

## Electron Microscopic Observation of Pulmonary Alveolar Structures of Laboratory Mammals

Kôji ITAGI\*

*From the Surgical Division (Director : Prof. Chūzo Nagaishi, M. D.)  
of the Tuberculosis Research Institute, Kyoto University.*

(Received for publication December 17, 1956)

### Introduction

The results of electron microscopic observations of the alveolar structures of the normal human lungs were reported in the Acta tuberculosea Japonica Vol. 5, No. 1-2, 1955, by the present author.

The purport of the report was to inform that the attenuated epithelial covering tissue continuously and entirely covered the surface of the alveolar walls, that the covering tissue was the attenuated and elongated cytoplasm of the nucleated alveolar epithelium, that the epithelium was always the nucleated one and there was not any of the so-called large-sized non-nucleated plaque, and that there always existed, even in the thinnest portion of the tissue, the epithelial cytoplasm and the endothelial cytoplasm with respective basement membranes between the air in the alveolus where air diffusion is carried out and the blood in the pulmonary capillary.

The present author has practised, as the second step, electron microscopic observations, trying to ascertain whether there is any difference between the alveolar structures of human lung and that of the lungs of a few laboratory mammals. The animals used were guinea-pigs, rabbits and dogs, all adult and normal.

### I) Methods :

To practise electron microscopic observations of the living tissues and cells, it is essential to have these materials as fresh as possible, and accordingly fixation and other necessary procedures were made immediately after the slaughter or vivisection.

Various procedures such as the fixations were made in the same way as in the case of the human lung, but it would be better to describe them in detail in order to have them understood more clearly.

---

\* 板 木 皓 二

Fixation : 1 % Osmium tetroxide, buffered by pH 7.4 phosphate, was used as fixative. The pieces of the lungs taken out were sliced into the size of about 1 mm<sup>3</sup> on the object-glass provided with a few drops of fixative and were put into the vessel full of fixative for about three hours and thirty minutes in the ice-room.

Washing and dehydration : Washing either with running water or distilled water was continued for one or few hours. Dehydration was performed with a seven-stage ethanol system ranging from 30 % to 100 %. The time of immersion was about 30 minutes respectively, except for 70 % and 100 % stages. The slices were immersed for about 12 hours, for convenience' sake of the time of operation, in 70 % ethanol and for about one hour in 100 % ethanol.

Embedding : Refined n-butylmethacrylate was used for embedding. The completely dehydrated slices were, first, as preliminary steps for embedding, immersed for an hour in the mixture of 100% ethanol and equal amount of monomer, and then in monomer, for an hour, in the ice-room and then again in fresh monomer, for more than 12 hours. After these procedures, these were embedded in the No. 00 gelatin capsules filled with monomer. Benzol peroxide was mixed at the ratio of 2 % in the monomer used for bedding in order to expedite polymerization.

Slicing of ultra-thin section and electron microscopic observation : A Kobayashi-type ultra-microtome made by the Shimazu Manufactory was used in slicing. The sections were cut into slices from 1/18 to 1/22 micron thick with glassknives, and were observed and photographed with a SM-K type electron microscope on the mesh with formvar-membrane, but some of the sections were observed and photographed with the embedding materials eliminated from them by means of acetic amyl.

## II) Observation

Any difference according to the kind of the animals could not be perceived by the electron microscopic observations of the fine structure of alveolar walls of the lungs, such as guinea-pigs, rabbits and dogs. All cases of these animals, therefore, can be summed up en bloc as follows.

### A) *Covering Tissue of Alveolar Wall :*

The covering tissues are seen continuously on the alveolar walls of the lungs of the several kinds of animals, as in the case of human lung, when observed with an electron microscope. In some cases, they are seen almost glued to the capillary walls, as if they cover the capillaries and in other cases they are seen in the condition as if they protect the so-called

septal stroma where several kinds of fibers and cells are seen. They are seen as the elongated cytoplasm of the nucleated alveolar epithelia, when the sections that have the alveolar epithelia are observed.

They are seen including mitochondrias and granules here and there. The thickness of them is not uniform and they seem to be a little thinner than those of human lungs. They are so thin that it is impossible to observe them with a light microscope. The so-called basement membranes are continuously seen on the base side of them.

*B) Nucleated Alveolar Epithelium and Alveolar Wall-cell as named by present author :*

The present author perceived, while he was engaged in the electron microscopic observation of the alveolar walls of the above-mentioned animals, some nucleated cells as well as the nucleated alveolar epithelia on the alveolar walls. These cells seemed to be quite different from the epithelia. Although details will be mentioned later on, a part or a greater part of them protruded into the air spaces like the epithelia. The author acknowledged, almost at the same time, the existence of these cells that should be distinguished from the original epithelia on the alveolar walls of the human lungs. Those of human lungs will be reported by the author under a separate title, but the present author will call them "Alveolar Wall-cells" for the time being.

The shape of the original nucleated alveolar epithelium of the animal lung is, as mentioned above, quite similar to that of the epithelium of human lung ; the cytoplasm of this cell abruptly becomes attenuated around the nucleus and extends on both sides and covers the surface of the alveolar wall, thus forming the epithelial covering tissue of the alveolar wall.

