Fine Structural and Enzyme Histochemical Observations on the Respiratory Epithelium of the Caecilian Lungs and Gills. A Contribution to the Understanding of the Evolution of the Vertebrate Respiratory Epithelium*

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Received July 30, 1980

Summary. The alveolar epithelium of larval and adult caecilians is composed of one cell type only, the cytoplasm of which, however, is divided into two divisions: an organelle-rich part containing the nucleus and a flattened peripheral part which covers the blood capillaries. Apically the cells bear variously shaped microvilli, which are sparse on the flattened areas of the cells. Among the cytoplasmic structures electron-dense bodies and lamellated bodies are prominent. Morphological observations suggest that in the adult the lamellated bodies are derived from the dense ones, which presumably represent lysosomes. Acid phosphatase and unspecific esterase are present in the entire alveolar epithelium. The lamellar material is extruded by exocytosis. A close structural relation between lamellated bodies and multivesicular bodies—as to be found in many mammals—does not exist. In the larvae among others there exists an extensive lateral labyrinth between neighboring alveolar cells and a considerable variability of the lamellated bodies. The latter frequently arise within or near fields of glycogen or presumably also within membrane systems.

The thickness of the air-blood barrier measures in the thinnest parts about 1 μm. In the subepithelial connective tissue, beside the spacious blood and lymphatic capillaries, smooth muscle cells, fibrocytes, collagen and elastic fibers, mast cells and nerve fibers (with granule-containing varicosities) are found. Within the low septa, which divide the inner surface of the wall of the lung into flat polyangular chambers (=alveoli), pieces of hyaline cartilage occur in the proximal parts of the lung. Macrophages are of rare occurrence in the caecilian lung. They contain acid phosphatase, β-glucosaminidase and unspecific esterase and are located in the connective tissue and within the alveolar epithelium near epithelial cells exhibiting structural alterations. The epithelium covering the gills of young larvae is composed of two layers of squamous cells. The cells of the surface layer contain large secretory granules.

The adult caecilians (=Gymnophiona, =Apoda) are vermiform subterranean tropical amphibians, which constitute an own order as opposed to the anuran and the urodele (Nieden, 1913; Taylor, 1968). They possess long tube-like lungs, of which the left

* Supported by the Deutsche Forschungsgemeinschaft (We 380/5)
one is largely reduced (Wiedersheim, 1879). According to Marcus (1928, 1937) the structure of the caecilian lung is particularly primitive and does not even show essential differences if compared with the lungs of the lungfishes (Dipnoi). The inner surface of the thin wall of the lung forms simple low septa, which anastomose forming a network of folds which delineate shallow alveoli. Caecilians possess only first order septa without cilia; other amphibians frequently form in addition septa of higher order which display numerous cilia (Marcus, 1937). The proximal septa usually contain hyaline cartilage, a trait which Marcus (1937) also judges to be a primitive one. The alveolar lining consists of flattened epithelial cells which cover the blood capillaries. In one caecilian genus, Ichthyophis, the presence of lysosomal and lamellated bodies has been demonstrated with the electron microscope in the alveolar cells (Welsch and Storch, 1973; Pattle et al., 1977).

In respect of the composition of the amphibian alveolar epithelium two opinions can be found. A number of authors find only one cell type (Krause, 1923, in Anura; Marcus, 1928, 1937, in all groups of Amphibia; Brooks, 1970, in Anura and Urodela; Weibel, 1973, in Anura; Meban, 1973, in Anura; Berezin and da Silva Sasso, 1974, in Anura; Pattle et al., 1977, in all groups of Amphibia). Other authors describe two cell types (Oppel, 1905, Binet, 1952; Welslaw, 1934 and Okada et al., 1982, all in Anura and Urodela). The latter authors have observed beside the flattened alveolar cells, additional small granulated elements which do not participate in covering the blood capillaries. These observations are of interest since similar granulated cells have been detected in the alveolar epithelium of the higher vertebrates.

