FULL PAPER **Anatomy**

Microvasculature in the Terminal Air Spaces of the Lungs of the Baird's Beaked Whale (*Berardius bairdii*)

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ABSTRACT. Lungs were obtained from five adult Baird's beaked whales (*Berardius bairdii*) and examined by means of light microscopy and scanning electron microscopy of corrosion casts. The alveolar septa of these whales are thick with a connective tissue core and a bi-layer capillary bed. A double capillary network is regularly found in the alveolar duct and alveolar septa. Occasionally, septa adjacent to alveoli and alveoli themselves show only a single capillary layer. The distance between the two capillary layers has a tendency to decrease toward the end of airspaces, suggesting an end result of capillary fusion. Vascular replicas of venous vessels have an nular furrows at regular intervals of 50 to 100 μm, which are caused by focal aggregations of collagen fibers circularly oriented and located immediately underneath the endothelium. The first valves appear in the collecting venules gathering alveolar capillaries. These valves are quite characteristic of flap-, funnel- and/or chimney like structures.

KEY WORDS: alveolar capillaries, Baird's whale, corrosion cast, lung, venous valves.

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The Baird's beaked whale (*Berardius bairdii*) is widely distributed around Japan, central California, U.S.A., and off Vancouver Island, Canada. They have been known to prefer deeper water, under the 1,000 meter line, and they can dive for over an hour. Small numbers of these beaked whales have been hunted off the Boso Peninsula in Japan, for several hundred years. Forty are taken annually under a government quota system since 1983.

An important part of understanding the respiratory function in diving mammals is having the knowledge of the structural features affecting ventilation and gaseous diffusion present in their terminal airways and alveoli. In contrast to what is known about the microanatomy of the lung [2–4], there is still little information about the microvascular system around the peripheral airways and alveoli of whales. It has been suggested that they have septa and alveoli with a thick sheet of connective tissue sandwiched between two capillary layers. Some reports have indicated that each alveolus has its own capillary bed and that these capillaries interconnect across the septal connective tissue [4, 10].

The aim of the present study is to visualize the alveolar microvasculature characteristic of cetaceans. In order to view the three-dimensional arrangement of the capillary network structure directly, we investigated corrosion casts of Baird's beaked whale pulmonary vasculature by means of light microscopy and scanning electron microscopy.

MATERIALS AND METHODS

Lungs from five Baird's beaked whales (adult males) were examined. All were harvested around Japan's coast by a whaling company in 2003. Samples of lung were taken 18–20 hr after they were sacrificed. These whaling were authorized by Fisheries Agency of Japan under the national regulation, and the biological surveys for each whale caught were conducted by the National Research Institute of Far Seas Fisheries (NRIFSF). Samples used in this study were authorized and obtained under the cooperation with NRIFSF, the whaling company and the Fisheries Agency of Japan.

Injection of resin: A cannula was tied into the pulmonary arteries. The vascular bed was flushed with 0.9% NaCl from the pulmonary artery. Through the same catheter a combination of methylmethacrylate monomer and Mercox (ratio in volume=8:2; Dainippon Ink & Chemicals, Tokyo, Japan) was subsequently injected. When resin was injected with a syringe under manual pressure, no attempt was made to measure the applied pressure. Then, the injected sector was placed in a water bath at 40°C for approximately 30 min to allow the resin to harden. Corrosion of the injected lung was accomplished by immersing it in 20% NaOH over a few days. After dissolution of the tissues, the corroded casts were thoroughly and repeatedly washed in running tap water. Individual pieces of larger lungs were placed in plastic cups and frozen in distilled water. The frozen blocks were cut into slices, 2–3 mm thick, with a fretsaw. After air being air-dried at room temperature, the casts were mounted on stubs, sputter-coated with gold and examined with a scanning electron microscope (ABT-32; Topkon Co., Ltd., Tokyo, Japan).

Histologic observation: Other pieces of lung were fixed in 10% formalin. Then the tissue was dissected out, dehydrated in ethanol and embedded in paraffin wax. A series of 5 μm sections was cut and stained with hematoxylin and eosin, and Azan Mallory.

