BLOOD GAS CHANGES ASSOCIATED WITH RESPIRATORY ALTERATIONS FOLLOWING BILATERAL VAGOTOMY IN GALLUS DOMESTICUS

by

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INTRODUCTION

Although studies of the effects of bilateral vagotomy in the chicken extend back to the early 1800's, changes in the blood gas tensions and pH during the drastic respiratory alterations which follow this procedure have not been monitored. It is generally agreed that the respiratory rate in the fowl is greatly depressed following bilateral, cervical vagotomy and that the respiratory amplitude is increased. Conflicting reports exist concerning the degree and time of acceleration in respiratory rate following the period of initial depression.

The purpose of this project was to answer the following questions by correlating the changes in respiratory movements with the changes in blood gas tensions; (1) To what degree does the increase in respiratory amplitude compensate for the decrease in rate in the maintenance of adequate pulmonary ventilation; and (2) if compensation does occur, how does the age of the bird affect it?

Before the above questions could be answered, it was necessary to determine the reliability of the PO_2 and PCO_2 sensing electrodes with chicken blood by making *in vitro* gas-blood equilibration comparisons. Such measurements were needed to determine whether these electrodes would give reliable recordings with the nucleated red blood cells of the chicken.

PART I. RELIABILITY OF THE BLOOD GAS SENSING ELECTRODE SYSTEM WITH CHICKEN BLOOD

Literature Review

With the introduction of the Clark oxygen electrode in 1956, polarographic determinations of PO_2 can be made directly from blood or other fluids with ease and rapidity. The maintenance of a constant polarizing voltage at the platinum cathode with respect to the silver anode causes the formation of an electrolytic current so that dissolved oxygen from the sample, which diffuses across a polyethylene membrane, is reduced at the cathode. The magnitude of the current is then directly related to the PO_2 in the material being analyzed.

The carbon dioxide electrode introduced by Stowe (1957) and later modified by Severinghaus (1958) measures the change in hydrogen ion concentration of an aqueous layer of sodium bicarbonate solution which is separated from the sample by a teflon membrane that is permeable to carbon dioxide. The measured pH is altered in direct proportion to changes in log PCO_2.

In mammalian blood, the accuracy, dependability and limitations of these blood gas sensing electrodes and their later modifications have been well documented. Severinghaus (1958) found identical PCO_2 readings between blood and its equilibrating gas. An excellent correlation between the readings of the carbon dioxide electrode and standard techniques (Van Slyke manometric technique) in determination of PCO_2 in human blood was reported by Purcell and Rodman (1965). They further reported that the

PO_2 electrode gave readings which were 10% lower than standard techniques at 70 mm Hg PO_2 and above, in excellent agreement with these techniques from 50 to 70 mm Hg PO_2, and 10% higher than these techniques at PO_2 levels below 50 mm Hg. They also found that the PCO_2 electrode was much more stable and dependable than the PO_2 electrode. An excellent correlation between the PO_2 in blood and in the equilibrating gas at levels ranging from approximately 150 mm Hg to 725 mm Hg in in vitro studies with human blood was described by Torres (1963). Rhodes et al. (1964), on the other hand, states that a perfect correlation between the PO_2 of blood equilibrated in a tonometer and the PO_2 of the equilibrating gas does not exist when measured with a PO_2 electrode. Instead, they state that at all tensions below 70 mm Hg, the tension reading in blood must be multiplied by a factor of 1.24 to equal the tension in the gas while at all tensions above 70 mm Hg, the tension reading in blood must be multiplied by a factor of 1.08 + 10 to equal the tension in the gas.

No publications can be found in which this work has been performed on nucleated red blood cells. Meaningful interpretations of blood gas determinations on chicken blood cannot be made until it is found what effect the respiring, nucleated red blood cells have on the electrode system.

Materials and Methods

A. Calibration of PO_2 and PCO_2 Electrodes. For measurement of PO_2 and PCO_2 in blood, saline, and gas, the Beckman

modular cuvette[1] with appropriate electrodes was used. The electrodes were the Beckman oxygen macroelectrode[1] and the Severinghaus PCO_2 electrode[1]. Since the values measured with the electrodes vary with the temperature, it was necessary to accurately control the temperature of the cuvette. A custom-made water bath incorporating a temperature controller[2], an immersible thermistor[3], a heater[4] (200 watts), and a recirculating pump[5] was used with the modular cuvette to hold the temperature of the water circulating through the cuvette at $40.00 \pm 0.07^{\circ}$ C.

Calibration of the oxygen and carbon dioxide electrodes required the use of calibrating gases.[6] Certified analysis of these gases showed purity to be 5.031% CO_2 in O_2 and 12.26% CO_2 in O_2. Nitrogen (1.3 molar PPM O_2) was used for zero oxygen. Air (20.93% O_2) was also used as an oxygen standard. Barometric pressure readings were made prior to each trial. The calibrating gases were bubbled through water in a test tube in order to humidify the gases before they reached the electrodes. The electrodes were connected directly to the appropriate couplers

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[1]Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

[2]Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio (Model 71).

[3]Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio (Number 403).

[4]Aloe Scientific, St. Louis, Missouri.

[5]Aloe Scientific, St. Louis, Missouri.

[6]Air Products of Minnesota, Inc., P. O. Box 176, Shakopee, Minnesota.

of a multichannel Beckman Type S recorder[7] and the number of millimeters of pen deflection was plotted against gas tension to produce the calibration curves.

B. Equilibration of Fluids with Gases.

1. Construction of the tonometer.

A tonometer capable of simultaneously equilibrating nine liquid samples with different gas mixtures was constructed. Twenty-three floating ball flowmeters[8] were arranged in groups of two or three to produce the gas mixtures shown in Table 1.

Table 1. Desired gas mixtures made by using the nine flow meter systems in the tonometer.

	High O_2	Medium O_2	Low O_2
High CO_2	Mixture #1 CO_2 - 20% O_2 - 80% N_2 - 0%	Mixture #2 CO_2 - 20% O_2 - 18% N_2 - 62%	Mixture #3 CO_2 - 20% O_2 - 0% N_2 - 80%
Medium CO_2	Mixture #4 CO_2 - 6% O_2 - 80% N_2 - 14%	Mixture #5 CO_2 - 6% O_2 - 18% N_2 - 76%	Mixture #6 CO_2 - 6% O_2 - 0% N_2 - 94%
Low CO_2	Mixture #7 CO_2 - 3% O_2 - 80% N_2 - 17%	Mixture #8 CO_2 - 3% O_2 - 18% N_2 - 79%	Mixture #9 CO_2 - 3% O_2 - 0% N_2 - 97%

[7]Offner Division of Beckman Instruments, Inc., 3900 River Road, Schiller Park, Illinois.

[8]Ace Glass, Inc., Vineland, New Jersey.

Gas flows to each flowmeter were controlled with a needle valve located at the entrance of the meter. Because of the coarse nature of these valves, difficulty occurred in placing the balls of the meters at exactly the desired location, especially when low flow rates were employed. Three cylinders of O_2, CO_2, and N_2 were used to supply gases to the three manifolds which supplied the various flowmeters. A flow of 2,000 milliliters per minute was used, and the manufacturer's calibration curves were used to obtain the coarse flowmeter settings. Fine adjustments of these settings were made on the basis of gas mixture analysis with the PO_2 and PCO_2 electrodes in the modular cuvette.

Each gas mixture was bubbled through a test tube of water located adjacent to the equilibrating chamber in a constant temperature ($40.0 \pm 0.5^{\circ}$ C), rotating water bath.[9] This served to heat and humidify the gas mixture before it made contact with the fluid. The gas mixture then flowed into the equilibrating chamber (a 250 ml. Erlenmeyer flask) through a tube in the top of the stopper and exited to the atmosphere through an adjacent hole in the stopper. Thus a continuously flowing, gaseous environment was provided for equilibration.

The equilibrating chamber was shaken in the rotating water bath during equilibration at a speed which caused the fluid to be forced up the edges of the flask and thus provide a large surface area. The fluid could be withdrawn from another tube

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[9]New Brunswick Scientific Co., New Brunswick, New Jersey (Model G 76).

attached to a hypodermic needle inserted through the stopper. This tube reached the bottom of the flask so that all of the fluid could be removed.

The repeatability of the desired flowmeter settings was determined for each of the nine gas mixtures with eight successive trials. The gases were turned on at the source tank and a pressure of five pounds per square inch was applied to the manifolds. The flowmeter valves were then adjusted until the desired settings were obtained. Ten minutes was allowed to assure that a complete turnover of gas in the flask had occurred. Sampling of the gas from the equilibration chamber was then initiated starting with chamber #1 and proceeding until all nine were sampled. Each sample was withdrawn into a 10 ml. syringe very slowly to prevent the formation of a subatmospheric pressure in the flask and thus possible contamination with air. The sample of gas was then immediately injected into the cuvette. About 45 seconds was allowed for complete response of the PO_2 electrode, and about 3 minutes was allowed for complete response of the PCO_2 electrode before readings were taken. The sampling was repeated to assure stabilization of the electrodes. The cuvette was periodically flushed with saline to prevent dehydration of the membranes. Between each trial of nine samples, all of the valves were closed on both the tonometer and the source tanks, thus requiring new settings for each trial.

C. Equilibrating Fluids Used. Two fluids, 0.75% saline and blood were used to determine the relationships between the readings of PO_2 and PCO_2 in the gas above the liquid and the PO_2

and PCO_2 in the liquid after equilibration. Comparisons were also made between the readings obtained with old chicken blood, fresh chicken blood, and fresh canine blood. Throughout this paper, old chicken blood refers to blood which was drawn from donor birds 1 to 4 days prior to use. Fresh chicken blood refers to blood which was placed in the tonometer immediately upon withdrawal from the donor.

Old blood was obtained via heart puncture from several breeds of adult male chickens on the Kansas State University Poultry Farm. Approximately 450 ml. of blood from many birds was pooled and stored in an Erlenmeyer flask which contained 10,000 units of heparin diluted in 50 ml. of saline. The blood was stored at refrigerator temperatures until used. Even these extremely high heparin levels failed, in some cases, to prevent clotting of the blood for the entire period.

Fresh blood was withdrawn into a heparin coated syringe from the cutaneous ulnar vein of an anesthetized bird or the femoral vein of an anesthetized dog in the laboratory. No clotting occurred during the short interval of this procedure.

D. Gas-liquid Equilibration. Two methods were used for the equilibration of either saline or blood with gas. The first method involved the use of the equilibration chamber described above, in which the gas flowed continuously over the liquid during the time of equilibration. The second method was similar to that used by Torres (1963) in which a 3 ml. of liquid was placed in a 50 ml. syringe and gas mixture from the appropriate flowmeter system was drawn into the remainder of the syringe. The

syringe was then placed in the water bath and shaken while the liquid was equilibrating.

1. The Flask Method.

To determine the length of time required for PO_2 and PCO_2 equilibration, the following procedure was performed six times. Gas mixtures 1, 5, and 9 from Table 1 were used as sources of high, medium, and low O_2 and CO_2. Fifty ml. of old chicken blood was placed into the equilibrating flask and shaken for 30 minutes in the water bath ($40.0 \pm 0.5^{\circ}$ C). At this time the gases were turned on and the blood was agitated at a speed fast enough to spin the blood up on the walls of the flask without foaming. Samples were withdrawn into a 10 ml. syringe at 30, 60, and 90 minute intervals and injected into the cuvette.

Upon preliminary analysis of the data for blood, it was noted that the high PO_2 samples were still increasing their values at 90 minutes equilibration time. The procedure was then repeated twice using gas mixtures 1, 4, and 7 for equilibration times of 30, 60, 90, 120, and 150 minutes.