It is the aim of the present communication to analyze the fine structure of the alveolar epithelium of two different caecilian species, Chthonerpeton indistinctum and Ichthyophis paucisulcus, originating from different continents. Their relationships are comparatively distant. In the Ichthyophis species material from before and after the final metamorphosis (Klumpp and Eggert, 1934) was available. Comparing the lung of these tropical amphibians with their numerous ethological and anatomical specializations, with the lungs of other lower vertebrates, special as well as common characters shall be pointed out.

**MATERIAL AND METHODS**

Lung tissue of the following caecilian species was investigated: 3 adult specimens of Chthonerpeton indistinctum (South America; special thanks for making this species available are due to Prof. Dr. J. Hauser, S. Leopoldo, Brazil), 3 larvae (12 cm long), 5 adult specimens of Ichthyophis paucisulcus (Sumatra, Indonesia), and 3 larvae (3.5 cm long) still within the egg shell. Cold, 3.5%, phosphate buffered glutaraldehyde (pH 7.5) initially was injected carefully into the lumen of the lungs; after about 10 min small pieces of lung tissue were immersed into the same glutaraldehyde solution for 1 hr and 30 min. Thereafter the tissue was repeatedly rinsed in cold phosphate buffer (pH 7.5), postfixed in 2% OsO₄, again rinsed in phosphate buffer, dehydrated in ethanol and embedded in araldite. For light microscopy 1 µm thick sections were stained with toluidine blue. For electron microscopy thin sections were stained with uranyl acetate and lead citrate to be observed in a transmission electron microscope (Philips EM 300).
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For scanning electron microscopy, glutaraldehyde and osmium-fixed tissues were processed according to the critical point procedure and observed in a Cambridge Stereoscan S4-10.

Enzyme histochemistry: cryostat sections of formaldehyde (pH 7.2) fixed tissues were processed for acid phosphatase, alkaline phosphatase, alpha naphthyl acetate esterase, and \( \beta \)-glucosaminidase (Pearse, 1968, 1972).

**OBSERVATIONS**

**I. Lungs**

**A. Enzyme histochemistry**

Adult specimens of *Ichthyophis paucisulcus*:

Concerning \( \alpha \)-naphthyl acetate esterase, there was a positive reaction of medium to strong intensity in the entire alveolar epithelium. In the subepithelial connective tissue individual positively reacting cells are demonstrated (Fig. 1a).

Concerning acid phosphatase, the entire alveolar epithelium gave a positive reaction of medium to weak intensity. Usually the type of reaction is of a more diffuse character, only rarely a clearly granular reaction is recognizable. Individual positive cells were found in the connective tissue.

In respect to \( \beta \)-glucosaminidase there was no unequivocal reaction in the alveolar epithelium. A few positively reacting cells occurred in the connective tissue and in blood vessels.

And finally concerning alkaline phosphatase, a strong positive reaction is demonstrated in the capillary endothelium as well as in the endothelium of larger blood vessels (Fig. 1b).

**B. Scanning electron microscopy**

Adult *Ichthyophis paucisulcus*:

At low magnifications a network of low septa was recognizable which outlined the shallow alveoli. The surface of the septa and of the alveoli exhibited a microrelief which was formed by the capillary network which bulged into the lumen of the lung. Only one cell type was recognizable, which bore variable amounts of microvilli or microridges. Usually that part of an epithelial cell which covers a blood capillary bears few or no microvilli, whereas that part of the cell which contains the nucleus and which is located in the meshes between the capillaries, is characterized by numerous microvilli, which frequently form small groups. The septa bore no cilia (Fig. 2 a–d).

