Development and Spatial Organization of the Air Conduits in the Lung of the Domestic Fowl, *Gallus gallus* variant *domesticus*

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KEY WORDS avian; lung; development; secondary bronchi; parabronchi

ABSTRACT We employed macroscopic and ultrastructural techniques as well as intratracheal casting methods to investigate the pattern of development, categories, and arrangement of the air conduits in the chicken lung. The secondary bronchi included four medioventral (MVSB), 7–10 laterodorsal (LDSB), 1–3 lateroventral (LVSB), several sacobronchi, and 20–60 posterior secondary bronchi (POSB). The latter category has not been described before and is best discerned from the internal aspect of the mesobronchus. The secondary bronchi emerged directly from the mesobronchus, except for the sacobronchi, which sprouted from the air sacs. Parabronchi from the first MVSB coursed craniodorsally and inosculated their cognates from the first two LDSB. The parabronchi from the rest of the LDSB curved dorsomedially to join those from the rest of the MVSB at the dorsal border. Sprouting, migration, and anastomoses of the paleopulmonic parabronchi resulted in two groups of these air conduits; a cranial group oriented rostrocaudally and a dorsal group oriented dorsoventrally. The neopulmonic parabronchial network formed through profuse branching and anastomoses and occupied the ventrocaudal quarter of the lung. There were no differences in the number of secondary bronchi between the left and right lungs. Notably, a combination of several visualization techniques is requisite to adequately identify and enumerate all the categories of secondary bronchi present. The 3D arrangement of the air conduits ensures a sophisticated system, suitable for efficient gas exchange.

INTRODUCTION

The avian lung is noted to be an efficient albeit functionally and morphologically complex organ (King and McLelland, 1984). Farner (1970) observed that, "historically the avian respiratory system is highly ranked among the controversial organ-systems." As recently noted by one of the leading avian morphologists, the functional design of the avian respiratory system remains abstruse, despite concerted efforts to unravel the mysteries of its architecture (Maina, 2003a). Notably, the lung is noncompliant and it takes two inspiratory and two expiratory cycles for the air to traverse the entire system and get out to the trachea (Fedde, 1980). The physiological and anatomical explanations for this mechanism remain elusive and the topographical organization of the air conduits is recondite. The aerodynamic valves formerly purported to be important in regulation of airflow are now known to be nonexistent (King and McLelland, 1989; Maina and Africa, 2000; Maina and Nathaniel, 2001).

The air sacs operate like synchronized bellows that ventilate the lungs continuously and unidirectionally in a craniocaudal manner (Fedde, 1980; Scheid, 1979). Despite recent reports on the development of the avian lung (Maina, 2003a,b, 2004a,b, 2005, 2006), descriptions of the three-dimensional arrangement of the air conduits remain equivocal. The latest detailed update indicates that in the domestic fowl, the intrapulmonary primary bronchus gives rise to four dorsomedial bronchi, four dorsobronchi, and three laterobronchi (Lopez et al., 1992). Earlier, Duncker (1974) reported four medioventral secondary bronchi, 7–10 laterodorsal secondary bronchi, and an indeterminate number of laterobronchi. A recent review by Duncker (2004) has not helped resolve the situation since it maintains the short form of the names (dorsobronchi, ventrobronchi, etc.) and has not clarified the number of the various categories of secondary bronchi, or even their three-dimensional arrangement. A comprehensive review of development, structure, and function furnished by Maina (2006) has not addressed the confusion in nomenclature of the secondary bronchi.

At the parabronchial level, the air conduits form a honeycomb-like structure and are separated by the interparabronchial septa, which carry blood vessels and nerves (King and McLelland, 1984; Maina, 1982, 1988). From these parabronchi, there emerge the atria, which give rise to infundibulae and the air capillaries (Maina, 1988; Makanya et al., 2006). The air capillaries

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Received 10 February 2008; accepted in revised form 9 April 2008

Contract grant sponsor: Swiss National Science Foundation; Contract grant numbers: 31-45831.95, 31-55895.98, 31-55895.98/2; Contract grant sponsor: Bernese Cancer League.

TABLE 1. Techniques used and number of secondary bronchi encountered

Technique/specimen used	No of specimens studied	Categories of bronchi studied	Number of bronchi counted		Technique description
			Right lung	Left lung	
Gross specimens	Eight adults	MVSB	4	4	Examination of openings in the mesobronchus
		LDSB	7–10	7–10	
		POSB	20–6	20–60	
SEM	Four embryos	MVSB	4	4	Examination of tissue under SEM
		LDSB	7–10	7–10	
		POSB	24–56	20–50	
Corrosion casting	Two adults	MVSB	4	4	Direct observation in adults, use of SEM in embryos
	Four embryos	LDSB	7–10	7–10	
		LVSB	1–2	1–2	
Silicon rubber casting	Four adults	MVSB	4	4	Direct observation
		LDSB	7–10	7–10	
		LVSB	1–2	1–2	

are the sites of gas exchange but unlike the mammalian alveoli, they do not expand but are rigid structures that are thought to render mechanical support to the conterminous blood capillaries (West et al., 2006).

In the current study, we have used macroscopic as well as microscopic techniques to study the development of the air conduits and their definitive 3D arrangement in embryonic and adult birds. We present unequivocal data showing the numbers, categories, and structure of the extant secondary bronchi and their relationship to the rest of the air conduits.

MATERIALS AND METHODS

Experimental Animals

Brown leghorn eggs were incubated at 37°C and a humidity of 65%. Embryos covering Hamburger and Hamilton (HH) stages 31 to 46 (embryonic days 8–21) were obtained and processed as detailed below. In addition, adult Rhode Island Red layers (*Gallus gallus* variant *domesticus*) already identified for culling were procured for the study. For late stage embryos from embryonic day 18 (E18, HH stage 43) and adult birds, anesthesia was achieved by intra-abdominal injection of Euthatal® (sodium pentobarbitone, injected i.p. at a dosage of 50 mg kg^{-1} body mass). The details on the number of specimens used and the techniques applied are provided in Table 1.

Macroscopic Observations of Lungs

Lungs from eight adult birds were used for macroscopic observations of the secondary bronchi. Birds were killed as described above, lungs were fixed in situ by intratracheal infusion with a solution of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4, 350 mOsm), carefully dissected out, and subsequently immersed into the same fixative. A longitudinal cut was made along the long axis of the intrapulmonary primary bronchus, thus resulting in two halves of the mesobronchus exposing the internal aspect. These halves were used to identify and enumerate the various categories of secondary bronchi emanating from the mesobronchus (see Fig. 1).

Scanning Electron Microscopy of Tissues

This was performed on lungs from E14 to E21 (HH stages 39–45) embryos. A total of four embryos were used for this procedure. The lungs were fixed as described above, dissected out, and opened through a longitudinal cut along the long axis of the primary bronchus. The resulting two halves were washed, dehydrated in ascending concentrations of ethanol and dried in a desiccator. Samples were mounted on aluminum stabs, sputter-coated with gold, and viewed under a Philips XL 30 FEG scanning electron microscope.

Intratracheal Resin Casting

Two adult birds and four late stage embryos were randomly selected for intratracheal casting. To assess the development and final pattern of the bronchial network, methyl methacrylate resin was introduced through the tracheae of anesthetized adult birds as well as selected embryos under slight pressure. The entire lung was filled with methyl methacrylate resin (Mercox, Vilene Hospital, Japan) containing 0.1 mL accelerator per every 5 mL of the resin or with Technovit® 3040 powder mixed with catalyst in the ratio 1:1 as per the manufacturer's instructions. One hour after perfusion, either the entire embryo or specific targeted organs were immersed in Ringer's solution for at least 2 h and subsequently transferred to a 15% potassium hydroxide solution for 2–4 weeks. After dissolution of the tissues, the embryos and organs were washed, dehydrated in ascending concentrations of ethanol, and dried in a desiccator. Samples were mounted on aluminum stubs, sputter-coated with gold, and viewed under a Philips XL 30 FEG scanning electron microscope. In addition the resulting casts from adult bird lungs were subjected to macrophotography using a SHARP VL-Z500 digital camera.

