# FACTORS INFLUENCING GAS EXCHANGE IN THE RAINBOW TROUT (*SALMO GAIRDNERI*)

by

GEORGE F. HOLETON

B. Sc., University of Alberta, Calgary, 1964

A thesis submitted in partial fulfilment of
the requirements for the degree of

MASTER OF SCIENCE

in the Department

of Zoology

We accept this thesis as conforming to
the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

June, 1966

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Department of Zoology

The University of British Columbia
Vancouver 8, Canada

Date Sept 22, 1966

## ABSTRACT

A study of factors affecting gas exchange at the gills of rainbow trout during hypoxia was made. Techniques for implanting cannulae in the buccal and opercular chambers, the dorsal aorta, and the ventral aorta are described. The responses of afferent and efferent blood and water pressures, $Po_2$, $PCo_2$, pH, and the hematocrit of efferent blood were made while the unanaesthetized free swimming trout was in a sealed respirometer. The fish were allowed to consume the oxygen in the respirometer to produce conditions of hypoxia. Determination of oxygen uptake and a knowledge of the solubility of oxygen in blood and water allowed indirect estimation of ventilation volume, cardiac output and stroke volume of the heart. With this knowledge, inferences on the function of circulatory and respiratory mechanisms during hypoxia were drawn. The rainbow trout possesses a number of homeostatic mechanisms which augment oxygen uptake during hypoxia. It was found that the trout could maintain a fairly uniform oxygen uptake in environmental oxygen tensions as low as 30 to 50 mm Hg. The major homeostatic responses to hypoxia affecting oxygen uptake were: an increase in ventilation volume, an increase in the functional capacity of the blood, and an apparent vascular shunting of blood closer to the surface of the respiratory epithelium.

No significant increase in cardiac output was observed. Since there was a pronounced bradycardia with hypoxia the stroke volume was apparently increasing. The role of the bradycardia, which has usually been associated with a reduction

in cardiac output, is not clear. Possible reasons and functions of the bradycardia are discussed.

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ACKNOWLEDGMENT

I wish to thank Dr. D. J. Randall for suggesting this this research problem and for his guidance and encouragement in the present study.

I am grateful to Drs. W. S. Hoar, J McFadden, and J. E. Phillips for their advice and suggestions in the preparation of this manuscript.

Thanks are also due to Dr. J. R. Brett for use of facilities and equipment at the Biological Station at Nanaimo, B. C., and to the technical staff at the station for their ready assistance, advice, and for procuring and caring for some of the fish used in this work.

I am indebted to Mrs. C. Beaumont for technical assistance in analysing blood samples and for assistance in preparing illustrations for this manuscript.

This research was assisted by the National Research Council of Canada through a grant to Dr. D. J. Randall and is gratefully acknowledged.

I wish to express my appreciation for the technical assistance of Miss Georgina Docherty in analysing blood samples for lactate.

# INTRODUCTION

Mott (1957) reviewed the cardiovascular system of fishes and stressed the limited nature of the present knowledge, and the need for more information. Much of the present information on circulatory and respiratory systems of fishes is of a fragmentary nature encompassing fish of widely differing habitat, habit, and species. There is a definite need for an integrated and comprehensive study of circulatory and respiratory characteristics on a single species of fish. There has been no study which has measured all the major factors governing gas exchange at the gills of fish in a simultaneous and integrated manner. The object of the present study was to attempt to measure, and to interpret the significance of, the factors affecting gas exchange at the gills of a fresh water teleost, the rainbow trout (Salmo gairdneri R.).

A major difficulty in measuring the circulatory and respiratory parameters of fish is that the fish must be able to breathe, a problem which limits the time or the conditions under which the various operations and measurements can be made. This, to a great extent, explains the limited and fragmentary nature of the current information. Rushmer and Smith (1959) emphasized the need for, and the importance of, making studies on the cardiovascular systems of animals which are intact, unrestrained, and unanaesthetized. This would suggest that much of the current knowledge of fish circulatory parameters may not be of physiological significance. Much of the information has been obtained from fish which are

not intact, (Berg & Steen, 1965; Goldstein, Forester, & Fanelli, 1964; Greene, 1904; Itazawa, 1957; Johansen, 1962; Satchell, 1960, 1962; Steen & Kruysse, 1964) or from fish which are either restrained or anaesthetized (Burger & Bradley, 1951; Itazawa, 1957; Mott, 1950; Randall & Shelton, 1963; Satchell, 1960, 1961, 1962).

The effect of hypoxia upon circulatory and respiratory parameters of fish is not well documented. The influence of hypoxia upon the heart rate, breathing rate, and breathing amplitude of tench (Tinca tinca L.) has been reported by Randall and Shelton (1963). However, little is known about changes in blood pressure and vascular resistance during hypoxia. Satchell (1962) demonstrated that the dogfish (Squalus acanthias) responded to anoxia by a transient decrease in heart rate and dorsal aortic blood pressure associated with an increase in the mean and pulse pressures in the ventral aorta. It was shown that an increase of peripheral resistance, an increase in pressure drop over the gills, and an increase in stroke volume of the heart were all implicated in the response of the dogfish to anoxia.

An insight can be obtained into the mechanism of regulation of gas exchange in fish by recording changes in gas tensions in the blood and water, afferent and efferent to the gills during hypoxia. Little information of this kind is available in the literature. Van Dam (1938), Itazawa (1957), and Saunders (1962) have listed some information on the effects of hypoxia on blood and water gas tensions, but there has been no integrated study of several of these parameters simultaneously.

The measurement of the flow of blood and water past the exchange surfaces, and changes in these flows in response to hypoxia would also allow an insight to be obtained into the gas exchange mechanisms. Changes in the flow of water past the gills in response to hypoxia have been determined previously by Van Dam (1938) and Saunders (1962) but the information is not sufficient for the present study. Although Burger and Bradley (1951), Goldstein, Forster, and Fanelli (1964), Hart (1943), Johansen (1962), and Mott (1957) have presented some information and estimates of gill blood flow of various fishes, there is, to my knowledge, no satisfactory information on changes in gill blood flow in response to hypoxia.

Hughes and Shelton (1962) and Hughes (1964) have suggested that perhaps the fundamental relationships derived from heat exchange technology would prove useful in obtaining information about the functional aspects of gas exchange and respiratory homeostatic mechanisms associated with the gills of fishes. Only fragmentary experimental information from widely varying sources was available to test these suggested relationships. A knowledge of the qualitative and quantitative aspects of the factors affecting gas exchange from a single species under a uniform condition would allow an examination of the applicability of the heat exchanger analogy relationships and possibly disclose some functional aspects of homeostatic mechanisms operating during hypoxia.

It is the object of this study to record the effects of hypoxia upon the rate of oxygen uptake and upon the pressures and gas tensions of the blood and water afferent and efferent

to the gills of unrestrained, unanaesthetized, and intact rainbow trout. This knowledge will then be utilized to estimate changes in the flow of blood and water past the exchange surface, which in turn will allow an examination of the theoretical aspects of the exchange process and possible homeostatic mechanisms.

Techniques have been worked out for cannulating the ventral aorta, the dorsal aorta, the buccal chamber, and the opercular chamber of rainbow trout with very little tissue damage. The experiments were carried out on trout which were intact save for the insertion of hypodermic tipped cannulae into the dorsal and ventral aortae from the exterior of the fish, and the small holes punched in the cartilaginous portions of the snout and one of the operculae. Thus it has been possible, by means of direct connections, to study continuously, responses to hypoxia of the circulatory and respiratory systems, efferent and afferent to the gills of unanaesthetized, free swimming, but confined trout.

## MATERIALS AND METHODS

The experiments were carried out on 86 rainbow trout obtained from the following sources:

A. A small private hatchery near Nanoose Bay, Vancouver Island, British Columbia. Age 3 years. Weights ranged from 300 to 450 grams.

B. Summerland Trout Hatchery at Summerland, B.C. Ages were varied and not determined. All were in excess of one year old. Weights ranged from 206 to 700 grams.

C. Sun Valley Trout Farm, Port Coquitlam, B.C. These fish had a history of furonculosis. They were quarantined and treated with sulphamerazine until several weeks after all manifestations of the disease were no longer observable. Ages were 2 and 3 years. Weights ranged from 300 to 400 grams.

D. Saltspring Island, B.C. These fish were raised in large outdoor ponds and consequently were larger than fish from the other sources. Ages were 2 and 3 years. Weights ranged from 350 to 1050 grams.

The fish were held in large tanks of at least 500 gallons capacity. All experiments were carried out at the same temperature as that of the water in the holding tanks. The fish were acclimated in the holding tanks to the experimental temperature for at least one week prior to experimentation.

The experiments were carried out at the Biological Station, Nanaimo, B.C. during May to September 1965, and at the University of British Columbia from September 1965 to February 1966.

A fish was selected from the holding tank and anaesthetized by immersion in water containing 1:10,000 tricaine methanesulphonate (MS-222). The anaesthetized fish was gently placed in a

moist cloth hammock, ventral side up. Care was taken to avoid injury to the fish. Water containing 1:10,000 to 1:15,000 MS-222 was perfused over the gills by means of a recirculating pump. Provisions were made for perfusion into either the buccal cavity or into the opercular cavities. With higher water temperatures it was found that cooling and oxygenating the recirculating water greatly extended the period of time that the fish could be kept under anaesthesia in good condition.

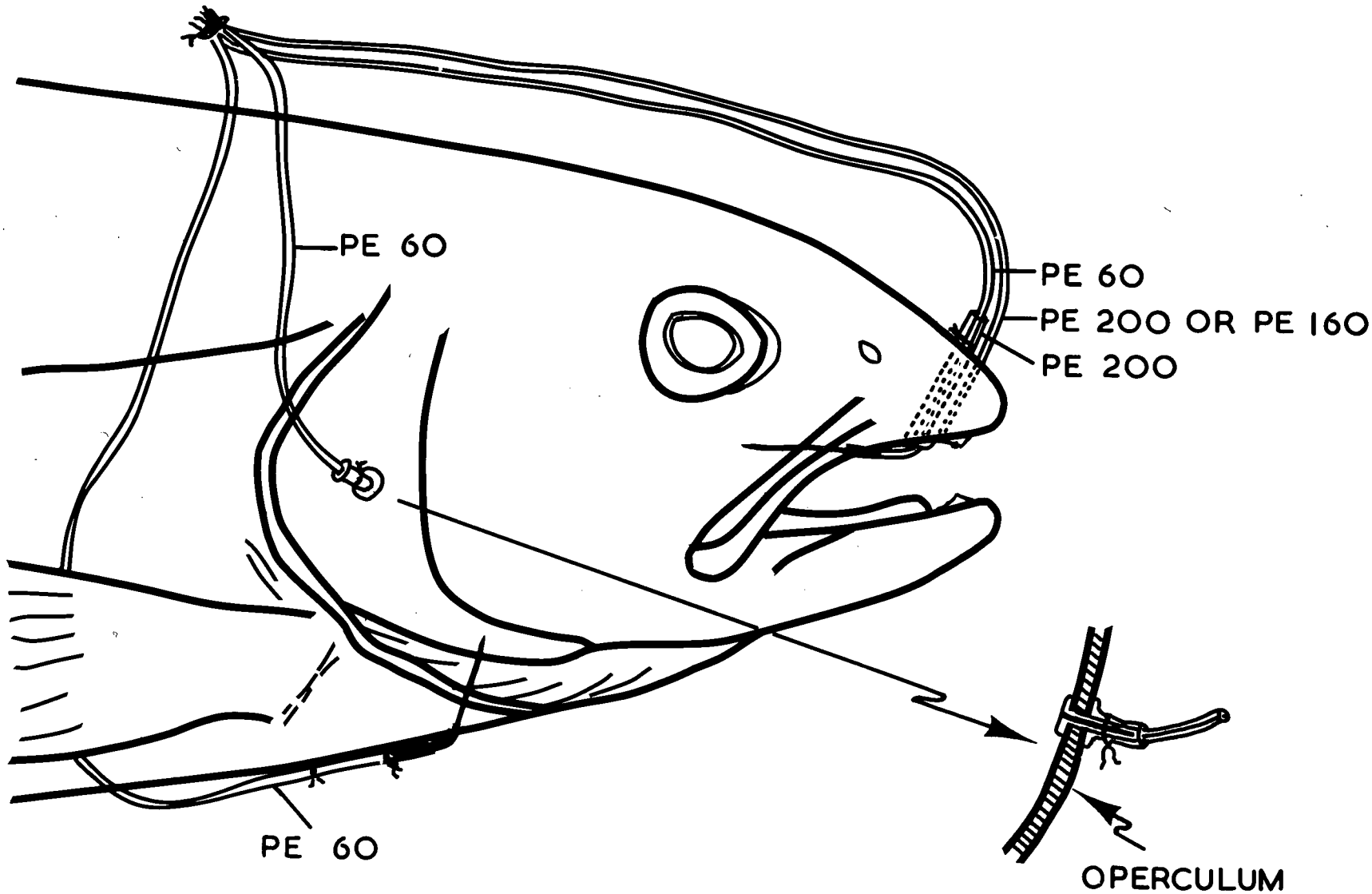
While anaesthetized the fish were cannulated at four sites as follows:

1. Buccal Cavity Cannulation

   The techniques was essentially the same as that described by Saunders (1961). Care was exercised to avoid damage to the buccal valves and the olfactory lobes. 80 cm of P.E. 200 or P.E. 160 polyethylene tubing was used (Fig. 1).

2. The Opercular Cavity Cannulation

   The cavity was cannulated (Fig. 1) by punching a hole in the center of the operculum with an 18 gauge hypodermic needle, and anchoring a heat flared 80 cm length of P.E. 60 polyethylene tubing in the hole (Saunders, 1961). A one cm length of heat flared P.E. 90 tubing was passed over the outside of the opercular cannula. This short piece of tubing was brought up snugly against the operculum and secured with thread, thus anchoring the cannula in place. P.E. 60 tubing was used in this application in order to maintain a high level of flexibility so as not to impair the normal functioning of the operculum.


PE 60
PE 60
PE 200 OR PE 160
PE 200
PE 60
OPERCULUM

Legend for Figure 1

Details of the head of a trout showing relative positions of the cannulae.

3. The Dorsal Aortic Cannulation

Cannulation of the dorsal aorta was carried out as described by Smith and Bell (1964). P.E. 60 polyethylene tubing, 80 cm long tipped with the end of a 21 gauge huber point hypodermic needle, was used. The point of cannulation was at the junction of the right and left supra-branchial arteries (Fig. 1).

4. The Ventral Aortic Cannulation

Cannulation was carried out using an 80 cm length of P.E. 60 polyethylene tubing, tipped with a 21 gauge short bevel hypodermic needle bent at an angle of approximately 60 degrees about 1-2 cm back from the point. The ventral aorta was usually visible from the lateral view as it passed through the isthmus. It was possible to pierce the ventral aorta from the ventral median surface while observing the progress of the cannula tip from a lateral view. The coronary artery which also passes in this region is sufficiently small and slippery that it is not cut by the cannula tip. It was found convenient to have a selection of cannula tips of various lengths and angles on hand before attempting a cannulation in order to accommodate the variability of position and angle of the ventral aorta that was found from fish to fish. It was found that the ventral aorta should be cannulated as far anteriorly as is practicable. Cannulae placed too near the bulbus are prone to leakage due to the

distensibility of the vessel in this region. Such leakage can fill the pericardial cavity with blood and death may ensue. Once the ventral aorta was pierced the cannula was anchored with two stitches, one and two centimeters posterior to the point of emergence of the cannula from the fish's isthmus. When the proper choice of tip length and angle were made, the cannula would lie flat on the ventral body surface. If the tip used was too long and the bend in the cannula tip extended beyond the point of emergence, a pad was placed under the bend to prevent the cannula from being pushed completely through the ventral aorta. This preparation was found to be flexible and resistant to sudden strains (Fig. 1).

There was no apparent effect of the ventral aortic cannulation on the action of the sternohyoideus muscles. The cannula passed between the paired sternohyoideus muscles and because of the proximity of the cannula to the point of insertion of these muscles onto the cleithrum, movement was minimal and had no effect on the position of the cannula.

The dorsal and ventral aortic cannulae were filled with heparinized (10 i.u. per ml) Courtland saline (Wolf 1963), and the ends plugged with tapered stainless steel pins.

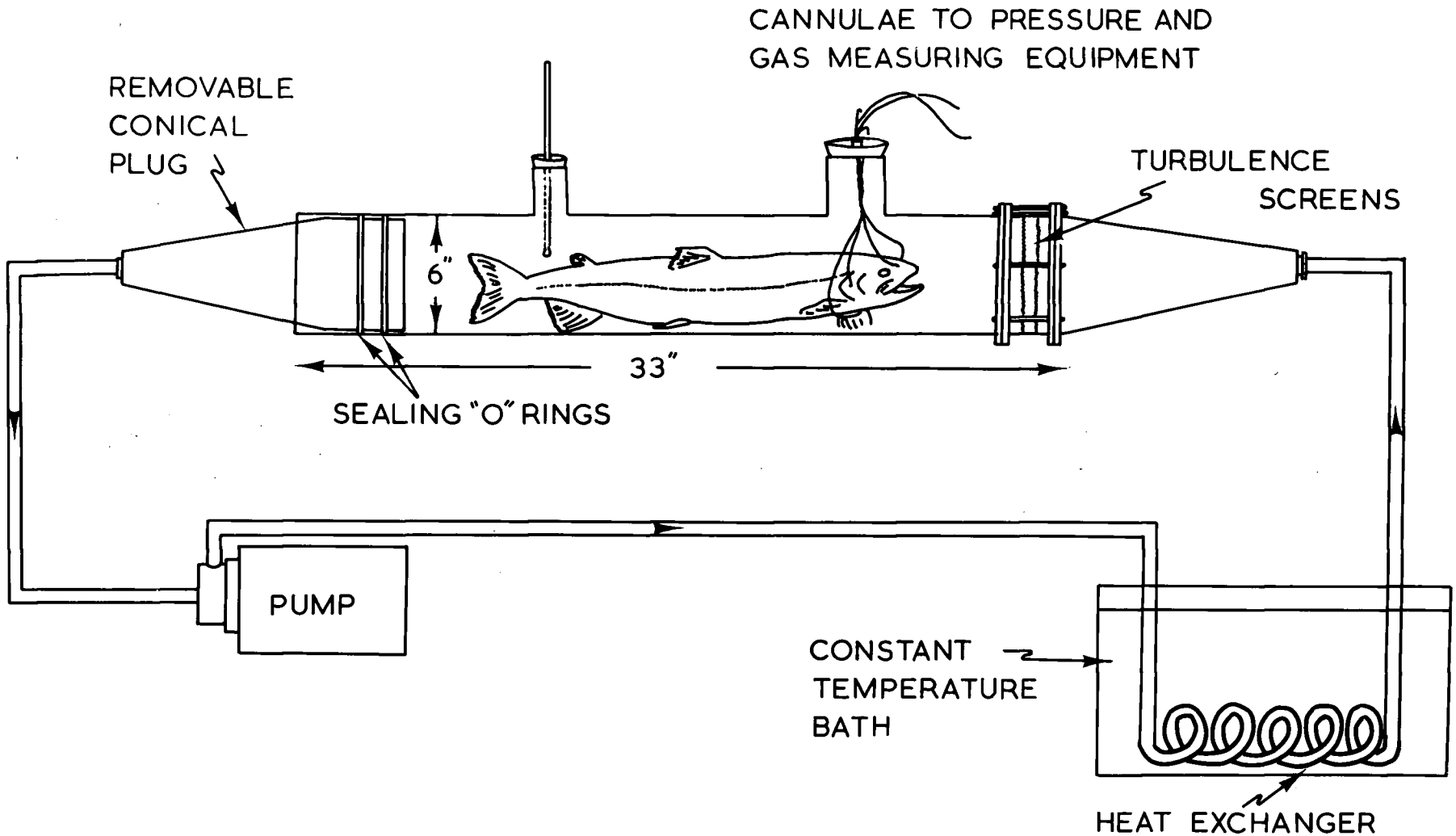
In many instances all four cannulae were not placed in a single fish, but with experience it became possible to place all four cannulae in position in 15 - 25 minutes.

After cannulation the fish were partially revived by

circulating fresh water over the gills. When regular breathing movements were observed the fish was quickly and gently transported to a respirometer (Fig. 2). The respirometer was flushed with fresh water and the fish was allowed to recover from the operation for at least eight hours. The respirometer was darkened by a black polyethylene cover during this period.

The respirometer consisted of an acrylic plastic tube (wall thickness 1.27 cm) with conical ends (Fig. 2). Its capacity was 12.23 liters. Water could be flushed or circulated through it from a tap supply or recirculated by means of a small electric pump. The capacity of the pump was approximately 13 liters per minute. Included in the recirculating water circuit was a coil of metal tubing immersed in a constant temperature bath, serving as a heat exchanger. This arrangement allowed control of the temperature of the respirometer. The upstream end of the respirometer chamber held a series of grids which produced small turbulence eddies in the incoming water (Brett, 1964). These reduced the laminar flow conditions which, if well developed, would result in unequal residence time and poor mixing of water in the respirometer. The cannulae passed out through one of a pair of exit tubes in the top of the respirometer. The respirometer was sufficiently large that all but the largest fish were able to turn around and swim from end to end of the chamber. The fish usually assumed a position near the inlet of the chamber facing upstream, and remained quiet.

