

5121

RESPIRATION AND CIRCULATION IN AMPHIUMA TRIDACTYLUM

by

DANIEL PETER TOEWS

B.Sc., University of Alberta, 1963

M.Sc., University of Alberta, 1966

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the department
of

ZOOLOGY

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

AUGUST, 1969

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and Study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology

The University of British Columbia
Vancouver 8, Canada

Date Sept. 8, 1969

ABSTRACT

Respiratory and circulatory changes which accompany submergence were studied in Amphiuma tridactylum, an aquatic urodele. All experimentation was performed at 15° C with the exception of oxygen consumption recordings taken at 25° C. The lungs, systemic arches, pulmonary arteries, pulmonary veins, post caval vein and dorsal aorta were cannulated. Serial sampling procedures enabled PO₂ and PCO₂ measurements to be made through several breathing cycles on all animals.

Oxygen consumption in Amphiuma at 15° C was the lowest recorded for any amphibian at a comparable temperature. It was found that the lungs were the primary respiratory exchange surface for oxygen consumption and were used very little for carbon dioxide elimination.

Oxygen tensions in the major vessels showed large fluctuations which were related to the breathing cycle of the animal. Amphiuma breathed about once every hour at 15° C and it was just after a breath that oxygen tensions in the lungs and major vessels were the highest. There was a definite gradient between the pulmonary artery and systemic arch which persisted throughout the breathing cycle.

Termination of inspiration in Amphiuma was shown to be controlled by a volume detection mechanism.

It was found that low oxygen tensions in the blood brought about a breathing response whereas there was no relationship between the carbon dioxide levels in the body and the breathing response.

The pulmonary artery had a lower diastolic blood pressure than did the systemic arch. There was a slight pulse lag in the systemic arch when compared to the pressure rise in the pulmonary artery such that a pressure rise was not recorded in the systemic arch until the blood pressure in the two circuits became equal. It was suggested that the pulse lag could account for deoxygenated blood being primarily shunted to the pulmonary circuit, and oxygenated blood being shunted to the systemic circuit.

TABLE OF CONTENTS

INTRODUCTION	1
GENERAL MATERIALS AND METHODS	5
A. ANIMALS	5
B. OPERATIONS AND CANNULATIONS	5
C. ANALYTICAL PROCEDURES	11
D. MEASUREMENT OF OXYGEN CONSUMPTION	12
E. ANIMAL SURVIVAL	15
PART I. GAS TENSIONS IN THE LUNGS AND MAJOR BLOOD VESSELS IN A FREE-MOVING AMPHIBIAN, <u>AMPHIUMA TRIDACTYLUM</u> .	
INTRODUCTION	16
MATERIALS AND METHODS	19
RESULTS	20
DISCUSSION	42
PART II. RESPIRATORY CONTROL IN <u>AMPHIUMA TRIDACTYLUM</u>	
INTRODUCTION	55
MATERIALS AND METHODS	57
RESULTS	58
DISCUSSION	73
PART III. SOME FEATURES OF THE CIRCULATION IN <u>AMPHIUMA TRIDACTYLUM</u>	
INTRODUCTION	80
MATERIALS AND METHODS	85
RESULTS	85

	iv
DISCUSSION	94
GENERAL SUMMARY	101
LITERATURE CITED	104

LIST OF TABLES

	Pages
I Types of cannulae and numbers of animals used.....	19
II Oxygen consumption measured in fifteen <u>Amphiuma</u> at 15° and 25° C.....	39
III Extension of the time between breaths by injecting atmospheric air into the lungs..	59
IV Air removal from the lungs during inspiration.....	60
V Injection of nitrogen into the lungs of <u>Amphiuma</u> which were about to surface to breathe	61
VI Carbon dioxide injections into the lungs of <u>Amphiuma</u>	65
VII Nitrogen injections into the lungs of <u>Amphiuma</u>	70

LIST OF FIGURES

		Pages
Figure 1	A drawing of the techniques used in the cannulation of <u>Amphiuma tridactylum</u>	8
Figure 2	The major blood vessels, lungs and heart of <u>Amphiuma tridactylum</u>	10
Figure 3	Apparatus used for the measurement of oxygen consumption in <u>Amphiuma tridactylum</u>	14
Figure 4	PO ₂ decrease in the lungs of four <u>Amphiuma tridactylum</u>	21
Figure 5	Alveolar "PO ₂ -PCO ₂ diagram" for six <u>Amphiuma</u>	23
Figure 6	The relationship between lung volume and body weight in <u>Amphiuma</u>	25
Figure 7	PO ₂ and PCO ₂ changes which occurred in the lungs and dorsal aorta in one animal during four breathing cycles	27
Figure 8	PO ₂ gradient between the dorsal aorta or systemic arch and the lung	28
Figure 9	Changes which occurred in the pulmonary vein and lung PO ₂ and PCO ₂ levels in one animal during five breathing cycles	29
Figure 10	PO ₂ gradient between the pulmonary vein and lung in <u>Amphiuma</u>	31
Figure 11	PO ₂ and PCO ₂ changes which occurred in the pulmonary artery, systemic arch and lung in one animal during one complete breathing cycle	32
Figure 12	PO ₂ gradient between the pulmonary artery and systemic arch in eight <u>Amphiuma</u>	33
Figure 13	PO ₂ levels in the systemic arch and pulmonary artery (from Fig. 12) converted to per cent saturation	35

Figure 14	PO ₂ and PCO ₂ gradient between the post caval vein and dorsal aorta in five <u>Amphiuma</u>	36
Figure 15	"The Standard <u>Amphiuma</u> ". A reconstruction of the probable PO ₂ fluctuations in the lungs and major blood vessels during three breathing cycles	38
Figure 16	Rates of oxygen consumption at 15° C in ten <u>Amphiuma</u> measured by a volume change technique	40
Figure 17	The probable mode of gas exchange in <u>Amphiuma tridactylum</u>	53
Figure 18	The removal of high concentrations of carbon dioxide and normal levels of oxygen from the lungs of four <u>Amphiuma</u>	63
Figure 19	Arterial PO ₂ levels through several breathing cycles in <u>Amphiuma</u>	67
Figure 20	Artificial increase in tank water PO ₂ and the corresponding increase in dorsal aorta PO ₂	69
Figure 21	PO ₂ levels in the dorsal aorta and systemic arch in five <u>Amphiuma</u> at the time of breathing	72
Figure 22	Diving bradycardia or breathing tachycardia in five <u>Amphiuma</u>	87
Figure 23	Breathing and lung inflation and deflation effects on the pulmonary artery (PA) and systemic arch (SA)	88
Figure 24	Injections of high concentrations of carbon dioxide into the lungs and the associated pressure changes in the systemic arch	90
Figure 25	Simultaneous pressure recordings in the pulmonary artery and systemic arch showing the pulse lag in the systemic arch	91

Figure 26	Pressure relationships in the pulmonary artery and systemic arch in;	
	a. slowly beating heart.	
	b. superimposed pressure recordings from the pulmonary and systemic arches in another <u>Amphiuma</u>	92

ACKNOWLEDGEMENTS

I wish to thank Dr. David Randall, Chairman of my committee, for his guidance and enthusiasm throughout the study. I also thank Dr. W. S. Hoar, Dr. A. M. Perks, Dr. J. E. Phillips, Dr. D. R. Jones and Dr. G. Shelton for their critical reviews of the manuscript.

I also wish to thank my wife, Lorette, for her constant encouragement throughout the study.

I am grateful also to the following persons: Dr. J. R. Ledsome, Physiology Department, U.B.C., for discussions pertaining to the Third section of this thesis; Dr. G. Shelton, Biology Department, The University of East Anglia, for his useful advice and assistance during the summer of 1968; and my colleagues, who provided not only useful ideas throughout my study, but also made my stay at the University of British Columbia an enjoyable one.

Financial support was provided by two Teaching Assistantships from the University of British Columbia and a bursary from the Fisheries Research Board of Canada. The research was supported by grants from the National Research Council, The Fisheries Research Board of Canada and the British Columbia Heart Foundation.

GENERAL INTRODUCTION

Interest in the respiratory and circulatory dynamics in the Amphibia dates back to the 19th century when Brücke (1852) in Germany and later Sabatier (1873) in France examined, with primitive techniques by our standards, the anatomy and physiology of frogs and other vertebrates. They postulated what is now known as the "Classical Hypothesis" of amphibian double circulation.

In brief, the hypothesis states that deoxygenated blood from the right atrium enters the ventricle and remains on the right side whereas oxygenated blood from the left atrium enters and remains on the left side of the ventricle. Second, there is very little mixing of the two types of blood as a result of the trabeculate nature of the ventricle. Third, the spiral valve in the conus directs the deoxygenated blood to the pulmonary circuit, aided by a lower pressure in the pulmonary vessels. The result of this channeling is that the pulmonary circuit contains primarily deoxygenated blood and the systemic circuit oxygenated blood.

The points of agreement today with this earlier hypothesis are that there is a separation of the two types of blood in the ventricle and that the blood which flows to the pulmonary circuit appears to be relatively

deoxygenated and comes from the right atrium. The oxygenated blood from the left atrium is thought to be primarily shunted to the systemic circuit. Recent work in this area has been done by Foxon (1947 and 1964), Hazelhoff (1952), De Graaf (1957), Sharma (1957), Simons (1959), DeLong (1962), Johansen (1963) and Johansen and Ditadi (1966). The actual mechanism whereby oxygenated blood enters the systemic circuit and deoxygenated blood shunted to the pulmonary circuit is obscure.

The amphibian, Amphiuma tridactylum, used in this study is an aquatic urodele found in freshwater drainage ditches, ponds and slow moving streams in the southeastern United States. The reasons for choosing this animal are that it is large, the blood vessels are large and accessible for cannulation and minimal maintenance is required to keep these animals in the laboratory. Recovery from operations is rapid and complete. The animal breathes very infrequently (at 15° C breathing occurs every 45 to 75 minutes (Darnell 1948)), and therefore changes in blood gas tensions between breaths can be easily monitored. Many amphibians take up oxygen through their skins as well as their lungs but Amphiuma, although aquatic and unable to survive on land, is dependent upon access to the atmosphere and cannot

remain submerged for more than four to five hours (Darnell 1948). This amphibian therefore probably takes up most of its oxygen via the lungs. The lungs are large, elongated sacs which extend at least 4/5 of the way along the body cavity. The circulatory system is typically amphibian except for the lack of a cutaneous branch from the pulmonary artery and the absence of the ductus Botalli (Baker 1949). The nucleated red blood cells are the largest known in any animal (70 to 80 microns in diameter).

In the first section of this study, oxygen and carbon dioxide tensions in the major blood vessels and lungs were measured in free-moving, unanaesthetized animals. Oxygen consumption at 15 and 25°C was also measured in several animals.

In the second section the animals' breathing responses to the injections of various oxygen and carbon dioxide concentrations into the lungs were determined. Termination of inspiration and the various stimuli involved were also studied.

In the third section experiments were performed to determine the mechanism whereby oxygenated blood was sent to the systemic circuit and deoxygenated blood to the pulmonary circuit. Experiments were also designed

to show the effects of inspiration and expiration on the circulatory system.

In summary, the objectives of this study were to obtain a more complete picture of the physiology of gas exchange in one species of amphibian and attempt to elucidate the mechanisms involved in the associated respiratory and circulatory processes.

GENERAL MATERIALS AND METHODS

A. ANIMALS

The amphibians used in this study, Amphiuma tridactylum, were obtained every four to six weeks from the North Carolina Biological Supply Company in Durham, North Carolina, U.S.A. All animals were maintained at the University of British Columbia in thirty gallon aquaria containing fresh dechlorinated water at 12-18°C. The animals were used for experiments within six weeks of arrival and feeding was not necessary. Survival was good and the animals did not show any sign of furunculosis if the water in which they were maintained was allowed to become somewhat "swampy", similar to their normal habitat.

All of the animals used were adults, ranging in weight from 250-1000 g and in length from 50-100 cm. Since actual metabolic rate was not an important parameter in the majority of experiments, male and female animals were used at random.

B. OPERATIONS AND CANNULATIONS

All operations were performed after animals had been anaesthetized by total immersion in a solution of MS 222 (tricaine methanesulphonate, Sandoz) at concentrations of 15 g/l. Anaesthesia occurred within 20-40

minutes and the animal remained anaesthetized for $\frac{1}{2}$ to $\frac{3}{4}$ of an hour at this dosage. Care was taken to keep the skin moist throughout an operation. After the operation the animals recovered fully in fresh water within one hour. In all experiments which required an operation, the right lung was cannulated and lungs were artificially ventilated with atmospheric air to speed up recovery.

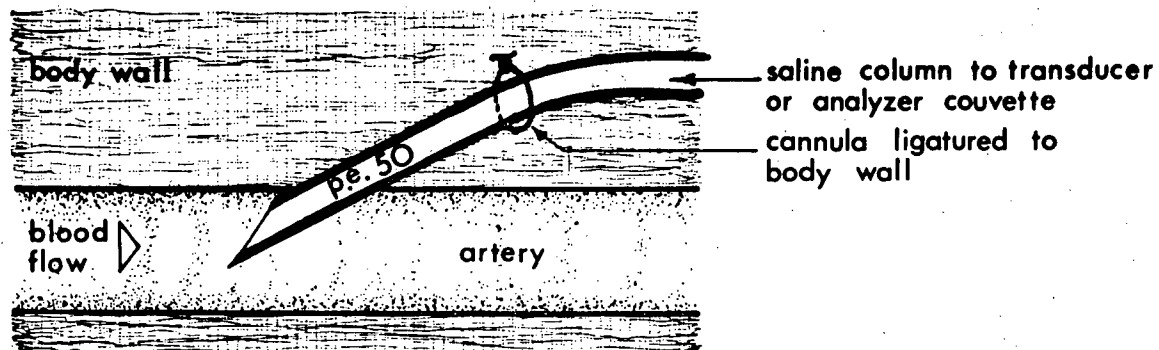
Various veins and arteries were cannulated and in all cases indwelling, chronic, polyethylene cannulae were used. Blood vessel and lung cannulae were all made from Clay-Adams P.E. 50 tubing (I.D. 0.023 inches, O.D. 0.038 inches). Blood cannulae were filled with heparinized saline (250 IU per ml) to prevent clotting.

Depending upon the type of cannulation desired, a 5 cm incision was made in the ventral musculature and body wall either at a point midway between the anterior appendages (for pulmonary artery, systemic arch and pulmonary vein cannulations) or 5 cm anterior to the vent (tip of the lung, post caval vein or dorsal aortic cannulations). After the operation the pericardium was sewn up and the incision in the body wall closed with Clay-Adams 9 mm wound clips.

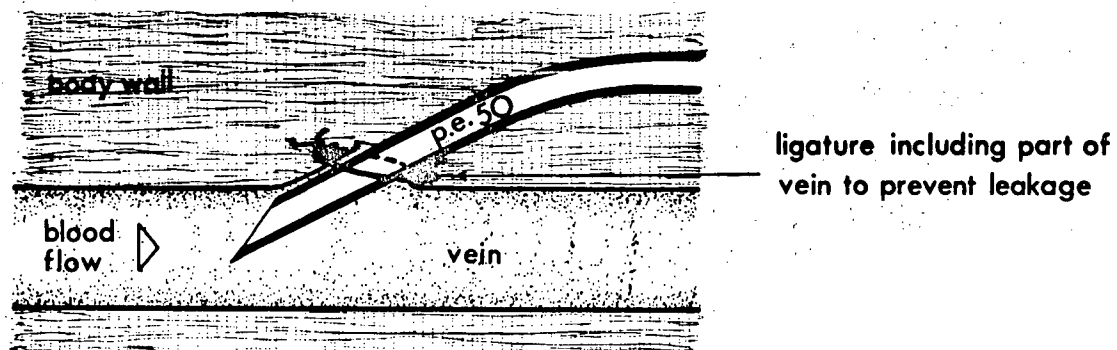
Cannulation of the pulmonary artery, dorsal

aorta and systemic arch are relatively easily performed by sharpening a 55-65 cm length of P.E. 50 tubing, filling it with heparinized saline, attaching a 1 ml hypodermic syringe to the unsharpened end and inserting the sharpened end into the artery (Fig. 1). It was important that cannulae inserted into the pulmonary artery and systemic arch were of a similar length such that meaningful comparisons of blood pressure in the two vessels could be made. After the artery had been exposed and a free 0.5 cm portion of the vessel clamped, a 23 gauge needle was used to puncture a small hole in the artery. The sharpened portion of the cannula was then forced into the vessel and the clamps removed. The muscular nature of the artery forms a tight seal around the cannula and there was no leakage of blood from the point of entry into the vessel. The size of the indwelling cannulae was never more than one third the size of the vessel, minimizing the effect of the cannulae on the pattern of blood flow in the vessel. After insertion the cannula was tied into place, usually to a piece of strong tissue beside the vessel. At the point of exit from the body, the cannula was also sutured to the skin of the animal so that minor movements of the animal would not result in the cannula being pulled from the vessel.

Figure 1 A drawing of the techniques used in the
cannulation of Amphiuma tridactylum.
a. arterial cannulation
b. venous cannulation.



A. ARTERIAL

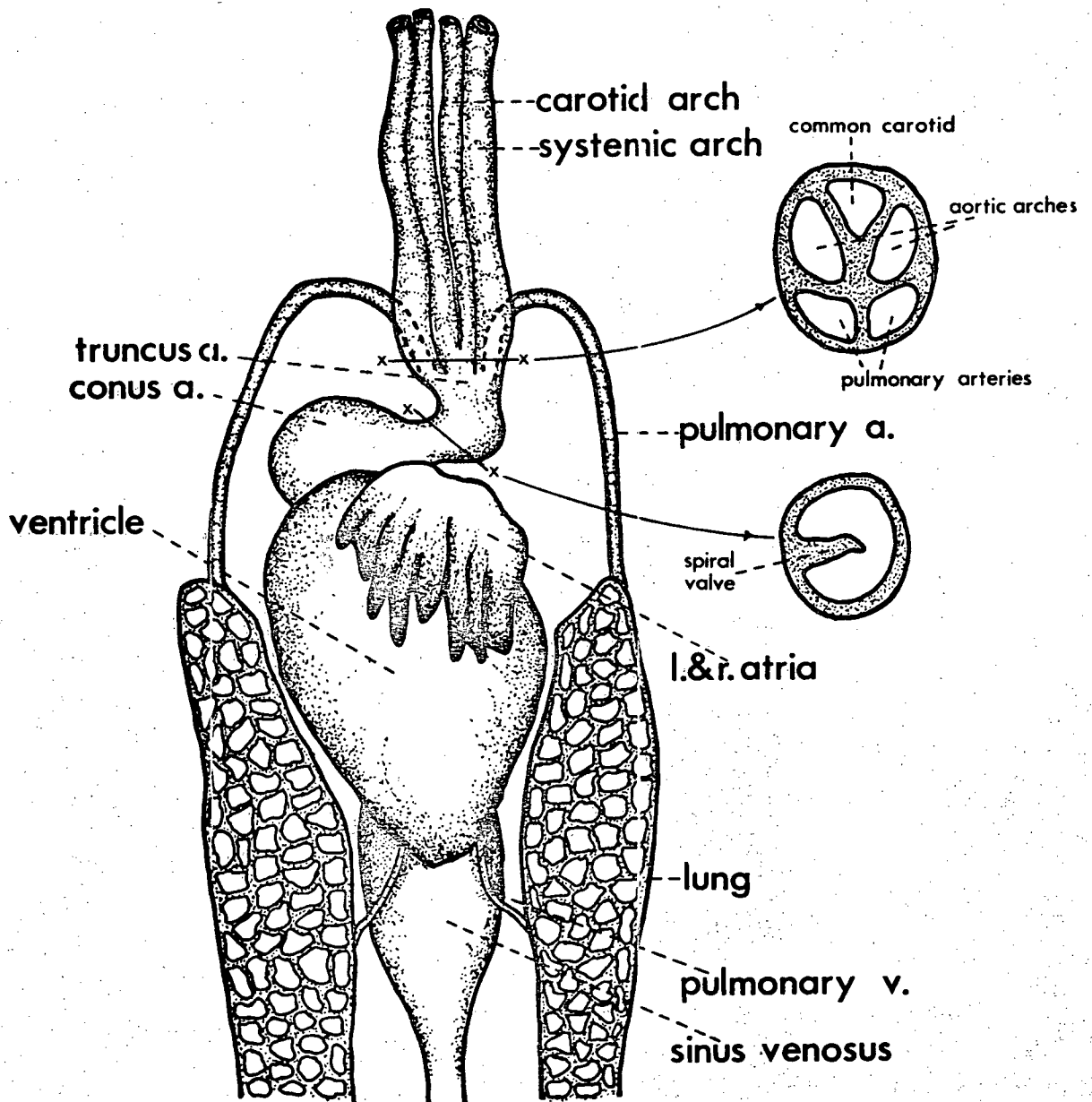


B. VENOUS

Venous cannulations, on the other hand, were more difficult. The pulmonary vein was cannulated at a point where the vein leaves the lung and before it fuses to the sinus venosus. Cannulation procedure was similar to that described for arterial cannulation with the exception that venous cannulae did not remain firmly in the vessel unless the cannula were sutured to the vessel (Fig. 1). In the cannulation of the post caval vein it was possible to enter the larger vessel by forcing the cannula through a small collateral vein. Small collateral veins used in this technique were ones which were draining small fat bodies and it was felt that this would in no way disturb the general circulation of the blood. By cannulation of the veins and arteries using the above mentioned techniques, it was possible to obtain serial samples of blood at any time desired with minimal disturbance to the animal. Figure 2 illustrates the major vessels cannulated and their spacial relationships with the heart.

The distal tip of the right lung of each animal was cannulated by inserting the flared end of a 50 cm length of P.E. 50 tubing into a small incision in the lung and then firmly suturing the lung around the cannula. It was possible to obtain small samples of alveolar gases

Figure 2 The major blood vessels, lungs and heart
of Amphiuma tridactylum.



(through the lung cannula) at any time during the experiments with minimal disturbance to the animal.

C. ANALYTICAL PROCEDURES

1. Blood Gases

Blood oxygen and carbon dioxide tensions were measured on a Radiometer Acid-Base Analyzer Type PMH 71. The PO_2 and PCO_2 electrodes were calibrated with saline equilibrated at the desired partial pressures. Blood was allowed to flow from the animals through the cannulae into the PO_2 and PCO_2 electrode systems; the total amount of blood in the cannulae from one sample never exceeded 0.20 ml. After the reading had been made (3-5 minutes) the blood was gently forced back into the animal, a negligible amount of blood was lost and the total blood volume of the animal was probably not disturbed.

2. Alveolar Gases

Alveolar gas samples of no larger than 0.20 ml were analyzed for oxygen, nitrogen and carbon dioxide on a Varian Aerograph Gas Chromatograph Series 200. The separation columns used in the chromatograph were Silica-gel (screen size 42/60) and Molecular Sieve 5A (screen size 42/60). The columns were arranged in series with the thermal conductivity detector. Standard gas samples used to calibrate the chromatograph were obtained

from The Matheson Company of Canada, Whitby, Ontario and the Canadian Liquid Air Company, Vancouver, B.C. The total amount of time required for the analysis of one sample was six minutes. Gas samples were taken from the lungs in a 0.25 ml Hamilton Syringe (Gastight # 1750 with a Chaney adaptor) and injected into the gas chromatograph.

3. Blood Pressure

To record blood pressure, the cannulae were connected to Statham 23AA (arterial), 23BB (venous), or 23 Db (small volume arterial) pressure transducers and displayed on either a Beckman Type R Dynograph or a Gilson Polygraph. The pressure transducers were calibrated with a column of saline prior to and during all experiments.

A square wave pressure change was applied to the complete system (cannulae, transducers, amplifier and pen recorder) in a method described by Shelton and Jones (1965a) and it was found that the response time of the equipment was 0.2-0.25 msec., ten times faster than any pressure change recorded from the experimental animals.

