Color:
Blue
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May 21, 1936.

To the Committee on Graduate Study:

I submit herewith a thesis written by Mr. Spurgeon Meek Wingo and entitled "The Relation of the Carbon Dioxide Tension of the Water to the Hemoglobin Content of the Blood, the Gaseous Content of the Swim-Bladder, and the Ability of the Fish to Extract Oxygen from the Water at Low Oxygen Tensions", and recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Zoology.

Edwin B Powers
Major Professor

At the request of the
Committee on Graduate Study,
we have read this thesis,
and recommend its acceptance.

Barton C.O. Ressler

Judson A. Robertson

Accepted by the Committee

J. S. Guest
Chairman

THE RELATION OF THE CARBON DIOXIDE TENSION OF THE WATER TO THE HEMOGLOBIN CONTENT OF THE BLOOD, THE GASEOUS CONTENT OF THE SWIM-BLADDER, AND THE ABILITY OF THE FISH TO EXTRACT OXYGEN FROM THE WATER AT LOW OXYGEN TENSIONS

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A THESIS

Submitted to the Graduate Committee
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

by

SPURGEON MEEK WINGO

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INTRODUCTION

Probably in no field of biology is reasoning from analogy depended upon more than it is in human physiology to answer the questions which constantly arise in the mind of the investigator. This is particularly true of that phase of respiration which attempts to explain the apparent secretion of oxygen into the blood at low oxygen tensions. The secretion theory has found much favor with certain physiologists and in briefly presenting their case in his Text Book of General Physiology (1932) Mitchell has said as a point in its favor, "Glands of the swim bladders of many species of fishes secrete oxygen under high pressure into the swim bladders". In a full discussion of this question Powers (1932) has shown that gases are not secreted into the swim-bladder in the true sense of that term, and that, "The secretion of oxygen into the lungs might still be an open question. But, on the other hand, the deposition of gases into the swim-bladder cannot be used as an illustration of a mechanism by which this is accomplished." Thus in this case, as with many other physiological problems, it will be necessary to extend our knowledge of the physiological process in other forms of animal life if conclusions based upon them are to be accepted as valid.

It has been shown by Powers and Hickman (1932) that the oxygen dissociation curve for the blood of certain fishes shows two depressions as the carbon dioxide tension to which the blood

is exposed increases. The first depression they attribute to the lack of affinity of hemoglobin for oxygen at its isoelectric point. This is shortly followed by a rise which continues until the carbon dioxide tension is about 1.2% of an atmosphere, after which there is a second depression. It is suggested by Powers and Hickman that, "The second depression is perhaps due to the actual breaking down of hemoglobin into its intermediate products."

The series of experiments which have been performed were designed to investigate the changes which take place in the swim-bladder gas and the blood of fish when exposed to small variations in carbon dioxide tension. It was thus hoped to learn something of the physiological activities of the fish when in a habitat whose carbon dioxide tension is close to the isoelectric point of the blood.

MATERIAL

One species of fish, Lepomis pallidus (Mitchill), the common blue bream, was used throughout this series of experiments. About five hundred were gotten from the Chilhowee Park lakes in the fall and brought to the University. Fifty of these were put in a small artificial pool at the Biology Building and the remainder in the swimming pool at the University Hospital. Experiments 0 through 29 were performed on the fish kept at the Biology Building.

During the very cold weather of February the hospital swimming pool remained frozen for four weeks to a depth of six to eight inches. Every effort was made to keep the ice broken around the edges of the pool so that fish could be obtained for continued experimentation. None of these efforts proved very successful; and when the pool was finally freed from ice, it was found that not more than fifty fish had survived and could be used. These were removed to the Biology Building and kept in the artificial pool already referred to. These were the fish used in experiments 30 through 50.

Experiments 51 through 94 were performed with fish obtained from the Chilhowee Park lakes in the middle of March; those used in the experiments were kept at the Biology Building.

