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RESPIRATORY FUNCTION IN EXERCISING FOWL FOLLOWING OCCLUSION OF THE THORACIC AIR SACS

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Summary

Oxygen consumption, respiratory evaporative water loss, respiratory rate and gas tensions in the clavicular and abdominal air sacs and in arterial blood were monitored after occluding either the cranial thoracic air sac only (CRT group) or the cranial and caudal thoracic air sacs together (CRT–CT group). Respiratory water loss was used to estimate minute ventilation. Both experimental groups were able to maintain control levels of ventilation at rest and during treadmill exercise at approximately three times the resting metabolic rate. The CRT group regulated blood and intrapulmonary P_{CO_2} and P_{O_2} normally, but there was a slight hypoxaemia/hypercapnaemia in the CRT–CT group, apparently as a result of parabronchial hypoventilation. The differential distribution of gas tensions between the cranial and caudal groups of air sacs was the same in control and experimental birds, suggesting that a normal intrapulmonary airflow pattern was preserved in the absence of the thoracic air sacs. The findings are discussed in the light of current models of the control of intrapulmonary airflow in birds.

Introduction

The avian respiratory system shows a functional division of labour between the lung, which carries out gas exchange, and the air sacs, which generate the airflow through the lung. Since each air sac has a direct connection to the non-gas-exchanging mesobronchus in addition to its numerous parabronchial connections, the potential exists for considerable wastage of inspired gas. The unidirectional airflow scheme which originated in the work of Brandes (1923, 1924) and Bethe (1925) and was refined by Dotterweich (1936) and Hazelhoff (1943), has provided a basis for understanding how potential dead-space shunts can be avoided. There appears to be little experimental evidence for the existence of physical valves in the lung (Jones *et al.* 1981) and the unidirectional airflow scheme relies on the operation of aerodynamic valves, one preventing entry of inspired air into the ventrobronchial openings and the other preventing leakage of expired air from the caudal sacs along the mesobronchus (see reviews by Scheid, 1979; Fedde, 1980;

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Powell *et al.* 1981; Scheid & Piiper, 1989). There is recent experimental evidence for the presence inside the lung of an inspiratory valve which has aerodynamic characteristics (Banzett *et al.* 1987; Kuethe, 1988; Wang *et al.* 1988). Furthermore, indirect evidence from flow measurements (Bretz & Schmidt-Nielsen, 1971, 1972) and from gas analysis at intrapulmonary sites (Powell *et al.* 1981) supports the notion of an expiratory valve in the caudal end of the mesobronchus.

These valves appear to be of critical importance in determining the most efficient use of air entering the lung, but it is not known whether the valvular effects are due in equal measure to the actions of all the air sacs, or whether some air sacs may play a more strategic role than others. There is evidence from unidirectionally ventilated geese that the inspiratory valve breaks down if the cranial group of air sacs is inactivated: this allows inspired air to enter the ventrobronchial orifices and to pass cranio-caudally across the parabronchi (Brackenbury, 1979). In the present study we attempted to obtain further information on the relationship between the actions of the individual air sacs and the control of intrapulmonary airflow, by monitoring the effects of blocking specific air sacs on ventilation and respiratory gas tensions. These experiments were carried out in resting birds and birds in which the demand for ventilation was increased by treadmill exercise.

Part of this study was presented to the Second International Congress of Comparative Physiology and Biochemistry, Baton Rouge, USA, 1988.

Materials and methods

Animals and experimental techniques

The study employed 16 male, adult White Leghorn chickens (body mass 1·59–2·47 kg) which had been selected from a larger group after preliminary running trials on an animal treadmill (Woodway England, Garrick Ltd, London). Before experimentation each bird received at least 5–6 sessions of exercise lasting for 20 min at a speed of 4·5 km h^{-1} (1·2 m s^{-1}). The birds were then divided into a control group ($N = 6$) and two experimental groups ($N = 5$ each). The experimental groups were anaesthetized (intravenous injection of 30 % urethane/pentobarbitone sodium, 60 mg ml^{-1}, 50:50 vols%) and either the cranial thoracic air sac alone (CRT group) or the cranial plus caudal thoracic air sacs together (CRT–CT group) were blocked on both sides of the body (Fig. 1). Access points for each air sac were carefully worked out on dead specimens. The cranial thoracic sac was punctured *via* the third from last intercostal space, at a point approximately 1 cm ventral to the junction between the sternal and vertebral parts of the ribs. The caudal thoracic sac was pierced *via* the last intercostal space at a point approximately 2 cm dorsal to the junction between the sternal and vertebral parts of the ribs. At each point, after reflecting the skin and fascia, a hole approximately 5 mm in diameter was made in the intercostal muscle and the underlying air sac was packed with small pieces of sterilized cotton wool approximately 0·25–0·5 cm^3 in volume. Approximately 90–100 pieces were required to pack the cranial