The nucleated alveolar epithelium invariably is of the above mentioned shape and the cell-body itself is never seen as round-shaped without having cytoplasmic extension. The shape of the cell is generally round or oval, except for the portion of so-called epithelial covering tissue formed by the attenuated elongation of the cytoplasm. The rim of the cell is rather smooth and the nucleus is also generally round or oval, with dents in a few places. The nuclear membrane is clearly observed, and sometimes a nucleolus is seen in the nucleus. The quantity of the cytoplasm is quite low and sometimes the nucleus occupies nearly the whole of cell-body. The structure of the cytoplasm is generally quite delicate and a few mitochondrias and granules are found in it as in the epithelial covering tissue as mentioned above. The shape and the structure of the nucleated alveolar epithelium mentioned above quite resemble the endothelium of pulmonary

capillary.

Now, we come to the alveolar wall-cell as named by the present author. All alveolar wall-cells are on the surface of the alveolar wall. A part or a greater part of the cell itself protrudes directly into the air space, and accordingly is not covered with the epithelial covering tissue. The cell is generally round or oval and the cytoplasm of it does not extend nor cover the alveolar wall as in the case of the nucleated alveolar epithelium. It means that a border line is clearly observed between the alveolar wall-cell and the epithelial covering tissue.

These wall-cells have such feature as tell us, at a glance, that they should be distinguished from the nucleated alveolar epithelia. One of the features is found in the structure of the cytoplasm. The cytoplasm of the alveolar wall-cell is generally of rough structure. And large and small vacuoles, large inclusions of mitochondria shape, some large, round, microscopically black lumps included in large vacuoles and some substances that are thought to be coal powders or dusts, are seen in it, in addition to the ordinary cell constituents, such as many large granules and mitochondrias, endoplasmic reticula and the object that looks like Golgi Komplex.

All these substances, however, are not always found in these cells. In other words, the constituents of the individual cell are varied. But considering that these cells have two or three foreign constituents alike and common morphological features, as described below, it may be safely asserted that they are of the same kind and should collectively be called alveolar wall-cells.

The second feature of the alveolar wall-cell is its margin facing to the air space. It means that the portion of the wall-cell facing to the air space generally has an irregular line and sometimes looks like the teeth of a saw, and that in most cases small round or spindle-shaped black dots can be seen near or a little apart from the irregular line when observed by an electron microscope.

The third notable feature is their relation with the adjoining epithelial covering tissues. Sometimes the covering tissue covers a greater part of the wall-cell and only a small part of the latter is exposed to the air space, and in other cases the covering tissue covers only a part of the wall-cell and a greater part of the wall-cell is exposed accordingly. In other words, some wall-cells are firmly fixed on the alveolar wall, while others attach themselves to the alveolar wall quite unstably.

Besides the three notable features of the alveolar wall-cell described above, there is the following feature still to be noted. It is that the wall-cell, unlike the epithelium, has rich cytoplasm and often two or three of

them are seen gathered together. It seems there are only two kinds of cells that expose themselves directly to the air spaces, one is the original nucleated alveolar epithelium and the other is the alveolar wall-cell as named by the present author.

**C) Basement Membrane :**

The basement membranes are continuously seen not only along basement of the alveolar epithelium, but also along the outside of the capillary, i. e. along the basement of the capillary endothelium. It can distinctly be seen where the epithelium and endothelium are contiguous.

The present author described, in his last report on the alveolar structures of the human lung, that "the basement membrane is electron dense and shows a line of a uniform size and an even quality". But it seems that the basement membrane of the human lung as well as the animal lung looks like a small line of a uniform size when it is cut in a right angle, but when cut diagonally, it looks, by a close observation, like a thing made of fine fibrils gathered together.

These membranes, existing ubiquitously along the basements of epithelium and endothelium, seem to perform their functions as supporting tissues of alveolar wall together with the various kinds of fibers in the stroma.

*D) The Relation between the Air Space and the Pulmonary Capillary :*

The capillary wall is formed by the attenuated and extended cytoplasm of endothelium. This is similar to the fact that the attenuated and elongated cytoplasm of the epithelium forms the epithelial covering tissue. The endothelium has its basement membrane on the outer side. No crevices of the capillary wall can be seen in any section of normal alveolus and the thickness of the capillary wall is almost the same with that of the epithelial covering tissue. Although the epithelium and the endothelium resemble each other, as mentioned before, they can easily be discerned, when the condition of their surroundings can, even a little, be observed.

As mentioned before, the continuous epithelial covering tissue is invariably seen on the surface of the alveolar wall. It is no doubt, therefore, that the epithelium and the endothelium always exist between the air in the airspace and the blood in the capillary, however thin the stratum of the tissue may be. Sometimes, however, collagen fibers and elastic fibers are observed between them.

The capillaries that do not seem to participate in diffusion, like those in the thick septal stroma or those that are always isolated, even though

they are on the alveolar wall and exist in the portion where there are many kinds of fibers, may belong to the bronchial blood-vessels. But the structures of these capillaries are not different from those of the capillaries that seem to take part in diffusion.

The alveolar wall-cell does not exist right over the capillary, and it seems that the nucleated portion of the nucleated alveolar epithelium also rarely exists right over the capillary.