The next step was to determine actual gas-blood equilibrations using the same procedure described above with the following exceptions: (1) all nine gas mixtures were used; (2) time of equilibration for high O_2 mixtures (1, 4, and 7) was 90 minutes while all other samples were equilibrated for 60 minutes; and (3) the gas above the blood was analyzed each time prior to the analysis of the blood itself.

2. The Syringe Method.

Several variations of this method were conducted using

different fluids.

(a) A 50 ml. heparinized syringe coated with silicone grease with a three-way stopcock attached was used as an equilibration chamber. Three ml. of old chicken blood was drawn into the syringe followed by 45 ml. of gas from a flowmeter system. The syringe was shaken in the rotating water bath ($40.0 \pm 0.5^{\circ}$ C) for 10 minutes. At the end of this time the gas above the blood in the syringe was injected into the cuvette. After a recording had been taken, the three ml. blood sample was injected into the cuvette. During the time between injections, care was taken to return the syringe to the water bath to prevent cooling. Six trials of gas mixtures 1, 5, and 9 were conducted.

(b) Fresh chicken blood was used as the equilibrating media in six trials with the following changes: (1) The sciatic artery of two 11-month-old male Single Comb White Leghorns weighing approximately 1.8 kg. was cannulated under sodium pentobarbital anesthesia for withdrawal of blood. An injection of three ml. of saline into the birds for each three ml. of blood drawn was used to maintain circulatory volume; (2) gas mixtures 2, 5, and 8, which contained varying levels of carbon dioxide at a constant oxygen level, were used to establish PCO_2 gas-blood relationships; and gas mixtures 4, 5, and 6, which contained varying levels of oxygen at a constant carbon dioxide level, were used to establish PO_2 gas-blood relationships; (3) the usage of heparin was limited to 200 units which was administered to the donor bird at the start of the withdrawal procedures, and the amount used to heparinize the syringes before the withdrawal

of blood.

(c) To determine the gross metabolic rate of nucleated red blood cells, the syringe method with fresh chicken blood was used with the following changes: (1) Immediately after the syringe containing the blood and gas mixture had been placed in the rotating water bath, a 5 ml. sample of gas was slowly withdrawn from the tonometer flask into a 10 ml. syringe and analyzed in the cuvette; (2) equilibration time was extended from 10 to 35 minutes; (3) at five-minute intervals from the start of equilibration, five ml. aliquots of the gas above the blood in the syringe were analyzed in the cuvette; (4) five minutes after the sixth gas mixture analysis, the blood in the syringe was injected into the cuvette; (5) the only heparin utilized in this procedure was the amount required to coat the syringe before the blood sample was withdrawn.

(d) The syringe technique as used for old chicken blood was then repeated six times using physiological saline instead of blood.

(e) The syringe technique was again repeated six times using fresh blood from a female canine. Cannulation of the femoral artery was accomplished under pentobarbital anesthesia. Three ml. blood samples were taken with no saline replacement.

E. Reporting of Data. Means and standard deviations were computed for all sets of data. Where applicable, regression analysis was performed to compare tension between gas and liquid media. T-tests of the hypothesis that the slope of the

regression curve was not different from one, which would represent a regression curve of perfect agreement between the tensions in the equilibrating gas above the liquid and the tensions of liquid, was performed according to the methods of Snedecor (1956) and Huntsberg (1961).

Results

The first procedure was to determine the repeatability of the flowmeter settings for the nine gas mixtures which would be utilized as gas sources throughout the succeeding experiments. The results of these trials are shown in Table 2.

Table 2. PO_2 and PCO_2 analyses for nine gas mixtures from the various flowmeter systems.

Gas mixture	PO_2 (Mean±S.D.)	PCO_2 (Mean±S.D.)
1	559 ± 9	122.4 ± 6.8
2	111 ± 14	143.2 ± 6.0
3	0 ± 0	134.4 ± 3.1
4	513 ± 6	40.8 ± 2.4
5	106 ± 1	38.0 ± 2.5
6	0 ± 0	37.2 ± 2.4
7	527 ± 14	13.5 ± 1.1
8	103 ± 4	10.7 ± 6.8
9	0 ± 0	10.8 ± 1.1

The reliability of the flowmeter systems in the tonometer is shown by the relatively small deviations from the means for each gas mixture. The only questionable readings are the PO_2 value for gas mixture 2 which has a mean of 110.6 and a standard deviation of 13.5, and the PCO_2 value for gas mixture 8 which has a mean of 10.7 and a standard deviation of 6.8. The large

standard deviations shown in these readings are indicative of the coarse nature of the needle valves used in the construction of the tonometer system as described in the methods. In spite of the relatively low variations of the gas mixtures, an analysis of the equilibrating gas mixture prior to all fluid determinations was made using the Beckman electrode system.

The period of time required for PO_2 and PCO_2 equilibration between the gas mixture and the fluid in the tonometer using the flask technique was then determined by analysis of the old chicken blood samples at 30, 60, and 90 minute intervals after the beginning of equilibration (Table 3). High O_2 equilibration periods were extended an additional hour since it was noted upon preliminary examination that the O_2 tension was still increasing at the 90-minute interval. After final analysis, however, the small standard deviation for all readings indicated that 30 minutes was sufficient equilibration time for the flask technique.

Table 3. Means of seven determinations of equilibration time of blood with gases by the flask technique.

Gas mixture		Time (minutes)					Mean±S.D. of means	
		30	60	90	120	150		
PO_2	High	420.0	403.0	430.5	415.8	409.0	415.7	10.5
	Medium	95.3	96.6	95.6	-	-	95.8	0.7
mm Hg	Low	0.0	0.0	0.0	-	-	0.0	0.0
PCO_2	High	113.5	114.9	112.7	-	-	113.7	1.5
	Medium	37.4	36.9	38.2	-	-	37.5	0.7
mm Hg	Low	16.0	16.3	13.2	-	-	15.2	1.7

To determine possible differences in gas tension readings between the equilibrating gas and the liquid after equilibration, the flask method was used to equilibrate old chicken blood with all nine gas mixtures. The comparison between gas and blood PO_2 and PCO_2 determinations is shown in Figs. 1 and 2. The dots on the graphs represent the individual observations. Slopes of both regression lines, 0.80 for PO_2 and 0.92 for PCO_2, are significantly different from one at the .001 level of probability. These data indicate that either the gas tensions in the blood, especially the PO_2, is considerably less than that in the equilibrating gas or that the electrode system is giving erroneously low readings for the blood gas tensions as compared to equilibrating gas tensions. If the former is true, the most probable reasons which would account for the discrepancy are incomplete equilibrating duration or inefficiency of the equilibrating method. If the latter is true, factors which could explain these erroneous readings are: (1) Age and condition of the equilibration media; (2) possible active cell respiration; (3) an inherent fallacy of the electrode system per se; or (4) unknown factors peculiar to chicken blood.

Data presented in Table 3 indicate that equilibration of the liquid with the gas is complete at the end of 30 minutes, hence tending to discount the possibility of incomplete equilibration. Thus, another tonometer system, the syringe technique described by Torres (1963), was employed to determine the efficiency of the equilibrating methods. Old chicken blood was equilibrated with gas mixtures 1, 5, and 9 utilizing this

Fig. 1. Comparison of oxygen tension measurements between old chicken blood and equilibrating gas mixtures using the flask method. Dashed line represents theoretically perfect regression between gas and blood tensions.

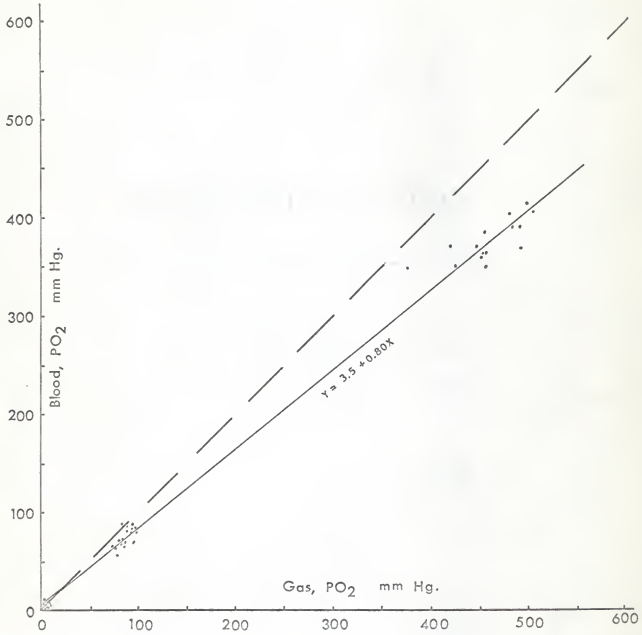
600
500
400
300
200
100
0
Blood, PO_2 mm Hg.
Y= 3.5 +0.80X
Gas, PO_2 mm Hg.
0
100
200
300
400
500
600

FIGURE 1

Fig. 2. Comparison of carbon dioxide tension measurements between old chicken blood and equilibrating gas mixtures using the flask method. Dashed line represents theoretically perfect regression between gas and blood tensions.

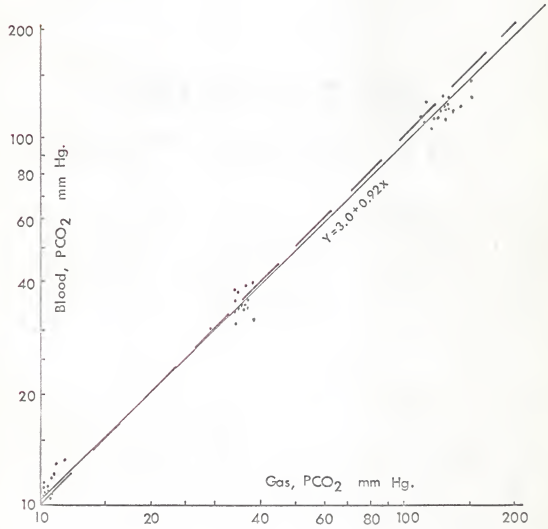
200
100
80
60
40
20
10
Blood, PCO_2 mm Hg.
Y=3.0+0.92x
Gas, PCO_2 mm Hg.
10
20
40
60
80
100
200

FIGURE 2

technique. The regression lines and individual observations for this study are plotted in Figs. 3 and 4. The slope of the regression line for PO_2 was 0.76, which is significantly different from one at the .001 level of probability, while the slope of the regression line for PCO_2 was 1.05, which was not significantly different from one. The differences in the measurements of tensions between the gases and the blood are very similar with the syringe method as compared to the flask method. Hence, these data further indicate that it is highly probable that equilibration had efficiently and completely occurred in both studies and that other factors were causing the low tension readings in blood as compared to the equilibrating gases.

One of these factors could be the age and condition of the chicken blood used as the equilibrating media. To determine the importance of this factor, the syringe technique was utilized with fresh chicken blood. The data from this study are presented in Figs. 5 and 6. The slopes of the regression lines were 0.90 for PO_2 and 1.17 for PCO_2, both significantly different from one at the .001 level of probability. When comparing these regression slopes with those obtained with old chicken blood, it can be noted that the slope of the regression line for PO_2 is directly related to the age of the cells since in fresh chicken blood it is closer to one than the corresponding value for old chicken blood. On the other hand, an inverse relationship is noted for PCO_2 between the slopes of the regression lines and the age of the blood. These data suggest that factors relating to the age of the blood are influential in

Fig. 3. Comparison of oxygen tension measurements between old chicken blood and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents perfect regression between gas and blood tensions.