METHOD

A. Determination of gas content of water.

1. Oxygen content. Dissolved oxygen was determined by the Rideal-Stewart (1901) modification of the Winkler method as approved by the American Public Health Association and the American Water Works Association (1933). The method was slightly modified in that two 100 ml. samples were titrated from each 250 ml. bottle, rather than titrating a single 200 ml. sample; and the sodium thiosulfate solution was made up approximately .01 N., instead of .025 N. When titrating a 100 ml. sample of water for oxygen content, no correction was made for the displacement of water by the addition of reagents as the error thus introduced was within that of the method.

The thiosulfate solution was standardized against potassium permanganate, its normality being determined as .0099. One milliliter of such a solution is equivalent to .0554 ml. of oxygen at standard conditions. Since 100 ml. samples were used, the total oxygen per liter is found by multiplying the number of milliliters of thiosulfate used by the factor .554.

2. Carbon dioxide content. The Van Slyke gas analysis apparatus (Van Slyke and Neill, 1924) was used for the determinations of total carbon dioxide. Samples of water were drawn slowly from the bottom of the experimental tanks into special tonometers fitted with two two-way stop-cocks. From these tonometers samples were delivered to the extraction chamber of the Van Slyke apparatus according to the procedure described by Brock. (1934).

Gas-free .015 N. HCl was used for the extraction of carbon dioxide. A small amount of thymol blue, pH range 1.2 to 2.8 and 8.0 to 9.6, was added to the acid when it was prepared. By noting the color of the solution in the extraction chamber during liberation and measurement of carbon dioxide (red) and the change in color upon introduction of 1 ml. of 5 N. NaOH for absorption of carbon dioxide (yellow to blue) no doubt existed in either case as to the reaction of the extraction chamber solution.

Throughout the experiments 5 ml. samples of water were used, 5 ml. of acid being used for extraction of carbon dioxide, thus maintaining a constant volume of solution, or "S", at 10 ml. All pressure readings were made with a gas volume of .5 ml.

With 5 ml. gas-free HCl in the extraction chamber, and a mercury seal at the top stop-cock, the 5 ml. sample was admitted through the side arm. A Torricellian vacuum was obtained by lowering the mercury in the extraction chamber to the 50 ml. mark. The solution was then shaken for three minutes to extract carbon dioxide. Mercury was re-admitted and the meniscus of the solution raised to the .5 ml. mark. At this point P_1 was read. The level of the solution was again allowed to fall near to the 50 ml. mark and 1 ml. of 5 N. NaOH was admitted slowly into the extraction chamber. The operation required from thirty to forty seconds; after which a mercury seal was again made at the top of the extraction chamber, and the level of the solution was returned to the .5 ml. mark by admission of mercury. At this point the P_2 reading was made.

A correction factor is necessary for the P_2 reading due to the addition of 1 ml. of 5 N. NaOH, which lowers the mercury level in the extraction chamber by increasing the volume of fluid above the mercury between the mercury meniscus and the gas mark. This in turn lowers the mercury level in the manometer. This correction is determined by blank analyses which give the difference in the actual P_2 reading and what it would have been had the volume not been increased by the addition of 1 ml. of 5 N. NaOH. This correction was found to be 3.7 mm. with the extraction chamber used in this work, when the gas volume is .5 ml. and the volume of solution 10 ml. By adding this correction to the P_2 reading and subtracting the total from P_1, P_{CO_2} is obtained. On multiplying this reading by the appropriate temperature factor, the volume per cent of carbon dioxide is obtained.

Since there were no factors available for calculating volume per cent of carbon dioxide under the conditions of these determinations, it was necessary to calculate a table of factors using the formula of Van Slyke and Neill (1924). These factors will be found in Appendix I.