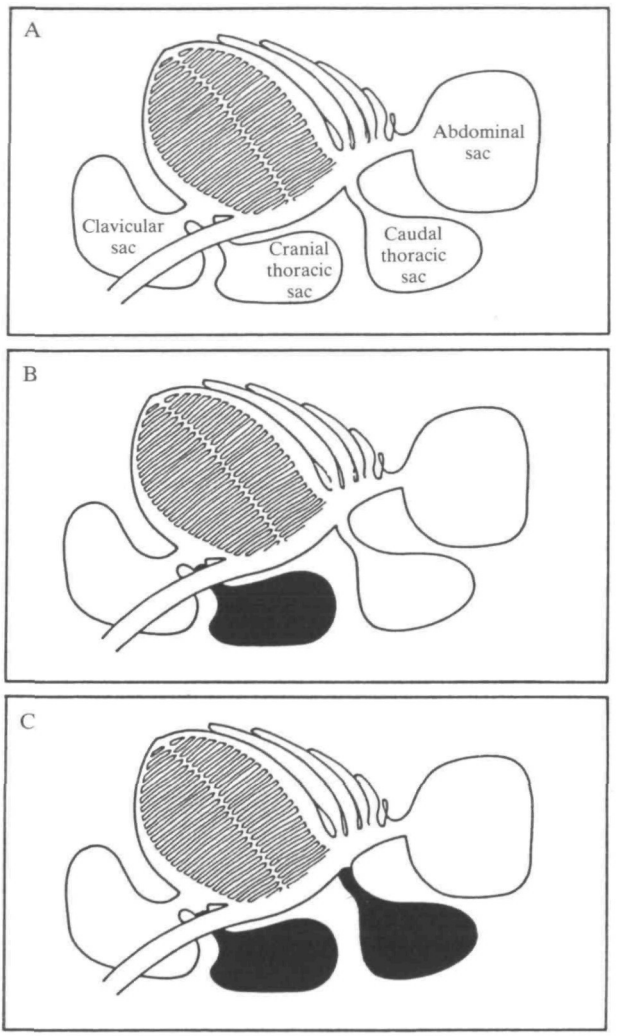

Fig. 1. Scheme of air sac blocking experiments. (A) controls; (B) CRT group; (C) CRT–CT group. The cervical air sac is not represented. The neopulmo is a small area of parabronchi connecting the dorsobronchi to the caudal air sacs and is not shown.

thoracic sac and 50–60 pieces to pack the caudal thoracic sac. The effectiveness of this technique for blocking the air sacs had been validated in preliminary experiments and was verified by *post-mortem* examination of the experimental birds (see Discussion).

After 2–3 weeks of recovery from surgery the experimental birds, plus the controls, were again anaesthetized and cannulae were implanted into one carotid artery, and into the clavicular and abdominal air sacs. The arterial cannula was externalized high on the neck at a point 2 cm ventral to the external auditory meatus. The clavicular sac cannula was tied securely to the clavicle. The

abdominal sac was cannulated immediately behind the base of the last rib and the cannula was tied to the rib in front. Approximately 50 units of heparin was administered throughout the operation, and thereafter the arterial cannula was flushed regularly with heparinized saline.

1 ml blood samples were removed anaerobically using a double-syringe technique and analysed for P_{O_2} and P_{CO_2} on a Radiometer BMS2 blood-gas analyser thermostatted at 41·5 °C. The electrodes were calibrated with a precision gas mixer after each measurement. The P_{O_2} and P_{CO_2} of clavicular and abdominal air sac gases were measured on Beckman OM-11 and LB-2 analysers, respectively, after drawing the samples through Drierite. The sampling flow rate was less than 50 ml min^{-1} and this was assumed to be small in comparison with the normal gas turnover in individual air sacs.