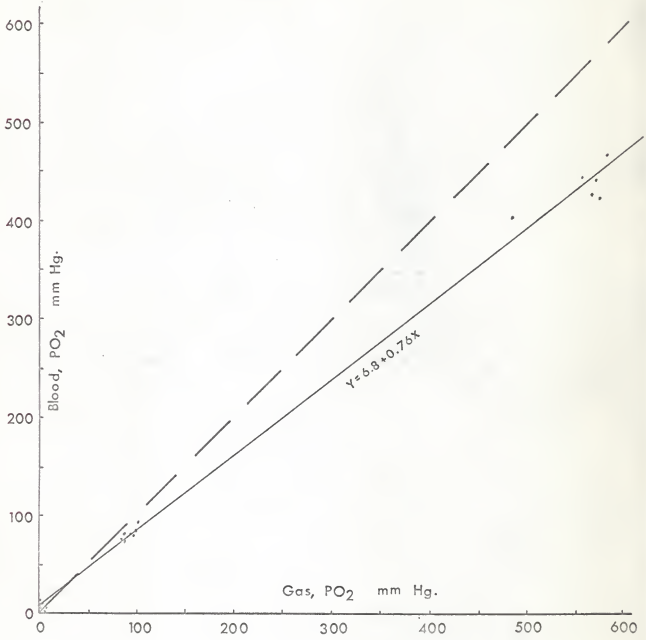
600
500
400
300
200
100
0
Blood, PO_2 mm Hg.
Y=6.8+0.76X
Gas, PO_2 mm Hg.
0
100
200
300
400
500
600

FIGURE 3

Fig. 4. Comparison of carbon dioxide tension measurements between old chicken blood and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

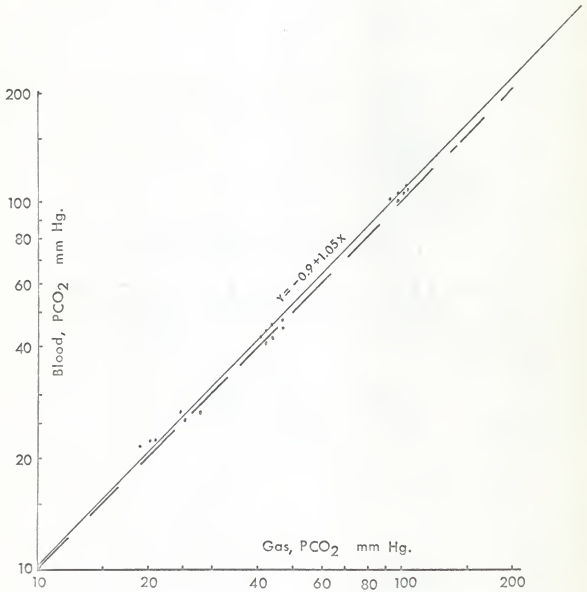
200
100
80
60
40
20
10
Blood, PCO_2 mm Hg.
Y= -0.9+1.05X
Gas, PCO_2 mm Hg.
10
20
40
60
80
100
200

FIGURE 4

Fig. 5. Comparison of oxygen tension measurements between fresh chicken blood and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

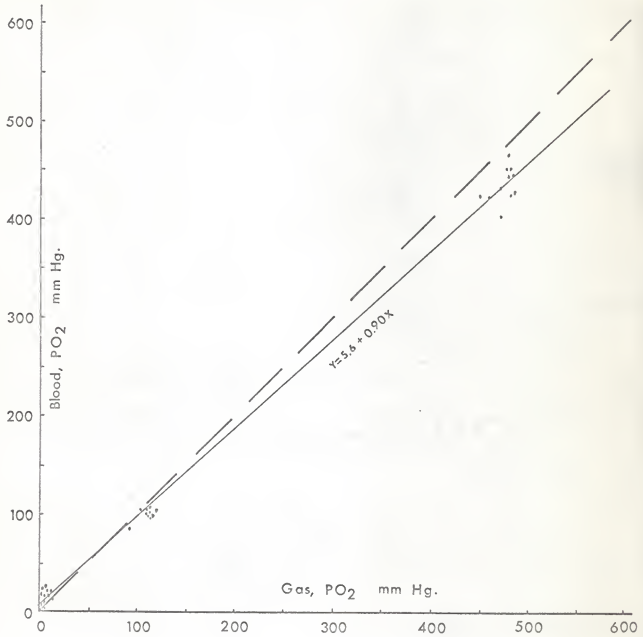
600
500
400
300
200
100
0
Blood, PO_2 mm Hg.
Y=5.6+0.90X
Gas, PO_2 mm Hg.
0
100
200
300
400
500
600

FIGURE 5

Fig. 6. Comparison of carbon dioxide tension measurements between fresh chicken blood and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

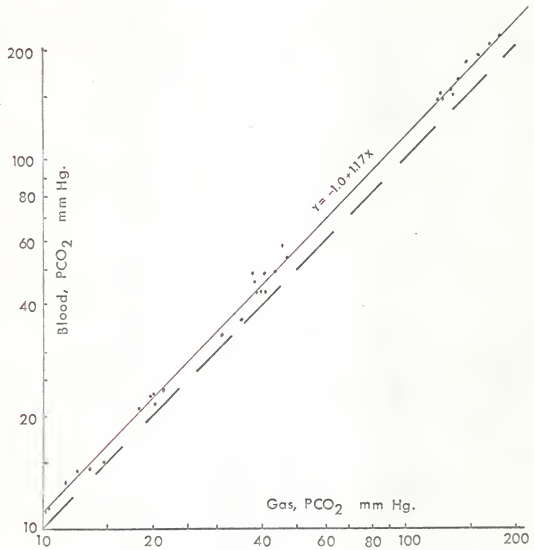
200
100
80
60
40
20
10
Blood, PCO_2 mm Hg.
Y= -1.0+1.17X
Gas, PCO_2 mm Hg.
10
20
40
60
80
100
200

FIGURE 6

determining the degree of variation between readings for tensions in the blood as compared to the tension in the equilibrating gas.

The possibility of active cell respiration by the nucleated red blood cells as a factor in producing the low PO_2 readings was grossly examined by allowing the equilibration period in the syringe technique to continue for 35 minutes instead of the usual 10 minutes. The equilibrating gas was sampled every five minutes for the first 30 minutes and the blood gas tension was analyzed at the end of 35 minutes. If the red cells were using a large amount of oxygen, a decrease in the PO_2 value and an increase in the PCO_2 value of the equilibrating gas should occur with time. The means and standard deviations of these readings are illustrated in Figs. 7 and 8. It is readily apparent from these graphs that the utilization of the O_2 and the buildup of CO_2 due to active cellular respiration was not of a sufficient magnitude to be detected by a method this gross in nature. The initial slight rise in CO_2 tension (Fig. 8) for low PCO_2 values was probably the result of addition of CO_2 to the gas mixture from the arterial blood which has been anaerobically drawn. The sensitivity of the PCO_2 electrode is very high in the low PCO_2 ranges since it is a logarithmetically responding electrode and a large pen deflection occurs for a small PCO_2 change in these ranges. Thus, when low PCO_2 equilibrating gases were used, a slight shift in PCO_2 due to CO_2 eminating from the blood would produce a readable pen deflection as seen in the initial readings in Fig. 8 for low PCO_2 values.

Fig. 7. Gross determination of PO_2 decay over a 30-minute period using the syringe technique. Means and standard deviations are shown for three successive trials.

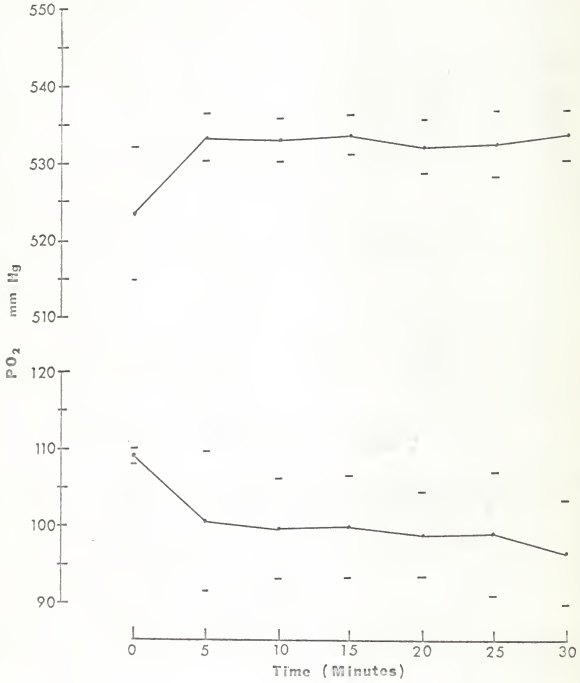
550
540
530
520
510
mm Hg
PO_2
120
110
100
90
0
5
10
15
20
25
30
Time (Minutes)

FIGURE 7

Fig. 8. Gross determination of PCO_2 increase over a 30-minute period using the syringe technique. Means and standard deviations are shown for three successive trials.

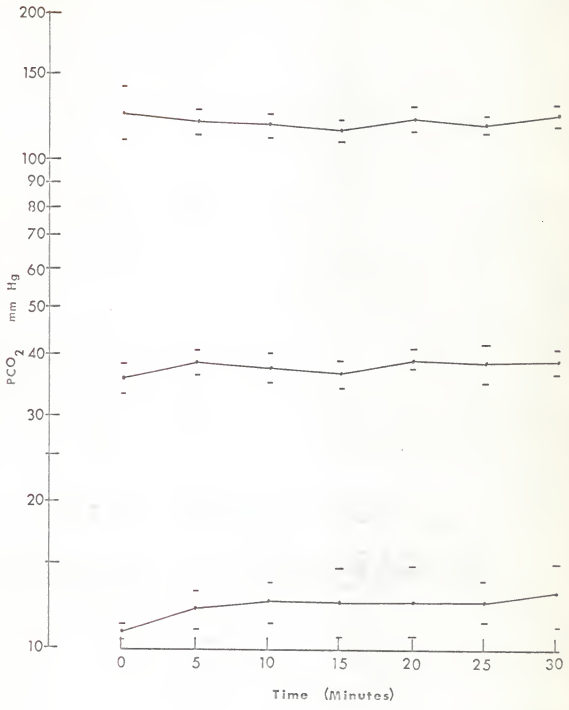
200
150
100
90
80
70
60
50
40
30
20
10
PCO_2 mm Hg
0
5
10
15
20
25
30
Time (Minutes)

FIGURE 8

The gas-blood tension comparisons at the end of 35 minutes are shown in Figs. 9 and 10. The slopes of the regression lines for both PO_2 and PCO_2 are not substantially different from those obtained after 10 minutes of equilibration as shown in Figs. 5 and 6. These data confirm the fact that 10 minutes is sufficient time for complete equilibration with oxygen and carbon dioxide even at higher tension levels using the syringe technique.

To determine if some idiosyncrasy of the electrode system was producing erroneous readings when a liquid, as opposed to a gas, was being analyzed, saline was substituted for blood in the equilibrating chamber and was equilibrated with gas by the syringe method for 10 minutes. This procedure would eliminate any peculiarities which the cellular components in blood might present in the response of the electrodes. The data for this study are presented in Figs. 11 and 12. No significant differences exist between the slope of the theoretically perfect regression line and the experimental regression line for either PO_2 or PCO_2. Hence, liquids, at least saline, do have the same gas tensions as the equilibrating gas and the electrodes are equally sensitive to both media.

