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THE DYNAMIC EFFECTS OF PULMONARY CO₂
ON TIDAL VOLUME IN A
UNIDIRECTIONAL VENTILATED AVIAN PREPARATION

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Robert Murray Weissberg, B.S.

The Ohio State University
1972

Approved by

[Signature]
Adviser
Department of Physiology
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PUBLICATIONS


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Exercise Physiology. Dr. Donald R. Mathews

Environmental Physiology. Drs. Harold S. Weiss, Edwin P. Hiatt, and Charles E. Billings

Application of Control Theory to Physiological System. Dr. Albert L. Kunz
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Here is Edward Bear, coming downstairs now bump, bump, bump, bump, on the back of his head, behind Christopher Robin. It is, as far as he knows, the only way of coming downstairs, but sometimes he feels that there really is another way, if only he could stop bumping for a moment and think of it.
INTRODUCTION

Carbon dioxide is normally produced in the tissues where it enters the capillaries and thence the venules, veins, and right heart. In the process of this confluence, its original point of entry loses its significance, and the mixed venous blood is recognizable only as having a load of CO₂. In passing through the lung, this blood, whatever its load, is processed in such a fashion that the issuing arterial blood has the uniformity of composition which we are accustomed to expect and which we include under the broad-family of regulatory actions called homeostasis. The problem which is of interest to respiratory physiologists is in the phrase "such a fashion," for in order to produce a constancy of arterial CO₂ tension, the ventilation must increase or decrease as a function of CO₂ production. How the respiratory system accomplishes this is an exciting problem that is only partially understood.
Steady-State Effects of CO₂. Most of the available information regarding the ventilatory response to CO₂ comes from observations made after prolonged periods of breathing CO₂. The arterial CO₂ tension or end expiratory CO₂ level is plotted against ventilation, and the slope of the resultant curve describes the "sensitivity" of the respiratory center. The graph that describes this relationship is represented by a constant slope over the physiological range of 30 to 50 mmHg (PaCO₂). However, some changes in ventilation that result from variation in PaCO₂ cannot be described by this linear relationship.

For example, the data from experiments during exercise (Grodins, 1950; Douglas and Haldane, 1909) show that while arterial pH, PCO₂ and PO₂ remain within normal bounds, dramatic increases in respiratory minute volume may occur. The theories advanced to explain the hyperpnea that develops with exertion compose a broad spectrum ranging from metabolites and hormones to temperature and irradiation from the cerebral cortex. Most of these factors have been adequately ruled out as the only cause of the hyperpnea of exercise, but none have been eliminated as one of the many causes.

The philosophy of control theory led Riley (1963) to suggest that the primary function of hyperpnea during exercise was to blow off CO₂. He postulated that this
was controlled by a servo-loop and showed that ventilation was linearly related to $P_{\text{vco}_2}$. He implied that there were chemoreceptors sampling mixed venous blood and relaying this information to the respiratory center. However, Heyman, Comroe and DeJours (1950) were unable to locate any chemoreceptors in the venous circulation or in the tissues.

Regulated Feedback System. Grodins (1954) described the respiratory system as a regulated system where arterial $PCO_2$ is the controlled quantity. The following is a brief description of Grodins' model (Figure 1). The complete regulator is composed of two sub-systems called the "controlling system" (respiratory center and pump) and the "controlled system" (lung-blood gas exchanger). The apparent goal of the respiratory system is to keep $PaCO_2$ (the controlled quantity) equal to or at least close to some described value (set point) despite disturbances produced by the body to satisfy different metabolic requirements. To accomplish this, the output of the controlled system ($PaCO_2$) is sensed, fed back, and compared to the desired value. The controlling system responds to the deviation from the set point by appropriately adjusting ventilation.
Figure 1. General Pattern of a Feedback Regulator
Dynamic Effects of \( \text{CO}_2 \). If \( \text{PaCO}_2 \) is the controlled quantity, does it provide a sufficient signal for its own homeostasis when it deviates from the set point? This is a crucial question that has not yet been resolved.

Yamamoto and Edwards (1960) infused rats intravenously with blood of high \( \text{CO}_2 \) content. The rats excreted the excess \( \text{CO}_2 \) by increasing ventilation in proportion to the infusion rate. During this process the animals were quiescent and showed no metabolic changes; therefore, they suggested that the effective signal for the regulation of arterial \( \text{CO}_2 \) is \( \text{CO}_2 \) itself. Dutton (1960) found that oscillations in \( \text{CO}_2 \) of the blood in the carotid arteries of dogs cause a greater elevation in ventilation than perfusing the same arteries with blood of higher mean \( \text{CO}_2 \) but without oscillations. This result, indicates that information coded in the oscillations of arterial \( \text{CO}_2 \) can affect ventilation. Dutton's experiments were tedious, could only be carried out for short periods of time, and were conducted on anesthetized animals (dogs).

A New Approach for Studying \( \text{CO}_2 \) as a Respiratory Stimulus.

An alternate method of studying the dynamics of respiratory control is to change rapidly the pulmonary gas concentration. The dead space and wash out volume of the mammalian lung make it difficult to systematically investigate and
analyze the dynamic effects of CO₂ on breathing movements. However, this objection does not apply to birds. These animals have lungs in which air flow can be made unidirectional. Air can be forced directly through the lung. This air passes the peripheral CO₂ receptors in the avian lung with apparently little frequency distortion (Osborne 1971). Therefore, the pulmonary gas concentration can be changed quickly and precisely.

History of Flow-Through Systems. Prior to 1968 observations of respiratory responses to variations in CO₂ were made on anesthetized birds that were restrained in dorsal recumbency (Burger and Lorenz, 1960; Fedde and Burger, 1962). Weissberg and Weiss (1968, unpublished) developed a flow-through preparation using an intact, awake and up-right bird. The carotid artery was cannulated, permitting not only continuous recording of blood pressure and heart rate, but also sampling of arterial blood.

These pilot studies showed that the preparation was functional, and that elevation of CO₂ in the flow-through gas increased respiratory frequency and tidal volume. Removal of CO₂ from the flow-through gas caused apnea. Blood samples drawn during different steady-state periods had PCO₂ and PO₂ values that corresponded to the gas concentrations of the flow-through mixtures. These
findings corroborated those of Ray (1966) who made extensive gas analyses measurements on blood drawn from anesthetized chickens.

Opening the Loop. Kunz, Morgan and Miller (1970) further modified the flow-through preparation developed by Weissberg and Weiss. The major advantage of their system was that air was forced through the system at 4-1/2 liters per minute. This high volume flow minimizes the effects of the amount of gases the chicken adds to or removes from the stream. The effect of this modification was to open the loop of the chicken's respiratory control, in that respiratory movements minimally change the concentration of the gases in the lung. Consider the closed information loop of the CO₂ receptor reflex as: $P_A^{CO_2}$ → CO₂ receptor → brain → respiratory movement → $P_A^{CO_2}$. This preparation opened the information loop at the point where respiratory movements affect $P_A^{CO_2}$ and, allowed independent forcing of the input CO₂.