#### *E) So-called Septal Stroma*

In most cases elastic fibers and collagen fibers, and sometimes various kinds of cells, are seen existing between the epithelium and the endothelium that have respective basement membranes. The alveolar wall consists commonly of the capillaries and in most cases, a few fibrillar substances are observed between these capillaries. It is rare, however, that the cells that belong to the stroma are seen between them.

It was impossible to ascertain how and where the elastic and collagen fibers, and silver-stained fibrils existed as the supporting tissues of the alveolar wall. Muscle fibers, however, were not observed in the portion of the alveolar wall that were provided with many capillaries.

It seems impossible under the present condition to conclude the kind of the cells that belong to the stroma as well as decide whether alveolar poles exist or not.

### **III) Generalization and Discussion**

In the fine structure of the alveolar wall, there is, as mentioned above, no fundamental difference between the human lung and the lungs of animals, such as guinea-pigs, rabbits and dogs. It means that in both cases there can be observed the continuous epithelial covering tissue on the surface of the alveolar wall, that it is a part of cytoplasm of nucleated alveolar epithelium, that the pulmonary capillary itself is not exposed to the air space. But many alveolar wall-cells, as named by the present author, are observed in the animal lung as mentioned before, whereas these are not observed so many in the human lung.

To avoid overlapping, it would be better here to have an explanation with regard to the epithelial covering tissue of the alveolar wall, centering our notice on the so-called alveolar wall-cell.

Some reports on the pulmonary structure of the laboratory mammals seem to have been made since the beginning of the nineteenth century, but Eberth first acknowledged the existence of the epithelium as the respiratory epithelium in 1863. It was done by the so-called silver-impregnation method,

Kölliker developed his study employing the same method and established "Kölliker's theory" later on. His theory was that the alveolar wall was entirely covered by large-sized non-nucleated plaques and small-sized nucleated cubic cells. Although his theory was based only on the doubtful silver-impregnation method, and caused many objections and confusions, with the advance of various methods of researches, e. g. researches by vital staining method, by foreign-body injections or infectious experiments and by tissue culture, it is still widely accepted even at present. Two or three reports of the researches by an electron microscope have been made quite of late, but the structure of the alveolar wall, chiefly concerned with the epithelium, has not been fundamentally clarified. As various theories and assertions about these problems were already reported in the previous essay of the present author, they are omitted here.

The present author's view on the epithelium of the human lung that reported in the previous report is still applicable to that of the animal lung. It means that there is only one kind of epithelium and it is always nucleated, and its cytoplasm is attenuated, elongated and constitutes the epithelial covering tissue and covers continuously the surface of the alveolar wall. Accordingly, *raison d'être* of the epithelium called non-nucleated plaques is not comprehensible, still less the evidence of its existence is recognized. Thus, although the individual cell-body of the nucleated alveolar epithelium is flat as the endothelium, it may be said to be a remarkably large-sized cell.

Here comes, however, a new problem to be confronted. It is the so-called alveolar wall-cell mentioned before. Is it really a kind of epithelium? If it is not, then it means that the epithelial tissue that should indispensably exist continuously is partly void. Then, is the alveolar wall-cell to be considered as a kind of epithelium? No, it is not.

As the present author already pointed out, the first of the three features of this cell is the characteristic structure of the cytoplasm. The cytoplasm of the alveolar wall-cell often includes vacuoles large and small, large granule-like substances, web-like substances and something that look like a foreign body, in addition to the normal constituents cytologically acknowledged. The formation of such vacuoles and the existence of strange substances, when taken into consideration alone, may be suspected to be the result of decrepitude or degeneration of some cell ..... the nucleated alveolar epithelium in this case. But the present author does not think it a proper surmise, because anything that may be supposed to be the intermediates between the alveolar wall-cell and the epithelium were not perceived and because there is too much morphological difference between the two.

The second feature is that the line or contour facing to the air space is irregular and sometimes looks like the teeth of a saw, and that in most cases micrographically round or spindle-shaped black dots can be seen near or a little apart from the cell. What does it mean? De Robertis and his colleagues showed an electron microscopic picture of polynuclear leucocyte with amoeba-like pseudopods in their "General Cytology". It was a leucocyte that was not cut into section and if it had been cut into section, the margin of the cell would naturally have showed irregular line and the pseudopods would have been observed as round or spindle-shaped pieces around the cell. The present author has often observed the irregular contoured leucocytes. The leucocyte is not the only one that protrudes the amoeba-like pseudopods ; if the cells that have phagocytic or migrating abilities are cut into section while doing their functions, they would surely show irregular contours.

The third feature is that the bordering condition between the alveolar wall-cell and the adjacent epithelial covering tissue is not uniform : sometimes the greater part of the wall-cell is covered by the epithelial covering tissue and sometimes only a small part is in contact with the epithelial covering tissue. Thus, there are many degrees of covering conditions.

It may be interpreted that such a phenomenon shows the process of the alveolar wall-cell coming out from below the epithelial covering tissue, breaking or pushing through it and at last occupying a part of the alveolar wall.

When the studies on these three features are put together, the nature of these cells becomes clarified of itself. They may be defined as a sort of cells that have phagocytosis and occupy their positions on the alveolar wall, performing their function of cleansing the air space. It is not easy to know their essential substance and origin, but the present author thinks that at least the epithelial nature of these cells is to be denied.

