NOTE *Anatomy*

Scanning Electron Microscopic Study on the Microarchitecture of the Vascular System in the Pigeon Lung

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ABSTRACT. The resin casts of the respiratory and vascular systems in pigeon lung were examined using a scanning electron microscope. The primary bronchi branched to form many secondary bronchi that anastomosed with each other via the parabronchi. Numerous infundibula protruded from the parabronchi via the atria and ramified into the air capillaries. The pulmonary artery entered into the lung and branched into three vessels that coursed the interparabronchial parts. The intraparabronchial arterioles penetrated the gas-exchange tissue to form the anastomosing networks of blood capillaries. The observation of the double casts of the respiratory and vascular systems revealed three-dimensional complicated networks of air capillaries and blood capillaries.
KEY WORDS: lung, pigeon, vascular system.

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The respiratory organs of birds are structurally different from those of mammals. The avian lung is devoid of lobulation, alveoli, and tree-like branching of the air ducts that terminate in alveoli [8]. Hence, it can be assumed that the mechanics of breathing of birds are quite different from those of mammals [4].

The respiratory system of the avian lung is composed of ramifying and anastomotic parabronchi (tertiary bronchi) [5]. These parabronchi that arise from the secondary bronchi occupy more than half of the avian lung volume in the form of dense bundles of interanastomosing tubes [8]. Some narrow columnar chambers (infundibula) protrude from the parabronchi via the dilated chamber or depression of parabronchial wall known as atria [15], and a number of air capillaries arise from the end of the infundibula [3, 5, 8, 16].

Based on the relationship between the direction of air flow and the direction of capillary blood flow within the alveolar walls, two systems for gas exchange in the avian lung have been theorized. These theories, namely, the counter (parallel) current system and the cross current system, are based on the anatomical and physical models as well as gas analysis [3, 14, 16].

The anatomical relationship between the air capillaries and blood capillaries in the chicken lung is studied by the examination of an injected cast by using scanning electron microscope (SEM) [3, 9, 10] and transmission electron microscope [7]. However, the detailed morphological relationships between the terminal parts of these capillaries have not been verified. The cast of the blood capillary system in the pigeon lung is studied by the SEM in detail [15] but the respiratory system is not described. In this study, the fine structure of the double resin casts of both the respiratory tract and blood vessels in the pigeon lung was observed using SEM in order to elucidate the structural relationship between them at the level of the terminal parts of air capillaries and blood capillaries.

All procedures were done in accordance with the "Guiding principles for the care and use of animals in the field of physiological sciences" of the *Physiological Society* of Japan. A total of 15 adult male pigeons were used in this study. After inducing anesthesia with chloroform, the breast of the bird was opened, and the pulsating heart was exposed. The apex of the heart was excised and a polyethylene tube was inserted into the pulmonary artery, and the vessels in the lung were perfused with saline until the fluid was observed to run clear from the transected pulmonary veins. Immediately after the blood had drained off, a casting resin mixture (Mercox, Dainippon Ink and Chemicals Incorporated, Tokyo, Japan) was injected into the vessels of the lung through the same tube by using a disposable syringe.

To prepare the resin cast of the respiratory system, the resin was injected into the bronchi via the trachea without perfusion. The double casts of the vessels and the bronchi were prepared by the injection of the resin separately into the artery and trachea at the same time.

After complete polymerization of the injected resin, the lung was removed from the body and immersed in NaOH solution (concentration approximately 1%) at 60°C for tissue digestion. Following maceration of the lung tissues, the specimen was gently washed under running water. After the tissues were cleaned, the specimen was frozen, sliced using a razor blade in order to expose the observation surface for examination under a binocular dissecting microscope. The resultant cast was air-dried, sputter coated with gold in a vacuum chamber (E-1030, Hitachi Co. Ltd., Tokyo, Japan), and observed under a scanning electron microscope (S-4100, Hitachi Co. Ltd., Tokyo, Japan) .

