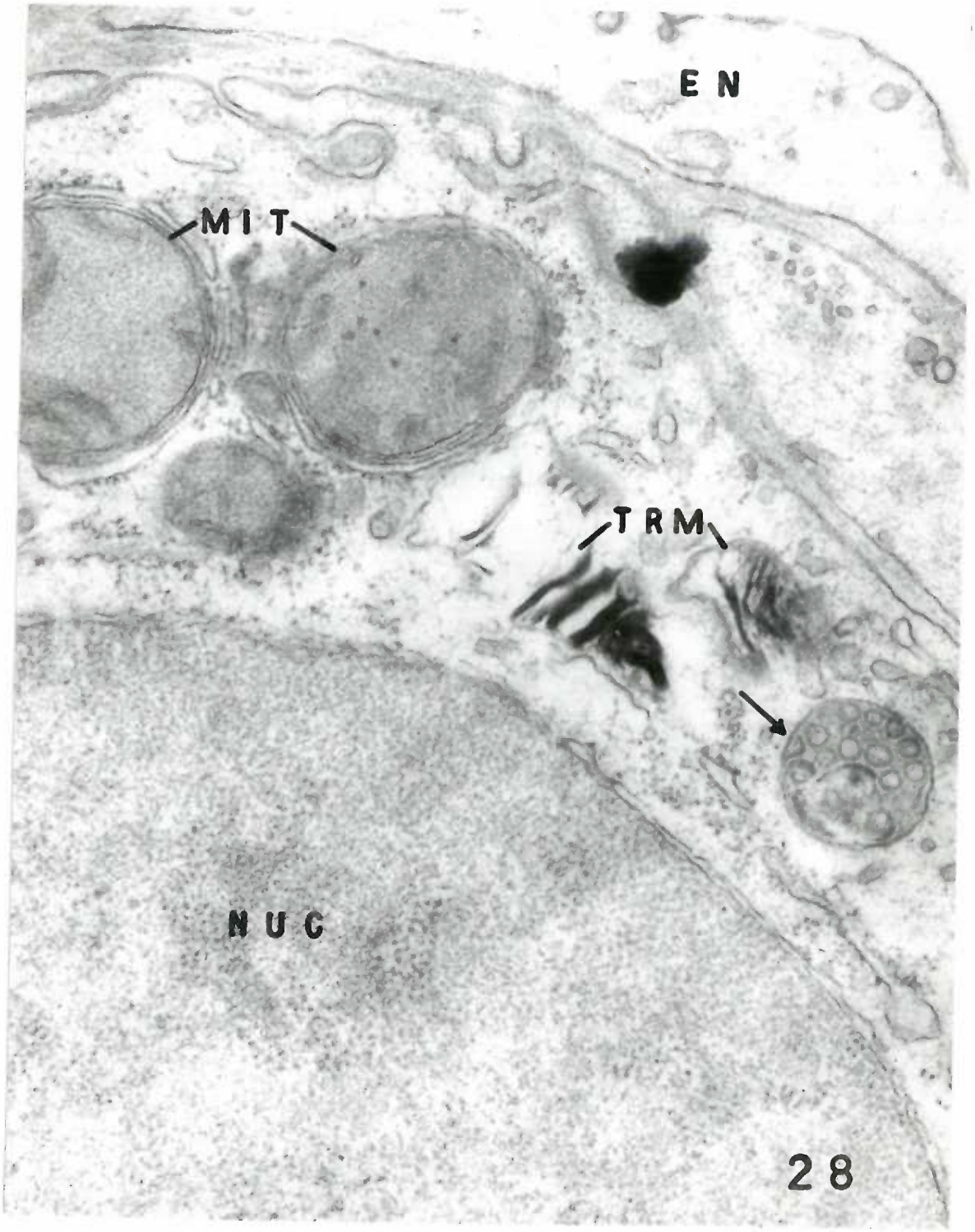