Closing the Loop. Miller, Kunz, and Weissberg (1970) first described the results of an externally closed-loop preparation whereby the bird's breathing movements affected the input CO₂ through the use of an analog computer. This method uses the plethysmograph output to continuously set the level of input CO₂ via the
Algorithm
\[
\text{CO}_2(t) \propto \int (Q - KV_I) \, dt
\]
where \(Q\) is the analog metabolic \(\text{CO}_2\) production and \(V_I\) is inspiratory ventilation rate. This equation is continuously solved on-line by the analog computer. The pattern of \(\text{CO}_2\) produced (Figure 2) is that when the animal is not breathing, the \(\text{CO}_2\) drifts up with a slope equal to \(Q\). With each breath the \(\text{CO}_2\) is lowered by a decrement proportional to the inspiratory tidal volume. This externally closed-loop preparation seeks a stable steady state in which the pulmonary \(\text{CO}_2\) fluctuates about a constant mean value. The important considerations of this closed-loop preparation are as follows: 1) it helps preserve homeostatic mechanisms, such as those for \(\text{CO}_2\) and acid-base balance; 2) it allows comparison of the transfer function of the biological component in and out of the loop; and 3) it can provide a method of investigating whether the respiratory controller can learn or adapt.

The Avian Respiratory System. The respiratory center in birds is located in the medulla, as it is in mammals. Like the mammalian respiratory center, it is sensitive to

*Algorithm: A mathematical statement of how the bird's breathing movements were manipulated to produce some input \(\text{CO}_2\).*
Figure 2. Closed-loop Preparation

\[ \%CO_2(t) \propto \int [Q - k \dot{V}_T(t)] \, dt \]

In SS: Avg. \( \dot{V}_T = \frac{\dot{Q}}{k} \)

Slope Up \( \propto \dot{Q} \)

Down \( \propto V_T \)
changes in pH, temperature, and blood gas concentrations of CO₂ and O₂. Expired air of birds contains about the same amount of O₂ and CO₂ as that of mammals. Chemosensitivity of the avian lung was first demonstrated by Berger (1968). Peterson and Fedde (1968) confirmed that in birds the receptors sensitive to CO₂ are located in the lungs.

The avian respiratory system provides an excellent model for the dynamic study of the control of respiration. By taking advantage of the unique characteristic of the avian lung, it is hoped to achieve some insight into the causal relationships between pulmonary CO₂ (t) changes and "ventilation" on an informational basis. First we must know what happens before we can say where and how it happens.

PURPOSE

The purpose of Part I is to investigate how the bird regulates CO₂ via an algorithm that incorporates a trigger or threshold level in the external feedback loop. Here the effect of tidal volume is an all-or-none phenomenon, and frequency is the variable the animal can manipulate. This mode should speak specifically to the separation of the frequency and tidal volume controlling functions. Will the system regulate within reasonable limits or will
it show a Cheyne-Stokes type of ventilatory pattern? 
If they can regulate, over what range of metabolic loads?
What is the shape of the load response curve? Does a lowering of CO₂ trigger the onset of expiration?

METHODS

Unidirectional Artificial Ventilation. The experiments were performed on unanesthetized white Leghorn chickens about four months old and having a mean weight around 2.2 Kg. Under a local anesthetic (Xylocaine) a trachectomy was made in the lower half of the trachea. The bird was then placed on its side, and the short oblique part of the caudolateral process of the sternum was palpated. A small skin incision was made from this point and continued caudal to it. By use of blunt dissection, the oblique abdominal externus and internus were separated until entry was made into the posterior thoracic air sac. A one-half inch ID clear plastic tube with a light fixed to the distal end was inserted into the posterior sac to view the air sacs. Heat cauterization was used to perforate the membranes of the air sacs. A section of gum rubber tubing containing holes along its last 1-1/2 inches was then placed in the now confluent air sacs and sewn in place. This process was repeated on the chicken's other side.
This procedure permits air to be forced in sequence through the tracheotomy, lungs, air sacs, and exit tubes to the outside. The 1/4 inch internal diameter of the exit tubes offer much less resistance to air flow than the bird's respiratory tubules, and consequently the bellows-like movements of the air sacs are unable to blow air back through the lung. Air flow through the system was kept continuous at 4.5 liters per minute which is about nine times the resting ventilatory flow. This high flow of air through the chicken minimized the effect of the amount of CO₂ the chicken added to the stream (about 20 ml per minute, Figure 3).

**Experimental Set-Up.** Figure 4 is a schematic of the experimental set-up used during the course of this study. After surgery the chicken was placed in a whole-body plethysmograph, and the tracheal cannula was connected to the input gas tubing through which the gas mixtures entered the bird's respiratory system. The exit tubes were each connected to tubes that conducted the flow-through gas to the outside of the chamber.

At rest, the temperature differential between ambient air and the bird's expired air (37°C) is about 14°C. Since the bird is ventilated at about nine times its resting value, the flow-through gas was kept at 37°C (before entry into the bird) in order to produce rates of
Figure 3: Schematic of Bird's Respiratory System
Figure 4. Schematic of Experimental Set-Up
heat loss comparable to those produced by normal breathing. The air was humidified first, by bubbling it through a water bath kept at 35° C. Since the input gas mixture was 2° C higher than the air passed through the bath, the bird could lose normal amounts of water by further saturating the flow-through gas. Heating the flow-through gas was accomplished by flowing DC current through a nichrome wire (36 gauge) that ran the length of the flow-through tubing. Separate circuits allowed temperature control of both tracheal input gas mixture as well as the gas that passed through the exit tubes. The latter was important because it prevented water from condensing in the exit tubes.

The input CO₂ was controlled by an electropneumatic transducer (Honeywell) that converted an electrical signal into a corresponding CO₂ concentration signal. This CO₂ signal served as the input during the experiments. The electropneumatic transducer (EPT) had a rise time (time for step change to go from 10% to 90% of 0.3 seconds and an absolute time delay of 0.4 seconds.

Monitoring Devices and Calibration: Temperature. Four separate YSI thermisters (number 401) were used to measure the following temperatures:

1) tracheal
2) chamber (plethysmograph)
3) water bath
4) body.

The thermisters were connected to a YSI (model 8423) Telethermometer and direct visual readouts were made.

**Plethysmograph.** A Grass (Model PT-5) low volumetric pressure transducer electrically coupled the bird's respiratory movements both to the Grass Model 7 recorder and analog computer. The plethysmograph was calibrated by injecting a known volume of air into the chamber with a syringe. Under normal operating conditions the calibration was set so that 1 cm of pen deflection (on the Grass recorder) was equal to a volume change of 5 ml. A small calibrated leak in the chamber allowed for measurement of rate and volume of respiratory movements while eliminating the troublesome effects of temperature and pressure changes and of inboard gas leaks from the preparation. At 8 cycles per minute the amplitude is only attenuated 10 percent.