Silicon Rubber Casting

Four (4) adult birds were randomly selected for this procedure. Silicon rubber was mixed with silicon oil at the ratio of 5:2 (volume per volume, vol/vol). Blue dye was then added and stirred until the required color was obtained. The resulting mixture was mixed with hardener at the ratio of 1:35 (hardener to mixture, vol/vol). The mixture was then injected into the tracheae of adult Rhode Island Red hens under deep barbiturate anesthesia (Euthatal®) administered as described above. The silicon was left to set for at least 15 min and the lungs (with or without air sacs) were dissected out and corroded in 15% potassium hydroxide. Photographs of the required parts of the resulting silicon

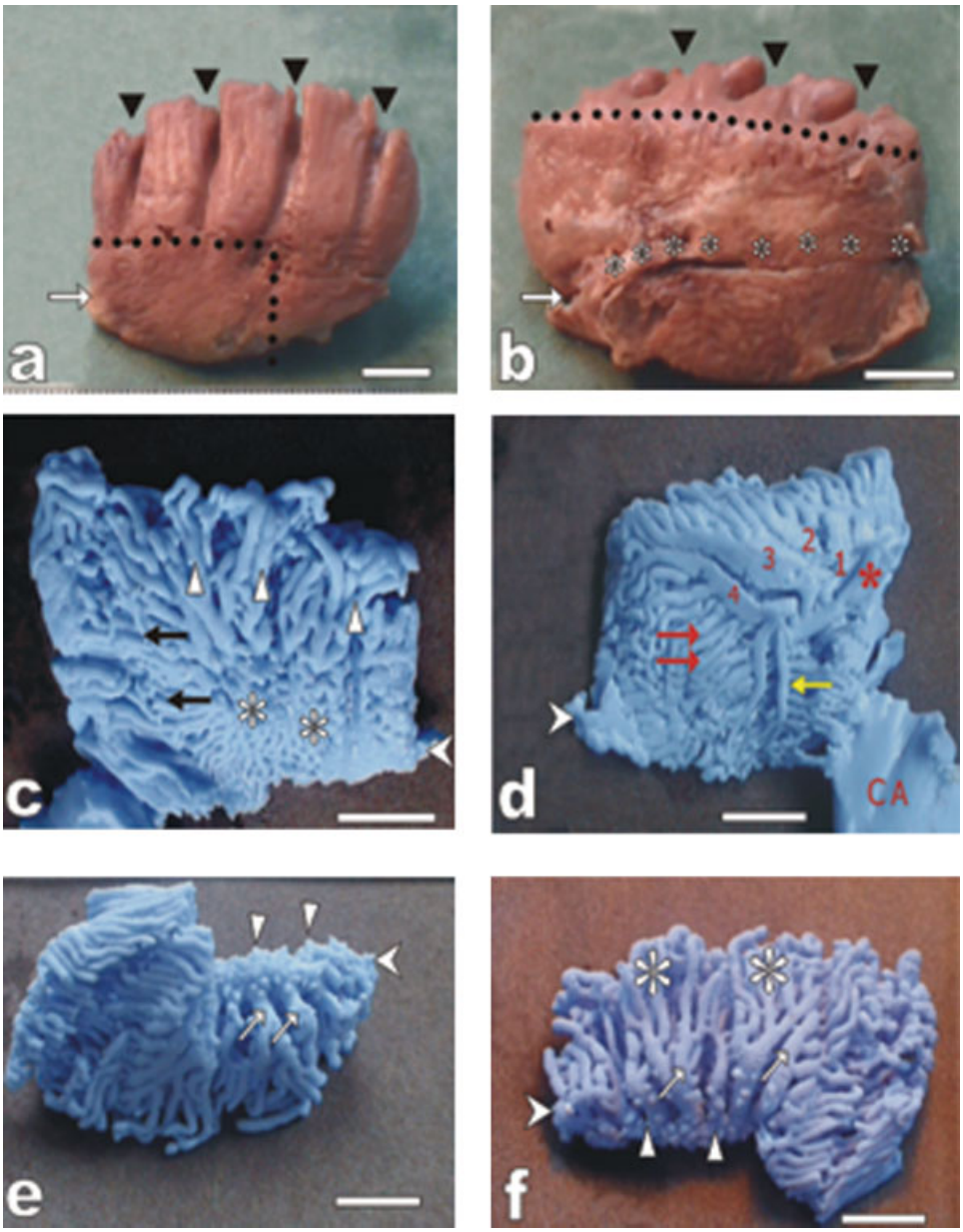

Fig. 1. Macrographs of fixed lung specimens (**a** and **b**), and silicon rubber casts (**c**–**f**) explicating the lung structure in the adult chicken. All Scale bar are 1 cm. a: On the lateral surface the rib impressions (arrowheads) extend down to the level of the long axis of the lung. Note the extent of the neopulmonic region (dotted line). The arrow indicates the position of the ostium of the abdominal air sac at the caudal part of the lung. b: The medial surface of the lung looks rather smooth, the ventral border (dotted line) is rounded, and the rib impressions at the dorsal border are deep (arrowheads). The arrow indicates the cranial extremity of the intrapulmonary primary bronchus. The trajectory of the primary bronchus is indicated by the asterisks (see Figs. 2a–2d for details). c–f: Silicon rubber casts showing the disposition of the parabronchi on the lateral surface (c, e, and f) as well as the arrangement of the secondary bronchi and parabronchi on the medial aspect (d). Horizontal arrowheads in all cases indicate the posterior aspect of the lung where the primary bronchus emerges and enters the abdominal air sac. c: A cranial group of paleopulmonic parabronchi runs parallel to the long axis of the lung (arrows). Caudal to the latter is a dorsal group, which is perpendicular to the long axis of the lung (arrowheads) and these are the direct branches of LD3–LD10. Below the latter secondary bronchi, lies the neopulmonic parabronchial network (asterisks). d: On the medial aspect, the primary bronchus (asterisk), the medioventral secondary bronchi (1–4), and the lateroventral bronchi (white arrow) are conspicuous. Note also the parabronchi emanating from the MV4 and the LV2 contribute to forming the ventral part of the neopulmonic region (double arrows). Part of the left cervical air sac (CA) is also indicated. e and f: Lateral view of silicon rubber casts with the parabronchi of the neopulmonic region removed to show the stumps of the severed POSB (vertical arrowheads). Arrows show the points where the LDSB curve dorsally and branch to form the dorsal group of the paleopulmonic parabronchi (see asterisks in f). Scale bar = 1 cm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 2. Names and numbers (mean ± SD) of secondary bronchi encountered in the lung of the domestic fowl

Older names	Proposed/preferred name	Numbers encountered	
		Left lung	Right lung
Ventrobronchi, Medioventral SB, Craniomedial SB, Anterodorsal SB	Medioventral secondary bronchi (MVSB)	4	4
Laterobronchi, Lateroventral SB	Lateroventral secondary bronchi (LVSB)	1.3 ± 0.46	1.4 ± 0.66
Dorsobronchi; Laterodorsal SB, Mediodorsal SB, Posterior dorsal SB	Laterodorsal secondary bronchi (LDSB)	9.5 ± 1.07	9.4 ± 1.05
Saccobronchi; Recurrent SB	Saccobronchi	Several	Several
Mediodorsal SB, Laterodorsal SB, Lateroventral SB, Posterior dorsal SB	Posterior secondary bronchi (POSB)	37.3 ± 12.4	36.1 ± 13.4

casts were obtained with a SHARP VL-Z500 digital camera.

Statistical Analysis

Number of various categories of the secondary bronchi were counted in both the right and left lungs for each individual. Adult birds and embryos within the targeted age ranges were picked randomly and processed for techniques that adequately revealed the structures under study. Notably, specific categories of secondary bronchi required unique techniques for their identification and enumeration. MVSB were accessible in all casts, that is, silicon and resin casts, (eight embryos, six adults), and all opened lung specimens (eight adults, four embryos). LVSB were counted only on cast specimens (four embryos, six adults). LDSB were counted on both cast and opened specimens, POSB were counted on opened specimen only (four embryos, eight adults). Data were presented as group means ± SD, (see Table 2). Differences between the left and right lung were analyzed with the paired student's *t*-test. In all cases $P < 0.05$ defined the level of statistically significant difference.

RESULTS

The macroscopic appearance of the adult chicken lung is demonstrated in Figures 1a and 1b. The rhomboid shape of the lung was distinct, with a sharp ventral and a rounded dorsal border. The dorsal half of the lateral surface and the dorsal border were marked by the very conspicuous costal sulci while the medial surface was generally smooth and flat. The lateral aspect of silicon rubber cast of the lung (Fig. 1c) clearly demonstrated the various categories of parabronchi. Directed toward the front and roughly occupying the rostral half of the lung was a set of parallel paleopulmonic parabronchi. The caudal dorsal quarter was taken by dorsally oriented paleopulmonic parabronchi, while the caudal ventral quarter was occupied by a network of profusely anastomosing neopulmonic parabronchi (Fig. 1c). On the medioventral aspect of the silicon rubber cast of the lung (Fig. 1d), the four medioventral secondary bronchi (MVSB) and the two lateroventral secondary bronchi (LVSB) and the parabronchi emanating from the latter two structures as well as the cervical air sac were vividly evident (see also Figs. 7a, 7b and 8a). Enumeration of the MVSB and the LDSB was made easier by cutting off the superficial parabronchi and especially the neopulmonic parabronchi to expose the secondary bronchi at their points of origin (Figs. 1e and 1f). The posterior secondary bronchi (POSB) were best discerned from the internal aspect of the opened mesobronchus (Figs. 2a–2d) and were demonstrated on silicon rubber casts by cutting off the entire neolpumonic parabronchial meshwork (Figs. 2e and 2f) thus leaving stumps of the POSB on the primary bronchus.

Development and establishment of the various categories of parabronchi was captured in semithin sections (Figs. 3a and 3b) and intratracheal casts (Figs. 3c–3f). At E8, large secondary bronchi with parabronchial buds invading the abundant mesenchymal tissue mantle were evident. At this time no other specific structures were evident in the vast mesenchyme, save for a few empty spaces, probably primitive vasculogenic sinusoids. The direction taken by the incipient secondary bronchi and parabronchi was studied in intratracheal resin casts (Figs. 3c–3f). The MVSB appeared to form first and gave rise to caudally oriented parabronchi that tended to approach the parabronchial buds from LDSB (Fig. 3c). By E17, the various groups of developing parabronchi were clearly discernible and a zone of parabronchial anastomoses (Figs. 3d and 3f) between the parabronchi from the first LDSB (LD1) and the second LDSB (LD2) on one hand and those from the first MVSB (MV1) was already established. The anastomoses were such that the abutting migrating parabronchial tubes formed one or more slender branches that inosculated those of approaching cognates. The growth of parabronchial branches from LD3 to 10 sprouted towards the dorsal border (Fig. 3e) where anastomoses with cognates from MV2 to MV4 were accomplished (see also Figs. 7 and 8).