**C. Light microscopical and transmission electron microscopical findings**

1. *Chthonerpeton indistinctum*

In light microscopical cross sections the tube-like lung was of almost circular outline. From the inner face of its thin wall 5–7 low septa projected into the wide central lumen. The diameter of the proximal cross section was about 2.5 mm, the distal cross section measured about 0.8–1 mm. In the proximal parts of the lung the septa contained pieces of hyaline cartilage and smooth muscle cells, while in the distally located septa, cartilage was lacking (Fig. 1c). The inner surface of the lung was
composed of a one layered flat epithelium. The septa showed no epithelial special-
izations. This epithelium, the alveolar epithelium, covered the wide blood capillaries
which formed a uniform layer. The nucleus containing part of the alveolar cells
was located between the capillaries, and projected into the underlying connective

Fig. 1. a and b. Ichthyophis paucisulcus, adult. a. Alpha-naphthyl acetate esterase, mainly in
the alveolar epithelium. × 350  b. Alkaline phosphatase in the capillary endothelium.
×350. c and d. Chthonerpeton indistinctum, adult. c. Proximal part of the lung with
two pieces of cartilage. ×220. d. Alveolar lining and blood capillaries. ×350. A alveo-
lar lumen, arrows: nuclear area of the pneumocytes.
Fig. 2. *Ichthyophis paucisulcus*, adult. 

a. General pattern of septa and alveoli. ×110. 

b. Alveolar surface, note the variable distribution of the microvilli and microridges. C capillary bulging into the lumen. ×2,200. 

c. Nuclear parts of the pneumocytes with numerous microvilli in the meshes of the capillary network. ×2,300. 

d. Higher magnification clearly showing the two subdivisions of the pneumocytes: densely arranged microvilli on the nuclear area and more of less smooth surface of the flattened area over the capillaries. ×5,250
Fig. 3. **a and b. Chthonerpeton indistinctum.** Supranuclear cytoplasm of pneumocytes with electron-dense and lamellated bodies. ×19,440
tissue and into the lumen of the lung. Usually one or two nuclei are found between the capillaries (Fig. 1d). The cytoplasm may contain individual dark staining granules. Occasionally single epithelial cells were particularly large, such cells could be partly detached from the basement membrane.

The main components of the subepithelial connective tissue are the blood capillaries, collagen fibres and smooth muscle cells. In addition, larger vessels, including lymphatic ones, fibrocytes, mast cells (metachromasia), and individual other free cells

Fig. 4. Chthonerpeton indistinctum. a. Supranuclear cytoplasm of a pneumocyte with transitory stages between electron-dense and lamellated bodies. \( \times 19,400 \). b. At the right of the lamellated body two small granules resembling mucus droplets. \( \times 28,500 \). c. Flattened part of a pneumocyte, air-blood barrier (on top: alveolar lumen, below: lumen of blood capillary). \( \times 23,600 \)
occur. The outer face of the lung is covered by flat coelomic epithelial cells, and a mesopneumonium is present dorsally over wide extensions.

Electron microscope observation also indicates a single type of alveolar epithelial cells (= pneumocytes). Their nucleus is of irregular outline, frequently exhibiting apically directed projections. Heterochromatin is concentrated in the nuclear periphery with a voluminous nucleolus ordinarily in the center. A medium sized Golgi apparatus is to be found in supranuclear position, and is surrounded by clear vesicles. Prominent constituents of the cytoplasm are irregularly shaped electron-dense bodies and lamellated bodies (Fig. 3a, b). The amount, size and shape of both types of bodies vary considerably among cells, occasionally they fill large parts of the cytoplasm (Fig. 3b). Exocytosis of the lamellar material can be detected regularly. Often it is difficult to recognize the outer limiting membrane of the lamellated bodies. It is easy to establish a morphological series between the electron-dense and the lamellated bodies (Fig. 4a).

Figure 4b shows another type of granule, which is relatively small and the contents of which seem to mature from a fine particulate consistency to a fine filamentous substructure. Such granules, which resemble small mucus droplets, are mainly found in the apical cytoplasm.

Further constituents of the cytoplasm are: infrequent multivesicular bodies, individual rough and smooth cisterns of the endoplasmic reticulum, free ribosomes, groups of mitochondria, and bundles of microfilaments found mainly in the apical cytoplasm (Fig. 4a). The apical plasma membrane of the nuclear area of the pneumocytes forms numerous irregularly shaped microvilli (Fig. 3a). The flattened parts of the epithelial cells which extend from the nuclear part and cover the blood capillaries, usually bear only a few microvilli (Fig. 4c) and contain reduced amounts of organelles (including lamellated bodies). Regular components are basally located light vesicles, which also occur in the nuclear part of the cell, and an apical layer of microfilaments. Neighboring cells are interconnected by zonulae occludentes and desmosomal junctions.

