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EFFECTS OF COMBINED ABDOMINAL AND THORACIC AIR-SAC OCCLUSION ON RESPIRATION IN DOMESTIC FOWL

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Summary

Ventilation and respiratory and blood gas tensions were monitored at rest and during running exercise, following bilateral occlusion of the cranial and caudal thoracic and the abdominal air sacs. This represents a removal of approximately 70 % of the total air-sac capacity. At rest, the birds were strongly hypoxaemic/hypercapnaemic. Ventilation was maintained at its control value but respiratory frequency was significantly increased and tidal volume diminished. The birds were capable of sustained running at approximately three times the pre-exercise metabolic rate. Minute ventilation during exercise was the same as that of the controls, but breathing was faster and shallower. Exercise had no effect on blood gas tensions in either the control or the experimental birds. There was no evidence of a detrimental effect of air-sac occlusion on the effectiveness of inspiratory airflow valving in the lung: hypoxaemia appeared to be due to the altered respiratory pattern, which resulted in increased dead-space inhalation.

Introduction

Present understanding of the mechanics of airflow within the avian lung air-sac system is based on the unidirectional airflow scheme which originated in the work of Brandes (1923, 1924), Bethe (1925), Dotterweich (1936) and Hazelhoff (1943) and which has been verified and elaborated by many later studies (see recent review by Scheid and Piiper, 1989). The unidirectional airflow scheme ensures that the palaeopulmonic parabronchi are aerated during both inspiration and expiration. Control of airflow direction within the lung is governed at least in part by aerodynamic valves: an inspiratory valve located at the cranial end of the mesobronchus and incorporating the origins of the ventrobronchi, and an expiratory valve in the caudal end of the mesobronchus near the entrances to the dorsobronchi. The operation of these valves depends on geometrical features and, as has been shown experimentally (Banzett *et al.* 1987; Kuethe, 1988; Wang *et al.* 1988), gas velocity and density. The contribution that the air sacs make to intrapulmonary airflow valving in normal conditions is not known, although there is evidence from anaesthetized artificially ventilated geese that the inspiratory

Key words: bird, respiration, airflow, exercise.

valve breaks down if the cranial air sacs are inactivated, but remains effective when the caudal sacs are inactivated (Brackenbury, 1979). Recently it has been shown that occlusion of both pairs of thoracic air sacs has surprisingly little effect on the ability of fowl to regulate intrapulmonary airflow or blood gas tensions either in resting conditions or when the birds were subjected to increased oxygen demand during exercise (Brackenbury *et al.* 1989). The cranial and caudal thoracic air sacs comprise approximately 25 % of the total lung/air-sac volume. In the present study we occluded both pairs of thoracic sacs, together with the abdominal sacs, which are the largest in the air-sac system. This operation inactivates approximately 70 % of the total lung/air-sac volume. Nevertheless, although blood gas regulation was strongly affected, there was no evidence that this was due to a failure of inspiratory airflow valving.

A synopsis of this study was presented at an International Symposium of the Society for Experimental Biology held in Tützing, West Germany, in 1989.

Materials and methods

Animals and surgical techniques

Eleven adult male White Leghorn domestic fowl (body mass 1.54–2.10 kg) were used in the study. The birds were subjected to a preliminary treadmill exercise programme during which they received five or six 20 min runs at speeds of up to 4.8 km h^{-1} (1.3 m s^{-1}, zero gradient) and were trained to wear a loose-fitting facial mask. The birds were divided into a control group of five and an experimental group of six and before further experimentation the latter were anaesthetized [intravenous injection of ethyl carbamate (150 mg ml^{-1}) and sodium pentobarbitone (30 mg ml^{-1})] and the cranial and caudal thoracic air sacs were bilaterally blocked. Access to the cranial thoracic sac was obtained *via* a 5 mm incision over the third from last intercostal space at a point lying approximately 1 cm ventral to the junction between the sternal and vertebral parts of the ribs. The caudal thoracic air sac was similarly pierced *via* the last intercostal space at a point 2 cm dorsal to the junction between the sternal and vertebral parts of the ribs. The underlying air sacs were packed with pieces of sterilized cotton wool each approximately 0.25–0.5 cm^3 in volume.

