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THE MOVEMENT OF GAS IN THE RESPIRATORY SYSTEM OF THE DUCK

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INTRODUCTION

The avian respiratory system is structurally quite complex, composed of components which perform different functions in respiration. The lungs, where gas exchange with the blood occurs, are thought to be relatively constant-volume blocks of tissue composed of interconnecting passageways through which air is pumped. The thin-walled air sacs, which apparently do not participate significantly in gas exchange, act as bellows to move respired air through the passage-ways of the lung (see Fig. 1).

A simple description of the pathways of respired gases through this system has proven very difficult to obtain, although several hypotheses about the patterns of air flow have been proposed (Dotterweich, 1933, 1936; Vos, 1934; Zeuthen, 1942; Hazelhoff, 1951; Shepard *et al.* 1959; Cohn & Shannon, 1968).

Bretz & Schmidt-Nielsen (1970, 1971) developed a fundamentally new approach to the problem by constructing devices suitable for determining the instantaneous direction of air flow at various points in the avian respiratory system. Their determinations of air-flow directions permitted them to propose the patterns of air flow through the system shown in Fig. 1 B. These patterns are consistent with earlier experimental data obtained from studies of deposition of particulate matter (Dotterweich, 1930; Vos, 1934; Graham, 1939; Hazelhoff, 1951), analyses of gas composition (Dotterweich, 1933; Vos, 1934; Makowski, 1938; Scharnke, 1938; Graham, 1939; Zeuthen, 1942; Shepard *et al.* 1959; Cohn, Burke & Markesbery, 1963; Cohn & Shannon, 1968; Schmidt-Nielsen *et al.* 1969) and pressure measurements (Baer, 1896; Francois-Frank, 1906; Victorow, 1909; Cohn & Shannon, 1968; Schmidt-Nielsen *et al.* 1969).

Scheid & Piiper (1971) also devised a means of directly determining the instantaneous direction of air flow in the caudodorsal secondary bronchi of the avian lung. The results of their experiments are in agreement with those of Bretz & Schmidt-Nielsen (1970, 1971).

With the patterns of air flow shown in Fig. 1 B in mind, we wanted to examine the time of arrival of an inspired marker gas at various points in the respiratory system during the time course of a single respiratory cycle and the subsequent cycles. According to the patterns illustrated in Fig. 1 B, inspired gas should appear first in the more posterior air sacs (the abdominal and caudal thoracic sacs), then at a later time in the anterior sacs (the cranial thoracic, cervical and interclavicular sacs). This can be tested by continuously monitoring the air sacs for presence of the marker after the bird has inspired a single breath of the marker gas. If the response time of the monitoring

system is rapid enough, it should be possible to resolve small differences in the times of arrival of the marker gas in the various air sacs.

This approach has been employed in the ostrich (Schmidt-Nielsen *et al.* 1969), which respires slowly enough (≈ 6 cyc. min^{-1} at rest) to permit the use of oxygen as a marker and an oxygen electrode as the monitoring system.

For the present study we used ducks because Bretz & Schmidt-Nielsen (1971) obtained the data on which they based their proposed patterns from ducks and Scheid & Piiper (1971) also used ducks in their experiments.

The respiratory rate of a normally respiring unanaesthetized duck is so rapid(≈ 15 cyc. min^{-1}) that it requires the use of a monitoring system with a rapid response time (< 500 msec). Certain mass spectrometers meet this requirement.