Positions and categories of the various secondary bronchi were further studied with the scanning electron microscope on resin casts and fixed lungs of late stage embryos (Fig. 4). Removal of some parabronchi to reveal the targeted secondary bronchi enabled actual enumeration of the latter air conduits (Figs. 4a–4c). Evidence for the numbers and categories of the secondary bronchi in the late stage embryos was further augmented by making longitudinal sections of the lungs along the long axis of the intrapulmonary primary bronchus. The resulting two halves of the intrapulmonary primary bronchus were used to count the various categories of emergent bronchi (Figs. 4d–4f). The POSB participated in the formation of the neopulmonic network and sent branches to the dorsal group of paleopulmonic parabronchi (Figs. 4a and 4c).

The topography of the various categories of the incipient air conduits was captured from the resin casts of late stage embryos (Fig. 5). As evident from Figure 5, the initial parts of the secondary bronchi had no atria, showing that these parts did not participate in gas

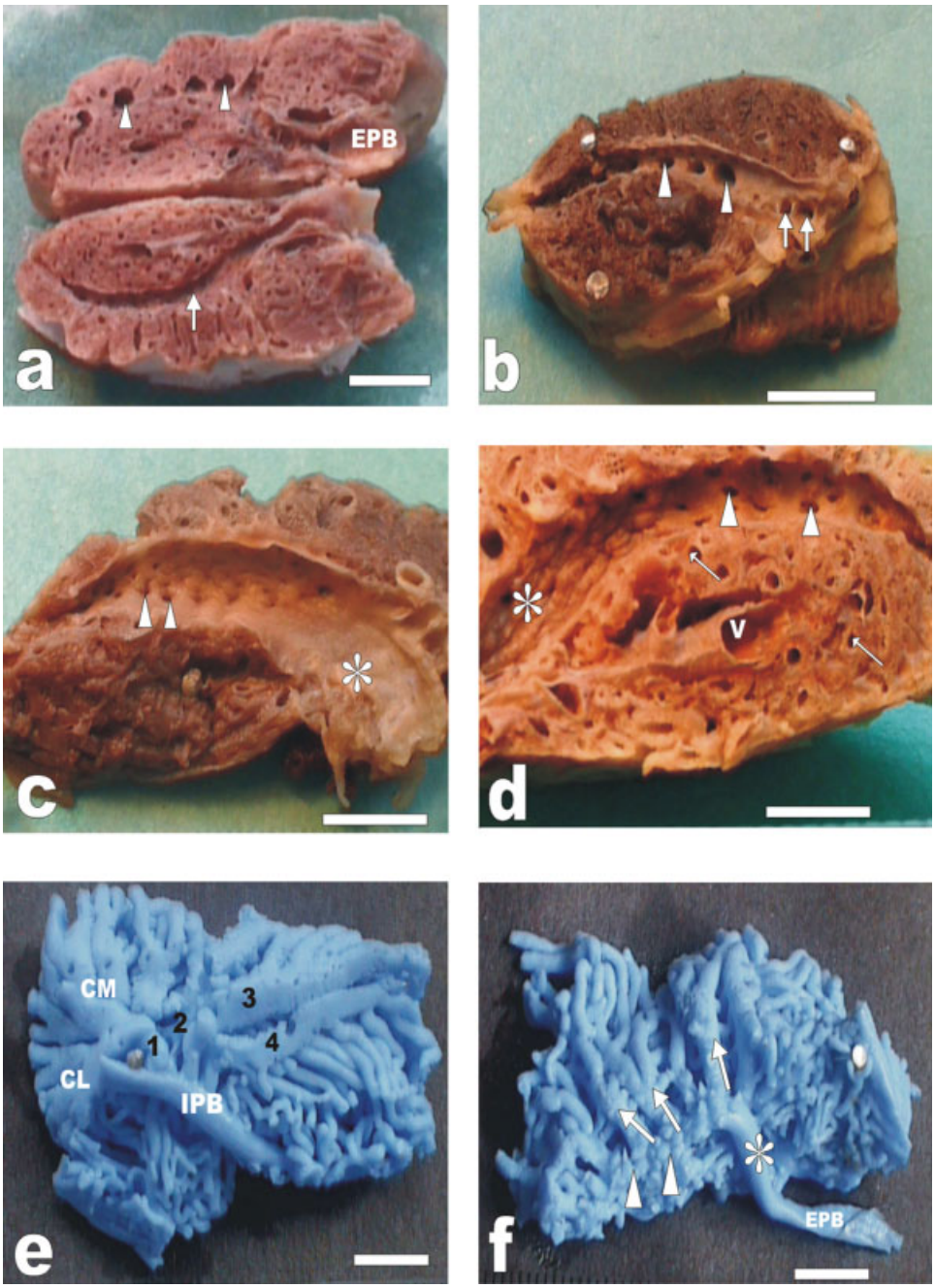

Fig. 2. Macrographs of fixed adult chicken lung specimens cut along the long axis of the primary bronchus to show the bronchial openings (**a**–**d**) and silicon rubber casts (**e** and **f**) showing the disposition of the secondary bronchi. Such specimens were used to count the numbers of the various categories of secondary bronchi. All scale bars are 1 cm. a: Sagittal section through the lung showing the curved primary bronchus on the lateral segment (arrow) and the openings of LDSB in the medial segment (arrowheads). The caudal part of the extrapulmonary primary bronchus (EPB) is also shown. b: Medial half of the lung showing the openings into the LDSB (arrowheads) and the last two MVSB (arrows). Note that the LDSB emerge from the mediodorsal aspect of the primary bronchus then course laterally (see also Figs. 3d–3f for further explanations). c and d: Lateral half of the lung exposing the internal surface of the primary bronchus. Notice the openings into the posterior secondary bronchi (arrowheads). The asterisks denote the cranial part of the intrapulmonary primary bronchus with no bronchial openings. The arrows in (d) point to typical parabronchi and a big blood vessel (V) is also indicated. e and f: Silicon rubber cast of the adult chicken lung with neopulmonic parabronchial network removed. e: On the medioventral aspect, the points of origin of the MVSB (1, 2, 3, and 4) emerging from the intrapulmonary primary bronchus (IPB) are evident. The branches of the first MVSB spread craniomedially (CM) and craniolaterally (CL). f: On the lateral aspect with neopulmonic parabronchi removed and LDSB (arrows) deflected, the stumps of the severed POSB (arrowheads) become evident. The lateral cranial third of the intrapulmonary primary bronchus (asterisk) and the extrapulmonary primary bronchus (EPB) have no secondary bronchi. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