To measure oxygen consumption, the exit tubes were sealed with a floating layer of liquid petrolatum and the


CANNULAE TO PRESSURE AND
GAS MEASURING EQUIPMENT
REMOVABLE
CONICAL
PLUG
TURBULENCE
SCREENS
6"
33"
SEALING "O" RINGS
PUMP
CONSTANT
TEMPERATURE
BATH
HEAT EXCHANGER

Legend for Figure 2

The respirometer used in the experiments. The fish illustrated is considerably larger than the average size of fish used in the experiments.

water movement inside switched from flushing to pump recirculation. Periodic oxygen determinations of the declining oxygen content of the recirculating water allowed computation of oxygen consumption (Brett, 1964; Brett & Sutherland, 1965; Randall, Smith, and Brett, 1965). During the period May 1965 to July 1965 oxygen was measured by means of the unmodified Winkler method as applied by Brett (1964). This method had the disadvantage that about 130 ml of water was withdrawn for each determination resulting in changes in the total volume of water in the respirometer. After August 1965 all oxygen determinations were made by means of a Beckman oxygen electrode. This method gave determinations of $PO_2$ which could be converted to oxygen content by application of solubility constants. The latter technique required only about 1 ml of water for a determination.

The temperature of the water varied from $9^{o}C$ to $19^{o}C$ during the course of the experiments. In any one experiment temperature changes never exceeded $1.2^{o}C$ and usually were less than $0.6^{o}C$. In all cases the temperature tended to increase during an experiment.

Experiments were carried out between 9:00 A.M. and 3:00 A.M.

All experiments consisted of sealing the respirometer and allowing the fish to consume the available oxygen. The rate of oxygen consumption was monitored continuously and a variety of parameters of the circulatory and breathing systems recorded. In this way the response of the circulatory and breathing systems to hypoxia was determined. When the fish showed signs of cardiac or respiratory failure due to hypoxia, flushing of the

respirometer with fresh water was resumed and the experiment terminated.

While this method is a convenient way of varying environmental oxygen, it has the disadvantage of a concomitant $CO_2$ buildup. However the solubility of $CO_2$ in water is more than 33 times that of oxygen and changes in the partial pressure of $CO_2$ were of the order of 1 - 2 mm Hg.

The recording of pressures from the circulatory and breathing systems was carried out in the following manner. Buccal and opercular cannulae were connected to Statham P 23 - AA pressure transducers. Dorsal and ventral aortic cannulae were connected to Statham P 23 - BB pressure transducers. Alternatively the dorsal and ventral aortic cannulae were connected to a Sanborn model 268B differential pressure transducer. The output from the transducers was recorded continuously with a Beckman Offner Type R Dynograph recorder.

Care was taken to ensure that all cannulae were free of constrictions and that all were the same length. All cannulae were of PE 60 polyethylene tubing except the buccal cannula which was PE 200 or PE 160.

All determinations of heart and breathing rates obtained from the pressure recordings were based on at least twenty seconds recording.

Experiments were carried out to test interaction effects that the dorsal and ventral aortic cannulae might have upon blood pressure. Fish with only one of the two cannulae in them were subjected to the same tests as were fish with both cannulae in place. No significant interaction effect or

blockage was detected as a result of the cannulations.

The $PO_2$, $PCo_2$, and pH of blood and water afferent and efferent to the gills was measured using a Beckman model 160 physiological blood gas analyser. The measurements were recorded simultaneously with measurements of blood and breathing pressures. Also at the same time serial determinations of the hematocrit and red blood cell count of the fishes blood were made. The samples for gas tension and pH analysis were conducted into modular cuvettes maintained at the same temperature as that of the respirometer. Oxygen determinations were made using a Beckman platinum - silver polarizing electrode covered with polypropylon membrane. The $PCo_2$ determinations were made using a Beckman Severinhaus type electrode covered with a teflon membrane. The pH of the samples was determined by means of a glass pH electrode. Only two of these three parameters were measured on any single experiment, but oxygen tension was measured in all cases.

The electrodes used were mounted in modular cuvettes manufactured by the Beckman instrument company, in which the sample volume required to cover the electrode tips was less than 1.0 ml.

Samples of blood or water were admitted directly into the cuvettes from the four cannulae which have already been described. The blood samples were admitted into the cuvettes by means of the fishes' blood pressure. The water samples were assisted into the cuvettes by applying a slight suction on the outlet of the cuvettes.

The analysis time for $PO_2$ was from 30 to 120 seconds,

for pH analysis time was 1 to 3 minutes, and for $PCo_2$ the time for analysis was 5 to 11 minutes.

The blood samples were returned to the fish immediately after each analysis.

All the electrodes were calibrated immediately prior to each experiment and checked at the end of each experiment for drift. In addition the pH electrode was checked and recalibrated after every 2 or 3 determinations using a freshly prepared buffer solution. Both the $PO_2$ and $PCo_2$ electrodes were calibrated with moist gas mixtures, nitrogen and atmospheric air in the case of the $PO_2$ electrode and standardized gas mixtures in the case of the $PCo_2$ electrode.

Hemolysis and clotting of the blood samples was prevented by flushing the cuvette chambers thoroughly with Courtland saline (Wolf 1963) containing a wetting agent (Tween - 80, one drop per liter) and 10 i.u./ml of sodium heparin. The cuvettes were flushed with this solution before admission of every blood sample.

Small quantities of blood were taken from the dorsal aortic cannula at the beginning of every experiment in order to determine the hematocrit value of the fish's blood. In some cases the hematocrit samples were taken periodically throughout the course of an experiment.

Blood samples were drawn from the dorsal aortic cannula periodically in order to determine the red blood cell count as well as the hematocrit values of the blood in order to determine whether or not there was any marked change in the characteristics of the blood during hypoxia.

As many measurements as possible were made during the course of an experiment.

A check was made on the lactate levels of the blood of the fish before and after experimentation. Four fish were examined. Blood samples obtained from the dorsal aortic cannulae (0.5 ml) were placed in aliquots of 10% trichloro-acetic acid immediately after withdrawal. The samples were then filtered and the filtrates were stored in a refrigerator (not frozen) until they were analysed for lactate by the method of Barker and Summerson (1941).

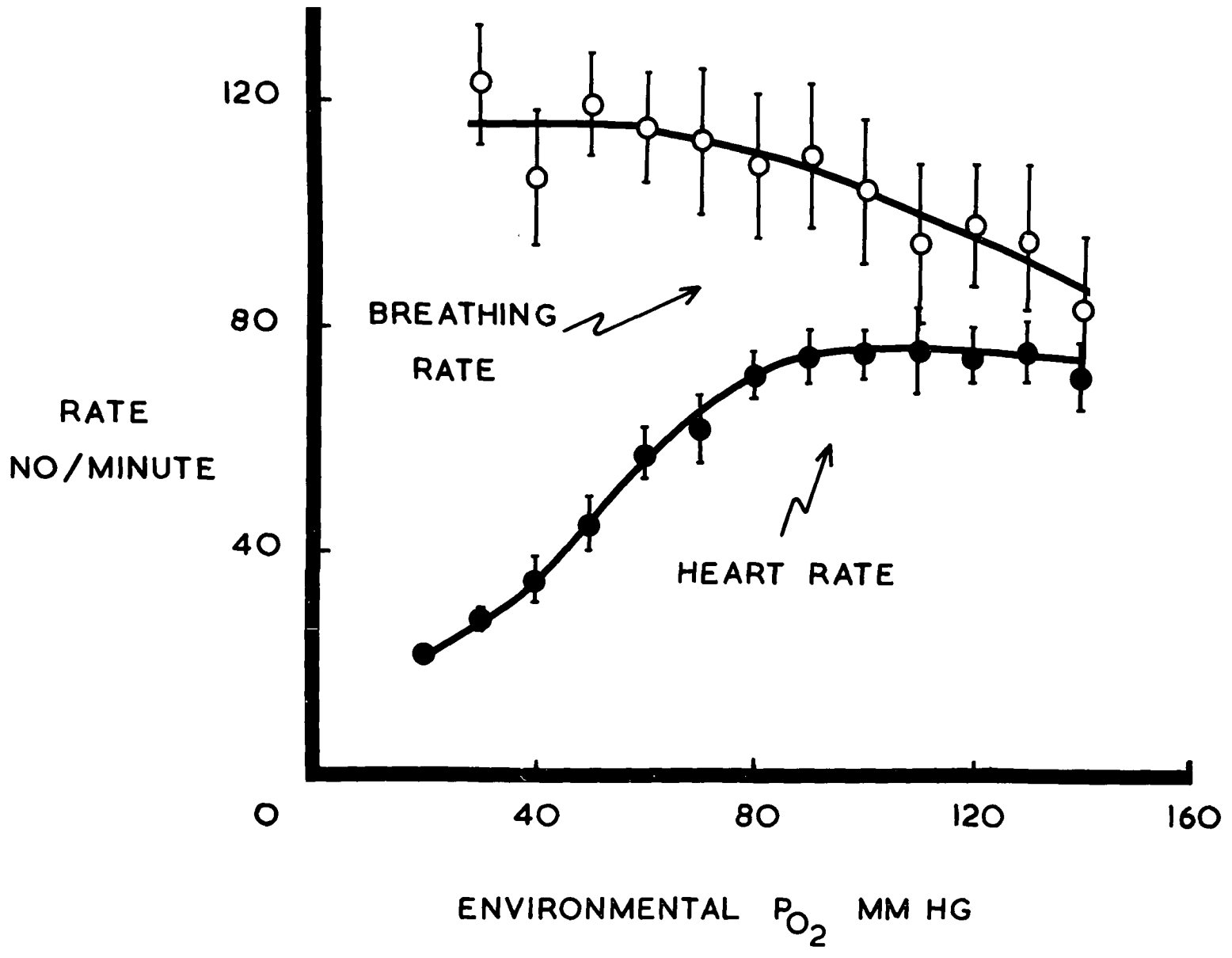
RESULTS AND DISCUSSION - I

The trout responded to hypoxia by increasing the rate and amplitude of breathing movements (Fig. 3). Breathing rate increased from 83 movements per minute to 123 per minute. Breathing rate was variable between fish owing largely to the diverse size of the fish examined. Large fish tended to respire at lower rates. Amplitude of breathing movements, as indicated by visual observations and recorded pressures, increased with lower environmental oxygen levels.

Changes in environmental $Po_2$ between 150 and 90 mm Hg produced no significant changes in heart rate. Between 90 and 30 mm Hg $Po_2$ the heart rate decreased from normal levels of 75 beats per minute to values of 25 per minute (Fig. 3). The onset of the bradycardia did not always occur gradually. In some cases the heart rate would drop abruptly.

The observed responses of heart rate, breathing rate and breathing amplitude (Fig. 3) are similar to those observed by Randall and Shelton (1964) in tench (Tinca tinca L.) but in tench the onset of bradycardia occurred at lower oxygen levels than in rainbow trout.

Ventral aortic systolic blood pressure was found to be about 54 mm Hg and the pulse pressure was found to be about 16 mm Hg, in the resting fish in well oxygenated water (Figs. 7 and 8). This value is considerably higher than those reported for elasmobranchs, 39/28 (Burger and Bradley 1951), and the eel, 37.5 mm Hg (Mott 1950), but lower than that reported for chinook salmon (74.6 mm Hg Greene 1904). There are two reasons which account, in part, for the difference in


120
80
40
0
RATE
NO/MINUTE
BREATHING
RATE
HEART RATE
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

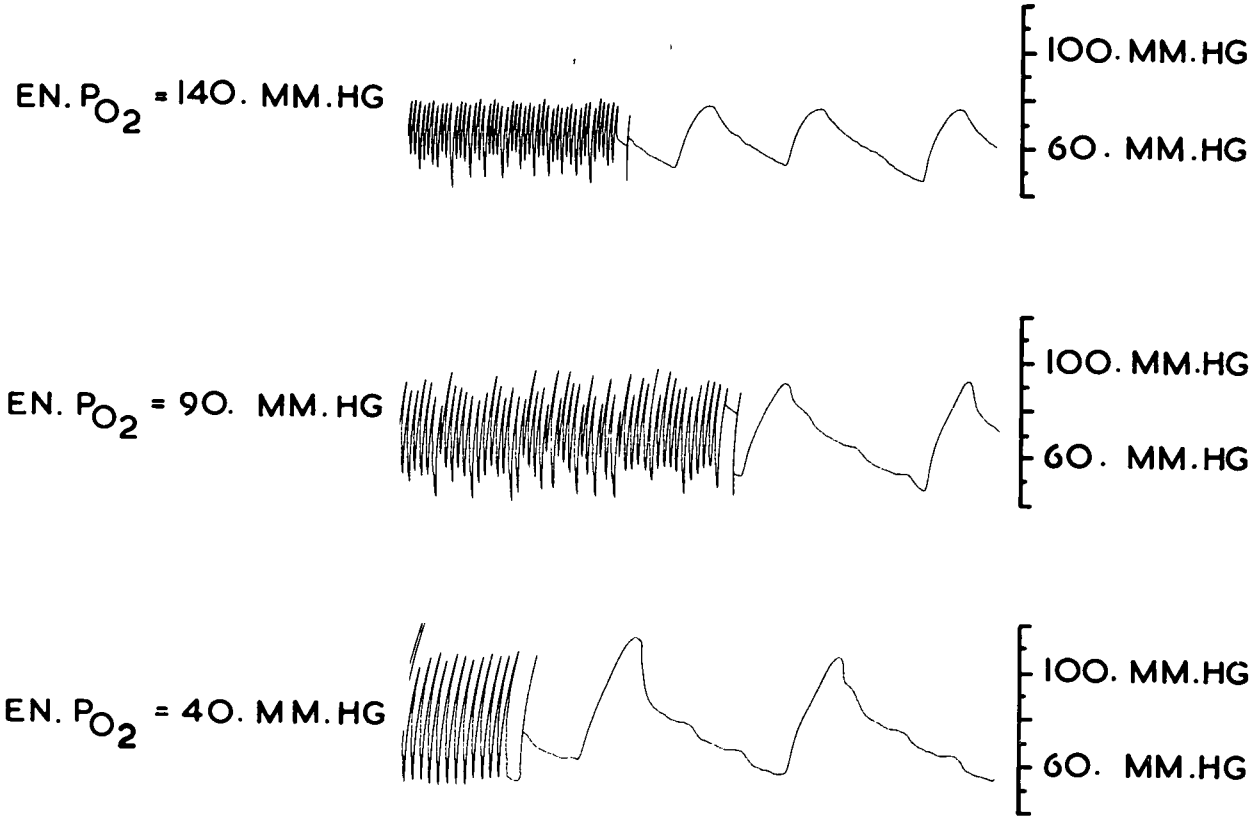
Legend for Figure 3

The effect of hypoxia upon breathing and heart rate. Each circle represents an averaged value for all determinations within a 10 mm Hg environmental oxygen tension interval. The vertical bars represent plus or minus 2 standard errors.

ventral aortic blood pressures reported here for rainbow trout and those pressures recorded in chinook salmon by Greene (1904). Both Greene (1904) and Burger and Bradley (1951) who worked with Squalus acanthias observed that larger fish had higher blood pressures. The salmon that Greene (1904) worked with were two to four times as long as the rainbow trout used in the present study. Secondly the salmon used by Greene (1904) were held restrained out of water with an artificial means of irrigating their gills. It is quite possible that these salmon were in an hypoxic state in which case the blood pressures developed would be comparable to the blood pressures obtained in this study when the rainbow trout were in an hypoxic condition.

The effect of increasing hypoxia upon the ventral aortic blood pressure of rainbow trout was a gentle increase in systolic pressures becoming increasingly pronounced at lower environmental oxygen levels (Figs. 4 and 7). The highest systolic pressures of 81 mm Hg were recorded at 40 mm Hg environmental $Po_2$. The results indicated that at environmental oxygen levels below 40 mm Hg $Po_2$, the ventral aortic systolic pressure decreased. This was symptomatic of the onset of respiratory and cardiac collapse. Few experiments were continued below environmental oxygen levels of 30 mm Hg $Po_2$. Pulse pressure increased as hypoxia developed, the increases becoming more intensified at the lowered environmental $Po_2$ levels. The highest pulse pressures of 40 mm Hg were recorded at an environmental oxygen level of 30 mm Hg $Po_2$. Unlike the systolic pressures, the ventral aortic pulse

VENTRAL AORTIC BLOOD
PRESSURE RECORDINGS



Legend for Figure 4

Pressure recordings from the ventral aorta under normal, partially hypoxic, and hypoxic environmental oxygen tensions. There is a slight distortion of the tracings due to curve-linear writeout from the recorder.

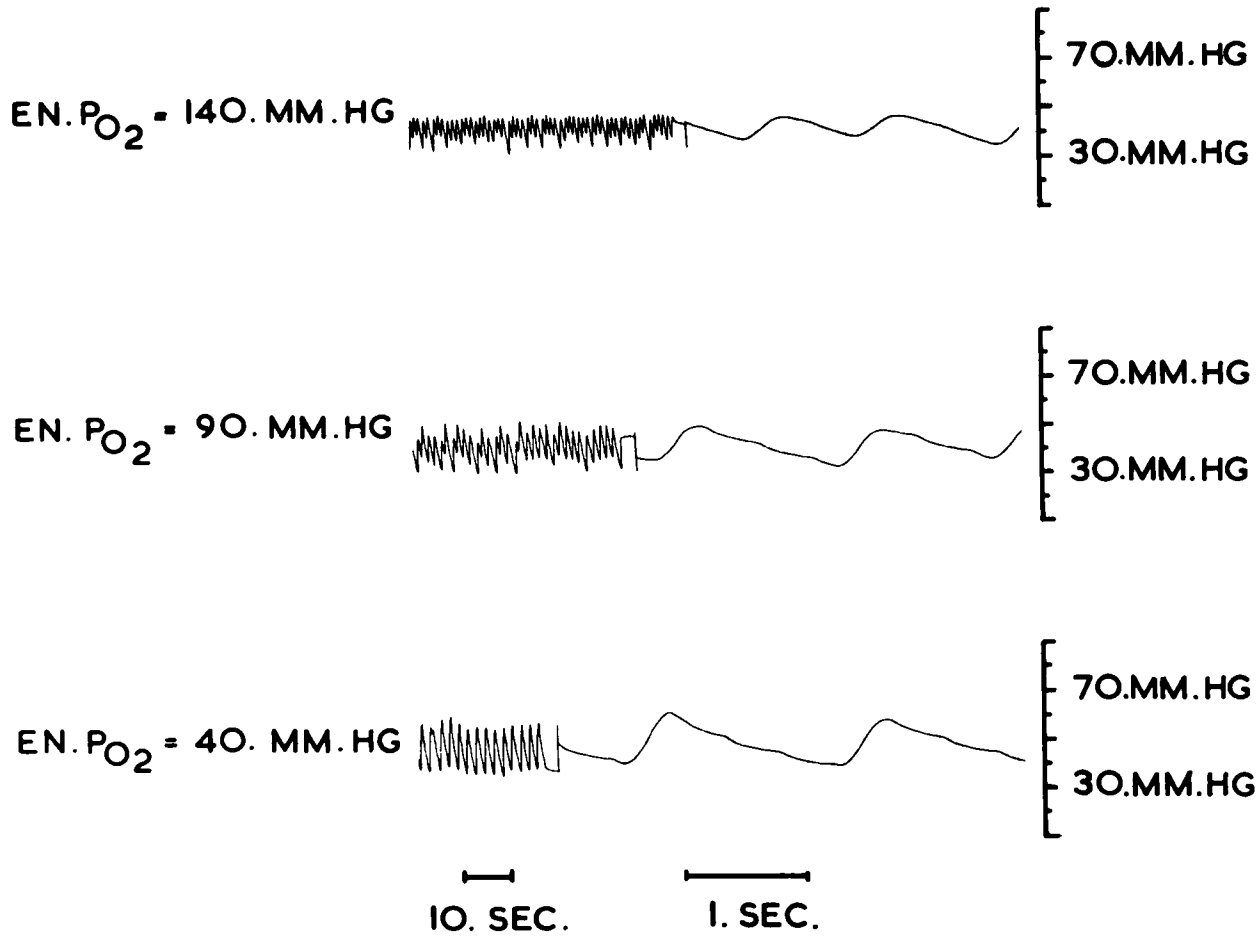
pressures did not show any marked tendency to decrease at very low environmental oxygen levels (Fig. 7).

During hypoxia there were increases in dorsal aortic systolic and pulse pressures (Fig. 5 and 8). Systolic pressures increased from a mean of 34 mm Hg when the resting fish was in well oxygenated water to 47 mm Hg at environmental $Po_2$ of 30 mm Hg. Pulse pressure in the dorsal aorta similarly increased from 6 mm Hg to 14 mm Hg at 30 mm Hg $Po_2$ in the water. No drop in either systolic or pulse pressure was observed in the dorsal aorta at the low environmental oxygen levels as was previously observed with ventral aortic systolic pressure.

The dorsal aortic blood pressure of 34 mm Hg agrees closely with the value of 38.7 $\pm$ 2.9 mm Hg in free swimming rainbow trout at approximately the same temperature reported by Randall, Smith, and Brett (1965). The dorsal aortic blood pressure for the rainbow trout is considerably lower than the value of 53.3 mm Hg in the dorsal aorta of chinook salmon reported by Greene (1904). The reasons for the difference are probably the same as those suggested for the corresponding discrepancy in ventral aortic pressures. Randall, Smith, and Brett (1965) noted that the blood pressures of rainbow trout (using the same technique as was used in this work) were, in general, higher than other teleost blood pressures reported in the literature. They suggested that the differences were a result of recording from a free swimming intact fish as opposed to restrained and in some cases anaesthetized animals.