B. Determination of carbon dioxide tension.

Total carbon dioxide content of a water as determined with the Van Slyke gas analysis apparatus is not a true measure of this factor as it affects the respiration of fishes. Neither can the alkali reserve be taken as an index of the gaseous tension to which the blood of the fish is exposed as it passes through the gills. A more satisfactory method of arriving at this important data is through application of the formula of Powers and Bond (1928) to three pH readings. These readings are made at two known

different carbon dioxide pressures and on the undisturbed water.

The mass action equation for dissolved carbon dioxide tension may be written as the equation of the Power Law and reduced to the linear form

$$\log C_H = n \log (Kk_{gas}P) \qquad (I)$$

Here C_H is hydrogen ion concentration, n the rate of change in log $(Kk_{gas}P)$ with a change in hydrogen ion concentration, K the primary ionization constant of carbonic acid, K_{gas} the solubility factor of carbon dioxide, and P the carbon dioxide tension of the liquid expressed in per cent of an atmosphere.

Kk_{gas} varies in absolute value for each natural water necessitating the introduction of a correction factor e_1. Furthermore, since hydrogen ion concentrations met with in biological work are less than unity it is convenient to express them as the log of the reciprocal, using the sign pH to indicate this. With these changes equation (I) becomes,

$$pH = -n(\log P + \log (Kk_{gas}) + e_1) \qquad (II)$$

Since both $\log(Kk_{gas})$ and e_1 are unknown and together may be taken as equal to e, this value may be substituted in equation (II) and by rearranging we have,

$$pH = -ne - n\log P \qquad (III)$$

Powers (1930) has shown that for waters of the Holston and Clinch rivers the value of n is equal to .9836. This is very probably close to the value of n for the laboratory tap water used in these experiments, as the source of this water is the Tennessee river formed by the junction of the Holston and French Broad a few miles from Knoxville. Assuming this value of n to be

correct for the water used, two equations may be set up thus,

pH(aerated) = -ne -.9836 log.00035

pH(unaerated) = -ne -.9836 logP

The value .00035 is substituted for P in the first equation because air is .035 per cent carbon dioxide. Subtracting the second equation from the first and rearranging,

.9836 logP = .9836 log.00035 + pH(aerated)-pH(unaerated)

Dividing through by .9836 and introducing the log of 760(barometric pressure in millimeters of mercury at standard conditions) on the right we have,

$$\log P_{mm} = -3.45593 + \left[\frac{pH(aerated)-pH(unaerated)}{.9836}\right] + 2.88081$$

where log Pmm is the log of the carbon dioxide tension in millimeters of mercury. This equation becomes,

$$\log P_{mm} = -.57512 + \left[\frac{pH(aerated)-pH(unaerated)}{.9836}\right]$$

and in this form was used to determine the carbon dioxide tensions of the experimental waters at the time of the death of the fish.

C. Alkali Reserve.

McClendon (1917) has described as a measure of the alkali reserve of a water the amount of .01 N. HCl required to bring the pH of a 100 ml. sample to 4.2. This method was followed, using Brom-Cresol-Green as indicator.

D. Determination of hemoglobin.

Hemoglobin estimations were made with the Newcomer hemoglobinometer. As originally described (Newcomer, 1919) it consisted of a small Duboscq Colorimeter in which the depth of the diluted blood sample being examined was varied until the light

transmitted by the sample matched that transmitted by a special yellow glass. In this work the Newcomer Hemoglobin attachment supplied by the Bausch and Lomb Optical Company for their Duboscq Colorimeter No. 2502 was used. This consists of a special yellow glass filter, the absorption curve of which runs as mean through that of acid hematin; and a special blue filter for the eyepiece which cuts off most of the light waves greater than 5000 Å , thus eliminating two weak bands forming a depression from 5000 Å to 5800 Å. A special mixing pipette for making blood dilutions of 1/500 and 1/250, and a conversion chart for translating millimeter readings into grams of hemoglobin are also supplied.

E. Swim-bladder gases.

Carbon dioxide and oxygen of the swim-bladder were determined with the large laboratory model of the Haldane gas analysis apparatus (Haldane, 1920 ed.) with some modifications as suggested by Carpenter (1915).