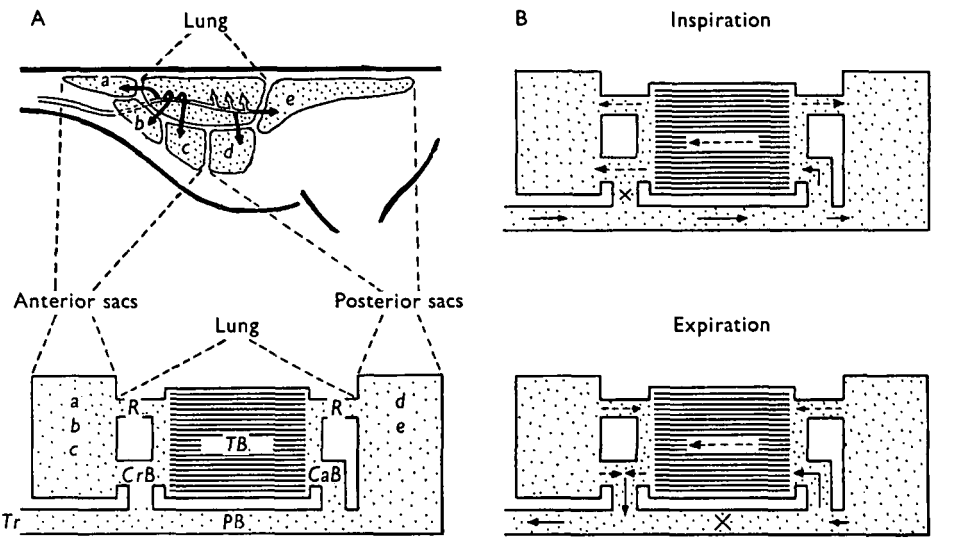

Fig. 1. (A) Diagrammatic view (top) and simplified schematic (bottom) of one lung and its associated air sacs. Anterior air sacs: *a* = cervical, *b* = interclavicular, *c* = cranial thoracic. Posterior air sacs: *d* = caudal thoracic, *e* = abdominal. The three anterior solid arrows indicate direct connexions between the primary bronchus and the anterior air sacs; the two posterior solid arrows indicate the direct connexions between the primary bronchus and the posterior air sacs; the three dorsally directed hollow arrows indicate caudo-dorsal secondary bronchi branching from the primary bronchus. Passage-ways: *Tr* = trachea, *PB* = primary bronchus, *CrB* = craniomedial secondary bronchi, *CaB* = caudo-dorsal secondary bronchi, *R* = recurrent connexions between air sacs and lung. *TB* = tertiary bronchi (represented by the heavy horizontal lines), which are the finest branches and form connexions between branches of *CrB* and *CaB*. (B) Proposed inspiratory and expiratory patterns of air flow in the avian lung. Arrows indicate air-flow directions; crosses indicate no flow. Solid symbols indicate experimentally determined directions; dashed arrows indicate inferred directions.

METHODS

Adult male mallard ducks (*Anas platyrhynchos*) weighing from 980 to 1490 g (mean, 1146 g) were used in this study. The birds were anaesthetized with 2·5 ml kg^{-1} Equithesin, I.M. injection. A simple Y-tube (Fig. 2) was inserted into the trachea in the mid-neck region to permit introduction of the marker gas. All birds were then allowed a minimum of 12 h to recover from the effects of the anaesthesia before the experiments.

During experiments the bird rested in a natural upright position in a cloth sling (Fig. 2). Polyethylene cannulae (20 cm long, 1·7 mm O.D.) were inserted into air sacs through 13-gauge hypodermic needles. At the end of the experiment the bird was painlessly killed and the position of each cannula was confirmed by dissection.

A differential pressure transducer (Sanborn Model 270) connected between one of the cannulated sacs and atmosphere (Fig. 2) was used to record air-sac pressure continuously during the course of an experiment. This pressure-recording was used to