D. MEASUREMENT OF OXYGEN CONSUMPTION

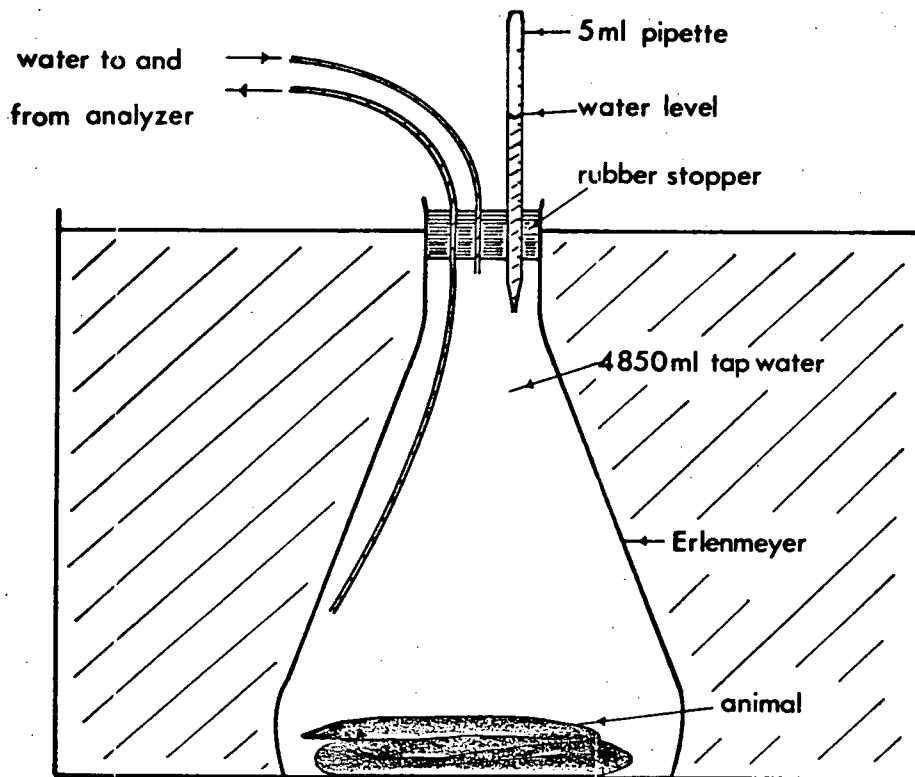
Oxygen consumption in Amphiuma tridactylum

at 15°C was measured in two different ways. In the first method an animal was put into a 4,850 ml Erlenmeyer flask and immersed in a water bath at 15°C. The flask was then filled with water, also at 15°C, and sealed with a rubber stopper perforated with a 5 ml pipette (Fig. 3). The tip of the pipette extended into the animal chamber and was also filled with water. It was assumed from information provided by Krogh (1904), Winterstein et. al. (1944), Jones (1967) and myself (obtained from the first section of this thesis) that very little carbon dioxide is released into the lungs of amphibians. Hence, a drop in the water level in the pipette would be indicative of a decrease in lung volume and therefore the animals volume and would represent the amount of oxygen consumed plus any nitrogen diffusing out of the lung. The animal was allowed to breathe once every hour at which time the flask was sealed again and the water level in the pipette set at zero. Oxygen and carbon dioxide tensions in the water surrounding the animal were continually monitored by pumping water from the chamber through silastic tubing, past the oxygen and carbon dioxide electrodes, and back into the animal chamber.

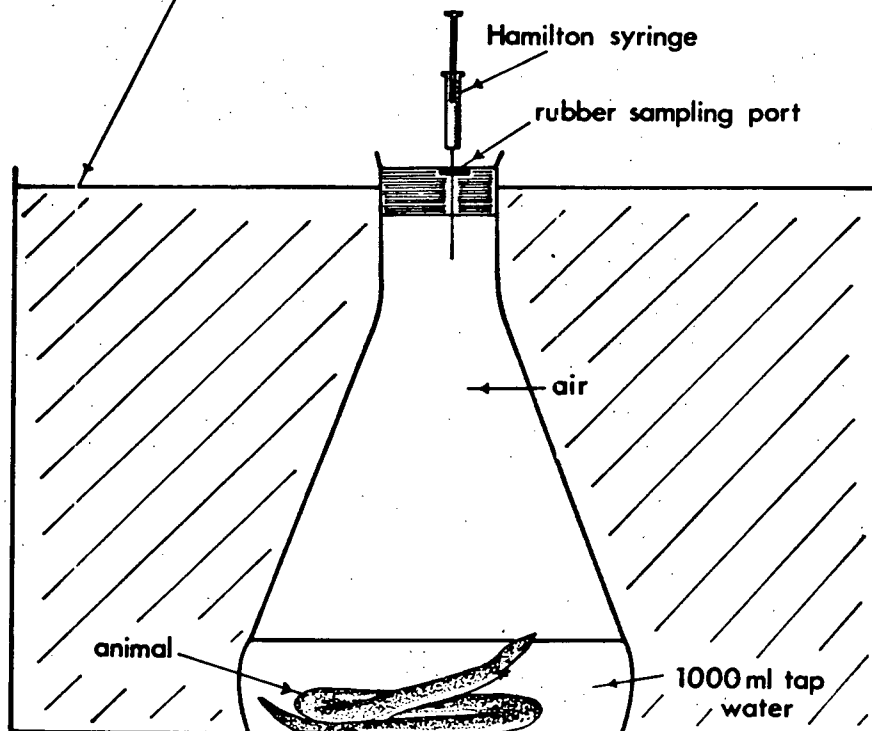
In the second method of oxygen consumption the animal was placed into a 4,850 ml Erlenmeyer flask

Figure 3 Apparatus used for the measurement of
oxygen consumption in Amphiuma tridactylum.
a. volume change method.
b. modified Scholander method.

A. VOLUME CHANGE



B. MODIFIED SCHOLANDER



containing 1,000 ml of fresh water. Air samples were taken every hour from the chamber through a tight rubber seal at the top of the flask and analyzed for oxygen, nitrogen and carbon dioxide concentrations on a gas chromatograph (Fig. 3). The Erlenmeyer flask was immersed in water at 15°C.

E. ANIMAL SURVIVAL

Animals with chronic indwelling cannulae survived for periods in excess of seven days. Death usually resulted from some violent movement which pulled the cannulae from the vessel. In most cases however, the animal was killed, after the experiments had been performed, in order to measure lung volume and the physical dimensions of the animal.

PART I. GAS TENSIONS IN THE LUNGS AND MAJOR BLOOD
VESSELS IN A FREE MOVING AMPHIBIAN,
AMPHIUMA TRIDACTYLUM.

INTRODUCTION

In the majority of investigations amphibian blood gas tensions and contents have been determined from terminal blood samples (DeLong, 1962; Johansen, 1963). Serial blood samples for gas analysis have been taken in only a few instances (Lenfant and Johansen, 1967; Shelton, personal communication on unpublished data). In the experiments reported here, serial blood samples were obtained for blood gas analysis from free moving, unanaesthetized animals.

DeLong (1962) found that in analysis of the oxygen content of terminal blood samples in several Rana pipiens "the carotids receive primarily left atrial blood, which is highly oxygenated, whereas the pulmonary vessels receive blood almost exclusively from the right atrium". He also found that there was considerable mixing of oxygenated and deoxygenated blood.

Johansen (1963) found in his analysis of the oxygen content of terminal samples in the major vessels of Amphiuma tridactylum that in most animals sampled, the aortic arch received blood of a higher oxygen content

than did the pulmonary arch. He also found that the oxygen content in the pulmonary vein was similar to that found in the aortic arch. The results he obtained however, could possibly be quite abnormal in that in most experiments pure oxygen was injected into the lungs prior to sampling. Johansen and Ditadi (1966), working on the giant toad, Bufo paracnemis, obtained results similar to those of Johansen (1963).

Terminal blood sampling is acceptable if only one blood sample from the animal is required or if the experimental animal is too small to take more than one sample. Terminal determinations have the disadvantage that the state of blood is known only at one particular time. Lengthy times between breaths must certainly affect the oxygen and carbon dioxide levels in the blood and it is difficult to determine from one sample the normal gas levels in the blood.

Determination of the oxygen or carbon dioxide content of blood is indeed useful for a complete understanding of the respiratory physiology of an amphibian; however, if content determinations are desired blood must be permanently removed from the body and serial sampling on one animal could drastically upset the normal physiology by lowering the total blood volume. Partial

pressure determinations have the advantage that micro blood samples are required and can be returned to the body after the determination has been made. Content can then be calculated from the blood dissociation curves.

Serial blood gas determinations have been carried out by Lenfant and Johansen (1967). They subjected three species of amphibians (Necturus maculosus, Amphiuma tridactylum and Rana catesbeiana) to prolonged periods in the air or under water. Although the effects on individual animals were not shown, they found that in Amphiuma tridactylum and Rana catesbeiana the overall effect of submergence was to lower the blood oxygen tensions and to slightly raise the blood carbon dioxide tensions and by keeping the aquatic Necturus maculosus exposed to the air, they found that blood oxygen tensions dropped and the carbon dioxide rose slightly.

The present experiments were designed to study the changes in gas tensions of oxygen and carbon dioxide in the major vessels and lungs of Amphiuma tridactylum during normal breathing cycles, and to determine the relationships between these levels. Oxygen consumption was also measured.

MATERIALS AND METHODS

These experiments were carried out on fifty-four Amphiuma weighing between 250 and 1000 g. Animals were not used for physiological experimentation if they had been at the university for a period longer than six weeks. The water temperature for all experiments other than oxygen consumption determinations was $15 \pm 0.05^\circ \text{C}$.

The Amphiuma were anaesthetized and cannulated as previously described. No more than three cannulae were inserted into the animal at any one time. In all cases a minimum of two hours was allowed to elapse before the start of the experiment proper. Table I indicates the types of cannulae used and the number of experiments done, contributing to the experimental results of this section.

Table I. Types of cannulae and numbers of animals used.

Type of cannulation	number of animals
lung alone	6
lung and dorsal aorta	9
lung & systemic arch & pulmonary vein	16
lung & dorsal aorta & post caval vein	5
lung & dorsal aorta & pulmonary vein	2
lung & pulmonary artery & pulmonary vein	1

Blood and lung gas tensions were measured as described in the General Materials and Methods. Oxygen consumption of fifteen Amphiuma at 15°C was measured using the volume change technique (10 Amphiuma) and modified Scholander technique (5 Amphiuma). The ten animals measured at 15°C by the volume change method were also measured at 25°C using the same method.

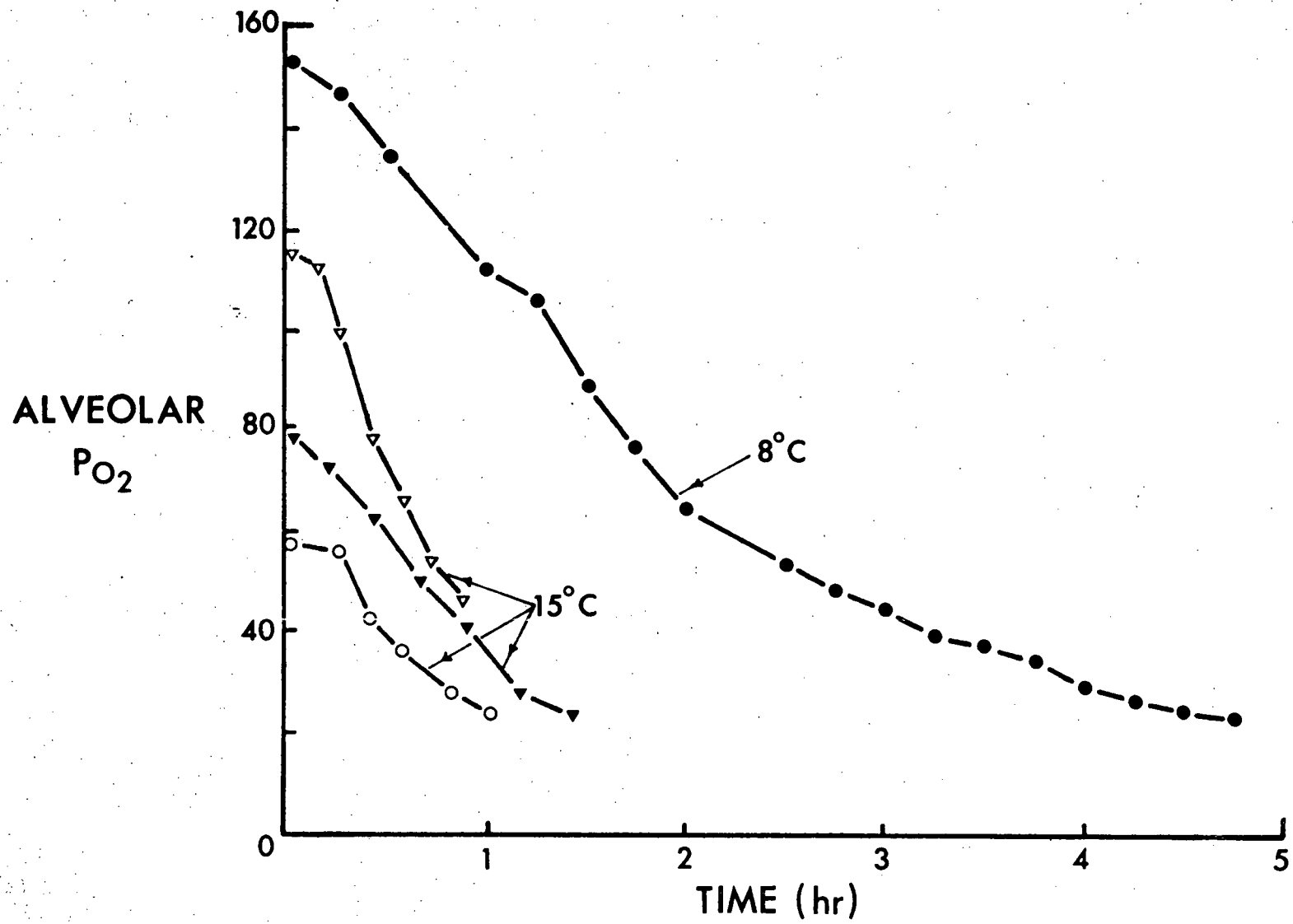
RESULTS

A. ALVEOLAR GASES

Amphiuma tridactylum remains submerged for long periods of time and surfaces to breathe. The Amphiuma in these experiments had a breathing interval of 55 ± 3 (S.E.) minutes at 15°C. Although critical temperature experiments were not done the one experiment performed at 8°C extended the breathing interval to 285 minutes.

P_O₂ in the lungs declined between breaths, falling from 101 ± 4.8 to 44 ± 2.9 mm Hg. The highest P_O₂'s are recorded immediately after a breath and the lowest immediately prior to a breath (Fig. 4). Where serial samples were taken closely enough to observe the shape of the P_O₂ disappearance curve, it was observed that oxygen concentrations dropped in a slightly reversed

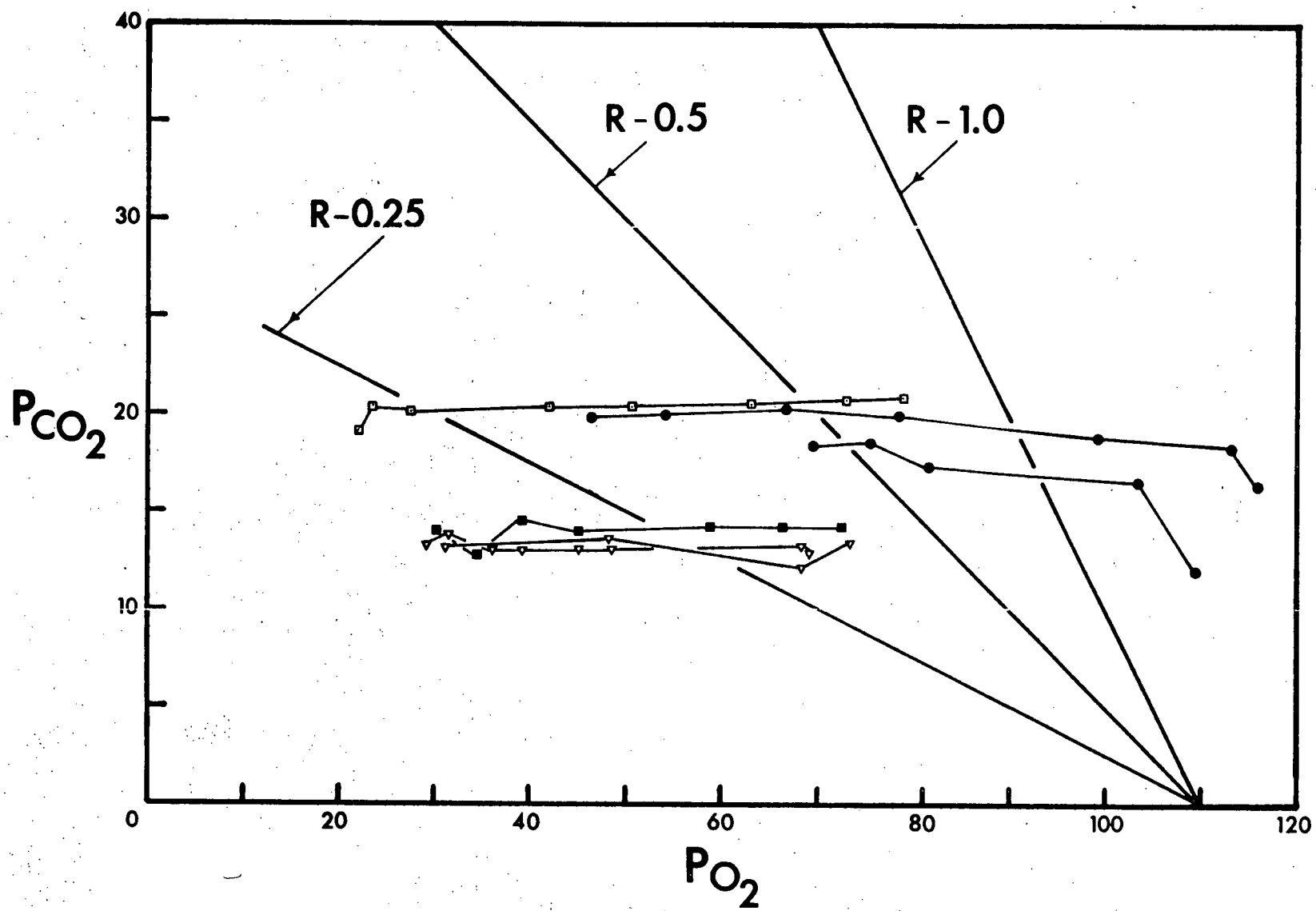
Figure 4 PO₂ decrease in the lungs of four Amphiuma,
The highest PO₂ levels occur immediately after
a breath and the lowest levels immediately
prior to a breath.



sigmoid-shaped curve. More simply, the rate of oxygen decrease from the lungs was not as rapid in the first fifteen minutes as it was for most of the remaining time. Figure 4 also illustrates the effects of a low temperature on the nature of the lung oxygen disappearance curve. At 8 C° the PO₂ reached 23 mm Hg within 285 minutes, when the animal breathed.

Alveolar PCO₂ varied little throughout the breathing cycle in all experiments. The first lung sample, taken immediately after a breath, tended to be slightly lower in carbon dioxide concentration than that found during the rest of the breathing interval. Within five minutes the alveolar PCO₂ returned to the mean level of 14.9 mm Hg after the recorded drop of about 16% (the percentage drop is a mean value calculated from 20 animals through 68 breathing cycles) and did not fluctuate appreciably thereafter. In only one out of twenty-two experiments in which the lung carbon dioxide was measured did the levels increase to any extent as the animal remained submerged. A "PO₂ and PCO₂ diagram" was constructed for breathing cycles of six Amphiuma (Fig. 5). The diagram describes the changes in PO₂ and PCO₂ in the lungs during a breathing cycle. The R line which would describe PO₂ and PCO₂ changes in the Amphiuma lung would

Figure 5 Alveolar " PO_2 - PCO_2 diagram" for six Amphiuma
The diagram illustrates the relationship between alveolar PO_2 and PCO_2 .



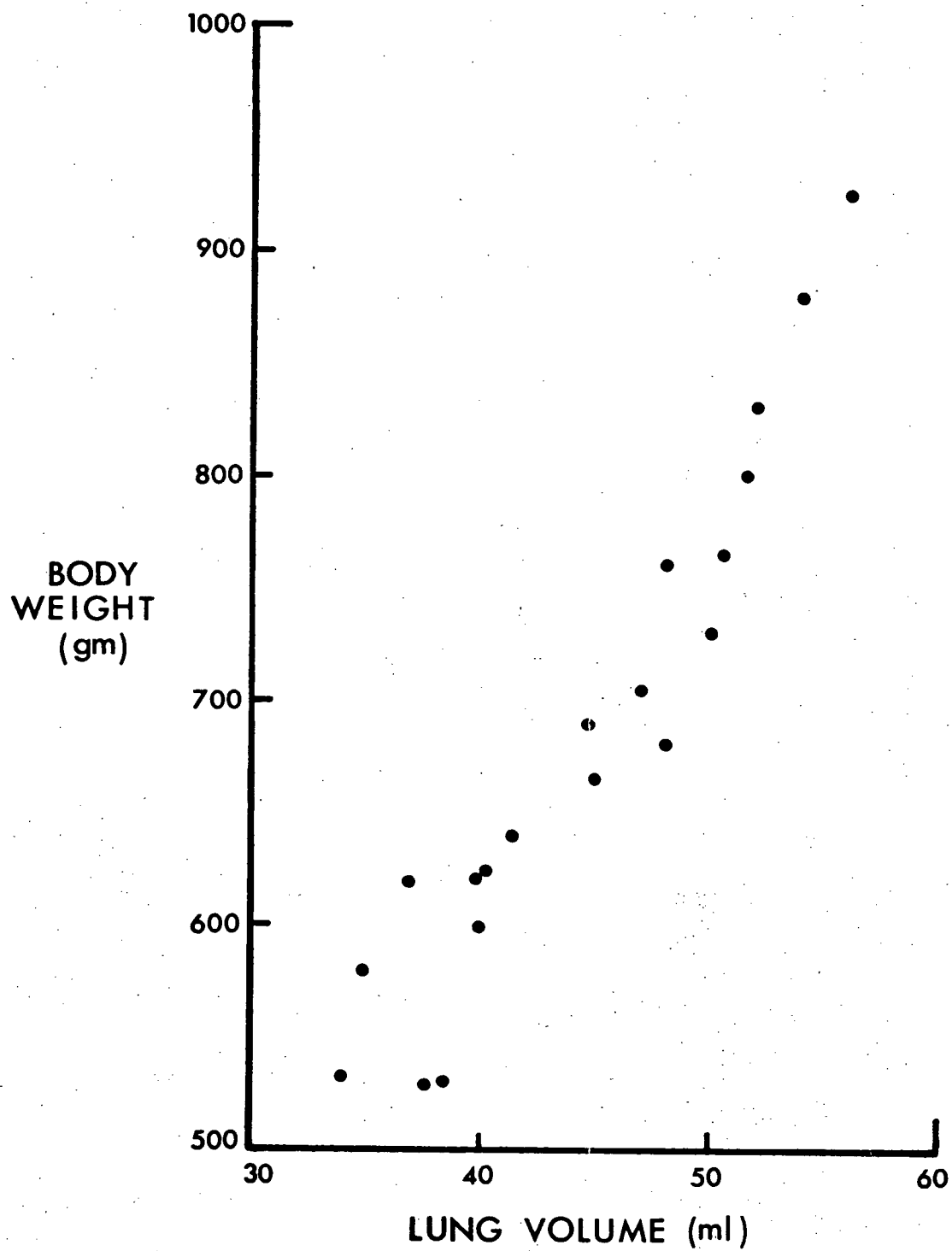
essentially be equal to zero during the majority of the breathing interval.

