Pertanika 11(2), 229-238 (1988)

Quantitative Studies of the Lung of the Domestic Fowl (*Gallus gallus* var. *domesticus*)

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Key words: Domestic fowl; quantitative studies; morphometry; lung

ABSTRAK

Kajian kuantitatif yang komprehensif telah dijalankan atas paru-paru ayam peli haraan (Gallus gallus *var.* domesticus). *Data yang diperoleh adalah VL/W 14.65 + 3.17 cm^3/kg; Sa/W 18.08 + 2.51 cm^2/g; τht 0.322 ± 0.01 um; Dto_2/W 12.79 ± 2.20 mlo_2/min/mmHg/kg; dan DLo_2/W 1.39 ± 0.36 mlo_2/min/mmHg/kg. Nilai-nilai ini telah dibandingkan dengan nilai-nilai yang telah dilaporkan oleh pengarang-pengarang lain, dan alasan-alasan yang munasabah tentang perbezaan di antara nilai-nilai ini dibincangkan. Nilai-nilai purata bagi kebanyakan parameter telah dihitung supaya nilai-nilai yang diperoleh ini dapat membentuk suatu asas kepada kajian-kajian yang selanjutnya.*

ABSTRACT

Comprehensive quantitiative studies on the lung of the domestic fow, (Gallus gallur *var.* domesticus). *have been made. The data obtained were VL/W 14.65 ± 3.17 cm^3/kg; Sa/W 18.08 ± 2.51 cm^2/g; τht 0.322 ± 0.01 um; Dto_2/W 12.79 ± 2.20 mlo_2/min/mmHg/kg; dan DLo_2/W 1.39 ± 0.36 mlo_2/min/mmHg/kg. These values are compared with those reported by other authors, and possible reasons for the differences are discussed. The mean values for many of the parameters have been calculated so that the values so obtained may form a basis for further investigations.*

INTRODUCTION

Comprehensive pulmonary morphometric parameters of the domestic fowl (*Gallus gallus var. domesticus*) have been reported only by Abdalla *et al.* (1982) and Maina (1982), and then in only 5 birds altogether (data from the same 5 birds were used by both group of authors). However, other more limited quantitative studies of the domestic fowl were also carried out by Duncker (1971, 1973), Abdalla (1977), Dubach (1981), and Abdalla and Maina (1981). An especially interesting aspect of the studies by Maina (1982) was that in terms of stereology, the lung of the domestic fowl seemed to be much less well adapted for gas exchange, than the lungs of birds in general.

A factor of particular importance in determining the sample variance in morphometric observations is the number of animals investigated, a major problem in biological experiments being variations between individuals (Fraher, 1980; Gundersen *et al.*, 1981; Mathieu *et al.*, 1981; Gupta *et al.*, 1983; Mayhew and Sharma, 1984. In fact Gupta *et al.* (1983) commented that precise measurements made on inadequate numbers of animals make little statistical or biological sense. Thus, there is a need to make further stereological observations on the lung of the domestic fowl in order to confirm and if possible to extend the findings of Maina (1982) and Abdalla *et al.* (1982).

The objective of this paper is to double the sample population size by extending the comprehensive stereological investigation of the lung of the domestic fowl to another 5 birds. By this means it is hoped that a reliable basis will have been achieved for further quantitative studies on the normal or pathological lung of this important experimental and commercial bird.

MATERIALS AND METHODS

Five female domestic fowls of a commercial layer strain (Euribred Hisex Brown) with mean body weight of 1.87±0.35 kg were obtained from a commercial poultry farm in England. The birds were in their first year of lay. They were subjected to a comprehensive stereological pulmonary examination.

The birds were killed by an intraperitoneal injection of sodium pentobarbitone. The technique of fixation of the lung, processing for light and electron microscopy, random multistage sampling and stereological measurements were the same as those described by Abdalla *et al.* (1982) and Maina and King (1982a), the essentials being as follows.