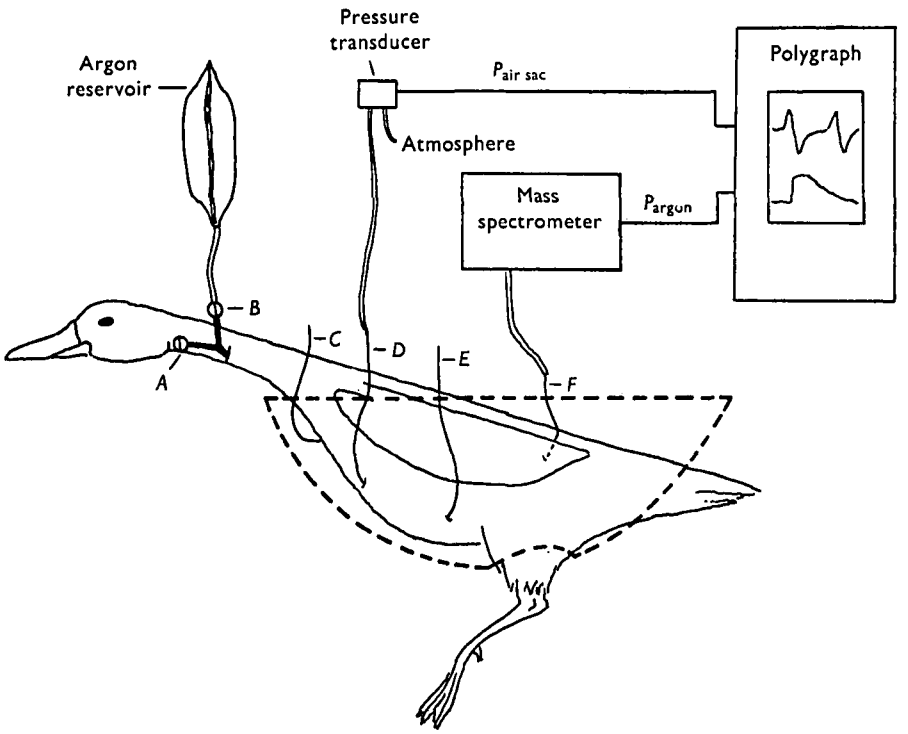

Fig. 2. Duck made ready for experiment. *A* = valve between Y-tube and atmosphere; *B* = valve between Y-tube and argon reservoir. Air-sac cannulae: *C* = interclavicular, *D* = cranial thoracic, *E* = caudal thoracic, *F* = abdominal. Dashed line indicates outline of a cloth sling used for support.

define precisely the initiation and duration of each phase of the respiratory cycle and thus provided the reference for determining the exact sequence of arrival times of the marker at the various sampling sites.

The bird was allowed to inhale the marker gas (100% argon) via the tracheal Y-tube cannula. During the marked inspiration the side of the Y-tube through which the bird normally respired atmospheric air was closed, while the side connected to a reservoir of argon was opened. The bird was then immediately returned to breathing atmospheric air. Opening and closing of the Y-tube arms was effected manually, and was easily synchronized by observing the instantaneous recording of air-sac pressure.

A Bendix time-of-flight mass spectrometer (Model Ma-1) equipped with an atmospheric sampling system was used to detect the partial pressure of argon in the cannulated air sacs (Fig. 2). Air was withdrawn continuously (mean sampling rate:

0·22 ml sec^{-1}) via the cannula of an air sac prior to, during, and following the administration of the marker, and was analysed for P_{argon} by the mass spectrometer.

The time course of marker appearance was determined for one sac over several successive tests (allowing complete washout of argon from the sac between trials); then a different sac was connected to the mass spectrometer and the process was repeated.

The time constant of the mass spectrometer for a change in P_{ar} of 0 to 100% was very rapid (300 msec to reach $1 - 1/e$ of the final value). The inlet system (consisting of the 20 cm polyethylene cannula plus 33 cm of 1·6 mm O.D. copper capillary tubing) introduced a time lag in the response of the mass spectrometer. This time lag was determined for each experiment on a different bird, and was constant for any given sampling rate. Calibrations of the sampling rates were made with Brooks Vol-U-Meter. Calibration for P_{ar} was based on only two values (0% and 100% argon).

Records were taken on a Sanborn polygraph (series 7700) using a carrier pre-amplifier (Model 350-1100C) for the differential pressure measurement, and a low-level d.c. pre-amplifier (Model 350-1500A) for the output of the mass spectrometer.

RESULTS

Fig. 3 is a series of recordings showing the arrival time of the marker gas in the interclavicular, cranial thoracic, caudal thoracic and abdominal air sacs. (The cervical sacs were never successfully cannulated by reason of their position and small size.) The mass-spectrometer recordings (bottom traces) have been shifted to the left appropriately to compensate for the time lag of the sampling system.

The marker arrived in the caudal thoracic and abdominal sacs (Fig. 3*a*, *b*) during the marked inspiration, the same inspiration during which it was administered, hereafter designated as the first inspiration (I_1). The magnitude of the increase of P_{ar} (ΔP_{ar}) from initial to final values during this inspiration indicates that the inspired gas flows directly to these sacs. The maximum ΔP_{ar} occurred during the marked inspiration (I_1), and during subsequent cycles of respiration there was a decline and gradual washout of the marker.