**CO₂ Recording.** The CO₂ concentration of the input gas mixture was continuously monitored by a Beckman LB-1 infrared CO₂ analyzer. The sampling flow rate for this was 500 ml per minute, drawn from the inflow tube just before it entered the chamber. A Beckman LB-1 Linearizer was connected between the LB-1 and the polygraph to provide a linear record of percent CO₂. Known gas mixtures
were preheated, humidified and passed through the system for calibrating the LB-1. Under normal conditions a 1 cm pen deflection was set to equal a one percent CO$_2$ change.

**Tracheal Pressure.** A Grass (PT-5) transducer was connected via a small polyethylene tube to the junction of where the tracheal cannula was connected to the input gas tubing. Since the flow-through gas rate was regulated, resistance changes of the bird's respiratory tract resulted in pressure fluctuations. The normal fluctuations fell between the range of 20 to 30 cm H$_2$O. Tracheal pressure was either directly recorded or read off a pre-calibrated voltmeter.

**Programming.** Control and signal manipulation was facilitated by the use of an analog computer (E.A.I.). The computer group 16-13 P is a general-purpose analog computer with forty amplifiers, and can be operated independently or coupled with other analog and digital equipment. Individual digital circuits (Schmitt triggers and monostable multivibrators) were added to the system. A 7 channel tape recorder (Data Tape CEC) was used in conjunction with the Grass recorder.

This experimental set-up permitted the following measurements to be recorded:
1) input CO₂ concentrations
2) frequency
3) tidal volume
4) inspiratory and expiratory flow
5) period between breaths
6) mean CO₂
7) tracheal pressure
8) temperature measurement

Diagnostic Criteria for the Quality of the Preparation.
The criteria used to judge the quality of the preparation was not entirely objective. Before the surgical procedure was performed, the normal resting ventilation was measured with the bird in the plethysmograph. If the bird's ventilation departed too much (10-20%) from the normal level, the preparation was considered poor. Another test measured the percent CO₂ in the exit tubes. If it fluctuated more than one half percent, this usually meant the tubes were improperly placed, and that the aeration of the lungs was poor. Tracheal pressure usually measured 20 cm H₂O. If the pressure increased above 30 cm H₂O, the trachea was aspirated to remove any clotted blood and/or mucous.

PROCEDURE

In the previously described feedback system, the animal can manipulate both frequency and tidal volume to
regulate CO₂. In contrast, this feedback system (Figure 5) uses a threshold or trigger level. This makes the effect of tidal volume an all-or-none phenomenon. Each suprathreshold (effective) tidal volume produced a fixed decrement (C) in the CO₂ signal while each subthreshold tidal volume produced no effect. Q is the analog of metabolic CO₂ production, and fₑ (effective frequency) is the number of breaths per minute which trigger CO₂ lowering. This algorithm permits simple and direct forcing of the bird's ventilatory response by parametrically changing Q, C, and threshold level.
Figure 5. Operational Schematic used for "Trigger" Experiments
A threshold level was effected by incorporating a Schmitt Trigger into the system. This is a bi-stable device that is either in the "on" or "off" state. The bird's breathing movements are coupled to this switching device via the electrical output of a whole-body plethysmograph. When the bird's inspiratory efforts generated an electrical voltage greater than or equal to a predetermined threshold level, the electrical output of the Schmitt Trigger increased from 0 to 5 volts. This voltage change served as the input into a monostable multivibrator. The integrated output of this device (a stereotyped pulse with a fixed amplitude and width) lowered the input CO$_2$ by a fixed amount. This triggering circuit makes the effect of tidal volume an all-or-none phenomenon. The pattern of CO$_2$ produced is that when the animal is not breathing or when its inspiratory tidal volume is below the threshold level, the CO$_2$ drifts up with a slope equal to Q, and with each effective breath the CO$_2$ is lowered by a constant decrement C.
RESULTS

**Frequency Regulation.** Figure 6 is a record of how the bird regulates CO₂ by frequency changes. The second tracing shows the plethysmograph signal; the peak-to-peak amplitude is tidal volume. In order for the bird to decrease the CO₂, it has to take an inspiratory tidal volume greater than or equal to the threshold level (20 ml). A synchronizing pulse marking the time at which inspiratory tidal volume crossed threshold was added to the plethysmograph record for ease of analysis and discussion. The top tracing shows the time course changes of pulmonary CO₂. Here every breath is greater than the threshold level and is therefore effective in decreasing CO₂. When Q (analog of metabolic load) is increased, a new steady state is reached with a corresponding increase in frequency.

**Intermittent Regulation.** Figure 7 shows the second mode of regulation. Here at a lower Q frequency does not slow down to the necessary rate for maintaining the steady state, but intermittently subthreshold breaths are taken. Pictured in this record are two separate runs. The trigger level was changed from 30 ml on the left to 20 ml on the right. Note that the CO₂ (top channel), though not as constant as before, still stays within reasonable limits.
Figure 6. Regulation of Input CO$_2$ by Frequency Changes
Figure 7. Regulation of Input CO₂ by Taking Subthreshold Breaths
The second channel is again tidal volume with a synchronizing pulse added marking the time when inspiratory tidal volume crossed threshold.

The tidal volumes of the effective breaths were so near the threshold level, that it was postulated that the onset of expiration was triggered by the lowering of CO₂. This made a certain amount of sense teleologically, in that the chicken apparently breathes to lower CO₂; when this is accomplished, further inhalation is unproductive.

The close coupling of tidal volume with threshold was also suggestive that feedback information might be coming from volume receptors in the lungs or stretch receptors in the respiratory musculature. However, Eaton, et al (1971) failed to demonstrate the presence of volume or stretch receptors in the respiratory system of chickens. He altered the intrapulmonary pressure (3 to 18 mmHg) at four points during the respiratory cycle (just before inspiration, during inspiration, at the end of inspiration, and during expiration) and produced little change from the normal rate. Likewise, releasing the pressure at corresponding points of the cycle did not alter respiration. Deflating the respiratory system with subatmospheric pressures (-5 to -10 mmHg) at various times during the respiratory cycle also produced no noticeable change in respiratory rate.
Continuously Changing Threshold Level. To test the hypothesis that expiration was triggered by CO₂ lowering, an experiment was designed whereby the threshold level would be continuously changed. Figure 8 is a section of a record from an experiment where threshold level was varied throughout. The lower channel depicts the trigger level as it changed from 10 ml to 30 ml and back again to 10 ml. In other words, the bird could lower the input CO₂ by taking a tidal volume of 10 ml at the beginning of the run, but half-way through it, an effective breath would have to be at least 30 ml. Channel two is again tidal volume with a sync pulse marking each effective breath. Channel one is percent CO₂.