Oxygen consumption, $\dot{V}_{O_2}$, was measured in four control birds to give an indication of work load. The method has been described previously (Brackenbury *et al.* 1981). Briefly, the subject wore a loose-fitting plastic mask into which air was drawn from the atmosphere at a measured rate of 20–30 l min^{-1} using a 1 m length of flexible tubing attached to a vacuum pump. A sample of the gas drawn through the mask was taken over Drierite into a Beckman OM-11 oxygen analyser. In individual experiments the flow rate of the gas collected from the mask was adjusted so that the analyser gave a displacement of approximately 1 % compared to atmospheric, thereby ensuring against loss of expirate from the mask. Measured values of $\dot{V}_{O_2}$ were corrected to STPD. The same mask was used to measure simultaneously respiratory evaporative water loss by drawing the collected gas through an airtight vessel containing a thermometer and a relative humidity detector (Vaisala, Finland) with an accuracy of ±2 %. The response time of the detector to a step-wise increase in relative humidity was approximately 90 s. The detector was calibrated with both dry and saturated air at known temperatures, and total respiratory water loss was calculated from the known gas flow rate, relative humidity and temperature. This value was converted to the corresponding value for expired minute ventilation, $\dot{V}_E$, assuming that the expired gas had a temperature of 38 °C. This indirect method of measuring ventilation has been validated by comparing values for estimated and directly measured ventilation in resting and exercising chickens (Brackenbury *et al.* 1982*a*). Respiratory rate was measured directly by drawing a sample of the collected gases directly into a Beckman LB-2 CO_2 analyser which was capable of following breath-to-breath variations in P_{CO_2}. Tidal volume was derived using the estimated $\dot{V}_E$ and measured respiratory frequency values.

Air sac gas tensions, $\dot{V}_{O_2}$ and the changes in relative humidity and P_{CO_2} of the gas collected from the mask were all displayed continuously on a Grass model 7D chart-recorder. Rectal temperature was measured in the control birds before and immediately after exercise using a mercury-in-glass thermometer. All experiments were carried out in an unheated laboratory at ambient temperatures of 14–17 °C to minimize any possible effects of thermal stress on the respiratory responses of the animals.

Experimental protocols

Each bird performed dual treadmill runs in two separate series of experiments. Except for the fact that different parameters were measured, the two series were carried out in identical conditions at a treadmill speed of $4{\cdot}5\,km\,h^{-1}$ (zero gradient), for a period of 10 min. In the first series, air sac gas tensions were monitored continuously before, during and for 10–15 min after the run. Arterial blood was sampled immediately after the cessation of the run and again approximately 10 min later when the air sac gas tension traces indicated a return to steady-state resting conditions. Preliminary trials had shown that measurements taken after a run generally gave a more stable indication of resting conditions than those taken before, when the birds tended to be more easily distracted by sounds and movements in the laboratory. The total delay between the end of a run and the final transfer of a blood sample into the blood gas analyser was less than 45 s. In the second series of runs, the birds were fitted with the mask and $\dot{V}_{O_2}$, respiratory water loss and respiratory rate were measured simultaneously. Mean values of all the measured variables from both series were estimated over the last 2–3 min of exercise, and 10–15 min after exercise. All data are quoted as mean values ± 1 S.E.M. Significance tests (Student's *t*-test) were carried out on paired values at the 5 % level.

Results

Minute ventilation increased by approximately 200 % in the control birds and this was mainly due to elevated respiratory rate, with little change in tidal volume. Exercise resulted in a slight fall in arterial P_{CO_2} and a slight rise in arterial P_{O_2} in the control birds, and similar changes were reflected in the composition of the clavicular and the abdominal sac gases. Rectal temperature increased only marginally during exercise in the control birds, from $41{\cdot}4 \pm 0{\cdot}17$ to $41{\cdot}8 \pm 0{\cdot}01$ °C.

Both experimental groups showed perfect compensation for the lack of function in the pertinent air sacs and their ventilatory characteristics at rest and during exercise were virtually indistinguishable from those of the controls (Table 1). Taken together, the control and experimental birds increased minute ventilation by approximately 250 % compared to the non-exercised state. The CRT group regulated blood and air sac gases normally. The CRT–CT group showed a mild but significant hypoxaemia/hypercapnaemia compared with controls and this was also observed in the abdominal, although not the clavicular, sac gas tensions. As with the controls and the CRT group, the CRT–CT group displayed a slight freshening of both air sac and blood gases during exercise.