There were slight increases in the differential pressure

DORSAL AORTIC BLOOD
PRESSURE RECORDINGS



Legend for Figure 5

Pressure recordings from the dorsal aorta under normal, partially hypoxic, and hypoxic environmental oxygen tensions. There is a slight distortion of the tracings due to curve-linear write-out from the recorder.

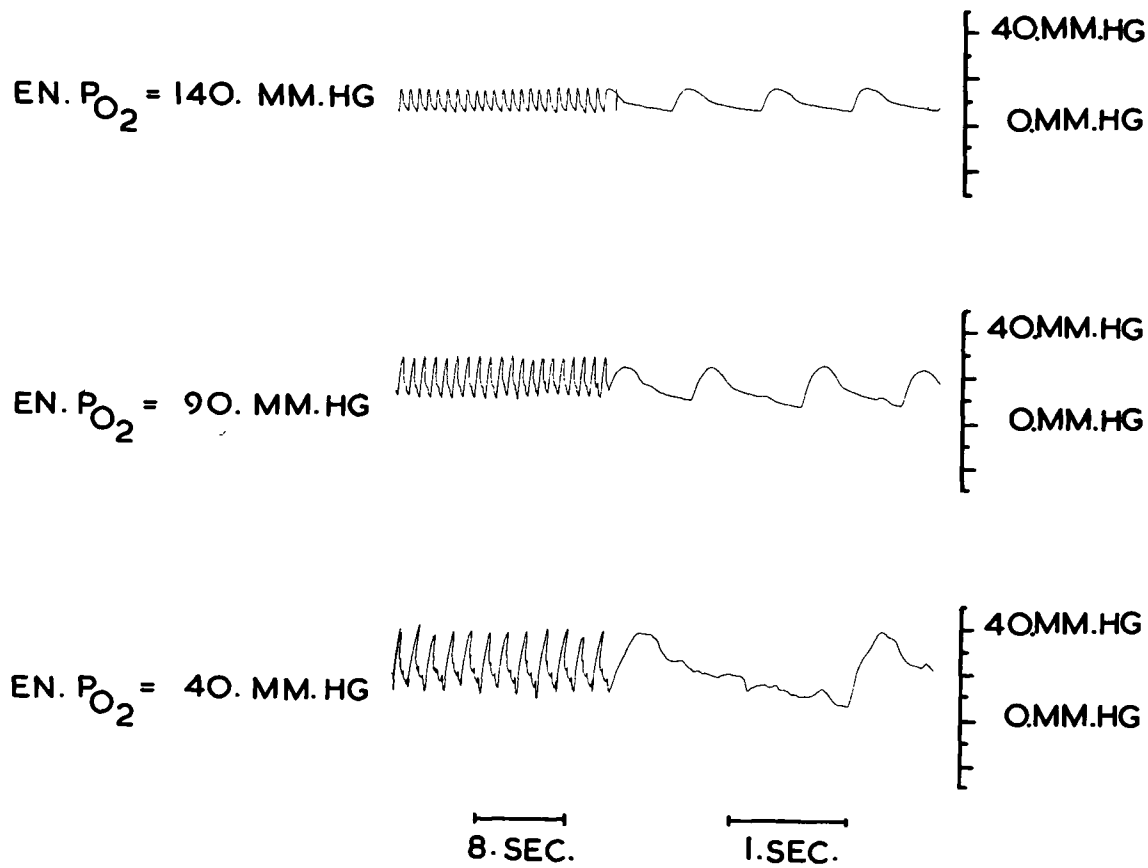
between the dorsal and ventral aortae in response to increasingly hypoxic conditions (Figs. 6 and 9). There was, as well, an increase in the pulsatility of the differential pressure. The general form and pattern of response of differential pressure was very similar to that of the dorsal aortic pressures. The pressure differential over the gills rose from a maximum of 17 mm Hg and a minimum of 6 mm Hg at an environmental oxygen $Po_2$ of 140 mm Hg, to a maximum of 35 mm Hg and a minimum of 9.5 mm Hg at an environmental oxygen level of 40 mm Hg $Po_2$ (Fig. 9).

The general increase in blood pressures could be caused by two factors; an increase in cardiac output, and by an increase in peripheral resistance to blood flow.

If it is assumed that the pressure rise is due to increased cardiac output alone, then the cardiac output would have to virtually double. The basis of this hypothesis is that a passive blood vessel will distend somewhat under increased pressure, and thus an increase in pressure would result in a considerably greater flow. Furthermore, if cardiac output alone were responsible for the increase in blood pressures during hypoxia, there would be at least a six-fold increase in stroke volume since heart rate decreases with increasing hypoxia. A six-fold increase in stroke volume of the heart seems rather large so it is likely that the increase in blood pressures with hypoxia are due, to some extent, to an increase in peripheral vascular resistance.

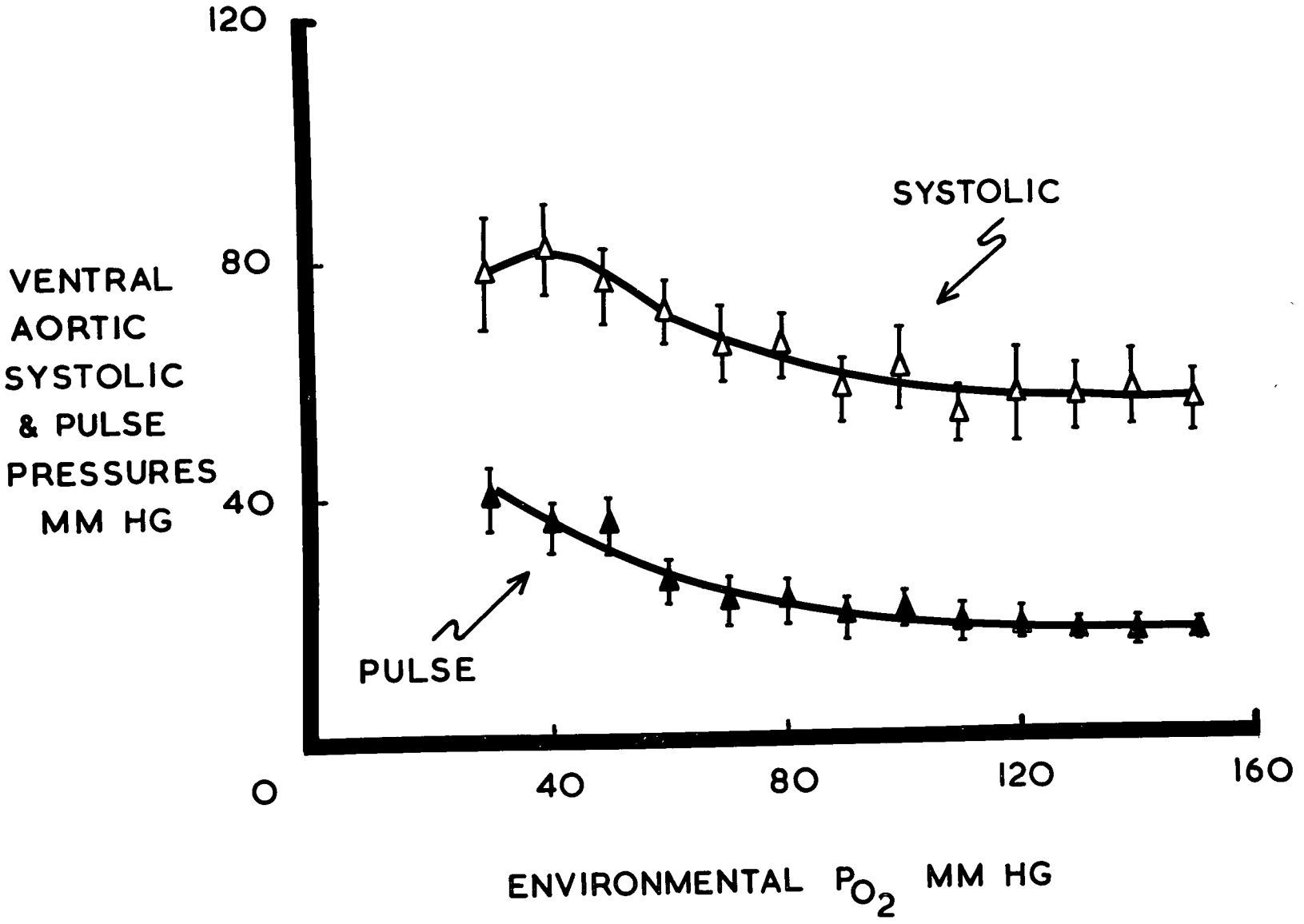
If an increase in vascular resistance is totally or in part responsible for the increase in blood pressure during

DIFFERENTIAL BLOOD
PRESSURE RECORDINGS



Legend for Figure 6

Pressure recordings of the blood pressure differential over the gills (ventral aortic - dorsal aortic) under normal, partially hypoxic, and hypoxic environmental oxygen tensions. Curvelinear write-out has again caused some very slight distortion of the tracings.


120
80
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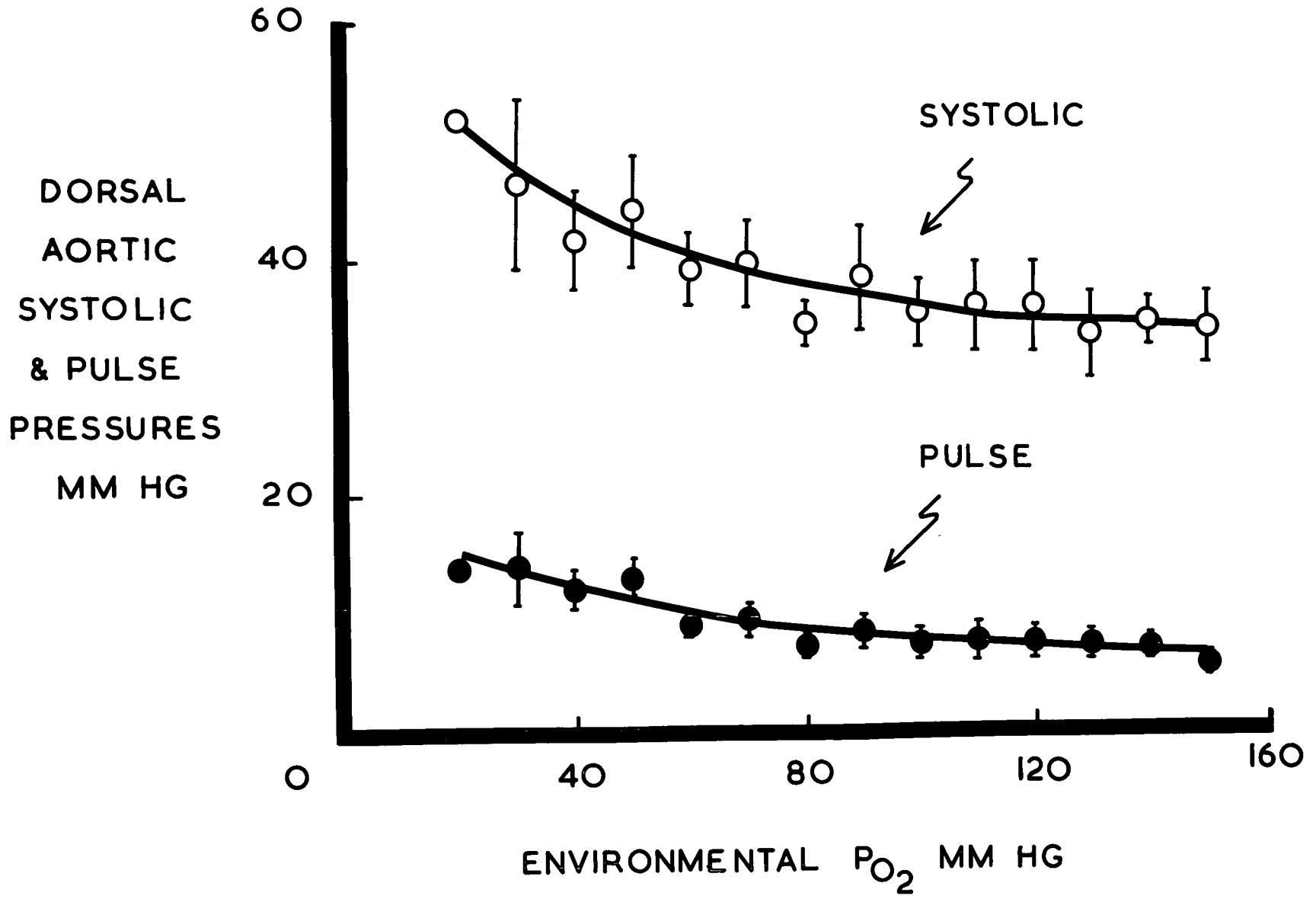
0
VENTRAL AORTIC SYSTOLIC & PULSE PRESSURES MM HG
SYSTOLIC
PULSE
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

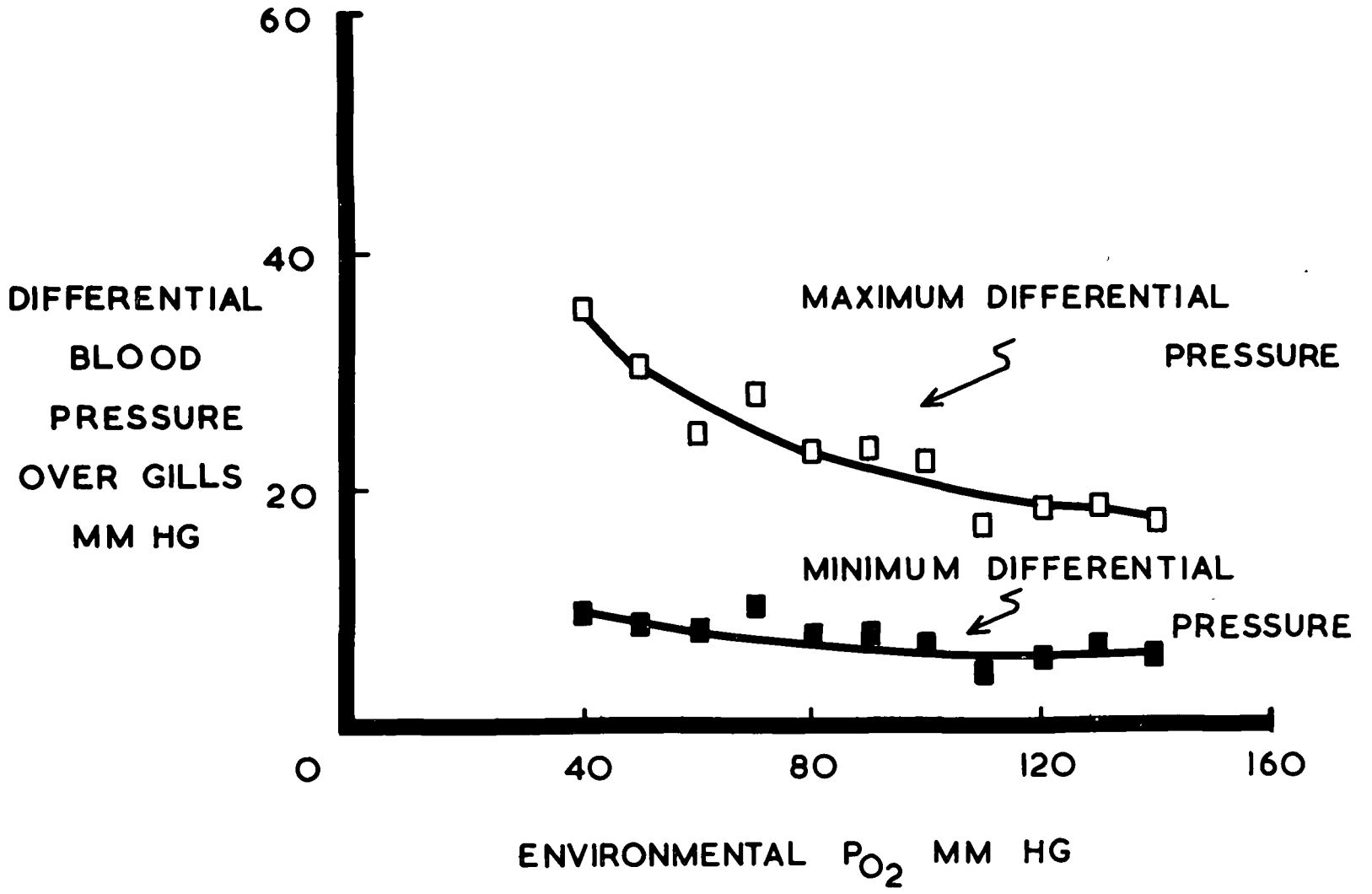
Legend for Figure 7

The effect of hypoxia upon ventral aortic systolic and pulse pressures. Each point plotted is an average value for all determinations within a ten mm Hg environmental oxygen tension interval. The vertical bars represent plus and minus two standard errors.


60
40
20
0
DORSAL
AORTIC
SYSTOLIC
& PULSE
PRESSURES
MM HG
SYSTOLIC
PULSE
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 8

The effect of hypoxia upon dorsal aortic systolic and pulse pressures. The vertical bars represent plus or minus 2 standard errors. Each point plotted is an averaged value for all determinations within a 10 mm Hg environmental oxygen tension interval.


60
40
20
0
DIFFERENTIAL
BLOOD
PRESSURE
OVER GILLS
MM HG
MAXIMUM DIFFERENTIAL
PRESSURE
MINIMUM DIFFERENTIAL
PRESSURE
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 9

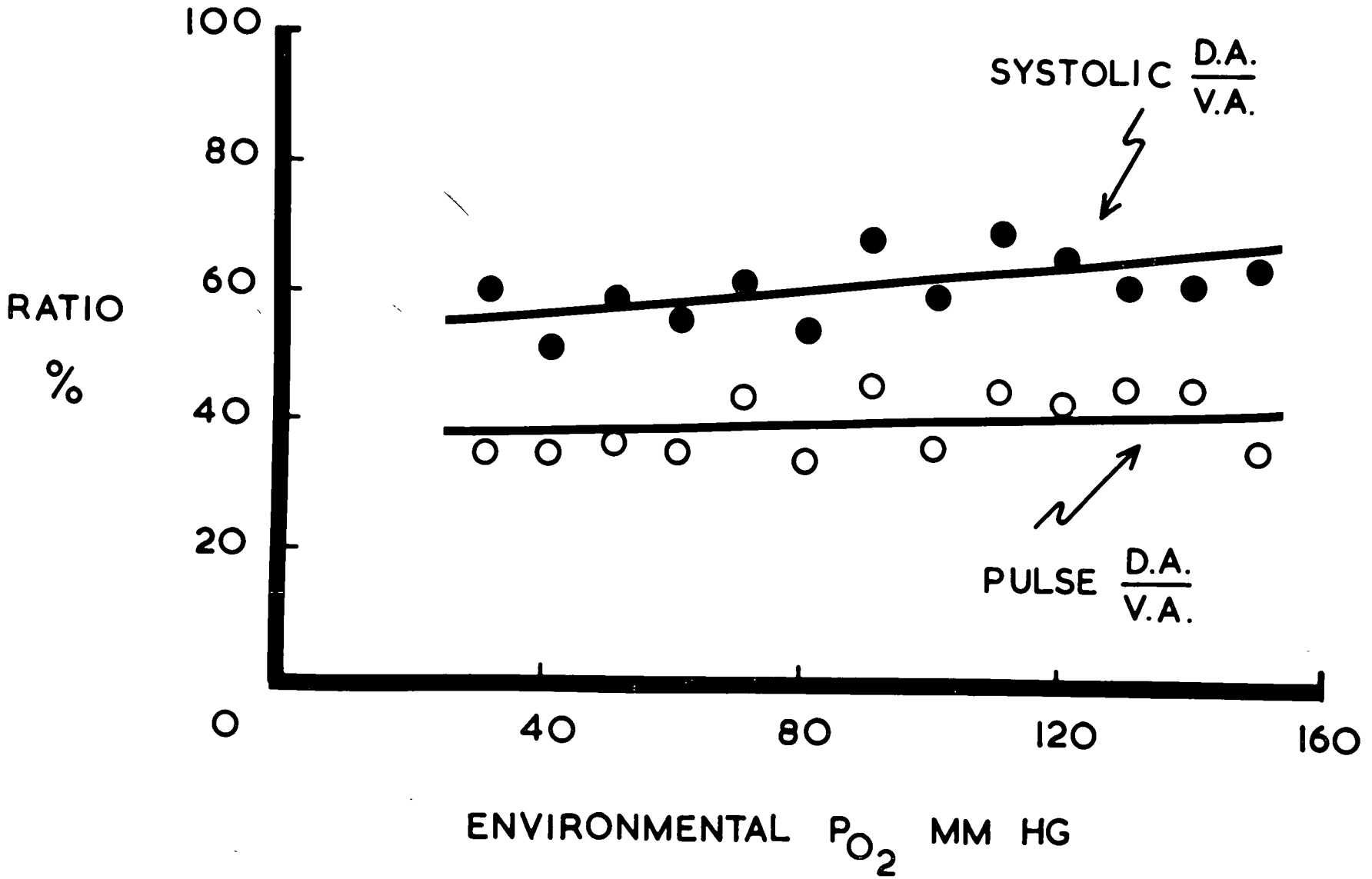
The effects of hypoxia upon blood pressure differential over the gills (ventral aortic - dorsal aortic). Each point plotted is an averaged value for all determinations within a 10 mm Hg environmental oxygen tension interval.

hypoxia, it should be possible to detect where the changes in resistance are occurring by examination of the pressure recordings. The ratio of dorsal aortic to ventral aortic pressure is fairly uniform at all environmental $Po_2$ levels (Fig. 10). This suggests that any resistance changes are occurring in gill and peripheral vessels at almost the same time.