Approximately 3 weeks later these birds were again anaesthetized to allow bilateral blocking of the abdominal air sacs (Fig. 1). Access to these sacs was made just behind the last rib and they were packed with cotton wool as previously described. Preliminary investigations had shown that total blockage of the abdominal sac was impracticable owing to its great distensibility. To have completely filled the sac would have placed unacceptable pressure on the abdominal viscera. Fortunately it proved sufficient to pack only the cranial 30 % or so of the resting volume of the sac, in the region of its connections to the mesobronchus and neopulmonic parabronchi, since the remainder of the air-sac wall subsequently became fused to the implant after a 2- to 3-week period, completely obliterating the lumen. The effectiveness of all air-sac blocks was

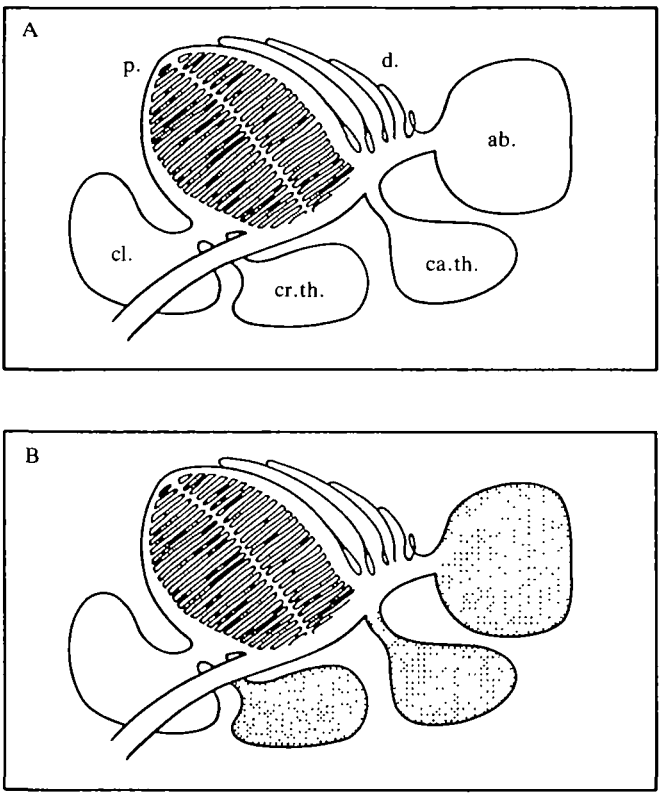

Fig. 1. Scheme of air sac occlusions used in the experiments. (A) Control; (B) experimental. cl., clavicular sac; cr.th., cranial thoracic sac; ca.th., caudal thoracic sac; ab., abdominal sac; d, dorsobronchi; p, parabronchi. The cervical air sac and the neopulmo are not indicated.

checked in *post-mortem* examination and documented photographically. Approximately 2–3 weeks after the second blocking procedure, cannulae were implanted under general anaesthesia into a carotid artery (cannula diameter 1 mm) and the clavicular air sac (cannula diameter 3–4 mm) of both the control and the experimental groups. The abdominal sac of the control birds was also cannulated immediately caudal to the base of the last rib, to which the cannula was tied. Approximately 50 i.u. of heparin was administered during the operation and the carotid cannula was flushed twice daily with heparinized saline. Post-operatively the experimental birds appeared healthy and were distinguishable from the control birds only in that they showed a slightly paler colour in the comb. They were able to run on the treadmill at 4.5 km h^{-1}, the speed used in the previous study, but could not maintain this pace for the necessary 10 min. Consequently, for the experimental runs the treadmill speed was lowered to 3.2 km h^{-1}.

Experimental methods and protocols

Experiments were begun after the animals had recovered for at least 24 h and

and in some cases 48 h. The techniques employed to measure blood and respiratory P_{O_2} and P_{CO_2}, oxygen consumption and ventilation have been described in a previous study (Brackenbury *et al.* 1989). Briefly, the birds wore a loose-fitting plastic mask from which a mixture of air and the expired gases could be drawn at a measured rate. Oxygen consumption was measured directly by analysing the concentration of oxygen in the collected gas stream. Ventilation was measured indirectly by monitoring the temperature and relative humidity of the collected gas which were then used to calculate expiratory water loss. From the latter value, ventilation could then be estimated assuming an expired gas temperature of 38°C. Data were collected in resting conditions and during treadmill exercise. To minimise discomfort to the animals, two identical sets of runs were performed, the first to monitor blood and air-sac gas tensions, the second to measure oxygen consumption and ventilation. Ventilation and oxygen consumption were averaged over the final 3 min of runs lasting 9–10 min. A blood sample was drawn anaerobically immediately after the run and was placed in the gas analyzer within 45 s. Preliminary trials showed that a more reliable indication of resting respiration could be obtained, not before a run, but 10–15 min after a run, by which time the birds had become relaxed and less liable to distraction from laboratory sounds and movements. Resting blood and air-sac gas tensions were therefore monitored 10–15 min into the post-exercise period.