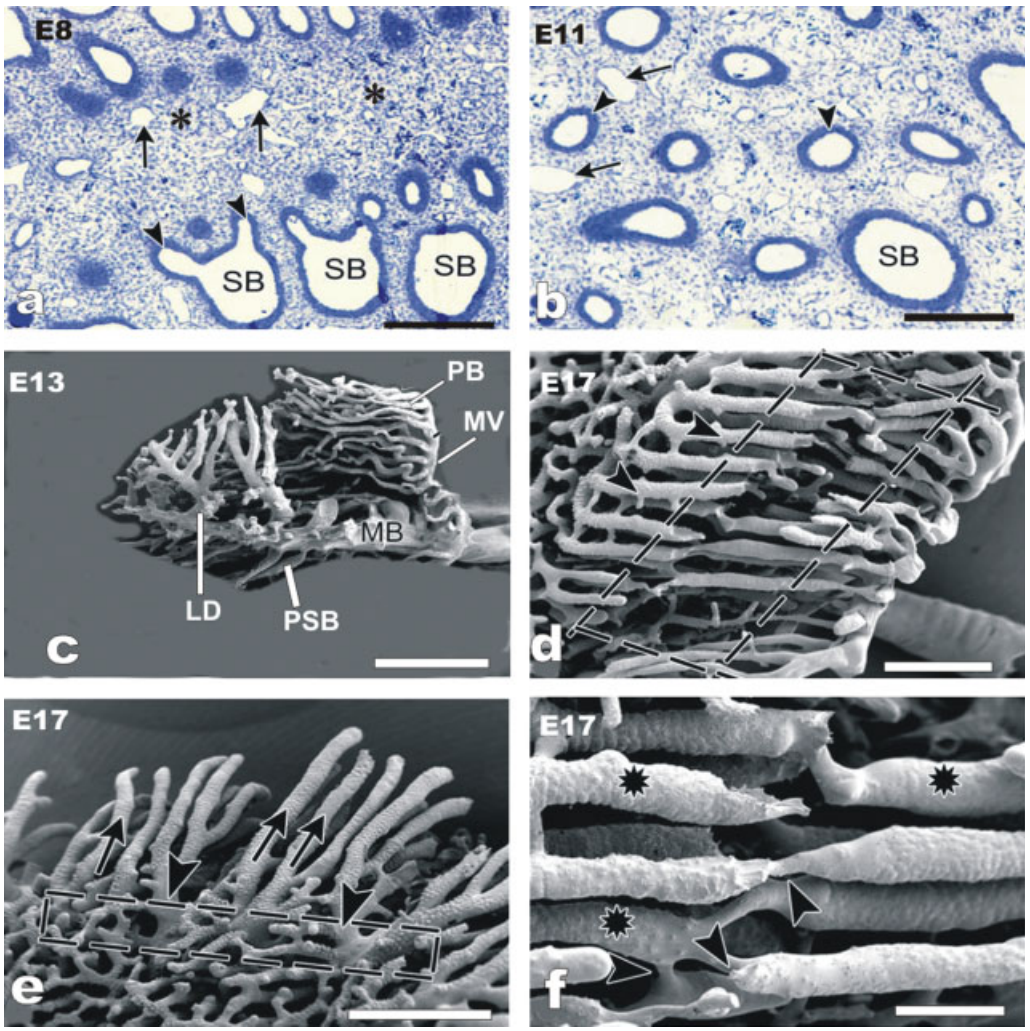

Fig. 3. Semithin sections (**a** and **b**) and SEM micrographs (**c**–**f**) illustrating the sprouting secondary bronchi present in the chicken embryo lung. Specimens were prepared from day 8–21 chicken embryos. a and b: Early at E8 (HH stage 31) the secondary bronchi (SB) with sprouting parabronchi (arrowheads) are already present (a). These are separated by abundant mesenchymal tissue (asterisks) with a few empty spaces (arrows). By HH stage 36 (E11) (b), the number of air conduits (arrowheads) increases at the expense of the mesenchyme. A few empty spaces (arrows) are also evident). Scale bars = 100 μm. c–f: Intratracheal mercox casts showing developmental patterns of the parabronchi and secondary bronchi. c: At E13 (HH stage 38), the laterodorsal secondary bronchi (LD), the posterior secondary bronchi (PSB), and the medioventral secondary bronchi (MV) are evident. The MV already have parabronchial branches (PB) at this time showing that they are the first to emerge. d: Parabronchial branches from MVSB grow caudally to anatomose with those of the first two LDSB. The hatched rectangle shows the zone of anastomoses. e: The rest of the LDSB, that is 3–10, grow dorsolaterally and form parabronchial branches (arrows) that grow toward the dorsal border where they anastomose with similar branches from MVSB. Note the boundary between the formative parabronchi of paleopulmonic region (arrowheads) and zone of parabronchial anastomoses between the two regions (hatched rectangle). f: Anastomoses of the paleopulmonic parabronchi occurs by approaching conduits (asterisks) sending slender branches (arrowheads) to one or more of the targeted cognates. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

exchange. Specifically, MV1 and its primary and secondary branches were entirely conducting conduits (Figs. 5a and 5b), as was the case with the sacobronchi (Figs. 5c and 5d). The future exchanging parts of the embryonic secondary bronchi at E17 were well covered by small mounds of sprouting atria, which later gave rise to infundibulae and air capillaries (Fig. 5f). The abdominal air sac opened to many sacobronchi while MV1 had the ostium of interclavicular air sac (Fig. 5a).

The nonexchanging nature of the initial parts of the secondary bronchi was also captured in the posterior secondary bronchi (POSB), whose initial parts were devoid of atria and were covered with a scantly ciliated epithelium (Fig. 6). The POSB were notably much smaller than the LDSB and were continuous with the parabronchi in both the paleopumonic and neopulmonic regions. Clear mucosal junctions delineating the nonexchanging ciliated parts and the exchanging parts of the POSB were evident.

The final pattern of the various categories of secondary bronchi was studied in resin casts of the adult chicken lung (Fig. 7) and is demonstrated schematically in Figure 8. A total of between seven and 10 LDSB emerged from the dorsomedial aspect of the intrapulmonary primary bronchus and coursed laterally forming parabronchial branches that grew toward

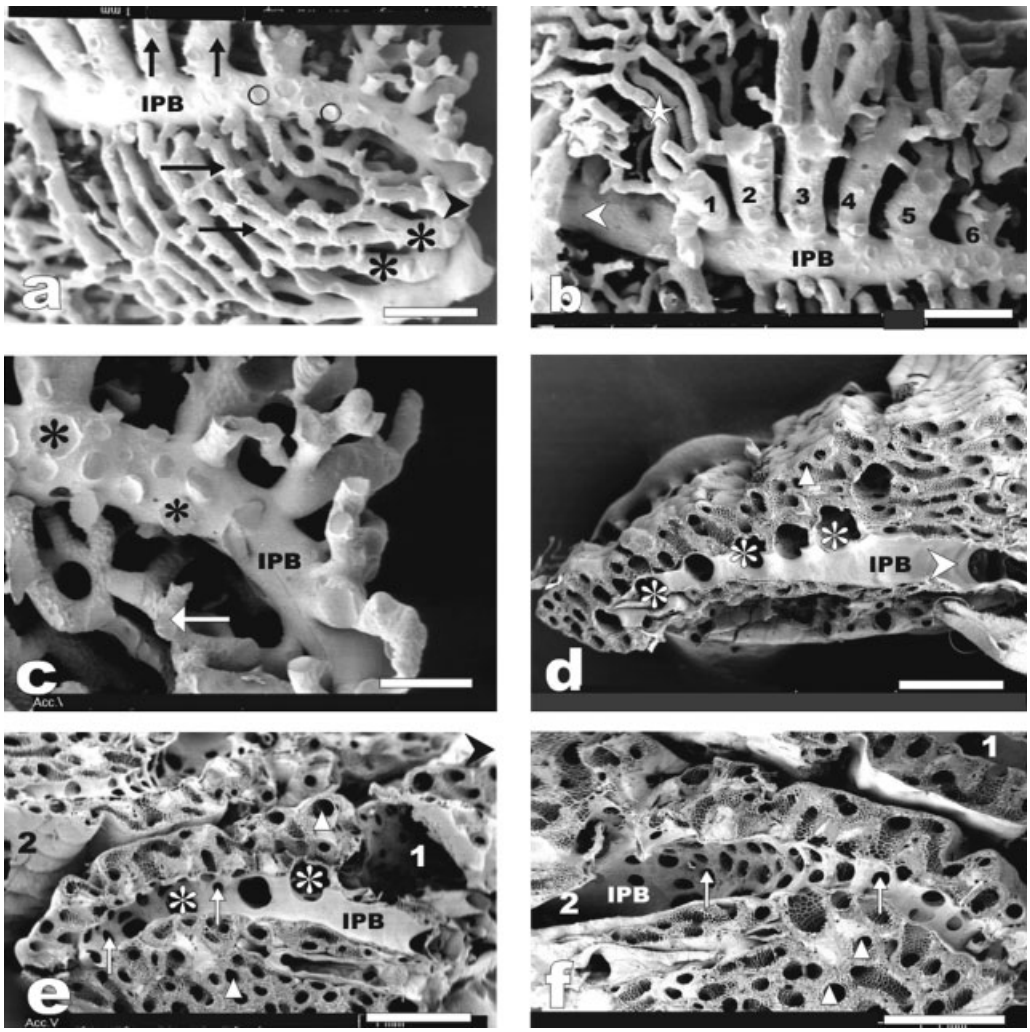

Fig. 4. SEM micrographs illustrating the types and number of secondary bronchi present in the chicken embryo lung. Specimens were prepared from day 18 to 21 (HH stages 43–46) chicken embryos. All scale bars = 1 mm. **a–c**: Intratracheal mercox casts showing the various categories of the secondary bronchi in the posterolateral aspect of the avian lung. Note that in all cases most of the parabronchi have been cut off to reveal the inner structures. a: The LDSB (vertical arrows) emerge from the dorsomedial aspect of the intrapulmonary primary bronchus (IPB). At the same time numerous POSB (horizontal arrows) emanate from the posterior two thirds of intrapulmonary primary bronchus and continue as parabronchi of the neopulmonic region. The latter parabronchi are continuous with the sacobronchi (asterisks) of the abdominal air sac. Several of the POSB have been removed (circles) to expose the LDSB. b: The first two LDSB (indicated 1, 2) contribute to the formation of the anteriorly oriented paleopulmonic parabronchi (asterisk) that anastomose with those from the medioventral secondary bronchi. The rest of the LDSB are indicted with numbers (3–6). The LDSB emerge from the mediodorsal aspect of the intrapulmonary primary bronchus (IPB). The arrowhead indicates the anterior lateral aspect of the intrapulmonary primary bronchus that has no secondary bronchi. c: Posteriorly the intrapulmonary primary bronchus (IPB) curves ventrally and ends up in the abdominal air sac. Note the remnants of the severed POSB (asterisks) and the neopulmonic parabronchi (arrow). **d–f**: SEM micrographs illustrating openings to the various types of secondary bronchi present in the embryonic chicken lung. The numbers 1 and 2 refer to the lateral and medial halves of the lung, respectively. Small arrowheads indicate openings into the parabronchi. d: Micrograph of the medial half of the lung showing the openings into the laterodorsal secondary bronchi (asterisks). Note that much of the medial aspect of the intrapulmonary primary bronchus (IPB) has no posterior secondary bronchi. e and f: The distribution of the various types of the secondary bronchi is revealed by observing the luminal aspect of the intrapulmonary primary bronchus by making a longitudinal section along the long axis of the primary bronchus. Note that in e and f, the two halves of the lung are placed side by side for ease of orientation. e: The anterior part of the primary bronchus (IPB) is devoid of the small openings. However, the posterior two thirds have a few openings (arrows), which lead to the posterior secondary bronchi. On the mediodorsal aspect are large holes that lead to the laterodorsal secondary bronchi (asterisks). f: This micrograph is taken from the lateral half of the lung; part of the lateral half is seen at the top right hand corner (2). On the lateral, laterodorsal and lateroventral aspects of the posterior two thirds of the intrapulmonary primary bronchus (IPB), large openings are absent but numerous tiny openings (arrows), which lead to the POSB are evident. A total of 40 such openings were counted on the two halves of the lung. Arrowheads indicate parabronchi.