It was found that there was an inverse relationship between the alveolar PO_2 and PN_2 such that if there was no increase in the alveolar PCO_2 over a period of time, the decrease in PO_2 would result in a similar increase in PN_2 .

Total lung volume in all animals was a function of the weight of the animal (Fig. 6). Lung volume in any animal was determined by successive filling and removal of air through the indwelling cannula with a calibrated syringe. The accuracy of this method was confirmed by several autopsies performed in which the lungs were removed and the volume measured. It was not possible to damage the lung in an animal by injections of air, in that, as soon as the lungs were full, the animal would open the spiracles on the antero-lateral part of the body and release excess air.

Tidal volume was estimated using an indirect method. Knowing that the mean alveolar PO_2 prior to a breath is 44 mm Hg and immediately after a breath is 101 mm Hg, and that the PO_2 of the inspired air is about 160 mm Hg (dependent upon the atmospheric pressure of the day), the tidal volume, based on a principle of

Figure 6 The relationship between lung volume and body weight in Amphiuma.



gas dilution, would be about 72% of total lung volume.

B. BLOOD GAS CONCENTRATIONS

1. Dorsal Aorta

Figure 7 illustrates the changes which occurred in the lung and dorsal aorta in one animal during four breathing cycles. As the oxygen tensions in the lung fell, the PO_2 levels in the dorsal aorta also fell. The rapid rise in the lung oxygen tensions immediately after a breath resulted in an almost simultaneous rise in the dorsal aorta PO_2 . The gradient established between the dorsal aorta and lung oxygen tensions was relatively constant within any animal and did not change markedly before, during or after a breath once the gradient had been established (Fig.8).

Dorsal aorta PCO_2 levels were always 1-10 mm Hg lower than lung PCO_2 levels. This phenomenon was observed in all experiments in which the lung and dorsal aorta PCO_2 was monitored.

2. Pulmonary Vein

Because of the difficulty of pulmonary vein cannulation, the number of determinations of gas tensions in blood from this vessel was limited. Nevertheless, in all experiments the pulmonary vein PO_2 levels were very close to those found in the lung (Figure 9 is an

Figure 7 PO_2 and PCO_2 changes which occurred in the lungs and dorsal aorta in one animal during four breathing cycles. Vertical arrows at the top of the diagram indicate breathing times.

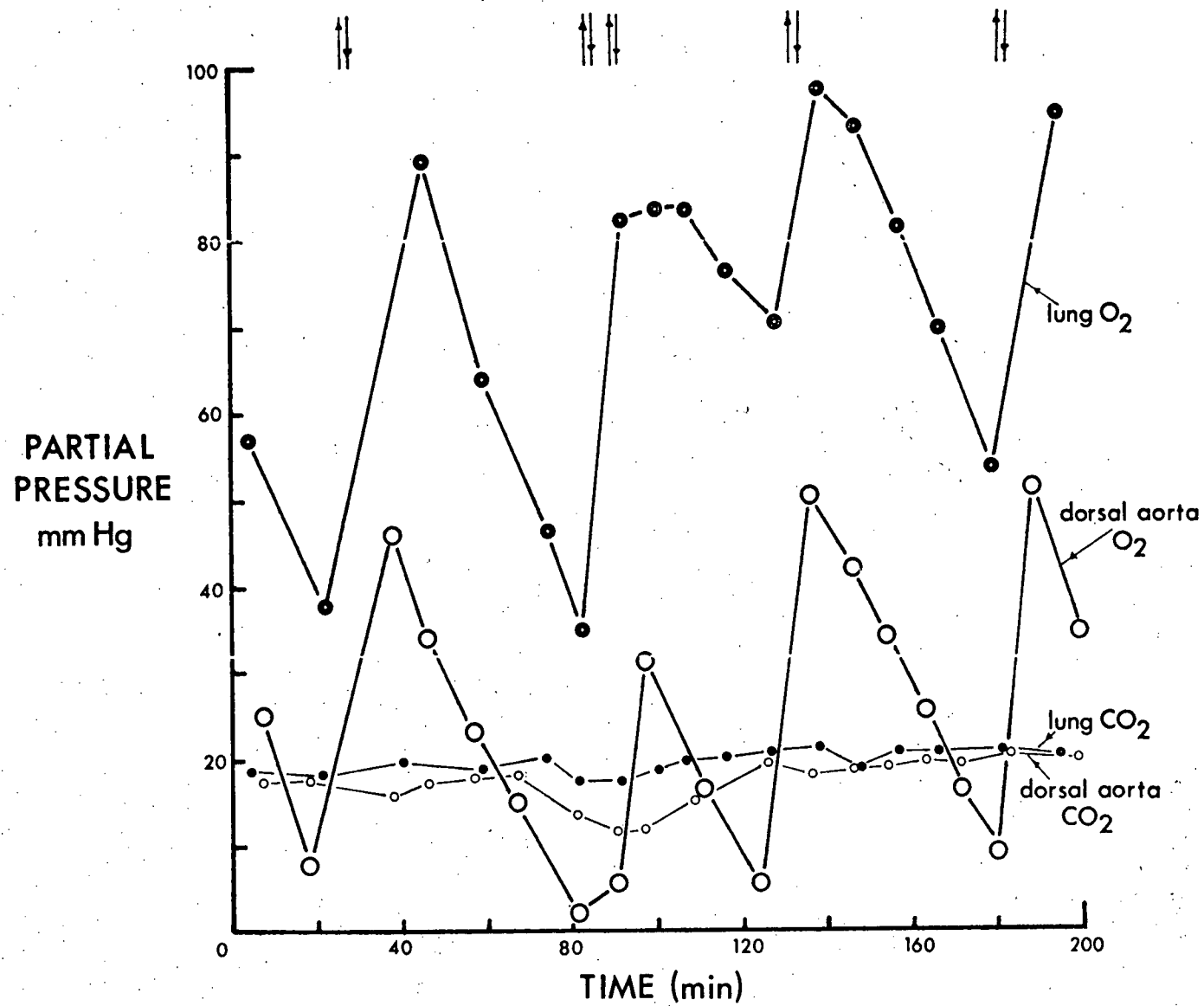


Figure 8 PO₂ gradient between the dorsal aorta or systemic arch and the lung.

The % time submerged on the abscissa of the graph equates all breathing intervals, i.e. 100% = interval between breaths

Means are given \pm S.E. for each 20% of the interval. Each mean represents not fewer than 10 gradient measurements.

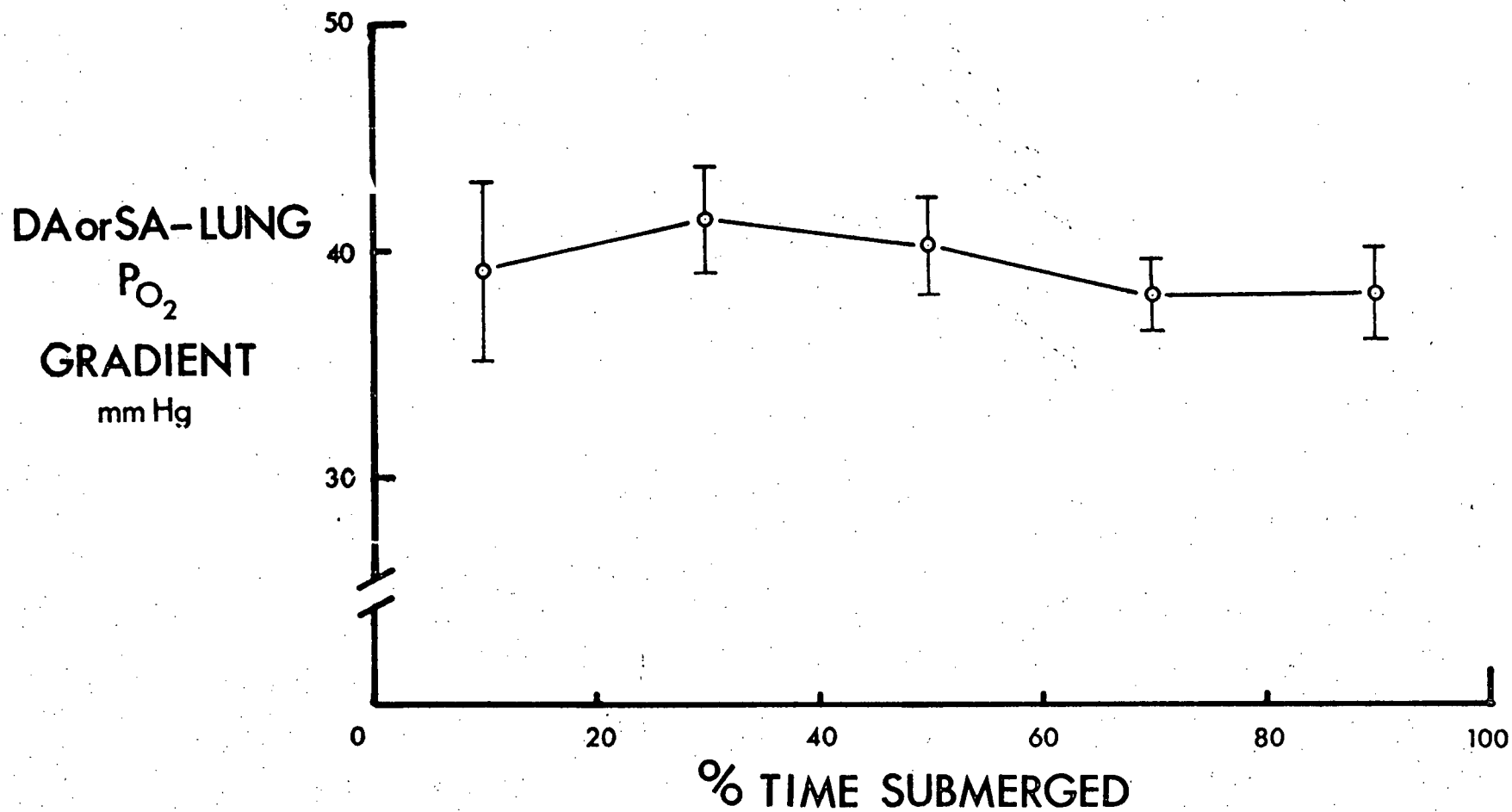
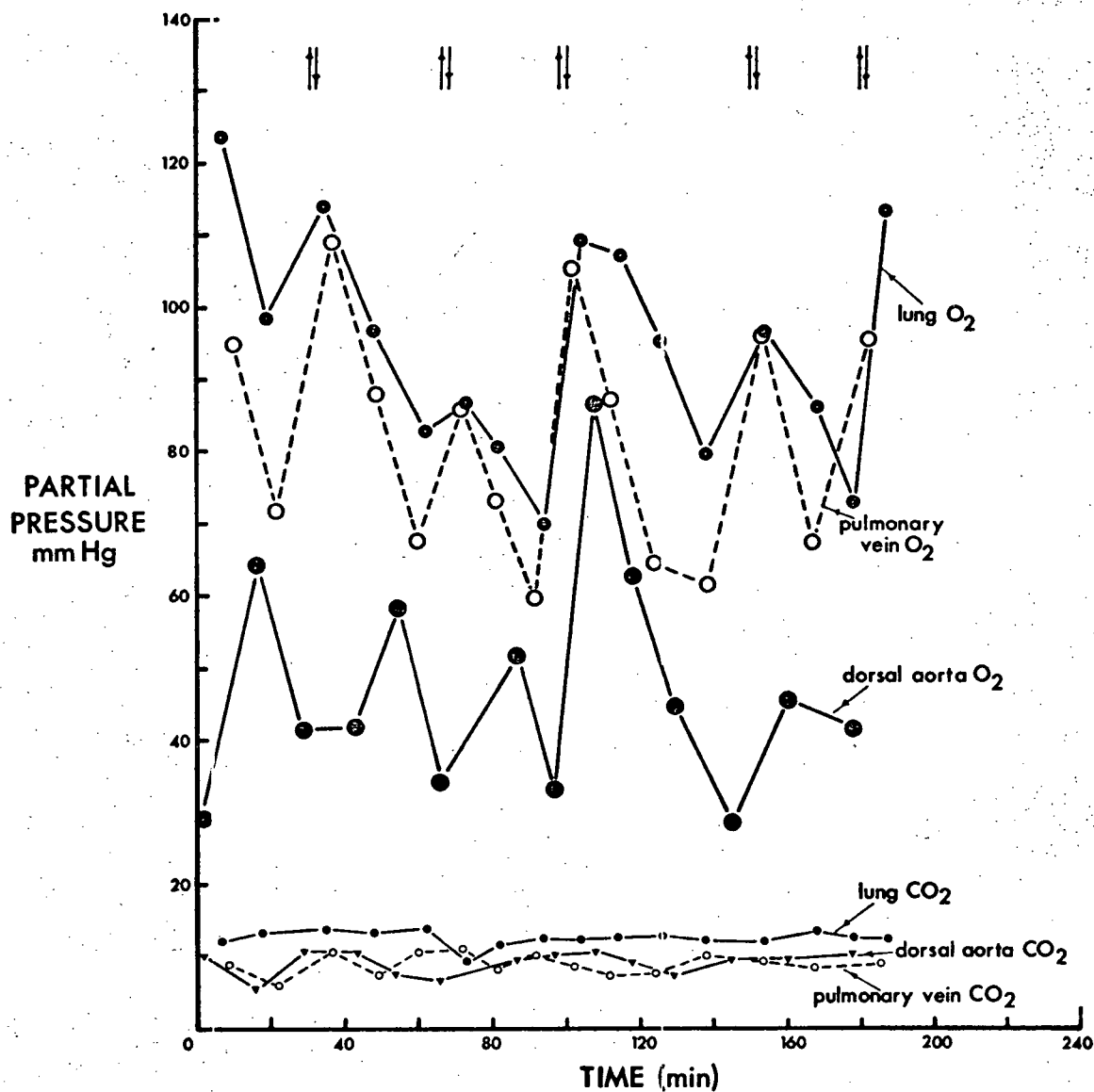


Figure 9 Changes which occurred in the pulmonary vein and lung PO_2 and PCO_2 levels in one animal during five breathing cycles. Vertical arrows at the top of the diagram indicate breathing times.



example of five breathing cycles in one animal). Immediately after a breath the PO_2 gradient between the lung and pulmonary vein was very small but increased as the animal remained submerged (Fig. 10). Pulmonary vein PCO_2 levels were, in almost every sample (one exception at 73 minutes, Fig. 9), lower than PCO_2 in the lungs.

3. Pulmonary Artery

PO_2 levels in the pulmonary artery are highest immediately after a breath and drop to the lowest levels just before a breath (Fig. 11). PO_2 gradients (the term gradient in this instance is essentially a PO_2 difference between two vessels) between the dorsal aorta and pulmonary artery were found in all experiments where these two vessels were cannulated*. The blood in the systemic circuit (systemic arch or dorsal aorta) is always more highly oxygenated than the blood flowing to the lungs via the pulmonary artery. Figure 12 illustrates the PO_2 differences between these two circuits in eight different Amphiuma. The gradient, immediately after a breath, decreased as the length of submerged time increased. The PO_2 gradient was initially as large as 25-30 mm Hg and fell to 1-5 mm Hg (Fig. 12).

* From the point of view of blood gas tensions, no distinction was made between the dorsal aorta, systemic arch or ascending aorta and the terms will be used interchangeably.

Figure 10 P_{O_2} gradient between the pulmonary vein and lung in Amphiuma. Gradient measurements were made at each point a P_{O_2} determination was made in either the lung or pulmonary vein. The % time submerged on the abscissa of the graph equates all breathing intervals in all animals, i.e. 100% = interval between breaths. The line was fitted to the data by eye.

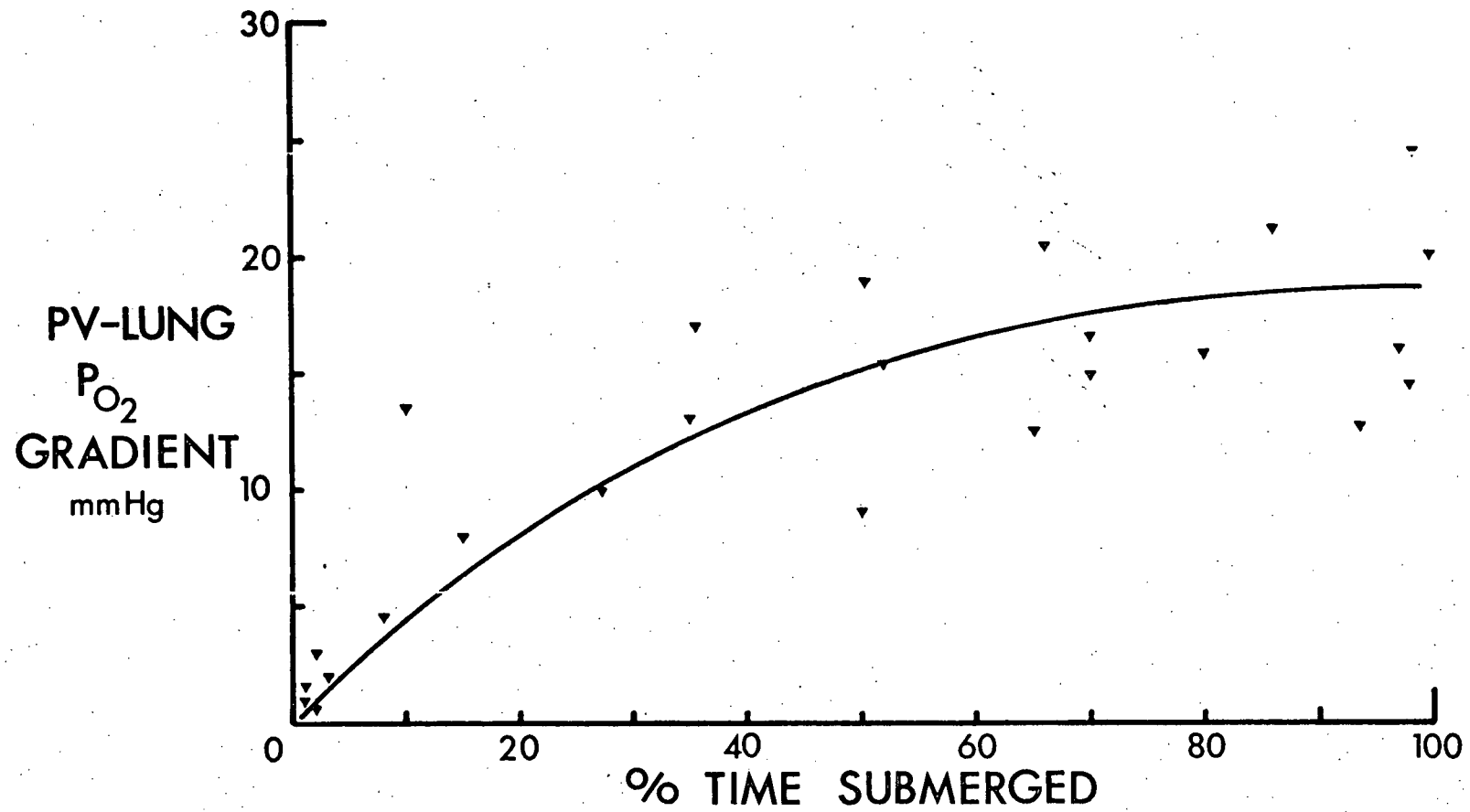


Figure 11 PO_2 and PCO_2 changes which occurred in the pulmonary artery, systemic arch and lung in one animal during a complete breathing cycle. Vertical arrows at the top of the diagram indicate breathing times.

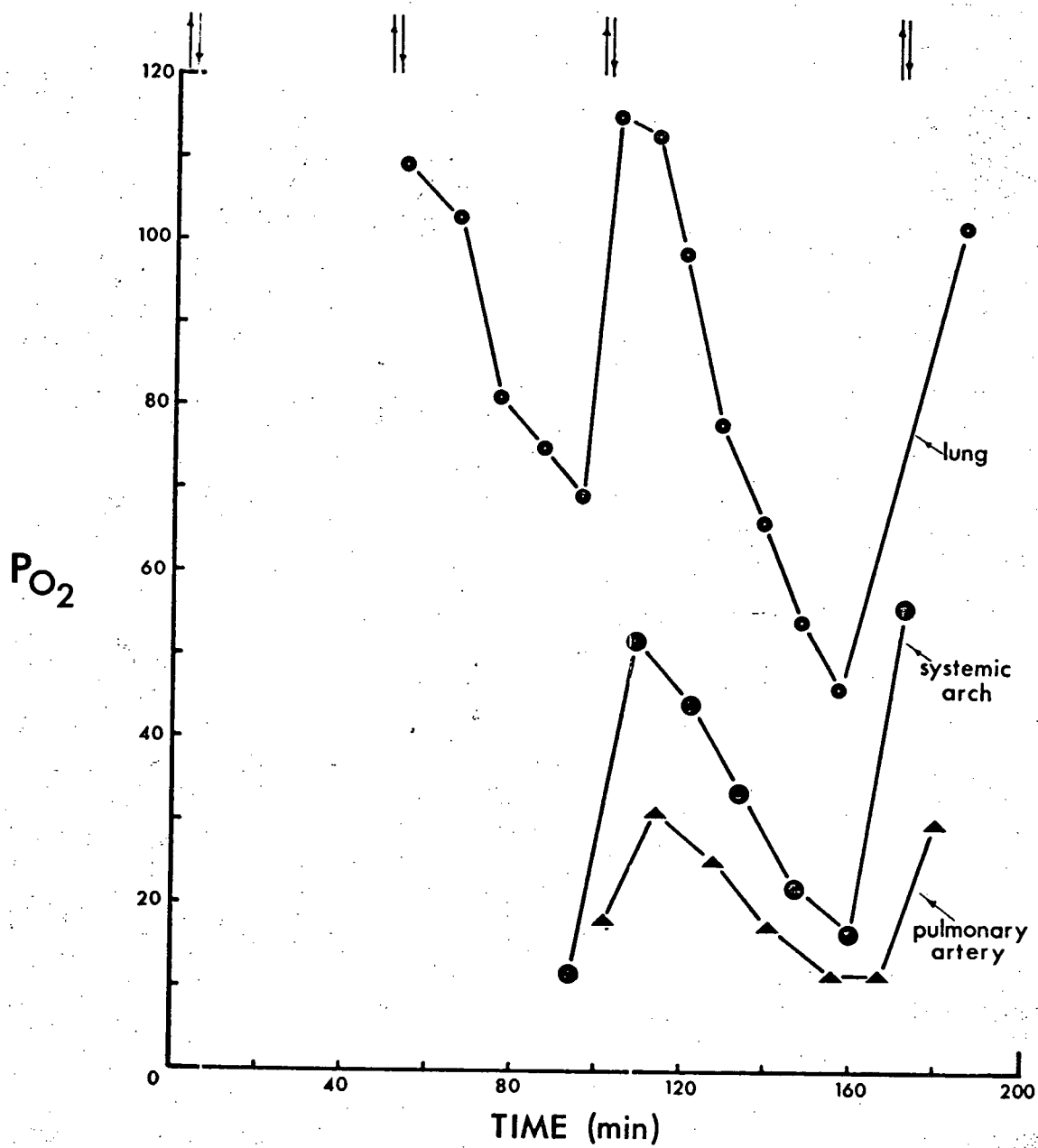
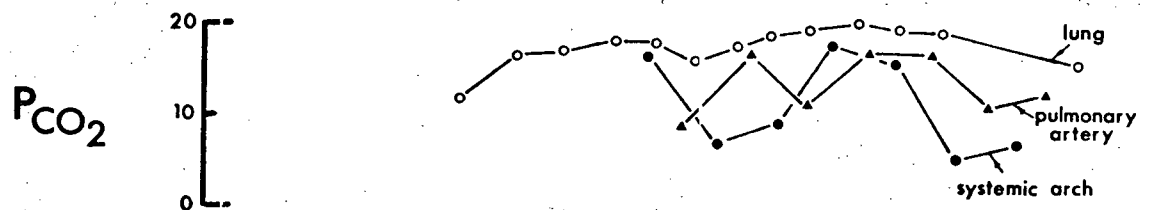
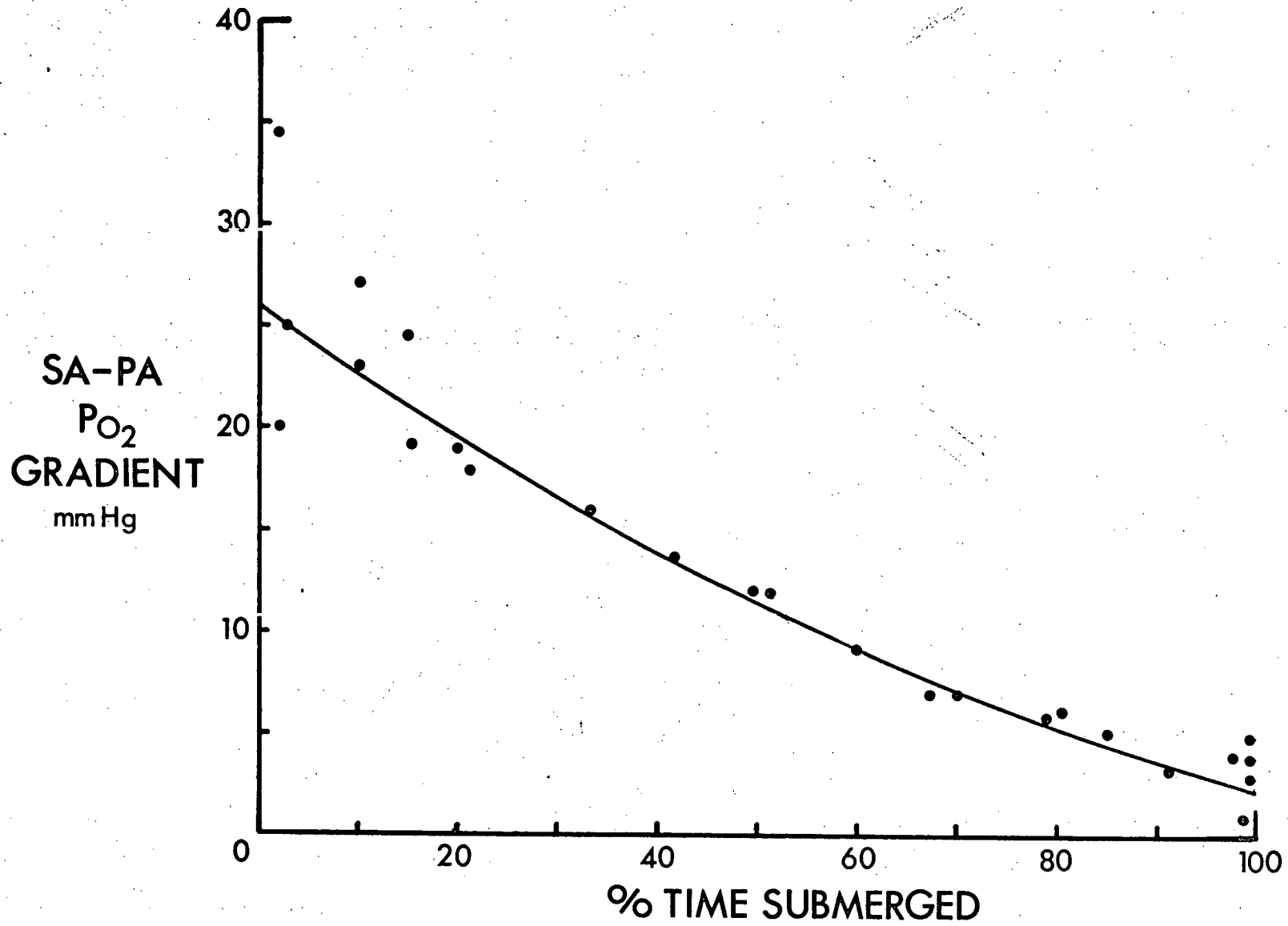