From the preceding experiments, it appears that blood has some component which produces erroneous readings by the electrode system. In order to isolate the component which was responsible for these variations, gas-blood comparisons were conducted using the syringe technique on non-nucleated, fresh canine blood. The comparisons are shown in Figs. 13 and 14. There was a significant ($P = .05$) difference between the slope of the PO_2 regression line

Fig. 9. Comparison of oxygen tension measurements between fresh chicken blood and equilibrating gas mixtures using the syringe technique for a 30-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

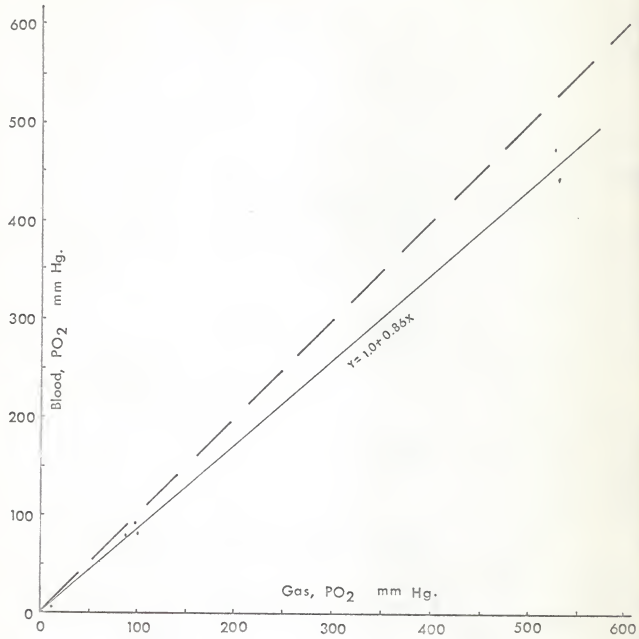
600
500
400
300
200
100
0
Blood, PO_2 mm Hg.
Y= 1.0+0.85X
Gas, PO_2 mm Hg.
0
100
200
300
400
500
600

FIGURE 9

Fig. 10. Comparison of carbon dioxide tension measurements between fresh chicken blood and equilibrating gas mixtures using the syringe technique for a 30-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

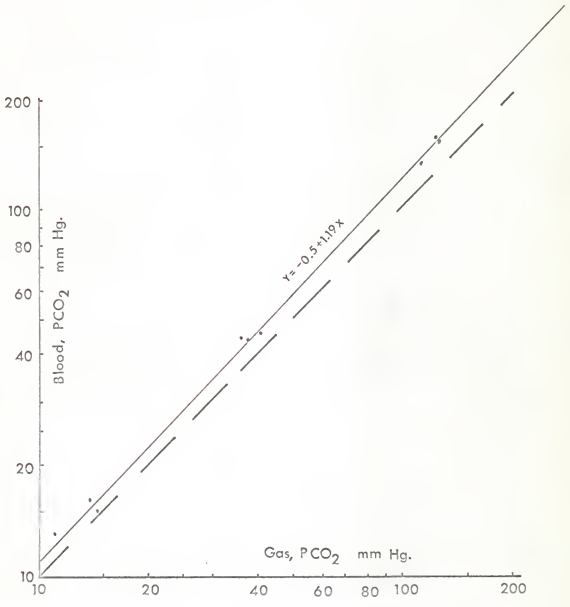
200
100
80
60
40
20
10
Blood, PCO_2 mm Hg.
Y= -0.5+1.19x
Gas, PCO_2 mm Hg.
10
20
40
60
80
100
200

FIGURE 10

Fig. 11. Comparison of oxygen tension measurements between saline and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and saline tensions.

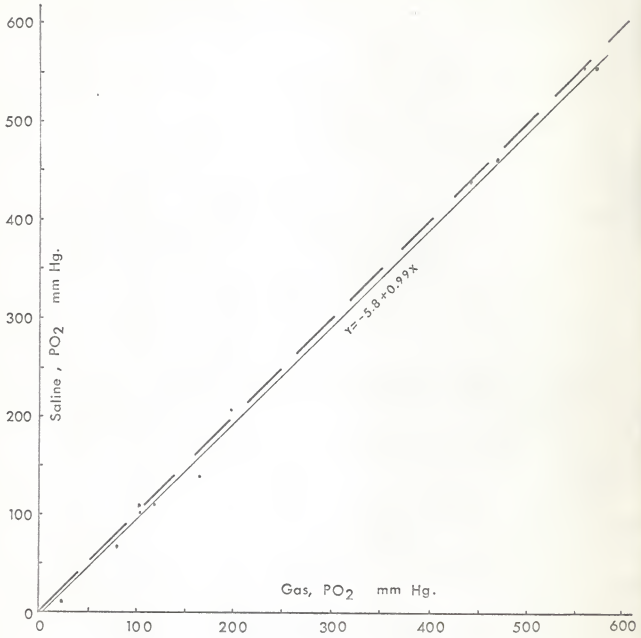
600
500
400
300
200
100
0
Saline , PO_2 mm Hg.
Y=-5.8+0.99X
Gas, PO_2 mm Hg.
0
100
200
300
400
500
600

FIGURE 11

Fig. 12. Comparison of carbon dioxide tension measurements between saline and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and saline tensions.

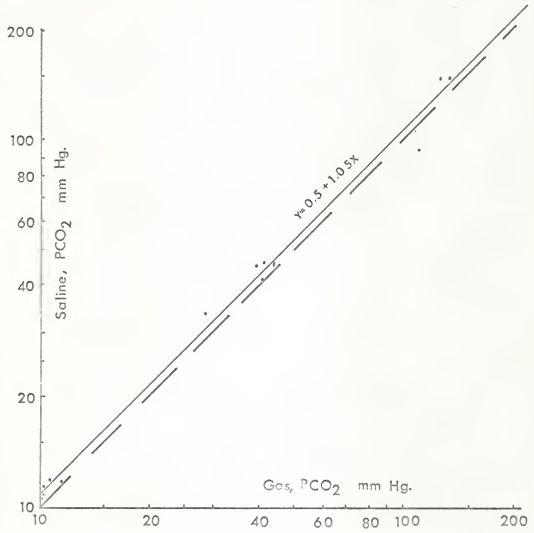
200
100
80
60
40
20
10
Saline, PCO_2 mm Hg.
Y=0.5+1.05X
Gas, PCO_2 mm Hg.
10
20
40
60
80
100
200

FIGURE 12

Fig. 13. Comparison of oxygen tension measurements between canine blood and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

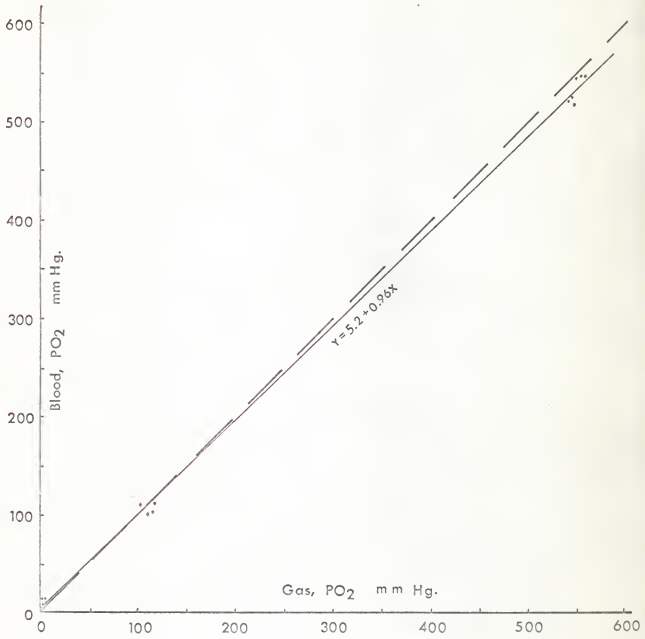
600
500
400
300
200
100
0
Blood, PO_2 mm Hg.
Y=5.2+0.98X
Gas, PO_2 mm Hg.
0
100
200
300
400
500
600

FIGURE 13

Fig. 14. Comparison of carbon dioxide tension measurements between canine blood and equilibrating gas mixtures using the syringe technique for a 10-minute equilibration time. Dashed line represents theoretically perfect regression between gas and blood tensions.

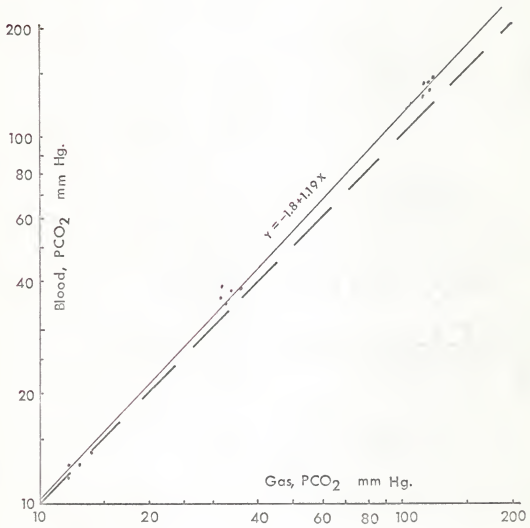
200
100
80
60
40
20
10
Blood, PCO_2 mm Hg.
Y = -1.8+1.19X
Gas, PCO_2 mm Hg.
10
20
40
60
80
100
200

FIGURE 14

and the theoretically perfect regression line and a highly significant difference (P = .001) between the slope of the PCO_2 regression line and the theoretically perfect regression line. However, the slope of the PO_2 regression line (0.96) was considerably closer to one than that of fresh chicken blood (0.90 in Fig. 5 or 0.86 in Fig. 9) indicating that some factor in the chicken blood is responsible for producing the greater portion of the error involved.

Discussion

In this gas-blood equilibration study, significant differences were found in both PO_2 and PCO_2 readings between chicken blood and equilibrating gas mixtures. The magnitude of these differences was increased by aging of the blood 1 to 4 days. Since no significant discrepancies could be found between saline and gas PO_2 and PCO_2 readings, it is assumed that the electrodes used to measure the PO_2 and PCO_2 values were not discriminating between gas tensions in liquids and gas mixtures per se. Hence, some factors, peculiar to chicken blood, appear to be responsible for the above stated differences.

The presence of active metabolism in mammalian RBC's has been shown by many investigators. Oxygen tension decay has been found by Lenfant and Aucutt (1965) to occur at a rate of 2.7 mm. Hg./min. in human blood samples stored at 37° C, while the PCO_2 increases at a rate of 0.11 mm. Hg./min. These rates are directly related to temperature by a factor of .063 mm. Hg./min. per °C for PO_2 and .003 mm. Hg./min. per °C for PCO_2. Thus,

for blood held at 40° C the decay rate for PO_2 would be 2.89 mm. Hg./min. and the rate of increase for PCO_2 would be .12 mm. Hg./min. The O_2 consumption at 37° C for washed human erythrocytes, buffy coat removed, is 0.020 uL/mg. dry wt./hr. while for chicken blood it is 0.136 uL/mg. dry wt./hr., constituting an increase in activity of the chicken erythrocyte of some 6.8 times that of the human erythrocyte (Hunter and Hunter, 1957). Hence, at tensions above 150-200 mm. Hg. where hemoglobin is completely saturated, oxygen tension in chicken blood might be expected to be reduced at a value of at least 19 mm. Hg./min. and probably even more if one considers that the white blood cells are highly active and are about 14 times more numerous in chicken blood than in human blood.

In the gas-blood equilibration studies, the approximate time lapse between the analysis of the gas and that of the blood was three minutes. Thus, a tension drop of some 8 mm. Hg. may have resulted in the canine blood, assuming its metabolic activity is similar to that of human. From Fig. 13, it can be calculated that at 300 mm. Hg., the oxygen tension in the blood is approximately 7 mm. Hg. lower than the tension in the gas. It is very possible that this reduction is associated with the cellular respiration. Since the oxyhemoglobin curve for the chicken has a much lower slope than that of man, it is likely that rather high tensions must be applied before the hemoglobin is completely saturated (Morgan and Chichester, 1935). At 500 mm. Hg. gas tension, the oxygen tension in the fresh blood was approximately 456 mm. Hg. (Fig. 5), a difference of 44 mm. Hg.

If the PO_2 decay is in the neighborhood of 19 mm. Hg./min., then in the three-minute period between measurements, a decay of 57 mm. Hg. would be expected. This compares favorably with the reduced value measured. It would therefore appear that the electrode system is providing reliable tension readings and that the rapidity of the measurement following blood withdrawal is very critical in the proper interpretation of the results.