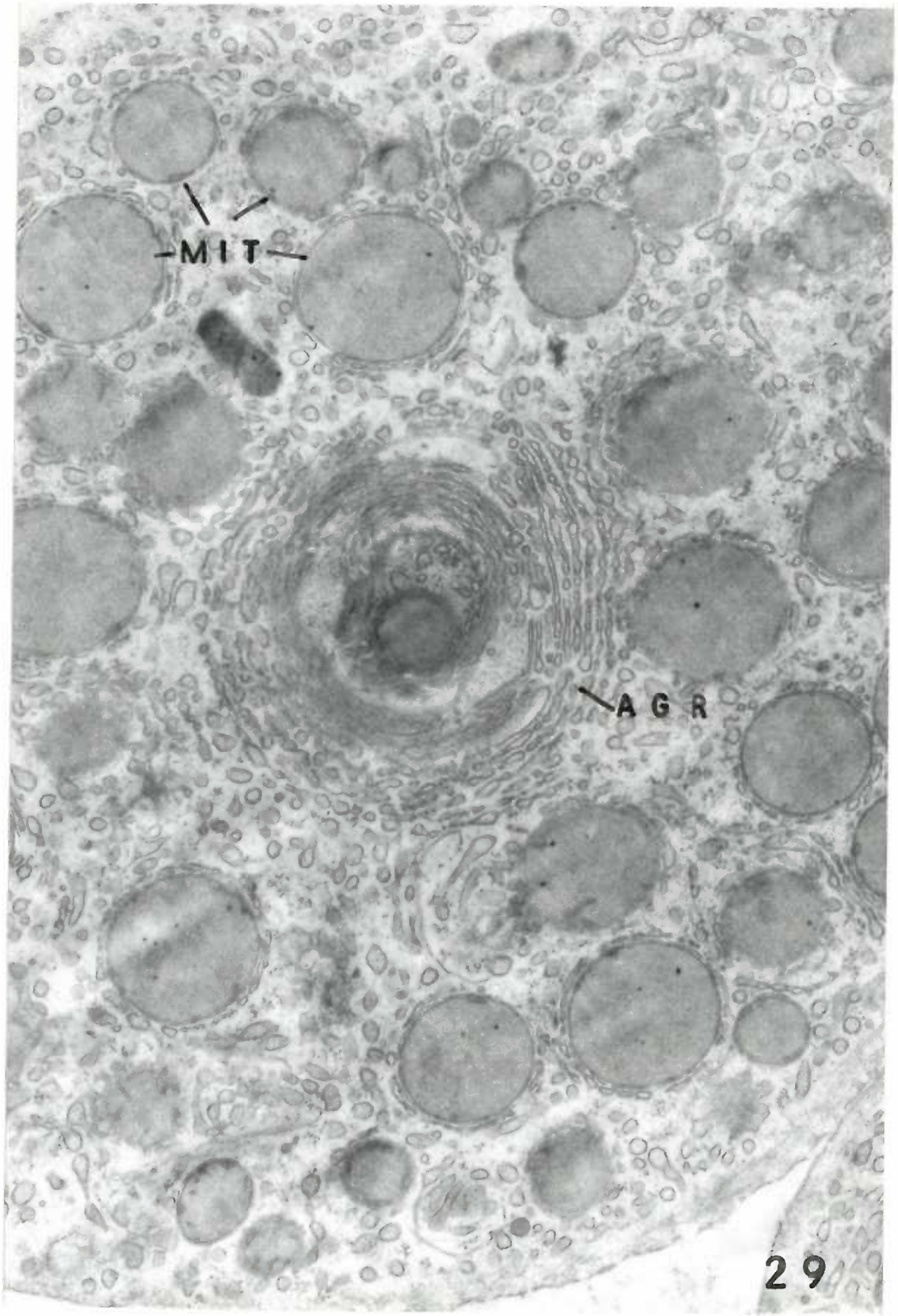