Now, the present author wishes to inquire into a few experiments on the structure of the alveolar wall performed by others, on the basis of the informations gathered by his own researches in this field.

Silver-impregnation method by the intratracheal injection of  $\text{AgNO}_3$ -solution seems to be the only basis of argumentation by the believers in Kölliker's theory, on the ground that it can dye the dividing lines of the epithelia. But the method itself has become the basis of the anti-Kölliker scholars on the ground that its dying is unreliable and the silver-impregnated line is uncertain and so on. The present author, to his regret has not yet practised the silverimpregnation method in addition to his various experiments. He can not, however, overlook a paragraph mentioned by



Ogawa in his essay, who is one of the followers of Kölliker's theory and has made a comprehensive and systematic morphological study on the pulmonary alveolar structure of the vertebrates. When he applied the silver-impregnation method, after injecting 0.5 cc of distilled water into the lung of rats through its trachea, with the purpose of studying the regeneration and restitution of the respiratory epithelium, he reported as follows : "While we see the smallest of the small-sized nucleated cells often dyed, we see, on the other hand, remarkably large ones also dyed. Sometimes, nucleated cells as large as non-nucleated plaques are seen. In short, the nucleated cells of various sizes are observable." Although he injected 0.5 cc distilled water into the trachea before applying the silver-impregnation method, he said he did not notice any effects of it, such as exudation into the air space or swelling of the epithelium by the stimulus. The present author does not think any change would be caused in the silver-impregnated line by such a simple operation. He thinks that Ogawa's "remarkable large nucleated cells" point the nucleated alveolar epithelia with the elongated and expanded cytoplasm, and "the small-sized nucleated cells" correspond to nothing but the alveolar wall-cells named by him, and Ogawa's experiments incidentally disclosed the real state of the alveolar wall.

The present author wishes to consider the following vital staining experiments performed separately by Majima, Westhues and Seemann etc. The common traits of their experiments are that some of the cells that seem to be the epithelia take pigment granules and some do not, when various kinds of pigments are intratracheally injected, and that some cells on the alveolar wall take pigment granules in one or two hours, that is, by the time the leucocyte and the histiocyte-like cells migrate into the air space. These phenomena seem, to the present author, to be easily understood, when we know that the alveolar wall-cells which have phagocytosis are exposed to the air space, in addition to the nucleated alveolar epithelia on the alveolar wall. Majima etc. construed that the histiocytes existed under the non-nucleated plaques on the alveolar wall. It is to be regretted that they could not find out the real state of the alveolar wall, as they had not the means to make a correct observation of the fine structure of it.

There are some who are engaged in the observation from a little different angle. They are Bertalanffy and Leblond. They observed the alveolar wall of rats light-microscopically, and divided the cells on the alveolar wall into four groups.....vacuolated alveolar cells, non-vacuolated alveolar cells, endothelium-like cells and migrating blood-cells, besides the peculiar cells that are on the bronchioli and the capillary walls. They reported the frequency of the observation of these cells in percentage. It is quite interesting that

two kinds of the cells which they reported to be alveolar cells suggest the alveolar wall-cells and the alveolar epithelia, even if there may be some exceptions. The percentage showed 17.8 of vacuolated alveolar cells and 13.0 of non-vacuolated. The present author can not report the percentage so precisely as they did, but it may be said that more alveolar wall-cells are observed than the alveolar epithelia as far as the animal lung is concerned. The alveolar wall-cells are comparatively few in the human lung.

As the results of these studies mentioned above, the present author thinks that the so-called alveolar wall-cell should be considered as of a separate kind independent of the alveolar epithelium.

### Conclusion

The present author practised, as mentioned above, electron microscopic observations of the pulmonary alveolar walls of the adult and normal guinea-pigs, rabbits and dogs and came to the following conclusion after comparing the results of the observations with those of the normal human lung he reported before.

1) No difference is observed among the fine structures of the alveolar walls of the laboratory mammals, such as guinea-pigs, rabbits and dogs.

2) There is no fundamental difference between the structures of the alveolar walls of the human lung and those of the laboratory mammals.

3) Thin, membraneous epithelial covering tissues continuously and entirely cover the surface of the alveolar walls as a general rule.

4) The epithelial covering tissue is not an independent cell, but an attenuated and elongated cytoplasm of the nucleated alveolar epithelium. There is no evidence to prove the existence of the so-called large-sized non-nucleated plaque.

5) A kind of the nucleated cells is observed on the alveolar wall besides the nucleated alveolar epithelium. This kind of cells is exposed directly to the air space and generally of round shape, and can not be considered as the epithelium. Accordingly it is to be considered that the alveolar wall is devoid of the epithelial covering tissue at the portion where this kind of cell exists. This kind of cell has been named "alveolar wall-cell" by the present author.

6) Fairly many alveolar wall-cells are observed in animal lung, but comparatively fewer in the human lung.

7) In most cases, two or three alveolar wall-cells make a group at a place. Considering their morphological condition, it is supposed that these cells probably have phagocytosis and perform the roles of cleansing the alveolar wall.

