Department of Anatomy (Prof. T. Ito),
Gunma University School of Medicine, Maebashi, Japan

Histological Studies on the Respiratory Tissue in the Dolphin Lung

Toshio ITO, Kan KOBAYASHI and Yoshiyuki TAKAHASHI

(伊東俊夫, 小林 寛, 高橋嘉幸)

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In whales, the aquatic mammals, it is expected that the lung should have several specific histological characteristics, since they have the capacity to make long-term divers into the depth of the sea. Until today there have been published a number of histological studies on the lungs in some species of the toothed and the baleen whale (KIKKAWA 1944, FIEBIGER 1916, NEUVILLE 1928, WISLOCKI 1929, BÉLANGER 1940, MURATA 1961, ENGEL 1966 etc.). In the present study the authors have histologically and partially cytologically observed the respiratory tissue of the dolphin lung and obtained some interesting results which may contribute to some extent to clarify the histological characteristics of the whale lung.

Material and Method

The material consisted of lungs from 6 adult dolphins, *Prodelphinus (Stenella) caeruleoalbus* MAYEN, captured in December at Futo on the Izu Peninsula. Histological specimens were taken from lung-peripheries and fixed as soon as possible in Levi's fluid containing osmic acid and Zenker-formalin. 4 μ thick serial paraffin sections were made and stained with hematoxylin (Hansen)-eosin, azan, periodic-acid-Schiff's reaction (PAS) and aldehyde-fuchsin-light green-orange G for elastica staining.

Results

As mentioned above, the specimens were taken from the lung periphery, so the large vessels and bronchial branches are not found. The largest bronchial branches observed in the present study are bronchioli lined with the pseudostratified columnar epithelium which, however, were lacking in the cilia and goblet cells. In the connective tissue beneath the epithelium the glands are completely absent. In the outer border of the mucous membrane of such somewhat thick bronchioli lineal arrays of many small rod-shaped cartilages are observed along the long axis of the bronchioli. However, the majority of the bronchioli found in the present study are bronchioli terminales and respiratorii, both bronchioli do not differ in general histological structures and in thickness except for the conspicuous difference in the lining epithelium of the mucosa. The epithelium of the bronchioli terminales represents one type of the simple cuboidal epithelium in which the arrangement and the shape of the epithelial cells are in some places irregular (Fig. 1). The epithelium of the bronchioli respiratorii resembles that of the terminales, but contains from place to place blood capillaries, the walls of which seem as if partially to face directly the bronchio-

Fig. 1. Simple cuboidal epithelium (*e*) of a bronchiolus terminalis. The epithelial cells possess some stainable granules in the supranuclear cytoplasm. *bd* Connective tissue layer of the mucosa, *c* capillary, *ef* elastic fibers, *l* lumen. Levi's fluid, aldehydefuchsin-light green-orange G staining. $\times 1,080$

lar lumen (Fig. 2, 20), although it might be probably lined by thin cytoplasmic extensions protruded from the neighboring epithelial cells. This histological finding suggests that the respiratory process may already take place in the epithelium of this most distal branching of the bronchial tree. In both the terminal and respiratory bronchioli the epithelium is devoid of cilia and goblet cells and the thin connective tissue layer of the mucosa contains no glands. The

walls of the bronchioli terminales and respiratorii are also reinforced by many small cartilages densely distributed beneath the mucosa along their long axis. As observed in the longitudinal sections of both parts of the bronchioli, numerous round, oval and rod-shaped profiles of the cartilages are densely aligned on both sides of the bronchiolar lumen (Fig. 3), and in the cross sections the profiles of the cartilages are rod-, crescent- and horse-shoe-shaped surrounding the lumen.

It is one of the noteworthy findings that the bronchioli terminales and respiratorii possess in their wall just beneath the thin epithelium, numerous well-developed sphincter muscles at regular intervals, so that the bronchiolar lumen forms be-

Fig. 2. Simple cuboidal epithelium (*e*) of a bronchiolus respiratorius. Capillaries (*c*) filled with erythrocytes appear within the epithelium and seem as if facing directly on the bronchiolar lumen (*l*). *bd* Connective tissue layer of the mucosa. Levi's fluid, azan staining. $\times 1,080$

Fig. 3. Longitudinal and partially cross section of a bronchiolus terminalis and respiratorius, containing in the wall numerous small cartilages (*k*) and sphincters (*s*). The lumen forms ampullar dilatations (*a*) between the sphincters, and constricted portions (*i*) corresponding to the sphincters. Zenker-formalin, PAS-hematoxylin. $\times 48$