EN
MIT
TRM
NUC
28

Figure 29

Non-ciliated terminal bronchiole cell. This picture of a cross-sectioned non-ciliated bronchiolar cell shows the extensive development of the agranular reticulum, AGR. The mitochondria of this cell resemble the mitochondria of the type B cell shown in the previous figure.

Magnification - 27,200 X



29

Figure 30

Non-ciliated terminal bronchiole cell. This higher magnification picture of the non-ciliated bronchiolar cell reveals the peculiar form of the short mitochondrial internal membranes. Also, the circumferential relation of the agranular reticulum to the mitochondria is well seen.

Magnification - 49,700 X

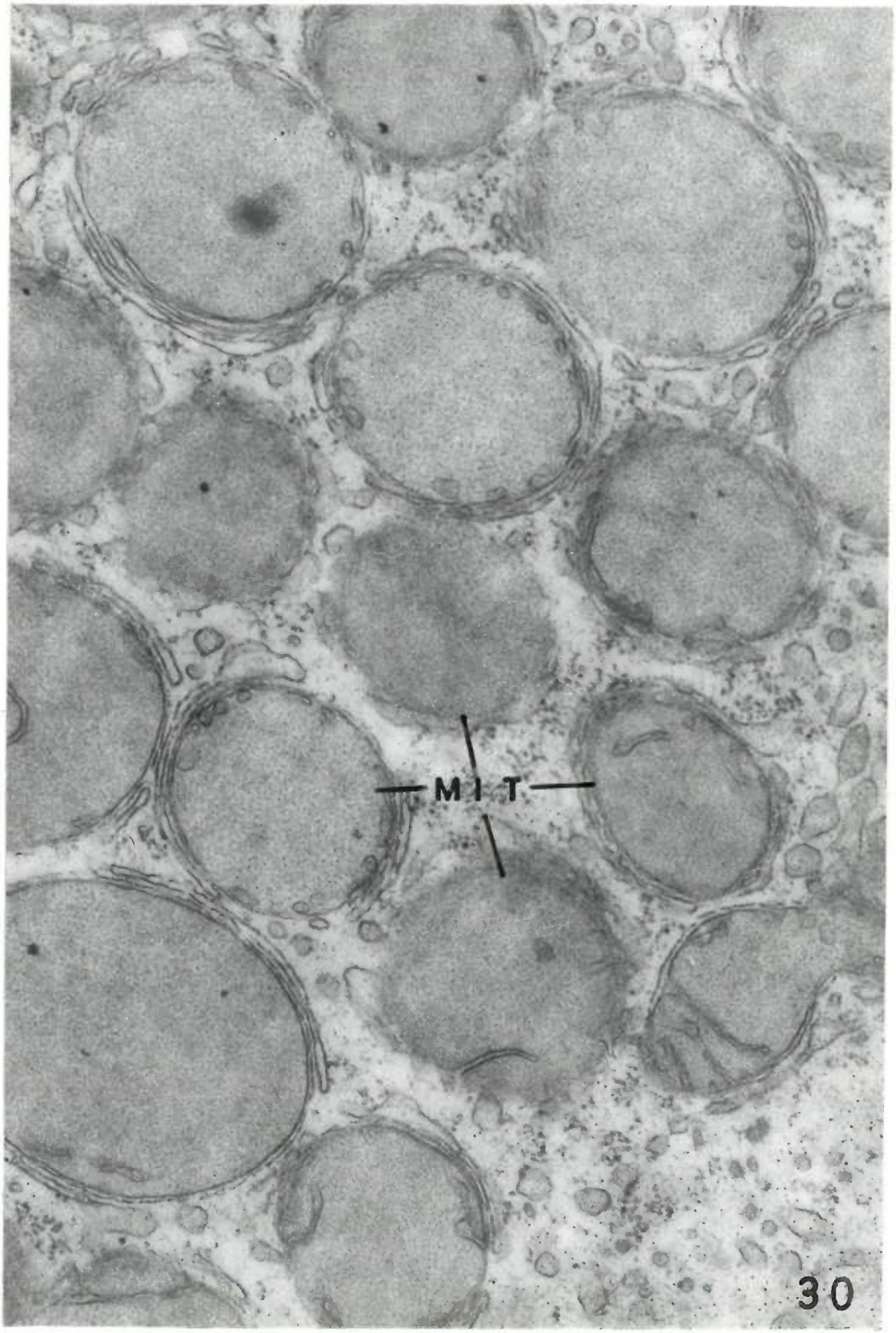

M
I
T
30

Figure 31

Normal lung. This picture, and the next, shows at higher magnification the pinocytotic activity of alveolar epithelium and capillary endothelium. Minimal pinocytotic activity is observed in this area. Relatively few pinocytotic vesicles, PV, are present in the small area of cytoplasm shown. In the endothelial cell, there appears to be an association between the pinocytotic vesicles and the endoplasmic reticulum, ER, (arrows).