The very large differences seen between the oxygen tension in old blood and the equilibrating gas, 113 mm. Hg. at 500 mm. Hg. PO_2 in gas (Fig. 3), cannot be accounted for by metabolic factors. When avian blood is hemolyzed, it separates into two phases upon centrifugation, the lower portion of which is a reddish jelly (Christensen and Dill, 1935). In this laboratory, while performing white blood counts, a similar gel was seen when 1% acetic acid and 0.1 N hydrochloric acid diluting fluids were used to hemolyze the erythrocytes. If a gel of this nature were to adhere to the electrode membrane forming a barrier which would decrease the permeability of the membrane to the gases, then a plausible theory could be formulated accounting for the variation in PO_2 and PCO_2 readings in chicken blood. Such a gel could very likely occur if a significant degree of hemolysis of the red blood cells occurred with aging due to increased fragility of the cells as suggested by Prankerd (1958). This would result in a proteinaceous barrier being formed over the membrane while sampling old blood as compared to fresh blood. Oxygen tensions would be much more effected than carbon dioxide tensions due to the fact that the relative rates of diffusion between

these gases is about 1:20.7 (Comroe, 1965). Thus, the barrier would tend to greatly retard the diffusion of oxygen from the sample to the inside of the membrane of the oxygen electrode but would have only a minor effect on the diffusion of carbon dioxide. The data in Figs. 1, 2, 3, and 4 tend to support these explanations.

The similar PCO_2 readings between mammalian blood (Fig. 14) and fresh chicken blood (Fig. 6) are expected when it is considered that the respiration rate of chicken blood acts to increase the PCO_2 values and the formation of a barrier on the membrane acts to decrease it. The antagonizing forces could leave the PCO_2 at a level comparable to mammalian blood cells even though the avian RBC's have a much more rapid respiration rate.

The slight but significant differences of the slope of the regression lines from one for the PO_2 and PCO_2 values obtained for canine blood can be explained by the increase in metabolism of the RBC. These readings are in disagreement with the results reported by Torres (1963) and Purcell (1965) who found a high degree of correlation between the tensions of blood and the equilibrating gases. The explanation for this disagreement could be that their work was conducted at 37° C while our work was carried out at 40° C. According to Lenfant (1965), this increase in temperature would cause an increase in PO_2 decay and PCO_2 accumulation, thus resulting in the slight difference between tensions measured in gas and blood.

The nature of the barrier caused some concern since it was

thought that an accumulation of the proteinaceous material might invalidate the continuous PO_2 and PCO_2 readings. However, since it was possible to perform final calibrations following a saline flush and obtain readings within a reasonable range of the initial calibrations, it was decided that the material is removed by a saline flush and does not accumulate forming an impenetrable barrier. This is also indicated by the fact that progressively lower readings were not seen to occur as the experiment progressed.

Summary

A comparison of gas tension readings of blood and equilibrating gas mixtures have been made to determine the reliability of the use of the PO_2 and PCO_2 electrodes in the measurement of blood gas tensions in nucleated chicken blood at 40° C.

The significant findings of this study are:

(1) Equilibration of blood with gases of various tensions was complete at the end of 30 minutes when the flask method was used and at the end of 10 minutes when the syringe method was used.

(2) The PO_2 and PCO_2 readings were significantly lower in old (1-4 days after withdrawal) chicken blood than in the equilibrating gas when the flask method was used to equilibrate the blood. When the syringe method was used to equilibrate the blood, the PO_2 readings were significantly lower in old chicken blood than in the equilibrating gas but no significant differences occurred for PCO_2 .

(3) The PO_2 readings were also significantly lower in freshly withdrawn chicken blood than in the equilibrating gas, but were much closer to that of the equilibrating gas than when old blood was used.

(4) The PCO_2 readings of freshly withdrawn chicken blood were significantly higher than that of the equilibrating gas.

(5) No reduction in either PO_2 or PCO_2 in the equilibrating gas mixture utilizing the syringe technique due to respiration of the cells in the blood could be detected over a 30-minute period.

(6) The PO_2 and PCO_2 readings of 0.75% saline equilibrated with various gas mixtures were not significantly different from the tensions of the gases in the mixtures indicating that the electrodes are capable of reliably detecting gas tensions in fluids.

(7) Slight, but significantly lower, PO_2 readings were obtained from canine blood than were obtained from the equilibrating gas while significantly higher PCO_2 readings were obtained from this blood than were obtained from the equilibrating gas.

The theory was proposed that these differences in gas tensions were due to: (1) a barrier being formed at the site of the sensing membrane by the nuclei of the chicken erythrocytes which had been freed by the hemolysis of the cells; and (2) active respiration of the nucleated chicken cells which tended to produce low PO_2 and high PCO_2 readings.

PART II. ALTERATIONS OF BLOOD PO_2 AND PCO_2 FOLLOWING BILATERAL, CERVICAL VAGOTOMY IN THE CHICKEN

Literature Review

After a comprehensive search of the literature, Fedde et al. (1963) indicated that general agreement exists concerning the alterations in the respiratory pattern following bilateral, cervical vagotomy in the fowl. The two characteristic respiratory changes in the vagotomized fowl are the immediate depression of the respiratory rate as a result of a prolongation of the expiratory portion of the cycle, and a profound increase in amplitude or depth of the respiratory movement. Following these initial changes, an acceleration in rate occurs from within 30 minutes to 2 or 3 days after vagotomy (Couvreur, 1891; Heistand and Randall, 1942). Fedde et al. (1963) reported that 32-week-old, male chickens showed an acceleration in rate approximately 30 minutes following bilateral vagotomy, and that this was not typical of younger birds, 16 weeks old. They also reported that death resulted within 3 hours following bilateral, cervical vagotomy in unanesthetized 32-week-old birds. Death was thought to be due to asphyxia resulting from massive pulmonary vascular congestion. Young cocks (16 weeks old) on the other hand, did not develop these pulmonary conditions and survived up to 10 days following vagotomy, finally dying from inanition due to paralysis of the crop.

The only study dealing with the efficiency of respiratory exchange following bilateral, cervical vagotomy was conducted by

Couvreur (1891). From the results of this study, he noted that immediately following bilateral vagotomy, the respiratory exchange for a given period of time is decreased. Second, during the first day following vagotomy, the respiratory exchange follows the variation in pulmonary ventilation. Third, on the last day before death, the pulmonary ventilatory rate remained at a constant level while a continued decrease in respiratory exchange was being noted. From the above results, especially the third point, Couvreur concluded that death following bilateral vagotomy was complicated by asphyxia as a result of pulmonary lesions and not due to inanition alone as was the popular belief of the time.

It is the object of this study to determine the effectiveness of pulmonary ventilation by determining the blood gas tensions following bilateral, cervical vagotomy and the variance of these tensions as related to age.

Materials and Methods

Two experiments were conducted. The first utilized cocks (Hy-line)[1], 30 months of age, while the second experiment utilized similar birds which were 11 months of age.

A. Old Birds. A diagrammatic presentation of the experimental preparation is shown in Fig. 15. Six cocks raised under standard flock conditions were restrained in dorsal recumbency. Procaine hydrochloride was injected subcutaneously in the

[1]Combes and Sons Hatchery, Sedgwick, Kansas.

Fig. 15. Experimental arrangement for the simultaneous recording of arterial blood pH, arterial blood PO_2, arterial blood PCO_2, arterial blood pressure, and respiratory period and amplitude during alterations in respiratory PO_2 and PCO_2.

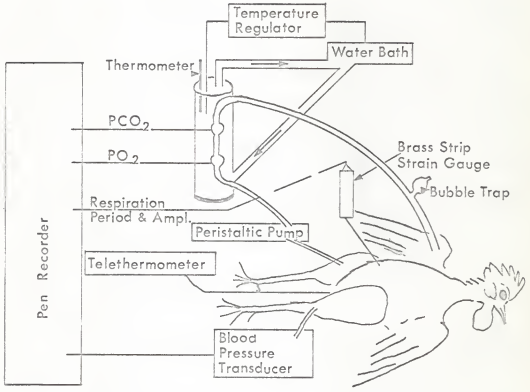
Temperature Regulator
Water Bath
Thermometer
PCO_2
PO_2
Brass Strip Strain Gauge
Bubble Trap
Respiration Period & Ampl.
Peristaltic Pump
Pen Recorder
Telethermometer
Blood Pressure Transducer

FIGURE 15

midventral, cervical region, in the popliteal region of both legs, and in the midventral bracheal region of the right wing. A midventral, cervical incision was made, the trachea was cannulated, and both vagi were isolated. The right and left sciatic arteries and the right cutaneous ulnar vein were isolated and cannulated with polyethylene cannulea. The left sciatic cannula was connected to Statham pressure tranducer[2] for measurement of systemic blood pressure and heart rate. The right sciatic artery and right cutaneous ulnar vein were connected to a Beckman modular cuvette extracorporeal blood gas sensing system for continuous monitoring of PO_2 and PCO_2. A peristaltic pump[3] was inserted into the arterial line between the subject and cuvette for maintenance of a constant blood flow rate through the cuvette of about 9 ml./min.

A description of the Beckman modular cuvette, the PO_2 and PCO_2 electrodes and their calibration procedures was presented in Part I of this thesis. This system was used in a continuous flow mode with blood passing from the sciatic artery of the bird, into the cuvette, past the sensing electrodes, and back to the bird via the cannulated cutaneous ulnar vein. To prevent the blood from clotting in the cuvette system, the bird was given an initial dose of 100 units of heparin, with 50 additional units each succeeding half hour. Also, the cuvette was flushed with

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[2]Stathum Laboratories, Inc., Hato Rey Industrial Subdivision, Hato Rey, Puerto Rico (Model P23GP).

[3]American Instrument Co., Silver Spring, Maryland.

0.75% saline every 15 minutes. In order to measure the flow rate of blood through the cuvette, a small air bubble was injected into the three-way stopcock between the cuvette and the return cannula. The movement of the air bubble along a marked length of the cannula was timed in order to provide an indication of the rate of flow of the blood through the cuvette. Satisfactory flow rates were on the order of 9 ml./min. At this flow rate, it required less than four seconds for blood to reach the sensing device from the bird. To prevent the air bubble from reaching the bird, a bubble trap was inserted into the cannula a few centimeters before the blood entered the vein. This trap was essentially a perpendicular extension from a small glass tube (through which the blood flowed) forming a bulb which had an opening at the top into which was inserted a small serum bottle stopper. When the bubble reached the trap, it rose into the bulb and thus did not enter the vascular system. The trapped air could occasionally be removed from the bulb by a syringe and needle inserted through the rubber serum bottle stopper.

Respiratory movements were measured with the aid of a strain gauge[4] device which was attached 2.5 cm. from the caudal tip of the sternal carina (Fedde et al., 1963). Respiratory amplitude (in terms of millimeters of sternal deflection) and period (seconds/respiration) were measured. Respiratory period was measured over two complete cycles and the arithmetic mean

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[4]Baldwin-Lima-Hamilton Corporation, Electronics and Instrumentation Division, Waltham, Massachusetts.

was converted to seconds per cycle.

All of the above parameters were simultaneously recorded on a multichannel Beckman Type S recorder.

Body temperature was monitored on a read out thermometer[5] connected to a rectal thermistor[6] inserted 10 cm. into the rectum of the bird.

Control readings of all parameters were taken 1.5 and 7 minutes after completion of the transducer connections. The right vagus was then isolated and a control reading was taken just prior to severing the nerve. After the right vagus was severed, recordings were taken at 30 seconds, 1, 5, and 10 minutes post-unilateral vagotomy. The left vagus was then isolated and again a reading was obtained prior to severance of the nerve. Following section of the left vagus, recordings were made at 30 seconds, 1, 5, 10, 20, and 50 minutes.