Fig. 4. High power view of the bottom part of the bronchiolus illustrated in Fig. 3. *a* Ampullar dilatation of the lumen, *e* epithelium, *i* constricted portion of the lumen, *k* cartilage, *sc* transverse section of a sphincter, *st* tangential section of a sphincter. Zenker-formalin, PAS-hematoxylin. $\times 120$

tween the sphincters numerous wide round dilations (ampullae) which are communicated by constricted portions corresponding to the sphincters (Fig. 3, 4), in the longitudinal section the round dilations and constrictions aligned successively exhibit a feature similar to a string of beads. Each sphincter is composed of a single bundle of densely packed smooth muscle fibers. The sphincters also occur in the wall of the above described thicker bronchioli with a pseudostratified columnar epithelium, in which, however, they are composed of a number of bundles of smooth muscle fibers. The bronchioli terminales and respiratorii show ramifications.

From the distal end of the bronchioli respiratorii constricted by the last bronchiolar sphincter, the alveolar duct arises. In the wall of the alveolar duct several thick bundles of the elastic fibers which do not contain the smooth muscle fibers are observed (Fig. 5). The sphincters become gradually thinner toward the end of the bronchiolus respiratorius and suddenly disappear in the alveolar duct wall.

Besides the well-developed elastic tissue in the alveolar duct wall, there occur elastic fibers immediately beneath the epithelium of the bronchioli (Fig. 1), in the outer border of the bronchiolar mucosa connecting the cartilages of the bronchiolar

Fig. 5. Longitudinal section of an alveolar duct (*ad*) with strong elastic strands (*ef*) in the wall. *al* Alveolus. Levi, aldehyde-fuchsin-light green-orange G. $\times 120$

wall, as well as in the sphincter intermingled among the densely packed smooth muscle fibers. The sphincters of the bronchioli protrude the connective tissue projection containing elastic fibers to the adjacent bronchiolar cartilages to anchor on the latter (Fig. 6).

Histological characteristics in the dolphin lung are also found in the structural properties of the alveolar septa or alveolar walls. In the axis of the alveolar septa there exists a continuous thin connective tissue layer containing elastic fibers

Fig. 6. Cross section of the bronchiolar sphincter (*s*), anchored with connective tissue extensions containing elastic fibers (dark) on two neighboring cartilages (*k*). *l* Bronchiolus lumen. Levi, aldehyde-fuchsin-light green-orange G. $\times 240$

of different thickness and a small number of connective tissue cells with oval nuclei, by which the two surfaces of each septum facing the neighboring alveolar lumina are completely separated (Fig. 7). In both surfaces independent blood capillaries are densely distributed and among them alveolar epithelial cells are scattered, they are, however, in contradiction to those in the terrestrial mammalian lung, never common to both alveolar surfaces (Fig. 8—11). Generally, cuboidal alveolar epithelial cells, scattered singly or making small

Fig. 7. Alveolar septa separated into two independent alveolar surfaces by the axial connective tissue layer (clear) containing elastic fibers (*ef*). For the both surfaces particular blood capillaries and alveolar epithelial cells are distributed respectively. In the alveolar lumina (*al*) a few free alveolar phagocytes are found. Levi, aldehyde-fuchsin-light green-orange G. $\times 480$

Fig. 8. High power view of a portion of the alveolar septum. *ac* Axial connective tissue layer containing elastic fibers (*ef*) and connective tissue cell (*bc*), *ae* large alveolar epithelial cell with granules in supranuclear cytoplasm. *c* capillary containing erythrocytes, *al* alveolar lumen. Levi, aldehyde-fuchsin-light green-orange G. $\times 1,080$

groups among the capillaries, rest with their basal surface on the axial connective tissue layer and face the alveolar lumen with their more or less convex free surface; they contain relatively large, round or oval, clear nuclei with small nucleoli. They are classified into large and small ones: The large alveolar epithelial cells (Fig. 8 and 9) are rich in dark cytoplasm containing in the supranuclear region several small granules stained by orange G or azocarmine (Fig. 8 and 15); by careful observation with high power a faint brush border can occasionally be found along the free surface

Fig. 9. High power view of portion of the alveolar septum. *ac* Axial connective tissue layer with connective tissue cells (*bc*), *ae* large alveolar epithelial cell rich in cytoplasm, *al* alveolar lumen, *c* capillary. Zenker-formalin, hematoxylin-eosin. $\times 1,080$