The gas samples were stored in tonometers under slight positive pressure until the analyses were run. All determinations on swim-bladder gases were made by Miss Lula Mae Shipe of the Department of Zoology.

PROCEDURE

The experiments were performed in groups of three. At least two hours, and usually much longer, before a series of experiments was begun the fish to be used were transferred from the small artificial pools at the rear of the Biology Building to a tank in the laboratory. The water in this tank was constantly aerated with a slow stream of compressed air and approximated room temperature.

In these experiments ten large glass bottles with a volume of about forty-eight liters each were used. The necks were cut off a few centimeters below the top so that they could be fitted with a number 15 rubber stopper. This gave an opening large enough to permit easily the admission of all fish. The total volume of each tank, minus the water displaced by its rubber stopper, was accurately determined.

Before a series of experiments was begun the tanks were thoroughly washed and rinsed several times. Tap water was allowed to run until its temperature had become relatively constant, after which the tanks were filled. The water in each tank was then aerated for thirty minutes, using a blower which drew air from the outside. Following aeration, the water in each tank was stirred for thirty minutes with a mechanical stirrer fitted to an electric motor. The same procedure was followed closely with each tank, which insured as nearly uniform conditions as possible as to gaseous content, pH, and temperature of the water.

Samples of water for oxygen, carbon dioxide, alkali reserve, and carbon dioxide tension determinations were taken from each tank. The oxygen of the samples was fixed at once; i.e., all reagents were added except concentrated sulfuric acid for iodine liberation. Carbon dioxide samples were stored under pressure and alkali reserve samples kept in Pyrex glass-stoppered bottles until they could be run. The pH's of two samples were determined colorimetrically, one sample being undisturbed and the other aerated. Two other samples were saved for comparison at the end. Depending upon the conditions of the experiment; fish were put in the tanks at once, or after known volumes of carbon dioxide or sodium hydroxide were added.

Tonometers, whose volumes had been determined gravimetrically with distilled water at 23° C., were used for the addition of carbon dioxide. These were first filled with water which was then allowed to flow out. This was to prevent variations in the amount of carbon dioxide added to the different tanks of one series of experiments due to vapor pressure effects. After the first tonometer of gas was added to the first tank this factor was necessarily introduced as the technique followed resulted in water being forced through the tonometer for absorption of the carbon dioxide. The tonometers were filled with carbon dioxide by displacement of mercury and the gas pressure brought to atmospheric pressure by opening one of the stop-cocks for a few seconds. The tonometer was then suspended in a tank of water, and water slowly forced through it with a rubber bulb fitted with valves so that the flow

was in one direction only. This was continued until a volume of water several times that of the tonometer had been forced through it. The tonometer was then taken almost completely from the water and the water in it allowed to run back into the tank.

The volume of carbon dioxide added was corrected to standard conditions after the vapor pressure corredtion was made. Variations in barometric pressure during the time required to add carbon dioxide to three tanks was negligible, as were temperature changes. The amount of gas added to each tank in each set of experiments was, therefore, assumed to be constant.

The first 15 experiments, 0 through 29, were performed with two fish in each tank; the rest with one fish in a tank. Neither carbon dioxide nor sodium hydroxide was added in experiments 0 through 5, 30 through 32, 42 through 44, and 51 through 53. Carbon dioxide in varying amounts from 27.8 ml. to 166.8 ml. (volumes uncorrected) was added to all other experiments exclusive of 72 through 94. The purpose of this was to increase the initial carbon dioxide tension of the water bathing the fish's gills. In other cases, in order to reduce the initial carbon dioxide tension of the water, and to alter its final tension, equal volumes of .098 N. NaOH were added to the three tanks of each experiment. The volumes varied from 10 ml. in experiments 72 through 74 to 60 ml. in experiments 87 through 89.