The lungs were fixed by the intratracheal infusion of 2.3% glutaraldehyde buffered with sodium cacodylate to a pH of 7.4, total osmolarity 350 mOsm, at a constant pressure head of 25 cm. After removal, the lungs were placed in the same fixative for 24 hours. The volume of the lung was measured by the method of Scherle (1970). Test measurements showed no difference between the volume of the right and left lungs, and therefore the volume of the one lung was doubled to give the total volume of both lungs together. For the reasons given by Abdalla *et al.* (1982) and Maina and King (1982a), it is believed that the procedures for fixation and for subsequent processing and measurement should yield estimates of volumes, areas, and thickness which are likely to represent approximately the values in life.

For light micrsocopy the fixed left lung was cut transversely along the costal sulci to yield six slices. All the slices were halved by cutting immediately dorsal to the primary bronchus. Each half was routinely processed for paraffin sections, and the first technically adequate section was completely analysed with a 100 point Zeiss integrating eyepiece graticule at x100 to obtain the volume density of the main components of the lung.

For electron microscopy, the right lung was cut into the same six slices and half slices as before. Each half slice was diced into small cubes which were processed routinely for electron microscopy. From each half slice, 10 resin blocks were prepared, and from these, one block was randomly selected. Ultrathin sections were counterstained with lead citrate and uranylacetate. Three electron micrographs were taken from predetermined corners of grid squares at a primary magnification of x2750 which were checked by diffraction grating. From each negative, two prints were made at a final magnification of x6875, one with a superimposed quadratic lattice (*Figure 1*) and the other with a random short-line test (*Figure 2*). A minimum number of 24 negatives were prepared from each lung. The sufficiency of point counts was checked by cumulative means and the graphs of Weibel (1963) and Dunnill (1968). The short-line grid was used for estimating arithmetic mean thicknesses, and the

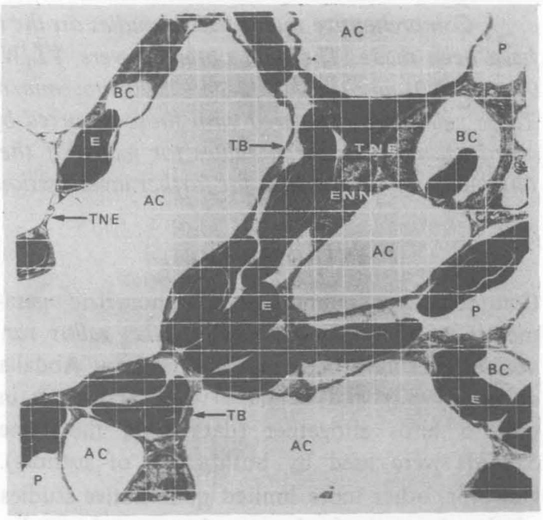

Fig. No. 1: An electron micrograph superimposed with a quadratic lattice grid. × 2309. AC, air capillaries; BC, blood capillaries; TB, tissue barrier; E, erythrocyte; ENN, endothelial nucleus; TNE, tissue not involved in gas exchange; P, plasma.

lattice grid was used for all other measurements, the surface area of the blood plasma layer being calculated as the mean of the surface area of the pulmonary endothelium and the erythrocytes (Weibel and Knight, 1964; Weibel, 1970/71). The anatomical diffusing capacities of the blood-haemoglobin pathway were estimated from Weibel's (1970/71) model. The physical coefficients for the tissue barrier (Kto_2), plasma (Kpo_2), and for oxygen uptake by whole blood (Θo_2) were those cited by Weibel (1970/71) for mammalian tissues, since no equivalent values for birds are available.

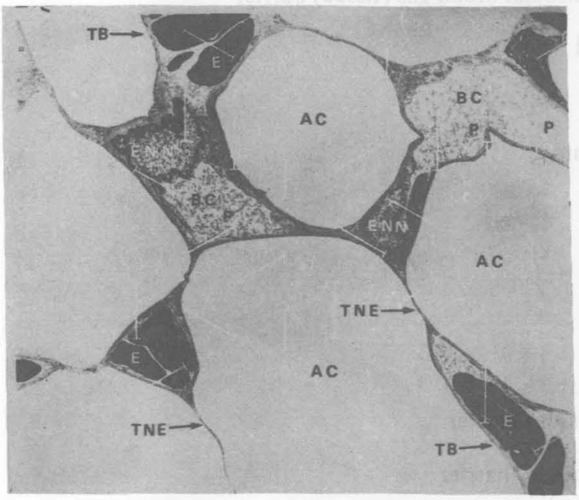

Fig. No. 2: An electron micrograph superimposed with a random short line test grid. × 2682. AC, air capillaries; BC, blood capillaries; TB, tissue barrier; E, erythrocyte; ENN, endothelial nucleus; TNE, tissue not involved in gas exchange; P, plasma.