the dorsal border of the lung (except the first two, see below). Numerous POSB emanated from the posterior aspect of intrapulmonary primary bronchus and continued as parabronchi of the neopulmonic region in the ventrolateral aspect of the lung and were directly continuous with the sacobronchi of the abdominal air sacs (Figs. 4a and 8b). The dorsally oriented POSB formed branches that anastomosed with the LDSB as well as the neopulmonic parabronchi. The LDSB were clearly much larger than the POSB (Figs. 4d–4f). A total of

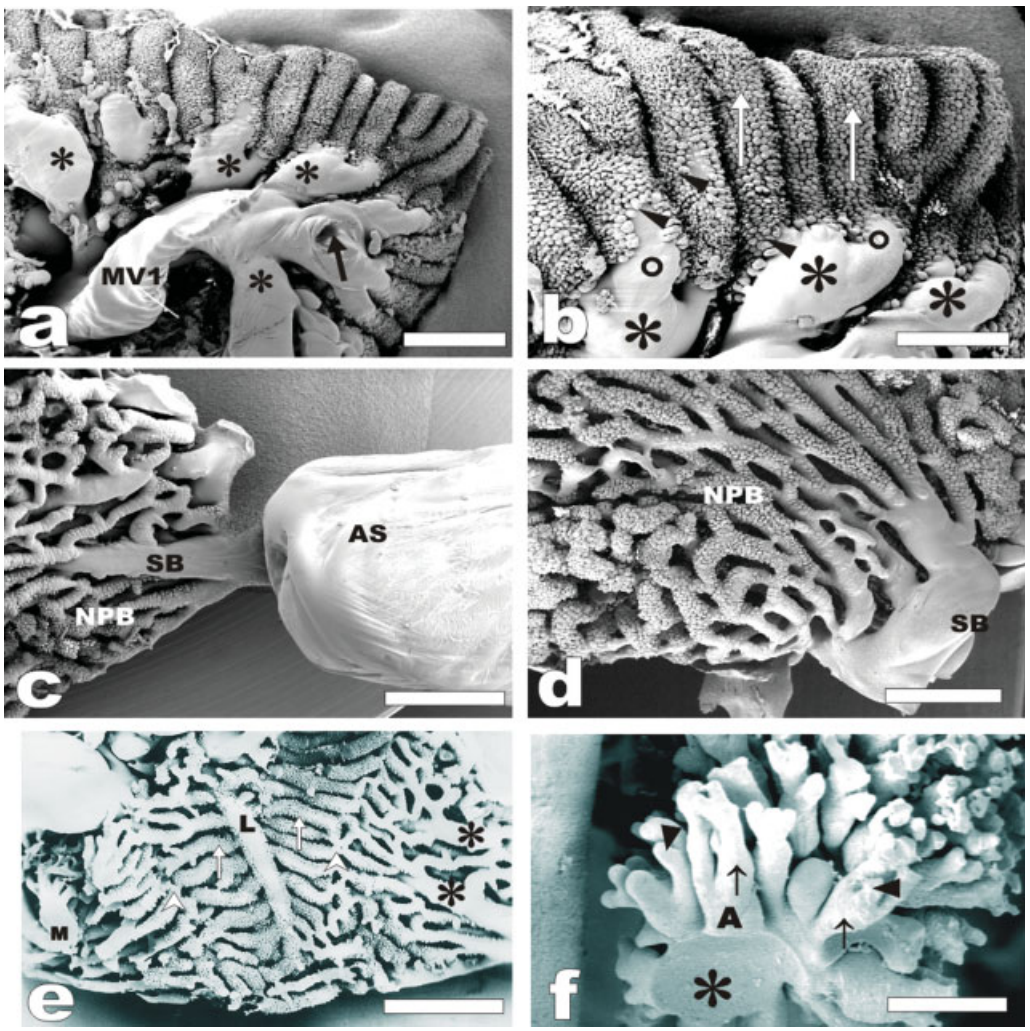

Fig. 5. Micrographs of intratracheal mercox casts of the developing airway and gas exchange system illustrating the development of the various groups of the parabronchi at HH stage 42 (E17). See also Figure 7 for anatomical explanations of the final morphology. All scale bars = 1 mm. **a**: A mercox cast showing the first medioventral secondary bronchus (MV1), which forms several short branches on the ventromedial and craniolateral aspects (asterisks). Each branch then forms several nonexchanging branches, which continue as parabronchi. The arrow indicates the ostium of the interclavicular air sac. **b:** A higher magnification of the mercox cast in (a) above showing the primary (asterisks) and secondary (circles), branches of MV1. Note that each primary branch forms several nonexchanging secondary branches (circles), which continue as parabronchi (arrows). The parabronchi are covered with the developing atria, which appear as small mounds (arrowheads), thus giving the external surface of the parabronchus a grainy appearance while the secondary bronchus appears smooth-surfaced. **c** and **d:** Mercox casts showing the posteroventral aspect of the lung with the dense network of neopulmonic parabronchi and prominent sacobronchi (SB). Note the rough surface of the parabronchi due to the developing atria as opposed to the smooth surface of the sacobronchi. The abdominal air sac (AS in c) is also shown. The sacobronchi branch and contribute to the neopulmonic parabronchial network (NPB). **e** and **f:** Mercox cast showing the posteroventral aspect of the lung with the dense network of neopulmonic parabronchi, sacobronchi (asterisks) and c a prominent LVSB (L). The arrows indicate the parabronchial branches from LVSB, which cranially anastomose with those from the medioventrals (M) and caudally with those the sacobronchi of the abdominal air sac. Arrowheads indicate the line of parabronchial anastomoses. f: At E20 (HH stage 45), the development of the air conduits is well advanced so that the atria (A) emerging from the parabronchi (asterisks) are well captured with intratracheal mercox casting. The arrows show infundibulae, which continue to form air capillaries (arrowheads). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

20–60 openings into the POSB (Table 1) were counted on the two halves of the primary bronchus. One to two lateroventral secondary bronchi (LVSB) were encountered and these sent out parabronchial branches that anastomosed with those from MV1, the sacobronchi and those from the neopulmonic region (Fig. 5e). The LVSB emerged from the ventral aspect of the intrapulmonary primary bronchus, in common with the fourth medioventral secondary bronchus (Figs. 1d, 7a, and 8a), and thus the opening of the LVSB was not visible on the opened mesobronchus.

The laterodorsal secondary bronchi (LDSB) arose from the dorsomedial aspect of the intrapulmonary primary bronchus; but immediately curved laterally so that they occupied the dorsolateral aspect of the lung (Figs. 1c, 1f, 2f, and 8b). The first LDSB was more laterally oriented (Figs. 4b and 8b) and formed the parabronchial conduits that occupied the cranial lateroven-