8) The basement membranes are ubiquitously observed on the respective

basements of the epithelium and endothelium. They look like fibrils gathered together and are worthy of notice as the supporting tissues of the alveolar wall cooperating with collagen fibers and elastic fibers.

9) The epithelial cytoplasm and the endothelial cytoplasm with respective basement membranes exist between the air in the alveolus where diffusion is carried out and the blood in the pulmonary capillary, however thin the stratum of the tissue may be.

### Acknowledgment

Before bring this brief report to the close, the present author wishes to extend his cordial thanks to Prof. Chûzo Nagaishi M. D., who has given kind instructions during the whole study and has kindly revised this report, and to Assistant Prof. Keinosuke Kobayshi of the Institute for Chemical Research, Kyoto University, who has favoured the author with guidance and assistance in electron microscopic observations, and to Naoyuki Nagasawa M. D. of the Surgical Division of the tuberculosis Research Institute, Kyoto University, who has given many kind advices to the author.

### Literature

- 1) Akasaki, K., On the epithelial covering of pulmonary alveolar wall. *Nissin-Igaku*, Years 32, No. 11, 1943.
- 2) Akasaki, K. and Hosaka, T., On the epithelial covering of alveolar wall in the human lung. *Medical Science and Biology*, Vol. 2, No. 10, 1942.
- 3) Akutsu, K., Studies on the fine structure of the pulmonary alveoli of the vertebrata. *Nissin Igaku*, Vol. 22, 1933.
- 4) Baker, R. F. & Modern, F. W. S., Controlled fixation with osmium tetroxide. *Anat. Rec.*, Vol. 114, No. 2, 1952.
- 5) Bensley, R. D. & Bensley, S. H., Studies of the pulmonary alveolus of normal lungs of adult animals. *Anat. Rec.*, Vol. 64, No. 1, 1935.
- 6) Bensley, S. H., On the presence, properties and distribution of the intercellular ground substance of loose connective tissue. *Anat. Rec.*, Vol. 60, No.1, 1934.
- 7) Bensley, S. H. & Groff, M. B., Changes in the alveolar epithelium of rat at birth. *Anat. Rec.*, Vol. 64, No. 1, 1953.
- 8) Bertalanffy, F. D. & Leblond, C. P., The continuous renewal of the two types of alveolar cells in the lung of the rat. *Anat. Rec.* Vol. 115, No. 3, 1953.
- 9) Dempsey, F. W., The chemical characterization and submicroscopic structure of elastic tissue. *Science*, Vol. 116, 1952.
- 10) Foot, N. Ch. Studies on endothelial reaktion. *J. of Exp. Med.*, Vol. 32, 1920.
- 11) Gale, J. G., Electron microscope studies of collagen from normal and diseased tissues. *Am. J. of Path.*, Vol. 27, 1.
- 12) Hayek, H. V., *Die Menschliche Lunge*. 1953.

- 13) Itagi, K., Electron microscopic observation of pulmonary alveolar structures. *Acta tuberculosea Jap.*, Vol. 5, No. 1-2, 1955.
- 14) Kajikawa, K. & Sumita, Y., Electron microscopic studies of connective tissue. *Transact. Soc. Path. Jap.*, Vol. 42, 1953.
- 15) Koelliker, A., cit. 21) Miller.
- 16) Lansing, A. L., et al, The structure and chemical characterization of elastic fibers as revealed by elastase and by electron microscopy. *Anat. Rec.*, Vol. 114, 1952.
- 17) Loosli, C. G., The rabbits lung after phrenicotomy and pneumothorax. *Anat. Rec.*, Vol. 62, No. 4, 1953.
- 18) Low, F. N., Electron microscopy of the rat lung. *Anat. Rec.*, Vol. 113, No. 4, 1952.
- 19) Low, F. N., The pulmonary alveolar epithelium of laboratory mammals and man. *Anat. Rec.*, Vol. 117, No. 2, 1935.
- 20) Majima, U., Pathological and histological studies of pneumonia. *Kyoto Med. J.*, Vol. 17, 1920.
- 21) Miller, W. S., The lung. 1947.
- 22) Miyata, S., Pathological and morphological studies of connective tissue. *Transact. Soc. Path. Jap.*, Vol. 42, 1953.
- 23) Morigami, S., Origin of pulmonary alveolar dust-cell. *Nisshin Igaku*, Years 32, No. 10, 1943.
- 24) Nakahata, T., Studies on the silver-impregnated fibrils. *Niigata Med. J.*, Years 63, No. 5, 1949.
- 25) Ogawa, Ch., Histological studies of pulmonary alveoli of the vertebrata. *Kyoto Med. J.*, Vol. 14, 1917.
- 26) Palade, G. E., A study of fixation for electron microscopy. *J. Exp. Med.*, Vol. 95, 1952.
- 27) Rinehart, J. F., Electron microscopic studies of sectioned white bloodcells and platelets. *Am. J. cl. Path.*, Vol. 25, No. 6, 1955.
- 28) De Robertis, E. D. P., Nowinski, W. W. & Saez, F. A., *General Cytology*. W. B. Saunders Co., Philadelphia, London, 1948, 1954.
- 29) Sakamoto, S., Studies of tuberculous pneumonia by the vital staining. *Osaka Med. J.*, Vol. 16, No. 4, 1917.
- 30) Seemann, G., cit. 1) Akasaki and 23) Morigami
- 31) Swigart, R. H. and Kane, D. J., Electron microscopic observation of pulmonary alveoli, *Anat. Rec.*, Vol. 118, No. 1, 1964.
- 32) Tamaru, K., On a few problem of the alveolar structure of the rabbits lung after pneumothorax. *J. Anat.*, Vol. 29, No. 1, 1953.
- 33) Tomita, H., The entity of the epithelioid cells appeared in the Pulmonary alveoli. *Osaka Med. J.*, Vol. 17, No. 11, 1918.
- 34) Watanabe, Y., Takamatsu, M. & Ōseko, R., Fine structure of plasma cell as revealed by electron microscope. *Electron Microscope*, Vol. 4, No. 3, 1956.
- 35) Westhues, H., cit. 23) Morigami.
- 36) Yamada, Y., Comparative histological studies on the cells of lungs of vertebrata, especially on its relation to capillaries. *J. Anat.*, Vol. 28, No. 1, 1953.