A study was made of the rate of pressure drop in differential pressure across the gills, at a chosen pressure of 10 mm Hg. Records from experiments on six fish were used. Four of the six fish showed a definite decrease in the rate of pressure drop across the gills in response to hypoxia (Fig. 11). The other two fish examined showed no significant change in the rate of pressure drop. This information suggested that there is an increase in gill resistance with increasing hypoxia.

If it is accepted that gill resistance increases then there must, when we consider the previous argument, be an increase in peripheral resistance as well.

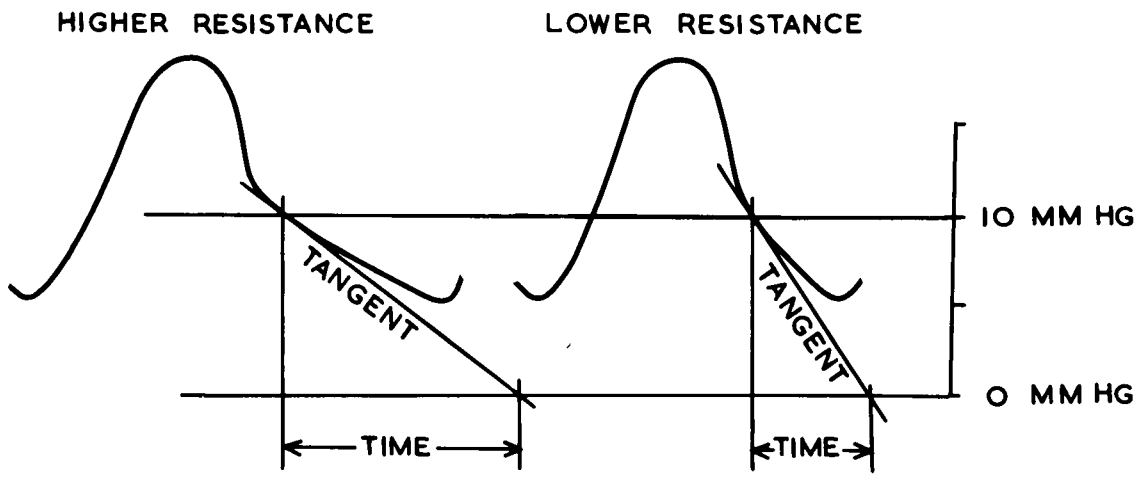
In some instances when flushing the respirometer at the end of an experiment Evans blue dye was injected into the oxygenated water. The front of the dye was observed carefully. Cardioacceleration took place in less than three seconds from the time that the dyed oxygenated water was observed to reach the hypoxic fishes mouth (Fig. 12). Intake of oxygenated water by the hypoxic fish was followed by a rise in ventral and dorsal aortic systolic blood pressure and a large decrease in pulse pressure (Fig. 12). The rise in dorsal aortic blood pressure was not as pronounced

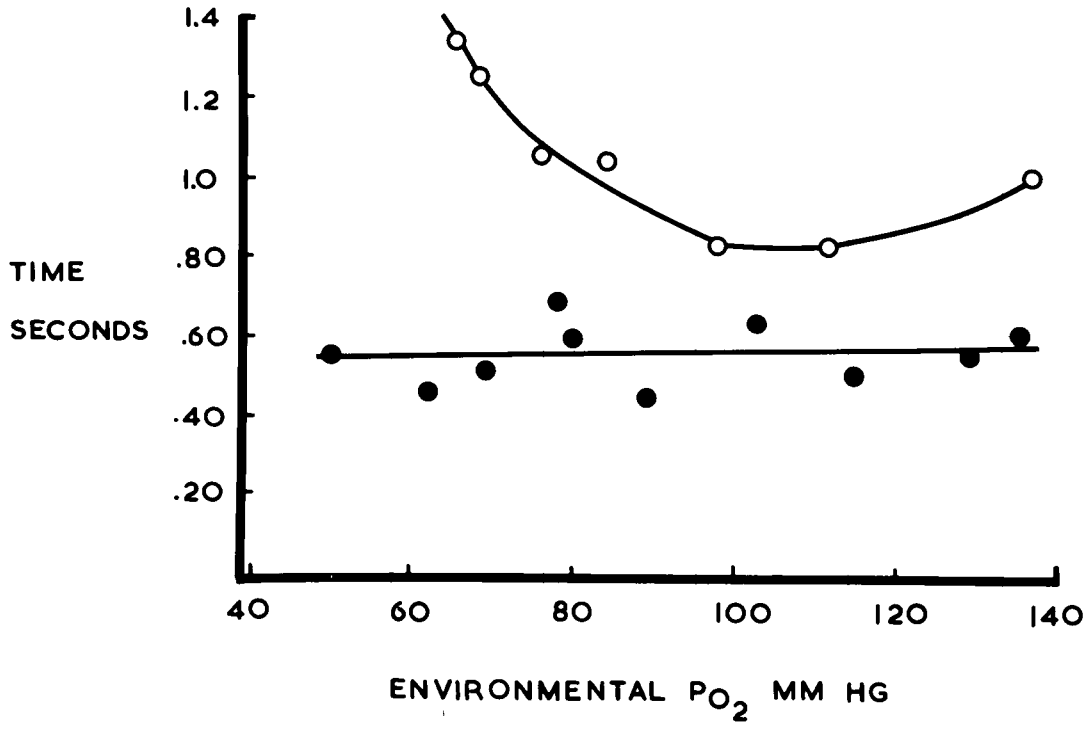

100
80
60
40
20
0
RATIO
%
SYSTOLIC D.A./V.A.
PULSE D.A./V.A.
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 10

The ratio of dorsal aortic systolic pressure to ventral aortic systolic pressure, and the ratio of dorsal aortic pulse pressure to ventral aortic pulse pressure at varying environmental oxygen tensions.

DIFFERENTIAL BLOOD PRESSURE RECORDS



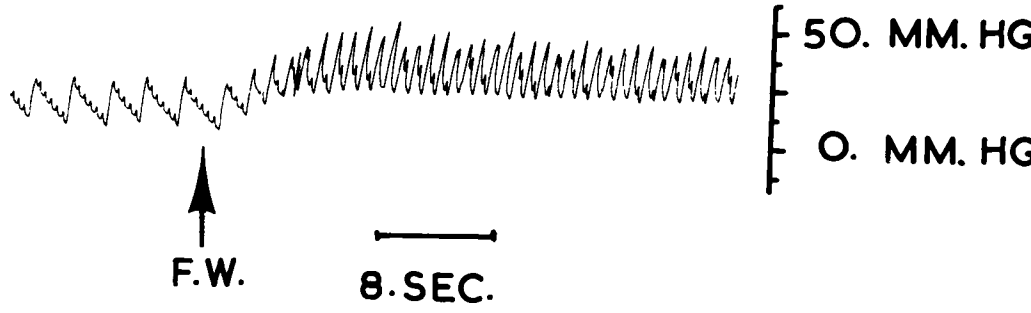



Legend for Figure 11

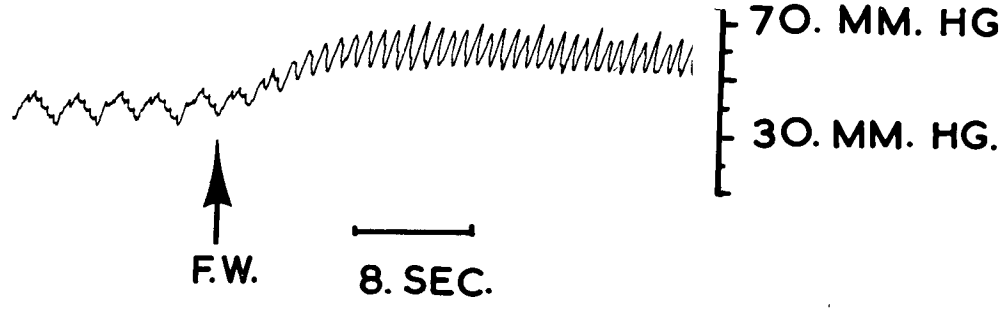
The upper illustration shows the method of determining the rate of differential blood pressure (ventral aortic minus dorsal aortic) decrease over the gills. The length of time for the pressure differential to decrease 10 mm Hg was used as a measure of the resistance to flow of blood through the gills. The rate was derived from an extrapolation of the rate of pressure drop which was found at a pressure differential of 10 mm Hg. Only recordings in which the flatter portions of the pressure pulse tracings included the 10 mm Hg level were used.

The lower figure illustrates the effects of hypoxia upon the rate of differential blood pressure drop obtained from two experiments. A total of six experiments were examined; four of which showed increases in time for pressure to drop 10 mm Hg indicating flow resistance increases with hypoxia such as shown in the upper line (open circles), and two of which showed no change such as the lower line (closed circles). The absolute values of time shown are not important but the relative changes within an experiment are useful as evidence of flow resistance changes within the gills.

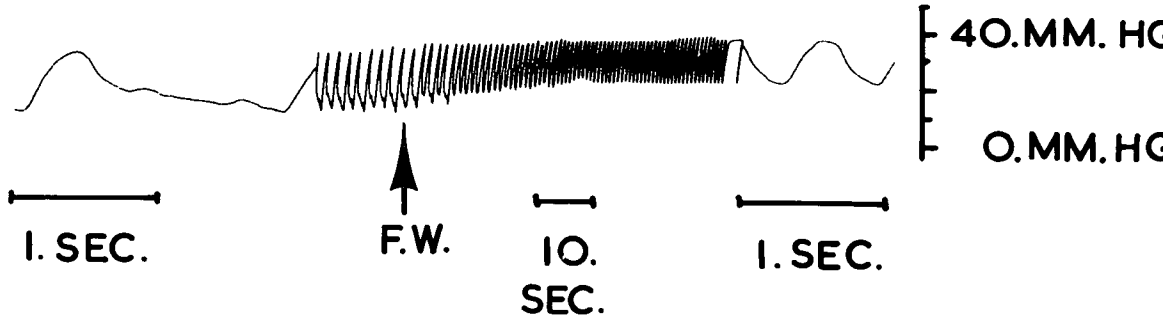
EFFECT OF FRESH WATER
UPON BLOOD PRESSURE IN
HYPOXIC RAINBOW TROUT




VENTRAL AORTA AUG. 23, 1965




DORSAL AORTA AUG. 26, 1965




DIFFERENTIAL SEPT. 2, 1965

Legend for Figure 12

The effect of flushing fresh oxygenated water into the respirometer at the end of an experiment when the environmental water in the respirometer was of low oxygen content, upon the heart rate and blood pressures of the rainbow trout.

as the rise in ventral aortic systolic blood pressure. Six of the fish examined had post-hypoxic recovery systolic blood pressures over 110 mm Hg in their ventral aortae. In one case, a 308 gram female developed a systolic blood pressure of over 128 mm Hg in her ventral aorta.

Since the dorsal aortic pressures did not increase as much as the ventral aortic pressures during this period vascular changes, either increased resistance of the gills or reduced peripheral resistance is implicated. It was not possible to compare the rate of decrease of differential pressure as done in the previous studies because the post hypoxic differential pressures were too high to compare on an equitable basis with hypoxic differential pressures.

Ventral aortic and dorsal aortic pressures declined slowly after their initial abrupt rise and returned to normal or original resting levels in about 1 to 1½ hours.

Some idea of the effects of vascular changes on pressures was obtained by injecting 0.25 mg of epinephrine into a fish weighing 497 grams. Blood pressure increased but the heart rate remained almost constant. The ratio of dorsal aortic: ventral aortic systolic pressures increased to 0.82 and the ratio of pulse pressures increased to 0.56 (compare with Fig. 8). In this case either the gill vessels decreased their resistance or peripheral resistance increased, or a combination of both factors occurred.

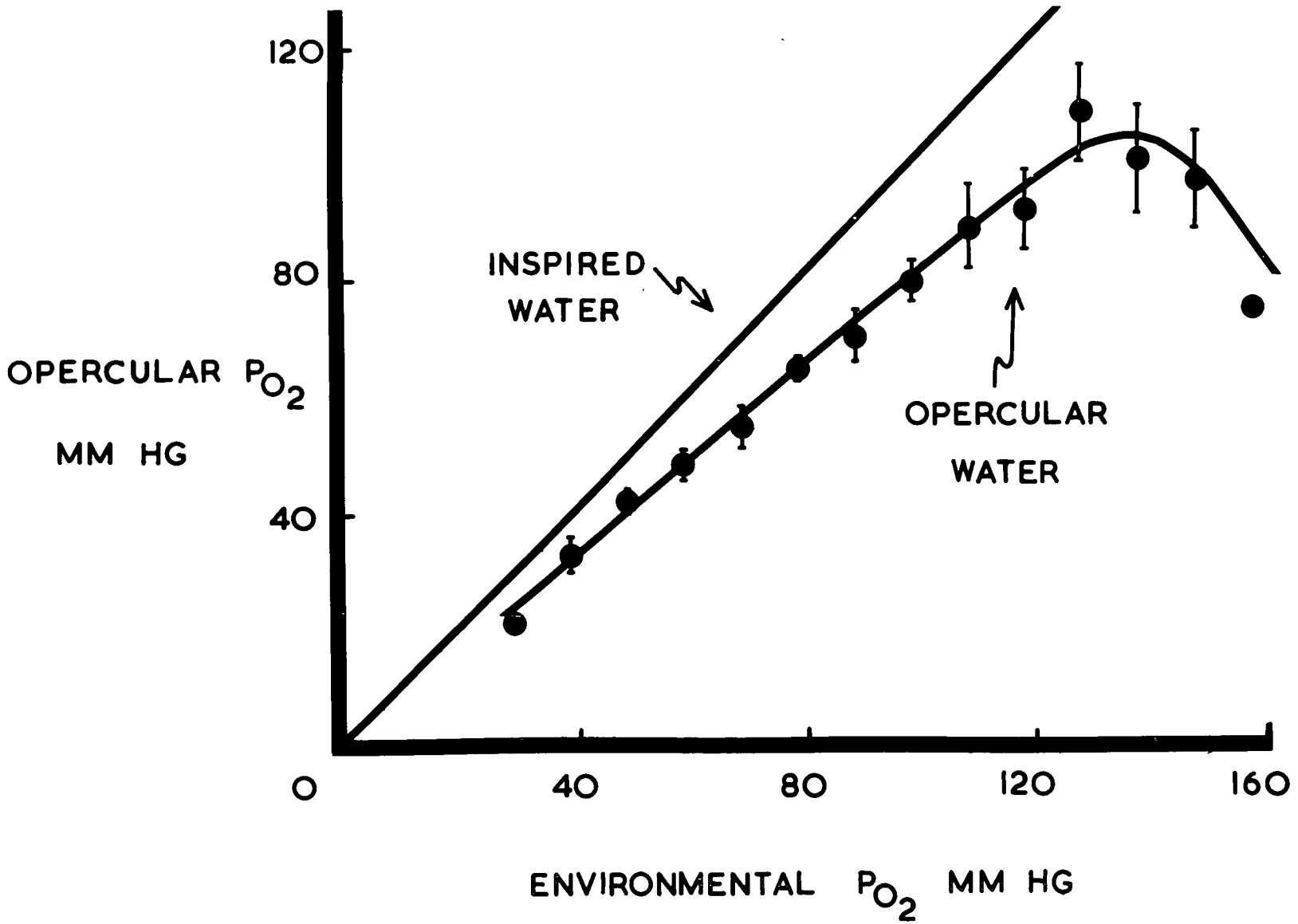
The introduction of fresh water resulted in a gradual (30 to 120 minute) decline in breathing rate and amplitude, back to normal resting levels.

RESULTS AND DISCUSSION II

Since a sample from only one of the four cannulae could be analysed at one time it was necessary to compare all other determinations of blood and water $Po_2$, $PCo_2$, and pH to interpolations between the serial determinations of environmental oxygen. The interpolations between the environmental oxygen levels with respect to time during the experiments are reasonably reliable as the large volume of water in the respirometer (approximately 12 liters) was resistant to sudden changes in oxygen level.

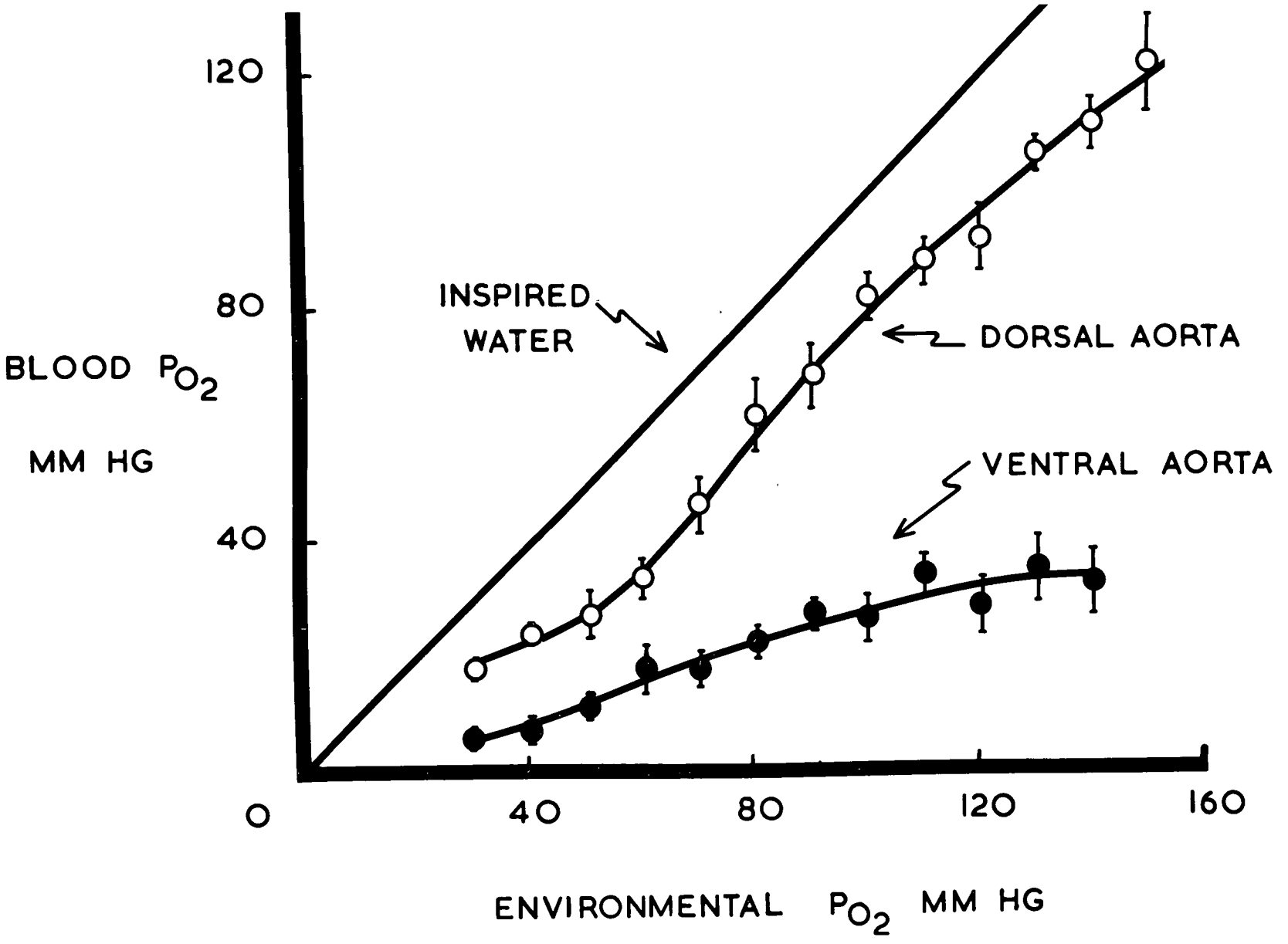
The effect of increasing hypoxia in the medium was to produce a rapid decrease in the percentage of the oxygen in inspired water that was utilized by the fish (Fig. 13). This effect was very pronounced between environmental $Po_2$ levels of 160 mm to 140 mm Hg. Below environmental $Po_2$ of 140 mm Hg there was no significant change in the percentage utilization of oxygen from inspired water. Both Van Dam (1938) and Saunders (1962) describe a decrease in percentage utilization of oxygen in inspired water with fish under hypoxic conditions.

The dorsal aortic blood of quiet fish was found to have an average $Po_2$ of 122 mm Hg at an environmental $Po_2$ level of 150 mm Hg. The dorsal aortic blood $Po_2$ decreased to 19 mm Hg at an hypoxic environmental oxygen level of 30 mm Hg $Po_2$ (Fig. 14). It is interesting to note that the dorsal aortic blood $Po_2$ at the highest levels of environmental oxygen is significantly higher than the $Po_2$ of expired water at the same environmental oxygen level. This verifies again the


120
80
40
0
40
80
120
160
OPERCULAR $P_{O_2}$
MM HG
INSPIRED
WATER
OPERCULAR
WATER
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 13

The effect of hypoxia upon oxygen tension of opercular water. Vertical bars represent plus or minus 2 standard errors of the average of each of the subsamples plotted.