Oxygen consumption in four of the control birds increased by 300 % from $16{\cdot}00 \pm 0{\cdot}95\,ml\,kg^{-1}\,min^{-1}$ in the non-exercised state to $66{\cdot}97 \pm 1{\cdot}43\,ml\,kg^{-1}\,min^{-1}$ during exercise.

Post-mortem examination of the experimental birds confirmed the effectiveness of the air sac block and this was documented photographically. In each case there had been a vigorous foreign body reaction by the walls of the air sacs concerned,

Table 1. *Respiratory characteristics of domestic fowl at rest and during exercise following occlusion of the cranial thoracic air sac alone (CRT) or the cranial and caudal thoracic air sacs together (CRT–CT)*

	Control ($N = 6$)		CRT ($N = 5$)		CRT–CT ($N = 5$)	
	Rest	Exercise	Rest	Exercise	Rest	Exercise
Respiratory evaporation ($mmol\,kg^{-1}\,min^{-1}$)	0·93 (0·09)	2·83 (0·11)	1·06 (0·07)	3·57* (0·12)	0·97 (0·05)	3·22 (0·17)
Minute volume ($ml\,kg^{-1}\,min^{-1}$)**	319 (32)	976 (39)	367 (24)	1231* (42)	335 (16)	1110 (59)
Tidal volume ($ml\,kg^{-1}$)	13·3	16·0	14·7	18·6	14·0	15·4
Respiratory frequency (min^{-1})	24 (0)	61 (3)	25 (1)	66 (1·5)	24 (1)	72 (5)
Arterial P_{CO_2} (kPa)	4·11 (0·09)	3·88 (0·08)	4·11 (0·05)	4·03 (0·05)	4·40* (0·09)	4·21* (0·08)
Arterial P_{O_2} (kPa)	11·01 (0·17)	11·34 (0·27)	10·61 (0·2)	10·58* (0·16)	10·30* (0·15)	10·53* (0·15)
Clavicular sac P_{CO_2} (kPa)	5·36 (0·08)	4·95 (0·09)	5·25 (0·11)	4·84 (0·15)	5·56 (0·07)	4·66 (0·09)
Clavicular sac P_{O_2} (kPa)	13·25 (0·13)	14·38 (0·12)	13·88* (0·16)	14·96* (0·12)	13·53 (0·13)	15·17* (0·12)
Abdominal sac P_{CO_2} (kPa)	2·68 (0·11)	2·37 (0·11)	–	–	3·08* (0·12)	2·93 (0·29)
Abdominal sac P_{O_2} (kPa)	16·82 (0·19)	17·52 (0·23)	–	–	16·26 (0·33)	16·64 (0·4)

Mean values (1 S.E.M.), except for tidal volume which was calculated from respiratory rate and minute volume.

N = number of birds.

* Significantly different from control values ($P < 0·05$).

** Estimated from respiratory evaporation using the method described in the text.

1 kPa = 7·52 mmHg.

resulting in the entire plug of cotton wool becoming invested with a thick layer of fibrous tissue which bound it to the surrounding walls. In a small minority of cases the lumen of the air sac had not been completely obliterated but the volume of the remaining air space was only a few percent of its original volume.

Discussion

Critique of experimental methods

Initial attempts to design a flowmeter for the direct measurement of ventilation based on the face-mask design used previously in running hens (Brackenbury *et al.* 1982*b*) proved impracticable, since the size and shape of the comb in the White Leghorn males made it impossible to obtain an airtight seal around the mask. We decided against the use of a helmet completely enclosing the head (Gleeson *et al.*

1985) since we felt that this would interfere with normal respiration in an exercising bird. For similar reasons, we discounted the idea of using an acute tracheostomy. The method finally adopted, although indirect, has been validated in a previous study in which it was shown that there was a linear relationship between respiratory evaporative water loss and directly measured ventilation over a range of respiratory flow rates up to $6\,l\,min^{-1}$, well above the maximum ventilatory flow rates encountered in the present study (Brackenbury *et al.* 1982*a*). The method, however, is sensitive to changes in temperature of the expired air since this governs the slope of the water loss *versus* ventilatory flow rate relationship. The assumption of an expired gas temperature of 38°C is based on the previously quoted study, but variations from a linear relationship would be expected if there were any significant alterations of expired gas temperature between rest and exercise. However, since rectal temperature hardly altered in the present experiments, it seems unlikely that there would be significant changes in expired gas temperature. To check on the reliability of the method, we compared ventilatory data in the present study with data obtained by direct measurement in hens running at the same mass-specific work loads (Brackenbury *et al.* 1982*b*) and found excellent agreement.