Table 1. *Changes in P_{ar} associated with a single inspiration of the marker*

(Cycle 0 includes inspiration and expiration prior to administration of marker; cycle 1 includes the marked inspiration and following expiration; cycles 2 and 3 include subsequent inspirations of atmospheric air and following expirations. 0 = P_{ar} prior to administration of marker; + = increasing P_{ar}; − = decreasing P_{ar}. Number of birds examined is in parentheses.)

Sampling site	Cycle 0	Cycle 1	Cycle 2	Cycle 3
Abdominal sac (6)	0	+	−	−
Caudal thoracic sac (5)	0	+	−	−
Cranial thoracic sac (5)	0	0*	+	−
Interclavicular sac (5)	0	0*	+	−

* In some experiments there was a small increase in P_{ar} due to sampling artifact (see Discussion).

The time of appearance of the marker gas in the cranial thoracic and interclavicular sacs (Fig. 3*c*, *d*) was different. Both of these sacs usually received a trace amount of the marker during the marked inspiration, but this was probably an artifact of the sampling technique (see Discussion). There was a substantial ΔP_{ar} in these sacs during the

second inspiration (I_2), and then during subsequent respiration P_{ar} decreased as washout progressed.

Table 1 summarizes the results, such as those shown in Fig. 3, from experiments on eight birds. In general, the posterior air sacs (the abdominal and caudal thoracic