RESULTS

Light Microscopy: Despite a careful observation of the experimental protocol, the tissue sections were far from

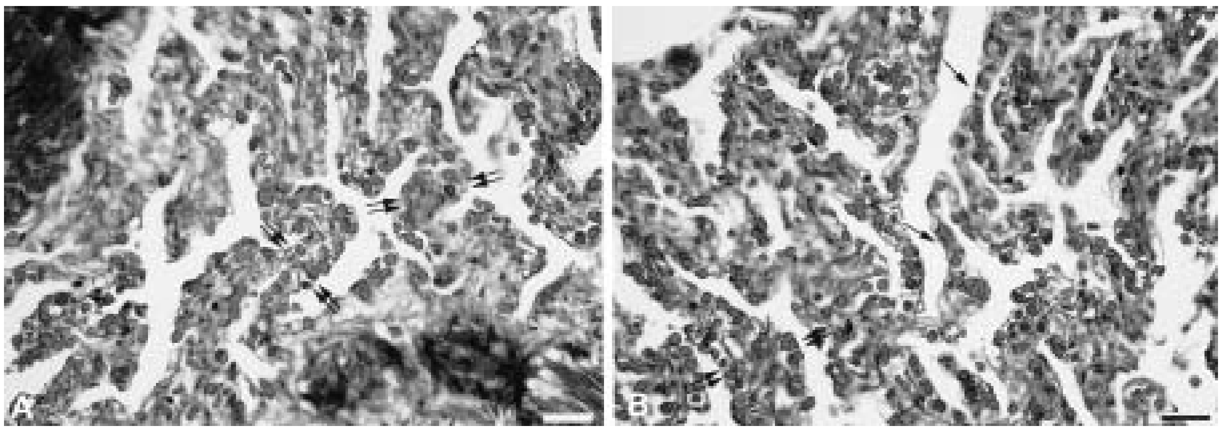


Fig. 1. Light micrograph of Baird's beaked whale lung. A. The alveolar septum possesses two capillary layers, one on either side (double arrows) of a connective tissue core. Azan Mallory stain. Bar=30 μm. B. The alveolar sacs exhibit one capillary network (arrows). Azan Mallory stain. Bar=30 μm.