The venous haemocrit was measured on five adult female domestic fowls of the same strain as that used for stereology, the value being 27.07%. The value for the domestic fowl was lower than the 31.5% obtained from the literature by Abdalla *et al.* (1982). The value of θo_2 was adjusted by subtracting the volume of the nuclei of the erythrocytes (Abdalla *et al.*, 1982; Maina and King, 1982b).

The terminology for the structure of the avian lung follows the Nomina Anatomica Avium (Baumel *et al.*, 1979). Symbols are defined in Table 1.

RESULTS AND DISCUSSION

The results are summarized in Table 3, and alongside them are the corresponding stereological data reported by other workers. These will be considered in sequence. The domestic fowls used by Maina (1982) and Abdalla *et al.* (1982) were heavier than those used in the present study, and their greater body weights partly explains the lower values for the weight-specific volume of the lung (VL/W).

There are differences in the estimates of the volume densities of some of the four components of the lung in Table 2, namely the exchange tissue (Vx), the lumen of the parabronchi and secondary bronchi (Vlb), the blood vessels larger than capillaries (Vb), and the primary bronchus (Vp). However, of these four parameters Vx is the most important because it is used subsequently as the reference volume for analysing the components of the exchange tissue. It is reassuring that the estimates of this parameter in Table 2 agree quite closely with the reported values. The discrepancy in Vp is not unexpected because the primary bronchus is small in area and is subject to sampling error. The variation in Vb can be attributed to the slightly different methods of interpretation of "blood vessels larger than capillaries". In the present study arterioles and venules contained within the mantle of exchange tissue were counted as exchange tissue (Vx), whereas Abdalla *et al.* (1982) and Maina (1982) included these in the category (Vb). The large discrepancy in Vlb cannot be explained by any known differences in anatomical criteria for identifying this tissues.

Of the components of the exchange tissue, the volume densities of the capillary blood (Vc) and the volume of the air capillaries (Va) are the most important functionally, and the estimates are quite closely similar. The volume densities of the tissues involved (Vt) and not involved (Vtn) in gas exchange vary because of known differences in the criteria for identification of tissues (compare Maina, 1982, with Vidyadaran, 1987). However, these parameters are of relatively little interest and anyway it is probably better to combine them together as was done by Duncker (1973).

Many of the ratios of volume to weight are reasonably close. The larger discrepancies tend

TABLE 1

Definition of symbols

Deo_2	diffusing capacity (conductance) of the erythrocyte for oxygen
DLo_2	total anatomical diffusing capacity for oxygen
Dmo_2	anatomical diffusing capacity of the membrane for oxygen
Dpo_2	anatomical diffusing capacity of the plasma for oxygen
Dto_2	anatomical diffusing capacity of the blood-gas (tissue) barrier for oxygen
Kpo_2	physical coefficient for oxygen permeation through plasma
Kto_2	physical coefficient for oxygen permeation through blood-gas (tissue) barrier
Θo_2	coefficient of oxygen uptake by whole blood
Sa	surface area of the air capillary epithelium
Sc	surface area of the blood capillary endothelium
Se	surface area of the erythrocyte
Sp	surface area of the plasma layer
St	surface area of the blood-gas (tissue) barrier
τhp	harmonic mean thickness of the plasma
τht	harmonic mean thickness of the blood-gas (tissue) barrier
$\bar{\tau}t$	arithmetic mean thickness of the blood-gas (tissue) barrier
Va	volume of the lumen of the air capillaries
Vb	volume of the wall and lumen of the blood vessels larger than capillaries
Vc	volume of the lumen of the blood capillaries
VL	volume of the fixed lungs (left lung x 2)
Vlb	volume of the lumen of parabronchi and secondary bronchi (including atria)
Vp	volume of the wall and lumen of the primary bronchus
Vt	volume of the tissue involved in gas exchange
Vtn	volume of the tissue not involved in gas exchange
Vx	volume of the exchange tissues of the lung
W	body weight

Weight-specific values are those standardized againts body weight. For example, VL/W is the values for the specific lung volume.