Resin cast of the respiratory tract: The trachea divided into two primary bronchi in the lung. These primary bronchi branched into many secondary bronchi that anastomosed with each other by the parabronchi (synonym: tertiary bronchi; Fig. 1). Many small branches (infundibula) protruded from the side of parabronchi via the atria (Figs. 2, 3). At

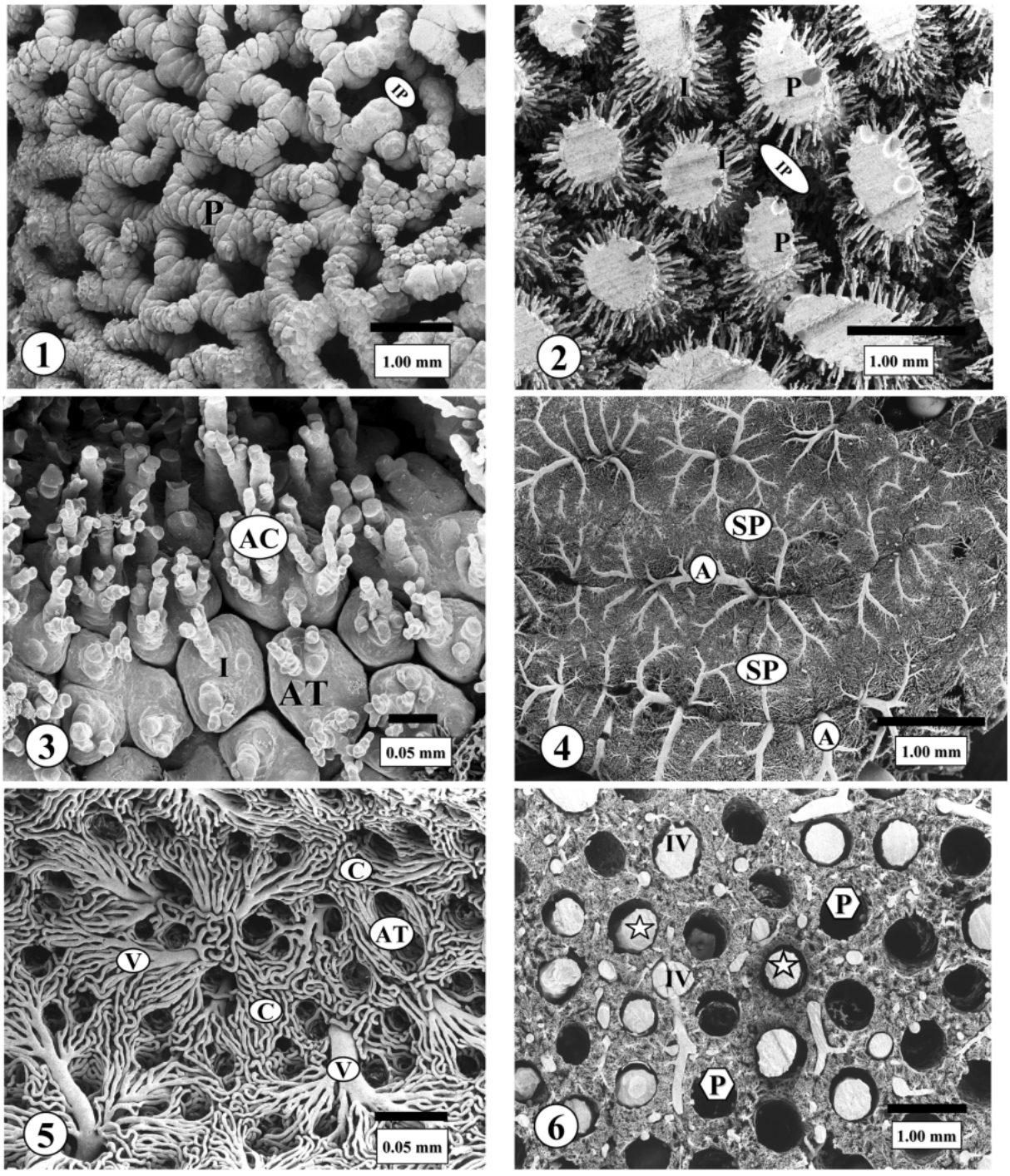


Fig. 1. Resin casts of the parabronchi in the parenchyma of the pigeon lung. The parabronchi anastomose and form a complicated labyrinth. In this specimen, the infundibula from the parabronchi are not observed due to incomplete injection of resin. IP: interparabronchial part, P: parabronchus.

Fig. 2. Cut surfaces of the parabronchial casts. Numerous infundibula protrude from the parabronchi. IP: interparabronchial part, I: infundibulum, P: parabronchus.