Fig. 10. High power view of the alveolar septum. *ac* Axial connective tissue layer with connective tissue cells (*bc*), *al* alveolar lumen, *as* small alveolar epithelial cell with thin cytoplasmic projections ($\uparrow$) covering the alveolar surface of the capillaries (*c*), *en* endothelial cell. Zenker-formalin, hematoxylin-eosin. $\times 1,080$

(Fig. 8). The small alveolar epithelial cells possess a small amount of cytoplasm surrounding as a thin cytoplasmic layer a relatively large nucleus. They contain no stainable granules, but protrude thin cytoplasmic projections covering continuously the alveolar surface of the adjacent blood capillaries (Fig. 10 and 11). In all dolphins examined in the present study the majority of the alveolar blood capillaries are filled with erythrocytes among which a number of leucocytes are intermingled. The latter are rich in cytoplasmic glycogen, the PAS staining is therefore most useful in detecting them.

The dark nuclei of the endothelial cells lining the alveolar blood capillaries are spindle- or rod-shaped. They are elongated along the inner surface of the capillary wall, but their location is variable

Fig. 11. Alveolar septum with thick bundles of elastic fibers (*ef*) in the axial connective tissue layer. *al* Alveolar lumen, *as* small alveolar epithelial cell with thin cytoplasmic projection lining the alveolar surface of the capillaries (*c*), *bc* connective tissue cell, *en* endothelial cell. Zenker-formalin, PAS-hematoxylin. $\times 1,080$

occurring sometimes in the capillary wall facing the alveolar lumen, sometimes on the contrary in the basal wall resting on the axial connective tissue layer and occasionally in the lateral wall (Fig. 10 and 11).

As mentioned above, it has been revealed that the cell components, which participate in the structure of the alveolar septa, are, as in common terrestrial mammals, the large and small alveolar epithelial cells, the connective tissue cells and the endothelial cells.

In all specimens from six dolphins more or less numerous large

Fig. 12. Large round alveolar phagocytes (*ap*) storing large fat droplets blackened by osmic acid in the alveolar lumen (*al*). *ae* large alveolar epithelial cells, *c* capillary. Levi, aldehydefuchsin-light green-orange G. $\times 1,080$

round cells, containing large fat droplets blackened by osmic acid, are frequently found in the alveolar lumina (Fig. 12). Their cytoplasm appears like that of the large alveolar epithelial cells dark, their single round or oval nucleus has been usually pushed by fat droplets to one side of the cytoplasm. In sections fixed by Zenker-formalin and stained by hematoxylin-eosin the fat droplets have been completely dissolved and remain as clear vacuoles in the dark cytoplasm, and among these fat vacuoles a small amount of black particles, probably dust particles, are scattered throughout the cytoplasm (Fig. 13). These large round cells found in the alveolar

Fig. 13. Large round alveolar phagocytes (*ap*) in the alveolar lumen (*al*), which contain fat vacuoles and a few dust particles (black). *ae* Large alveolar epithelial cell, *bc* connective tissue cell in the axial connective tissue layer, *c* capillary. Zenker-formalin, hematoxylin-eosin. $\times 1,080$

lumina may probably be alveolar phagocytes or macrophages storing fat droplets and dust particles which should have been taken up by them by means of phagocytosis. In dolphin lungs the amount of fat droplets stored in them is generally large, on the contrary that of dust particles small.

Now the origin of the alveolar phagocytes released in the alveolar lumen must be investigated. On the alveolar wall the similar large round dark cells containing large fat droplets are occasionally found in the location of the alveolar epithelial cells and their bulky round cell body project extensively into the alveolar lumen (Fig. 14); they might represent alveolar phagocytes fixed to the alveolar wall. On the other hand, there occur not infrequently such large alveolar epithelial cells which contain a number of small fat droplets in the apical cytoplasm (Fig. 15). The transitional forms are observed between these large alveolar epithelial cells with small fat droplets and the alveolar phagocytes fixed to the alveolar wall. The following presumption may therefore be probable, that the large alveolar epithelial cells may store fat droplets taken up from the blood capillaries and be enlarged as the stored fat droplets increase in size and number and finally be transformed into the large

round alveolar phagocytes which project more or less into the alveolar lumen. In sections fixed by Zenker-formalin and stained by hematoxylin-eosin it has been revealed that the large alveolar phagocytes fixed to the alveolar septa also contain, among fat vacuoles, a small number of dust particles which have probably been phagocytized from the inhaled air in the alveolar lumina (Fig. 16).