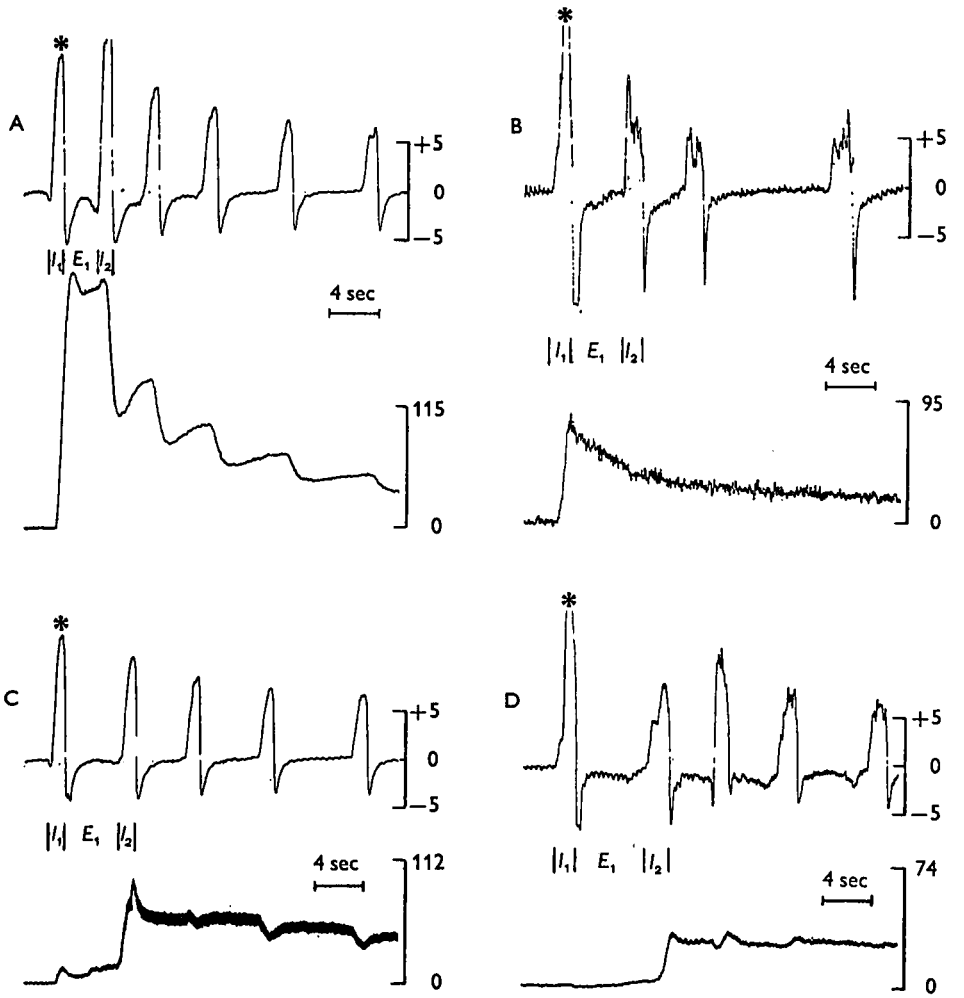

Fig. 3. Recordings of the market gas (argon) arrival in the air sacs. Air-sac pressures (top traces) and P_{ar} (bottom traces) are from different air sacs. (A) Air-sac pressure = interclavicular sac; P_{ar}^{1} = caudal thoracic sac. (B) Air-sac pressure = interclavicular sac; P_{ar} = abdominal sac. (C) Air-sac pressure = caudal thoracic sac; P_{ar} = cranial thoracic sac. (D) Air-sac pressure = abdominal sac; P_{ar} = interclavicular sac. I_1 = inspiration during which marker gas was inhaled. The recordings from the mass spectrometer (P_{ar}, bottom traces) have been shifted to the left to compensate for the time lag in the sampling system. Air-sac pressure expressed as mmH_2O; P_{ar} expressed as mmHg. Asterisk denotes inspiration of marker gas.

sacs) received the inspired marker during the respiratory cycle in which it was administered, while the marker appeared in the anterior sacs (the cranial thoracic and interclavicular sacs) during later cycles of respiration. The time of appearance and the rate of change of P_{ar} were quite constant for the posterior sacs, while there was more variability for the anterior sacs.

After the marker gas has arrived at an air sac, each subsequent inspiration of atmospheric air will reduce the P_{ar} in the air sac by dilution. The rate of decrease of P_{ar} in, or washout rate of argon from, an air sac is a direct function of the ventilation of that air sac. Table 2 compares the washout rates of argon from each cannulated sac, the rates being expressed as the number of inspirations required to reduce P_{ar} to 0·50 of the maximum P_{ar} attained in the same sac. The number of respiratory cycles required to reduce P_{ar} to this level is similar for posterior and anterior sacs.

Table 2. *Marker washout from air sacs, expressed as the number of inspirations required to reduce P_{ar} to one-half of maximum P_{ar}*

(Each entry is a mean value, followed by two standard errors of the mean. The number of observations for each entry is in parentheses.)

Sampling site	No. of inspirations	Respiratory rate (cyc. min^{-1})
Abdominal sac (16)	3·4 ± 1·2	10·3 ± 2·3
Caudal thoracic sac (18)	2·8 ± 1·0	11·3 ± 1·1
Cranial thoracic sac (11)	2·1 ± 0·8	9·3 ± 1·7
Interclavicular sac (7)	3·4 ± 1·4	8·3 ± 1·3

DISCUSSION

Previous related work

Experiments involving the inhalation or injection of a foreign gas mixture as a means of examining the nature of avian respiration have been conducted in the past. Early work done by Vos (1934), Scharnke (1938) and Zeuthen (1942) depended on chemical analyses of spot samples of gas withdrawn from the respiratory system by hand. Experiments based on this method, because of the time relationships involved, were not very successful as a means of exploring events as rapid as the distribution of a respired gas during a single respiratory cycle.

Shepard *et al.* (1959) pioneered the use of the mass spectrometer as a research tool in avian respiratory physiology. The results of their experiments permitted some general statements regarding the complex pattern of air flow and also demonstrated that in the chicken the anterior air sacs are ventilated equally as well as the posterior air sacs.

Scheid & Piiper (1969) made elegant use of the mass spectrometer to analyse the washout of a marker gas from the entire body (respiratory system, blood and tissues) of the chicken. They described a three-compartment system with different ventilation/volume ratios for each compartment, but did not seriously attempt to identify the theoretical compartments with specific anatomical components.

Schmidt-Nielsen *et al.* (1969) examined the arrival time of an inspired marker gas in the air sacs of the ostrich. The slow resting respiratory rate of the ostrich permitted the use of pure oxygen as a marker while its arrival at various sites in the respiratory system was monitored with an oxygen electrode. This study was the first attempt to study the distribution of respired gas over short time-intervals approaching a single respiratory cycle.

Discussion of present study

The present study examines the distribution of an inspired volume of gas in the respiratory system of the duck, phase by phase, over several successive respiratory cycles.