120
80
40
0
BLOOD $P_{O_2}$
MM HG
INSPIRED
WATER
DORSAL AORTA
VENTRAL AORTA
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 14

The effect of hypoxia on the oxygen tension of ventral aortic and dorsal aortic blood. Vertical bars represent plus or minus 2 standard errors of the subsample averages plotted.

existence of a functional counter current flow arrangement of blood and water past the respiratory exchange epithelium of the gills previously documented by Krogh (1941).

The $Po_2$ of venous blood of rainbow trout is surprisingly higher than values estimated by Mott (1957), and Saunders (1962), or the levels reported by Itazawa (1957) for carp. The resting rainbow trout were found to have an average ventral aortic $Po_2$ of 30 - 35 mm Hg at environmental oxygen levels of 140 to 110 mm Hg $Po_2$. Ventral aortic $Po_2$ levels dropped gradually to an average 6 mm Hg at environmental oxygen tensions of 30 mm Hg $Po_2$ (Fig. 14).

The partial pressures of $CO_2$ in the blood of quiescent rainbow trout resting in well oxygenated water were found to be very low. The $CO_2$ electrode employed was calibrated to measure a range of $CO_2$ partial pressures from 3 mm to 30 mm Hg. In all cases the dorsal and ventral aortic blood, and the buccal and opercular water $PCo_2$ levels were less than 3 mm Hg $PCo_2$. It was possible to gain an estimate of some of the $PCo_2$ levels in the samples by empirical means from offsetting the measuring range of the instrument. Since the instrument scale was logarithmic, the estimates of $PCo_2$ are not as accurate as they would be if they were taken in the middle of the instrument range.

The ventral aortic $PCo_2$ levels were the closest to registering on the calibrated range of the instrument and were estimated to be 2.5 mm Hg $PCo_2$, with resting fish in a well oxygenated environment. With the onset of hypoxic conditions, the ventral aortic $PCo_2$ levels rose to levels of 4.5 to 5 mm Hg.

The $PCo_2$ of dorsal aortic blood of the quiet trout in well oxygenated water was lower than the $PCo_2$ of the ventral aortic blood. It was estimated to be 1 - 1.5 mm Hg. During a decline in environmental oxygen tensions from 80 mm Hg to 30 mm Hg the dorsal aortic blood $PCo_2$ increased to 3.5 to 4 mm Hg.

Haning and Thompson (1965) have stressed that the blood $PCo_2$ levels in fish are much lower than in land vertebrates or amphibians. They reported a value of 9.2 mm Hg $PCo_2$ for arterial blood of the catfish, Ictalurus punctatus. Irving, Black, and Safford (1941) estimated arterial blood $PCo_2$ levels in trout to be near 2 mm Hg. Black, Kirkpatrick, and Tucker (1966) reported venous blood samples from Brook trout had $PCo_2$ levels of 5.6 to 6.5 mm Hg. Ferguson and Black (1941) listed venous $PCo_2$ levels in rainbow trout of 8 to 10 mm Hg but the samples were of very low oxygen content, a different condition than observed in the present study. The present findings are close to the values reported by the previous workers and affirm that the $PCo_2$ levels in trout blood are considerably lower than the $PCo_2$ levels of the blood of terrestrial vertebrates. This difference is probably due to the low $CO_2$ content and high capacity of the environmental water which most fish breath.

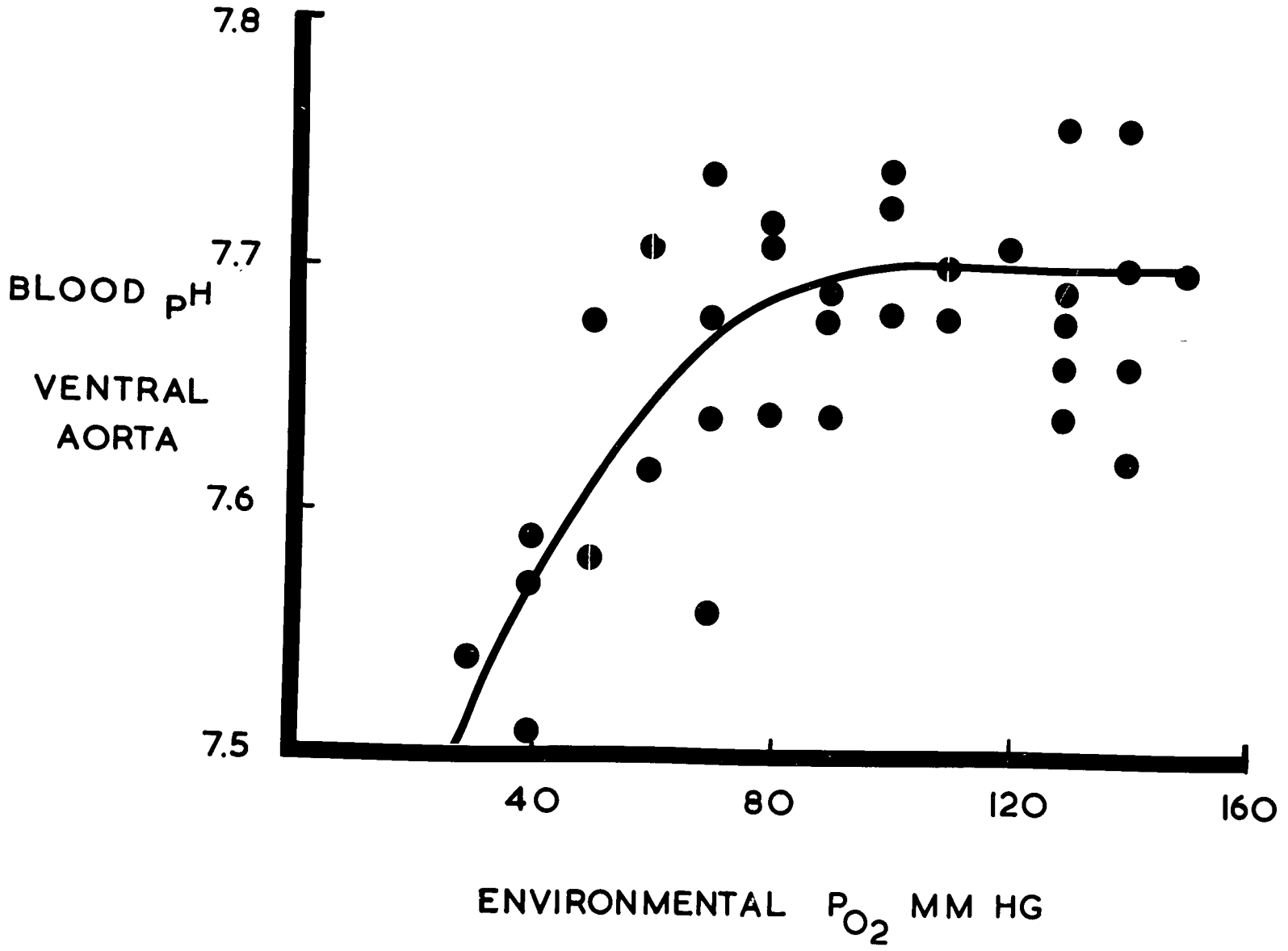
The $PCo_2$ levels of buccal and opercular water were considerably lower than those of the blood. The water $PCo_2$ levels were not very accurately determined but were estimated to be less than 2 mm Hg $PCo_2$.

The pH of ventral and dorsal aortic blood was found to be very close to 7.7 in the quiet trout in well oxygenated environmental conditions. The pH of the blood of the trout

changed very little with the onset of hypoxic conditions and only under the most extreme hypoxia (30 mm Hg environmental $Po_2$) did the blood pH drop to a level of 7.4 (Figs. 15 and 16).

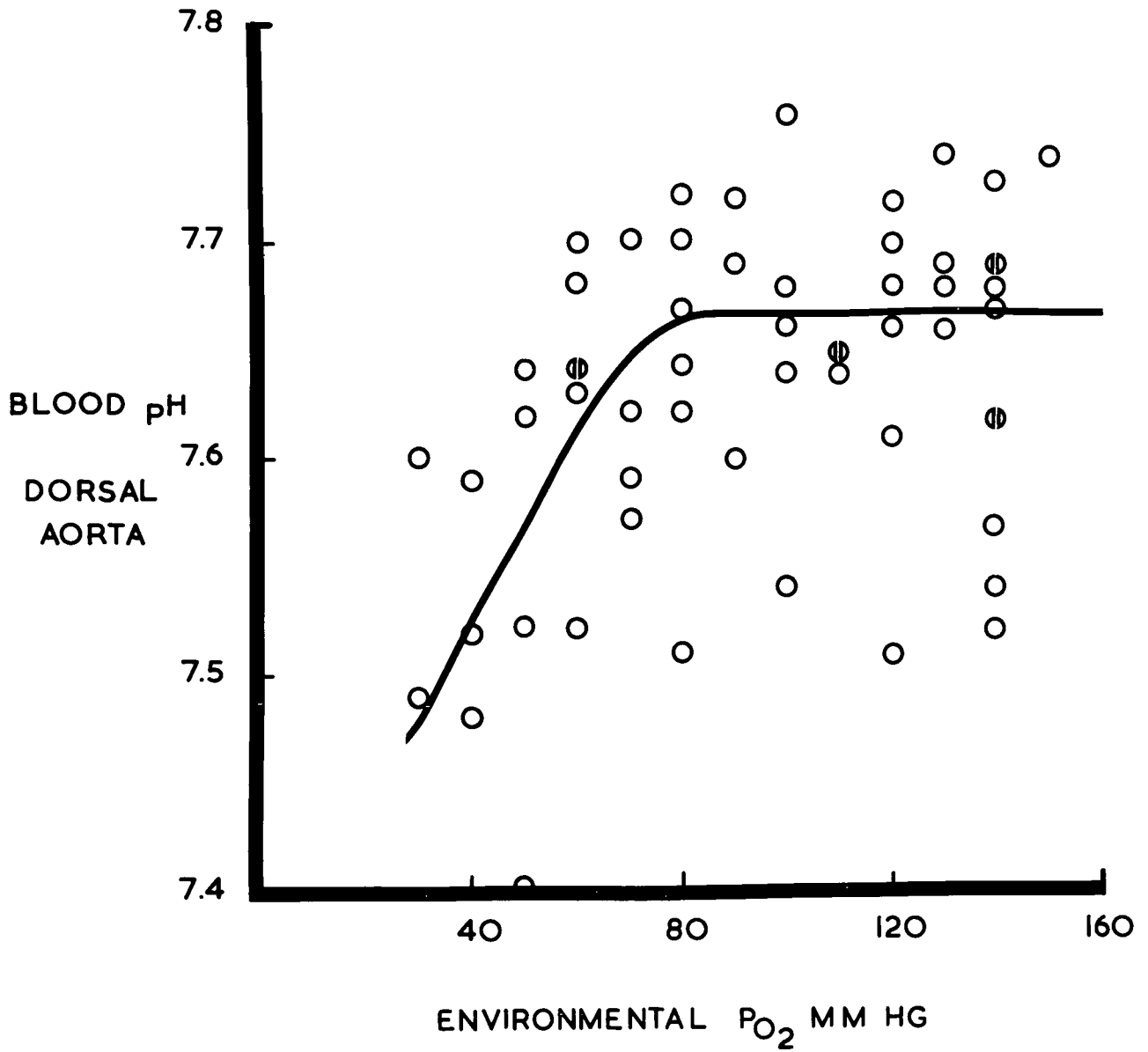
The pH of the water sampled from the buccal and opercular chambers dropped during the course of each experiment. This was probably due to the formation of carbonic acid from $CO_2$ expired by the fish. This hypothesis is further strengthened by the observation that the pH of water sampled from the opercular chamber of the fish was about .2 pH units lower than that of water samples taken from the buccal chamber at similar environmental oxygen tensions. This difference again was presumably a consequence of expired $CO_2$ from the gills forming carbonic acid (Fig. 17).

It was observed that in most cases the hematocrit of the blood of the fish increased with the onset of hypoxia. This increase was most pronounced in fish with initially high hematocrit values; fish with low hematocrits generally did not show such a response. The increase in hematocrit could have been a result of cellular swelling or an increase in cell numbers in the blood. The red blood cell count of the blood of five fish was taken serially with the onset of hypoxia. In each of these experiments there was a marked increase in hematocrit with deep hypoxia (15% to 50% more than the initial values), but there was no significant increase or decrease in the number of red blood cells in the blood of these fish. From this it would appear that the observed increase in hematocrit concomitant with deepening hypoxia is primarily a


7.8
7.7
7.6
7.5
BLOOD pH
VENTRAL
AORTA
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

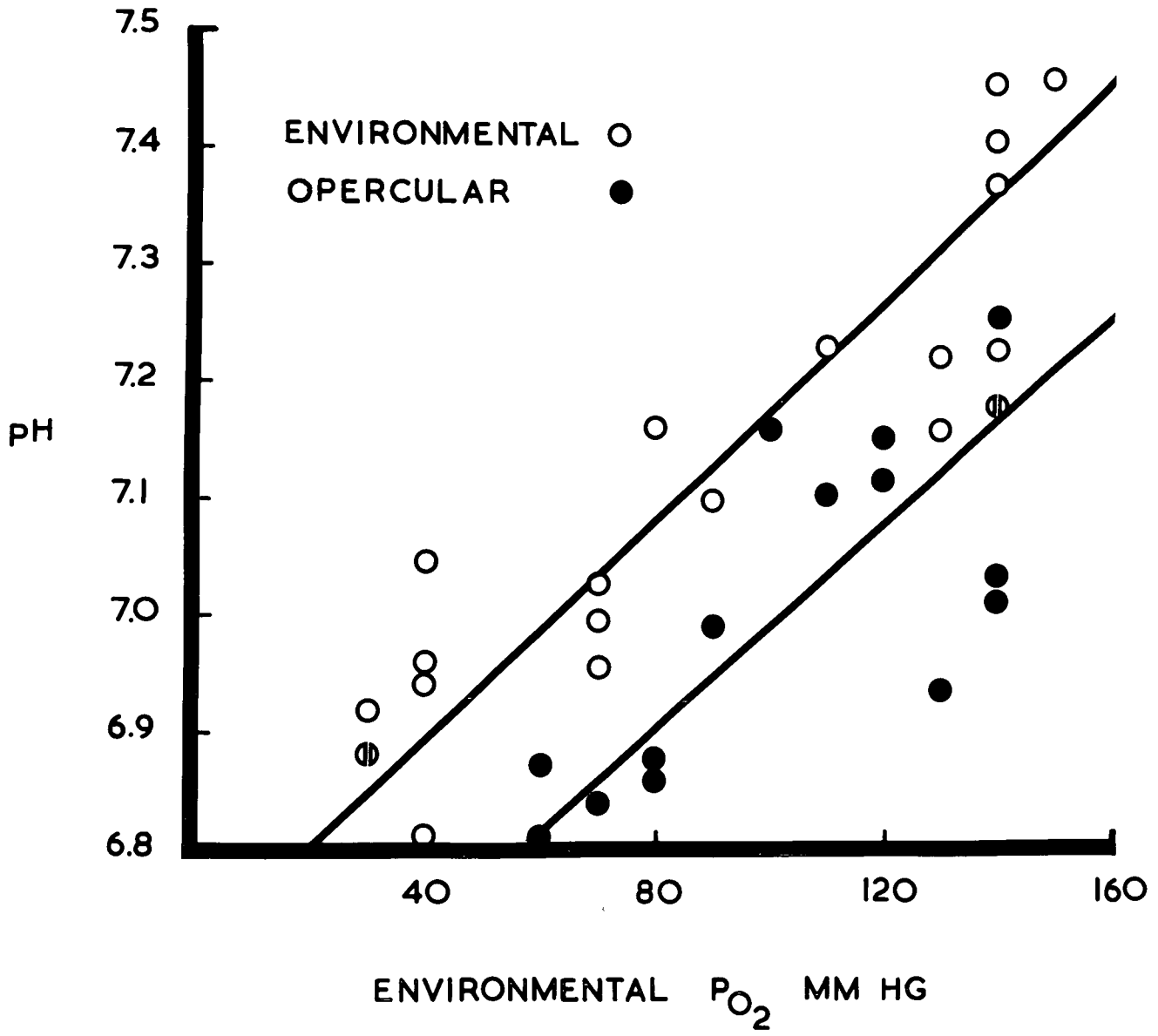
Legend for Figure 15

The effect of hypoxia upon the pH of ventral aortic blood. Split circles represent 2 values at that point.


7.8
7.7
7.6
7.5
7.4
BLOOD pH
DORSAL
AORTA
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 16

The effect of hypoxia upon the pH of dorsal aortic blood. Split circles represent 2 values at that point.

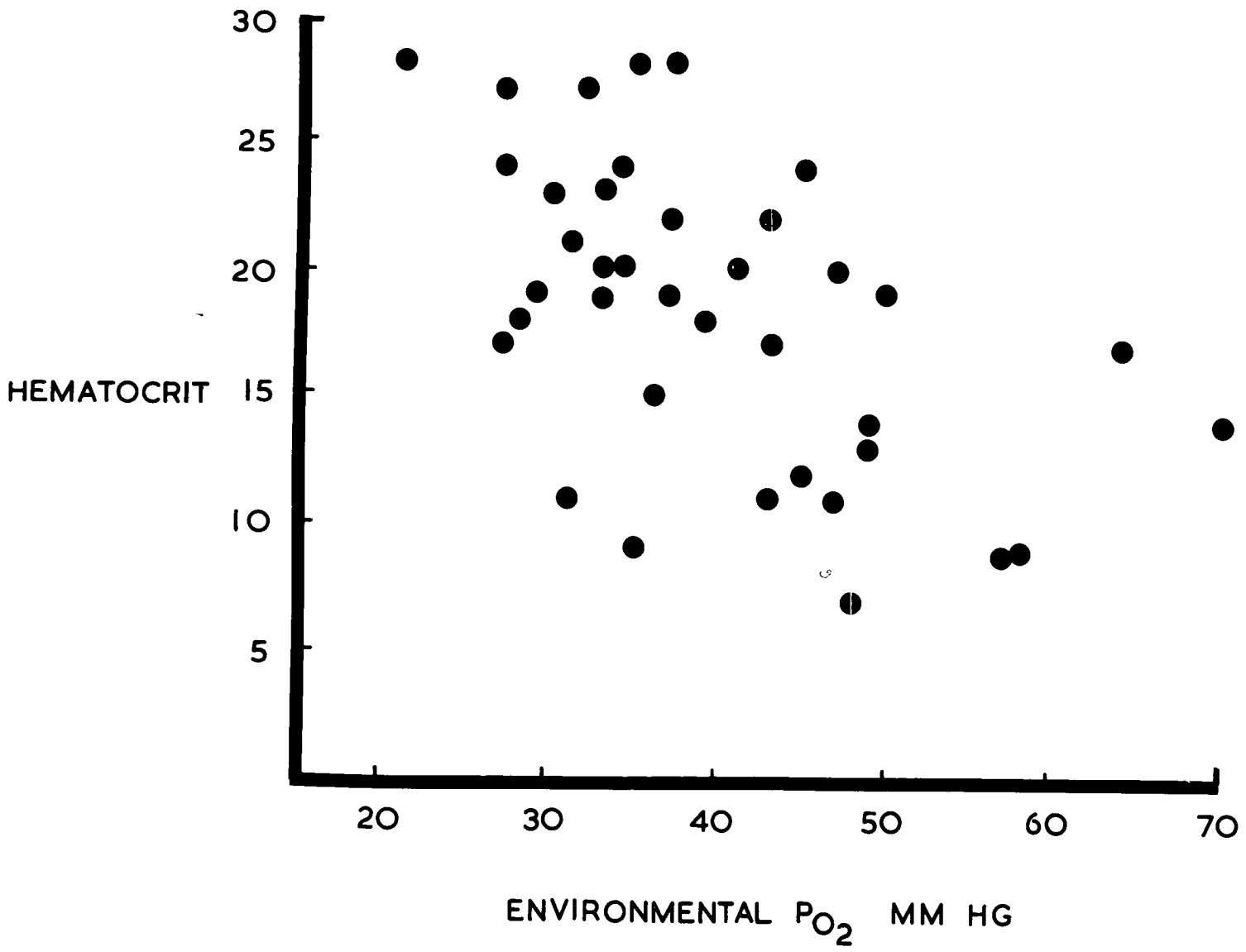

7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
pH
ENVIRONMENTAL
OPERCULAR
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 17

The pH of environmental and opercular water during the course of progressively severe hypoxia. Split circles represent two values at that point.

result of cellular swelling. Ferguson and Black (1941) have shown *in vitro* that the red blood cells of rainbow trout swell considerably with rises in $CO_2$ in the blood.

There was a suggestion of a loose inverse correlation between the hematocrit of the fish and the oxygen tension at which the fish began to show signs of respiratory failure. Figure 18 shows the relationship between the initial hematocrit of the fish and the oxygen tension at which the various experiments were terminated. It appears that the initial hematocrit and correspondingly the capacity of the blood for oxygen is of some importance to the fish for survival or at least resistance to hypoxic conditions in the environment.