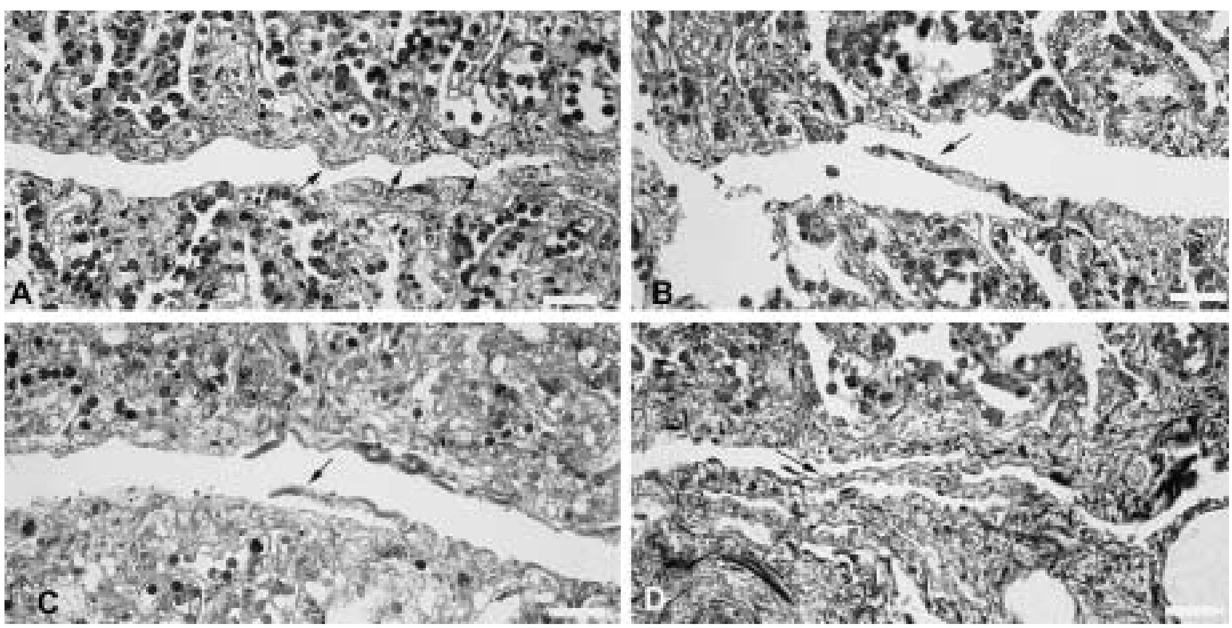


Fig. 2. Light micrographs of collecting venules. Azan Mallory stain. Bar=30 μm. A. A venule with focal tufts of collagen fibers (arrows). B. A venule with a single leaf-like valve (arrow). C. A venule with a funnel-like valve (arrow). D. A venule with a chimney-like valve (arrow).

being complete. The fine structure of the septa and alveoli was inconspicuous due to autolysis; swelling and/or sloughed endothelial and epithelial cells were frequently observed. The lung of the whale resembles that of terrestrial animals. In favorable sections, the capillaries are seen to be covered by a thin layer of alveolar epithelium. One suspects that two types of pulmonary epithelium are present there. The alveolar ducts and alveolar septa consisted of a thick connective tissue core 3–10 μm thick with fibroblasts, lymphatics and capillaries on each surface forming a double capillary bed (Fig. 1-A). Occasionally, septa abutting against the pleura, the larger vessels, or the terminal airways showed only a single layer. In some areas, the alveoli consisted of a thin wall, a single capillary bed and an alveolar epithelium lacking the connective tissue core (Fig. 1-B). Some collagen fibers aggregated and formed bundles encircling the wall of the pulmonary venules, and located in a series at short intervals of 50–100 μm (Fig. 2-A). Some were very well-developed and extended along the direction of blood flow to form thin flap-like (Fig. 2-B) or funnel-like valves (Fig. 2-C). The flaps consisted of a single sheet of valve and were 90–150 μm in length. The funnel was relatively short (30–90 μm) and with openings with 25–40 μm in diameter. Others were more prominent, forming long (150–200 μm) and slender chimney-like valves with narrow openings with 10–15 μm in diameter (Fig. 2-D). These valves were 10–20 μm in thickness and consisted merely of an endothelial layer accompanied by a thin connective tissue, quite similar to venous valves found in terrestrial animals. These venous valves were found only in the collecting venules, while ordinary venous valves were not found in any thicker intrapulmonary veins. Well-developed venous plexuses, consisting of many thin-walled veins up to 40 μm in diameter, were seen between the cartilage and the internal elastic lamina immediately beneath the epithelium of respiratory airways ranging from the trachea to the terminal bronchi (Fig. 3).