The sequence of arrival times of an inspired marker (Fig. 3; Table 1) indicates that the posterior air sacs are ventilated during a given inspiration (I_1) with a portion of the inspired volume of gas, while the anterior air sacs do not receive any significant portion of this inspired volume of gas. Then, during the second inspiration (I_2), a portion of the gas inspired during I_1 appears in the anterior sacs, while the posterior sacs received fresh air inspired during I_2.

The appearance of a small amount of the marker during I_1 in the cranial thoracic sac (Fig. 3*c*) and, to a lesser extent, in the interclavicular sac (Fig. 3*d*) is considered an artifact of the sampling technique. Gas was being continuously withdrawn for analysis at a rate of 0·22 ml sec^{-1}. The lung passage-ways and air-sac connexions are open and interconnected and contain no demonstrable valves.

If air is continuously withdrawn at some point in the system, it creates a region of relative low pressure thus causing a small artificial flow within the system. An indication that this occurred is that, if the sampling rate was changed, there was a proportional change in this low-level rate of appearance of the marker in the anterior air sacs during I_1 and E_1. Furthermore, the inspired marker arrived in a discrete front in the posterior sacs during I_1, and a similar discrete front arrived in the anterior sacs during I_2. The low-level trace appearance of the marker in the anterior sacs during I_1 cannot represent real ventilation of these sacs directly by gas inspired during I_1, considering the discrete arrival of the marker during I_2.

Zeuthen (1942) thought, on the basis of his experiments and calculations, that the anterior air sacs of the chicken and the duck are poorly ventilated, compared to the posterior sacs. The results of the experiments by Shepard *et al.* (1959) show that in the chicken the anterior air sacs are well ventilated, and the work by Schmidt-Nielsen *et al.* (1969) show that this is also the case for the ostrich.

The data presented in Table 2 of this paper show that in the duck the anterior air sacs are ventilated to an extent comparable to that of the posterior air sacs. However, it is clear from data on the composition of gas in the anterior air sacs and the direction of air flow in the lung passage-ways (see Bretz & Schmidt-Nielsen, 1971) that while the posterior sacs are ventilated by fresh inspired air, the anterior sacs are ventilated by air which has been elsewhere in the respiratory system prior to arrival in the anterior sacs.

On the basis of these experiments, the air-flow directions determined by Bretz & Schmidt-Nielsen (1970, 1971) and by Scheid & Piiper (1971), and from other experimental data (see Bretz & Schmidt-Nielsen, 1971), the movements of respired gas in the respiratory system of the duck appear to be as follows.

During the inspiratory phase (I_1) of a given respiratory cycle, the posterior air sacs (the caudal thoracic and abdominal sacs) receive fresh inspired gas. The anterior air sacs (the cranial thoracic and interclavicular sacs) do *not* receive any significant portion of the gas inspired during I_1, but are filled by gas from the lung.

During the expiratory phase (E_1) gas moves from the posterior air sacs into the lung

passage-ways, while gas from the anterior air sacs flows to the main bronchus, the trachea, and out.

During the second inspiration (I_2) fresh inspired gas again flows to the posterior sacs. The anterior sacs receive gas which entered the system on the preceding inhalation, was mixed with residual gas in the posterior sacs, and then was moved into the lung passage-ways (where it was exposed to gas exchange with the blood).

Thus the avian respiratory system appears to operate as a two-cycle pump. During inspiration air is drawn into the posterior air sacs; during expiration and the following inspiration this air is moved unidirectionally through the tertiary bronchi of the lung and into the anterior air sacs; and finally, this air is exhaled from the anterior air sacs during the second expiration.

The posterior air sacs can thus be considered as mixing chambers, functioning to maintain a gas mixture of relatively constant composition for flow over the exchange surfaces of the lung. The anterior air sacs can be considered as holding chambers which during inspiration receive the air moving over the exchange surfaces.

SUMMARY

1. A single inhalation of marker gas (argon) was administered to unanaesthetized ducks. P_{argon} was continuously monitored in the interclavicular, cranial thoracic, caudal thoracic and abdominal air sacs with a mass spectrometer during the marked inspiration and subsequent respiratory cycles.

2. The sequence of arrival times of the inspired marker at the various sampling sites was determined; ventilation rates of the different air sacs were compared by examining the rate of decrease of P_{ar} during washout of each sac.