Fig. 3. Surfaces of the parabronchi. Some infundibula protrude from the atria and branch to form the air capillaries. These air capillaries ramify into several branches. AC: air capillary, AT: atrium. I: infundibulum.

Fig. 4. Casts of the blood capillary system. Surfaces of the parabronchi (basal view). The arteries in the parenchyma (A) enter into and branch to supply the surface of the parabronchi. SP: superficial vessels of parabronchus.

Fig. 5. Vascular cast of the parabronchial wall demonstrating the arrangement of a dense capillary plexus around the atria (luminal view). AT: atrium, C: vascular capillaries, V: venule from the capillaries.

Fig. 6. Cast of both the respiratory tract and vascular system (luminal view). A cut surface of the parabronchial part. Resin casts of parabronchi (stars) are observed in the hole surrounded by the dense capillary system. IV: interparabronchial vein, P: parabronchus.

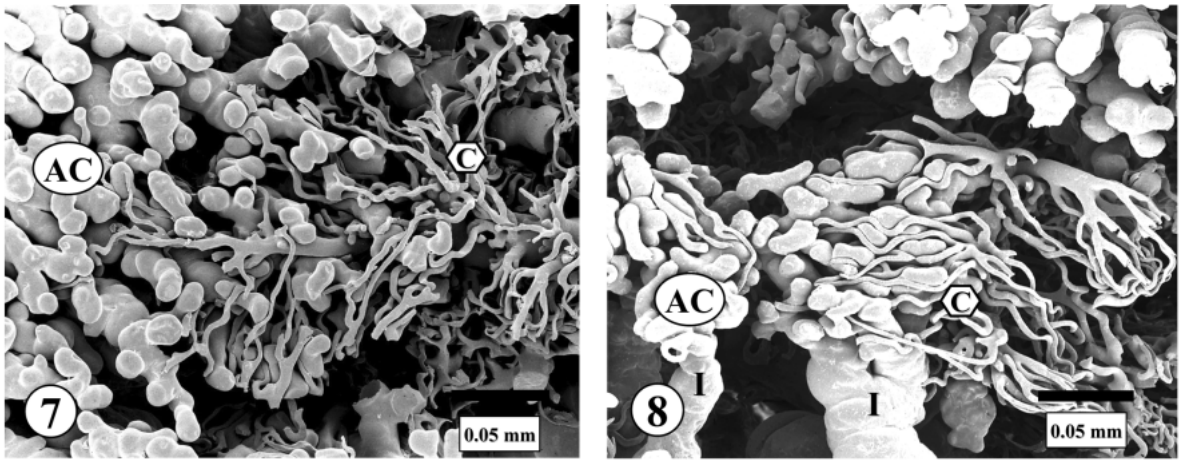


Fig. 7. Cast of the terminal parts of the respiratory tract and vascular system. The air capillaries ramify and have blind ends. The vascular capillaries branch like a tree and course between the air capillaries. AC: air capillary, C: vascular capillary.

Fig. 8. Cast of the terminal parts of the respiratory tract and vascular system. The air capillaries protrude from the infundibulum, and small vascular capillaries form the complicated respiratory plexus with the vascular capillaries. AC air capillaries, C: vascular capillaries, I: infundibulum.

their ends, the infundibula formed the air capillaries (synonyms: bronchioli, flute tube, or alveolar sac) [5] that branched like a tree. The terminal part of an air capillary had a blind end (Fig. 3).

Resin cast of the vascular system in the lung: The pulmonary artery entered into the lung to branch into three vessels (data not shown). These large arterial branches divided immediately and eventually formed many small arteries that coursed the interparabronchial parts. The interparabronchial arteries divided into some branches on the surface of the lung to surround the parabronchi (Fig. 4). The small intraparabronchial arteries from the interparabronchial arteries penetrated the gas-exchange tissue surrounding the parabronchial lumen (Fig. 4). These arterioles branched to form anastomosing networks of blood capillaries in a random manner, and they traveled in a predominantly circumferential direction around the openings (atria) of the air capillaries, which formed the bulk of the lung parenchyma. At the inner wall of the parabronchus, the continuous network of blood capillaries interdigitated with each other and surrounded the atria and infundibula (Fig. 5).