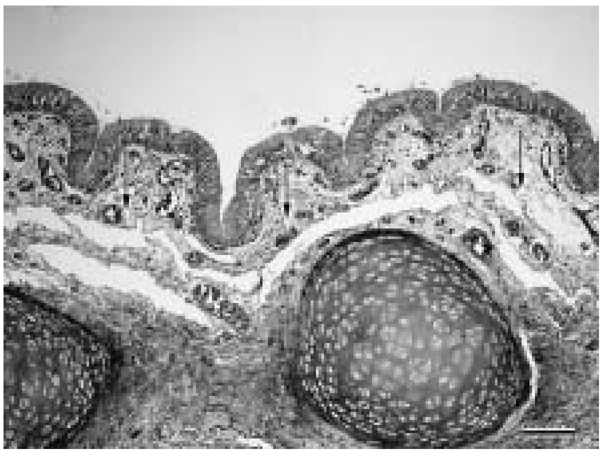
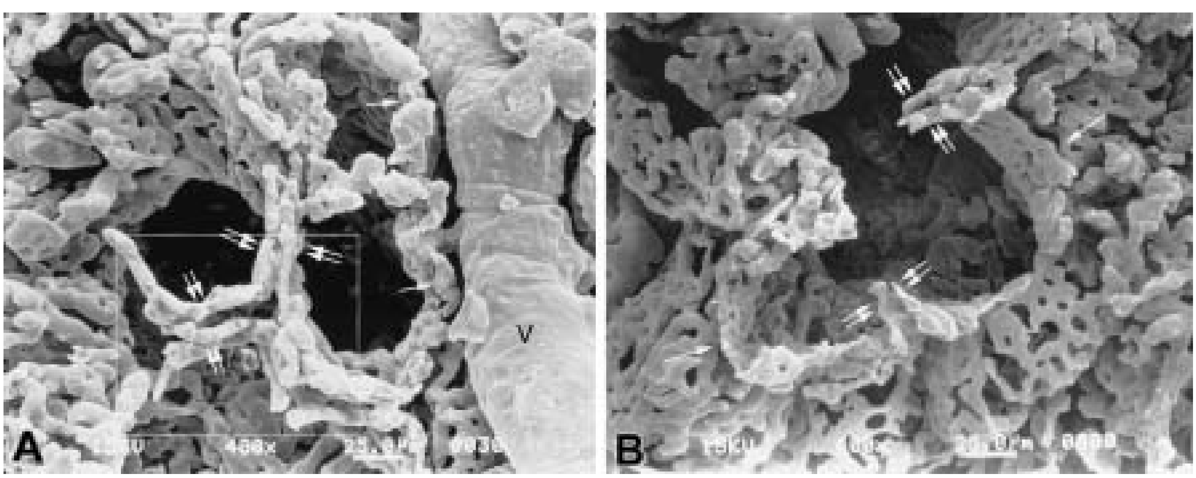


Fig. 3. Light micrograph. A terminal bronchus approximately 1.5 mm in diameter with a prominent venous plexus (arrows). Azan Mallory stain. Bar=120 μm.

Scanning electron microscopy of casts: Scanning electron microscopy of corrosion casts of the alveolar septum corroborated the observations made using light microscopy with respect to the double capillary bed. The bronchial vasculature was rarely filled; in some cases, a few vessels running longitudinally in the peribronchial and periarterial sheaths could be seen. The pulmonary arteries and their branches followed the bronchial tree to supply blood to the peripheral margin of the lobes. The capillaries of the lung parenchyma formed characteristic baskets in relation to the alveolar structures. The surface of the capillary casts did not show any constrictions or bulging areas. The mean diameter of the capillaries was 9–11 μm. In cross-sectional views of the pulmonary casts, the capillaries encircling the alveoli had a polygonal arrangement that gave them a honeycomb appearance as a whole (Fig. 4-A). The alveolar ducts and large parts of the septal microvasculature appeared as trilay-