3. The experimental results agree with previously proposed patterns of air flow in the duck respiratory system.

4. It is proposed that the movement of gas in the respiratory system of birds is a two-cycle event. During the inspiratory phase of the first cycle, inhaled air is drawn into the posterior air sacs; during the expiratory phase of the first cycle, this air (having mixed with the residual air in the posterior air sacs) is pumped into the secondary and tertiary bronchi of the lung; during the inspiratory phase of the second cycle this air in the lung passage-ways is drawn into the anterior air sacs; and finally, during the expiratory phase of the second cycle, this air is exhaled from the anterior air sacs and the respiratory system.

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REFERENCES

BAER, M. (1896). Beiträge zur Kenntnis der Anatomie und Physiologie der Athemswerkzeuge bei den Vögeln. *Z. wiss. Zool.* **61**, 420–98.

BRETZ, W. L. & SCHMIDT-NIELSEN, K. (1970). Patterns of air flow in the duck lung. *Fedn Proc.* **29**, 662.

BRETZ, W. L. & SCHMIDT-NIELSEN, K. (1971). Bird respiration: flow patterns in the duck lung. *J. exp. Biol.* **54**, 103–18.

COHN, J. E., BURKE, L. & MARKESBERY, H. (1963). Respiration in unanesthetized geese. *Fedn Proc.* **22**, 397.

COHN, J. E. & SHANNON, R. (1968). Respiration in unanesthetized geese. *Resp. Physiol.* **5**, 259–68.
DOTTERWEICH, H. (1930). Versuch über den Weg der Atemluft in der Vogellunge. *Z. vergl. Physiol.* **11**, 271–84.
DOTTERWEICH, H. (1933). Ein weiterer Beitrag zur Atmungsphysiologie der Vögel. *Z. vergl. Physiol.* **18**, 803–9.
DOTTERWEICH, H. (1936). Die Atmung der Vögel. *Z. vergl. Physiol.* **23**, 744–70.
FRANCOIS-FRANK, M. (1906). Études graphiques et photographiques de méchanique respiratorie comparée. *C. r. Séanc. Soc. Biol.* **61**, 174–6.
GRAHAM, J. D. P. (1939). The air stream in the lung of the fowl. *J. Physiol., Lond.* **97**, 133–7.
HAZELHOFF, E. H. (1951). Structure and function of the lung of birds. *Poult. Sci.* **30**, 3–10. (Reprinted from *Versl. gewone Vergad. Afd. Natuurk. K. ned. Akad. Wet.* **52**, 391–400, 1943.)
MAKOWSKI, J. (1938). Beitrag zur Klärung des Atmungsmechanismus der Vögel. *Pfluger's Arch. ges. Physiol.* **240**, 407–18.
SCHARNKE, H. (1938). Experimentelle Beiträge zur Kenntnis der Vogelatmung. *Z. vergl. Physiol.* **25**, 548–83.
SCHEID, P. & PIIPER, J. (1969). Volume, ventilation and compliance of the respiratory system in the domestic fowl. *Resp. Physiol.* **6**, 298–308.
SCHEID, P. & PIIPER, J. (1971). Direct measurement of the pathway of respired gas in duck lungs. *Resp. Physiol.* **11**, 308–14.
SCHMIDT-NIELSEN, K., KANWISHER, J., LASIEWSKI, R. C., COHN, J. E. & BRETZ, W. L. (1969). Temperature regulation and respiration in the ostrich. *Condor* **71**, 341–52.
SHEPARD, R. H., SLADEN, B. K., PETERSON, N. & ENNS, T. (1959). Path taken by gases through the respiratory system of the chicken. *J. appl. Physiol.* **14**, 733–5.
VICTOROW, C. (1909). Die kühlende Wirkung der Luftsäcke bei Vögeln. *Pflüger's Arch. ges. Physiol.* **126**, 300–22.
VOS, H. J. (1934). Über den Weg der Atemluft in der Entenlunge. *Z. vergl. Physiol.* **21**, 552–78.
ZEUTHEN, E. (1942). The ventilation of the respiratory tract in birds. *K. danske Vidensk. Selsk. Biol. Medd.* **17**, 1–51.