TABLE 2

Summary of the observed stereological values by various authors

Parameters	Units	Present study n = 5	Abdalla, 1977 Abdalla *et al.* 1982 n = 5	Maina 1982 n = 3	+Mean values	Dubach 1981	Duncker, 1973	++Pooled means
W	kg	1.873 ±0.36	2.06 ±0.58	2.483	2.27	–	–	2.14
VL	cm^3	26.59 ±2.10	25.02 ±2.6	27.0 ±4.8	26.00	–	–	26.20
VL/W	cm^3/kg	14.65 ±3.17	12.14	13.00	12.57	–	–	13.26
Vx	%	49.66 ±0.88	46.35 ±1.60	46.35 ±1.60	46.35	–	–	47.45
Vlb	%	38.01 ±0.98	30.56 ±0.99	30.56 ±0.99	30.56	–	–	33.04
Vb	%	6.64 ±0.64	13.65 ±1.08	13.65 ±1.08	13.65	–	–	11.31
Vp	%	5.69 ±1.02	9.34 ±2.07	9.34 ±2.07	9.34	–	–	8.12
Vc	%	27.89 ±2.77	30.32 ±1.70	27.92 ±5.54	29.12	–	28	28.53
Va	%	55.59 ±0.66	55.76 ±3.40	60.90 ±6.60	59.26	–	56	57.53
Vt	%	7.44 ±0.65	9.25 ±1.16	6.30 ±3.15	7.78	–	[16, for combined value of Vt and Vtn]	7.76
Vtn	%	9.09 ±2.33	2.82 ±0.90	4.88 ±1.99	3.85	–		5.60
Vx/W	mm^3/g	7.29 ±1.69	5.63*	5.05*	5.34	–	–	5.99
V1b/W	mm^3/g	5.59 ±1.23	3.71*	3.33*	3.52	–	–	4.21
Vb/W	mm^3/g	0.96 ±0.14	1.66*	1.49*	1.58	–	–	1.37
Vp/W	mm^3/g	0.82 ±0.20	1.13*	1.02*	1.08	–	–	1.69

Table 2
(continued)

Parameters	Units	Present study n = 5	Abdalla, 1977 Abdalla *et al.* 1982 n = 5	Maina, 1982 n = 5	+Mean values	Dubach, 1981	Duncker, 1973	++Pooled means
Vc/W	mm^3/g	2.07 ±0.64	1.70*	1.41*	1.56	–	–	1.73
Va/W	mm^3/g	4.06 ±0.97	3.25*	3.08*	3.17	–	–	3.46
Vt/W	mm^3/g	0.54 ±0.11	0.52*	0.32*	0.42	–	–	0.46
Vtn/W	mm^3/g	0.64 ±0.10	0.16*	0.25*	0.21	–	–	0.35
Sa/W	cm^2/g	18.08 ±2.51	13.20*	11.16*	12.18	–	–	14.15
St/W	cm^2/g	12.46 ±1.96	10.09 ±1.1	8.70 ±1.1	9.40	–	18	12.31
Sc/W	cm^2/g	14.82 ±2.64	11.85	9.75*	10.80	13.6	13.6	12.51
Se/W	cm^2/g	16.05 ±3.67	15.24*	14.46*	14.85	–	–	15.25
Sp/W	cm^2/g	15.43 ±2.88	13.54*	12.12*	12.83	–	–	13.70
St/Vx	mm^2/mm^3	172.84 ±8.88	179.5 ±8.8	172 ±6.0	175.75	192	192	179.09
Vc/St	cm^3/m^2	1.65 ±0.24	1.69	1.62	1.66	–	–	1.65
τht	μm	0.322 ±0.01	0.314 ±0.02	0.318 ±0.02	0.316	0.346	0.346	0.325
τhp	μm	0.300 ±0.06	0.342 ±0.05	0.306 ±0.02	0.324	–	–	0.316
τt	μm	0.459 ±0.11	1.20 ±0.06	1.24 ±0.04	1.22	0.494	0.494	0.848
$\bar{\tau}$t / τht	ratio	1.44 ±0.37	3.87*	3;90	3.89	1.43*	1.43*	2.66