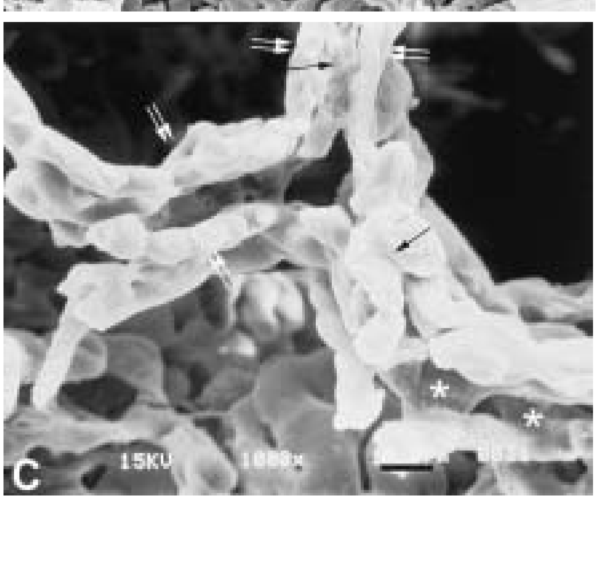


Fig. 4. SEM views of corrosion casts of Baird's beaked whale lung. A. Alveolar septa with double capillary network (double arrows). Septa abutting directly onto a large vessel possess only one capillary layer (arrows). v: venule. Bar=25 μm. B. Alveoli with one capillary layer (arrows). Capillary meshes are very irregular in size and shape. Alveolar septa with double capillary network are seen as well (double arrows). Bar=25 μm. C. Enlargement of an outlined area in Fig. 4A showing the two capillary layers of the septum (double arrows). Capillaries connecting each capillary network (asterisk) and capillary fusion (arrows) are clearly visible. Bar=10 μm.

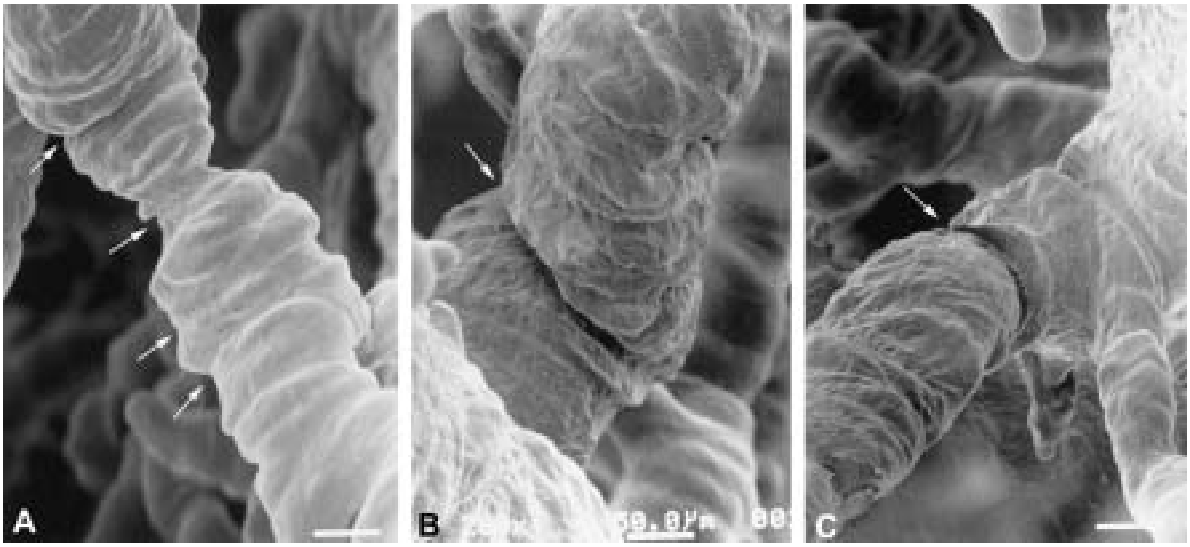


Fig. 5. SEM views of corrosion casts of collecting venules. Bar = 50 μm. A. Constrictions representing tufts of collagen fibers seen in Fig. 2A (arrows). B. An oblique cleavage presumably representing a leaf-like valve seen in Fig. 2B (arrow). C. A circular cleava ge presumably representing the base of funnel- and/or chimney-like valves seen in Fig. 2C and 2D (arrow).

ered structures with two distinct capillary networks, sandwiching the connective tissue core, which had been digested away. Capillaries formed fine vascular baskets around the alveoli. They often ran parallel and interconnected across the thick septum to form a ladder-like structure. The two capillary layers showed a tendency to decrease in the septa adjacent to alveoli, abutting against the pleura, the larger vessels, or the terminal airways, showing only a single capillary layer (Figs. 4-A, B). Our casts regularly revealed bridging or fusing segments between the two capillary networks (Fig. 4-C). The frequent incidence of the septa with a single capillary layer suggests an end result of capillary fusion. Collecting venules, being jointed by the capillaries, gradually increased in luminal diameter from 10 to 25 μm. The vascular replicas of collecting venules had distinctive and characteristic structures with ring furrows at regular intervals of 30 to 100 μm, which represented focal tufts of collagen fibers running circularly observed by light microscopy. Occasionally, marked strangulations were observed in these venules (Fig. 5-A). Casts of the collecting venules revealed slit-like cleavages running obliquely, which correspond to the flap-like valves (Fig. 5-B). Another impressive set of finding in the vascular casts of the venules was the narrow and deep annular cleavages, which represented the base of the funnel- and/or chimney valves (Fig. 5-C). These collecting venules joined gradually with other venules to form larger pulmonary veins, which had an average luminal size of 200 μm. These pulmonary veins failed to show valves and ring furrows, as did the greater pulmonary veins.