Immediately after adding gas or alkali to a tank a fish was put in and the tank tightly stoppered to prevent exchange of gases with the atmosphere by diffusion. The time was noted and an effort

made to obtain some idea of the physiological state of the fish as indicated by its respiratory rate. It was found that the respiratory rate increased as the death point approached, until a few hours (one to four) before it died the rate might be three or even four times as great as the normal rate. About an hour before the death point the fish would begin violent movements around the tank and the respiratory rate would rapidly drop. Through noting these points, an effort was made to remove all fish from the water as soon as their last respiratory movements had ceased but before the heart had stopped beating.

Upon the death of a fish (or upon the death of the first fish in experiments 0 through 29) oxygen, carbon dioxide, carbon dioxide tension, and alkali reserve samples were obtained from the water. These were treated as were the same samples at the beginning. In order to determine whether the pH change in the water was due solely to changes in carbon dioxide tension or to other substances (e.g., organic material) a sample of water saved from the beginning was aerated along with one procured at the end. Almost invariably there was found to be no difference in the pH of the two samples.

The fish was removed from the water and a sample of its blood obtained by introducing a needle into the conus arteriosus and withdrawing the blood into a small syringe. The syringe had been oxalated previously with 1% potassium oxalate to prevent clotting; oxalate solution was drawn into the syringe and as much of it as possible ejected. That remaining on the walls of the

syringe was allowed to evaporate. The blood so drawn was expelled into one of the cups of a porcelain outside-titrating plate. The blood was then drawn into the diluting pipette and diluted to a volume of 1/500 or 1/250 with 1% HCl. The acid hematin solution in the pipette was allowed to run into a clean test tube and stand for at least thirty minutes before the determination of hemoglobin was made.

The final operation was that of obtaining the gas of the swim-bladder. the scheme used consisted of two tonometers attached to the same ringstand and connected with each other by heavy pressure tubing. A needle was firmly fixed to the free outlet of the lower tonometer. During the actual operation of obtaining the gas the entire system was immersed in water to insure the admission of no air with the gas sample.

The lower tonometer was filled with mercury from the upper one, the final filling being done with the top stop-cock of the upper tonometer closed. This established a negative pressure in the system. When the needle of the lower tonometer was introduced into the swim-bladder, either from the front through the swim-bladder membrane after removal of the viscera, or through the side (which proved a more satisfactory method), and the stop-cock of the free end of the lower tonometer (now under water) opened, the gas rushed into the lower tonometer. The stop-cock was then closed and the upper tonometer raised as high as possible on the ringstand. The stop-cock of the free end of the upper tonometer was opened and a very definite positive pressure was

thus established on the trapped gas. The lower tonometer was cut off from the upper one by closing the stop-cock at the end connedted to the heavy pressure tubing. The tonometer was disconnected and set aside for gas analysis.

The fish was finally weighed and all data recorded.

DISCUSSION OF DATA

The results of the experiments performed are given in Tables I to V. Five tables have been prepared inasmuch as five variable factors have been considered, viz.: carbon dioxide tension and oxygen content of the experimental water at the time of the death of the fish, the per cent carbon dioxide and oxygen of the swim-bladder, and hemoglobin of the blood in grams per 100 ml.

Each variable factor appears as the base for one table; the data for it having been arranged according to increasing values, with all other data arranged in reference to the base. Figures have been prepared for all tables. Thus it is possible to compare easily any one factor with all other factors, both in tabulated form and graphically. The advantage of this arrangement is that an apparent relation between two factors can be tested by examining the figures for both. If a real correlation does exist, it will be shown by a similar trend in the points plotted along the ordinate in the figures for both factors. This is more fully explained by the discussion of the following paragraphs.

The data of the factor used as the base for any particular table is plotted along the abscissa; the four remaining variables are plotted along the ordinate. Thus Table I is arranged according to increasing values in carbon dioxide tensions of the water, as determined at the conclusion of the experiments; and in Figure I carbon dioxide is represented along the abscissa all other factors along the ordinate.