A sham vagotomy was performed on one bird in which all procedures were executed except the severing of the two vagi.

B. Young Birds. Identical procedures as listed in Section A were repeated on ten 11-month-old Hy-line males with the following exceptions: (1) A Beckman Model GS pH meter[7] was used in an attempt to analyze arterial pH. This procedure required removel of 2 ml. of blood from the exit point of the cuvette for

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[5]Yellow Springs Instrument Co., Yellow Springs, Ohio (Model 44TD).

[6]Yellow Springs Instrument Co., Yellow Springs, Ohio (Model 401).

[7]Beckman Instruments, Inc., Fullerton, California (Model GS).

each reading. Due to technical difficulties, the data from this measurement has been deemed unreliable and has not been included in the results. In three birds, the blood was not replaced but in seven other birds, including a sham vagotomized bird, an equal amount of blood was replaced into the bird. (2) The peristaltic pump was removed from the arterial line leading to the cuvette so that the birds' arterial pressure was used to force blood through the extracorporeal system. (3) A heating pad[8] was used to help maintain body temperature at a normal level. (4) The initial dose of heparin was increased to 200 units, with subsequent doses of 100 units at 30-minute intervals. Every 30 minutes the flow rate through the cuvette was also checked and if it fell below 9 ml./min. the cuvette was flushed with saline.

C. Reporting of Data. Since one of the old birds died prior to 50 minutes post-bilateral vagotomy, means of the various parameters could not be computed. For this reason, the data for the individual birds are presented in graphic form.

Means and standard deviations of the various parameters were calculated for the group of 11-month-old birds for each paramater.

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[8]Gorman-Rupp Industries, Inc., Bellville, Ohio.

Results

A. Old Birds. Alterations in the respiratory and circulatory parameters were noted at specific time intervals following bilateral, cervical vagotomy in birds 30 months old. The results for each individual bird are illustrated in Figs. 16 through 20, with the control bird, which was the subject of a sham vagotomy, illustrated in Fig. 21. Time intervals for obtaining readings are noted on the horizontal axis in minutes. It should be noted that the first two control readings, 1.5 and 7 minutes, are measured from the time of completion of the experimental hook-up. The breaks in the time line immediately following the 7-minute control and the RV 10-minute readings are used to represent the varying time required for final isolation of the right and left vagi.

Since the results for the first four vagotomized birds (Figs. 16 through 19) are somewhat similar, they will be summarized and discussed together. Bird 5 (Fig. 20) will be discussed separately with comparisons being made to the first four birds whenever possible. The mean body temperature for these 5 old birds was $40.2 \pm 1.0^{\circ}$ C.

Immediately after the left vagus was cut, a large increase in respiratory period and amplitude occurred. From 10 to 20 minutes post-bilateral, cervical vagotomy a slight acceleration in respiratory period was noted while the respiratory amplitude was seen to remain constant or slightly elevated.

A mirror image relationship between PO_2 and PCO_2 was present

Fig. 16. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from a 30-month-old cock (Bird 1) prior to and following bilateral, cervical vagotomy. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

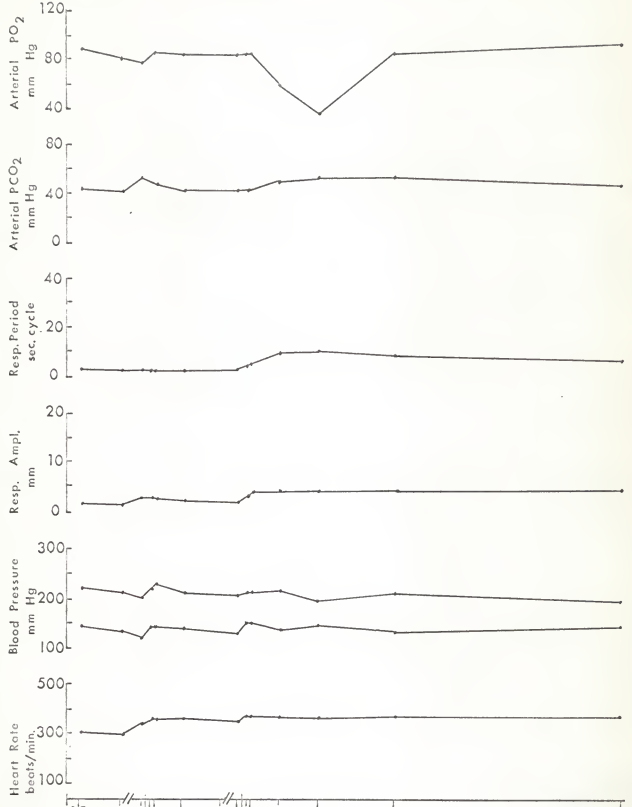

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec. cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 16

Fig. 17. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from a 30-month-old cock (Bird 2) prior to and following bilateral, cervical vagotomy. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

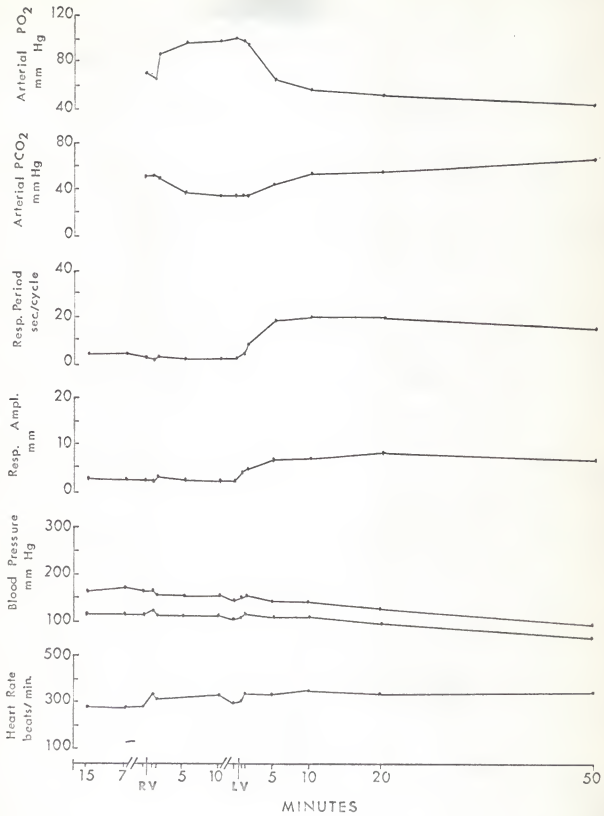

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec./cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
15
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 17

Fig. 18. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from 30-month-old cock (Bird 3) prior to and following bilateral, cervical vagotomy. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

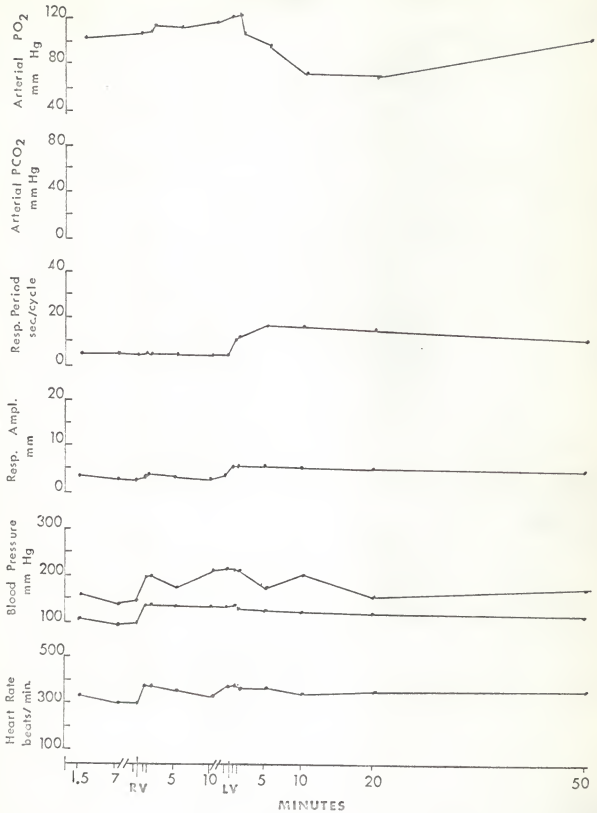

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec./cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 18

Fig. 19. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from a 30-month-old cock (Bird 4) prior to and following bilateral, cervical vagotomy. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

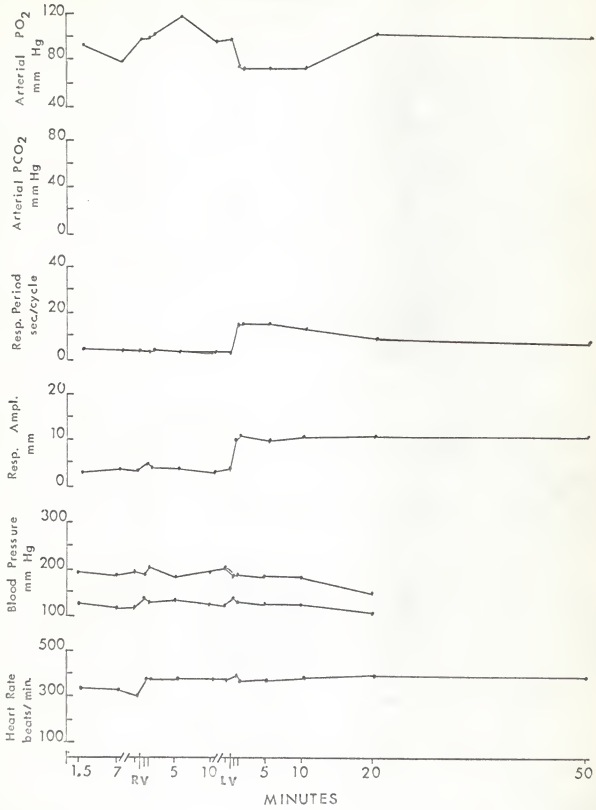

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec/cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 19

Fig. 20. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from a 30-month-old cock (Bird 5) prior to and following bilateral, cervical vagotomy. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves. Bird died at 10 minutes after left vagus was severed.