Magnification - 52,300 X

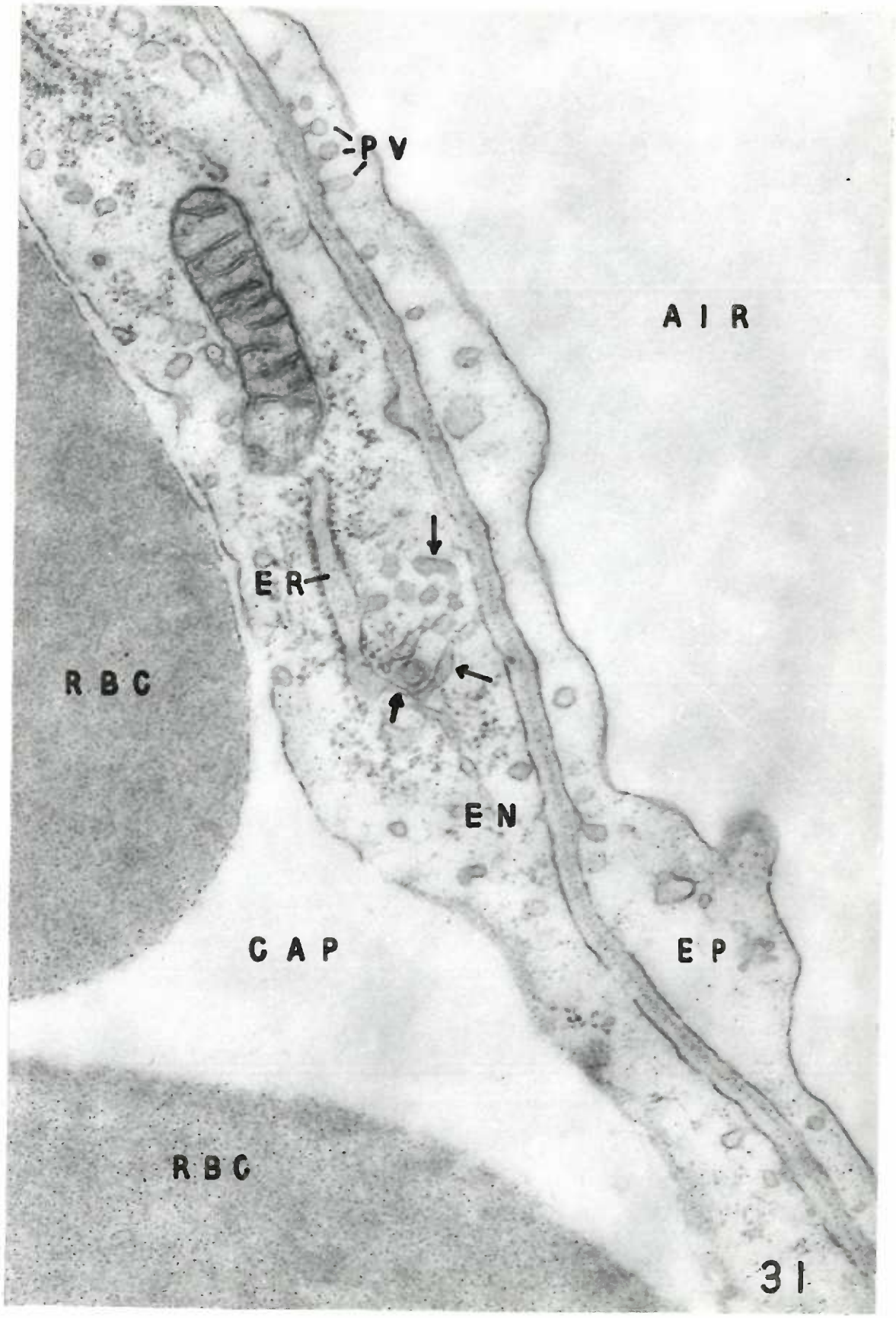

PV
AIR
ER
RBC
EN
CAP
EP
RBC
31

Figure 32

Normal lung. This picture, from an area close to the one shown in the previous figure, illustrates pinocytotic activity in a region where there is a connective tissue space between endothelium and epithelium. Here, both endothelial cell and epithelial cell show a slight increase in the number of pinocytotic vesicles and cell membrane invaginations as compared to the previous picture.