The thickness of the air-blood barrier measures in thin areas about 1 μm; regularly a few collagen fibers occur between the alveolar epithelium and the capillary endothelium (Fig. 4c). In the connective tissue, in addition to those components which could be seen already in the light microscope, nerve fibers in the vicinity of smooth muscle cells and elastic fibers have been observed. The nerve fibers often contain electron-dense granules. A striking feature of the flat coelomic epithelium is the presence of individual lamellated bodies.

2. Ichthyophis paucisulcus

a. Adult animals: The histology and fine structure of the Ichthyophis lung closely resemble that of Chthonerpeton. A minor difference seems to concern the morphology of the microvilli of the epithelial cells. In Ichthyophis they are of a more irregular structure, often hand- or tree-shaped microvilli can be seen. In this species, as shown in the light microscope, very voluminous alveolar epithelial cells have occasionally been found, which partly are detached from the rest of the epithelium. Similar to the other pneumocytes these cells contain lamellated bodies and desmosomes. Their cytoplasm is characterized by the following peculiarities: a dense layer of apical clear vesicles including many coated ones, below this layer a broad zone of small, elongated
Fig. 5. *Ichthyophis paucisulcus*, adult. 

a. Supranuclear cytoplasm of enlarged, altered epithelial cell. ×12,300. 

b. Macrophage (M) below altered epithelial cell (E). B basement lamina. ×9,600
mitochondria, the number of which is markedly increased if compared with the ordinary pneumocytes, and an increased number of bundles of microfilaments. Furthermore, large vacuoles with very heterogeneous contents occur (Fig. 5a, b). Beside or below these cells macrophage-like cells and lymphocytic cells can be found, still within the epithelium. In one case in the extended extracellular space of such a group of cells a section of a heavily ciliated cell has been seen which is interpreted to be a ciliated protozoon, presumably a parasitic organism. Identical macrophages occur in the subepithelial connective tissue (Fig. 5b, 6).

b. Larval animals: The alveolar epithelium of the larva is composed of one cell type like that of the adult animal, and in identical fashion shows a division into a nuclear and a flattened region. A number of peculiarities, however, can be observed: 1) The base of the cells extends numerous long projections into the underlying connective tissue. 2) The lateral plasma membranes form below the apical junctional complex complicated interdigitations, forming a sort of lateral labyrinth (Fig.
Fig. 7. *Ichthyophis paucisulcus*, larva. a. Lateral labyrinth between neighboring pneumocytes. $\times 37,500$. b. Lamellated bodies (L) near glycogen. $\times 28,500$. c. Two lamellated bodies with double membranes in their periphery (arrows), suggesting origin from endoplasmic reticulum, note the halo, which is clearly to be recognized and which may correspond to the halo of the dense body on Fig. 8c. $\times 82,000$
1) The apical microvilli are relatively long and slender. 4) The nucleus does not only form apical, but also basal projections. 5) The cytoplasm contains large fields of glycogen. 6) The lamellated bodies are of greater morphological variability than in the adult, a) their shape is often very irregular, even rod-shaped lamellated structures have been observed (Fig. 8a); b) as in the adult, electron dense-bodies seem to transform into lamellated bodies (Fig. 8c, d), the dense bodies however are smaller than in the adult and in their periphery exhibit a characteristic halo; c) often lamellated bodies occur within or near the fields of glycogen (Fig. 7b); d) rather often one can observe that the outer delimitation of the lamellated bodies obviously is composed of two closely opposed membranes, occasionally also such double membranes can be discerned in the interior of the bodies (Fig. 7c, 8d).