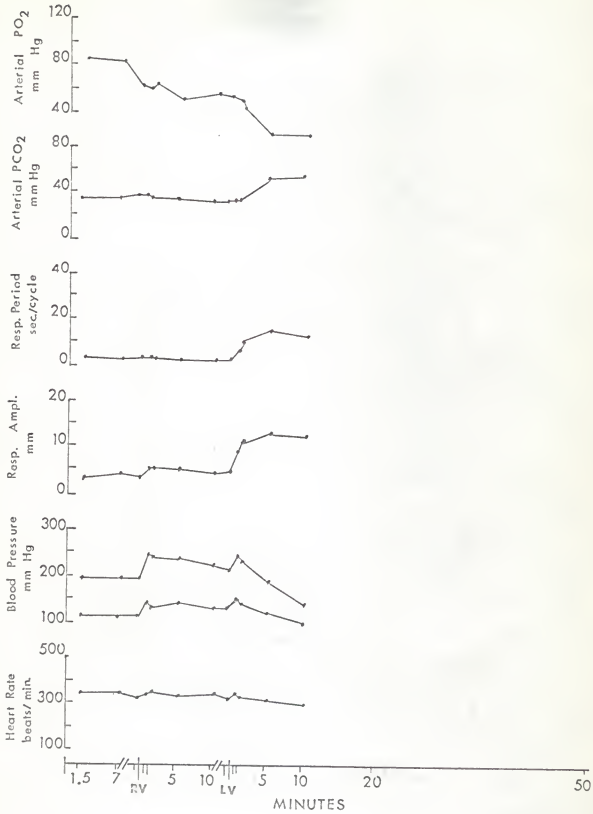

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec./cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 20

Fig. 21. Values of arterial blood PO_2, arterial blood PCO_2, respiratory amplitude, arterial blood pressure, and heart rate from a 30-month-old cock (Bird 6) in which a sham bilateral, cervical vagotomy was performed. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

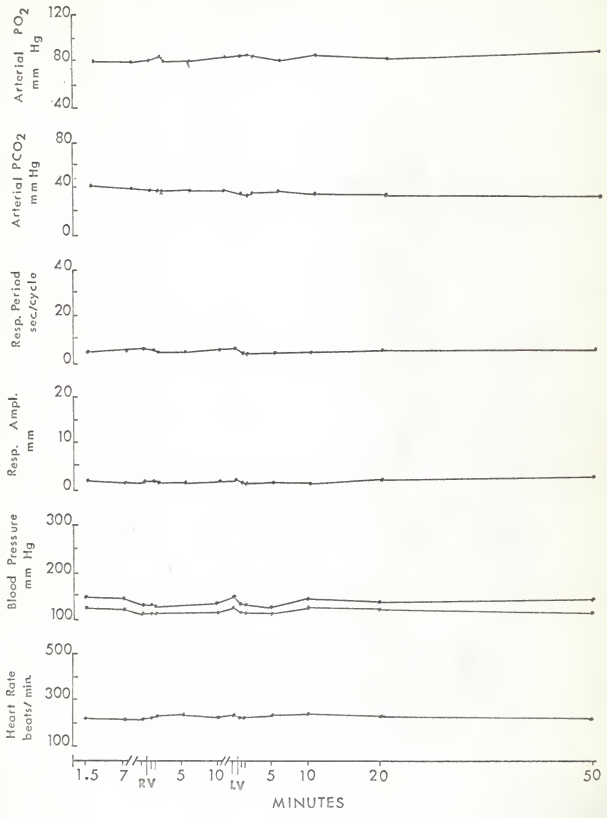

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec./cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 21

for the first two birds. Unfortunately, the membrane for the PCO_2 electrode was disrupted during experiments 3 and 4 (Figs. 18 and 19), so that no final calibrations could be obtained, and the results of the PCO_2 measurement for these two experiments had to be eliminated. The increase in PO_2 coupled with the decrease in PCO_2 following sectioning of the right vagus can be correlated with the slight increase in respiratory amplitude and no change in respiratory period, thus indicating that a slight degree of hyperventilation was occurring following unilateral, cervical vagotomy. A profound decrease in PO_2 and an increase in PCO_2 was seen to occur 1 to 5 minutes following sectioning of the left vagus. Stabilization or a trend back towards normal for the PO_2 and PCO_2 values occurred in birds 1, 3, and 4 (Figs. 16, 18, and 19) at 10 to 20 minutes after the vagi were severed. However, for bird 2 (Fig. 17) a continuous fall in PO_2 and elevation in PCO_2 was noted even though the respiratory period and amplitude remained constant at their elevated levels. It should be mentioned at this time that bird 2 expired shortly after the LV 50-minute readings were acquired, whereas the other three birds were still vigorous when the experiments were terminated.

No consistent trends could be found in circulatory changes following unilateral and bilateral vagotomy with the possible exception of a slight increase in heart rate within 30 seconds post-sectioning of the nerves. Whether this was due to excitation caused by the final isolation of the nerves, or due to severance of the nerves per se, is difficult to say. It can be

noted that following the slight rise after severance of each nerve, the faster rate was maintained, indicating a possible lack of vagal tone.

Blood pressure alterations following unilateral and bilateral vagotomy were so erratic and transitory that it is difficult to draw any conclusions. A slight systolic and diastolic pressure increase can be found immediately following the severance of the nerves, though again, the actual cause for this is hard to differentiate.

The data obtained for old bird 5 which expired 10 minutes after bilateral vagotomy are depicted in Fig. 20. The profound decrease in PO_2 occurring just prior to sectioning of the right vagus was unexplainable since it was not accompanied by any comparable alterations in the other parameters. An abrupt drop in blood pressure and PO_2 and an increase in PCO_2, respiratory rate and amplitude following sectioning of the left vagus were seen in the terminal stages of this experiment.

A sham vagotomy was performed on one old bird (Fig. 21) to show the normal response pattern to this procedure while the vagi were left intact. The lack of a measurable change in all parameters indicates that indeed all alterations recorded in the succeeding experiments were due to sectioning of the vagi. The slight increase noted in blood pressure during the isolation of the left vagus nerve was probably due to excitement stemming from manipulation of the subject. The mean body temperature of the bird throughout the procedure was $39.4 \pm 0.2^{\circ}$ C.

Postmortem examination of the lungs of all five vagotomized

old birds showed extensive vascular congestion, whereas the lungs of the sham vagotomized bird appeared to be normal.

B. Young Birds. To determine the relevance of age to the alterations seen in respiratory and circulatory patterns following bilateral, cervical vagotomy, nine birds 11 months of age were vagotomized and readings were taken at the same intervals of time as for the old birds in the preceding study. A composite graph for the nine birds showing the means and standard deviations is shown in Fig. 22. Due to the close proximity of the means for the diastolic and systolic blood pressures, only the positive standard deviation is shown for the systolic pressure and only the negative standard deviation is given for the diastolic pressure.

Alterations in respiratory amplitude and period were very similar to those measured in the old bird studies. The slight changes following unilateral vagotomy were apparent in both studies as were the profound increases immediately following bilateral vagotomy. In both sets of birds the increase in respiratory period and stabilization of respiratory amplitude occurred at 5 to 10 minutes after the vagi were severed.

A comparison of the blood gases for the two groups revealed a larger decrease in the arterial PO_2 for the young birds as compared to the old birds after sectioning of the left vagus. However, it is difficult to make exact comparisons between the two sets of data since the data for the old birds is presented on an individual basis and that for the young birds is shown in composite form with wide standard deviations being present. The

Fig. 22. Means and standard deviations of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from nine, 11-month-old cocks prior to and following bilateral, cervical vagotomy. Only positive standard deviations for systolic blood pressure and negative standard deviations for diastolic blood are shown. Right and left vagi were severed at RV and LV. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

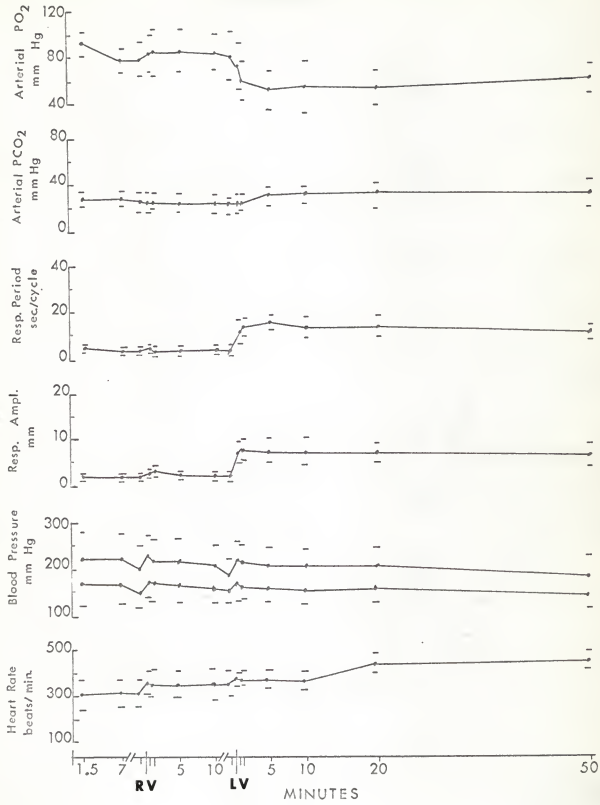

Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec/cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/ min.
500
300
100
1.5
7
5
10
5
10
20
50
RV
LV
MINUTES

FIGURE 22

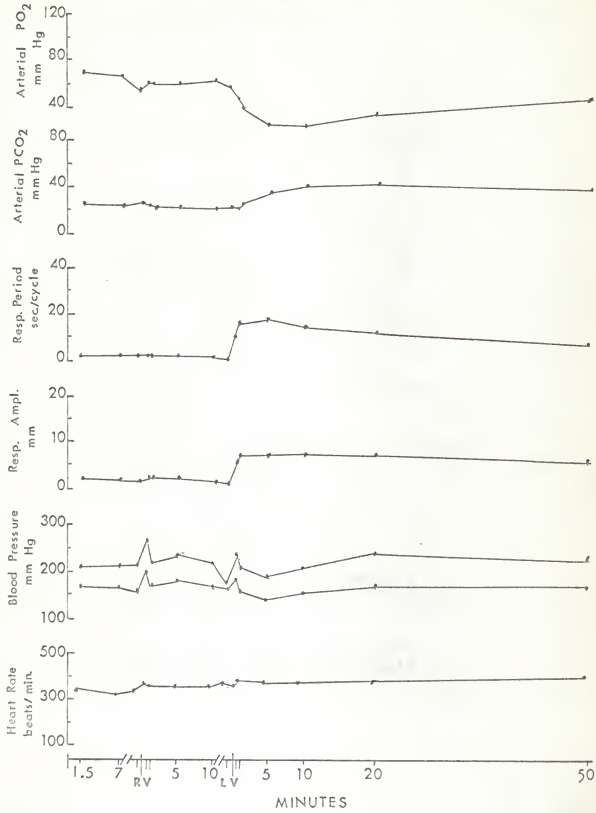
PCO_2 values appeared to be just opposite, with the old birds showing the higher accumulation of CO_2.

The outstanding difference in the alteration in the circulatory patterns between old and young birds was the large increase in heart rate occurring between 10 and 20 minutes after the left vagus was cut in the young birds. This increase was not apparent for the old birds. Changes in blood pressure were very similar between the two sets of birds with the possible exception that the young birds showed higher levels of pressure than did the old birds.

A Beckman model GS pH meter was used in an attempt to monitor the pH in the young birds. This required the withdrawal of two milliliters of blood for each reading which was immediately replaced in six of the birds by an injection of an equal amount of blood obtained by exsanguating the previous research subject. In three birds no replacement of the blood occurred. The results for a representative individual of each group are shown in Fig. 23 (transfused) and Fig. 24 (non-transfused). When these two graphs were compared with each other and with the composite graph in Fig. 22, little difference was noted. The graph in Fig. 24 for the non-transfused group did show a slight decrease in blood pressure.

A sham vagotomy was again performed on a young bird, to show the normal response pattern to this procedure when the right and left vagi were not cut. The results of this experiment are shown in Fig. 25 and indicate that all changes recorded in succeeding experiments were due to the bilateral, cervical

Fig. 23. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from 11-month-old cock prior to and following bilateral, cervical vagotomy. Two milliliter blood sample for pH determination was replaced immediately with 2 ml. whole blood. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.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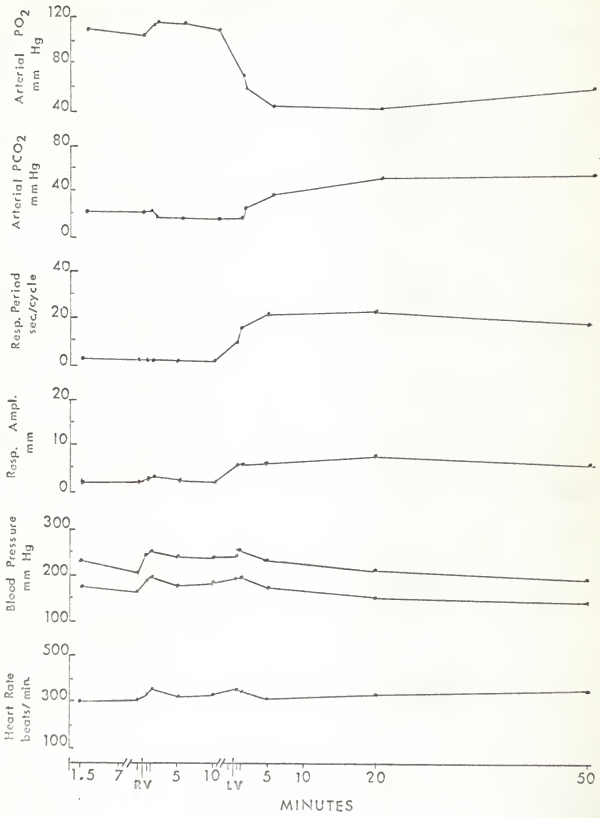
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec/cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 23

Fig. 24. Values of arterial blood PO_2, arterial blood PCO_2, respiratory period, respiratory amplitude, arterial blood pressure, and heart rate from 11-month old cock prior to and following bilateral, cervical vagotomy. Two milliliter blood sample for pH determinations was not replaced immediately with 2 ml. whole blood. Right and left vagi were severed at RV and LV, respectively. Breaks in time line prior to control reading before severance of the vagi represent variable time intervals required for final isolation of nerves.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120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec./cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/ min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 24

Fig. 25. Values of arterial blood PO_2, arterial blood PCO_2, respiratory amplitude, arterial blood pressure, and heart rate from 11-month-old cock in which a sham bilateral, cervical vagotomy was performed. Breaks in time line prior to control readings before severance of the vagi represent variable time intervals required for final isolation of nerves.