Magnification - 52,300 X

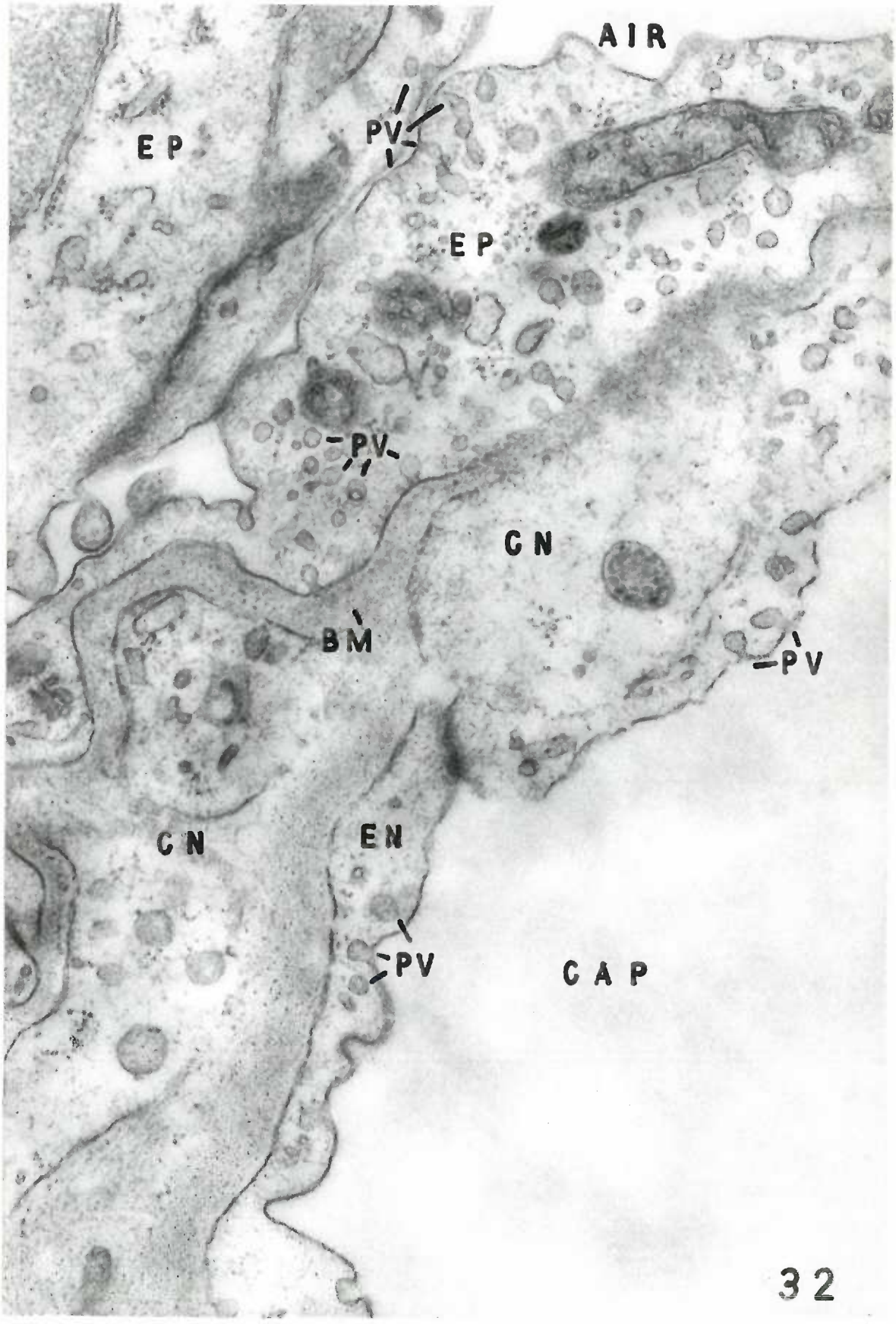

AIR
EP
PV
EP
PV
CN
BM
PV
CN
EN
PV
CAP

Figure 33

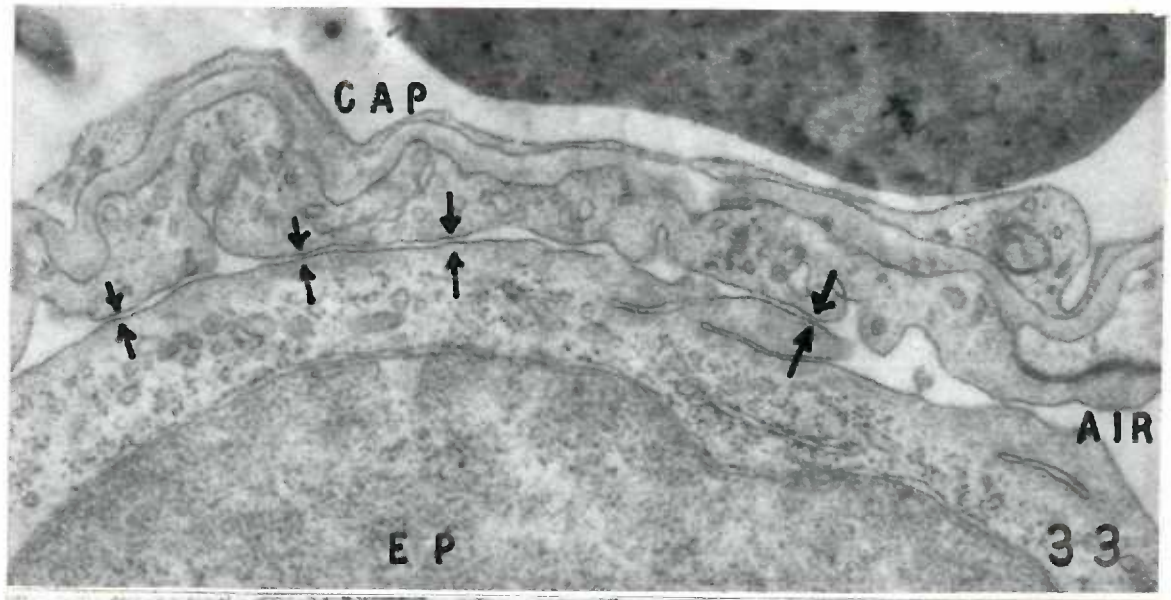
Normal lung. In a search for a hypothetical non-cellular surface film lining the alveolar cells, it is necessary to look in areas where opposing cells almost make contact. It is in such areas where the cell membranes of the opposing cells can be mostly clearly seen and where any layer that covers these cells should most readily be detected. If there was a layer covering each cell, then a total of four lines, including the cell membranes, should be seen. Such close contacts are shown at various points (arrows), but no four-line combination is observed.