Figure 12 PO_2 gradient between the pulmonary artery
and systemic arch in eight Amphiuma.

Graphic presentation is the same as in Fig. 10.



Using an oxygen dissociation curve for Amphiuma tridactylum, derived from data of Lenfant and Johansen (1967) and Scott (1931), the PO_2 tensions in the systemic arch and pulmonary artery shown in Figure 11 were converted to % saturation (Fig. 13). This was done to describe the pulmonary artery-systemic arch gradient in terms of oxygen content as well as partial pressure differences. After a breath the gradient in this case (Fig. 13) was about 25% and fell in 50 minutes to 10% saturation difference.

There was no visible PCO_2 gradient between the pulmonary artery and systemic arch in any experiment. The mean systemic arch or dorsal aorta PCO_2 of ten Amphiuma was 11.9 mm Hg and from the same animals the mean pulmonary artery PCO_2 was 12.2 mm Hg.

4. Post Caval Vein

From simultaneous cannulations of the dorsal aorta and the post caval vein it was possible to determine the PO_2 difference between these two vessels through several breathing cycles. The composite of this data is shown in Figure 14. Immediately after a breath the PO_2 difference is quite large (17-30 mm Hg), after which the gradient decreases as the length of time the animal remains under the water increases (1-10 mm Hg immediately before

Figure 13 PO_2 levels in the systemic arch and pulmonary artery from Figure 12 converted to percent saturation.

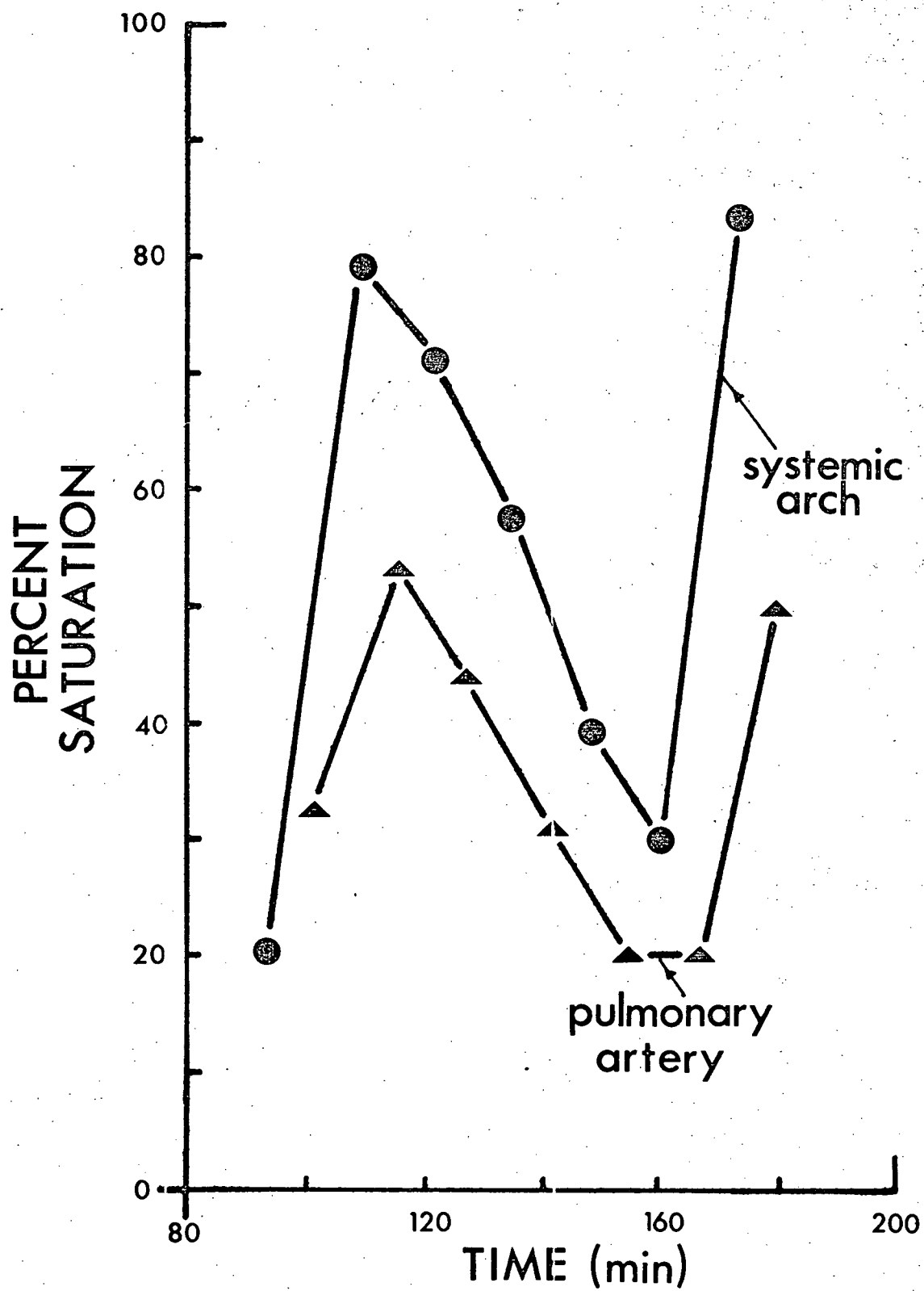
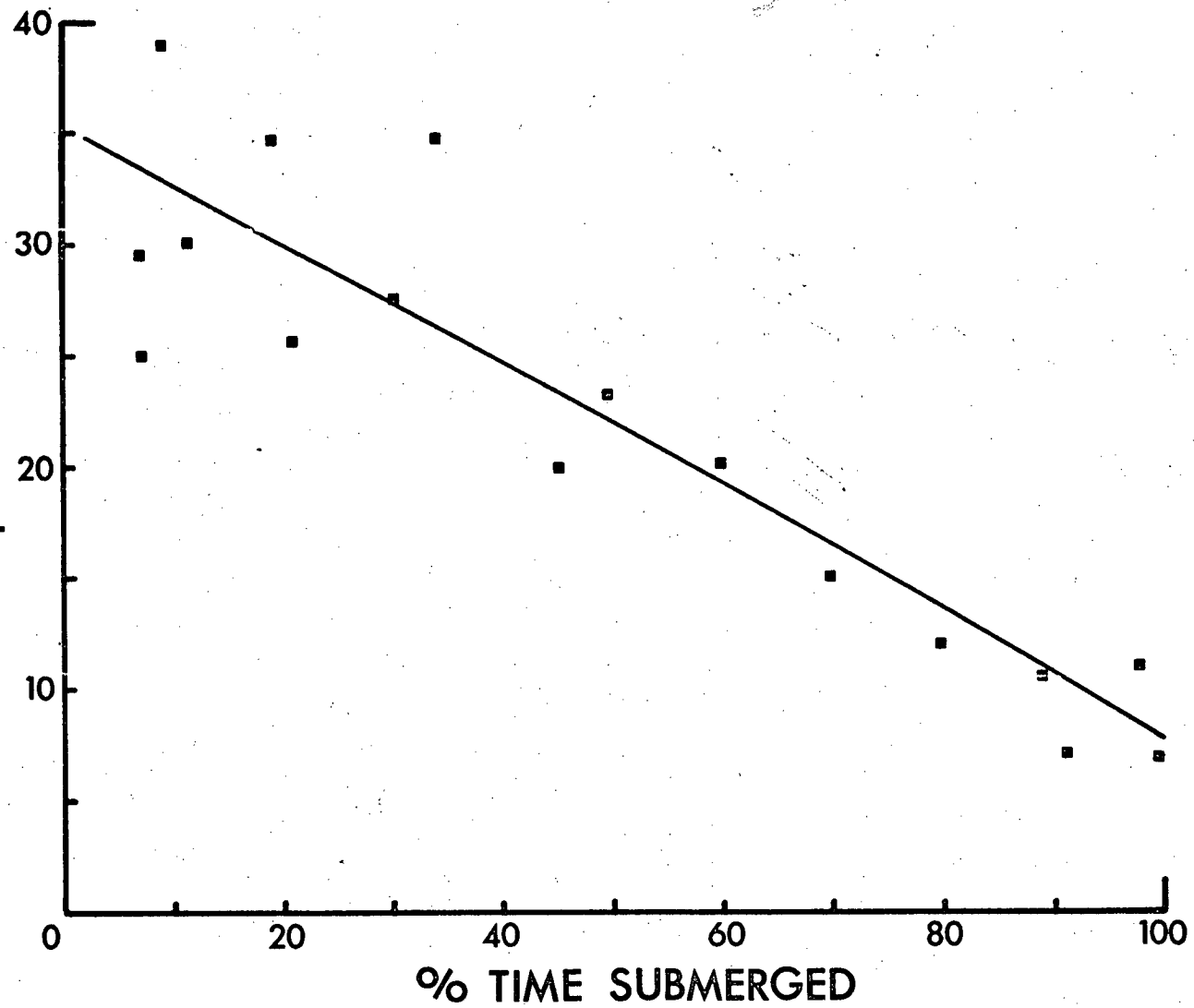


Figure 14 PO_2 and PCO_2 gradient between the post caval vein and dorsal aorta in five Amphiuma.

Graphic presentation is the same as in Fig. 10.

PCV-DA
 P_{O_2}
GRADIENT
mm Hg



breathing). Oxygen tensions in the post caval vein are always lower than those recorded in the dorsal aorta.

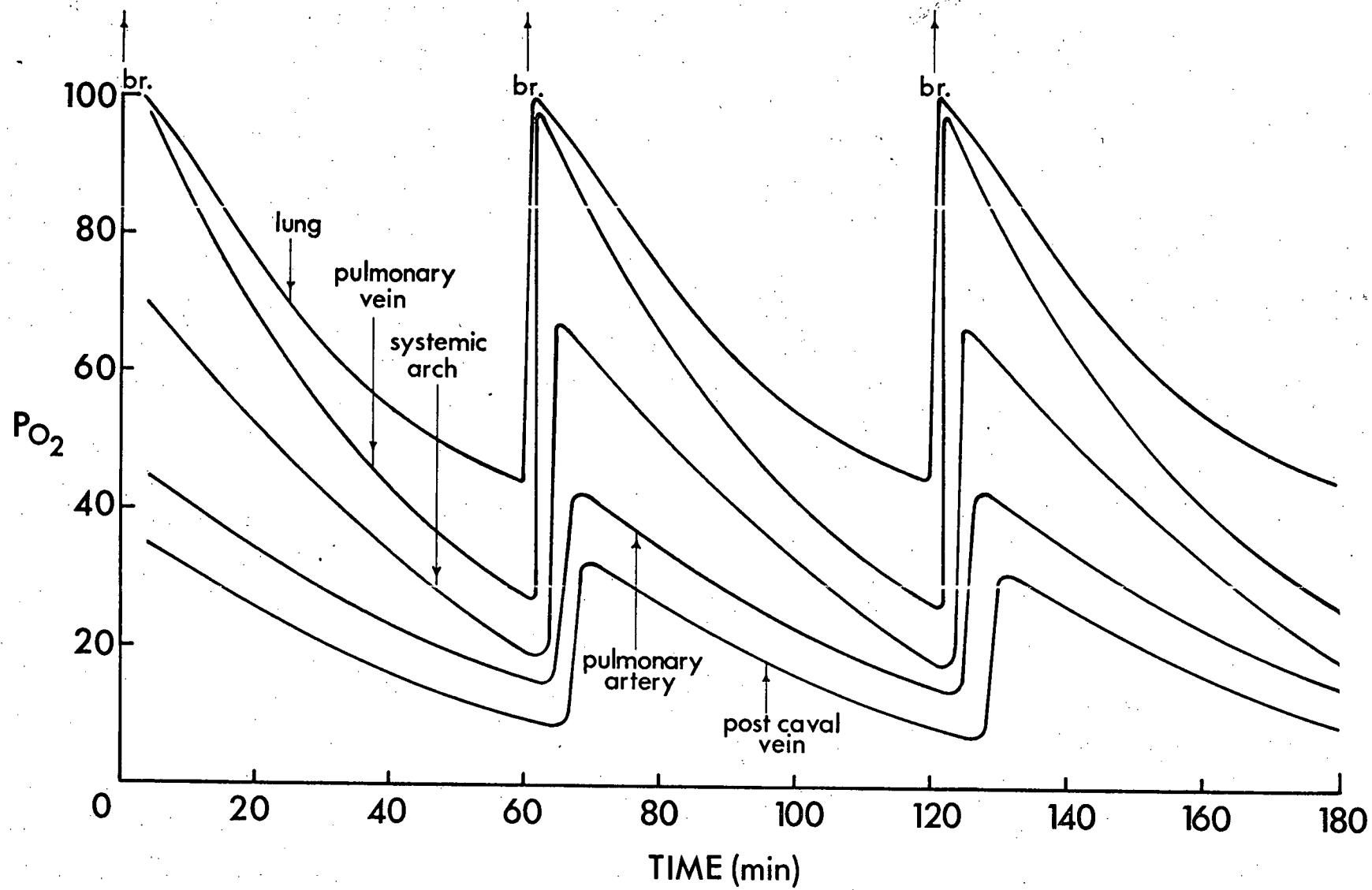
Mean PCO_2 levels in the post caval vein of five Amphiuma (46 samples) was 12.6 mm Hg. In the same animals, the dorsal aorta PCO_2 tensions had a mean of 11.6 mm Hg (individual samples from the two vessels were taken within ten minutes of each other).

5. Standard Animal.

It was impossible to obtain blood from all major vessels and lung gas measurements simultaneously. It was however possible to construct a generalized outline of the probable oxygen tensions in the major vessels during a breathing cycle. Knowing the pulmonary vein-lung gradient, the dorsal aorta-lung gradient, the pulmonary artery-systemic arch gradient and the post caval vein-dorsal aorta gradient, as well as knowing the mean oxygen tensions in the lungs and vessels immediately before and after a breath, it was possible to combine this information to form a representative or "standard animal" (Fig. 15).

PO_2 fell most rapidly between breaths in the pulmonary vein (Fig. 15) and least rapidly in the pulmonary artery and post caval vein (which fall at the

Figure 15 "The Standard Amphiuma". A reconstruction of the probable PO_2 fluctuations in the lungs and major blood vessels during three breathing cycles. Estimates obtained from available gradient data and mean blood and lung measurements.



same rate). Rapid rises in PO_2 occurred in the blood and lungs immediately after a breath.

C. OXYGEN CONSUMPTION IN AMPHIUMA TRIDACTYLUM

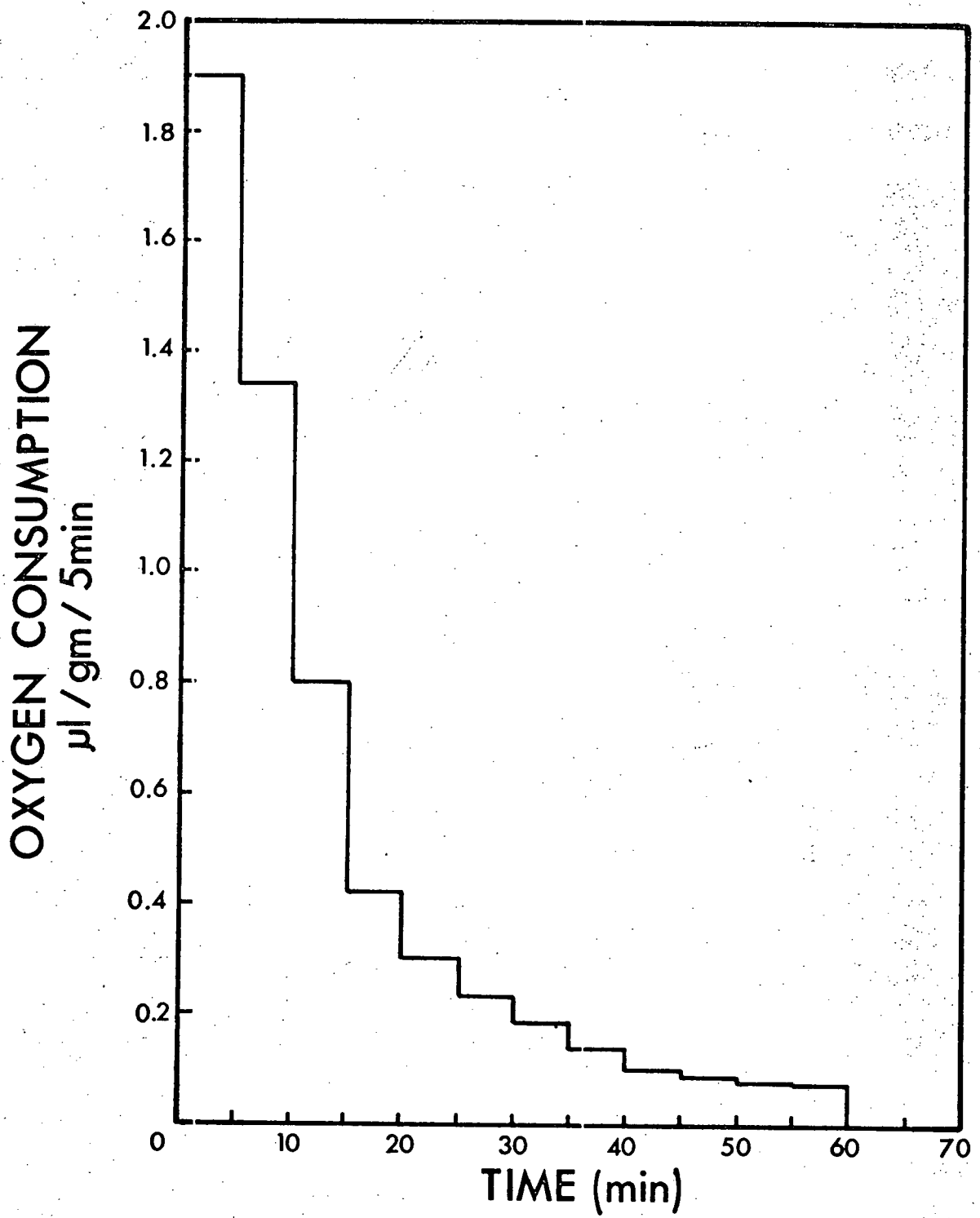
Oxygen consumption was measured in Amphiuma by two methods described earlier. Table II shows the mean consumption for fifteen animals used.

Table II. Oxygen consumption measured in fifteen
Amphiuma at 15 and 25°C.

Method	Number of animals	Temp°C	Mean oxygen consumption ul/gm/hr	Time measured per animal
Volume change	10	15	6.67 ± 0.14 (S.E.)	3 hours
Modified Scholander	5	15	6.89 ± 0.039 (S.E.)	22-28 hours
Volume Change (Same animals as at 15°C)	10	25	19.0 ± 0.23 (S.E.)	3 hours

Using two respirometry techniques, the mean oxygen consumption differed very little. The rate at which oxygen was consumed over a one hour period was not regular. Figure 16 illustrates the mean oxygen consumption for ten animals for a period of one hour measured at five minute intervals. Each experiment was repeated

Figure 16 Rates of oxygen consumption at 15°C in ten
Amphiuma measured by a volume change technique.
Means are given for each five minute interval.



three times on each animal. The most rapid oxygen consumption occurred within the first fifteen minutes of submergence; compared to this, consumption within the last forty-five minutes is very slow. Oxygen consumption in Amphiuma measured at 25°C increased to 19.0 ul/gm/hr, almost a threefold increase from the consumption at 15°C.

DISCUSSION

Amphiuma tridactylum breathes about once every hour almost completely replacing all air within the lung at each breath. Before taking a breath, the animal will raise its snout slightly above the surface of the water and will remove most of the residual air from the lungs by lowering the floor of the mouth with the external nares closed. The floor of the mouth is then raised and the external nares opened and the air in the mouth is expelled into the air above the water surface. This type of breathing movement is characteristic of amphibians which utilize pulmonary respiration as a method of gas exchange. Under normal circumstances all of the expired air is removed above the water surface such that bubbling does not occur in the water.