A C Coupling of Input CO₂. The hypothesis that expiration was triggered by CO₂ lowering was further supported by the finding that tidal volume followed changes in threshold levels with little overshoot. However, changing the threshold level altered the mean CO₂ level as well. Therefore, it could be argued that tidal volume was determined by the CO₂ level rather than expiration being triggered by the lowering of CO₂. To decide between these two arguments feedback generated by the bird's tidal volume was A C coupled about a constant mean CO₂ (Figure 9). Under this system, when an effective breath was taken, a pulse was generated that lowered the CO₂ one percent for
Figure 8. Triggering the Lowering of CO₂ with Continuously Changing Threshold
Figure 9. AC Coupling at Different Threshold Levels
0.8 seconds. If CO₂ lowering triggers expiration, then lowering the threshold should decrease tidal volume.

In the first third of this record the threshold level was kept at 30 ml, the second third at 25 ml, and the last third at 15 ml. There are no concomitant changes in tidal volume. (For clarity the plethysmograph signal was duplicated on the lower channel without the synchronizing pulse). The hypothesis that expiration was triggered by CO₂ lowering was, in fact, only the bird's rapid response to changes in mean CO₂.

**Frequency Range vs. CO₂ Disturbances.** The points on graph in Figure 10 were taken from a section of record where the trigger level was kept constant, and Q (metabolic CO₂ production) varied. At higher values of Q regulation was accomplished by frequency changes alone. The range of frequency change spans about one octave. There was a concomitant increase in CO₂ and tidal volume with increasing Q over this range. At lower values of Q regulation was accomplished by interspersing ineffective breaths; the circles mark the frequency of effective breaths per minute. In other words, as Q in the algorithm \[ \% CO₂ \propto \int (Q - cf_e) \, dt \] decrease, \( f_e \) must decrease to maintain the steady state condition (\( Q/C = f_e \)). If it does not, \( \% CO₂ \) drifts up or down as the difference accumulates.
Figure 10. Frequency Range vs. CO₂ Disturbances

\[ \dot{Q}(\text{CO}_2 \text{ LOAD}) \]
DISCUSSION

This external feedback control of alveolar CO₂ via respiratory frequency showed that the system regulates its CO₂ concentration in two ways, by frequency modulations and by taking subthreshold breaths. It was also demonstrated that a pulse of low CO₂ air does not trigger the onset of expiration, and that there appears to be close coupling between CO₂ concentration and tidal volume, almost on a breath-by-breath basis.

The trigger algorithm used in these experiments changed the controlled system (lung-blood gas exchanger) from a linear to a nonlinear component. In the range where the bird regulated CO₂ by taking ineffective breaths the threshold level determined both the amplitude of the tidal volume and the CO₂ level around which the system fluctuated. For example, when the threshold level was increased, the CO₂ began to increase and the bird's tidal volumes reflected this by becoming deeper. Eventually a tidal volume greater than or equal to threshold was reached, and the input CO₂ was decreased a fixed amount. A new equilibrium results where both CO₂ and tidal volume have increased. Figure 11 shows the relationship between threshold level and tidal volume.
Figure 11. Tidal Volume as a Function of Threshold Level

\[ V_T \text{ (ml)} = \text{MEAN OF 20 } V_T \]
Close Coupling. How well the bird regulated about the threshold level was previously described by the term close coupling. Figure 12 is a typical record of intermittent regulation (here some breaths are ineffective in lowering CO₂). The top channel is percent CO₂, and the center channel is again a record of tidal volume changes. The lower channel is percent CO₂ at twice the sensitivity (1 cm = 1/2% CO₂). A spike marking the beginning of inspiration was added to CO₂ on the lower channel. The threshold level was kept constant at 20 ml.

A simple model which rationalizes the breathing pattern in Figure 12 is that if CO₂(t) is above a certain critical value at the beginning of inspiration, the tidal volume will be suprathreshold. Conversely if CO₂(t) is below that critical value at the beginning of inspiration, a subthreshold breath is taken. In Figure 12, the critical value which would minimize exceptions to this model is calculated to be 4.1 percent. Those tidal volumes with dots over them represent exceptions. The first two and last dots single out those tidal volumes that were effective even though the CO₂(t) was lower than 4.1 percent, while the third dot marks a tidal volume that should have been suprathreshold but was not.

These irregularities can be explained, at least in part, by the following: Firstly, even at a constant CO₂
Figure 12. Close Coupling
level, tidal volumes will show some variance. Secondly, the trigger level was set and measured with respect to the center line of the plethysmograph channel on the Grass recorder. The plethysmograph signal was floating in the sense that all inspiratory efforts did not start from the same point. Therefore, a large tidal volume beginning from a lower value might not reach the triggering level. Thirdly, as will be discussed in the Part II, fluctuations in CO₂ may also effect tidal volume.

One might expect the bird to have some difficulty in regulating CO₂ due to the nonlinear component introduced into the respiratory loop and the variability produced by the experimental design. However, the CO₂ level remained remarkably constant, almost as if the bird was regulating on a breath-by-breath basis. Fedde and Peterson (1970) showed that CO₂ sensitive receptors fire in phase with respiratory movements during spontaneous breathing, but when the bird was ventilated unidirectionally, the receptors fired continuously. Intrapulmonary receptors appear to be important in regulating respiration in birds, because respiration is profoundly depressed immediately after bilateral, cervical vagotomy. Death of these birds generally occurred within hours following this operation.

The bird's system anatomically and physiologically could accommodate breath-by-breath modulations of tidal
volume. The sampling rate of the levels of CO$_2$ should be much higher in birds than in mammals which utilize the vascular system to carry the carbon dioxide signal to the chemoreceptors. Osborne (Doctoral thesis, 1971) has shown, using single afferent neurons from chemoreceptors in the chicken lung, that the neurons are capable of following CO$_2$ oscillations of up to 160 c.p.m. His results also indicate that these receptors display both proportional and bi-directional rate sensitivity. Peterson and Fedde (1968) also showed that when CO$_2$ was abruptly removed from the ventilatory gas mixture, apnea began within 0.5 seconds. After a bi-lateral thoracic vagotomy, the apneic response time increased to 4.5 seconds.