II. Gills from a larva still within the egg shell

In order to round off the analysis of respiratory epithelia of the caecilians, a brief description will be given of the epithelium covering the long and branched gills which are shed after leaving the transparent egg shell. The gills are the breathing organ within the egg. The thin epithelium is composed of two layers of squamous cells, the lower layer being markedly thinner than the upper one. The individual cells are interconnected by punctuate desmosomal contacts, as typical for epidermal cells (for the Ichthyophis epidermis see WELSCH and STORCH, 1973). The upper cell
row bears a few microvilli and cilia. The most characteristic component of the cytoplasm of the upper cells are large, frequently elongated apical granules, the long axis of which often is perpendicularly orientated in respect to the apical plasma membrane. The morphological evidence suggests that they arise in the Golgi apparatus as elongated electron-dense granules which transform into elements with a

Fig. 9. *Ichthyophis paucisulcus*, gill-bearing larva from within an egg capsule. Gill epithelium, composed of two cell layers. On the left cross sections of cilia in the fluid containing space of the egg, note specific elongated granules with electron-dense contents (on top of the figure) or fibrous substructure. ×24,000
fibrous substructure. Rough ER, mitochondria and bundles of filaments are well-developed (Fig. 9).

DISCUSSION

The present investigation has shown that the alveolar epithelium of two caecilian species is composed of a single cell type. This finding is in agreement with the observations on the caecilian species Hypogeophis rostratus by Marcus (1928, 1937) and on Ichthyophis orthoplicatus by Pattle et al. (1977). Since also in many other amphibians only one cell type has been described (see Introduction) it seems justified to postulate that indeed the primitive vertebrate alveolar epithelium is composed of only one cell type. This cell type, however, has rather complicated fine structure: it consists of an organelle-rich part around the nucleus and a flattened peripheral part, poor in organelles, which covers the blood capillaries. These two divisions of one cell are strongly reminiscent of the two alveolar cell types which line the alveoli or related structures in reptiles (for literature see Welsch and Müller, 1980), birds (for literature see Pattle, 1978) and mammals (for literature see Meyrick and Reid, 1977). The perinuclear cytoplasm with its densely placed microvilli in the caecilian and anuran (Meban, 1973; Weibel, 1972; Berezin and da Silva Sasso, 1974; Pattle et al., 1977) and urodele (Brooks, 1970; Pattle et al., 1977) alveolar epithelial cells corresponds to the pneumocytes II of the higher vertebrates; the flattened peripheral part of the amphibian alveolar cells—poor in organelles and microvilli—corresponds to the pneumocyte I of the higher vertebrates. Obviously in the course of evolution one large and rather complex cell type has developed into two simpler cell types, each of which has taken over one of the main functions of the precursor cell. The pneumocyte I of reptiles, birds, and mammals exclusively takes part in the formation of the air-blood barrier, the pneumocyte II is chiefly concerned with the production of the surfactant (for review of the literature see Meyrick and Reid, 1977) and the regeneration of the alveolar lining (Gaynor, 1971). Such an evolutionary pattern of specialization with a division of function conforms with generally accepted patterns of evolution within the fields of comparative anatomy (Remane, 1956).

In regards to the specific position of the caecilians within the amphibians, electron microscopy has not added important arguments to those offered by Marcus (1937). This author considers the caecilian lung to be primitive among amphibians and vertebrates in general (simple—but not simplified—pattern of septa, presence of cartilage, unspecialized epithelium of the alveoli). At the ultrastructural level the amphibian pneumocytes are all rather similar, those of the urodele (Brooks, 1970; Pattle et al., 1977) with their frequent paucity of lamellated bodies seem to represent a specialized line of evolution. Thus, differences of habitat, mode of life, and systematic position in this case are reflected more clearly at the light microscopic level than at the ultrastructural one.

The present study has revealed a number of differences of the alveolar epithelium in larvae and adults of Ichthyophis. A chief difference concerns the periphery of the cells; in the larvae the plasma membrane extends microvillus-like structures into all directions, and the lateral labyrinth is a striking feature. This indicates a high surface activity at all sides of the cell, and especially intercellular transport
processes. The significance of these specializations is not clear, since the larva in spite of its aquatic mode of life, breathes air. In some aquatic turtles, however not in all, a similar lateral labyrinth as well as a basal one has been found by Welsch and Müller (1980).