The large alveolar epithelial cells themselves do not contain dust particles, although they have, as mentioned above, the capacity to store fat droplets. Concerning the origin and properties of the alveolar phagocytes the following view can be proposed on the basis of the above observations: The alveolar phagocytes arise from the large alveolar epithelial cells; the latter store fat droplets and hypertrophy

Fig. 14. A large round alveolar phagocyte (*ap*) situated on an alveolar septum in the same location as alveolar epithelial cells, it contains large and small fat droplets. *al* Alveolar lumen, *ac* axial connective tissue layer containing elastic fibers in the alveolar septa. Levi, aldehyde-fuchsin-light green-orange G. $\times 1,080$

to be gradually transformed into the large round cells, the alveolar phagocytes, which have acquired the ability to phagocytize dust particles from the inhaled air. The alveolar phagocytes situated originally in the location of the alveolar epithelial cells have presumably a property to be easily detached by mechanical affections from the alveolar septa, so the tissue contraction induced by fixation and external pressure added to the specimens at the time of tissue resection can probably result in artificial detaching and make numerous free alveolar phagocytes in

Fig. 15. Large alveolar epithelial cell containing small fat droplets ($\uparrow$). Several large alveolar epithelial cells (*ae*) with stainable granules in supranuclear cytoplasm are seen. *al* Alveolar lumen, *ac* axial connective tissue layer in the alveolar septa, *c* capillary with erythrocytes. Levi, azan. $\times 1,080$

the alveolar lumen. In the areas where the alveoli have been fixed in rather dilated condition, no free alveolar phagocytes were found in the alveolar lumina.

Besides these epithelial phagocytes there occur in the axial connective tissue layer of the alveolar septa, in the adventitia of the blood vessels as well as in the connective tissue layer of the bronchiolar mucosa the mesenchymal histiocytes which are able to phagocytize and store fat droplets and dust particles. The histiocytes of the axial connective tissue layer of the alveolar septa scarcely store fat droplets, while they sometimes contain a considerable amount of dust particles (Fig. 17). The

Fig. 16. Large round alveolar phagocytes (*ap*) fixed on the alveolar septum and containing large fat vacuoles and a few dust particles (black) like the alveolar phagocytes in the alveolar lumen (Fig. 13). *al* Alveolar lumen, *bv* blood vessel. Zenker-formalin, hematoxylin-eosin. $\times 1,080$

Fig. 17. A mesenchymal histiocyte (*h*), storing considerable amount of dust particles, in the axial connective tissue layer (*ac*) of an alveolar septum in which elastic fiber (*ef*) and connective tissue cell (*bc*) are also seen. *al* Alveolar lumen, *c* capillary. Levi, aldehyde-fuchsin-light green-orange G. $\times 1,080$

most prominent storage of dust particles was observed in the histiocytes found within a small accumulation of the lymphocytes (lymphoid tissue) in the outermost layer of a bronchiolar mucosa (Fig. 18). As compared with other mammalian lungs, in the dolphin lung the histiocytes storing dust particles were in general scant and the amount of storage dust in each histiocyte was small, though some individual variations were noted.

As just mentioned above, though the connective tissue histiocytes of the alveolar septa store scarcely any fat droplets, those of other connective tissue areas often show storages of large amount of fat; they have been enlarged and rounded and contain several fat droplets of variable sizes (Fig. 19). The question, whether they

may migrate into the alveolar lumen to become free alveolar phagocytes or not, can not be answered in the present study.

The epithelial cells of the simple cuboidal epithelium of the terminal and respiratory bronchioli resemble in some cytological properties the large alveolar epithelial cells; they contain in the supranuclear area of the dark cytoplasm variable numbers of stainable granules like the latter (Fig. 1). Among these bronchiolar epithelial cells there appear not infrequently cells containing fat droplets (Fig. 20). They may probably be epithelial cells, which, similar to large alveolar epithelial cells, possess the capacity to take up fat from the blood and to store it as fat droplets. From these fat containing epithelial cells large round phagocytes may presumably arise, which contain large fat droplets and may be supposedly easily detached from epithelial lining into the bronchiolar lumen. In

Fig. 18. Small lymphoid tissue found in the outer layer of a bronchiolus mucosa. A number of histiocytes storing dust particles ($\uparrow$) are seen. *lv* Lymphatic vessel, *e* epithelium, *k* cartilages in the bronchiolar wall, *l* lumen of the bronchiolus. Zenker-formalin, hematoxylin-eosin. $\times 1,080$

the bronchiolar lumen there exist sometimes such free phagocytes which might consist of local phagocytes detached artificially or physiologically and alveolar phagocytes transported by air stream from the alveolar lumen.