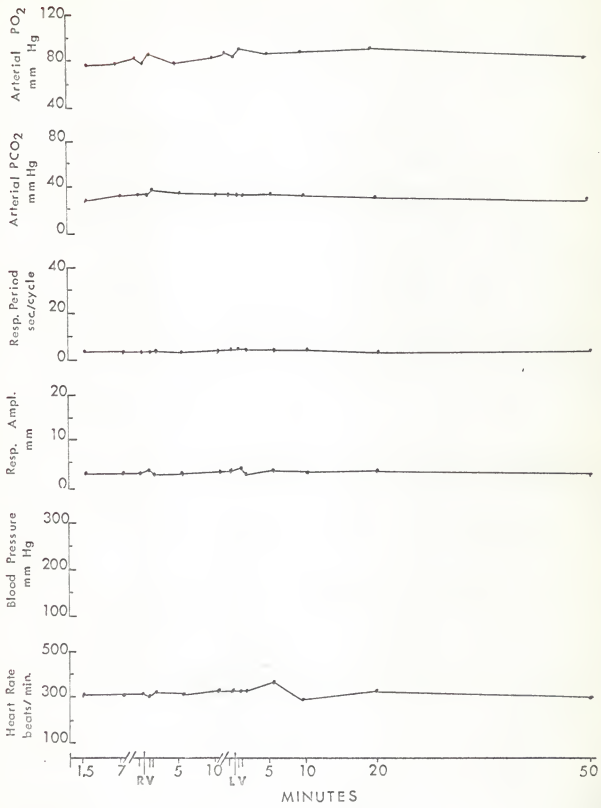
Arterial PO_2 mm Hg
120
80
40
Arterial PCO_2 mm Hg
80
40
0
Resp. Period sec./cycle
40
20
0
Resp. Ampl. mm
20
10
0
Blood Pressure mm Hg
300
200
100
Heart Rate beats/min.
500
300
100
1.5
7
RV
5
10
LV
5
10
20
50
MINUTES

FIGURE 25

vagotomy. Blood withdrawn for attempted pH readings was replaced immediately in this control bird. Mean body temperature for this bird was 40.97 ± .03° C.

Mean body temperature for the transfused bird (Fig. 23) was 41.0 ± .5° C., for the non-transfused bird (Fig. 24) was 40.6 ± .01° C., and for all nine vagotomized birds was 41.0 ± .09° C.

Postmortem examination of the lungs of all nine vagotomized young birds revealed a slight vascular congestion in only one bird. The lungs of the sham vagotomized bird appeared normal.

Discussion

On the basis of the present studies, age related differences could be noted in the changes in carbon dioxide and oxygen tensions following bilateral, cervical vagotomy. The PCO_2 and PO_2 levels were higher in the older birds than they were in the younger ones. Since all five of the vagotomized old birds showed extensive pulmonary vascular congestion while only one of the nine vagotomized young birds exhibited a slight degree (in agreement with Fedde et al., 1963a and 1963b); the higher PCO_2 values in the older birds were expected due to a reduced respiratory exchange. Following this line of reasoning it also would be expected that the PO_2 values would be lower in the older birds than in the younger ones after bilateral vagotomy. Unfortunately, the latter was not the case, since the younger birds showed a much greater decrease in oxygen tension. Considering the absence of pulmonary vascular congestion in the younger birds, the validity of these low PO_2 readings was

questionable.

From the results of the first study (Part I) it was noted that metabolism of the nucleated erthrocyte and the formation of a barrier due to the adhering of the nuclei from hemolyzed RBC's to the electrode membrane could both account for erroneously low PO_2 values. In the discussion of the first study (Part I) it was computed that PO_2 values for nucleated, chicken blood held at 40° C decayed at the rate of approximately 19 mm. of Hg./min. because of metabolism of the cell. At the flow rate of 9 ml./min. which was used in this study, it would require approximately six seconds for blood to traverse the distance from the thoracic area of the chicken to the oxygen electrode. During this time, a decrease in PO_2 of approximately 1.9 mm. Hg. would result due to metabolism of the red blood cell. Due to the minute decrease in PO_2 in this time interval, it was readily apparent that the metabolism factor could be eliminated as a reason for the low PO_2 values recorded for younger birds following bilateral, cervical vagotomy.

It was also theorized in the first study (Part I) that the barrier comprised of nuclear material from hemolyzed RBC's was apparently removed by flushing saline through the cuvette since the initial and final gas calibrations were very similar. The older birds were given 30 units of heparin in every 30 minutes and the cuvette was flushed every 15 minutes to prevent clotting; while in the younger birds, the dosage of heparin was increased to 100 units every 30 minutes at which time the flow rate was checked and the system was flushed if necessary. On

examination of the records from the study involving younger birds, it was noted that the post-flushing PO_2 readings were approximately 20 mm. Hg. higher than the pre-flushing readings. This lack of systematic flushing of the cuvette could result in erroneously low PO_2 values. Since CO_2 diffuses at a much greater rate than O_2 (20.7 to 1), the increased barrier would not affect CO_2 to the degree it does O_2 (Comroe, 1965). Additional studies in which systematic flushing of the cuvette system would be accomplished are required before it can be determined if the exceedingly low PO_2 values were valid results of bilateral, cervical vagotomy in the young birds.

The moderate circulatory effect of bilateral vagotomy was in agreement with results reported by Stubel (1910), who found that in birds with small hearts in relation to their body size (i.e., the chicken) that sectioning the vagi had little effect on the heart rate. These results are in contrast to data reported by Fedde et al. (1963) in which they found that anesthetized birds had a profound heart rate increase following bilateral vagotomy. Considering the fact that all manipulations in the present study were accomplished under local anesthesia instead of general anesthesia, it would be feasible that the state of excitement of the birds resulted in high initial heart rate which could mask the sharp increase sited by Fedde.

One of the purposes of this study was to determine whether the increase in respiratory amplitude could offset a reduction in ventilation due to the increase in respiratory period. If the decision is based upon the PCO_2 changes alone, this

compensation did not occur. The rise in PCO_2, which was directly correlated on a time basis with the alterations in respiratory period and amplitude, indicated a lower degree of ventilation. The fact that the PCO_2 values stabilized at a higher than normal level by 10 to 20 minutes following bilateral, cervical vagotomy could be accounted for by (1) a decrease in respiratory period, (2) an increase in stabilization of respiratory amplitude, and/or (3) a possible decrease in the degree of pulmonary, vascular congestion.

Summary

In this study, alterations in blood gas tensions, respiratory rate and amplitude, blood pressure and heart rate following bilateral, cervical vagotomy in 11 and 30-month-old cocks were recorded to determine the effect of age on the efficiency of pulmonary ventilation after bilateral vagotomy.

The conclusions drawn from this study were as follows:

(1) To monitor accurately continuous PO_2 tensions in chicken blood, frequent flushing of the cuvette module to remove nuclei from hemolized RBC's which were adhering to the sensing membrane was required.

(2) The incidence of pulmonary vascular congestion was an age related phenomena with older birds being the more susceptible.

(3) Carbon dioxide tension values in the blood were higher in older cocks than they were in younger ones.

(4) A typical bilateral vagotomy breathing pattern,

increased amplitude and period, was recorded in both age groups showing accelerative changes in this pattern with time.

(5) The circulatory effect of bilateral vagotomy was an increased heart rate with little effect on diastolic or systolic blood pressure.

(6) The increased respiratory amplitude did not increase pulmonary ventilation sufficiently to overcome the increase in respiratory period so that elevated levels of PCO_2 and lowered levels of PO_2 occurred following bilateral, cervical vagotomy.

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BLOOD GAS CHANGES ASSOCIATED WITH RESPIRATORY ALTERATIONS FOLLOWING BILATERAL VAGOTOMY IN *GALLUS DOMESTICUS*

by

RICHARD ALTON BOSTER

B. S., Kansas State University, 1960
D. V. M., Kansas State University, 1960

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Physiology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

Arterial PO_2 and PCO_2 were continuously monitored in 10 and 30-month-old male chickens with a Beckman oxygen macroelectrode and a Severinghaus PCO_2 electrode to determine: (1) Whether the increase in respiratory amplitude following bilateral, cervical vagotomy compensates for the increase in respiratory period in maintaining normal pulmonary ventilation; and (2) what effect the age of the bird had on this possible compensation.

Prior to the above study the reliability of the PO_2 and PCO_2 sensing electrodes in the measurement of blood gas tensions in nucleated chicken blood at 40° C. was determined by in vitro gas-blood equilibration comparisons using old and fresh chicken blood, saline and fresh, mammalian (canine) blood. The significant findings of the study were: (1) The PO_2 readings were lower in freshly withdrawn chicken blood following equilibration than in the equilibrating gas, but were much closer to that of the equilibrating gas than when old blood was used; (2) The PCO_2 readings of freshly withdrawn chicken blood following equilibration were higher than that of the equilibrating gas, whereas a significant difference did not occur for old chicken blood; (3) The PO_2 and PCO_2 readings of equilibrated physiological saline were not significantly different from the tensions of the equilibrating gases indicating that the electrodes were capable of reliably detecting gas tensions in fluids; (4) Following equilibration, slight but significantly lower PO_2 readings were obtained from canine blood than were obtained from the equilibrating gas; while significantly higher PCO_2 readings were obtained for the blood as compared to the equilibrating gas.

From the above findings the theory was proposed that the differences in gas tensions between blood and equilibrating gases were due to: (1) A barrier being formed at the site of the sensing membrane by the nuclei of the chicken erythrocytes which had been freed by the hemolysis of the cells; and (2) active respiration of the nucleated chicken cells which tended to utilize oxygen and produce carbon dioxide in the sample during the analysis.

Data from the study involving alterations in blood gas tensions, respiratory rate and amplitude, blood pressure and heart rate following bilateral, cervical vagotomy indicated the following: (1) The incidence of pulmonary vascular congestion was an age related phenomenon with older birds being the more susceptible; (2) carbon dioxide tension values in the blood were higher in older cocks than they were in younger ones; (3) a typical bilateral vagotomy breathing pattern, increased amplitude and period, was recorded in both age groups with both groups showing accelerative changes in this pattern with time; (4) the increased respiratory amplitude did not by itself offset the increased respiratory period which results from bilateral, cervical vagotomy. This was evidenced by the rise in $P^{CO}{}_2$ levels in both sets of birds; and (5) the circulatory effect of bilateral vagotomy was an increased heart rate with little effect on diastolic or systolic blood pressure.