In regards to the cellular origin of the lamellated bodies, so far no information exists concerning the caecilians and other amphibians. The present findings suggest differences in larval and adult animals. In the adult the lamellated bodies seem to arise from electron-dense lysosome-like bodies. In the larvae the situation is more complex. On the one hand, there is often a close spatial relation of the lamellated bodies and glycogen. O'Hare and Sheridan (1970) made a similar observation in the rat and postulated glycogen to be the source of the lamellated bodies. In the caecilians, however, these relations may be incidental, since a number of images suggest their origin from dense bodies as in the adult. Looking at the matter more closely, the dense bodies of the larva and the adult exhibit marked differences. In the larva they are usually small and have a characteristic halo, composed of fine floccular material (Fig. 7c, 8c), and also, they very often have an outer limitation which is composed of a double membrane. Double membranes are also to be found within the bodies and in their immediate surroundings. A similar observation must have been made by Pattle et al. (1977), who, without presenting a photomicrograph, remark that the lamellated bodies in amphibian may arise from the endoplasmic reticulum by coiling. If the observed double membranes indeed are to be derived from the endoplasmic reticulum, the electron-dense material (Fig. 8c, 8d) and the floccular material of the halo seem to accumulate outside the cisterns of the ER, between the double membranes, the lumen of the ER cisterns being almost obliterated. The exact mode of origin of the various components of the lamellated bodies certainly needs further investigation. The bodies shown in Figures 8a and 8b may represent different structures altogether. Finally, it seems noteworthy that no close relationship exists between multivesicular and lamellated bodies as has been described in some mammals (Sorokin, 1966; Kikkawa and Kaibara, 1972). The small granules with fine particular or filamentous contents are interpreted to be mucus droplets. As in reptiles, where they are more frequent (Welsch and Müller, 1980), their contents presumably take part in the composition of the surface film of the alveoli. Since such granules so far have not been described in mammals, their presence indicates considerable differences in the composition of the surface film in lower and higher tetrapodes.

Strikingly, in lower vertebrates including the caecilians, macrophages can be found only rarely in the alveoli (Bargmann, 1936). Possibly the pneumocytes themselves have a phagocytic ability, an assumption which is supported by the presence of acid phosphatase in the alveolar epithelium and the highly variable substructure of the lamellated bodies, which is particularly evident in reptiles (Welsch and Müller, 1980). Under certain conditions, however, macrophages seem to be able to invade the alveolar epithelium, as could be observed in Ichthyophis. In this species macrophages and lymphocytic cells have been found near enlarged epithelial cells, which presumably are in an activated state (numerous apical vesicles, large amounts of mitochondria and big vacuoles resembling voluminous lysosomes). The causative agents for this activation and the presence of macrophages and lymphocytes within the alveolar epithelium may be ciliated protozoan in this special case. At any rate,
the observed alterations show that defense mechanisms may operate in the amphibian lung, too.

Finally, brief mention will be made of the second cell type, which has been found in the alveolar lining of some amphibians, especially urodele (see Introduction). Since these cells have all the characteristics of pneumocytes II (Okada et al., 1962) their presence may be interpreted to be an expression of a particularly highly evolved alveolar epithelium within the group of amphibians, which appeared independent from reptiles, birds and mammals. On the other hand, they may be replacement cells, which in mammals, correspond to pneumocytes II as studies in the regeneration processes have shown (Adamson and Bowden, 1974). Evidently such cells are not present as a distinct cell type in the majority of amphibians, as has been mentioned before. If they are present, they may indicate a high cellular turnover possibly due to damage or growth processes.

Note added in proof: Upon investigating the lung of the African caecilian *Afrocaecilia taitana*, we have recently found that in this species the pulmonary septa are characterized by individual ciliary cells. Thus, the general statement of Marcus (1937) saying that the caecilian lung is devoid of cilia is no longer true, although, indeed, they seem to be of rather rare and irregular occurrence.

REFERENCES