Discussion

By observing some thick serial celloidin sections (20μ and 100μ thick) MURATA (1951) could in detail elucidate the ramification of the terminal portion of the bronchial tree in the lungs from

Fig. 19. Large round mesenchymal histiocytes (*h*) containing a large amount of fat droplets blackened by osmic acid in the connective tissue of the dolphin lung. Levi, azan. $\times 1,080$

Fig. 20. An epithelial phagocyte (*ep*) found in the simple cuboidal epithelium (*e*) of a bronchiolus respiratorius. The epithelium contains blood capillaries (*c*) filled with erythrocytes which seem as if facing directly on the lumen (*l*). *bd* Connective tissue layer of the mucosa, *k* cartilage with some chondrocytes containing small, osmium-blackened fat droplets. Levi, azan. $\times 1,080$

six species both of Odonto- and Mysticoceti. According to his observations especially in the lungs of the dolphin, *Prodelphinus caeruleoalbus*, bronchiolus terminalis gives off several branches from which arise respiratory bronchioles. The main stem of the respiratory bronchiole sends out side branches, each of which gives off 6 separate alveolar ducts through clear-cut openings. Each alveolar duct again gives off 4 alveolar sacs which protrude 20 alveoli. In the present histological study on the peripheral areas of the *Prodelphinus* lungs we could not satisfactorily clarify the detailed ramification mode of the terminal portion of the bronchial tree, since the paraffin sections used were too small and too thin to observe the three dimensional composition of the respiratory tissue. We could, however, identify bronchioli, bronchioli terminales, respiratorii and alveolar ducts among the numberless alveoli densely distributed throughout the sections. There occurred relatively numerous bronchioli terminales and respiratorii with thin mucous membrane lined by the simple cuboidal epithelium, the somewhat thicker bronchioli with thicker mucosa lined by the pseudostratified columnar epithelium were rarely encountered. The mucosa of these terminal branches of the bronchial tree contained no gland and the lining epithelium intermingled neither ciliated nor goblet cells, although KIKKAWA (1944) and MURATA (1951) proposed the occurrence of ciliated pseudostratified or stratified columnar epithelium and simple ciliated epithelium in the bronchiolar branches of the cetacean lungs.

The unique histological difference between bronchiolus terminalis and respiratorius consists in that in the latter the simple columnar epithelium contain from place to place blood capillaries which appear as if to face the bronchiolar lumen directly, although the luminal surface of them might presumably be lined by thin cytoplasmic projections of the adjacent epithelial cells. This histological finding suggests that this most distal portion of the bronchial tree may participate already

to some extent in respiratory process, therefore we have proposed for it the name "bronchiolus respiratorius", KIKKAWA (1944) reported both in toothed whales including dolphin and baleen whales the absence of bronchiolus respiratorius, while MURATA (1951), as mentioned above, confirmed the presence of it, and stated in agreement with us that in the respiratory bronchiole the non-ciliated cuboidal epithelium is altered here and there into the very thin respiratory epithelium, to which blood capillary are directly attached.

One of the most conspicuous histological characteristics of the dolphin lung consists in that the wall of bronchioli, down to the most distal end of the bronchioli respiratorii, is supported by numerous small cartilages regularly aligned along their longitudinal axis. The same finding has been reported by all previous workers engaged in the histological study on the cetacean lung (FIEBIGER 1916, NEUVILLE 1928, WISLOCKI 1929, BÉLANGER 1940, KIKKAWA 1944 and MURATA 1951), MURATA (1951), however, confirmed the disappearance of the bronchiolar cartilage in the intralobular bronchioli in the bottle-nose (*Beardius bairdii*). The presence of the bronchiolar cartilages in the cetacean lung should be the sign of the adaptation to the hydraulic pressure experienced in the time of the deep diving and moreover they may probably serve for the bronchiolar sphincter muscles as supporting points.

As widely known, in the mucous membrane of the bronchioli of the common mammalian lung a circular layer of the smooth muscle is observed down to the bronchioli alveolares between the lamina propria and the tela submucosa, instead of such a continuous circular layer there occur in the mucosa of bronchioli terminales and respiratorii of the dolphin lung, contrary to the result of KIKKAWA (1944), numerous well-developed sphincters at regular intervals, so that the bronchiolar lumen forms between sphincters many wide round dilations (ampullae or chambers) which are communicated with each other by constricted parts corresponding to the sphincters, showing a pattern similar to a string of beads. This second conspicuous histological characteristic, that the sphincter muscles occur up to the termination of the respiratory bronchiole, has been already reported by FIEBIGER (1916), NEUVILLE (1928), WISLOCKI (1929), BÉLANGER (1940) and MURATA (1951) in several species of cetacea. MURATA (1951) was the first who noted, as to the bronchiolar sphincter or circular muscle, a great difference between Ondonto- and Mystacoceti, proving that in the former including dolphin the sphincters are well developed, whereas in the latter they are in general poor, except for those in the fin whale which has many of them. According to him the formation of the successive ampullar dilatations or chambers along the bronchiolar lumen was scarcely seen in Mystacoceti. The functional significance of bronchiolar sphincter muscles in the cetacean lung may presumably consist in that at the time of long-term diving the considerable amount of air stored in the ampullar dilatations should be supplied by regulation of bronchiolar sphincters gradually via alveolar ducts to alveoli to be utilized there for respiratory process proceeding in the alveolar wall.