Table 2
(continued)

Parameters	Units	Present study n = 5	Abdalla, 1977 Abdalla *et al.* 1982 n = 5	Maina, 1982 n = 3	+Mean values	Dubach, 1981	Duncker, 1973	++Pooled means
Dto_2/W	mlo_2/min/ mmHg/kg	12.79 ±2.20	–	9.0 ±1.0	–	–	–	10.90
Dpo_2/W	mlo_2/min/ mmHg/kg	19.31 ±2.49	–	14.2 ±4.4	–	–	–	16.76
Deo_2/W	mlo_2/min/ mmHg/kg	1.76 ±0.52	–	1.29 ±0.41	–	–	–	1.53
Dmo_2/W	mlo_2 /min/ mmHg/kg	7.61 ±1.03	6.22*	5.40 ±0.96	5.81	–	–	6.41
DLo_2/W	mlo_2/min/ mmHg/kg	1.39 ± 0.36	1.23*	1.01 ±0.29	1.12	–	–	1.21
Vol. of air in the air capillaries	%	38.74	40.22*	41.45*	40.84	–	–	40.14
Vol. of air in the other airways	%	61.26	59.78*	58.55*	59.17	–	–	59.86
Vol. of blood in the larger blood vessels	%	32.18	49.28*	51.00*	50.14	–	37.6	42.52

Table 2
(continued)

Parameters	Units	Present study n = 5	Abdalla, 1977 Abdalla *et al.* 1982 n = 5	Maina, 1982 n = 3	+Mean values	Dubach, 1981	Duncker, 1973	++Pooled means
Vol. of blood in the blood capillaries	%	67.82	50.72	49	49.86	–	62.4	57.49
Rel. proportion of the lung occupied by blood	%	21.71	28.0	26.0	27	–	18.1	23.45
Rel. proportion of the lung occupied by air	%	71.27	66.0	68.0	67	–	75.4	70.17

\+ Mean values of Abdalla *et al.* (1982) and Maina (1982)
++ Mean values of all the studies
* Values calculated by us from the authors' data

to involve components where different methods of counting have already been identified above, i.e. Vlb/W, VB/W, Vp/W and Vtn/W.

The estimates of harmonic mean thickness of the tissue barrier and even of the plasma barrier, are reasonably close. This is helpful since the estimates of the anatomical diffusing capacity of the barrier for oxygen are much influenced by these parameters. The arithmetic mean thickness varies greatly according to different authors. The values reported by Abdalla *et al.* (1982) and Maina (1982) agree well with each other, as do those of Duncker (1973), Dubach (1981), and the present investigation. Maina (1982) discussed this problem fully, but could find no explanation.

The weight specific anatomical diffusing capacities of the blood-gas pathway for oxygen (Dto_2/W, Dpo_2/W, Dmo_2/W, Deo_2/W, and DLo_2/W vary to a greater of lesser degree. Since all these estimates utilize several structural parameters, errors become compound, and under such conditions the degree of agreement may be regarded as acceptable. Finally, the estimates of the relative distribution of air and blood throughout the various parts of the lung agree fairly well for air but less well for blood.

In an attempt to take full advantage of the data collected by the various investigations, means of the pooled data have been calculated. The procedure has been first to obtain means of the values reported by Maina (1982) and Abdalla *et al.* (1982), since they shared birds and some of the measurements. We have then calculated the means of the values obtained in the present study, by Maina (1982) and Abdalla *et al.* (1982) together, and by Duncker (1973) and Dubach (1981) where available. It is hoped that pooled means so obtained may form a helpful foundation for further investigations.

ACKNOWLEDGEMENTS

We thank the British Council and Universiti Pertanian Malaysia for making our collaboration possible. We are grateful to Mr. P. Ganesamurthi for technical assistance, and to Mrs. M.M. Thompson and Mrs. Shamala for typing the manuscript. M.K. Vidyadaran particularly thanks Dr. J.N. Maina of the University of Nairobi for advice on stereological techniques when this investigation was initiated.

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(Received 6 June, 1987)