The technique used to block the air sacs was simple in design and crude in operation but had been arrived at only after fairly extensive exploration of alternatives, including the implantation of inflatable balloons and the injection of inert foams. None of these methods proved as reliable as packing the sac with cotton wool. The only pathological reaction to the cotton wool was the fibrocytic response which not only isolated the implant but also sealed any small pockets of space that may have existed at the time of the implantation. The foreign body reaction appeared to be complete within 2–3 weeks and none of the birds showed chronic side-effects from the procedure. Although there was partial infiltration of the implants with tissue fluid, the additional mass was estimated to be less than 50 g or approximately 2 % of the mean body mass. No correction for this additional mass was made in the calculation of respiratory variables.

The dorsal wall of the cranial thoracic air sac is effectively the ventral surface of the lung underlain by the pulmonary aponeurosis. It could therefore be argued that direct pressure from the cotton wool implant might compress the pulmonary bronchi or the blood vessels. Such direct pressure could affect both airflow and blood perfusion, potentially creating ventilation/perfusion mismatches and leading to reduced O_2 diffusion capacity (Powell & Wagner, 1982; Hempleman & Powell, 1986). However, as well as possessing intrinsic tensile strength, the pulmonary aponeurosis is also tensioned by the pulmocostales muscles and both of these would resist compression of the lung. *Post-mortem* examination showed that although the cotton wool implant was firmly bound to the aponeurosis by fibrous tissue, there was no sign of haemostasis or necrotic tissue in the lung.

Well-trained male domestic fowl are capable of considerably higher work loads than those employed in the present study and it is possible that the use of a faster treadmill speed to stress the respiratory system would have elicited more

pronounced deficiencies in the experimental birds, particularly the CRT–CT group. Evidently, at the work load used, the birds were able to compensate for the lack of respiratory capacity by increasing the tidal excursion of the rib-cage, thereby enhancing the turnover of gas in the available air sacs. A higher work load might demand an excursion much closer to the mechanical limits of the rib-cage and might also incur a significant increase in the energy cost of ventilation. In view of the various instrumental attachments to the birds, however, it would be much more difficult to obtain smooth runs at higher treadmill speeds.

Ventilation, gas tensions and intrapulmonary airflow

The measured ventilatory responses of the birds to exercise were essentially the same as those previously documented: increased minute volume was achieved primarily by alteration of respiratory rate, with little change in tidal volume (Brackenbury *et al.* 1982*b*). The slight fall in arterial P_{CO_2} and rise in arterial P_{O_2} as a result of exercise (Table 1) are in keeping with the idea that there was a small degree of parabronchial hyperventilation, and this is supported by the freshening of gases that occurred in the air sacs. Parabronchial hyperventilation in exercising chickens is due, amongst other things, to thermal state and, at very high work loads, metabolic acidosis (Brackenbury & Gleeson, 1983), although other unidentified non-thermal factors may also be involved in the control of pulmonary ventilation (Gleeson & Molony, 1989). In the present study, it seems unlikely that either hyperthermia or lactacidosis would have contributed significantly to parabronchial hyperventilation.

According to the unidirectional airflow scheme, gas tensions in the cranial air sacs (i.e. the cervical, clavicular and cranial thoracic sacs) should all be identical (Bouverot & Dejours, 1971), although this assumption holds true only if the so-called inspiratory and expiratory valves in the lung work perfectly. There is considerable evidence of the effectiveness of these valves during resting respiration; this comes from direct flow recordings (Scheid & Piiper, 1972; Bretz & Schmidt-Nielsen, 1971, 1972), from gas sampling at intrapulmonary sites (Powell *et al.* 1981) and, more recently, from plotting the time of appearance of injected tracer gases in the cranial air sacs (Banzett *et al.* 1987). Nothing is known about the effectiveness of the valves during exercise hyperpnea, although Banzett *et al.* (1987) demonstrated that the inspiratory valve was more efficient at high flow rates and this suggests that exercise should, if anything, increase the efficiency of the aerodynamic valves.