Stability is dependent upon a short reaction time between CO$_2$ sensing and adjustment of tidal volume. This is almost certainly due to peripheral CO$_2$ receptors in the lung rather than central receptors in the brain or spinal fluid. Furthermore, the mechanism for this close coupling may involve CO$_2$ oscillations around the frequency of breathing. To investigate these possibilities, the studies in Part II were undertaken.
INTRODUCTION

Breath-to-Breath Control. The hypothesis that information stemming from peripheral receptors is important in maintaining ventilation on a breath-to-breath basis was postulated by Scott (1908) even before the discovery of the aortic chemoreceptors by Heymans and Heymans (1927). From studies of rabbits under chloral anesthesia, Scott showed that respiratory frequency did not increase in response to CO₂ after the vagi had been divided in the neck. He concluded that the depth of respiration is controlled by the tension of CO₂ in the blood and that the rhythm of respiration is controlled chiefly by impulses reaching the respiratory center along the vagi. There are a number of papers in the literature (Priban, 1965; Band, Cameron and Semple, 1969; Brisioe and Purves, 1967) whose results promote the thesis that impulses coming from peripheral chemoreceptors continuously modify respiratory period and tidal volume; the experiments of Black and Torrence (1971) and Kunz, Weissberg, and Miller (1971) have dramatically shown this, and have served as the impetus for the work discussed in this section.
Black and Torrence (1971) found that in anesthetized cats injections of saline equilibrated with CO$_2$, given in retrograde down the external carotid artery, caused an increase in the tidal volume of the breath taking place at the time. In order to produce this response, the injection had to be timed to reach the chemoreceptor during inspiration. If given early in an inspiration, it increased the depth of that inspiration, but if given late in an inspiration, it might not affect that inspiration. An injection of CO$_2$ saline given early in expiration prolonged the expiratory pause. These results illustrate a new feature of the problem of control: that the effect of a stimulus may depend upon its timing in relation to the respiratory cycle.

By carefully picking a frequency of input CO$_2$ oscillations near the chicken's normal respiratory rate, it was possible to pace the respiratory rhythm with CO$_2$ oscillations (the bird "breathes" with each CO$_2$ cycle). The range of frequencies over which the respiratory rate could be entrained was about two octaves (Kunz and Miller, 1971).

Kunz, Weissberg, and Miller (1971) have also shown that the bird can be paced by pulses of low CO$_2$. When these pulses were delayed, the chicken responded by increasing the period of his breath by the amount of that delay.
In all probability, the physical feature which determines this behavior of the system is that the respiratory pacemaker is a ring oscillator consisting of an information loop between the brain and a peripheral CO₂ receptor located in the lung (Kunz, personal communication). The main elements of the ring oscillator are 1) a center in the medulla, 2) mechanical system of the lung, 3) CO₂ receptors in the lung, and 4) the vagus nerve. The action pulse that passes from one element to another is described by the following sequence of events:

1) The brain "telling" the respiratory muscles to take a breath.
2) The breath causing lowering of CO₂ in the lung.
3) The lung receptors detecting the CO₂ changing and sending information up the vagus nerve to the brain, which determines the time of the next breath.

Under normal conditions the respiratory period equals the total time for an action signal to travel around the ring. In this manner CO₂ oscillations pace respiration.

This model implies that the pacemaker for ventilation is not anatomically located just in the brain stem, and that maintaining the integrity of the vagal input is important for fixing respiratory frequency and probably for regulation of CO₂. Marley and Stephenson (1967)
showed that respiration in chickens was depressed by halothane; the reduction of rate being more marked than that of amplitude. Vagal (central) stimulation restored respiratory rate to normal during halothane anesthesia, and further slowing of respiratory rate was not obtained with halothane in chronically vagotomized fowls, suggesting that respiratory slowing was due to an action of halothane on vagal pathways.

System Stability. Neither the mechanisms via which $CO_2$ modulates the bird's tidal volume nor the parameters that describe the relationship are completely understood. It is the thesis of this research that tidal volume, like frequency, is dependent on some pattern of fluctuations in the pulmonary $CO_2$ signal. A system where pulmonary $CO_2$ was sampled and fed back to affect tidal volume on a breath-to-breath basis, might enable the animal to regulate with greater precision than one where delays were present. (The delay implied here is the time it takes the blood to circulate from the lungs to the respiratory center). Milhorn and Guyton (1965) were able to produce an unstable breathing pattern in dogs (Cheyne-Stokes breathing) by artificially increasing the lung to brain circulation time. The bird with the chemoreceptor located in the lung is well suited for monitoring pulmonary $CO_2$. If information
residing in the dynamic behavior of CO₂ is important in maintaining CO₂ homeostasis, then sampling at the lung level should provide for greater resolution than sampling further down the line after mixing has occurred in the left heart.

Black and Torrence (1971) intermittently injected saline equilibrated with 100 percent CO₂ past the carotid body of cats at different times during the respiratory cycle. The cats' tidal volume was affected only if the CO₂ stimulus occurred during the first two-thirds of the respiratory cycle. Since there are oscillations in the pulmonary CO₂ during a respiratory cycle, it may be important to sample the CO₂ concentration at a fixed time during the cycle (intermittent sampling) to avoid these cyclic variations. Also, if derivative (slope) information is what is important to sample, it may be advantageous to sample only during a "time window" in the respiratory cycle. The up-slope of the pulmonary concentration signal should be proportional to the rate of CO₂ production. The ring oscillator may be important in synchronizing the sampling time with other events in a respiratory cycle.

If pulmonary CO₂ is sampled during a "time window", what kinds of information are sampled by the CO₂ receptor and how does it affect tidal volume? Is tidal volume
affected by the mean CO₂? Or is it affected by only the mean during the sampling window? Is it affected by the slope of the CO₂ signal during the window? Is it the average slope or the maximum slope? How long is the window? What event is it synchronized with? Inspiratory movements? Expiratory movements? Or some event in the CO₂ signal? Is tidal volume information sampled at a different point in time than frequency information?

PURPOSE

The purpose of this section is to show that tidal volume is affected not only by mean CO₂, but also by such factors as how fast the CO₂ is increasing or decreasing, and/or when in the cycle these changes occur.

METHODS AND PROCEDURES

The instrumentation and methods used in these experiments are the same as in the closed-loop chicken preparation described in Part I. Only the feedback algorithm has been changed. To investigate the effects of dynamic changes in CO₂ on tidal volume, AC coupling was used in the feedback loop. This mode of coupling permitted oscillatory information to be fed back into the system while filtering out changes in the mean CO₂.
The first algorithm used the plethysmograph output to produce the pattern of the CO₂ oscillations on the input signal. Mean CO₂ (measured over 2 or 3 cycles) was held constant at about 4 percent. In the second algorithm a 2 percent CO₂ step was sequentially moved through a respiratory cycle. Again the mean CO₂ was held constant at about 4 percent. Tidal volume was then plotted as a function of where in the respiratory cycle the step occurred.

RESULTS

AC Coupling Via Tidal Volume. This record (Figure 13) contrasts tidal volume with and without CO₂ oscillations. The top channel is the CO₂ concentration of the gas being blown through the chicken. The lower tracing is the recording of the volume changes of the bird as measured by the whole-body plethysmograph. The mean CO₂ is predetermined, and, in this run, is kept constant at 4 percent both in the oscillatory and non-oscillatory modes. The feedback is of the bird's tidal volume and not the mean CO₂ level. A delay in the feedback loop increases the amount of time it takes the breathing movements to effect the input CO₂.