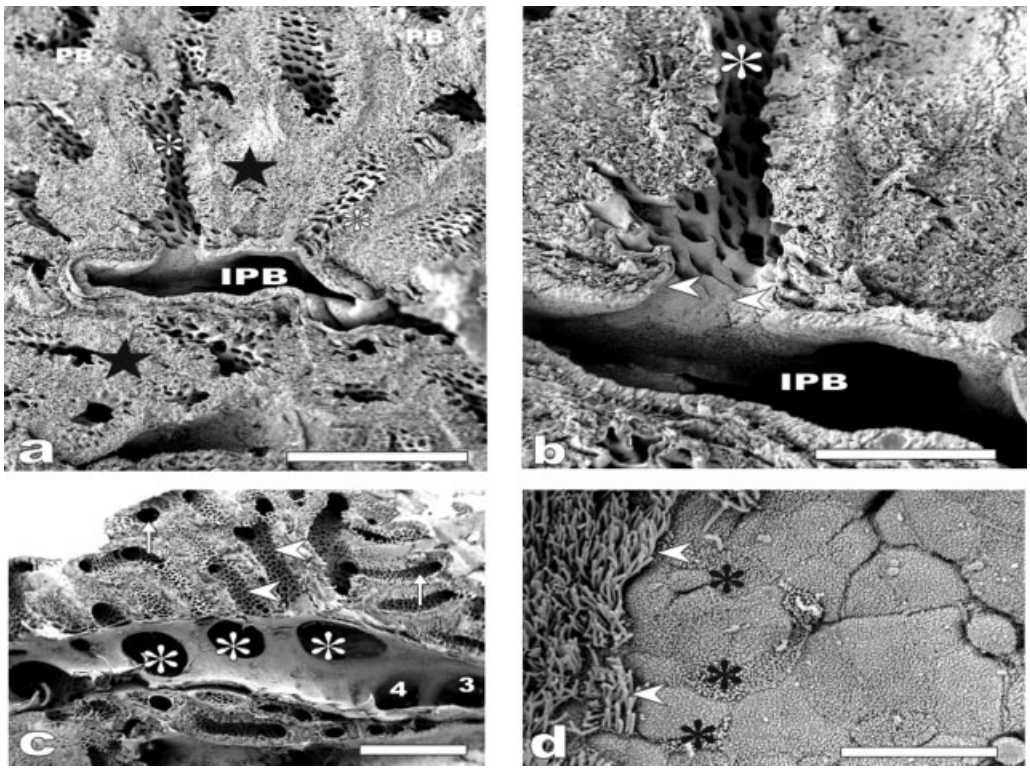

Fig. 6. **a–d:** SEM micrographs of the chicken embryo lung showing the patterns and categories of the developing air conduits emanating from it. a and b: E14 (HH stage 39) chick embryo lung showing the POSB (asterisks) emerging directly from IPB. The initial part of the emergent POSB has no atria (arrowheads in b). Note that the POSB (asterisk) closely resembles the parabronchi (PB). The star in (a) indicates the undifferentiated tissue intervening between the POSB. Scale bars = 200 μm. c: By E19 (HH stage 44), the interparabronchial septal tissue is much reduced and the POSB (arrowheads) as well as the parabronchi (arrows) are closely packed. Asterisks indicate openings to the LDSB on the mediodorsal aspect, while numbers 3 and 4 indicate the openings of 3rd and 4th MVSB, respectively. Scale bar = 500 μm. d: The mucosal transition from the ciliated epithelium of the IPB to the nonciliated part of the POSB at E14 is demonstrated. Notice the transition margin (arrowheads) with only a few ciliated cells (asterisks) on the side of the POSB. Scale bar = 20 μm.

tral quarter of the lung (Figs. 1c, 7d, and 8b). The second LDSB (LD2) was more dorsally oriented and formed the parabronchial branches that curved cranially and filled the rostral dorsolateral quarter of the lung (Figs. 1c, 1f, 7d, and 8b). The parabronchi from LD1 and LD2 inosculated their cognates emanating from the branches of the first MVSB (MV1). The rest of the LDSB formed parabronchial branches that grew dorsomedially to meet their cognates from the medioventrals 2–3 at the level of the dorsal border of the lung (Figs. 7 and 8). These inosculations resulted in the formation of cranial and dorsal groups of paleopulmonic parabronchi.

The secondary bronchi and the parabronchi gave rise to a complex system of air conducting and gas exchange channels characteristic of the adult avian lung depicted in Figures 7 and 8. The secondary bronchi included four medioventral secondary bronchi, named as 1st, 2nd, 3rd, and 4th (MV1-MV4) and several sacobronchi emerging from the various air sacs, 7-10 laterodorsal secondary bronchi, 1–2 lateroventral secondary bronchi, and 20–60 posterior secondary bronchi. MV1 was directed cranially and gave rise to the ostium of the interclavicular air sac. The rest of the MVSB were generally caudally oriented. MV1 gave rise to dorsally oriented branches, which further formed smaller branches that gave rise to parallel paleopulmonic parabronchi. The latter group of parabronchi grew laterally to meet those from the LDSB at the level of the dorsal border. MV2 and MV3 formed parabronchial branches that also curved dorsally in the posteromedial part of the lung to meet their cognates from the laterodorsal secondary bronchi. MV4 gave rise to parabronchial branches that coursed lateroventrally and anastomosed with those from the posterior sacobronchi and LVSB to form the neopulmonic network (Figs. 7 and 8).

The two categories of parabronchi described in the avian lung, i.e., the parallel paleopulmo and the networked neopulmo were distinctly identifiable (Figs. 7 and 8). The paleopulmo occurred in two groups: the cranial group emanating from the MV1 and LD1 and LD2 on one hand and the dorsomedial group emanating from MV2 and MV3 and LD3-10 on the other. The parabronchi gave rise to branches that were smaller in caliber that joined other adjacent parabronchi. Such branches were particularly conspicuous at the planum anastomosica and their formation was captured in the E17 embryo (Figs. 3d and 3f). The neopulmo comprised parabronchi from the MV4, LV1 and LV2, sacobronchi, and POSB and occupied the caudal ventral quarter of the lung (Figs. 1d, 4a, 7, and 8). A summary of the preferred names of the various categories of secondary bronchi is provided in Table 2.

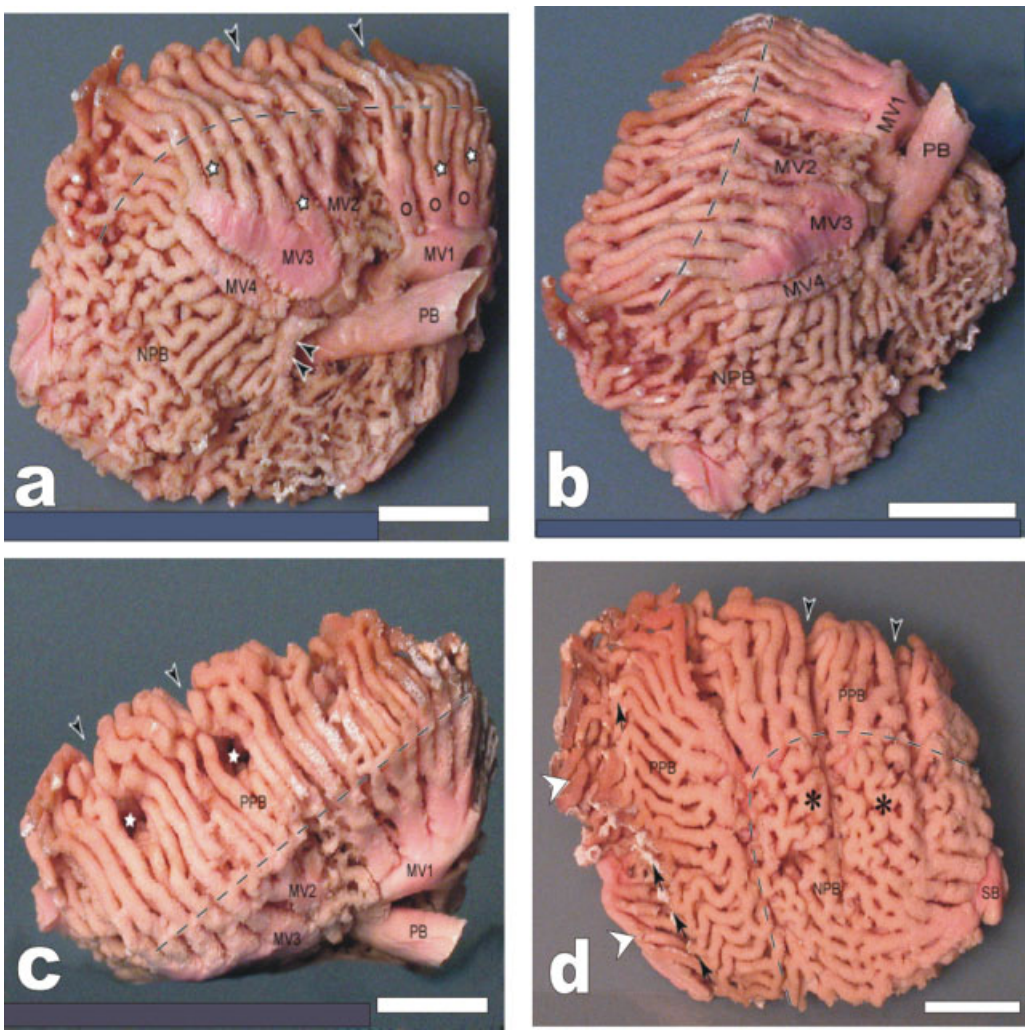

Fig. 7. **a–d**: SEM micrographs illustrating the types and number of secondary bronchi present in the adult chicken lung demonstrated with intratracheal mercox casts. All scale bars = 1 cm. a and b: Medioventral aspect of the lung showing the primary bronchus (PB), the medioventral secondary bronchi (MV1, MV2, MV3, MV4), the latero-ventral secondary bronchus (double arrowheads in a), and the neopulmonic parabronchi (NPB). The dashed line denotes the ventral border. Circles in (a) indicate nonexchanging branches of MV1 and stars denote the medial group of parabronchi emanating from the MVSB. The costal sulci are indicated by the arrowheads. c and d: Micrographs showing the medial (c) and lateral (d) aspect of the chicken lung. c: Notice the primary bronchus (PB), medioventral secondary bronchi (MV1, MV2, MV3), and the paleopulmonic parabronchi (PPB). The ventral border where the branches of MVSB curve dorsally is marked by the hatched line while the stars and arrowheads denote the costal sulci. d: On the lateral aspect, the cranial and dorsal groups of the paleopulmonic parabronchi (PPB) are evident. The arrows indicate the planum anastomosica, where the PPB from LD1 and LD2 meet those from MV1. The hatched line delineates the region of neopulmonic parabronchi (NPB) and asterisks indicate regions of intense anastomoses. Dark arrowheads denote costal sulci while the white ones indicate the parabronchial branches from MV1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Distribution of ostia to the various air sacs, or even air sacs themselves were captured in gross specimens, or latex casts, or even in resin corrosion casts (Fig. 5). The ostia are demonstrated schematically in Figure 8. The clavicular air sac was directly connected to the first MVSB; the posterior thoracic air sac was continuous with the LVSB while the abdominal air sac opened directly into the mesobronchus. The cervical and anterior thoracic air sacs did not appear to have direct continuity with the main secondary bronchi but were supplied by branches from the MVSB (Fig. 8). Notably, MV1 was nonexchanging and had primary and secondary nonexchanging branches that were continued as the paleopulmonic parabronchi on the craniolateral and craniomedial aspects of the lung as were the sacobronchi from the various air sacs (Figs. 5, 7, and 8).