DISCUSSION

The entity of the double capillary bed in the alveolar septum in whales has been the subject of much debate. Our study confirmed that the alveolar septa contain two partially interconnected capillary networks and alveoli exhibit a single capillary meshwork. Caduff *et al.* [1] observed developing microvasculature in the postnatal rat lung, and was led to the following concept of microvascular restructuring. During postnatal growth the alveolar septa of the lung undergo restructuring. While immature septa are thick and contain two capillary layers, mature septa are thin and contain a single microvascular network. This restructuring of the capillary network occurs by fusing and is practically complete after 1 month of age. With the dilation of the airspaces during postnatal maturation, the mass of intervening mesenchyme decreases and the capillaries are arranged to fuse. As a result, the septal wall contains two partly interconnected capillary layers. It is quite interesting that the adult whale lung has mostly the same configurations as the immature rat lung. Similar reconstructing principles may go on in the Baird's beaked whale. Fanning and Harrison [2] suggested that the thick septum with connective tissue core and lymphatics may serve to strengthen alveolar walls and help then to adapt to fluctuating alveolar air-pressure on quick and frequent diving and emerging, especially during violent inspiration and expiration. Clearly, evidence in support of this suggestion is not yet overwhelming, and further studies with a different methodological approach are needed.

The circular connective tissue bundle in the pulmonary venules is similar to that in dog pulmonary veins [5], dog hepatic veins [6], human splenic veins [8] and dog penile cavernous veins [7]. Fluctuations in pulmonary blood volume may cause repeated expansion and contraction in the peripheral capacitance vessels. Vascular connective tissue components such as collagen and elastic fibers provide the stabilizing framework for vascular structures. This framework must be sturdy enough to maintain the potency of the peripheral vascular structure, yet flexible enough to permit the expansion and contraction of circulatory movements. Blood pressure in the pulmonary vein is known to increase during cardiac failure in terrestrial animals when venous return is impeded. When the heart as well as the lung is compressed on deep dives, a similar situation may occur in the whale lung. The circular connective tissue bundle may serve as hoops to preserve the integrity of the vessel wall, resist during the elevation of venous pressure, and to maintain dynamic blood circulation within the lung when submerged.

Venous valves first appear in the pulmonary venules just after the capillaries. The venous valves seen in whales are quite different from those in terrestrial mammals. If the retrograde of blood into capillaries must certainly be prevented in the elevated venous pressure caused by lung compression at depth, a series of long and slender single leaf-, funnel- and/or chimney-like valves is preferable to ordinary valves with double short leafs in other mammals. It is probable that the system of valves with unique structures is as an adaptation to gaseous changes in alveoli of the compressed lungs when submerged with bradycardia.

The well-developed venous plexus lying in the connective tissue beneath the bronchial epithelium, which is characteristic of the airways of Cetacea, has been seen in the Baird's beaked whale, as well by light microscopy of tissue sections in the present study. Fanning and Harrison [2] stated that engorgement of the plexus regulates the diameter of the trachea and/or bronchus and serve to expand contained gases in alveoli in a sudden, maximal flow of tidal air. Goudappel and Slijper [3], and Henk and Haldiman [4] suggested that the plexus may play a role in heating inspired air, or may act as aerodynamic cushions. Ridgeway *et al.* [9] stated that the venous plexus could well become engorged on deep dives and could help prevent complete collapse of the trachea and intra-pulmonary bronchi.

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