Lung volume in Amphiuma is related to the size of the individual animal and represents about 6-7% of the total volume of the animal. For comparison, the total lung volume of man represents 7-8% of the body volume (Lambertsen, 1961). An Amphiuma weighing 500-700 gm would have, on average, a lung volume between 30-40 ml (Fig. 6).

The removal of air from the lungs is not complete because oxygen tensions do not approach atmospheric

air tensions. In fact, tidal volume would appear to be about 72% in Amphiuma. This is much higher than in man (tidal volume of about 12% at rest) but the rather infrequent method of breathing would necessitate an almost complete change of air within the lungs.

The amount of air taken into the lungs is related to the length of time the animal remained at the surface, the amount of air previously removed from the lungs and the levels to which the arterial levels dropped during the time submerged. Quantification of the results were not possible but it did appear, from observations, that the lower the arterial PO_2 levels were, the longer the animal remained at the surface breathing and the more "gulps" of air swallowed.

Most air samples were taken from the posterior portion of the lung. Initially, after a breath, PO_2 levels in the lung did not change rapidly even though the animal was consuming oxygen at a high rate compared with that occurring towards the end of a breathing cycle. PO_2 levels in posterior portions of the lung may have been maintained by mixing air of a higher PO_2 , from the more anterior portions of the lung, with that in the posterior portions of the lung. This mixing could have been aided by contractions of the lung and movements of the animal.

In support of this statement, Czopek (1962) has shown in Amphiuma means, a closely related species to A. tridactylum, that there are a great number of smooth-muscle cells in the pulmonary walls and ridges and suggests that "the lungs are able to contract and thereby promote aeration".

The oxygen and carbon dioxide concentrations in the lung in Amphiuma are such that gas exchange is difficult to analyze using the O_2 - CO_2 diagram. Hughes (1966) suggests that oxygen and carbon dioxide in the lung of Rana catesbiana, when plotted on an O_2 - CO_2 diagram, would fall on the $R=0.4$ line. In Amphiuma the first one or two lung samples after a breath may fall on the $R=0.4-0.5$ line but as the animal remains submerged the R line essentially becomes zero and the usefulness of the O_2 - CO_2 diagram in describing gas exchange in this amphibian becomes limited. It is quite possible that if Hughes had continued the study to show the gas concentrations in the lung after the animal had been submerged for a longer period of time (characteristic of the species (Noble 1931)), the R line might have decreased considerably such as that found in Amphiuma.

Oxygen consumption in Amphiuma tridactylum is the lowest recorded of any amphibian measured at a comparable temperature. (Brown, 1964; Jones, 1967).

The rate of oxygen uptake of a submerged animal between breaths decreases rapidly after the large initial consumption within the first 5-10 minutes after a breath of 2-3.5 ul/gm body weight. The rate of decrease in oxygen consumption is similar to the drop in alveolar PO_2 between breaths. In the volume change experiments on oxygen consumption, the water PO_2 was monitored continuously throughout the three hour experimental period. The PO_2 drop in the water over this period was never more than 1-2 mm Hg. This drop in PO_2 would account for no more than a 0.2-0.4 ml loss in oxygen in the whole system. It is clear from this evidence that the animals rely very little on oxygen in the water to supply or supplement metabolic demands. Since oxygen consumption measurements using the two techniques were almost identical, it appears that a volume change in Amphiuma while submerged is indicative of the amount of oxygen being consumed, and all oxygen is taken up via the lungs. By the same reasoning, if the two methods give similar results, nitrogen must not leave the lung as the animal consumes oxygen. If nitrogen, which built up in the lung, was removed into the blood and water, there should have been a much larger volume change than was actually measured.

The lack of increase in lung PCO_2 between

breaths is interesting when one considers the volume changes which must occur in the lung as oxygen diffuses into the blood. As air enters the lung during a breath the lung PCO_2 is slightly diluted but returns to normal within 4-6 minutes. The resultant increase in lung PCO_2 after a breath must result from carbon dioxide entering the lung from the blood. If this initial amount of carbon dioxide were to remain in the lung, the lung PCO_2 should increase by 2-4 mm Hg simply as a result of the concentrating effect of oxygen leaving the lung. This however was not observed and the conclusion can be made that carbon dioxide which entered the lung from the blood immediately after a breath, must in part return to the blood between breaths. The result of this phenomenon then is to have lung PCO_2 concentrations higher than PCO_2 levels in any of the major blood vessels. Once the PCO_2 gradient is established between the blood and the lungs, no more carbon dioxide enters the lungs unless blood PCO_2 levels increase sharply.

The oxygen gradient between air and blood across the lung wall is initially small but increases with time between breaths. The calculated volume of oxygen leaving the lung decreases with time after a breath. The transfer factor of the lung ($\dot{V}O_2/\Delta PO_2$) is therefore falling

during the interval between breaths. The change in transfer factor could be related to many factors including the volume and pattern of blood flow to the lungs, and the distribution of air within the lungs. A change in transfer factor indicates that one or more of these parameters is altered and is affecting gas transfer rates across the lung.

The high PO_2 levels in the pulmonary vein immediately after a breath would indicate, for a short period of time at least, the blood leaving the lung is fully saturated with oxygen. Using an oxygen dissociation curve for Amphiuma tridactylum blood constructed by Lenfant and Johansen (1967), 100% saturation occurs at PO_2 levels above 90 mm Hg. PO_2 in the pulmonary vein often exceeded this level just after a breath, indicating that during this period, blood leaving the lungs was fully saturated with oxygen.

Somewhat more interesting is the PO_2 gradient established between the pulmonary artery and the systemic arch. The problem of whether the single ventricle of an amphibian can maintain a divided stream of oxygenated and deoxygenated blood and somehow direct the deoxygenated blood to the lungs and the oxygenated blood to the systemic circulation has been a topic of discussion and

experimentation since the mid-19th century. The experiments in this study show that there is a definite oxygen gradient between these two circuits over a long period of time, even during long intervals between breaths. DeLong (1962), Johansen (1963) and Johansen and Ditadi (1966) have all made measurements of oxygen and carbon dioxide contents in the two vessels but did not obtain serial samples over a long period of time. In the three published works just mentioned, oxygen content gradients were found to exist between the body and lung circuit in Rana pipiens (DeLong, 1962), Bufo paracnemis (Johansen and Ditadi, 1966) and Amphiuma tridactylum (Johansen, 1963).

In this investigation it was shown that the gradient between the pulmonary arch and the systemic arch is present throughout the breathing cycle. The gradient decreases as the animal remains submerged, but does not disappear completely. The gradient is definitely greater immediately after the animal has taken a breath (20-25 mm Hg) and this is probably because the pulmonary vein (oxygenated blood) and the venous return (primarily post caval vein, deoxygenated blood) gradient is greatest at this time. Although actual PO_2 gradients were not measured between the pulmonary vein and the post caval vein it can be seen from the reconstruction of the standard

animal (Fig. 15) that the PO_2 gradient between the vessels becomes smaller as the length of time submerged increases. This in turn appears to result from the increase in the lung-pulmonary vein gradient as the time after breathing increases. Therefore, the decrease in the pulmonary artery-systemic arch gradient between breaths is a function of the decrease in the pulmonary vein-post caval vein (venous return) gradient and probably not a loss in the separation capabilities of the heart and associated vessels.

It is important to point out that oxygen tensions in the pulmonary artery and systemic arches are usually below 70 mm Hg. The changes in blood PO_2 will cause marked changes in percent saturation as it is in this portion of the Amphiuma dissociation curve that changes in PO_2 result in large changes in percent saturation (Compare Figs. 11 and 13).

As has been described earlier (see General Materials and Methods), continuous measurements in the venous return (in this case the post caval vein) are difficult in that cannulation procedures are much more complex. Nevertheless, sufficient data was obtained to ascertain the levels of PO_2 in the venous return in relation to the levels in the dorsal aorta. The post

caval vein-dorsal aorta PO_2 gradient was between 20-30 mm Hg immediately following a breath and decreased to between 5-10 mm Hg immediately before a breath. Rather than reflecting an increase in tissue utilization of oxygen, this decrease in gradient simply reflects the decrease in total oxygen entering the circulation as the lung PO_2 drops, i.e., dorsal aorta level falls, VO_2 falls between breaths.

All of the fluctuations in blood PO_2 levels in all the major vessels occurred in free-moving Amphiuma with access to the surface. The fluctuations are definitely related to the intermittent type of breathing which this animal exhibits. Lenfant and Johansen (1967) suggest, on data collected from six Amphiuma, that normal arterial PO_2 levels vary from 72-100 mm Hg (9 samples) and do not mention individual variation within one animal between breaths. If their animals were "prevented from surfacing" for 43 minutes, they found that arterial PO_2 dropped somewhat, although it is impossible to ascertain from their data the effect of submersion on the individual animal. The implication is made by them that fluctuations in arterial PO_2 are not normal in Amphiuma. This is in total disagreement with the present study.

Lenfant and Johansen (1967) have also shown

that if Amphiuma were prevented from surfacing for periods of 43 minutes the PCO_2 in arterial blood rose. In all of the experiments in the present study, where arterial or venous PCO_2 levels were monitored, there was no indication that submergence resulted in an increase in blood PCO_2 levels. Albeit, Lenfant and Johansen's experiments were performed at $20^\circ C$ ($5^\circ C$ higher than the present study), but it is doubtful that this increase in experimental temperature would result in this difference in response to submergence.

Because PCO_2 levels do not increase in either the lungs (with the exception of the slight lung PCO_2 rise shortly after a breath) or the blood between breaths, we can conclude that carbon dioxide produced by metabolism is removed via the skin into the surrounding water. This concept is not new and has been shown as early as 1904 by Krogh (on anuran amphibians).

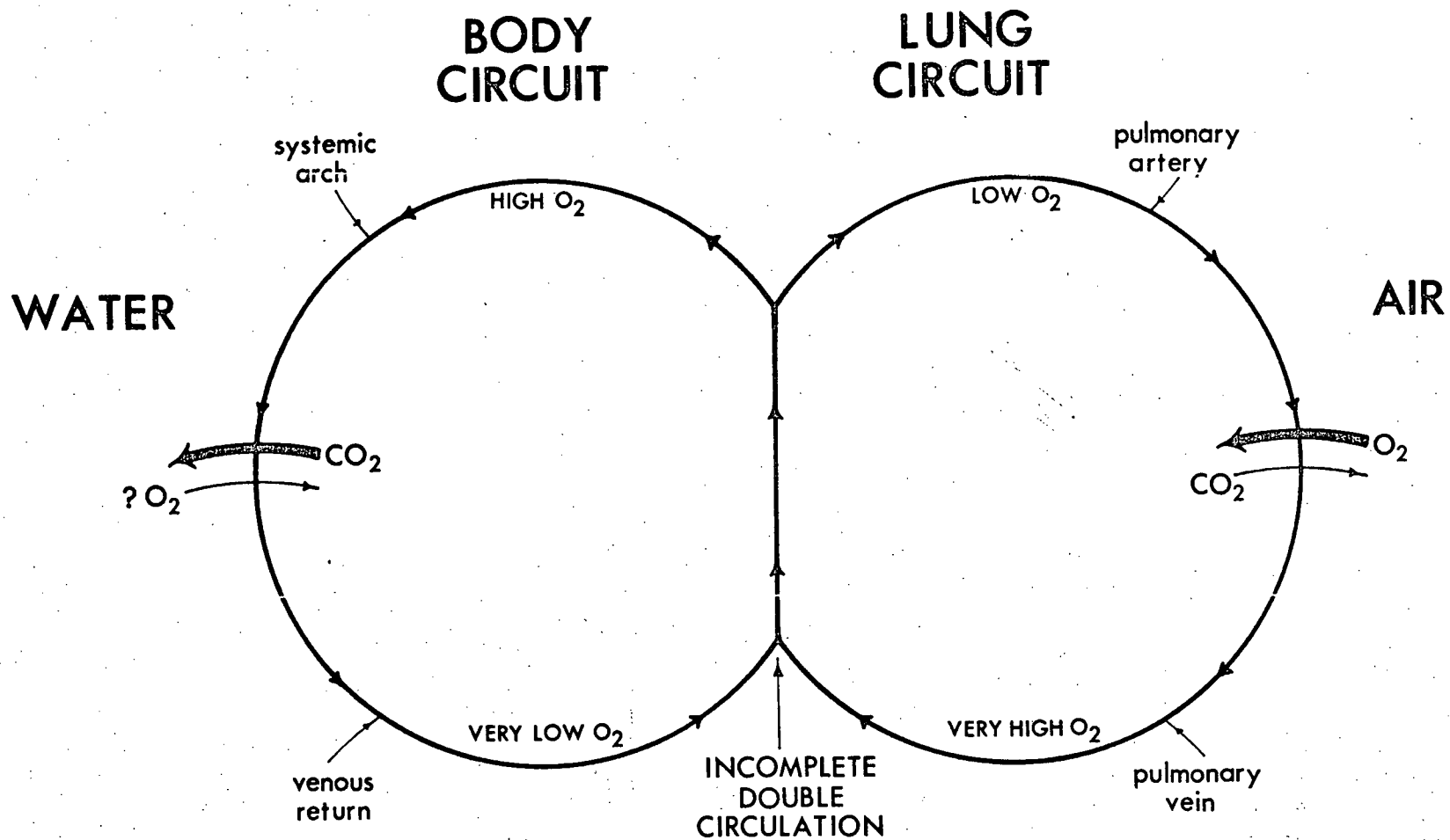
Carbon dioxide transfer from the blood to water will depend upon the dimensions of the exchange surface, i.e., the surface area of the skin, the volume and pattern of blood flow through the skin, and the concentration gradients between the blood and the water. There is a much larger PCO_2 gradient existing between the blood and the water than between the blood and the lungs. Carbon

dioxide will enter the lung until the gradient is small enough to effectively eliminate further passage of carbon dioxide into the lungs, whereas the amount of carbon dioxide which can pass into the water is large because of the high solubility of the gas in water as well as the fact that the volume of water surrounding an animal is very large.

The presence of carbonic anhydrase in blood increases the rate of formation of free carbon dioxide from bicarbonate and will help to maintain high carbon dioxide levels in the blood as carbon dioxide diffuses into the water. Carbonic anhydrase levels have not been measured in Amphiuma, but it is known that this enzyme is absent in the skin of Rana climatans and Rana Catesbiana (Maren, 1967). Hence, in the presence of low or non-existent levels of this enzyme, the rate of formation of carbon dioxide from bicarbonate may limit the rate of carbon dioxide transfer to the water across the skin.

The Haldane effect is small in Amphiuma (Lentant and Johansen, 1967) and therefore is not very important in augmenting the removal of carbon dioxide from the blood into the lungs. Figure 17 illustrates the probable mode of gas exchange in Amphiuma tridactylum. The buccopharyngeal mucosa of this species was not considered to

Figure 17 The probable mode of gas exchange in
Amphiuma tridactylum.



be a significant respiratory exchange surface primarily because of its small size in comparison to the total body size and secondly, Czopek (1962) found that in a closely related species, Amphiuma means, vascular supply to the mouth region was poorly developed.

PART II RESPIRATORY CONTROL IN AMPHIUMA TRIDACTYLUMINTRODUCTION

Work in the field of amphibian respiratory control has not been extensive. Neil and Zotterman (1950) and de Marneffe-Foulon (1962) investigated the neurophysiological basis of some respiratory reflexes in the frog. Jones and Shelton (1964) and Jones (1966) were particularly interested in factors affecting the recovery from diving bradycardia and how submergence influenced heart rate in the frog. Jones (1966), working on a variety of frogs (Bufo bufo, Rana pipiens and Rana temporaria), allowed them to surface into different concentrations of oxygen, carbon dioxide and nitrogen, and suggested "that the frog is sensitive to oxygen lack both during development of bradycardia and prolonged recovery from it". Although Jones has shown that diving bradycardia and recovery from diving bradycardia are more sensitive to oxygen lack than to the presence of carbon dioxide, the question of what were the stimuli (or stimulus) involved in the control of breathing remained unanswered.

Taglietti and Casella (1966) have presented neurophysiological evidence demonstrating that stretch receptors in frog's lungs are involved in the termination

of the lung filling process. More recently (1968) they have also produced evidence for the presence of deflation receptors in frogs' lungs. It is possible, from this evidence at least, that the stimulus for inflation and the termination of inflation could be related to the volume of air which the frog has in its lungs.

Since Amphiuma tridactylum remains submerged for extended periods of time (about 54 minutes at 15°C), it was convenient to design experiments in which not only gas tensions in the blood and lungs were monitored but also to inject varied concentrations of oxygen, nitrogen and carbon dioxide into the lungs and to observe the breathing responses of the animal. Experiments designed to determine the animal's ability to detect volume changes in the lungs were also carried out.

MATERIALS AND METHODS

Twenty-two Amphiuma tridactylum were used for experimentation in this section. Additional information was obtained from 17 animals used in Part I of this thesis.

Experiments to determine the animals response to varied gas concentrations were of four types; i, the oxygen tension in the lungs was lowered by injecting quantities of nitrogen into the lungs; ii, the carbon dioxide tension in the lungs was raised by injecting quantities of carbon dioxide into the lungs; iii, the carbon dioxide tension in the surrounding water was raised and the corresponding effects on blood and lung carbon dioxide observed, and iv, the oxygen tension in the lungs was raised by injecting pure oxygen into the lungs. Gases were injected into the lungs through a cannula (P.E. 50) inserted into the lungs as previously described.

Termination of inspiration experiments were performed by either injecting nitrogen into the lung as the animal was attempting to surface or to remove air from the lungs as the animal was breathing. The breathing response was recorded after the nitrogen injection and the amount of air "swallowed" was measured

if air was mechanically being removed from the lung during inspiration.

Lung gas concentrations were measured on a Varian Aerograph Gas Chromatograph. Water and blood PO_2 and PCO_2 were determined using a Radiometer Acid-Base Analyzer. Standardized gas mixtures used for lung injections were obtained from The Matheson Company of Canada and the Canadian Liquid Air Company. Prior to injection of gas mixtures into the lung, the gases were saturated with water. All experiments were performed on animals freely moving in a twenty litre glass aquarium containing ten litres of water. The water temperature in all experiments was maintained at $15^\circ C \pm 0.5^\circ C$.

RESULTS

A. BREATHING RATES

In all experiments in which blood and lung oxygen and carbon dioxide tensions were measured, disturbances to the normal breathing cycle (i.e. movement of tank, loud low frequency noises, or rapid movements above the water surface) were minimal. In these experiments the mean interval between breaths was 55 ± 3 minutes, the range from 26 to 120 minutes.

An extension of the time between breaths could

be accomplished by injecting atmospheric air into the lungs. Table III illustrates experiments on four different animals.

Table III. Extension of the time between breaths by injecting atmospheric air into the lungs.

Animal No.	Freq. of air injection	Time under water	Time from last injection to breath
1	20 ml/20-25 min	356 min	56 min
2	20 ml/20-40 min	303 min	41 min
3	20 ml/20 min	247 min	52 min
4	20 ml/20-24 min	345 min	62 min

In animals 1, 3 and 4 there were no apparent signs of agitation or attempts to surface until injections had been stopped. In animal 2 the time between injections was varied from 20-40 minutes and occasionally after 35-40 minutes had elapsed the animal moved around the bottom of the tank as though it was about to breathe. When these movements occurred, air was injected into the lungs and the animal characteristically coiled itself on the bottom of the tank and remained submerged. If the volume of continuous air injections exceeded the total lung volume, the animal removed the excess air as

bubbles through the spiracular openings while submerged.

An extension of the time between breaths could also be accomplished by injecting pure oxygen into the lungs. Twenty ml of pure oxygen per animal was injected into the lungs of five Amphiuma. The mean time between breaths in these animals was increased to 135 ± 6 minutes, the total range extending from 108-170 minutes.

B. TERMINATION OF INSPIRATION

In a free-moving Amphiuma, inspiration is terminated after 3-5 large "gulps" of air have been swallowed. After this period the animal will submerge and return to the bottom of the tank. It was found that if all the air entering the lungs while breathing was simultaneously removed, with a syringe through the lung cannula, the animal would continue breathing for an extended period of time. Table IV illustrates five such experiments performed where the actual amounts of air removed from the lung was measured before inspiration was terminated.

Table IV. Air removal from the lungs during inspiration.

Animal No.	Normal Lung Volume	Volume removed before termination of inspiration
1	40 ml	78 ml
2	47 ml	70 ml
3	34 ml	76 ml
4	37 ml	106 ml
5	45 ml	95 ml

The alternate experiment was performed on several other Amphiuma. As an animal raised its neck and snout towards the water surface to breathe, pure nitrogen was injected into the lungs before the animal reached the surface. It was found that after an injection of nitrogen, the animal did not breathe, but returned to the bottom of the tank for a short period of time and then resurfaced to breathe. Table V shows the amount of nitrogen injected as four animals surfaced and the time elapsed until they resurfaced to breathe. If more than one experiment was performed on one

Table V. Injection of nitrogen into the lungs of Amphiuma which were about to surface to breathe.

Animal No.	Amount of N ₂ injected as animal was surfacing	Time until breath
1	20 ml	5 min
	40 ml	11 min
	50 ml	9 min
	60 ml	5 min
2	40 ml	5½ min
	20 ml	2½ min
3	40 ml	6 min
4	40 ml	5 min

animal, at least two hours were allowed between injections of nitrogen. Any experiment in which the animals snout actually came above the water surface before or during

the injection of nitrogen was disregarded in that oxygen may have entered the lung.

C. BREATHING ONSET AND ITS RELATION TO BODY PCO₂ LEVELS

Figure 18 represents a series of experiments in which carbon dioxide/air mixtures were injected into the lungs of four animals and the rate of removal of these gases from the lungs monitored until the time of breathing. The removal of carbon dioxide from the lung, which was present initially in concentrations 3-5 times that of normal levels, was slightly faster than that of oxygen. It was assumed that, since the animals were under water, the carbon dioxide was being removed from the lung, into the blood and then into the surrounding water. In all cases the lung PCO₂ levels (Fig. 18) had returned to a base level (15-25 mm Hg) 30-70 minutes before the animal breathed.

Various concentrations of carbon dioxide in air were injected into the lungs. After injection, the time to onset of breathing was noted, and in several instances the concentration of gases in the lung, as near to the time of breathing as possible, were also measured. In these experiments a minimum of three hours was allowed between injections if the same animal was being used.

Figure 18 The removal of high concentrations of carbon dioxide and normal levels of oxygen from the lungs of four Amphiuma.

Breathing times are marked with vertical arrows and the abbreviation "br.".