30
25
20
15
10
5
HEMATOCRIT
20
30
40
50
60
70
ENVIRONMENTAL $P_{O_2}$ MM HG

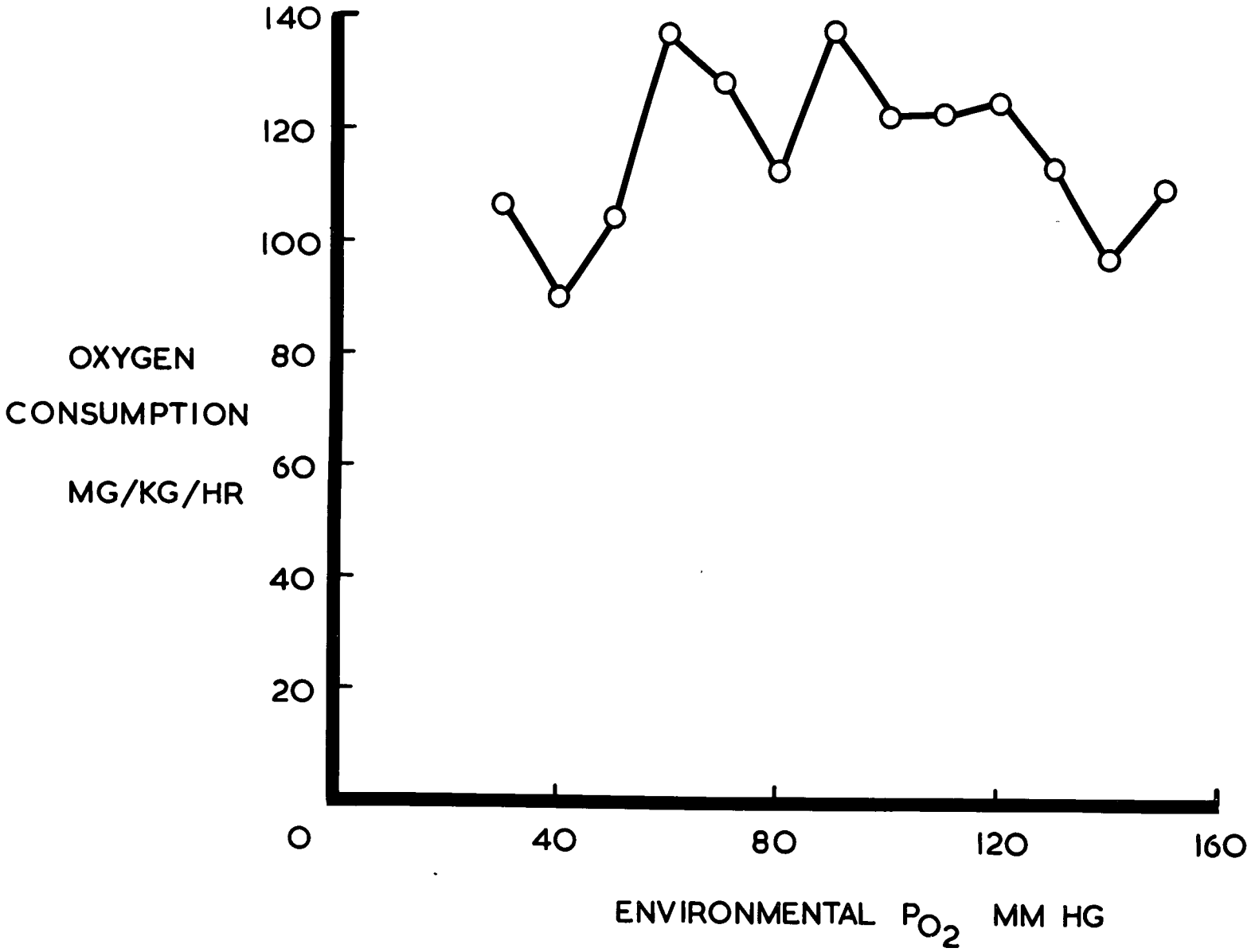
Legend for Figure 18

The hematocrit of the fish and the environmental oxygen tension at which the experiment with hypoxia was terminated due to signs of respiratory or cardiac failure. Split points indicate two values for that point.

RESULTS AND DISCUSSION III

The rate of oxygen uptake of the fish was recorded in all experiments (Fig. 19). The changes in oxygen uptake with hypoxia (Fig. 19) in the medium are not statistically significant and are probably related to spontaneous activity of the fish. No effort was made to either restrain the unanaesthetized fish during the experiments or to apply any correction for activity, in order to standardize the oxygen uptake results. However, the average rate of oxygen consumption (100 - 125 mg/Kg/hr) was only slightly higher than the standard levels of oxygen consumption for brook trout of similar size and at the same temperature reported by Beamish (1963 - C). This would support the evidence obtained from the sensitive buccal and opercular pressure recordings that the activity level of fish in the respirometer was generally quite low.

The oxygen uptake is a measure of the exchange between the water and the fish, not the blood and cells. Oxygen dissolved in the blood and muscle at the start of the experiment is not taken into consideration and does not represent a source of error. It has been assumed that oxygen uptake is the quantity of gas passing through the respiratory epithelium. Obviously the major source of error in this assumption is gas exchange which does not occur through the respiratory epithelium. Fry (1957) suggests that although the body surfaces of all but the most heavily armoured fish are permeable to respiratory gases, cutaneous respiration is normally of no appreciable significance in aquatic respiration of most fishes. Berg and Steen


140
120
100
80
60
40
20
0
OXYGEN
CONSUMPTION
MG/KG/HR
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 19

The effect of hypoxia upon the rate of oxygen uptake. The circles represent the averages of subsamples from each 10 mm Hg oxygen tension interval.

(1965) state that the eel, which has a considerable cutaneous respiratory capacity, receives about 85 to 90 per cent of its oxygen uptake in water through its gills. These figures would indicate that with some fish, such as the eel, there would be an appreciable error in assuming that oxygen uptake was exclusively via the gills. However it is felt that this error would be quite small in rainbow trout, which, aside from the lack of exceptional cutaneous vascularization, have a rather thick layer of slime over their body surface (Van Dam, 1938).

To obtain indications of the degree of anaerobic metabolism that the fish were using during the experiments, blood samples were taken before and after the experiments on four fish which weighed between 520 and 1015 grams. The blood sample of 0.7 ml if taken from a fish weighing 500 grams would represent approximately 5% of the blood volume of the fish assuming a blood volume of 2.8% of the total weight of the fish (Prosser and Brown 1961).

The average level of lactate in the fish before the experiments is quite close to the values of blood lactate level in rainbow trout reported by Black (1955), and Black et al (1962). In the present study it was found that lactate levels in the blood increased by a factor of 2 to 3, after the fish had been subjected to a period of hypoxia in the medium (Table I). The increase is presumably due to diffusion of lactate from muscle where there can be considerable anaerobic glycolysis of carbohydrate (Black 1955). The source of the lactate could be branchial muscles which obviously increase their output, or possibly it could come from a general increase in muscular activity.

TABLE I

BLOOD LACTATE CHANGES

| EXPT. | LACTATE BEFORE | LACTATE AFTER | % CHANGE |
|---|---|---|---|
| #1 | 22.52 mg% | 50.66 mg % | 225 |
| #2 | 10.60 | 30.46 | 288 |
| #3 | 9.27 | 36.75 | 396 |
| #4 | 8.69 | 21.56 | 248 |
| Avg. | 12.77 | 34.86 | 273 |

Legend for Table I

Level of lactic acid in the blood (mg/100 ml of blood) of rainbow trout before and after subjection to hypoxia.

Changes in ventilation volume during hypoxia were determined by application of the Fick principle, using the following equation:

$$\text{Ventilation volume: } V_w = \frac{R\ O_2}{C_w \times O_{2in} - C_w \times O_{2out}}$$

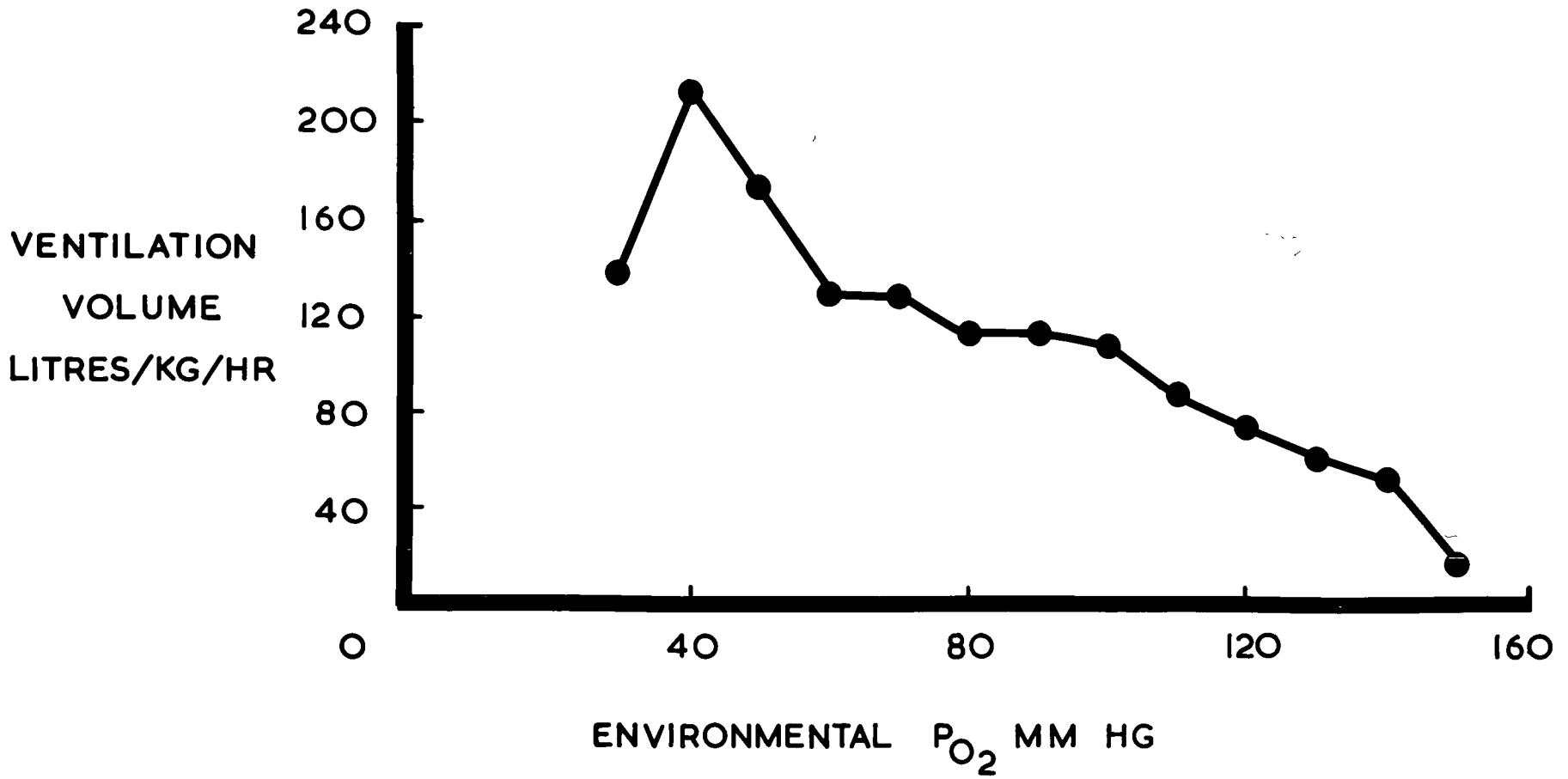
Where $R\ O_2$ = $O_2$ uptake ml/minute/Kg.

$C_w\ O_{2in}$ = Oxygen content of water afferent to the gills.

$C_w\ O_{2out}$ = Oxygen content of water efferent to the gills.

Oxygen content in the water was determined by multiplying the recorded partial pressure of oxygen in the water by the solubility coefficient for oxygen in the water at the prevailing experimental temperature.

The large increase in ventilation volume which was indicated from pressure and rate studies is shown also by the Fick principle estimates (Fig. 20) which showed an increase from 274 ml/min/Kg at an environmental level of 150 mm Hg $PO_2$ to a maximum of 3560 ml/min/Kg at 40 mm Hg environmental $PO_2$. The drop in ventilation volume with environmental levels of oxygen below 40 mm Hg was due primarily to respiratory failure. From Figure 20 it is obvious that the rainbow trout has a wide scope for increasing its ventilation volume; in this study the increase in average ventilation volume was 13 fold. The ventilation volume of the quiescent dogfish (Mustelus californicus) was reported by Ogden (1945) to be 500 ml/min/Kg which is quite similar to the trout in a well oxygenated environment in this study. Van Dam (1938) reported a five fold


240
200
160
120
80
40
0
VENTILATION
VOLUME
LITRES/KG/HR
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 20

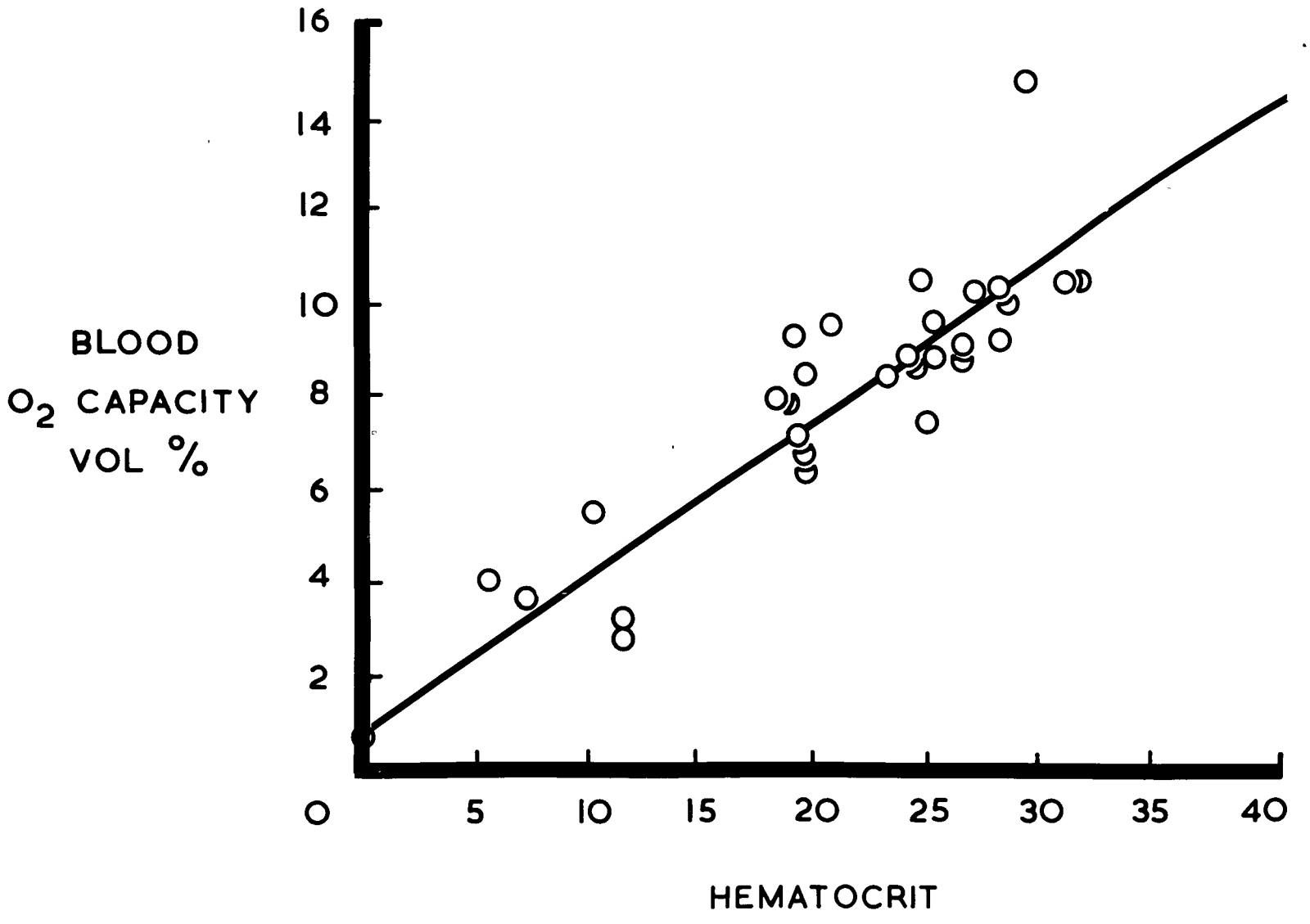
The effect of hypoxia upon ventilation volume. Each point is the average of several values within plus or minus 5 mm Hg environmental oxygen tension of that point.

increase in ventilation volume in response to lowered environmental oxygen in rainbow trout which is less than half the increase observed in the present study. The difference may, in part, be due to interference by Van Dam's apparatus with the normal breathing action of the fish. Saunders (1961) reported increases as high as 82 fold for the sucker, 70 fold for the bullhead, and 31 fold for the carp. These values are certainly far higher than the relative increase found in this study for rainbow trout, but represent extreme values, whereas the values for increase in respiratory volume in the present study are averaged.

An estimate of cardiac output can be obtained in the same manner as the ventilation volume; however, application of the Fick principle to blood flow is considerably more difficult owing to the solubility characteristics of blood. The capacity of blood varies in a non-linear manner with changes in oxygen and carbon dioxide tensions. The dissociation curves required to convert the recorded blood $PO_2$ levels to oxygen content were constructed in this laboratory (Randall, Beaumont, and Holeton; unpublished). The curves obtained are similar to those reported by Irving, Black, and Safford (1941) and Black, Kirkpatrick, and Tucker (1966) for brook trout, but are all at 14-15$^{o}$C and cover $PCo_2$ ranges between 0-1 mm and 10 mm Hg. The capacity of the blood for oxygen also varies with the hematocrit. In these experiments the hematocrit varied in the rainbow trout from 9 to 28.5 per cent. Measurements were made of the oxygen capacity of the blood of 35 fish of differing hematocrits (range

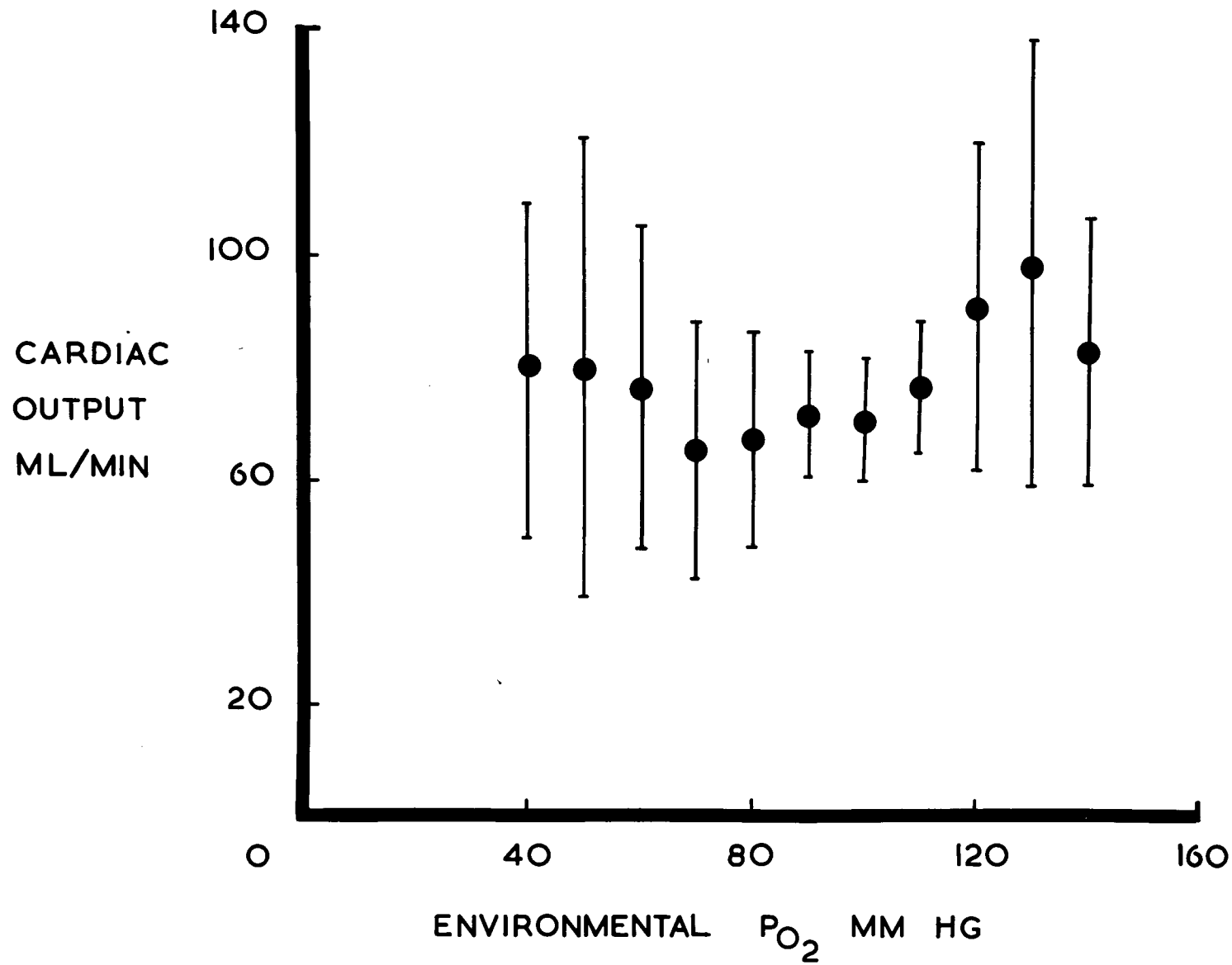
0 to 32%) and from this data it was possible to obtain a linear regression equation (capacity = .311 hematocrit + 0.7) (Fig. 21) relating the hematocrit to the capacity of the blood in volumes per cent of oxygen. The percent saturation of the blood afferent and efferent to the gills of the fish was determined from the recorded blood $PO_2$ and $PCo_2$ levels and the oxygen dissociation curves. The per cent saturation was then converted into oxygen content using the calculated oxygen capacity. Thus the oxygen content of the blood afferent and efferent to the gills was estimated. The volume of oxygen entering the blood was known, and therefore by application of the Fick principle the cardiac output was calculated. No significant changes in cardiac output were observed when the fish was subjected to an hypoxic environment (Fig. 22).