The first arrow marks the time when the preparation was changed from the oscillatory to the non-oscillatory
Figure 13. AC Coupling via Tidal Volume
mode. Both tidal volume and period increased. Note, however, that this change is not instantaneous; it takes three or four breaths to reach a new steady state. Conversely, when the oscillations were restarted (second arrow), the bird's tidal volume decreased while its breathing rate increased. Apparently mean CO₂ is not the only factor that affects the bird's breathing movements.

**Self-Paced Variable Upstep.** Figure 14 is a typical record showing how the bird's breathing movements were AC coupled to the input CO₂ by a different feedback arrangement. When the bird inspires, the CO₂ is depressed by 2 percent (closed-loop pacing) and stays at that level for a predetermined amount of time (top channel). The CO₂ then returns to the baseline (upstep) and remains at this level until the cycle is repeated with the next inspiration. (s represents the time from CO₂ lowering to the beginning of the upstep). The third channel is the mean CO₂ (filtered output of the first channel with the sensitivity increased by a factor of four). The lower channel is the plethysmograph record with a synchronizing pulse added to it marking the time when the upstep appears in the respiratory cycle. Note that in the section on the left the upstep comes during the latter part of
Figure 14. Self-Paced Variable Upstep
inspiration, while on the right it occurs late in expiration. The upstep was slowly moved through the cycle in time increments of about 0.2 seconds. The event marked on the time scale records when these increases took place.

Some of the experiments involved observing the response to a sudden change in where the upstep comes in the respiratory cycle. Figure 15 shows how this was accomplished. The top channel is percent CO₂, the middle channel is the plethysmograph recording, and the lower channel is again the plethysmograph recording with a sync pulse added marking the time when the upstep appears in the respiratory period. In Figure 14 the upstep was advanced into the cycle by a small (0.2 second) increment; however, in this record (Figure 15) the time of the upstep was changed by 1.5 seconds. Note the difference in tidal volume as a function of where in the cycle the upstep comes. (The tidal volumes in this record are smaller when the upstep comes in the expiratory portion of the breath; where s equals 1.8 seconds). This effect on tidal volume is not an instantaneous one. It takes three or four breaths before tidal volume changes are apparent. It was difficult to analyze the tidal volumes in periods of transition, because changing the width of
Figure 15. Rapid Forcing of Upstep
the step caused transient deviations in CO₂. The time course of these transients were dependent on the RC time constant used for AC coupling.

By plotting tidal volume as a function of where in the cycle the upstep occurs (s/period), it was hoped to delineate the type of CO₂ stimuli the system was responsive to and where in the period it displays this sensitivity. That tidal volume responded to more than just mean CO₂ was demonstrated in the experiments where the CO₂ step was systematically advanced through the bird's respiratory period. The data from these experiments show that a step increase in CO₂ has an effect on tidal volume, and this effect is a function of when in the cycle the upstep appears. The graph in Figure 16 represents a typical stimulus-response curve of how an increasing upstep affects the bird's tidal volume as it moves through a respiratory period. It was not possible to produce upsteps at the very beginning or very end of a respiratory cycle.

Polarity Change. When the step was changed so that the step moved from a higher CO₂ to a lower CO₂ (downstep), the bird's ventilatory pattern changed abruptly. Figure 17 shows a typical record of the effects of this polarity change. The top channel is percent CO₂, and the center channel is tidal volume. The lower channel is an index
Figure 16. Tidal Volume vs. Timing of Upstep
Figure 17. Self-Paced Variable Downstep
of the bird's respiratory period; and increasing amplitude of the saw-tooth pattern represents an increasing respiratory period. In the first quarter of the record, as before, the CO$_2$ decreases on inspiration, and an up-step occurs in $s$ amount of time later. The arrow marks the time when the polarity changed. Now CO$_2$ increased when the bird inspires, and a downstep comes down later in the cycle as a function of $s$. This mode caused the bird's tidal volume to decrease and the respiratory period to increase. The respiratory pattern varied so much with changes in $s$ that careful and systematic moving of the downstep into the cycle did not appear to be practical. When the polarity was changed back so that CO$_2$ was lowered by inspiration, the bird's ventilatory rate and tidal volume increased and the respiratory pattern stabilized.

DISCUSSION

The results of these experiments support the hypothesis that the dynamic behavior of pulmonary CO$_2$ can affect tidal volume. Apparently, there are two frequency bands of CO$_2$ oscillations to which the chickens' respiratory system is sensitive. One is the very slow changes which we call the mean CO$_2$, and the other is around the frequency of breathing. To investigate the effects of the latter, experiments were designed whereby the bird's breathing movements were AC coupled to the
input CO₂. This permitted higher frequency oscillation information to be fed back into the system while filtering out the low frequency changes in mean CO₂.

**Tidal Volume Feedback.** In the experiments where the bird's tidal volume was fed back to affect the input CO₂ (Figure 13), the results showed that CO₂ oscillations can influence tidal volume. Black and Torrence (1971) suggested that the phase relationship between oscillations in chemoreceptor discharge at the frequency of lung ventilation and the discharge of the respiratory center was of importance in determining ventilation in exercise.

Initially, to examine the effects of the phase relationship between tidal volume and CO₂ oscillations, the plethysmograph signal was delayed. Analysis of the raw data was difficult because as the delay was increased, there were attendant changes in both the rate and depth of breathing. Furthermore, the shape of the expiratory portion of the breath became irregular, and this produced fluctuations in the input CO₂ signal. Therefore, the shape of the CO₂ signal changed with delays. These variations made it difficult to assess cause and effect relationships.

The step experiments were designed to circumvent some of these problems (Figure 14). This feedback
arrangement permitted finer control over the shape of the CO₂ stimulus between breaths. By moving the time of the upstep into the cycle, it was hoped to find the type of dynamic information the system is responsive to and when in the cycle this sensitivity occurs. Figure 16 shows that tidal volume is a function of when in the cycle the upstep appears. How might the CO₂ step exert an influence on the neural mechanism of ventilation? The results reported do not indicate the exact nature of the mechanism responsible for this phenomenon. However, three models will be discussed that could explain the data in Figure 16:

1) Mean sampling during a fixed part of the respiratory cycle
2) Derivative sampling during a fixed part of the respiratory cycle
3) The clipping effect due to the nonlinearity of the CO₂ receptor

**Mean-Sampling Hypothesis.** This assumes that pulmonary CO₂ is sampled over a definite period ("window") each respiration, and the mean CO₂ during that "window" is what is being sensed. Figure 18 A, B, and C illustrate a CO₂ upstep occurring at three different times in the respiratory cycle. This sampling period (window) is labeled W in this illustration. It "sees" different
Figure 18. Intermittent Sampling of Pulmonary $\text{CO}_2$
levels of CO₂ depending on where in the cycle the upstep comes. In 18 A the mean CO₂ during the sampling period W would be greater than in C. When, as in 18 B, the upstep comes during the sampling period, the mean CO₂ sampled will depend on how early in the "window" the step change appears.