### Explanation of the plates

Bar on electron micrographs are equivalent to 1 micron. Fig. 4~Fig. 12 are micrographs of the sections with the embedding chemical.

[These plates were reduced in about a quarter dimension of the original size]  
[for printing convenience with understanding of the present author (Editor)]

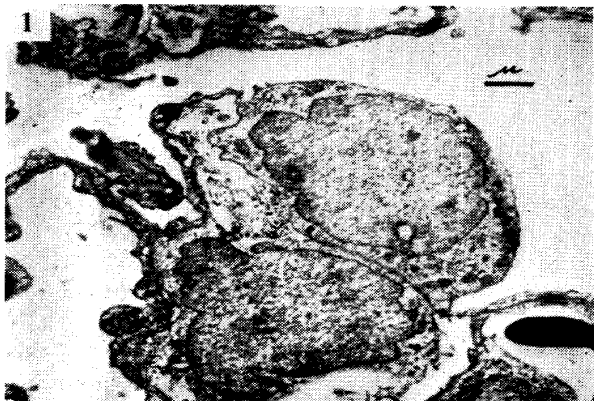


Fig. 1. Nucleated alveolar epithelium, lung, guinea-pig ( $\times 13000$ )

A nucleated alveolar epithelium is seen at the center of this picture. The attenuated and elongated cytoplasm of this cell covers the surface of alveolar wall.

The cell observed at the central lower part exists in the stroma. A capillary is seen at the lower left corner and the other at the lower right corner.

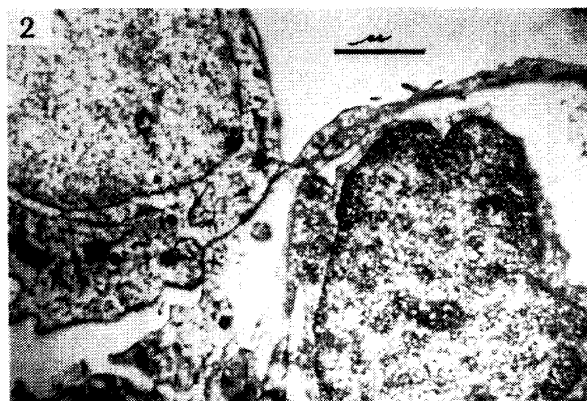


Fig. 2. Nucleated alveolar epithelium, lung, guinea-pig ( $\times 24000$ )

A nucleated cell observed at the upper left part of this picture is the alveolar epithelium, and a nucleated cell at the lower right part is the endothelium. The cytoplasm of the epithelium and the endothelium form respectively the epithelial covering tissue and the capillary wall, attenuating and elongating themselves.

The blank of upper right part is the air space and the lower left corner is the stroma.



Fig. 3. Nucleated alveolar epithelium, lung, dog. ( $\times 14000$ )

In this picture, two nucleated cells are seen. The upper cell of them is the epithelium, and the other one is the endothelium. The cytoplasm of the epithelium is attenuated and elongated. Note the continuous epithelial covering tissue covering the surface of alveolar wall.



Fig. 4. Nucleated alveolar epithelium and so-called alveolar ( $\times 5700$ ) wall-cell named by the present author, lung, guinea-pig

Two nucleated cells observed respectively at the left-hand and right-hand side of this picture are so-called alveolar wall-cell named by the present author. In the center, the nucleated alveolar epithelium is seen. Attenuated and elongated cytoplasm of the epithelium covers the surface of alveolar wall, but the alveolar wall-cells are generally round and their cytoplasm is not elongated.

Note the differences of size, quantity and cytoplasmic structures between the epithelium and the wall-cell. It may be perceived at a glance that these two kinds of cells are not the same.



Fig. 5. Nucleated alveolar epithelium and alveolar wall-cells, lung, guinea-pig ( $\times 7100$ )

Five nucleated cells in total are seen in this picture. Among those cells, the central left cell is the epithelium and the one at central right is the wall-cell. The former has a poor perinuclear cytoplasm, but the latter has abundantly. The central lower blank is the air space.