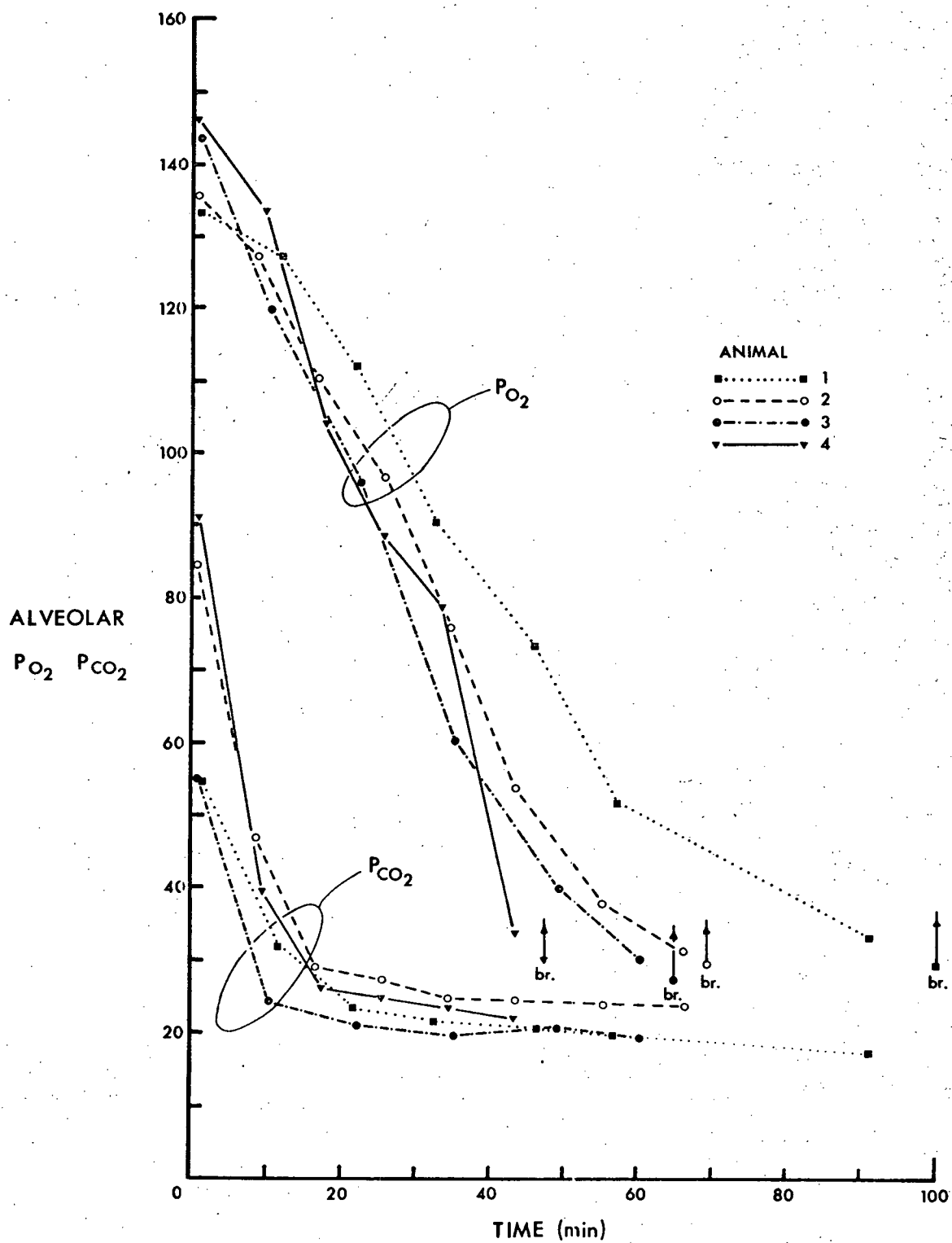


Table VI illustrates the results obtained. The breathing times after injection of 10% carbon dioxide in air were quite variable, ranging from 1 minute to 83 minutes with a mean of 43.1 ± 7.4 minutes. After injections of 15% carbon dioxide in air, the response was less variable, the time until breathing ranged from 41 to 72 minutes with a mean value of 51.8 ± 4.1 minutes. After injection of 20% carbon dioxide in air, the mean breathing time dropped to 20.6 minutes. The number of experiments done in which more than 20% carbon dioxide was injected were fewer in number and it can only be said that injections of high concentrations of carbon dioxide in the lungs (over 20% CO_2) resulted in the animal coming to the surface to breathe within a short time (less than 29 minutes).

It was impossible to ascribe any relationship between the onset of breathing and the blood PCO_2 levels. Figure 19 illustrates the results from four animals in which the systemic arch PCO_2 levels were followed through several breathing cycles. It cannot even be said that breathing occurred as the blood PCO_2 rose, for in fact breathing sometimes occurred as the blood PCO_2 fell.

If the blood PCO_2 levels in an animal were artificially raised by increasing the PCO_2 in the

Table VI. Carbon dioxide injections into the lungs of Amphiuma.

Time since last breath	Lung PO ₂ prior to injection	Lung PCO ₂ prior to injection	Type of injection	Time after injection until next breath
1 min	-----	-----	60 ml 10% CO ₂	63 min
40 min	27.5 (13)*	20.4 (13)	60 ml 10% CO ₂	15 min
41 min	28.0 (13)	14.0 (13)	60 ml 10% CO ₂	1 min
52 min	-----	-----	60 ml 10% CO ₂	43 min
4 min	53.6 (9)	20.6 (9)	20 ml 10% CO ₂	30 min
5 min	92.7 (47)	22.8 (47)	40 ml 10% CO ₂	83 min
10 min	25.8 (1)	21.4 (1)	20 ml 10% CO ₂	43 min
4 min	146.6 (3)	42.7 (3)	60 ml 10% CO ₂	6 min
8 min	-----	-----	50 ml 10% CO ₂	1 min
7 min	33.6 (6)	17.4 (6)	70 ml 10% CO ₂	85 min
10 min	30.6 (5)	19.7 (5)	80 ml 10% CO ₂	62 min
5 min	-----	-----	20 ml 10% CO ₂	61 min
3 min	-----	-----	30 ml 10% CO ₂	46 min
15 min	-----	-----	60 ml 10% CO ₂	64 min
64 min	31.1 (4)	23.9 (4)	60 ml 15% CO ₂	72 min
41 min	-----	-----	15 ml 15% CO ₂	45 min
57 min	-----	-----	30 ml 15% CO ₂	41 min
3 min	-----	-----	30 ml 15% CO ₂	48 min
4 min	-----	-----	100 ml 15% CO ₂	55 min
8 min	33.7 (4)	21.9 (4)	30 ml 15% CO ₂	50 min

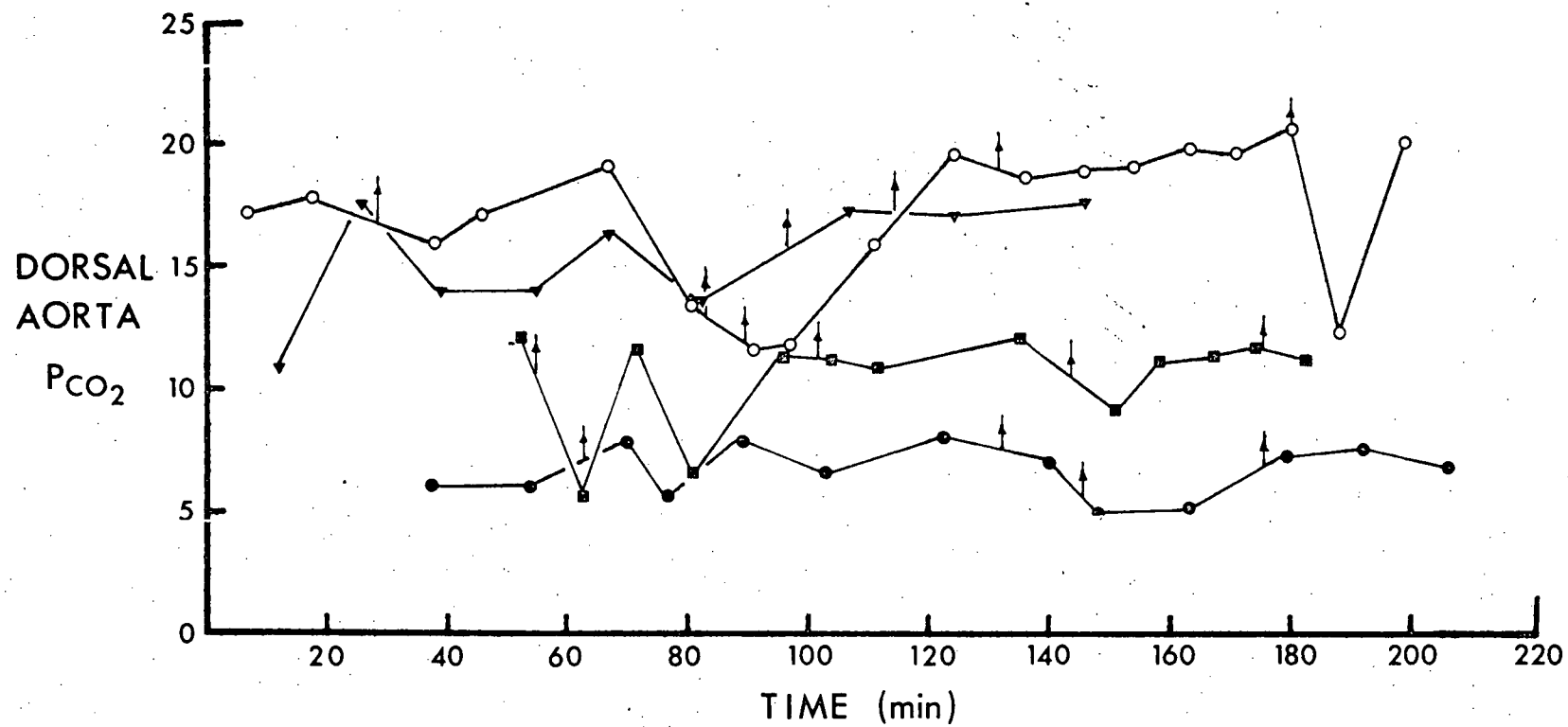
* Bracketed numbers indicate the time (minutes) that had elapsed between the injection of CO₂ and the lung measurements.

Table VI. (Continued)

Time since last breath	Lung PO ₂ prior to injection	Lung PCO ₂ prior to injection	Type of injection	Time after injection until next breath
59 min	-----	-----	60 ml 20% CO ₂	20 min
40 min	-----	-----	60 ml 20% CO ₂	17 min
3 min	-----	-----	60 ml 20% CO ₂	25 min
8 min	-----	-----	10 ml 30% CO ₂	1 min
51 min	-----	-----	10 ml 40% CO ₂	29 min
40 min	-----	-----	10 ml 50% CO ₂	3 min
23 min	65.0 (9)	16.0 (9)	10 ml pure CO ₂	6 min
30 min	-----	-----	10 ml pure CO ₂	2 min
57 min	-----	-----	20 ml pure CO ₂	3 min
3 min	-----	-----	20 ml pure CO ₂	5 min

Figure 19 Arterial PCO_2 levels through several breathing cycles in Amphiuma.

Vertical arrows extending above the graphed line for a particular animal, indicate the time of breath.



surrounding water, it can be seen (Fig. 20) that as the PCO_2 of the water rose to and remained at 80 mm Hg, the dorsal aorta PCO_2 rose to a level between 40-45 mm Hg. When the water was replaced (low PCO_2) the dorsal aorta PCO_2 fell. PO_2 fluctuations in the dorsal aorta did not appear to be affected by the increase in dorsal aorta PCO_2 levels (Fig. 20). A similar experiment was performed on another animal and it was found that although the dorsal aorta PCO_2 rose to 40 mm Hg the animal did not breathe until the oxygen tension in the dorsal aorta dropped to between 30-40 mm Hg (followed through three breathing cycles varying in length from 42-61 minutes).

D. BREATHING ONSET AND ITS RELATION TO BODY PO_2 LEVELS

Removal of oxygen from the lungs was accomplished by flushing large amounts of nitrogen through the lungs via the lung cannulae. In Table VII, eighteen experiments performed on five animals are shown to illustrate the effect of oxygen removal from the lung on breathing rate in Amphiuma. Since only pure nitrogen was being injected into the animal, it was important to consider the time spent submerged before the nitrogen injection was made. If the time elapsed between the previous breath and the injection time was not considered, the mean time to breathing was 9.0 ± 1.22 minutes. If injections were made

Figure 20 Artificial increase in tank water PCO_2 and the corresponding increase in dorsal aorta PCO_2 . Vertical arrows at the top of the diagram indicate the breathing times.

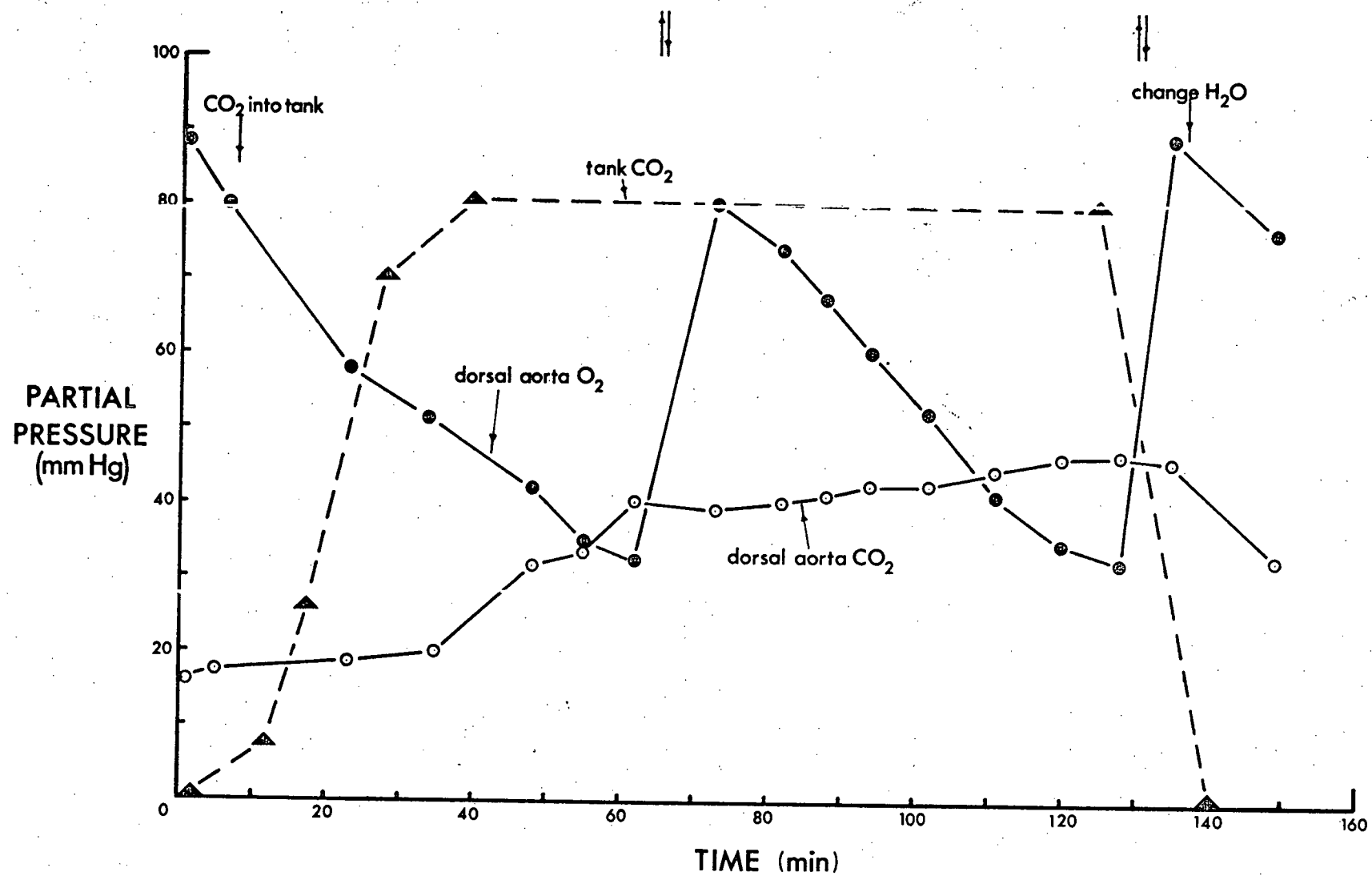


Table VII. Nitrogen injections into the lungs of Amphiuma.

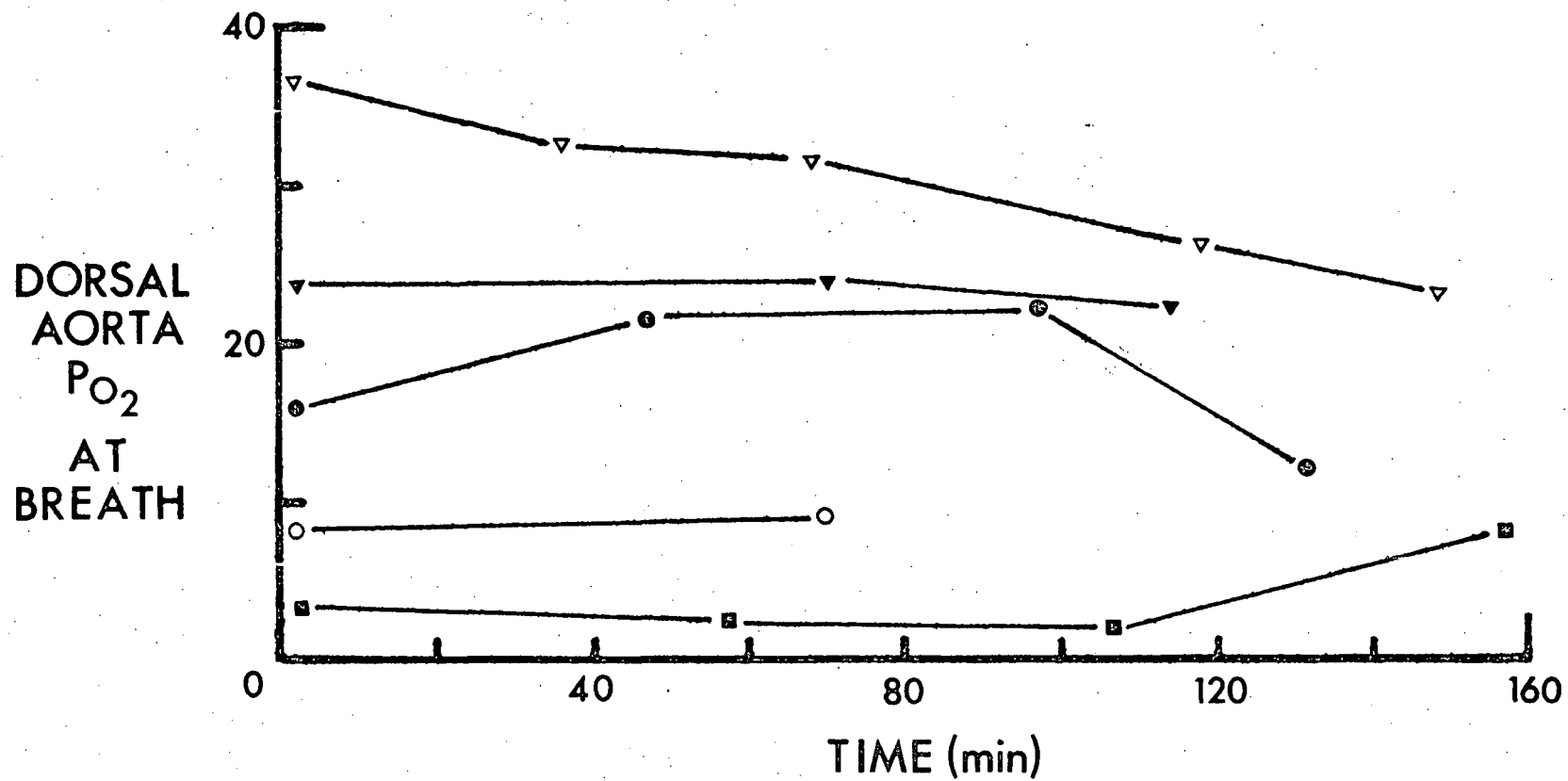
Time since last breath	Lung PO ₂ prior to injection	Lung PCO ₂ prior to injection	Type of injection	Time after injection until next breath
1 min	7.7 (3)*	10.2 (3)	40 ml N ₂	11 min
2 min	-----	-----	100 ml N ₂	8 min
3 min	-----	-----	100 ml N ₂	12 min
4 min	-----	-----	40 ml N ₂	4 min
3 min	-----	-----	100 ml N ₂	8 min
5 min	-----	-----	60 ml N ₂	21 min
6 min	-----	-----	40 ml N ₂	5.5 min
12 min	-----	-----	60 ml N ₂	3 min
13 min	7.8 (5)	8.9 (5)	140 ml N ₂	16 min
14 min	-----	-----	50 ml N ₂	7 min
2 min	3.8 (1)	4.9 (1)	100 ml N ₂	8 min
15 min	13.0 (10)	16.0 (10)	60 ml N ₂	17 min
15 min	8.1 (1)	6.6 (1)	80 ml N ₂	3 min
17 min	-----	-----	100 ml N ₂	13 min
20 min	-----	-----	50 ml N ₂	5 min
27 min	8.14 (1)	10.0 (1)	75 ml N ₂	3 min
37 min	-----	-----	60 ml N ₂	5 min
46 min	16.6 (6)	14.8 (6)	15 ml N ₂	13 min

* Bracketed numbers indicate the time (minutes) that had elapsed between the injection of N₂ and the lung measurements.

within 15 minutes after a breath, the mean time between breaths was 15.3 minutes, or on the average 9.4 minutes after the injection. If injections of nitrogen were made 15 minutes or more after the animal had taken a breath, the mean time to breathe after the injection was 8.4 minutes (the times of 9.4 and 8.4 minutes are not significantly different at the 5% level).

Using data from Part I of this thesis, PO_2 levels in the dorsal aorta and systemic arch at the time of breathing were obtained from five different animals (Fig. 21). As it was not possible to obtain blood PO_2 measurements at the exact time of breathing on all occasions, it was necessary to extrapolate "breathing values" by extending the removal curve for oxygen in the blood to the time of breathing. When illustrated in this manner (Fig. 21) there was as much as 14 mm Hg difference in arterial breathing values during five breathing cycles in one animal and as little as 1 mm Hg through three breathing cycles in another animal.

Figure 21 PO_2 levels in the dorsal aorta and systemic arch in five Amphiuma at the time of breathing.



DISCUSSION

Amphiuma tridactylum breathes about once every hour (55 ± 3 minutes). The animal becomes agitated, rises to the surface and fills its lungs. It then returns to the bottom of the tank. The mean time between breaths, recorded in this study, is in agreement with data reported by Darnell (1948). It is apparent (Part I of this thesis) that the primary function of surfacing in Amphiuma is to replenish the air in the lungs. What stimuli determine the cessation of inspiration?

In four experiments in which air was withdrawn from the lungs as the animal was breathing, lack of termination of the inspiratory process indicates that filling the lungs in Amphiuma terminates breathing. Termination of inspiration, after a certain volume of air had passed through the lungs and had been removed through the cannula, was probably because the speed of extraction of air did not equal the rate of lung inflation by the animal. The important observation is not the exact amount of air removed from the lungs but that, as air was withdrawn from the lungs, more was forced into the lungs by the animal than would normally fill the lungs. The inspiratory event is not based on a certain number of "gulps" of air but rather on the volume of air in the lungs which in

turn probably triggers stretch receptors in the lung. These have been shown to be present in other amphibians (Neil et.al., 1950; Paintal, 1963; Widdicombe, 1964; Taglietti and Casella, 1966; Shimada, 1966; Jones, 1966).

The nature of the gas mixture seems to be unimportant in regulating the termination of inspiration. The behavior of the animal prior to inspiration is very characteristic and it was possible to inflate the lungs with nitrogen during this period. Filling the lungs with nitrogen was sufficient to terminate the inspiratory response and the animal returned to the bottom of the tank for a short while. This "appeasement" period was shortlived and in all cases the animal returned to the surface to breathe within $2\frac{1}{2}$ -11 minutes. A similar type of response was recorded by Jones (1966). He found that frogs surfacing into nitrogen nearly recovered from diving bradycardia, however only lung inflations with air and "release of anoxia" brought about complete recovery. Because of the difficulty of recording an "E.C.G." through the thick skin and musculature of Amphiuma, heart rate was not monitored in most of the experiments in this part of the study. It is possible however, that there might be a relationship between heart rate and breathing

since (as will be shown in Part III of this study) there is a gradual slowing down of the heart rate as the animal remains submerged.