Figure 18 D illustrates how tidal volume would vary with s if it were dependent upon mean CO₂ sampled during the "window". As the step was advanced from early in the cycle, the window would be exposed to a higher CO₂, and tidal volume would be expected to increase until the step reached the front edge of the window. As the step moved through the window, the apparent CO₂ would decrease until it reached the back edge of the window. Therefore, the sampling period would be ascertained from where the tidal volume reached a maximum and minimum value. When the step no longer appeared in the window, the CO₂ sampled would once again increase.

The data from a typical step experiment (Figure 16) show only a minimum value. This result could be explained by the following:

a) The sampling of pulmonary CO₂ starts after CO₂ passes through a maximum (when the CO₂ begins to decrease).
b) The sampling period lasts for a fixed period of time.

c) The integration of vagal discharge frequency reflects the mean CO₂ level sampled during the "window."

d) An increase in the integrated discharge frequency inhibits tidal volume.

Tidal volume would decrease as the upstep was moved through the window, because the mean CO₂ sampled would decrease due to the effects of AC coupling. However, after the upstep passes by the window, the mean CO₂ sampled will begin to increase as the upstep is moved later into the cycle. The end of the sampling period would coincide with an increasing tidal volume (mid-expiration).

This model could, in part, explain the gross effects on the bird's breathing movements when the polarity is changed (Figure 17). When the bird inspires, the CO₂ is displaced to a higher CO₂ level, remains there for s amount of time, and then returns to the base level. In this mode the sampling period will "see" a low CO₂ level, particularly when s is at a minimum. The discharge frequency of the CO₂ receptor would increase, and this effect on breathing movements would be inhibitory.
Eventually the sampling period would be exposed to a constant mean CO$_2$ level, and a steady state ensues.

Eaton (1971) failed to demonstrate the presence of stretch receptors in the respiratory system of chickens. Therefore, it seems reasonable to suggest that sampling is synchronized to pulmonary CO$_2$, rather than to inspiratory or expiratory movements. Figure 19 shows the average stimulus-response relationship between CO$_2$ and percent maximal discharge frequency of a single CO$_2$ receptor (Osborne, 1971). Osborne suggests that an increasing discharge frequency exerts an inhibitory influence on breathing movements, since low CO$_2$ produces decreased ventilation.

This interpretation of the data is quite speculative, and additional experimental evidence is needed. An experiment that could help substantiate this model would involve delaying the triggering of the upstep so that it comes later in the respiratory cycle. If sampling of pulmonary CO$_2$ is synchronized to when CO$_2$ passes through a maximum, then the shape of the curve in Figure 16 should remain the same, but shifted to the right. How much it shifts would be a function of where in the cycle CO$_2$ lowering occurred.

**Derivative-Sampling Hypothesis.** Figure 18 E illustrates how tidal volume would vary with s if it were dependent
Figure 19. Avian (pulmonary) Chemoreceptor CO₂ Response
upon derivative \( \frac{d\text{CO}_2}{dt} \) information sampling during the "window" labeled W. Tidal volume would only deviate from the normal level when the CO\(_2\) is increasing or decreasing during the window, as in Figure 18 B. While the mean CO\(_2\) during the sampling period (W) in Figure 18 A and C has changed, the CO\(_2\) level remains constant during the window; therefore, it would not be sensed by the system.

The plot of the experimental data (Figure 16) resembles the theoretical curve for derivative sampling (Figure 18 E), except the effect of an increasing upstep appears to be inhibitory. This interpretation implies that the chemoreceptors are capable of sensing rapid changes in pulmonary CO\(_2\), coding it as discharge frequency, and transmitting this information to higher centers. Osborne (1971) has shown that most pulmonary afferent neurons respond to both the rate of change as well as the static level of CO\(_2\). An "off" response (lowering of CO\(_2\)) is characterized by a high frequency onset transient (overshoot) which is a function of the rate of decrease in F\(_1\)CO\(_2\). The "on" response is characterized by low frequency onset transients (undershoot), which then increase to a more or less steady rate of discharge determined by the fractional carbon dioxide content of the ventilatory gas. The amplitude of the overshoot or undershoot is dependent on the magnitude of the step changes in F\(_1\)CO\(_2\).
If derivative information was sensed, then the sampling period should be delineated by where in the cycle abrupt changes in tidal volume appeared. The curve generated by plotting tidal volume as a function of s/p shows that the maximum effect of the upstep occurred in the mid-portion of expiration. The length of the sampling period is difficult to assess due to the inadequate resolution of the data.

This lack of resolution could be explained by the following. Firstly, lowering of CO₂ was triggered off inspiration (in the upstep mode), but since there was an absolute time delay of 0.4 seconds in the electropneumatic transducer, the first two-thirds of inspiration was not exposed to the upstep stimulus. If part of the sampling period was located in this section of the cycle, then the exact length of the window would remain ambiguous. Secondly, even in the absence of precise information, it is reasonable to assume that the bird's pulmonary structure represents a low-pass filtering system of two wash-out compartments: the air volume of the lung (about 6.4 ml/Hg—Dunker, 1972; Burton and Smith, 1968; and King and Payne, 1967) and the intracellular fluid space that surround the CO₂ receptor. The CO₂ step has high frequency components associated with it, particularly when s came very early or late in the cycle. As the CO₂ step passed through the bird's lung, the filtering effect could further round off and attenuate
the amplitude of the CO$_2$ step. Therefore, the intrapulmonary CO$_2$ receptor would be exposed to different peak CO$_2$ concentrations. Osborne's (1971) data on phase angle versus frequency supports the contention that the lung acts as a low-pass filter. This filtering effect of the lung and the rounding-off of the input CO$_2$ step by the electropneumatic transducer would tend to give the illusion of a wider sampling period.

If the lung does filter the input CO$_2$ "signal," then changing the rate of the flow-through gases while keeping the CO$_2$ stimuli uniform, should result in different effects on tidal volume. Manipulating both the amplitude and the slope of the CO$_2$ step, particularly when the upstep appears in the mid-portion of expiration, could provide additional information that would help resolve the question of whether or not the rate of change of CO$_2$ is a factor in determining tidal volume.

**Nonlinearity of the CO$_2$ Receptor (Clipping Hypothesis).** Another possible explanation for the shape of the curve in Figure 16 might involve the nonlinear output of the intrapulmonary CO$_2$ receptor. Figure 19 shows the average stimulus-response relationship between CO$_2$ and percent
maximal discharge frequency of a single CO₂ receptor (Osborne, 1971). Osborne suggests that an increasing discharge frequency exerts an inhibitory influence on breathing movements, since low CO₂ produces decreased ventilation. The clipping hypothesis approximates that below 4 percent this receptor response is roughly linear to percent CO₂, and above the cut-off value of 4 percent, little further decrease in discharge frequency occurs.