As has been already revealed by MURATA (1951) especially in the fin whale, and WISLOCKI (1929) in the porpoise (*Tursiops truncatus*), the alveolar septa of the dolphin lung are separated by a continuous thin axial connective tissue layer containing elastic fibers and a few connective tissue cells into two completely isolated surfaces facing on the neighboring alveolar lumina, and on both alveolar surfaces particular

blood capillaries and alveolar epithelial cells are distributed respectively. The finding that each alveolar septa possess two independent respiratory surfaces facing the neighboring alveolar lumina, represents the third conspicuous histological characteristic of the dolphin lung. In this way the alveolar septa of the dolphin lung are thicker measuring approximately 13 to 20 μ in width and possess in general more numerous blood capillaries and epithelial cells as compared with those of the common mammalian lung.

According to MURATA (1951) the interalveolar septum of the fin whale is about 40 μ thick and has capillary beds separately on each surface. The cuboidal epithelial cells are crowded corresponding to meshes of the blood capillaries in various numbers according to the size of the mesh.

In the dolphin lung the cuboidal alveolar epithelial cells, which are distributed among the blood capillaries and rest on the axial connective tissue layer, are classified into small and large ones, the latter of which are rich in dark cytoplasm, containing stainable granules in the supranuclear region. The recent light microscopic observations on the mammalian lung (detailed references are found in AOKI 1964, ITO 1965) have discovered the important evidence that the alveolar surfaces of the capillaries distributed in the alveolar septa are lined by thin cytoplasmic projections protruded from the alveolar epithelial cells. By electron microscopic studies on the lung of several mammals (see detailed references in ITO and SHIBASAKI 1965) the same finding has been confirmed and the further evidences have been elucidated that the alveolar epithelial cells can be distinguished in fact into large and small ones and the thin cytoplasmic projections covering the blood capillaries are protruded exclusively from the cell body (perikaryon) of the small alveolar epithelial cells. In his light microscopic study on the cetacean lung MURATA (1951) has reported the finding that the cuboidal epithelial cells of the alveolar septa, which are densely crowded in corners or in capillary meshes, protrude the thin continuous cytoplasmic projection over the blood capillaries, representing seemingly the so-called non-nucleated plates of the respiratory epithelium, although he did not distinguish large and small epithelial cells. In the present study the authors have fortunately succeeded in revealing the evidence by light microscopy that the small alveolar epithelial cells exclusively send the cytoplasmic projections covering the blood capillaries.

The problem concerning the origin of the alveolar phagocyte has not been solved in spite of large number of light and electron microscopic studies on the lung alveoli. Generally speaking, authors opinions are summarized either in the epithelial or in the mesenchymal theory (see detailed literature references in AOKI 1964, ITO 1965, ITO and SHIBASAKI 1965). AOKI, ITO, ITO and SHIBASAKI were of opinion, that the dust cells (alveolar phagocytes) should originate from large alveolar epithelial cells; thus they are supporters of the epithelial theory. AOKI has observed in his light microscopic study on the bat lung that large alveolar epithelial cells (alveolar phagocytes) store in autumn besides dust particles fat droplets probably taken up from the blood. HIGASA (1958) revealed by experimental studies that the alveolar phagocytes should have the capacity to take up lipid administered into blood and thereafter to break down or discharge partially into the alveolar lumen.

In the present study the authors have confirmed that the large round alveolar macrophages of the dolphin lung should arise from the large alveolar epithelial cells

and that they contain usually considerable amount of fat droplets and small amount of dust particles. As has been revealed by ITO (1965) in the study of normal human lung, the large alveolar epithelial cells of the dolphin lung themselves have never the capacity to take up dust particles from inhaled air; after differentiation into large round phagocytes containing fat droplets they might gain for the first time the ability to phagocytize and store dust.