The major question to be considered in this part of the study is what stimuli are involved in determining the onset of breathing in Amphiuma. Continuous injections of air into the lungs result in animals being "satisfied" to remain below the water surface. Submerged times in excess of four hours clearly indicate that the stimuli or stimulus involved in triggering the animals breathing response could be overridden or negated by periodic air injections. Such lengthy times beneath the water surface also indicate, a priori, that carbon dioxide must be satisfactorily eliminated by respiratory surfaces other than the lungs. There is the possibility however, that carbon dioxide was removed as air bubbled out of the lungs at the time of air injection.

Injection of pure oxygen into the lungs of Amphiuma also extended the interval between breaths. Injection of 20 ml pure oxygen extended the breathing interval to slightly more than twice the normal breathing interval. Since the pure oxygen/air mixture in the lungs would not exceed the normal lung capacity, the lengthy submergence time would necessarily decrease the volume of air in the

lungs more than would occur if normal breathing had taken place. This alone would indicate that deflation receptors, found by Taglietti and Casella (1968) in the frog lung, did not provide the breathing stimulus in Amphiuma.

The chemical regulation of respiration by carbon dioxide in mammals is a well documented phenomenon (review by Hornbein, 1965) and it was thought that carbon dioxide might play an important role in the regulation of breathing in Amphiuma. Carbon dioxide/air mixtures were injected into the lungs. The results of these injections were in no way conclusive. For example the mean breathing time after injection of 15% carbon dioxide was 8-9 minutes longer than if 10% carbon dioxide were injected. Injection of 20% carbon dioxide resulted in the animal breathing within about 21 minutes, a definite shortening of the interval between injection and breathing. In this particular instance the amount of oxygen in the injected sample would be lowered to 16-17% simply by the presence of the carbon dioxide in the gas mixture. It seems doubtful however, that a 5% drop in oxygen concentration in the lung would shorten the interval between injection and the next breath to 21 minutes. Higher concentrations of carbon dioxide (30%-pure CO₂) injected into the lungs pro-

duces an even shorter interval between breaths.

Amphiuma tridactylum, under normal environmental conditions, would never encounter carbon dioxide concentrations as high as those injected into the lungs. Mammalian alveolar concentrations rarely exceed 10% carbon dioxide (76 mm Hg S.T.P.) and it has been shown earlier in this study that the mean alveolar PCO_2 for Amphiuma is about 15 mm Hg. In conclusion, it appears that Amphiuma has some detection mechanism whereby breathing onset will occur more rapidly if unphysiological doses of carbon dioxide are injected into the lungs.

If the arterial PCO_2 is raised to twice the normal level by increasing the PCO_2 of the surrounding water, no change in the normal breathing pattern results. In normal, free-moving Amphiuma in fresh water, no relationship was found between the PCO_2 levels in the blood and lungs and the onset of breathing (data used from Part I of this study). PCO_2 in the major vessels might either be falling slightly, rising slightly or more frequently, be constant at the time of breathing.

Elimination of carbon dioxide from the body is very rapid in Amphiuma tridactylum. Carbon dioxide injected into the lungs, raising alveolar PCO_2 to 3-5 times the normal levels, is removed from the lungs within

ten minutes after injection. The rapid removal of carbon dioxide from the lungs, if lung PCO_2 is raised, and the rapid rise in blood PCO_2 , if water PCO_2 levels are increased, indicate that the Amphiuma skin is capable of rapidly transferring large amounts of carbon dioxide across this respiratory surface.

Injecting nitrogen into the lungs of Amphiuma, thereby lowering the alveolar PO_2 levels, is somewhat more physiological in that when an animal has remained submerged for periods up to an hour, the lung is normally filled with 90-95% nitrogen. The effect of lowering alveolar PO_2 is that the times between breaths is definitely shortened. The actual response time is variable and is related to the fact that, if one postulates the presence of an oxygen chemoreceptor (which triggers the breathing response), injections of nitrogen into the lung will lower blood PO_2 at a rate which is related to the oxygen reserves (hemoglobin bound) in the body and the metabolic rate of the animal. The rate of oxygen consumption of Amphiuma, as shown earlier, is very low and is related to the blood oxygen content. The mean time to breathing of nine minutes after injection is not an unreasonable length of time for blood PO_2 levels to drop to levels similar to those in the lung. It seems quite

reasonable to suggest at this point that the onset of breathing is much more sensitive to oxygen deprivation than to increases in carbon dioxide concentrations.

These observations indicate that oxygen chemoreceptors may be involved in the initiation of breathing in Amphiuma. Relative consistency between the PO_2 in the dorsal aorta and systemic arch and the onset of breathing indicates that the receptor sites might be located in the arterial circulation. In an oscillating system, such as breathing in Amphiuma, it does not seem unreasonable to postulate a control mechanism triggered by another oscillating parameter (body PO_2 levels) rather than a system in which oscillations do not normally occur (body PCO_2 levels). High carbon dioxide levels however, do alter the interval between breaths. Carbon dioxide may have either a direct or an indirect effect on breathing in Amphiuma. Increased levels of carbon dioxide may stimulate oxygen consumption, which would shorten the breathing interval. Other alternatives might be that carbon dioxide may effect the relationship between PO_2 and breathing directly or perhaps carbon dioxide simply has a direct effect on breathing in the classical mammalian sense.

PART III. SOME FEATURES OF THE CIRCULATION IN

AMPHIUMA TRIDACTYLUMINTRODUCTION

Noble (1931) and Foxon (1964) have reviewed the work done up to five years ago on the circulatory dynamics of Amphibia. Shelton and Jones (1965 a,b and 1968) and Johansen and Hanson (1968) provide more recent accounts of research being done in this area. In view of the extensive reviews provided, only a brief resumé on subjects pertinent to the present study will be given here.

The "classical hypothesis" of blood flow through the amphibian heart was first put forward by Brücke (1852) and later modified by Sabatier (1873). They stated that oxygenated and deoxygenated blood remained unmixed in the ventricle, the oxygenated blood positioned on the right side of the ventricle was the first to leave the heart upon ventricular contraction. The direction of flow of deoxygenated blood through the conus was aided by the spiral valve and because of the lower pressure in the pulmonary circuit, deoxygenated blood preferentially flowed into the lung circuit. As pressure in the pulmonary and systemic circuits became equal the spiral valve was then thought to shut off flow to the pulmonary circuit and the oxygen rich blood

leaving the ventricle would enter the systemic and carotid vessels.

Since Brücke and Sabatier, Vandervael (1933) completely discarded the classical hypothesis and stated that blood in the ventricle and major vessels was completely mixed. Noble (1925), Acolat (1931, 1938), Foxon (1951), Simons and Michaelis (1953), de Graaf (1957), Simons (1959), DeLong (1962), Sharma (1957), Johansen (1963), Jchansen and Ditadi (1966) and Shelton (pers. comm.) have shown, by a variety of techniques on several species of amphibians, that there is a selective distribution of oxygenated and deoxygenated blood to the pulmonary and systemic circuits.

In discussion of this topic the two points of agreement (with the exception of Vandervael) are:

- i. the trabeculate nature of the amphibian ventricle does enable the blood to remain relatively unmixed.
- ii. blood from the right atrium (least oxygenated) is found in the right side of the ventricle, closer to the semilunar valves than the oxygenated blood from the left atrium.

Much disagreement occurs in the literature on the sequence

of events after ventricular systole that facilitate the movements of deoxygenated blood to the lungs.

Differences in the pulse pressure between the pulmonary and systemic circuits has been recorded by several people. Acolat (1938) found a 1-3 mm Hg lower diastolic pressure in the pulmocutaneous branch in eight species of anurans. De Graaf (1957), working on Xenopus laevis, found that diastolic pressures in the pulmocutaneous artery were on the average 7 mm Hg lower than in the other two arches. DeGraaf also found that there was a "lag" in the rise in pressure in the systemic circuit, in that the pressure in the pulmocutaneous circuit rose 0.10-0.15 seconds before the pressure increased in the systemic circuit. Johansen (1963) cannulating vessels at a greater distance from the heart in Amphiuma tridactylum recorded lower diastolic pressures in the pulmonary artery than in the systemic arch and also recorded a "slightly earlier pressure rise in the pulmonary artery". Neither de Graaf nor Johansen attached any significance to the pressure lag in the systemic circuit.

Shelton and Jones (1968), working on three species of Anura and one urodele, found consistently lower pressures in the anuran pulmocutaneous artery than were recorded simultaneously in the systemic arch, but found

similar systolic and diastolic pressures in the urodele. Similar pulse pressures in the urodele were thought to occur because of the ductus Botalli in this particular animal. Shelton (pers. comm.), in recent blood flow studies on Xenopus laevis, has evidence for a slight increase in blood flow to the pulmonary circuit prior to flow in the systemic. Shelton and Jones (pers. comm.) have data which suggests that in some anurans there is an increase in blood flow to the lungs for a period of time following a breath but that blood flow to the two circuits is equal throughout the greater portion of the breathing cycle.

Johansen (1963) was the first to examine the cardiovascular dynamics in Amphiuma tridactylum and he offers a partial explanation to the blood flow patterns in the major vessels in this animal. In general he recorded blood pressure and blood flow in the major vessels and also recorded cinefluorographically, the movement of blood through the Amphiuma heart into the arterial circulation. He stated that shunting of deoxygenated blood to the pulmonary circuit and oxygenated blood to the systemic circuit was accomplished by "laminar outflow patterns from the ventricle with a right-hand spiral movement through the undivided bulbus cordis". Johansen was also

aware that diastolic and systolic pressure changes in the major vessels could alter the selective passage of blood, but specific details with regards to this phenomenon were not given.

The objectives in this part of the study were to record blood pressures simultaneously in the body and lung circuits of Amphiuma tridactylum and to determine if there were any pulse pressure differences. The possible presence of pressure "lags" in the systemic circuit and the effects of breathing and air injections upon blood pressure in the major arteries was also investigated.

MATERIALS AND METHODS

The experiments in this study were performed on 23 adult Amphiuma tridactylum. The animals were cannulated by a method described previously in this thesis (General Materials and Methods).

Blood pressure was monitored with Statham 23AA, 23BB or 23 Db pressure transducers which were in turn connected to a Beckman Type R Dynograph. Pressure transducers were calibrated with a column of saline. The response time of the pressure recording equipment was 0.20-0.25 msec.

The experiments were carried out in a 20 litre glass aquarium which was partially filled with 10 litre of fresh water held at 15°C. All animals were free-moving and unanaesthetized; records were not taken until 4-6 hours after the operation.

RESULTS

A. HEART RATE

Heart rate in any particular animal was variable. The lowest heart rate recorded at 15°C was 5 beats/min and the highest 19 beats/min. In general there was a tachycardia immediately following a breath and a gradual slowing down of the heart rate as the submerged time increased.

The mean fluctuation in heart rate between breaths was 5.1 beats/min. Figure 22 illustrates the "diving bradycardia" or "breathing tachycardia" in several Amphiuma.

B. LUNG FILLING AND CIRCULATORY CHANGES

The tachycardia, which occurred when Amphiuma breathed was, in most cases, associated with a slight drop in blood pressure in both the pulmonary and systemic circuits. Figure 23a illustrates the breathing tachycardia as well as the pressure drop in the two circuits. It is important to note that the pulse pressure in the pulmonary circuit does not decrease to the same extent as does the pulse pressure in the systemic circuit. A similar phenomenon could be observed by artificially filling the lung with air or nitrogen (Fig. 23b). Figure 23c further illustrates the extent to which lung volume affects blood and pulse pressures. Injections of air into the lung raised the systemic diastolic pressure by about 3 mm Hg. After a short period of time the air was removed and the diastolic pressure in the systemic arch returned to the original level.

Injectons of low concentrations of carbon dioxide (5-15%) produced effects on blood pressure and heart rate similar to those of air injections. If the particular

Figure 22 Diving bradycardia or breathing tachycardia in five Amphiuma. All animals breathed at time "0" and breathed again at a point marked by a vertical arrow.

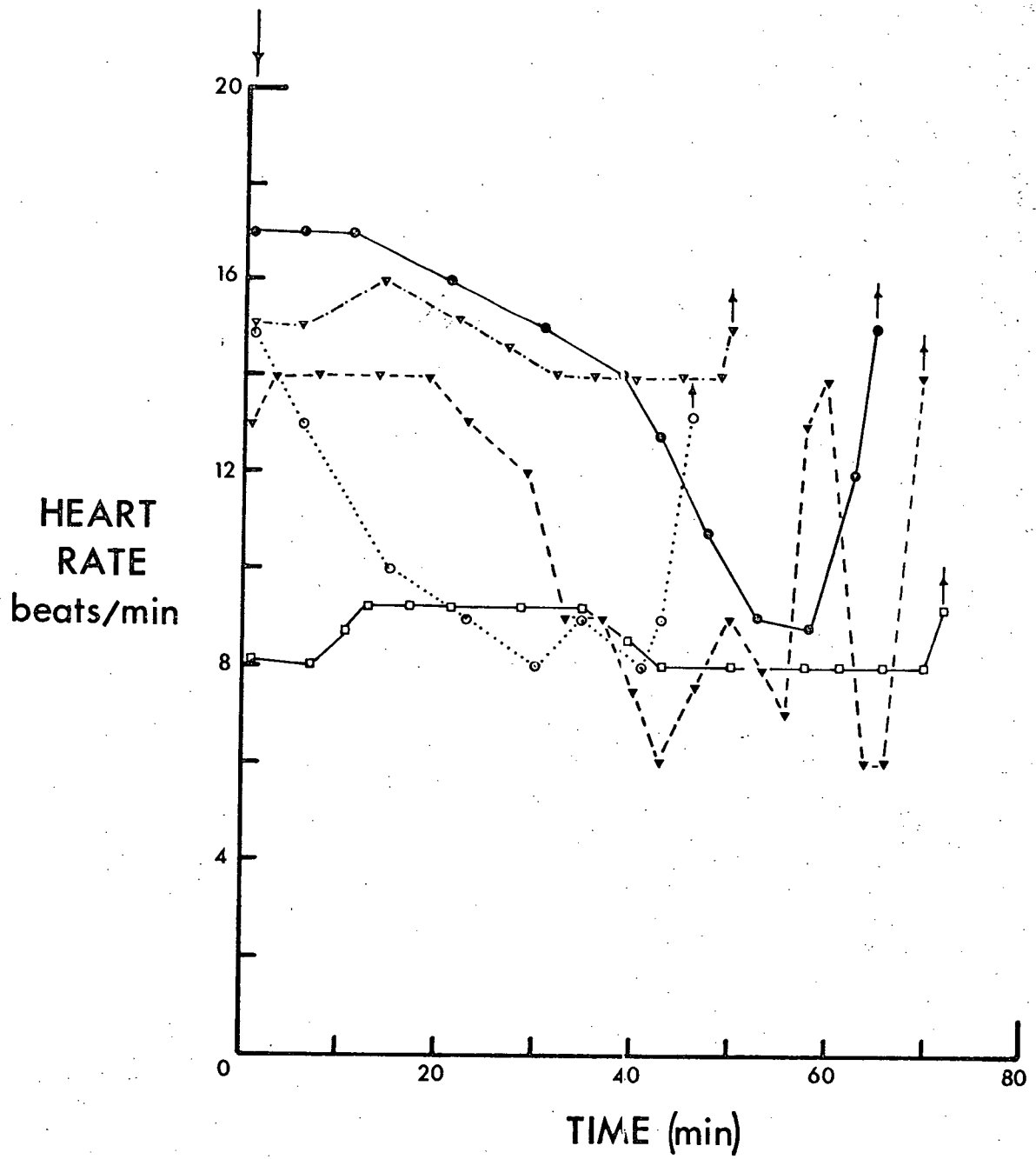
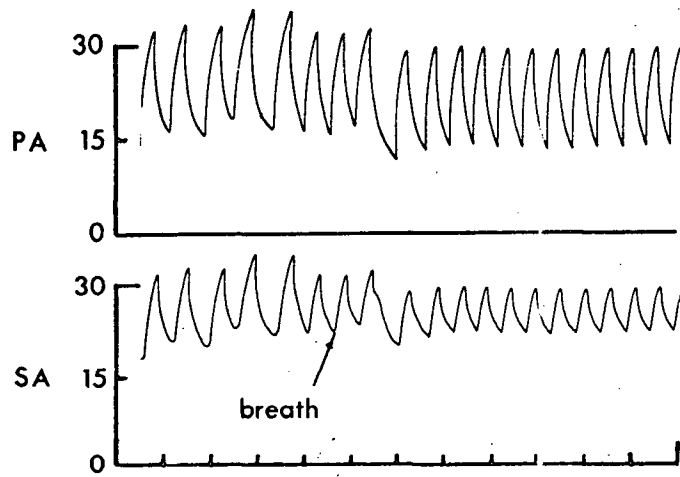


Figure 23 Breathing and lung inflation and deflation effects on the pulmonary artery (PA) and systemic arch (SA). Recordings obtained simultaneously.

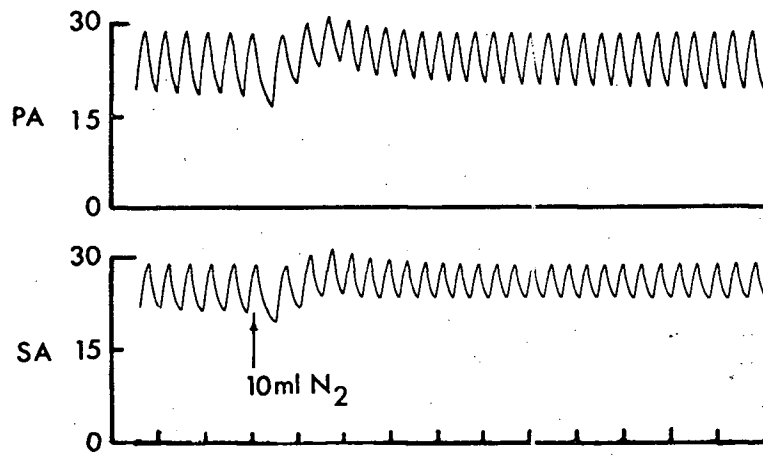
- a. Breathing tachycardia and the associated blood pressure drop in the pulmonary and systemic blood circuits.
- b. Artificial filling of the lungs with nitrogen
- c. The effects of lung deflation on pulse pressure.

A.

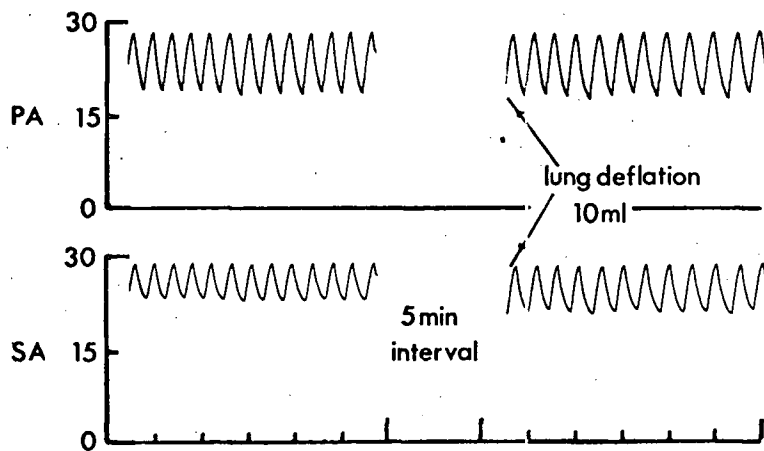


B.

BLOOD PRESSURE
mm Hg



C.



TIME (10sec. interval)

animal was in a state of bradycardia, a breathing type bradycardia would result, diastolic pressures would fall more in the pulmonary artery than in the systemic arch and there was not a general decrease in blood pressure. Injections of very high concentrations of carbon dioxide (25, 50 and 100%) into the lungs produced an almost immediate drop in blood pressure and heart rate (Fig. 24).

C. THE LAG PHENOMENON

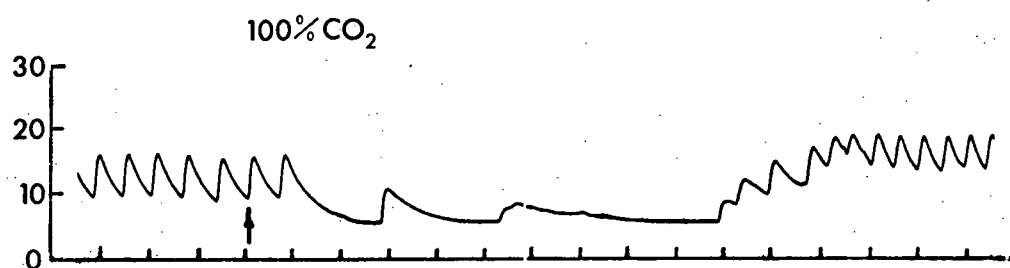
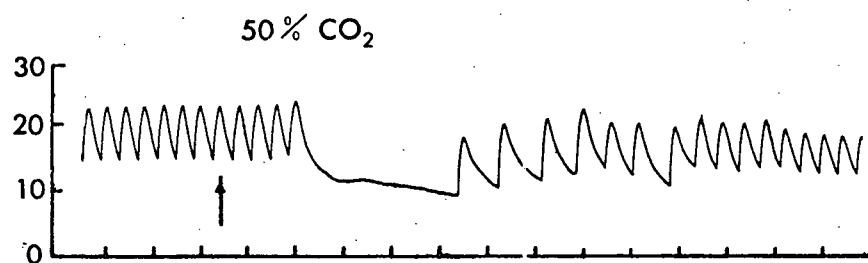
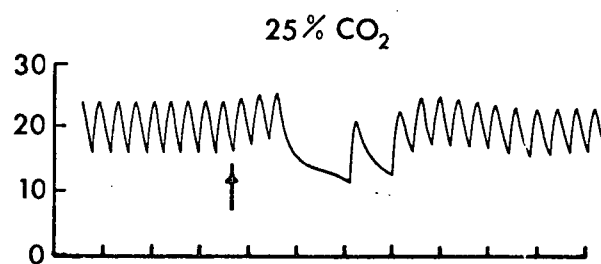
Diastolic pressures in the pulmonary arteries were on the average 3-5 mm Hg lower than those recorded simultaneously in the systemic arch at a point 1 cm anterior to the ventricle. Figure 25 illustrates this plus the fact that there was a definite lag in blood pressure rise in the systemic arch. The pressure rise in the pulmonary arch was very rapid at the start of ventricular systole and it was only at the point where the blood pressure in the two circuits was equal that the pressure rose in the systemic circuit. In the animal described in Figure 24, the lag was calculated to be 0.18-0.20 seconds in duration.

Figure 26a shows the pressure relationships in the two main arches in a very slowly beating heart (about 8 beats/min). The calculated lag is 0.20-0.25 sec

Figure 24 Injections of high concentrations of carbon dioxide into the lungs and the associated pressure changes in the systemic arch.

DORSAL AORTA
BLOOD PRESSURE

mm Hg



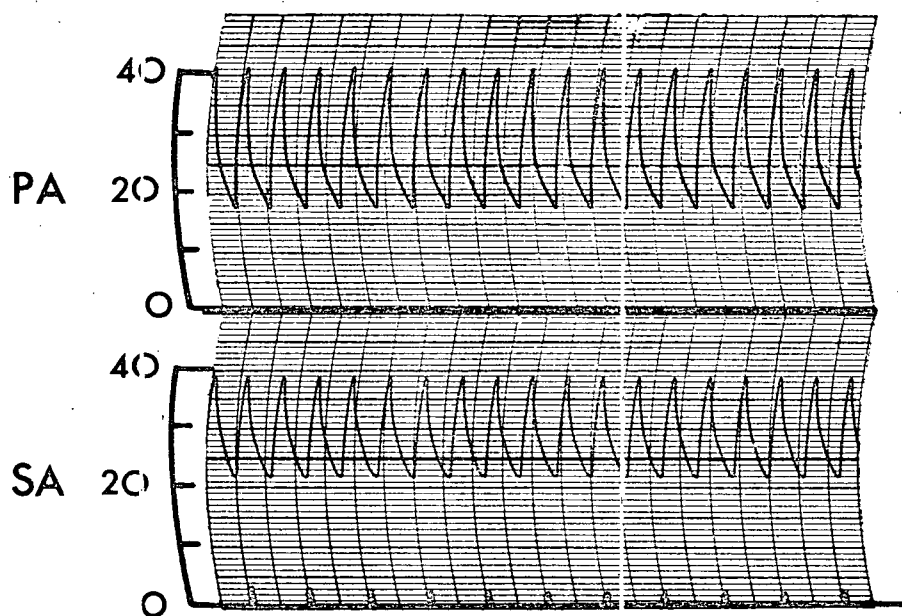
TIME (10 sec.interval)

Figure 25 Simultaneous pressure recordings in the pulmonary artery (PA) and systemic arch (SA) showing the pulse lag in the systemic arch.