How this clipping property of the CO₂ sensor could influence tidal volume must be considered in terms of what AC coupling does to the CO₂ input used in these experiments. Figure 20 A, B, and C shows how AC coupling affects the input CO₂ signal as the upstep is moved through a respiratory cycle. Although the step height (h) remains constant as s is increased, the step starts and ends at a higher CO₂ level. This is necessarily so, because if the mean CO₂ is to remain constant, the total area above the dashed line (mean CO₂) must be equal to the area below it. The amount the AC coupled step would be clipped would, therefore, be dependent upon s.

The amount of area clipped as the CO₂ step is moved into the cycle is described by equation (1)*

*Derivation of equations appears in Appendix 1
\[ A + \left(-\frac{h}{p}\right)s^2 + (h + d)s - dp \quad \text{Eq.}(1) \]

A = area clipped

\( d \) = the difference between the mean value of the input \( \text{CO}_2 \) and the cut-off value

\( p \) = the respiratory period

\( h \) = the height of the \( \text{CO}_2 \) step

\( s \) = the time from \( \text{CO}_2 \) lowering to the beginning of the upstep

The graph of this quadratic equation is a parabola opened downward. The time in the period the amount of area clipped is at a maximum is described by equation (2):

\[ \frac{s}{p} = \frac{1}{2} + \frac{d}{2h} \quad \text{Eq.}(2) \]

Figure 20 D illustrates how the area clipped (normalized by \( p \)) varies for two values of \( d \) (zero and \( 1/2 \) \( h \)). As \( d \) increases, the total amount of area clipped is reduced, and the maximal value occurs later in the cycle. At times, the entire step will fall below the cut-off value of the \( \text{CO}_2 \) receptor as in Figure 19 A. When this happens, the area clipped equals zero, and the mean discharge frequency will be determined by the input mean \( \text{CO}_2 \). The mean discharge frequency can be obtained from equation (3):

\[ \text{mean discharge frequency} = K(\text{mean } \text{CO}_2 - \frac{A}{p}) \quad \text{Eq.}(3) \]
\( \bar{CO}_2 \) = mean value of the input \( CO_2 \)

\( A \) = the amount of area clipped

\( p \) = the respiratory period

It is the increasing of mean discharge frequency that produces smaller tidal volumes. The curves generated by plotting discharge frequency as a function of s/p for two values of d (zero and 1/2 h) are illustrated in Figure 20 E.

In most of the step experiments, the mean value of the input \( CO_2 \) was kept close to the cut-off value (4%) of the \( CO_2 \) receptor; therefore, d would be close to zero. The amount of area clipped and the mean discharge frequency as the step is moved into the cycle would be similar to Figures 20 D and E (d equal zero). The clipping hypothesis suggests that tidal volume varies inversely with discharge frequency; therefore, tidal volume vs. s/p should be the inverse of Figure 20 E. The graph of the actual data (Figure 16) resembles the predicted curve.

SUMMARY

The chicken was chosen as an experimental animal because it is necessary to change the \( CO_2 \) gas concentration in the lungs quickly to study the dynamic effects of \( CO_2 \) on breathing movements. This study used a unidirectional ventilated avian preparation with a computer in the
feedback element. This externally closed-loop configuration permits the experimenter to interact in the \( \text{CO}_2 \) information loop of the bird's respiratory system. Using the computer to change one variable while holding the others constant allows the experimenter to informationally dissect the bird's control system.

The first algorithm investigated made the effect of depth of breathing an all-or-none phenomenon. This revealed the following:

1) There was a range of \( Q \) (analog of \( \text{CO}_2 \) production) over which the bird could frequency modulate to obtain a steady state \( \text{CO}_2 \).

2) Above this range a stable \( \text{CO}_2 \) could not be maintained.

3) Below this range a second mechanism was utilized; that of taking ineffective, subthreshold breaths.

4) Stability was dependent upon a short reaction time between \( \text{CO}_2 \) changes and adjustment of tidal volume and frequency. This is almost certainly due to peripheral \( \text{CO}_2 \) receptors in the lung rather than central receptors in the brain or spinal fluid.

To investigate the effects of \( \text{CO}_2 \) oscillations around the bird's frequency of breathing, AC coupling was used. This
mode of coupling permitted oscillatory information to be fed back into the system while filtering out changes in mean CO\textsubscript{2}. This technique revealed the following:

1) Comparisons between an oscillatory and non-oscillatory CO\textsubscript{2} signal (at the same mean CO\textsubscript{2}) showed both tidal volume and period decreased as a result of the CO\textsubscript{2} oscillations.

2) When a 2 percent (decreasing) step was moved through a respiratory cycle, the bird's tidal volume decreased and its respiratory period increased. The respiratory pattern varied so much with step changes, that careful and systematic moving of the downstep into the cycle did not appear to be practical.

3) When a 2 percent increasing step was moved through a respiratory cycle, tidal volume varied with where in the cycle the upstep occurred. The maximum effect on tidal volume resulted when the upstep came in mid-expiration. Three models were presented that explained this phenomenon:
   a) Derivative sampling of CO\textsubscript{2} during a fixed part of the respiratory cycle.
   b) Sampling of mean CO\textsubscript{2} during a fixed part of the respiratory cycle.
c) The clipping effect due to a nonlinear property of the CO₂ receptor.
Figure 21 represents an idealized CO\textsubscript{2} upstep. AC coupling maintains a constant mean CO\textsubscript{2} (dashed line) as s is moved into the respiratory period (p). Therefore, the area above the dashed line must equal the area below it.

\[ A_1 = A_2 \]  
\text{Eq. (1)}

The amount of area clipped as the CO\textsubscript{2} step is moved into the cycle \( A_3 \) is described by:

\[ A_3 = (p - s)(Z - d) \]  
\text{Eq. (2)}

Z can be described in terms of s, h, and p by the following equality:

\[ sh = Zp \]  
\text{Eq. (3)}
Dividing equation (3) by $p$,
\[ Z = \frac{sh}{p} \quad \text{Eq.}(4) \]
and substituting equation (4) into equation (2), one obtains
\[ A_3 = (p - s) (\frac{s[h/p]}{p} - d) \quad \text{Eq.}(5) \]
For simplicity, equation (5) is stated in the following form:
\[ A_3 = (-\frac{h}{p})s^2 + (h + d)s - Pd \quad \text{Eq.}(6) \]
Equation (6) is a parabola open downward. The time in the period the amount of area clipped is at a maximum is found by differentiating equation (6) with respect to $s$,
\[ \frac{dA_3}{ds} = \frac{-2 sh + h + d}{p} \quad \text{Eq.}(7) \]
and setting equation (7) equal to zero
\[ -2sh/p + h + d = 0 \quad \text{Eq.}(8) \]
For simplicity, equation (8) is stated in the following form:
\[ s/p = 1/2 + d/2h \quad \text{Eq.}(9) \]
Note that as $d$ increases (equation 8) the maximum amount of area clipped appears later in the cycle.
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