The number of secondary bronchi between the left and right lungs were not significantly different among all categories enumerated. The details of the average number of the various categories of secondary bronchi are provided in Table 2. The MVSB were the least and were invariably 4, the LDSB were 9.5 ± 1.07 (mean ± SD) in the left lung and 9.4 ± 1.05 in the right lung. Majority of the animals had a single LVSB with a maximum of 3 being encountered only once. The POSB were the most numerous and showed the highest variation at 37.3 ± 12.4 in the left and 36.1.4 ± 13.4 in the right lung.

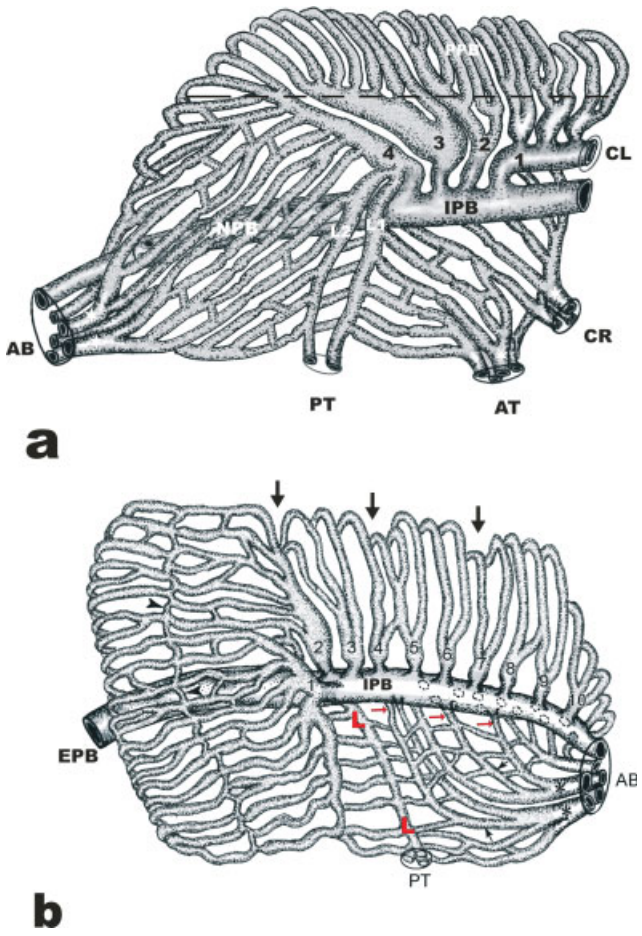

Fig. 8. Simplified schematic drawings of the adult chicken lung illustrating the patterns of the secondary bronchi and the parabronchi. **a:** Medioventral aspect of the lung showing the intrapulmonary primary bronchus (IPB), the medioventral secondary bronchi (1, 2, 3, 4), the LVSB (L1 and L2), and the sacobronchi (stars). Notice the smooth nature of the intrapulmonary primary bronchus, MV1, the sacobronchi, and the initial parts of the secondary bronchi showing that they have no atrial openings. The ostia to the various air sacs are: interclavicular (CL), cervical (CR), anterior thoracic (AT), posterior thoracic (PT), and abdominal (AB). The various categories of parabronchi are neopulmonic (NPB) and paleopulmonic (PPB). The dashed line shows the medial border. **b**: The lateral aspect of the lung shows the pattern of the various groups of secondary bronchi and the parabronchi emanating from them. The LDSB are labeled 1–10. Notice that the first two LDSB furnish the parabronchi that inosculate with those from the first MVSB. The plane of anastomoses is marked by smaller parabronchial branches (arrowheads). Notice the position of the LVSB (L) and the various POSB (thin horizontal arrows) that continue to form the sacobronchi (asterisks) to the abdominal air sac. AB and PT are the ostia of the abdominal and posterior thoracic air sacs, respectively, EPB is extrapulmonary primary bronchus and IPB is intrapulmonary primary bronchus. The thick arrows denote costal sulci and dashed circles denote stumps of POSB. The neopulmonic parabronchi (small arrows) are continuous with the sacobronchi and the POSB. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

DISCUSSION

Despite several studies on the avian lung by contemporary investigators, its development and functional morphology remain frustratingly enigmatic. The nomenclature and the number of secondary bronchi, for example, reported in literature show an alarming disparity. In the domestic fowl, Lopez et al., (1992) report only eleven (11) secondary bronchi, while Duncker (1974) reports over 17. Earlier reports indicated an even greater number with Akester (1960) reporting 21 and Payne and King (1960) reporting 42–51. The names provided for the various groups include ventromedial secondary bronchi (Payne and King, 1960), ventrobronchi (Akester, 1960; Duncker, 1971, 1974), and medioventral secondary bronchi (King and McLelland, 1984; Maina and Nathaniel, 2001) for the ventral group. Other categories reported include dorsomedial secondary bronchi (Akester, 1960), laterodorsal secondary bronchi (Duncker, 1974; Lopez et al., 1992), laterobronchi (Duncker, 1974; Lopez et al., 1992), mediodorsal and lateroventral secondary bronchi (King and McLelland, 1984; Maina and Nathaniel, 2001). King and McLelland (1984) reported four medioventral secondary bronchi, 7–8 mediodorsal secondary bronchi, eight lateroventral secondary bronchi, and up to 30 laterodorsal secondary bronchi. The remarkable differences in both the numbers and names used may be attributed to the methods used in the respective studies. Maina (2005) noted that the secondary bronchi are named according to the area of the lung they supply. However, and as is apparent from this study, any one region of the lung is supplied by more than one category of secondary bronchi, hence the confusion in nomenclature. Recent reports (Duncker, 2004; Maina, 2005, 2006; Woodward and Maina, 2005) have not clarified the arrangement and the names of the secondary bronchi. In the current study, this confusion has been resolved with the notion that a combination of several techniques needs to be employed to resolve problems identification, enumeration, and topography. Resin casting allows the rigidity that outlines and transfixes lumina of hollow structures in situ, silicon rubber casting proffers the flexibility to reveal outlined, albeit hidden structures and, in both cases, some of the casts can be removed without damaging the specimens.

Nomenclature and Number of the Secondary Bronchi

The avian trachea bifurcates to give rise to the two primary bronchi also known as extrapulmonary primary bronchi. On gaining entry into the pulmonary tissue, these become the mesobronchi (Bellairs and Osmond, 1998). In the domestic fowl, the first MVSB is directed cranially while the last three are directed caudally. The extents of the nonexchanging secondary bronchi and their branches was only apparent after intratracheal mercox casting since the regions bearing no atrial outpouchings remained smooth and could easily be discerned (see Figs. 5 and 8). Gross pictures from mercox or from latex rubber casting (see Figs. 1 and 7; also Akester, 1960; Duncker, 1974) do not furnish the necessary resolution to discern atria and hence the extents of the nonexchanging parts of the secondary bronchi could not be discerned by the methods used in the previous studies. The many and disparate number of secondary bronchi reported (see for example Lopez et al., 1992) were as a result of the disposition of the various secondary bronchi, their branches, and the resultant parabronchi. Silicon rubber is pliable and allows manipulation of the casts of the tubules so that

fine details can be observed (Akester, 1960). Resin casting on the other hand results in rigid structures that depict the in situ situation. Observation of the mesobronchial lumen on longitudinally bisected intrapulmonary primary bronchus and viewing under the scanning electron microscope reveals virtually all the openings to the secondary bronchi (Figs. 4d–4f), except those to the LVSB, which open into the MV4. Using a combination of the techniques mentioned above, 4 MVSB, 7–10 LDSB, 1–3 LVSB, and up to 60 POSB were discerned, as described below. Notably, many categories of secondary bronchi have previously been named mediodorsal secondary bronchi (see King and McLelland, 1984; Maina 2000, 2006; Maina and Nathaniel, 2001; Reese et al., 2006), but as described here, no group of secondary bronchi fits this name perfectly. Notably, once the primary bronchus enters the lung tissues, it is no longer visible since it is entirely covered by pulmonary tissue. Often, MV3, which is a large air conduit, has been confused with the mesobronchus (Cevik-Dermikan et al., 2006) and contemporary authors give same structures for different names.