All data are expressed as mean values ± S.E.M.. Differences between values obtained during rest and exercise were compared for significance at the 5 % level using the Student's *t*-test.

Results

In resting conditions the experimental birds showed a pronounced increase in arterial P_{CO_2} and decrease in P_{O_2} and a corresponding hypercapnia and hypoxia in the clavicular air-sac gas tensions (Table 1). Minute ventilation was not significantly different from that of the controls, but the respiratory rate was significantly higher, by 50 %, and the tidal volume was lower. During exercise, ventilation and oxygen consumption increased approximately threefold in both groups, although the experimental group appeared more fatigued by their efforts. Most of the increase in ventilation was due to an increased rate of breathing, together with, in the case of the experimental birds, a significant increase in tidal volume. There was no significant shift in arterial blood gas tensions compared to rest, and, in the case of the control birds, no change in either clavicular or abdominal sac gas tensions. The experimental birds suffered a significant 0.76 kPa increase in clavicular sac P_{CO_2} and a non-significant fall in P_{O_2} during exercise.

Discussion

Critique of experimental methods

A detailed critique of the method for estimating ventilation has been given

Table 1. *Respiratory characteristics of domestic fowl at rest and during exercise*

	Control		Experimental	
	Rest	Exercise	Rest	Exercise
Oxygen consumption ($mmol\,kg^{-1}\,min^{-1}$ STPD)	0.84±0.04	2.46±0.09*	0.79±0.04	2.29±0.04*
Ventilation ($l\,kg^{-1}\,min^{-1}$ BTPS)	0.32±0.03	0.93±0.06*	0.27±0.02	0.82±0.03*
Tidal volume ($ml\,kg^{-1}$ BTPS)	15.7±1.1	20.0±1.3	8.9±0.8	13.4±0.6*
Respiratory frequency (min^{-1})	20.2±1.1	47.5±2.6*	31.5±0.8	62.0±3.3*
Arterial P_{CO_2} (kPa)	4.2±0.09	4.0±0.13	6.2±0.52	6.6±0.47
Arterial P_{O_2} (kPa)	10.1±0.32	10.2±0.55	8.1±0.29	8.5±0.23
Clavicular sac P_{CO_2} (kPa)	5.08±0.13	5.12±0.15	7.09±0.15	7.85±0.24*
Clavicular sac P_{O_2} (kPa)	14.14±0.12	14.42±0.12	12.21±0.23	11.90±0.31
Abdominal sac P_{CO_2} (kPa)	2.68±0.12	2.37±0.13	NA	NA
Abdominal sac P_{O_2} (kPa)	16.82±0.17	17.52±0.24	NA	NA

Mean values±S.E.M.
Values marked by an asterisk are significantly different from rest.
NA, not applicable.

previously (Brackenbury *et al.* 1989). The method for blocking the air sacs relied on a vigorous but highly localized 'foreign body' reaction by the air-sac lining, which resulted in the implant becoming surrounded by fibrous tissue which fused it to the boundaries of the air sac and obliterated any small air spaces remaining between the pieces of cotton wool after the initial insertion. The foreign body reaction appeared to be complete within 2 weeks, and probably took place within the first few days of the operation. There were no other signs of pathological reaction either within the lung/air-sac system or in organs adjacent to the affected air sacs. Fortuitously, the foreign body reaction was particularly helpful in the case of the abdominal sac occlusions. It was only feasible to pack the cranial one-third or so of this sac during the initial operation, but *post-mortem* examination showed that the remainder of the air-sac wall collapsed around and adhered to the implant. In a few cases a small amount of tissue fluid remained associated with the implant but its weight was small compared to the total body weight and was ignored in the calculation of ventilation.

Control of ventilation and intrapulmonary airflow

In the previous study (Brackenbury *et al.* 1989) it was shown that occlusion of both pairs of thoracic air sacs has little effect on the respiration of domestic fowl either at rest or when the demand for oxygen is raised by treadmill exercise. According to figures given by King (1975), the combined volume of the thoracic air sacs in male chickens amounts to 25–30 % of the total lung/air-sac volume. In the present study a much larger fraction of the total volume, approximately 70 %, was

obliterated and this resulted in a pronounced effect on respiratory function. Despite having a similar minute volume to the controls both at rest and during exercise, the experimental birds were strongly hypoxaemic, indicating that there had been a fall in effective parabronchial ventilation. This could have been due either to a failure in inspiratory intrapulmonary airflow valving or to an increase in dead-space ventilation. Failure of the inspiratory valve would have resulted in inspired air entering the cranial sacs directly *via* their ventrobronchial connections, and this would have led to a lowering of clavicular sac P_{CO_2}, instead of the elevation that was actually observed (Table 1). It seems much more likely that hypoxaemia/hypercapnaemia resulted immediately from changes in breathing pattern: an increase in respiratory frequency would automatically increase anatomical dead-space ventilation. Since the total ventilation remained the same as in the controls, parabronchial ventilation must have been reduced, giving rise to the hypercapnia and hypoxia.