Double resin cast of the respiratory and vascular systems: The parabronchus was surrounded by the dense plexus of blood capillaries (Fig. 6). The infundibula were surrounded by many layers of capillaries (not shown). The intraparabronchial artery coursed between the infundibula and branched like a tree to form the blood capillaries (Fig. 7). The air capillaries were much more tortuous and ramified repeatedly, and they intimately interlaced with the blood capillaries. The small branches of the intraparabronchial artery passed through the spaces among the air capillaries and interdigitated with each other (Fig. 7). At the terminal part of the air capillaries, the vascular capillaries formed a three-dimensional network, and they intermingled with the air capillaries (Fig. 8).

The patterns of arterial supply and respiratory ducts in the pigeon lung are the same as those of other birds [1, 2, 6, 16]. The trachea divides into two primary bronchi, and these primary bronchi ramify into many secondary bronchi that further branch into parabronchi (tertiary bronchi) [4, 5, 8]. The parabronchi form a freely anastomosing network, and the air passages were continuous and had no blind ends; this was similar to the structure in other birds [6, 12, 13].

The pulmonary arteries were not closely associated with the bronchi within the lung. The large arterial branches divided and eventually formed many small arteries that coursed the interparabronchial parts. The terminal arteries in the alveolar wall formed anastomosing networks of capillaries that interdigitated in a random manner, and they traveled in a circumferential direction around the openings of the air capillaries.

The resin casts of the blood vessel in the pigeon [16] and chicken [3, 9, 10] lungs were observed by SEM, and the fine structures of these vascular capillary systems in the gas-exchange tissues were demonstrated. West *et al.* [15, 16] presented the schematic diagram of the relationship between the blood capillaries and infundibula in the pigeon lung. However, they could not elucidate the relationship between the vascular capillaries and air capillaries by using the single injection method. The double (of both the vascular and respiratory tracts) injection casting method was applied to chicken lungs [9, 10] in order to study the relationship between the air passages and vasculature. However, in these studies, latex rubber was used as the injection medium, and the injection technique is difficult because the artifacts are bound to arise from the extravasates as a result of the excessive pressure [10]. The latex rubber might not be able to pass through the small capillaries into the vein. In

the fowl lung, which are injected with latex rubber, almost all the casts of the arteries have blind ends and vascular capillaries are wider in diameter than the air capillaries [10]. This might be caused by the incomplete injection which can cast only large arteries. In this study, the double injection of the methacrylate compound (Mercox) into both the vascular system and air passages of the pigeon lung enabled the formation of fine casts.

The avian lung cannot alter its volume during either inspiration or expiration due to its position, rudimentary diaphragm, and the absence of a pleural cavity [1, 4, 5, 8]. The air sacs are filled and emptied via the bronchi [4], and they expand and contract similar to the bellows. The inspired air enters the air sacs through the parabronchi and air capillaries. During expiration, the air is exhaled from the air sacs via other parabronchi and air capillaries; thus, the gas exchanges in the avian lung are performed twice, i.e., during inspiration and expiration [5, 8, 16]. The air can circulate through these passages (anastomosed parabronchi and air capillaries) in contrast to the periodic inflow and outflow of air in the mammalian lung [11].

In this study, the interparabronchial artery coursed toward the parabronchial lumen perpendicularly (cross current system), similar to that observed in the previous reports [10, 15]. Circumferential capillaries surrounding the atria and the infundibula were observed (cross current system), which have been described by West *et al.* [15] as the gas-exchange capillaries. However, the air capillaries and vascular capillaries and both the cross and counter current systems might also be observed in the terminal gas-exchange tissues. The terminal part of the air capillaries branched irregularly, and the blood capillaries coursed between these branches. These interlaced capillaries formed a three-dimensional intricate structure. The transmission electron microscopic observation of pigeon lung revealed the presence of squamous respiratory cells between the air capillaries and blood capillaries; this ensures the integrity and stability of the gas-exchange tissues [7]. In conclusion, although the lung of the pigeon is restricted in volume during both phases of respiration, similar to that in other birds, the elaborate anatomical structure of the respiratory and vascular systems and the relationship between the flows of these gas-exchange media might be more complicated than the cross and counter current systems and might be able to efficiently perform the respiratory gas exchange.

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