The most significant finding of the present study is that functional ablation of the thoracic air sacs has little effect on the ability of the chicken to regulate either intrapulmonary or arterial blood gas tensions, even when the demand for gas exchange is raised by exercise. This was a surprising result since, on anatomical grounds, the thoracic air sacs would seem to be the best placed to influence intrapulmonary airflow. They are almost completely confined within the rib cage, and hence are most directly influenced by the movements of the body wall during ventilation. In contrast, the remaining air sacs, the cervical and clavicular lying to

the front, and the abdominal to the rear, lie outside the rib-cage. According to figures presented by King (1975), the combined volumes of both cranial and both caudal thoracic air sacs in the male chicken account for 25–30 % of the total air-sac volume, and presumably their contribution to the tidal volume is at least a comparable fraction, and probably a greater fraction in view of their mechanical advantage. Despite this, the CRT–CT group of experimental birds showed little sign of respiratory distress, even under challenge from exercise, apart from a minor hypoxaemia/hypercapnaemia (Table 1).

Although respiratory movements were not measured directly, a simple calculation indicates that to bring about an additional increase in volume of the body cavity by 30 %, experimental birds need only increase the linear excursion of the rib-cage by a further $(0{\cdot}3)^{1/3}$, or less than 10 %, compared to controls. The measured increase in arterial P_{CO_2} of the CRT–CT group (Table 1), although small, would probably have been sufficient to elicit this level of response. Respiratory pattern did not differ significantly between the control and experimental birds and this, in turn, may be related to the similarity in arterial and intrapulmonary P_{CO_2}, both of which are involved in the control of avian breathing pattern. Mechanoreceptors, capable of responding to changes in volume of the air sacs or the body cavity, may also play a role, but the absence of any experimental data on respiratory movements in the present study makes it impossible to comment further on this subject.

The interesting question remains: to what extent did the pattern of intrapulmonary airflow differ in the experimental and control groups? We have no direct evidence, but the fact that the differential distribution of gas tensions between the cranial and caudal air sacs (Table 1) was the same in control and experimental birds suggests that the unidirectional pattern of intrapulmonary airflow remained intact even after effective removal of the thoracic air sacs. The clear implication is that a normal unidirectional airflow pattern can be generated without the participation of the thoracic air sacs. It is logical to enquire whether other air sacs can be experimentally inactivated without influencing intrapulmonary airflow. In a preliminary attempt to answer this problem we tried blocking first the abdominal, then the clavicular air sacs, in separate series of experiments. Unfortunately, the distensibility of the abdominal sac is practically unlimited, and it is not possible to block this sac fully without imposing considerable pressure on the abdominal viscera. In a few cases we succeeded in blocking the cranial 30 % or so of the abdominal air sac, in the area of its connection to the lung, and *post-mortem* examination showed that the entire air sac wall had collapsed around and fused with the implant, completely preventing inflation. The respiratory characteristics of these birds, including blood gases, were normal, although the data were too fragmentary to justify formal inclusion in the results.

The evidence from the foregoing studies strongly points to an important role for the clavicular sac in the regulation of intrapulmonary airflow. Direct blocking was carried out in three birds, but all showed severe signs of respiratory distress upon recovery from surgery and had to be killed forthwith. Distress was probably

caused more by the pathological reaction to the implant than the physiological consequences of removal of air sac function. *Post-mortem* examination revealed copious secretions in the clavicular air sac and secondary bronchi of the lung, resulting from irritation of the mucosal coverings of the lung and the nerves, blood vessels and pericardium contained within the air sac. Further, there was evidence of a direct compression of the syrinx by the cotton wool. An alternative technique to direct physical block will have to be designed before the role of this sac can be investigated.

Finally, it is possible that blockage of the caudal thoracic air sac may interfere with normal airflow through the neopulmo, which is said to account for a significant fraction of gas exchange in the resting bird (Duncker, 1971, 1972; Piiper, 1978; Fedde *et al.* 1986). It is noteworthy that the resting P_{CO_2} in the abdominal air sac of the experimental birds was slightly but significantly higher than in the controls (Table 1). This may indicate that air that normally flows through the neopulmo to both the caudal thoracic and abdominal air sacs is now passing entirely to the abdominal sac, with a resultant increase in P_{CO_2}. These speculations, however, must also be weighed against evidence that caudal air sac gas composition in the penguin, which does not have a neopulmo, is similar to that in birds, such as the chicken, which have a well-developed neopulmo (Powell & Hempleman, 1985).

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