In sections of the dolphin lung, besides the alveolar phagocytes fixed to the alveolar wall like the alveolar epithelial cells, there occurred frequently a considerable number of them in the alveolar lumina. This evidence suggests, as has been proposed by MACKLIN (1951) and ITO (1965), that they might be easily detached from the alveolar septa by external affections, for example by tissue contraction induced by fixation and by pressure added at the time of tissue resection etc. The reason why the alveolar phagocytes are easily detached from the alveolar wall, was recently clarified by ITO and SHIBASAKI (1965) in the electron microscopic observation on the bat lung, in which they have revealed, that the large alveolar epithelial cells, at the time of transformation into phagocytes, are separated from the basement membrane of the alveolar wall by the thin cytoplasmic projection of the small alveolar cells which extend between the basal surface of them and the basement membrane. However, the entire alveolar phagocytes found freely in the alveolar lumina should not be regarded as artificially detached cells, at least some of them are taken for physiological ones, which may participate, as proposed by HIGASA (1958), in the discharge of fat via the air passage.

In the present study findings have been often observed which might suggest that the epithelial cells of the bronchioli terminales and respiratorii might also possess, concerning fat metabolism, similar properties and functions as large alveolar epithelial cells or alveolar phagocytes.

In the dolphin lung there exist, besides these epithelial phagocytes, several mesenchymal histiocytes in the connective tissue. Among them histiocytes in the axial connective tissue layer of the alveolar septa and those in small lymphoid tissue found in a bronchiolar wall stored almost exclusively dust particles of considerable amount. The histiocytes in other connective tissue portions stored mainly fat droplets like alveolar phagocytes. Thus, in the dolphin lung the amount of dust particles stored in alveolar phagocytes and mesenchymal histiocytes is in general very small as compared with that in the common mammalian lung. This fact may suggest, that in the sea surface, where dolphins live, the air contamination must be minimal. On the contrary the large amount of fat stored in alveolar phagocytes and mesenchymal histiocytes of the dolphin lung may support the view that the lung should participate to a large extent in fat metabolism as proposed by HIGASA (1958).

In his study on the porpoise lung WISLOCKI (1929) has already noted that within the lumen of alveoles and in smaller bronchioles, free mononuclear cells are encountered, the majority of which contain a fine stippling of black pigment. He has also encountered pigment in small clumps, here and there, in the fibrous tissue of the lung, especially in the major septa and in the adventitial coat of the bronchioles. His mononuclear cells with pigment stippling may correspond to the free alveolar phagocytes in the alveolar and bronchiolar lumina containing fat droplets and dust particles which we have observed in the present study. The pigment in small clumps

found by him in the connective tissue corresponds probably to the histiocytes storing dust particles as described by us. Further, BÉLANGER (1940) also has reported that in the lung of humpback whale (*Magaptera nodosa*) there occur in the lumen of the air spaces groups of epithelial cells that seem much larger than the lining cells and whose cytoplasm shows signs of vacuolization. These large cells correspond certainly to the large epithelial alveolar phagocytes containing the fat droplets described in the present study. He could not, however, answer the question as to whether or not these cells are desquamated lining epithelial cells or macrophages that have migrated through the alveolar wall. In the present study the only question left unanswered, is whether or not the mesenchymal histiocytes in connective tissue which store large fat droplets may migrate into the alveolar lumen.

The previous authors have agreed with each other in the observation that in cetacean lungs the elastic fibers are in general abundant (FIEBIGER 1916, WISLOCKI 1929, BÉLANGER 1940, MURATA 1951, ENGEL 1966 etc.). According to our observation on the dolphin lung, the development of the elastic tissue, as compared with that in other common mammalian lungs, seems to be not so prominent. The most well-developed thick elastic strands or bands were found in the wall of the alveolar duct. MURATA (1951) could confirm a species difference as to the composition of this elastic tissue proving that in the baleen whales the elastic bands contain a great amount of muscle fibers and might be called "myoelastic", while in the toothed whales the muscles contained in the bands are very few. The recent report of ENGEL (1966), however, does not completely agree with him, saying that in typical mammals the elastic strands of the alveolar ducts are mainly composed of muscle fibers and, to a lesser degree, of elastic fibers ("myoelastic" type), while in the lungs of large baleen whales elastic fibers predominate ("elasticomyal" type). Nevertheless, in the present study we have made it clear that the smooth muscle fibers were almost completely absent in the elastic bands of the alveolar duct of the dolphin lung.