The relationships between respiratory pattern in the air-sac-blocked birds and blood and intrapulmonary gas tensions differ from those observed in other experimental situations. For instance, normal birds subjected to thermal stress also show rapid, shallow breathing but this is associated with and dependent upon a lowering of arterial and intrapulmonary P_{CO_2} (Barnas *et al.* 1981; Brackenbury and Gleeson, 1983). In contrast, arterial and clavicular air-sac P_{CO_2} values in the air-sac-blocked birds were abnormally high, owing to the attendant parabronchial hypoventilation. Experimental hypercapnia, induced by CO_2 inhalation, produces an increase, not a decrease, in tidal volume (Milsom *et al.* 1981; Tallman and Grodins, 1982). Hypoxaemia in fully conscious chickens breathing hypoxic gas mixtures may be associated with rapid, shallow breathing (Bouverot and Sebért, 1979), although in hypoxic, isocapnic conditions tidal volume does not diminish (Brackenbury, 1986). These instances indicate that the relationships between respiratory pattern and blood gas tensions are governed by different factors in the two types of experimental situation. In the first case (inhalation experiments), a change in blood gas tensions is the primary stimulus; in the second (air-sac blockage), hypercapnaemia and hypoxaemia are brought about secondarily by a primary change in ventilation.

Two further considerations should be taken into account when trying to understand the respiratory responses of the air-sac-blocked birds. First, the relationship between hypercapnia and breathing movements may have been upset by simultaneous effects of air-sac blockage on mechanoreceptor activity. Electrophysiological recordings from the vagus have revealed the presence of fibres which fire in phase with changes in air-sac volume, and which may originate from mechanosensitive receptors in the air-sac walls (Molony, 1974). A role for these non-CO_2-sensitive receptors in the control of avian breathing movements is becoming increasingly acknowledged and the current synthesis of this subject postulates a convergence of inputs from intrapulmonary chemoreceptors and extrapulmonary mechanoreceptors into the brain stem (Gleeson and Molony, 1989). Air-sac blockage would clearly interfere with the normal functioning of

mechanoreceptors located within the walls and would therefore alter the relationship between feedback from CO_2-sensitive receptors and respiratory drive. However, such an effect was not apparent in the previous study employing blockage of the thoracic sacs alone (Brackenbury *et al.* 1989), implying that any involvement of mechanoreceptors, if present, must be restricted to the abdominal air sacs.

The second consideration is that tidal volume in the air-sac-blocked birds may no longer be a reliable measure of rib-cage expansion and therefore of respiratory muscle power and respiratory drive. This results from the fact that 70% of the normal air-sac volume has been inactivated, forcing the remaining air sacs, principally the clavicular, to compensate by increasing their own tidal volumes. This can only be achieved by increasing the excursion of the rib-cage; consequently, even though the measured tidal volumes of the experimental birds were smaller than those of the controls, the tidal movements of the rib-cage may have been equal to or even greater than those of the controls. An interesting parallel to this situation has already been described in the literature: that of normal, unanaesthetized chickens, placed on their backs (Fedde, 1987). This inversion results in collapse of the abdominal air sacs, which are normally expanded under the influence of gravity. The result is that breathing accelerates, tidal volumes are reduced by as much as 50%, but the amplitude of sternal movements doubles. These changes in respiratory pattern coincide with increases in intrapulmonary, and presumably arterial, P_{CO_2}. Thus, the altered respiratory mechanics of the supine chicken leads to the same kind of anomalous relationships between breathing movements and gas tensions that were observed in the air-sac-blocked chickens.

It has long been argued that intrapulmonary airflow valving in the avian lung is governed by the geometrical configuration of the junctions of the mesobronchus with the ventrobronchus and dorsobronchus. There is also recent evidence that the efficiency of inspiratory valving is dependent on aerodynamic variables, mainly the gas velocity in the mesobronchus (Banzett *et al.* 1987; Wang *et al.* 1988). None of these factors is governed by the position, number or size of the individual air sacs connected to the lung. This study has demonstrated that inspiratory airflow valving is not dependent on the thoracic and abdominal air sacs, although a role for the clavicular air sac, which cannot be blocked using the present techniques (Brackenbury *et al.* 1989), is not discounted.

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