- a. slow chart drive on recorder.
- b. rapid chart drive on recorder.

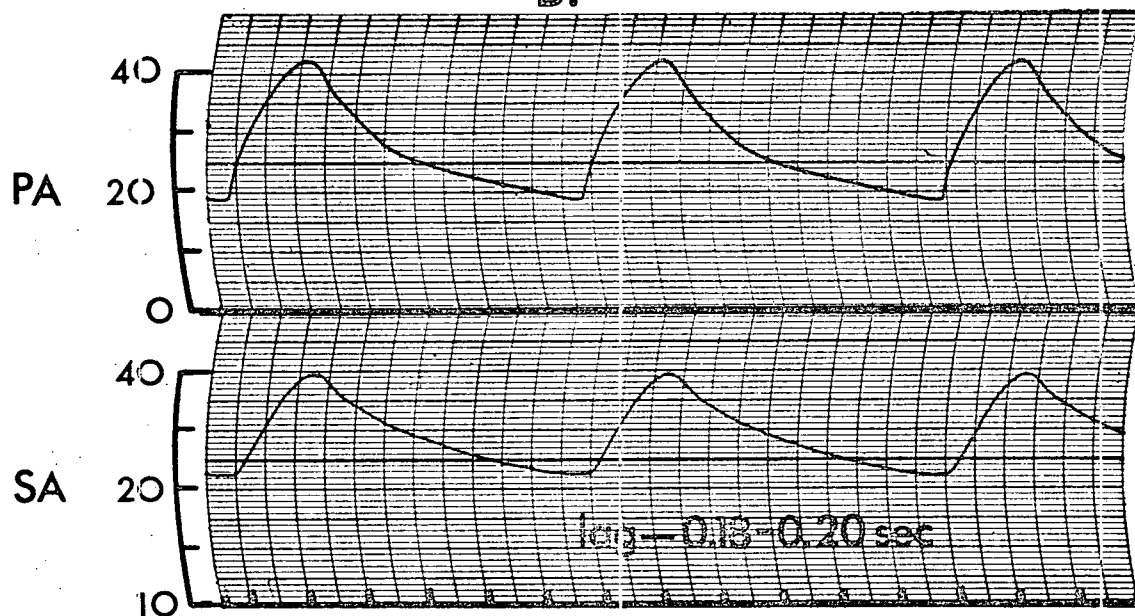
BLOOD PRESSURE
cm H₂O

A.



TIME INTERVAL — 10 sec

B.

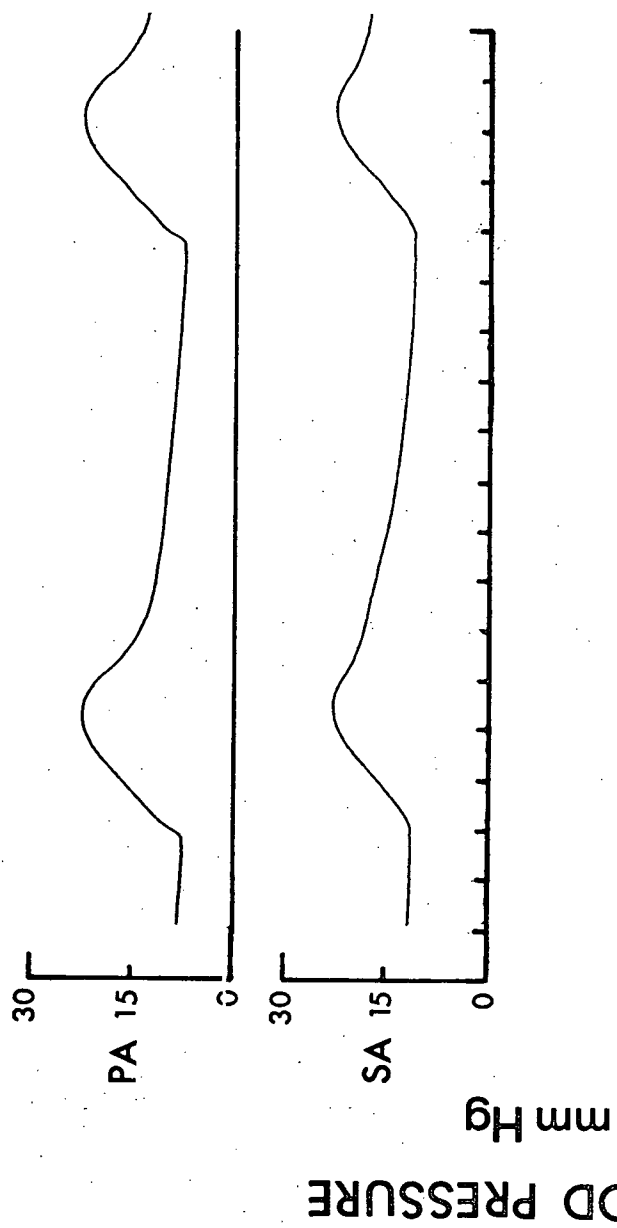


TIME INTERVAL — 1 sec

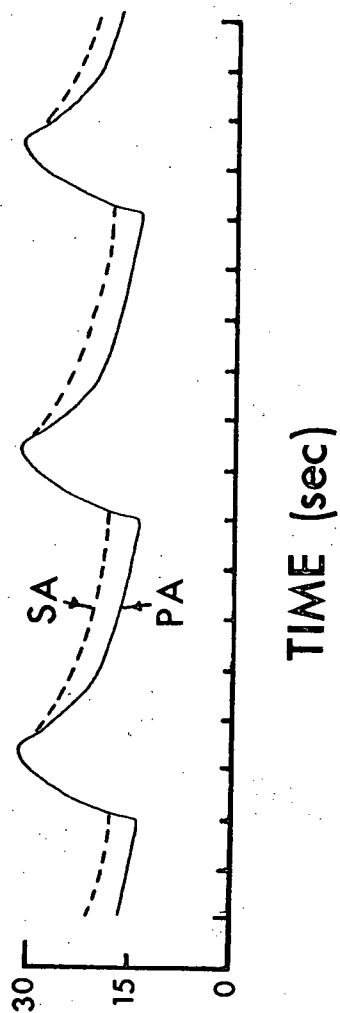
Figure 26 Pressure relationships in the pulmonary artery (PA) and systemic arch (SA) in

- a. slowly beating heart:
- b. Superimposed pressure recordings from the pulmonary and systemic arches in another Amphiuma.

A.



B.



and the diastolic pulse pressure difference between the two arches is 3.8 mm Hg. Figure 26b shows the difference in outflow pattern in the two circuits. There is a more rapid fall in blood pressure after incisura in the pulmonary artery than in the systemic circuit. Peak systolic pressures in this particular animal were reached simultaneously in the two arches, which was always the case. Systolic pressures rarely differed by more than 0.0-1.0 mm Hg in the two arches.

DISCUSSION

Jones and Shelton (1964) and Jones (1966; 1968) have discussed diving bradycardia in several species of anuran amphibians. They have shown that diving bradycardia is very pronounced in the Anura. Amphiuma tridactylum, an aquatic urodele, does not show a rapid drop in heart rate upon submergence, which may either reflect the fact that submergence was "voluntary" or that, because a submerged habitat is normal for Amphiuma, the diving bradycardia is not very pronounced.

The stimuli involved in bringing about diving bradycardia in amphibians is still unclear. Leivestad (1960), working on the toad, Bufo bufo, has shown that submergence for two hours and the resultant diving bradycardia does not result in an oxygen debt being built up. Jones (1967) has shown that during submergence in three species of anuran amphibians, the relationship between heart rate and oxygen uptake is simply; "if one is low then the other is generally low". In Amphiuma the relationship is also that lower oxygen consumption during the latter part of the submerged period usually coincides with lower heart rates.

When Amphiuma breathes, or the lungs are artificially inflated with air, there is a greater increase in

pulse pressure in the systemic arch than in the pulmonary artery. This is very indicative of an increased flow to the pulmonary circuit for a short period of time after a breath. Recent blood flow studies done by Shelton (pers. comm.) on Xenopus laevis and Jones (pers. comm.) on Rana pipiens, indicate that for a short period of time after a breath there is increased blood flow to the pulmonary circuit. After the initial increase the blood flow to the pulmocutaneous dropped and in Rana pipiens there was less flow to the pulmocutaneous than the systemic arches and in Xenopus laevis the blood flow to the two arches was more or less equal. Therefore, in Amphiuma, if pressure falls as flow increases in the pulmonary artery, there must be a substantial fall in lung peripheral resistance during breathing. To account for this phenomenon there must be increased vasoconstriction during the submerged period and vasodilation during the breathing process and for a short period of time thereafter.

 Injections of nitrogen into the lungs produced the same effects as those described by Jones (1966) in that there is a "release of the bradycardia" but the effects on blood pressure of nitrogen injection are different from those which occur when the animal breathes normally. Systolic and diastolic pressure increase in the pulmonary

artery but return to normal within 1-2 minutes. Systolic and diastolic pressures increase in the systemic arch for 1-2 minutes but the diastolic pressures remain elevated, resulting in a decrease in pulse pressure overall. The explanation for this might be that with nitrogen injection there may be no vasoconstrictive or vasodilatory responses and the decrease in pulse pressure might be due to the increase in heart rate or possibly the increase in resistance in the body circuit as a result of the physical displacement in the body caused by lung inflation.

The rapid drop in heart rate and blood pressure after injections of high concentrations of carbon dioxide into the lung is difficult to explain without some further experimentation in this area. Explanations of this phenomenon are complicated by the fact that to ellicit such a response, extremely high, unphysiological concentrations of carbon dioxide were necessary.

Peak systolic pressures recorded simultaneously in the pulmonary and systemic arches are, in the majority of cases, equal and occur at the same time. If the frequency response time in the recording system is adequate (this has already been shown to be true in this study) and pressures are recorded within 2 cm of the ventricle, pressure pulses

from the ventricle will arrive at the recording sites simultaneously (Shelton and Jones, (1968); Womersley, (1955)). Diastolic pressures and the rate of runoff are on the other hand related to peripheral resistance, compliance and heart rate. Runoff has been shown by Shelton and Jones (1968) and de Graaf (1957) to be more rapid in the pulmonary arch and it was suggested by de Graaf that this was a result of the lower resistance in the lung capillary beds. The pulse lag recorded in the present study, between the pressure rise in the pulmonary artery and systemic arch, could not occur unless there was some occlusion to flow in the systemic circuit for a short period of time.

In an attempt to elucidate the anatomical functioning of the spiral valve and associated structures, ten Amphiuma were anaesthetized after normal experimentation and the ventricle, conus, truncus and pulmonary portions of the circulatory system were dissected free of the body. In saline solutions, incisions or "windows" were made in the anterior portion of the conus and posterior portion of the truncus such that the functioning of the spiral valve could be observed with a dissecting microscope. It appeared that the spiral valve in Amphiuma was a triangular ridge of muscular tissue extending the length of the conus and pro-

truding into the conus lumen to the extent of $2/3$ of the conus diameter. At the anterior end of the conus the triangular ridge becomes a round, rather lobular structure which appears to occlude the entrance to the systemic circuit during the initial ventricular outflow. This tissue could occlude the vessel briefly as the blood pressure rises or it could simply be forced into this position as the conus is mechanically elongated as it becomes turgid with blood. Of the two alternatives, I would prefer the "occlusion as a blood pressure phenomenon" in that the pressure lag in the systemic circuit is very brief and a pulse pressure is recorded there at the same time as the pressures in the two circuits are equal.

A pulse lag of 0.2 seconds does not strike one as being significant when compared to a heart beat which extends for 5-6 seconds. However, if one considers that ventricular output occurs from the start of systole to the incisura, the lag time can amount to 10-15% of the ventricular output time. Could the amount of time, which primarily deoxygenated blood is flowing to the pulmonary circuit, be long enough to set up the gradients between the two circuits reported earlier in this study (Part I)? Speculating on the data available it would appear that, if the

first 10-15% of the blood leaving the ventricle was mixed venous and entered the pulmonary circuit. the gradients between the pulmonary artery and systemic arch in Amphiuma could have been obtained by this phenomenon.

Shelton and Jones (1968) have shown in the urodele Salamandra salamandra that there are no pulse pressure differences between the pulmonary and systemic circuits, and suggest that the presence of a ductus Botalli equalizes pressure in the two circuits. Simons (1959), working on Triturus cristatus and Salamandra maculosa, found that there was no differential distribution of blood demonstratable by injection of dye. Interestingly enough, both of these animals possess a ductus Botalli. This embryonic blood vessel is retained in most urodeles; Amphiuma provides one exception. It follows then, if all terrestrial amphibians dependent upon pulmonary respiration, lack a ductus Botalli (to my knowledge all adult anurans lack this vessel) the pressure difference created by having a separate pulmonary and systemic circuit could be strongly implicated in the separation of the two types of blood.

It has been shown that there are pulse pressure differences in the two circuits, a possible explanation

has been given as to how the diastolic pressure differences in the two circuits could result in blood from the body being in part sent to the lung circuit and the reason for such a phenomenon I think is best stated by Foxon (1964) when he said, "perhaps evolutionary selection has acted in favour of those animals which possessed, not some hypothetical mechanism for the selection of blood for the head region, but some mechanism which prevented blood which had returned from the lungs from being immediately sent there again".

SUMMARY

1. The experimental animal of this study was Amphiuma tridactylum, an aquatic urodele. Amphiuma breathed about once every hour and almost completely replaced all air within the lungs at each breath.

2. While Amphiuma remained submerged between breaths, oxygen was removed from the lungs but carbon dioxide levels did not increase. The R line for alveolar gases in this animal was therefore zero.

3. Oxygen consumption in Amphiuma at 15°C was the lowest recorded for any amphibian at a comparable temperature. Most of the oxygen was consumed within the first fifteen minutes of submergence. The primary respiratory surface for oxygen consumption was the lungs.

4. Oxygen tensions in the major vessels oscillated with the breathing cycles. There was a definite gradient between the pulmonary artery and systemic arch which persisted throughout the breathing cycle. The gradient decreased with time submerged, being caused by the decrease in gradient between the oxygen tensions in the pulmonary vein and venous return.

5. After each breath in Amphiuma the oxygen tensions, in all the vessels studied rose rapidly, the tensions in the pulmonary vein increased to levels found

in the lungs, and were usually completely saturated.

6. Termination of inspiration was shown to be controlled by a volume detection mechanism. Animals were shown to continue the breathing process if air was simultaneously removed from the lungs through a lung cannula. Injections of nitrogen into the lungs terminated inspiration for a short time but breathing occurred a short time after.

7. Carbon dioxide in the lungs in doses 3-5 times the normal levels were removed from the lungs rapidly and did not result in the onset of breathing if the oxygen tensions were sufficiently high. Very high concentrations of carbon dioxide in the lungs resulted in a shortening of the time between breaths. Increased levels of carbon dioxide in the dorsal aorta did not bring about the breathing response.

8. Removal of oxygen from the lungs brought about a rapid breathing response. The presence of an arterial oxygen chemoreceptor was postulated as a mechanism for controlling breathing in Amphiuma.

9. The diving bradycardia response in Amphiuma was not very pronounced and was quite irregular.

10. When Amphiuma breathed there was a greater increase in pulse pressure in the systemic arch than in

the pulmonary artery. If pressure fell as flow increased, there must have been a substantial fall in lung peripheral resistance when the animal breathed.

11. There was a lower diastolic pressure in the pulmonary artery than in the systemic arch. Pulse pressure was generally greatest in the pulmonary artery. There was a slight pressure lag in the systemic arch compared to the pressure rise in the pulmonary artery. It was suggested that the first blood to leave the ventricle would flow to the pulmonary artery initially because of the lower pressure in the lung circuit and possibly because the entrance to the systemic circuit appeared to be blocked during the initial phase of ventricular output. The pressure lag phenomenon in the systemic arch was thought to account for the PO_2 difference between the pulmonary artery and the systemic arch.

LITERATURE CITED

- Acolat, M.L. 1931. Recherches anatomiques relatives à la separation du sang veineux et du sang arterial dans la coe de la Grenouille. C.R. Acad. Sci. Paris. 192: 767-769.
- _____ 1938. Étude comparé de la pression sanguine dans le circuit pulmonaire et dans le circuit general chez lez Batracien et les Reptiles. C.R. Acad. Sci. Paris. 206: 207-209.
- Baker, C. L. 1945. The natural history and morphology of Amphiumae. J. Tennessee Acad. Sci. 20: 55-91.
- Brown, G.W.Jr. 1964. The metabolism of Amphibia. In Physiology of the Amphibia. Edited by J.A. Moore. Academic Press, New York and London. pp. 1-98.
- Brucke, E. von. 1852. Beiträge zur vergleichanden anatomie und physiologie des gefäss-systems der amphibien. Denkschr. Akad. Wiss. Wien. 3: 335-367.
- Czopek, J. 1962. Vascularization of respiratory surfaces in some Caudata. Copeia. 1962: 576-587.
- Darnell, R. M. 1948. Environmental factors which determine the habitat of Amphiuma. J. Tennessee Acad. Sci. 23: 3-12.
- DeLong, K. T. 1962. Quantative analysis of blood circulation through the frog heart. Sci. 138: 693-694.

- Foxon, G.E.H. 1964. Blood and respiration. In Physiology of the Amphibia. Ed. J.A. Moore. Academic Press, New York and London. pp. 151-209.
- de Graaf, A. R. 1957. Investigations into the distribution of blood in the heart and aortic arches of Xenopus laevis. J. Exp. Biol. 34: 143-176.
- Hazelhoff, E. H. 1952. Die trennung der blutmassen mit verschiedenem sauerstoffgehalt im froscherzen. Experientia. 8: 77.
- Hornbein, T. F. 1965. The chemical regulation of ventilation. In Physiology and Biophysics. Ed. T. C. Ruch and H. D. Patton. W.B. Saunders Co. Philadelphia and London. pp. 803-819.
- Hughes, G. M. 1966. Species variation in gas exchange. Proc. Roy. Soc. Med. 59: 494-500.
- Johansen, K. 1963. Cardiovascular dynamics in the amphibian, Amphiuma tridactylum, Cuvier. Acta. Physiol. Scand. Suppl. 217: 1-82.
- Johansen, K. and A.S.F. Ditadi. 1966. Double circulation in the giant toad Bufo paracnemis. Physiol. Zool. 39: 140-150.
- Johansen, K. and D. Hanson. 1968. Functional anatomy of the hearts of lungfishes and amphibians Am. Zoologist. 8: 191-210.

- Jones, D. R. 1966. Factors affecting the recovery from diving bradycardia in the frog. *J. Exp. Biol.* 44: 397-411.
- _____ 1967. Oxygen consumption and heart rate of several species of Anuran Amphibia during submergence. *Comp. Biochem. Physiol.* 20: 691-707.
- _____ 1968. Specific and seasonal variations in development of diving bradycardia in Anuran Amphibia. *Comp. Biochem. Physiol.* 25: 821-834.
- Jones, D.F. and G. Shelton. 1964. Factors influencing submergence and the heart rate in the frog. *J. Exp. Biol.* 41: 417-431.
- Krogh, A. 1904. On the cutaneous and pulmonary respiration of the frog. *Skand. Arch. Physiol.* 15: 328-419.
- Lambertsen, C. J. 1961. Respiration. In Medical Physiology, Part V. Ed. P. Bard. C.V. Mosby Co., St. Louis. pp. 559-710.
- Leivestad, H. 1960. The effect of prolonged submersion on the metabolism and the heart rate in the toad. *Arbok. Univ. Bergen. (Mat.-nat. serie)* 5: 1-15.
- Lenfant, C. and K. Johansen. 1967. Respiratory adaptations in selected amphibians. *Resp. Physiol.* 2: 247-260.

- Maren, T. H. 1967. Carbonic anhydrase: chemistry, physiology and inhibition. *Physiol. Rev.* 47: 595-781.
- de Marneffe-Foulon, C. 1962. Contribution à l'étude du mécanisme et du contrôle des mouvements respiratoires chez Rana. *Ann. Soc. Zool. Belg.* 92: 81-132.
- Neil, E., L. Strom and Y. Zotterman. 1950. Action potential of afferent fibres in the IX and X cranial nerves of the frog. *Acta. Physiol. Scand.* 20: 338-350.
-
- _____ 1950. Cardiac vagal afferent fibres in the cat and frog. *Acta. Physiol. Scand.* 20: 160-165.
- Noble, G. K. 1925. The integumentary, pulmonary and cardiac modifications correlated with increased cutaneous respiration in the Amphibia: a solution to the "hairy frog" problem. *J. Morph.* 40: 341-416.
- Noble, G. K. 1931. *The Biology of the Amphibia* McGraw Hill, New York.
- Paintal, A. S. 1963. Vagal afferent fibres. *Ergebn. Physiol.* 52: 74-156.
- Sabatier, A. 1873. Études sur la coeur et la circulation centrale dans la série des Vertébrés. Montpellier, Paris.

- Scott, W. J. 1931. Oxygen and carbon dioxide transport by the blood of the urodele, Amphiuma tridactylum. Biol. Bull. 61: 211-222.
- Sharma, H. W. 1957. The anatomy and mode of action of the heart of the frog, Rana tigrina. J. Morph. 100: 313-344.
- Shelton, G. and D. R. Jones. 1965a. Pressure and volume relationships in the ventricle, conus and arterial arches of the frog heart. J. Exp. Biol. 43: 479-488.
-
- 1965b. Central blood pressure and heart output in surfaced and submerged frogs. J. Exp. Biol. 42: 339-357.
- Shelton, G. and D. R. Jones. 1968. A comparative study of central blood pressures in five amphibians. J. Exp. Biol. 49: 631-643.
- Shimada, K. 1966. Mechanical properties of the smooth muscle in the lung of the toad. Acta. Med. Biol. (Niigata) 14: 23-33.
- Simons, J. R. 1959. The distribution of the blood from the heart in some Amphibia. Proc. Zool. Soc. Lond. 132: 51-64.
- Simons, J. R. and A.R. Michaelis. 1953. A cinematographic technique using ultra-violet illumination for amphibian blood circulation. Nature. Lond. 171: 801

- Taglietti, V. and C. Casella. 1966. Stretch receptors stimulation in frog's lungs. *Pflugers Arch. ges. Physiol.* 292: 297-308.
- Taglietti, V. and C. Casella. 1968. Deflation receptors in frog's lungs. *Pflugers Arch. ges. Physiol.* 304: 81-89.
- Vandervael, F. 1933. Recherches sur le mécanisme de la circulation du sang dans la coeur des amphibiens Anoures. *Arch. Biol. Paris.* 44: 571-606.
- Widdicombe, J. G. 1964. Respiration reflexes. *In* Handbook of Physiology, sec. 3, vol. 1. Amer. Physiol. Soc., Washington. pp. 585-630.
- Winterstein, H., F. Alpdoğan, and M. Başoğlu. 1944. Untersuchungen über die alveolarluft des frosches. *Istanb. Univ. Fen. Fak. Mecm.* 9: 171-180.
- Womersley, J. R. 1955. Method for the calculation of velocity, rate of flow and viscous drag in arteries when the pressure gradient is known. *J. Physiol.* 127: 553-563.