Summary

In the terminal portion of the bronchial tree of the dolphin lung the bronchioli, bronchioli terminales, respiratorii and alveolar ducts were identified. The simple cuboidal epithelium of the bronchioli respiratorii contained blood capillaries which appeared as if exposed directly to the bronchiolar lumen. In the wall of the bronchioli, down to the distal end of the bronchioli respiratorii, there existed numerous small cartilages. Numerous well-developed sphincters occurred especially in the wall of the bronchioli terminales and respiratorii at regular intervals and the bronchiolar lumen formed many large round dilations (ampullae) between the sphincters, which were communicated by constricted portions (isthmi) induced by the sphincters.

Each alveolar septum of the dolphin lung possessed two independent respiratory surfaces facing the neighboring alveolar lumina, which were completely separated by a thin continuous axial connective tissue layer. Both respiratory surfaces have their proper blood capillaries and alveolar epithelial cells respectively.

The cuboidal alveolar epithelial cells were classified into small and large ones, of which only the former protruded the thin cytoplasmic projection covering the alveolar surface of the blood capillaries. The large cytoplasm-rich cells might be transformed into round alveolar phagocytes storing a large amount of fat droplets

and scant dust particles which they might have probably taken up from the blood and inhaled air respectively. Similar transformation and fat storing were also sometimes observed in the simple cuboidal epithelium of the bronchioli. These epithelial alveolar phagocytes were easily detached from the alveolar wall into the alveolar lumina. Besides, there existed mesenchymal histiocytes; those in the connective tissue of the alveolar septa and in the small lymphoid tissue found in the bronchiolar mucosa stored mainly dust particles, while those in other connective tissue portions stored mainly fat droplets as did the alveolar phagocytes. Thus the amount of fat droplets stored in epithelial and mesenchymal phagocytes exceeded in general by far that of dust particles, the latter being as compared with that in other common mammals, extraordinarily small. These morphological facts suggested on the one hand that the dolphin lung may participate much in fat metabolism and on the other that the air contamination on the sea surface, where the dolphin lives, may be very low.

In the dolphin lung the development of the elastic tissue was not particularly good. The thick elastic strands found in the wall of the alveolar duct contained few smooth muscle fibers. The functional significances of the bronchiolar cartilages and sphincters were also discussed.

イルカ肺の呼吸組織の組織学的研究 (内容自抄)

イルカの肺の気管支分岐の終末部には 細気管支, 終末細気管支, 呼吸細気管支および肺胞管が区別された。呼吸細気管支の単層立方上皮は あたかも細気管支腔へ露出するとく見える毛細血管を含む。細気管支壁には 呼吸細気管支の遠位端に到るまで 多数の小軟骨があり, とくに終末細気管支と呼吸細気管支壁には 多数のよく発達した括約筋が 規則正しい間隔をおいて存在し, 細気管支腔は 括約筋の間で 大なる円形の拡大部を形成し, 括約筋によつて生ずる狭窄部によつて連絡する。

各肺胞中隔は 連続性の薄い中軸結合組織層によつて 隣接する肺胞腔に臨む2つの独立した呼吸面に分けられる。各呼吸面には各々別の毛細血管と肺胞上皮細胞が相接して分布する。

立方形の肺胞上皮細胞は大, 小2種に分類される。小肺胞上皮細胞は毛細血管の肺胞腔面を覆う薄い細胞質突起を出す。細胞質に富む大肺胞上皮細胞は 多量の脂肪滴と少量の塵埃顆粒を貯える大なる円い肺胞食細胞に転化する。これらは それぞれ血液と吸気から摂取されたものである。同様な転化と脂肪貯蔵が 終末および呼吸細気管支の単層立方上皮細胞にも時に認められた。これらの上皮性肺胞食細胞は 肺胞壁から容易に離れて肺胞腔へ脱落する。これらのほかに なお間葉性組織球があつて, 肺胞中隔の中軸結合組織内と細気管支粘膜の小リンパ組織内に認められた組織球は 主として塵埃顆粒を貯蔵していたが, 他部の結合組織にあるものは 上皮性肺胞食細胞のごとく主として脂肪滴を貯える。かくして一般に上皮性および間葉性食細胞に貯えられた脂肪量は塵埃顆粒量を遙かに凌駕する。塵埃量は一般哺乳類に比して非常に少ない。これらの形態学的事実は一方ではイルカの肺が脂肪代謝に高度に関与することを示し, 他方イルカの生息する海面の空気の汚染が非常に低いことを暗示する。

イルカの肺の弾性組織の発達の特記するほど良好ではない。肺胞管壁の厚い弾性線維束はほとんど平滑筋線維を含まない。そのほか細気管支軟骨と括約筋の機能的意義について論じた。

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