Stroke volume, estimated by dividing minute cardiac output by minute heart rate, increased markedly with hypoxia in the medium (Fig. 23). This would be expected if cardiac output remains constant and heart rate falls. The stroke volume at environmental oxygen levels of 40 mm Hg $PO_2$ is about twice that calculated for fish in water of oxygen levels near air saturation. At oxygen partial pressures below 40 mm Hg and at lower heart rates, however, there are apparently much larger changes in stroke volume. Only a single determination of stroke volume at 30 mm Hg $PO_2$ is available, but this value of 5.2 ml for a one Kg fish is some five times greater than the average stroke volume of 1 ml/Kg for fish in aerated water. Another estimation of stroke volume was made by utilizing averaged data from Figure 14 on blood $O_2$ levels and Figure 19 on oxygen uptake


BLOOD
O2 CAPACITY
VOL %
16
14
12
10
8
6
4
2
0
5
10
15
20
25
30
35
40
HEMATOCRIT

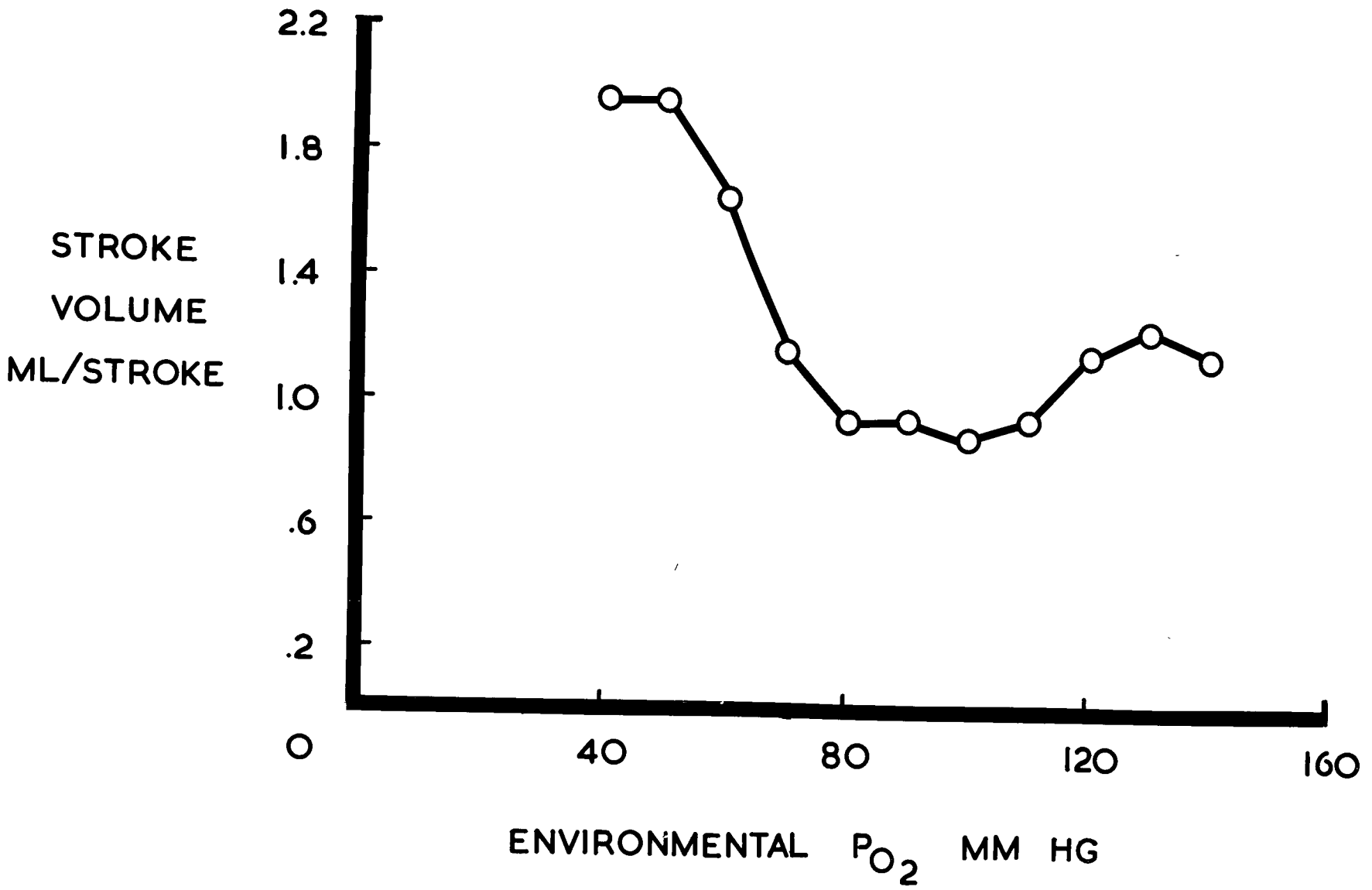
Legend for Figure 21

The relationship between hematocrit and blood oxygen capacity in volumes per cent.


140
100
60
20
0
CARDIAC
OUTPUT
ML/MIN
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 22

The effect of hypoxia upon cardiac output calculated by the Fick principle. Vertical bars are plus or minus two standard errors of the average values plotted.


STROKE
VOLUME
ML/STROKE
2.2
1.8
1.4
1.0
.6
.2
0
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 23

The effect of hypoxia upon stroke volume.

TABLE II

| ANIMAL | TEMP. | CARDIAC OUTPUT ML./KG./MIN. | STROKE VOLUME ML./KG. | METHOD USED | REFERENCE |
|---|---|---|---|---|---|
| Bowfin, Carp Sucker, Catfish | 10° | | .44, .36, .22, .52 | Ligation and subsequent weighing of heart chambers | Hart 1943 |
| *Squalus* sp. | 11-17° | 9.0 - 33.0 | .25 - .93 | Ligations, natural flow from cut ventral aorta. | Burger & Bradley 1951 |
| *Opsanus* sp. | | 10.1 | | Fick principle, minimum theoretical values. | Mott 1957 |
| *Tetraodon* sp. | | 15.5 | | | |
| *Stenotomus* sp. | | 15.7 | | | |
| *Gadus morhua* | | 9.3 | .31 can double | Electromagnetic flowmeter | Johansen 1962 |
| *Amphiuma* sp. | | 40.0 | .96 | | Johansen 1963 |
| Sculpin | 15-18° | 21.4 - 34.2 | | Fick principle | Goldstein *et al* 1964 |
| Octopus | 7-9° | 5.0 - 32.2 | .41-2.0 | Fick principle | Johansen 1964 |
| Frog | 20° | 57 | 1.0 approx. | Ventricular displacement | Shelton & Jones 1964 |
| Human | | 60 - 100 | | | Prosser & Brown 1961 |
| Cat | | 690 | | | |
| Trout | 12-18° | 65 - 100 | .85 - 2.0 | Fick principle | Present study |

Legend for Table II

A summary of some of the available information on cardiac output and stroke volume of various vertebrates.

rates. In this case the stroke volume of a fish, at partial pressures of oxygen of 30 mm Hg in the water was 2.47 ml/Kg of fish and the cardiac output was 69 ml/min/Kg. The final estimations of stroke volume at 30 mm Hg $PO_2$ in the medium were derived from a number of single determinations and not from paired or matched arterial-venous gas tension measurements, as were the other calculations of cardiac output and stroke volume. One might expect considerable variation in the data, especially between the two estimates of stroke volume at 30 mm Hg $PO_2$ in the water.

A comparison between the values for cardiac output and stroke volume obtained in the present study with values listed in the literature has been made in Table II. The values for cardiac output reported in the literature are generally a good deal lower than those of the present study. The literature values for stroke volume are also lower than the estimates of stroke volume of the present study. It is noteworthy that the estimates of Hart (1943), Burger & Bradley (1951), Johansen (1962), and Goldstein et al (1964) all were obtained from fish that were not intact, unanaesthetized, and free swimming. It is quite likely that failure to provide these previously mentioned requirements could have a marked effect upon blood circulatory characteristics, including cardiac output and stroke volume. The minimum theoretical cardiac output values for three marine fishes of rather sluggish behaviour, reported by Mott (1957) are actually quite close to what would be expected in rainbow trout if the venous blood were completely deoxygenated. A good deal of caution must be exercised however

when considering differences in physiological features of animals of different species, of differing size, and which have been examined under differing conditions.

Hughes and Shelton (1962) and Hughes (1964) presented a comparison of gas exchange at the gill surface with known relationships derived from studies upon compact heat exchangers (Kays & London 1958). They show how several heat exchanger relationships are probably of use in analysing gas exchange systems. One such relationship is the effectiveness of transfer. Effectiveness is the ratio of the actual gas transfer ($RO_2$) compared with the maximum amount of gas (Rmax) that could be transferred if the exchange were complete, a condition for which Hughes (1964) presented the following expressions for effectiveness:

$$\text{Effectiveness of transfer} = \frac{RO_2}{RO_2\ max} \times 100$$

$$= \frac{Vb\ Sbo_2\ (Tbo_2\ out - Tbo_2\ in)}{Vb\ Sbo_2\ (Two_2\ in - Tbo_2\ in)} \times 100$$

$$= \frac{\text{Amount of } O_2 \text{ taken out of water (entering blood)}}{\text{Amount of } O_2 \text{ that could possibly be taken up by blood}}$$

$$= \frac{\text{Rate of } O_2 \text{ uptake ml/min/Kg}}{Vb\ Sbo_2\ (Two_2\ in - Tbo_2\ in)}$$

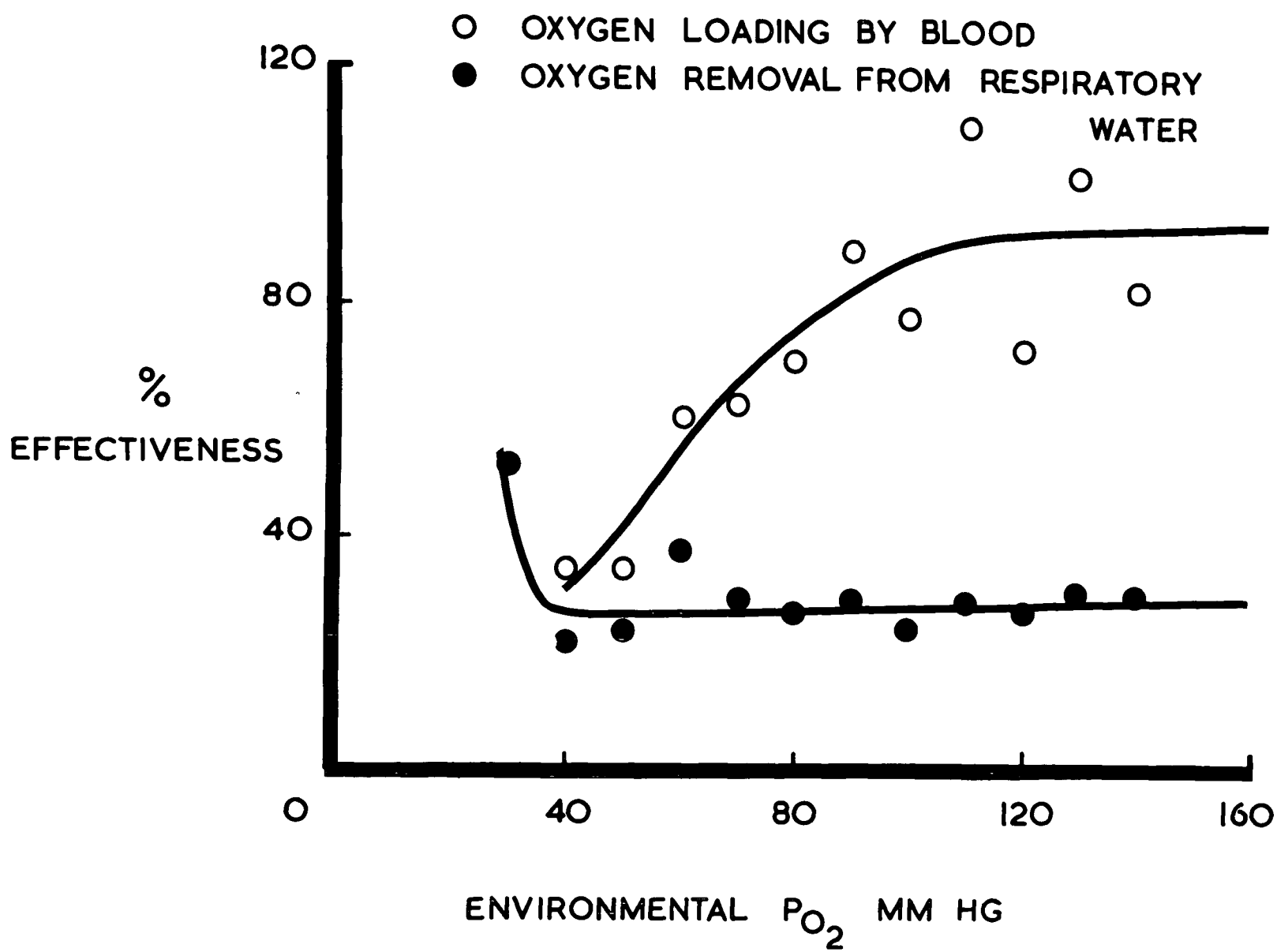
Where Vb and Vw are the volumes of the flow of blood and water respectively and Sw and Sb are the solubility or absorption coefficients of $O_2$ in water and blood. Sb will vary with the slope of the dissociation curve of blood. Tw and Tb are the partial pressures of oxygen in water and blood respectively.

The effectiveness of gas exchange across the gills has been calculated for rainbow trout using the information obtained

in this study. The effect of hypoxia upon effectiveness of exchange is presented in Figure 24 (open circles). Although there is limited accuracy in such estimates, the results would indicate that under well oxygenated conditions (90 to 140 mm Hg $PO_2$) the effectiveness of the gills is very near 100%. As hypoxic conditions develop, the effectiveness decreases, reaching values of 30% - 40% at environmental oxygen tensions of 40 mm Hg $PO_2$. This decrease in effectiveness is a result of the $RO_2$ max (maximum possible amount of $O_2$ the blood could carry away) increasing. Throughout the experiment the oxygen consumption has not changed appreciably so the "$RO_2$ max" has to increase. From this one could infer that at high oxygen tensions in the environment, the area, diffusion characteristics, and the amount of water presented to the gills all are sufficient to load almost completely the blood passing through the gills. Under hypoxic conditions there appears to be a large increase in blood capacity. Since it has been demonstrated that Vb does not change appreciably, the blood capacity increases must be dependent upon the dissociation characteristics of hemoglobin. Thus the decrease in effectiveness is related to the nature of the oxygen dissociation curve of the blood.

The previous discussion considered effectiveness from the aspect of blood oxygenation. Another consideration is the effectiveness of oxygen removal from the water. The effectiveness of oxygen removal can be obtained from the following expression:

$$\text{Effectiveness of removal} = \frac{RO_2}{RO_2 \text{ max pres.}}$$


O OXYGEN LOADING BY BLOOD
● OXYGEN REMOVAL FROM RESPIRATORY WATER
120
80
40
0
%
EFFECTIVENESS
40
80
120
160
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 24

Changes in the effectiveness of blood oxygenation and the effectiveness of oxygen removal from water occurring with increasing hypoxia.

$$= \frac{\text{Rate of } O_2 \text{ uptake ml/min/Kg}}{Vw\ Swo_2\ (Two_2in - Tbo_2in)}$$

Where $RO_2$ max pres is the maximum or total useable amount of oxygen presented to the gill surface.

The effects of hypoxia upon effectiveness of oxygen removal from inspired water with the rainbow trout have been calculated using the information obtained in this study and are illustrated in Figure 24. With the exception of very high (150 mm Hg $PO_2$) and very low (30 mm Hg $PO_2$) environmental oxygen tensions where a paucity of data renders the values less reliable, the effectiveness of oxygen removal does not vary greatly from 30%. The constancy of the effectiveness of the removal of oxygen from inspired water, coupled with the knowledge that $RO_2$ (oxygen uptake) does not change appreciably, indicates that a fairly uniform quantity of useable oxygen is being presented to the gill surface. This is accomplished by the increase in ventilation volume (Vw).

The percentage of available oxygen in inspired water that is utilized by the fish serves as an index of the efficiency of the gills for oxygen uptake (Van Dam 1938). This statement is incomplete without the added stipulation that the gills must still be taking up sufficient oxygen to keep the fish alive. With the rainbow trout in this study the average utilization of inspired water decreased from 55% at an environmental $PO_2$ of 160 mm Hg to 20% at an environmental $PO_2$ of 100 mm Hg. Below 100 mm Hg environmental $PO_2$ there was no significant departure from a utilization of 20%. This effect can be readily seen by comparing opercular and buccal water tensions illustrated in Figure 13. It was observed that the highest

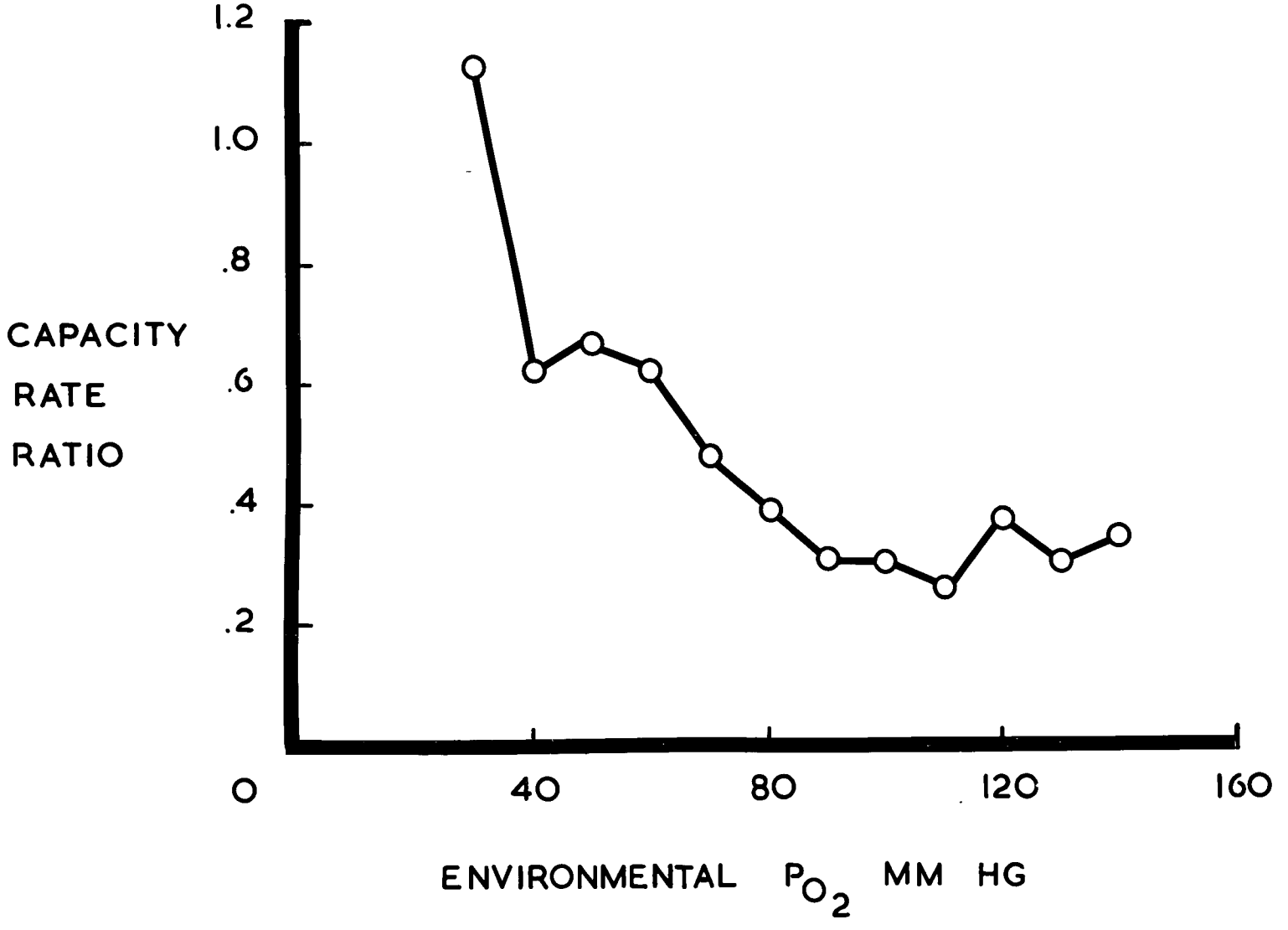
utilizations were obtained by large fish which were exceptionally quiet. A fright or burst of activity resulted in a large drop in utilization and correspondingly a two to four fold increase in ventilation volume. Van Dam (1938) reported a utilization as high as 80% in a rainbow trout resting quietly in well oxygenated water. A four fold increase in ventilation volume resulted in a drop in utilization from 80% to 60%. These observations were based upon determinations at 10°C upon a single 900 gram trout which was kept in a small (60 liters) holding tank prior to testing. In the present study a 945 gram fish tested August 29, 1965 at 16°C was observed to have a resting utilization of 78%. The lowering of environmental oxygen to 70 mm Hg $PO_2$ resulted in an increase from 7.75 to 38.2 liters/hr/Kg in ventilation volume and a decrease in utilization from 78% to 34%. Thus the behaviour of this particular fish was very similar to the behaviour of the particular fish upon which Van Dam (1938) experimented.