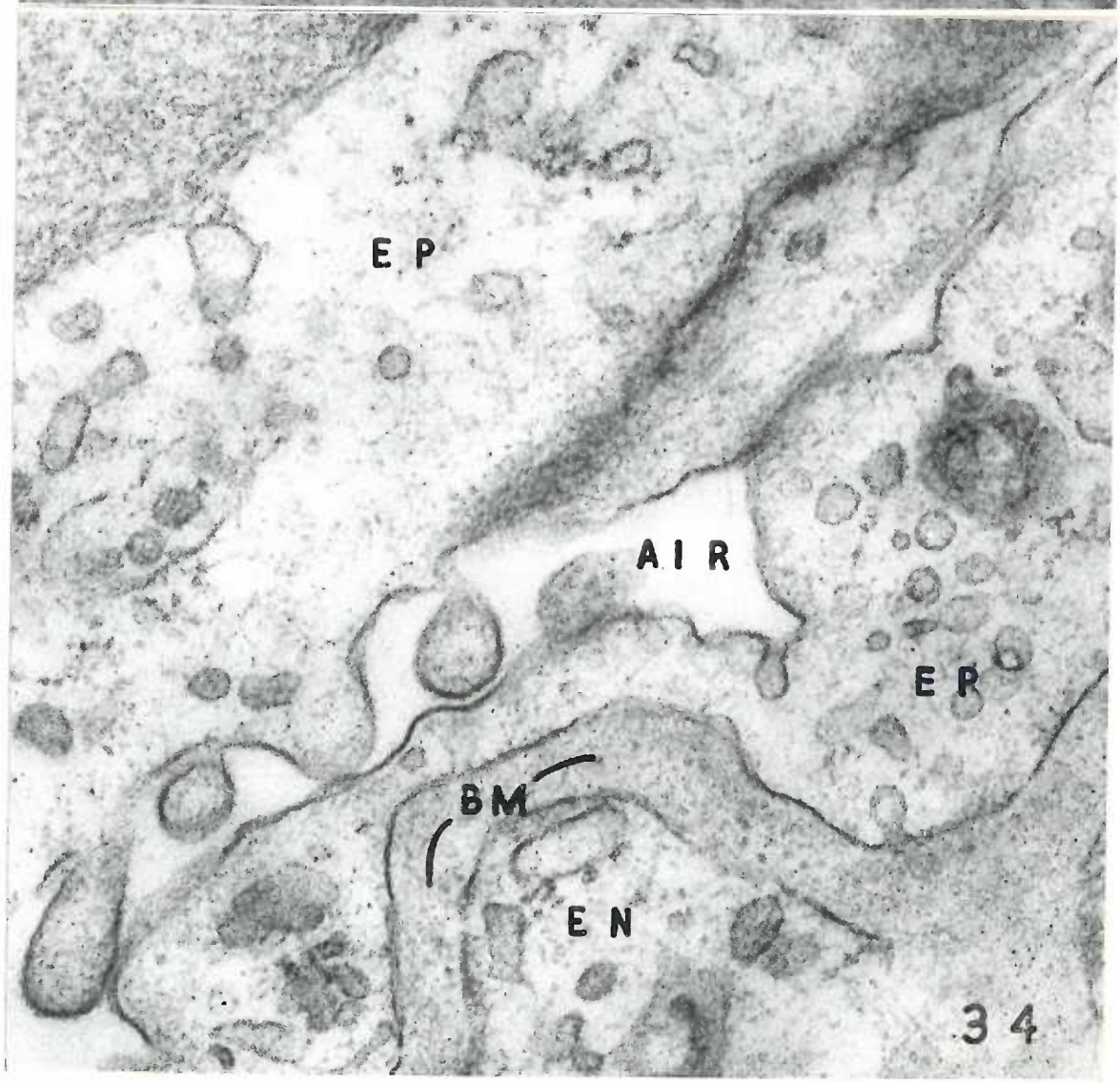
Magnification - 31,500 X

Figure 34

Normal lung. At higher magnification, cell membranes (arrows) of opposite cells are almost in contact. No interposed lines can be observed. At this magnification, the 100 Å thick cell membranes do not appear to include a hypothetical adherent thinner layer.

Magnification - 87,700 X


CAP
AIR
EP
33


EP
AIR
EP
BM
EN
34