At the right lower part of this picture, the cell (showing only cytoplasm and adjoining to the wall-cell) is seen. The present author regards it as the wallcell, although the cell apparently is not exposed to the air space in this section.



Fig. 7. Alveolar wall-cell, lung, guinea-pig ( $\times 8500$ )

A nucleated cell in this picture is alveolar wall-cell, and two capillaries are seen in this picture. The blanks in the upper and lower parts are both air spaces.

The margin of this alveolar wall-cell, facing on air space, looks like the teeth of a saw, and a right part of this cell is covered by the epithelial covering tissue. Note a big, black substance (electron-microscopically) in the cytoplasm of this cell.

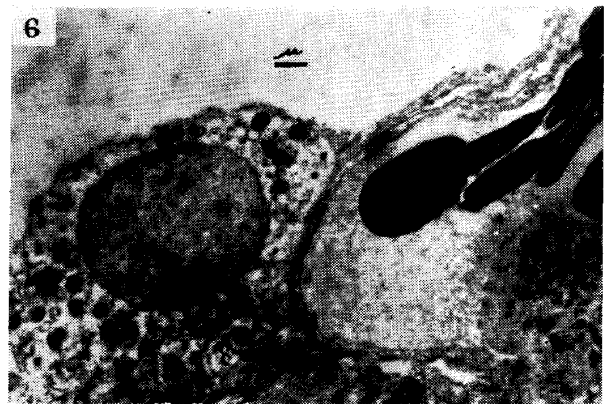


Fig. 6. Alveolar wall-cell lung, guinea-pig ( $\times 8500$ )

A nucleated cell in this picture is alveolar wall-cell. It looks apparently that the epithelial covering tissue is penetrated into the place between this wall-cell and the capillary wall.

The cytoplasm of this wall-cell contains many big and small granules, some net-work substances and a few irregular objects which are regarded as foreign-bodies, and besides the element that is considered to be so-called endoplasmic reticulum is observed, and a part of it consists of the nuclear membrane, grown like a pouch.

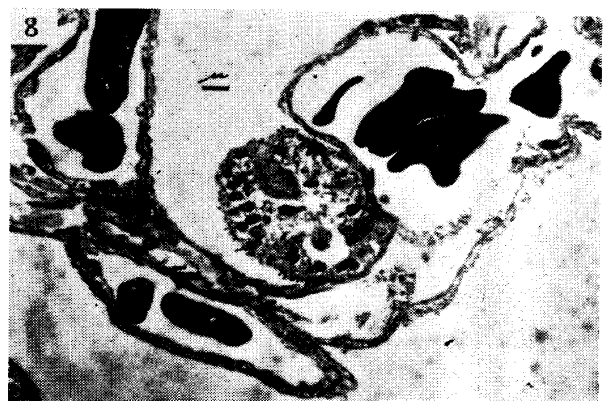


Fig. 8. Alveolar wall-cell, lung, guinea-pig ( $\times 7000$ )

In this picture, three capillaries which respectively have a few erythrocytes are seen in succession. Note the continuous epithelial covering tissues.

The alveolar wall-cell is seen at the center of this picture. This cytoplasm and the margin of this cell facing on air space are characteristic. There is the dividing line between such a wall-cell and the epithelial covering tissue



Fig. 9. Alveolar wall-cells, lung, guinea-pig ( $\times 7000$ )

Two alveolar wall-cells are seen abreast. The cytoplasm of these cells are metamorphosed owing to the formation of vacuoles and contain some arachnoid element, and accordingly the granules are pushed to several part of each cytoplasm.

The blank of upper part is the air space and the lower part in the picture is the stroma.

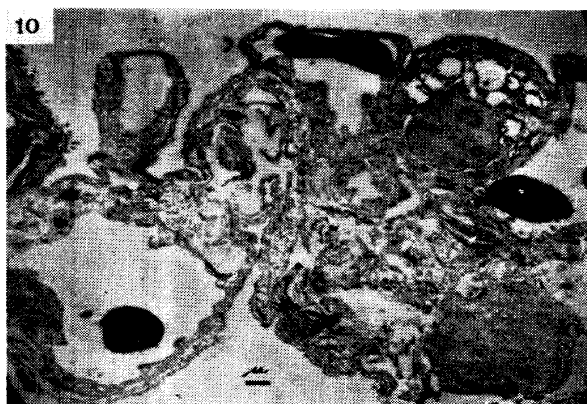


Fig. 10. Alveolar wall-cell, lung, rabbit ( $\times 6000$ )

Note the epithelial covering tissues that continuously cover the surface of alveolar walls

A nucleated cell seen at the upper right part of this picture is the alveolar wall-cell in rabbit lung. There are many big and small vacuoles and granules in its cytoplasm. The surface facing on air space of this cell is, for the most, covered by the epithelial covering tissue.

A nucleated cell observed at the right lower part exists in the stroma.