Many of the cardiovascular and respiratory characteristics of fish appear to be highly variable. The techniques used to measure these characteristics are often pushed to the limits of their accuracy. Under such circumstances the best interests of accuracy are served by pooling the information from a large number of experiments. Information obtained from a single or few determinations of a highly variable parameter can often be misleading. To illustrate this point, Saunders (1962) reported an 82 fold increase in ventilation volume for the sucker in response to hypoxia. This is based on a calculated increase in ventilation volume from 1.8 ml/second to 143.0 ml/second. The sucker weighed less than 250 grams and yet

was reportedly able to move 143 ml/sec with its respiratory pumps. This works out to approximately 70 to 100 ml each breathing cycle! This high ventilation volume is based in turn upon a determined utilization of 3.3%. If the accuracy of the oxygen determination technique was only plus or minus 1.5% it would be easy to incur an error over ten fold in the ventilation volume.

Summarizing, it is suggested that the branchial pump of the rainbow trout manages, though at progressively higher energy costs, to maintain a uniform supply of oxygen to the gill surface in the face of increasing hypoxia. The circulatory system responds to the hypoxia by increasing its ability to carry the oxygen away from the gills.

A change in the capacity rate ratio of blood ($Vb \times Sbo_2$) and water ($Vw \times Swo_2$) would alter the effectiveness of gas exchange (Hughes & Shelton 1962, Hughes 1964). The effect of hypoxia on the capacity rate ratio of blood and water was calculated and is summarized in Figure 25. It was observed that the capacity rate ratio increased from about 0.30 at environmental oxygen tensions from 140 to 100 mm Hg to more than 0.60 at environmental levels less than 60 mm Hg $PO_2$. As Hughes and Shelton (1962) have pointed out, the capacity rate ratio can have a strong influence upon effectiveness. As the capacity rate ratio increases the effectiveness of oxygen loading of the blood will decrease (Hughes and Shelton 1962, Hughes 1964). During hypoxia the capacity rate ratio of the trout increases (0.3 to 0.6) and as might be expected the effectiveness of transfer decreases.


1.2
1.0
.8
.6
.4
.2
0
40
80
120
160
CAPACITY
RATE
RATIO
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 25

Changes in capacity rate ratio of blood and water occurring with increasing hypoxia.

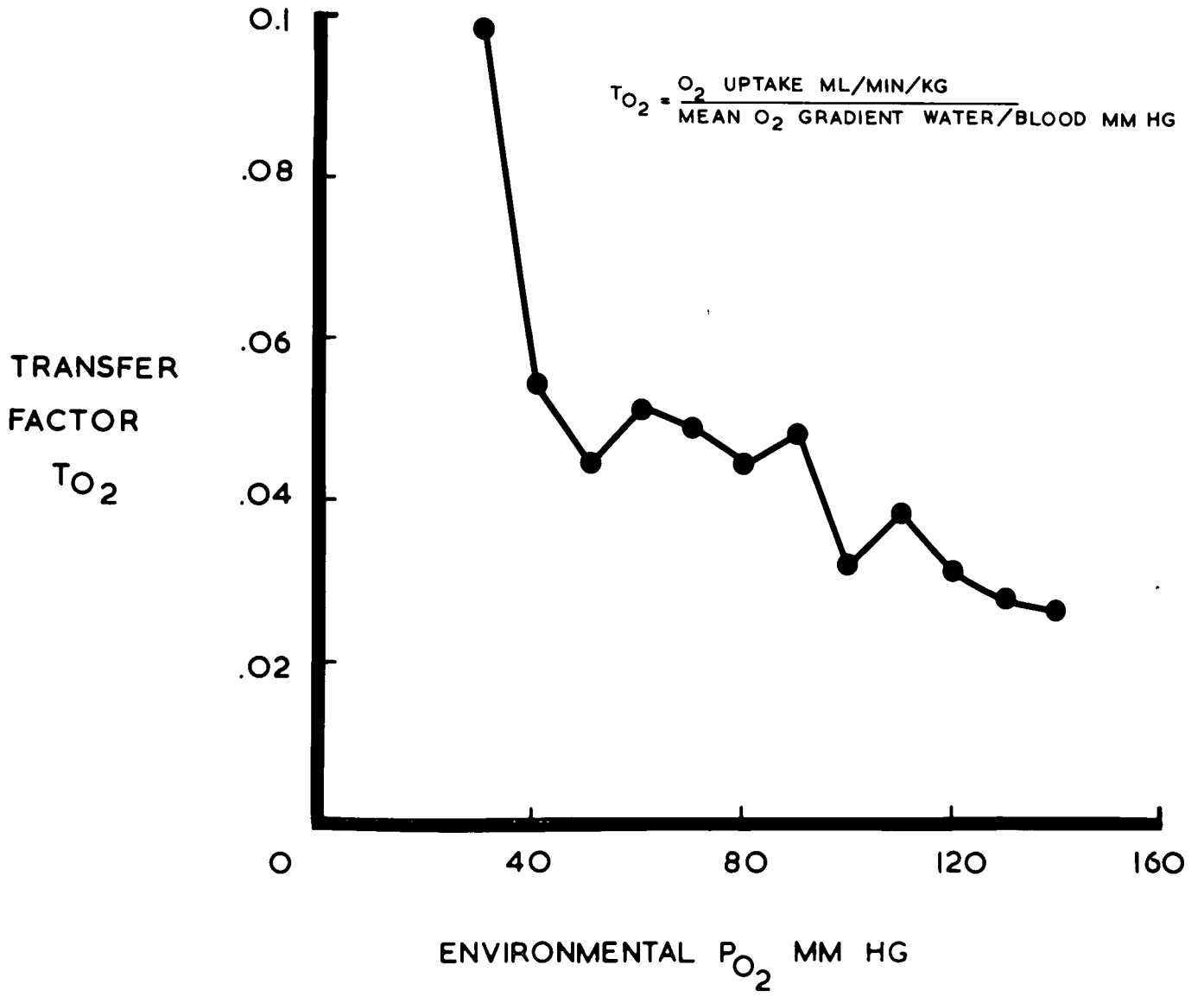
The diffusion capacity or transfer factor (Ruch and Patton 1965) will also affect the rate of gas exchange across the gills. If the surface area of the gills available for gas exchange increases, or if the diffusion distance between blood and water decreases, the effectiveness of oxygen loading of the blood will be increased. The term transfer factor (Ruch & Patton 1965) is defined as:

$$To_2 = \frac{\text{Rate of } O_2 \text{ uptake ml/min/Kg}}{\text{Mean pressure gradient across gill epithelium}}$$

$$To_2 = \frac{RO_2}{\frac{(Two_2in + Two_2out)}{2} - \frac{(Tbo_2in + Tbo_2out)}{2}}$$

The effect of hypoxia in the medium on the transfer factor was calculated and is summarized in Figure 26. There is a marked increase in the transfer factor of the gills of trout in response to hypoxic conditions in the environment.

Steen and Kruysse (1964) demonstrated the presence of alternate circulatory pathways in the gills of the eel and ten other teleosts, including the rainbow trout. They demonstrated that shunting changes could affect the degree of oxygenation of the blood of the eel. They intimate that most if not all teleost fish have the capacity to change the functional capacity of their gills for gas exchange by means of vascular changes within the gills. The increased transfer factor is probably related to a decrease in the diffusion distance between blood and water rather than an increase in the gill area available for gas exchange. A change in the diffusion distance between blood and water would be indicated by a


0.1
.08
.06
.04
.02
0
40
80
120
160
TRANSFER FACTOR $T_{O_2}$
$T_{O_2} = \frac{O_2 \text{ UPTAKE ML/MIN/KG}}{\text{MEAN } O_2 \text{ GRADIENT WATER/BLOOD MM HG}}$
ENVIRONMENTAL $P_{O_2}$ MM HG

Legend for Figure 26

Changes in transfer factor ($To_2$) of the gills during increasing hypoxia.

change in the pattern of blood flow through the gills (Fig. 11), and indeed this is indicated by the changes in vascular resistance associated with hypoxia, concluded from an examination of the changes in blood pressure recorded efferent and afferent to the gills of the trout. An increase in the area for diffusion rather than a decrease in the distance of diffusion is unlikely because the increase in water velocity associated with hypoxia would most likely disturb the arrangement of the gill lamellae and so reduce the area of the gills presented to the water for gas exchange.

The increase in transfer factor during hypoxia is probably a low estimate. Bijtel (1947, 1949) stated that during quiet respiration the tips of the gill filaments were in contact and that all respiratory water circulated over lamellar regions. At high ventilation, Saunders (1961) showed that the tips of the filaments of several fresh water teleosts separated somewhat. This would mean that some portion of the respiratory water was passing by the gills without coming in close contact with the respiratory surface. If this is the case, then water efferent to the lamellar surface is probably of lower tension ($O_2$) than is the efferent water sampled in the opercular chamber. This in turn would mean that at higher ventilation levels the oxygen tension gradient over the gill surface is likely smaller than estimated and the transfer factor is correspondingly larger than estimated.

There was a loose inverse correlation between the hematocrit of the fish and the oxygen tension at which the fish

began to show signs of respiratory or cardiac failure. Figure 18 shows this relationship between the initial hematocrit of the fish and the oxygen tension at which the various experiments were terminated. It would appear that the hematocrit and correspondingly the unit capacity of the blood of the fish is of considerable importance to the fish in survival in hypoxic conditions.

SUMMARY OF PART THREE

1. There were no significant changes in oxygen uptake during hypoxia in the medium. Values of between 100 - 125 mg/hr/Kg were obtained for the rainbow trout during these experiments.
2. The blood lactate of four fish examined increased 2.7 fold during the course of the experiments.
3. Ventilation volume increased 13 fold in response to hypoxia. The average ventilation volume increased from a resting level of 274 ml/min/Kg to a level of 3560 ml/min/Kg in response to hypoxia.
4. Cardiac output estimated by the Fick principle did not change significantly. The average value for cardiac output varied from 65 ml/min/Kg to 100 ml/min/Kg.
5. Stroke volume of the heart increased from approximately 1 ml/stroke to 2 - 5 ml/stroke in response to hypoxia.
6. Effectiveness of blood oxygenation decreased from approximately 100% to approximately 30-40% as a

result of the blood capacity increase which occurred at lower oxygen tensions.

7. Effectiveness of oxygen removal from inspired water tended to remain fairly uniform at about 30% indicating that a uniform quantity of useable oxygen is being presented to the gills.
8. Percentage utilization of oxygen from inspired water decreased, between oxygen levels in the environment (from 160 mm Hg $PO_2$ to 100 mm Hg $PO_2$, to 20% to 55%). Below environmental $PO_2$ levels of 100 mm Hg the percentage utilization did not vary significantly from 20%.
9. Capacity rate ratio (Capacity of blood/Capacity of water) increased from approximately 0.30 to more than 0.60 in response to hypoxia.
10. The transfer factor of the gills increased by a factor of at least three, and it is likely that this estimated increase is low. There is a strong suggestion that there is a shortening in the distance in the diffusion pathway for oxygen across the gill membrane as a result of vascular changes within the gills. This suggestion is supported by indications of resistance changes to blood flow through the gills.

## GENERAL DISCUSSION

In the present study the fish were undergoing only transient tests of their response to hypoxia. It is likely that most of the fish tested could not have survived at the lower oxygen levels, if subjected to these for a long period of time. The main purpose of the experiments was to examine the short term responses of the cardiovascular and breathing systems to hypoxia. The buildup of carbon dioxide in the respirometer from the fish's respiration was a complicating factor, but the levels were low (of the order of a few mm Hg). It is possible that the carbon dioxide may have affected the tensions at which the various responses occurred, but it is highly unlikely that the nature and general order of occurrence of the responses would be greatly affected.

Teleosts are a very diverse group and the responses of the rainbow trout to hypoxia are not necessarily representative of the whole group. The trout is a rather unspecialized fish which lives, generally, in environments of high oxygen content. Since it appears that trout are active, and their close relatives, the salmon, demonstrate tremendous ability for activity, it is reasonable to assume that the trout probably have a wide scope for increasing their oxygen uptake.

The present examination has demonstrated that the uptake of oxygen can, at least in part, be maintained despite lowered environmental oxygen levels. The fish were to some extent resorting to anaerobic means for their metabolic energy supply as is evidenced by the rise in lactate levels in the blood, between the beginning and the end of the experiments.

It was assumed that under hypoxic conditions the fish would need some oxygen and would take steps to obtain it. Unless the minimum amount of oxygen required to sustain the fish is very small, the fish would have to increase their effort to extract oxygen from the environment. If this is the case the extracting machinery will require increased oxygen to run it. This would create a problem of diminishing returns per unit effort for extraction as the environmental oxygen decreases.

Fundamentally the fish can take compensatory measures in two manners; passive and active. Firstly the fish can passively decrease the amount of oxygen uptake needed for sustenance. Secondly measures can be taken to maintain the amount of oxygen taken in by the fish.

To counteract the effects of hypoxia by passive means, the fish could do the following:

a. Reduce their metabolism generally, resorting to anaerobic metabolism where necessary.
b. Shut down oxidative metabolism in non-vital tissues.
c. Decrease muscular activity, especially activity not associated with oxygen extraction.

The rise in lactate in the blood of the experimental fish would suggest that the fish were resorting to anaerobic respiration to some extent. There was no reliable way of measuring whether or not the fish were taking advantage of the last two measures.

Active measures taken by the fish which will increase the rate of diffusion of oxygen across the respiratory membranes can be listed under the following categories:

a. The tension gradients could be increased.

b. The area of the exchange surface could be increased.

c. The distance for diffusion could be decreased.

d. The timing and arrangement of flows of liquids past the respiratory epithelium could be related so as to obtain an optimal flow arrangement for the exchange of gases.

Variables which will affect the tension gradient for oxygen across the gill membrane are; the tension of the environmental oxygen, the oxygen tension of venous blood, the flow rates of blood and water past the exchange surface, and the oxygen capacity of the blood and the water.

The maximum gradient which can be achieved, is the difference in oxygen tension between the environment and venous blood. With progressively deepening hypoxia the maximum possible gradient is diminishing. Increasing the flows of blood and water past the gill surface will serve to maintain the gradient at a level as near the theoretical maximum as possible. However, increasing the flows indefinitely will bring steadily diminishing returns as the gradient nears its limiting maximum. An increase in the capacity of the liquids for oxygen will allow the oxygen tension gradient to be maintained with lower flow rates.

With the present study, it is obvious that the oxygen tension of the environmental water is dropping throughout the course of the experiment. In other words, the fish is presented with the problem of continually decreasing maximum

possible diffusion gradients for oxygen over its respiratory surface. To counteract this the fish have no means of altering the capacity of the water, but they can, and do, increase the flow of water over the gills some thirteen fold. The arterial and venous oxygen tensions and the dissociation characteristics of the blood are such that the functional capacity of the blood is increased with the hypoxia even though the total capacity is diminished. As pointed out previously in this discussion, it is energetically more economical to increase the capacity of the fluids than to increase the flows. The trout studied in this work were observed to increase the functional capacity of their blood; at the same time there was no significant change in the estimates of cardiac output. Thus it appears that at least with a specialized fluid, the blood, the fish are able to observe the economy resulting from capacity increases in maintaining as high a gradient as possible for oxygen tensions over the respiratory membrane.

It is interesting to note that with the trout studied, although there is a large increase in the amount of water presented to the gills in response to hypoxia, the amount of useable oxygen in the water had in the same interval become proportionately less. With the blood, the situation was the reverse. The flow did not change significantly but the unit capacity increased as a result of the dissociation characteristics of the blood. Thus, as Hughes and Shelton, 1962, and Hughes, 1964, suggested, there is a fairly close coupling of the capacity x rate ratio between the blood and the water passing over the gills, in response to hypoxia.

The principle of functionally shortening the diffusion path through the gill membrane represents a very effective way of augmenting the rate of gas movement through the gill membrane. The suggestion of Steen and Kruysse (1964) that blood can be channelled closer to the surface of the gills is very interesting. Steen and Kruysse (1964) have demonstrated that the structural requirements for such a shunting system are present in a variety of fresh water teleosts. They also demonstrated that adrenalin could increase the degree of oxygenation of efferent blood. Evidence from the present study suggests that there are vascular changes in the gills associated with hypoxia which are causing an increase in resistance over the gills to the flow of blood. This occurrence, coupled with the increase in transfer factor of the gills with increasing hypoxia, provides good evidence for the functioning of a system whereby the diffusion characteristics of the gills are changed. There is a strong possibility that blood is being presented closer to the water.

One possible reason that the fish does not leave the transfer factor of the gills at the higher levels associated with hypoxia is that the supposedly shorter diffusion path probably increases the ionic or osmotic problems that the fish has to contend with, a problem which would throw a heavy work load upon the kidneys and the gills. The increase in transfer factor is likely to be an emergency response to augment the uptake of oxygen under the penalty of increased ionic or osmotic problems.

There is a counter current flow arrangement of blood and water passing the exchange surface which aids in maintaining the highest gas tension gradients under most of the reasonable flow rates. Evidence of such a system has been previously discussed in the second part of the present study. Rainbow trout apparently do have a counter current flow arrangement of blood and water past the respiratory surface.

Another means by which fish augment the exchange process is by a marked synchrony between the breathing movements and the heart beats. This has been observed in a wide variety of fish during hypoxia (Hughes, 1961; Shelton and Randall, 1962; Satchell, 1960). Hughes 1964, has amply pointed out the value of coupling the blood and water movements.

With the rainbow trout the point at which the homeostatic mechanisms can no longer compensate for the reduced oxygen supply is the beginning of a cataclysmic collapse. The respiratory and heart muscle will begin to fail if oxygen supplies fall too low, and to do so will further reduce the uptake of oxygen which in turn will further reduce the effectiveness of the extracting machinery even more so.

An interesting functional aspect of the blood pigment of the rainbow trout is that it serves as a reservoir for functional blood oxygen capacity increases. The venous blood pigment of the rainbow trout is practically all oxyhemoglobin when the environmental oxygen tensions are high. This is in sharp contrast with the assumptions of some (Mott, 1957; Saunders, 1962) that the venous blood is, in most cases, completely deoxygenated. In general the hemoglobin is not

used to much extent when the fish is resting in a well oxygenated environment. With some species of Chaenocephalus from antarctic regions there are no manifestations of blood pigments at all (Ruud, 1954). Anthony (1961) reported that goldfish could survive in a medium with high tensions of carbon monoxide, and concluded that hemoglobin was not absolutely necessary to the resting goldfish. It would appear from the present study and earlier work that the blood pigment of rainbow trout does not serve as a major carrier of oxygen except under conditions of stress or high oxygen demand. Instead it appears that the pigment serves to carry some of the increased loads upon the gas uptake mechanism rather than the heart. It would be of advantage to avoid increasing cardiac output. It would seem reasonable to assume that the heart does not have a very large scope for increasing its work load, since it must work continuously. There already is an increased work load on the heart during hypoxia, since the cardiac output does not change and the blood pressures nearly double.

The amount of hemoglobin in the blood would reflect the capability for increasing the capacity of the blood, and the ability to ease the work done by the heart. This suggestion is supported by the loose correlation observed between the hematocrit of the blood and the tension of the water at which the various experiments were terminated. Fish with higher hematocrits were generally able to sustain lower environmental tensions than fish with low hematocrits.

The bradycardia associated with the hypoxia remains an interesting problem. The bradycardia is a very wide-

spread response observed in diving birds, mammals, and many poikilotherms. It has more or less been assumed that the bradycardia results in a decrease in the heart output. Hughes (1964) suggests that the bradycardia serves to lengthen the residence time for blood in the gills, by merit of decreased output. This would provide for more complete loading of the efferent blood and maintaining the tissue oxygen levels. What renders the present study interesting is that there does not appear to be any decrease in cardiac output concomitant with the hypoxia-induced bradycardia. There is instead, a marked increase in stroke volume which is of about the same magnitude as the severity of the bradycardia. Why then is there a bradycardia at all? Some known factors are, that the work load on the heart is increasing and the oxygen unloading tensions at the tissue level are lower. In other words the heart will need more oxygen and at the same time the diffusion gradients driving the oxygen are lower. Possibly the bradycardia serves to provide a longer period for oxygen and metabolites to be restored in cardiac muscle between each working stroke. The suggestion is that cardiac muscle of fish, and possibly other vertebrates, may be able to operate more efficiently through long spaced strokes than through short spaced ones. At any rate it is hard to visualize any benefit from the increased pulsatility in pressures and presumably gill blood flows, which result from the bradycardia.

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