The Medioventral Secondary Bronchi

Interestingly, the MVSB emerge from the mediodorsal surface of the cranial third of the intrapulmonary primary bronchus but immediately curve ventrally so that at a glance they appear to emerge from the medial surface. The MVSB give rise to several primary branches, which are inclined mediododorsally. These branches give rise to several short secondary branches that form parabronchi. The primary and secondary branches of the first MVSB are all nonexchanging as seen in mercox casts (Figs. 5a and 5b). These branches turn sharply at the medial border to the dorsal direction and go to inosculate their cognates from the LDSB. The rest of the MVSB are oriented posteriorly but their branches as well turn dorsally at the medial border of the lung and anastomose with parabronchi from the LDSB. The initial parts of the latter MVSB are nonexchanging since they lack atria. Some branches from the first MVSB run laterally to anastomose with those from the LDSB (see below). Branches of the first three MVSB pass dorsally on the medial aspect of the lung and this category has sometimes been referred to as mediodorsal (Maina, 2000, 2006; Reese et al., 2006), ventrobronchi (Duncker, 1971, 1972), craniomedial or anterodorsal (Hodges, 1974), or just anterior secondary bronchi (Akester, 1960). However, King and McLelland (1984), correctly identify them as medioventral secondary bronchi (MVSB). The commonly used names and the proposed names of the various categories of secondary bronchi have been summarized in Table 1. The name tertiary bronchi, formerly used for parabronchi is untenable since the initial branches of some secondary bronchi are of the tertiary category and are nonexchanging.

The Laterodorsal Secondary Bronchi

Seven to 10 laterodorsal secondary bronchi arise from the dorsomedial aspect of the intrapulmonary primary bronchus but immediately curve laterally so that they appear to emerge from the dorsolateral surface. The first LDSB is more laterally oriented and forms the parabronchial conduits that occupy the cranial lateroventral quarter of the lung. The second LDSB is more dorsally oriented and forms the parabronchial branches that curve cranially and fill the rostral dorsolateral quarter of the lung. The latter two groups inosculate their cognates emanating from the branches of the first MVSB. The rest of the LDSB form parabronchial branches that grow dorsomedially to meet their cognates from the medioventrals at the level of the dorsal border of the lung. These inosculations result in the formation of cranial and dorsal groups of paleopulmonic parabronchi. This category of secondary bronchi has been referred to as dorsobronchi (Duncker, 1971, 1972; Nickel et al., 1977) or mediodorsal secondary bronchi (King and McLelland, 1984; Maina, 2005). Although the bronchi arise on the dorsomedial aspect of the lung, their bulk is in the laterodorsal orientation and the name mediodorsal would mainly describe their points of origin or their parabronchial branches, some of which curve dorsomedially. Generally the parabronchial branches of LDSB curve cranially (LD1 and LD2) and dorsomedially (LD5–LD10) to form the respective groups of paleopulmonic parabronchi. Therefore, the name laterodorsal describes this category of secondary bronchi more appropriately.

The Lateroventral Secondary Bronchi

One or two lateroventral bronchi are encountered emerging with the 4th MVSB and continue laterally on the ventral aspect of the lung to the posterior thoracic air sac. The lateroventral bronchi send parabronchial branches that anastomose with those from the neopulmonic parabronchi and the sacobronchi (see Figs. 1d, 7a, 8a, and 8b) and become part of the neopulmonic network. This category has been confused with POSB (see below) but clearly the diameter is much greater than that of the POSB. Duncker (1971) correctly identifies the course of LVSB and notes that the number varies between 2 and 3. Noncasting methods are unlikely to capture LVSB since they do not open directly into the mesobronchus.

The Posterior Secondary Bronchi

Up to 60 posterior secondary bronchi emanate from the posterior two thirds of the mesobronchus mainly on the lateral aspect, but also on all the other aspects of the mesobronchus (Figs. 2, 3, 4, 7, and 8). This category of the secondary bronchi has been named posterior secondary bronchi (POSB) because they emerge from all surfaces of the posterior two thirds of the intrapulmonary primary bronchus and run in all directions and as such do not fit in any described category. Duncker (1971) notes that some parabronchi emanate from the ventral and lateral aspects of the primary bronchus. The POSB observed in the current study are similar in size to parabronchi but have an initial nonexchanging part without atria as they emerge from the intrapulmonary primary bronchus, a phenotype that does not fit that of the parabronchi. Furthermore, the fact that they emerge directly from the primary bronchus places them in the category of secondary bronchi. Indeed, King and McLelland (1984) note that the secondary bronchi include all those bronchi of the second order, that is, all those that emerge directly from the primary bronchus. It is this group of secondary bronchi that has often caused confusion in the nomenclature and num-

ber of the secondary bronchi and is probably the category referred to as laterodorsal with variable distribution in King and McLelland (1984). The term posterior secondary bronchi was first floated by Akester (1960). This author reported that only two categories of secondary bronchi (anterior and posterior) were present in the lung of the domestic fowl. The investigation employed only one technique, latex rubber casting, and described 8–9 posterior dorsal bronchi, 7–8 posterior ventral secondary bronchi, and about 20 posterior lateral bronchi emerging from the mesobronchus. The latter category probably refers to the POSB but the former do not fit the POSB described here. His method of description, no wonder, was dismissed by subsequent authors. The POSB are most numerous on the lateral and ventral aspects of the mesobronchus.

The Recurrent Secondary Bronchi; Sacobronchi

Secondary bronchi originating from the intrapulmonary primary bronchus form the air sacs. The air sacs then send out sacobronchi (recurrent bronchi) that form parabronchial branches. The latter branches anastomose with the rest of the parabronchial system. With the exception of the abdominal air sacs, each air sac is connected directly to a secondary bronchus (King and McLelland, 1984). The cranial air sacs (clavicular, cervical, and anterior thoracic) are connected to the 1st, 2nd, and 3rd MVSB, respectively, the caudal thoracic is connected to the lateroventral secondary bronchus (LVSB) and the abdominal air sac opens directly into the posterior part of the intrapulmonary primary bronchus (King and McLelland, 1984; Fig. 8). In addition each air sac gives off several short recurrent bronchi, which are generally nonexchanging (Figs. 5c–5e; Fig. 8a). The latter bronchi give rise to branching and anastomosing parabronchi, which join the rest of the parabronchial system. It has been observed that ontogenetically, air sacs develop from the secondary bronchi and as such may be considered to be extensions of the airway system (Maina, 2003b). Indeed, Bezuidenhout (2005) has described portions of ciliated and cuboidal epithelium lining the air sacs that resemble the epithelia found in the primary and secondary bronchi.

Airflow Mechanics and Functional Architecture of the Avian Lung

Ventilation of the noncompliant avian lung is facilitated by the bellow-like action of the air sacs. The clavicular air sac is directly connected to the first MVSB; the posterior thoracic sac is continuous with the LDSB while the abdominal air sac opens directly into the mesobronchus. The cervical and anterior thoracic air sacs are supplied by branches from the MVSB. These details are captured in Figure 8 and well documented in King and McLelland (1984).

The fact that ventilation in the avian lung entails two inspiratory and expiratory cycles for air to pass through the entire lung has not been disputed. The current study has elucidated the topographical arrangement of the various categories of air conduits to generous details. Inspired air moves past and completely bypasses the openings of MVSB and moves into the caudal air sacs, a process termed inspiratory aerodynamic valving (Banzett et al., 1987, 1991; Wang et al., 1988, 1992). The latter process is facilitated by the presence of a constriction of the EPB termed segmentum accelerans (Maina and Africa, 2000; Wang et al., 1992). Doubtlessly, the intricate arrangement and orientation of the secondary bronchi in 3D has a strong implication on gas flow mechanics. All the MVSB emerge from the mediodorsal aspect of the IPB and are initially of narrow caliber, are oriented cranially before they take their definitive directions. The latter category of secondary bronchi communicates with the laterodorsals through paleopulmonic parabronchi. The actual role of the said arrangement in control of flow mechanics, however, remains to be investigated.

Nearly a century ago, Locy and Larsell (1916) noted that the morphology of the avian lung could only be elucidated by observations of its development. We have used this cue together with several visualization techniques to unravel the intricacies of the air conduits and parabronchi in the domestic fowl. A detailed account of the topographic arrangement of the avian bronchial system covering 155 species in 47 families was presented by Duncker (1972), using mainly injection techniques. The resolution applied then, however, could not resolve the fine topographical details reported in the current study.

Recent insights into the development of the avian lung (Anderson-Berry et al., 2005; Maina 2005, 2006; Makanya et al., 2006, 2007) have revealed promising results regarding the understanding of the architecture of the avian lung. Cell attenuation during establishment of the avian blood-gas barrier, for example follows unique processes of cell cutting (secarecytosis) and cell pinching (peremerecytosis) (Makanya et al., 2006). Development of the pulmonary vasculature is both by vasculogenic (Anderson-Berry et al., 2005) and nonvasculogenic mechanisms and developing airways participate in vascular patterning (Makanya et al., 2007). However, it remains to be clarified whether the new structures described above are common in all avian species.

ACKNOWLEDGMENTS

The authors thank Messrs Christoph Lehmann, J.M. Gachoka, Ms Krystyna Sala, Ms Christine Ndegwa, Ms Barbara Krieger, and Amos Tangai for their excellent technical assistance, and Prof. Dr. HR Duncker for helpful discussions.

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