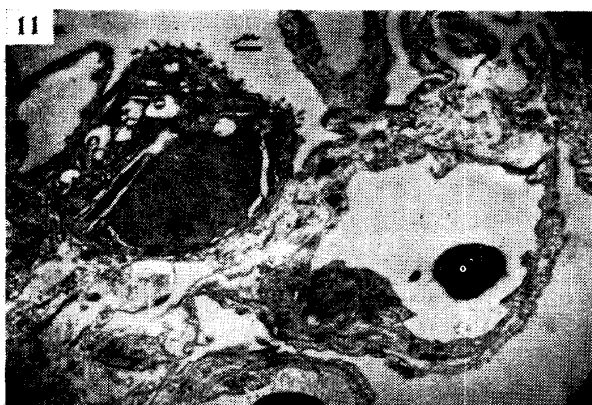


Fig. 11. Alveolar wall-cell, lung, rabbit ( $\times 7000$ )

An alveolar wall-cell is seen at the upper left part of this picture. The cytoplasm of this cell looks darker than that in the lung of guinea-pig, but it contains alike many big and small vacuoles and some granule-like substances, and besides the shape of the margin facing on air space of this cell draws the attention of the present author.



Fig. 12. Alveolar wall-cell, lung, rabbit ( $\times 14700$ )

This is an alveolar wall-cell. This wall-cell is especially characteristic as compared with the other same kinds of cells. Namely it has some big and small vacuoles and granules, and besides the greatest vacuole adjoining to the nucleus occupies about a half part of the cell-body, and the arachnoid structure is seen in it. I cannot tell the reason why such the metamorphosis has been caused.



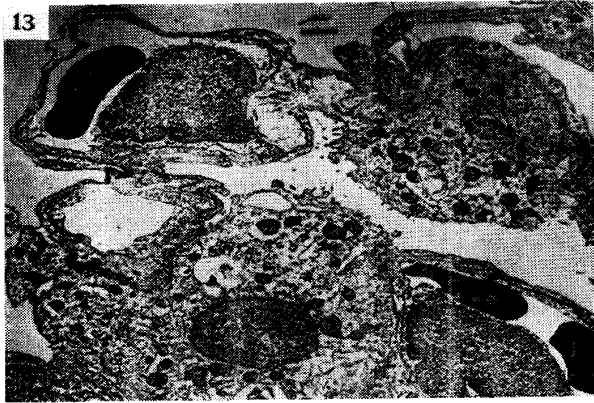


Fig. 13. Alveolar wall-cell, lung, guinea-pig ( $\times 9700$ )

Two alveolar wall-cells are seen in the picture. One of them has a nucleus, but the other does not show the cross-section of the nucleus.

In the cytoplasm of these wall-cells, some big and small vacuoles, many mitochondria-like and granule-like objects are seen. Note the continuous epithelial covering tissue.

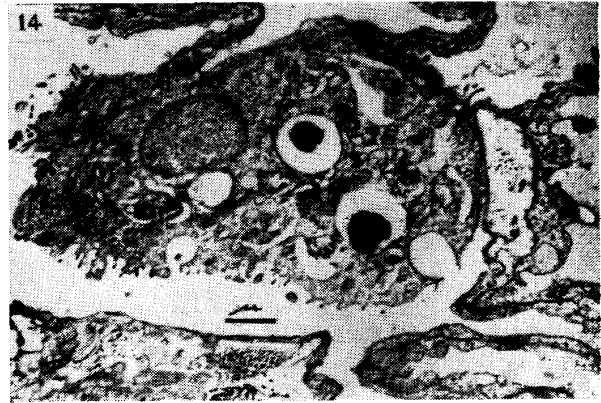


Fig. 14. Alveolar wall-cell, lung, guinea-pig ( $\times 13700$ )

An alveolar wall-cell is seen at the center of this picture. The blanks of upper central part, central right part and lower right corner are the all capillaries, and the other blanks are all the air spaces.

The nucleus of this wall-cell is small. Note the margin of this cell and the cytoplasmic structure that has some big and small vacuoles. In two big vacuoles, a round and black lump (electron-microscopically) is seen respectively.



Fig. 15. Two-nucleated alveolar wall-cell, lung, guinea-pig ( $\times 7500$ )

The two-nucleated cell is seen at the center of this picture. This is undoubtedly an alveolar wall-cell, judging from its morphological view. Sometimes such two-nucleated alveolar wall-cells are found on the alveolar walls.

The blank at the central right part is the air space. The left area that contains two nucleated cells is the stroma.

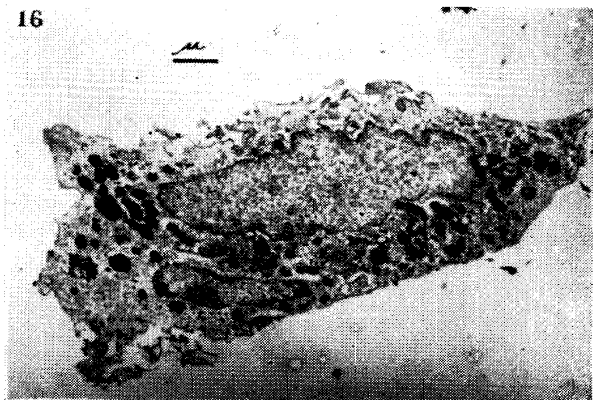


Fig. 16. Free-cell in the air space, lung, guinea pig. ( $\times 11800$ )

Such free-cells, as shown in this picture, are often found in the lung of the guinea-pig. I am not sure of its origin and its substance, but this kind of cells may have some relation to the alveolar wall-cells.