In the figures the points plotted represent the averages of all points which fall within a certain range along the abscissa. It will be seen upon inspection of the figures that this range has been kept as .5 of the abscissa unit in every case. Thus in Figure 1, all ordinate values for the points of each variable between carbon dioxide tensions of 0 to .5 mm., .5 to 1.0 mm., 1.0 to 1.5 mm., etc., have been averaged and these points only plotted. This has been done to simplify the figures, since the variables are represented by 80 to 94 points each; and to more clearly show the trends indicated in these experiments. The points plotted have been joined with solid or broken lines in different colors or in black. This has been noted on each figure. Beside each point plotted is a small figure. This indicates the number of points along the ordinate which were averaged to obtain the plotted point.

TABLE I.

This table is arranged on the basis of increase in carbon dioxide tension of the experimental water. Oxygen content of the water in milliliters per liter is given in the second column, hemoglobin in grams per 100 ml. of blood in the third, and per cent carbon dioxide and oxygen of the swim-bladder in the fourth and fifth columns. Blank spaces in this table, and in the others to follow, indicate failure to obtain blood or swim-bladder gas. In the case of hemoglobin estimations, 10 of the 94 fish used were lost. This was due chiefly to the fact that in these cases the fish had been dead several hours. Gas analyses were run on

samples from 81 fish, 13 being lost through faulty technique in obtaining the gas or in analyzing it, chiefly the former.

An examination of Figure 1 shows that with an increase in the carbon dioxide tension of the water there was: (1) a decrease in the amount of oxygen left in solution, (2) an increase in the carbon dioxide of the swim-bladder, (3) an increase in the hemoglobin, (4) and perhaps an indication that there is an increase in the oxygen of the swim-bladder. The figure also shows that the carbon dioxide tensions of the waters in these experiments cover a very narrow range, 1 to 6 mm. mercury. This corresponds to a carbon dioxide partial pressure range of .13 to .74 % of an atmosphere as compared with the normal carbon dioxide partial pressure of .035 % of an atmosphere.

Powers and Hickman (1932) found that the isoelectric point of the blood of the yellow cat, Letops olivaris (Rafinesque), was passed when the carbon dioxide content of the blood was increased from .2% to .6% of an atmosphere. Observations on the blood of the blue cat, Ictalurus punctatus (Rafinesque), and the German carp, Cyprinus carpio Linnaeus, indicate that the per cent carbon dioxide of the blood increases with an increase in carbon dioxide tension of the water (Powers, Hopkins and Hickman, 1932). With the carbon dioxide tensions met with in these experiments we are dealing with fish whose blood is close to or slightly on the acid side of the isoelectric point. This is of particular interest when the curve for oxygen left in solution at the time of the fish's death is examined, Figure 1. These results show that as the carbon dioxide tension of the water increases the

amount of oxygen extracted from the water becomes greater, i.e., the fish could more readily obtain oxygen at the high carbon dioxide tensions than at the low tensions. Hickman (1929) has shown that as the carbon dioxide tension of the water increases the amount of oxygen extracted becomes less, i.e., the fish could more readily obtain oxygen at low carbon dioxide tensions. The apparent difference is understandable when the conditions of the two series of experiments are compared.

The carbon dioxide tensions Hickman employed were considerably higher than those met with in these experiments; thus perhaps the hemoglobin of the fish she was working with was actually broken down into its intermediate products and the oxygen capacity of the blood reduced. On the other hand, in these experiments the increase in carbon dioxide was of such a degree that apparently the hemoglobin was carried a small way on the acid side of the isoelectric point. Under these conditions the blood increased its oxygen carring capacity, which it does not begin to lose again with increases in carbon dioxide tension until the tension is about 1.4% of an atmosphere (Powers and Hickman, 1932).

The increase in carbon dioxide of the swim-bladder which is evident in Figure 1 is doubtless due to the increase in carbon dioxide tension of the water, and therefore of the blood. Since the diffusion of gases into the swim-bladder of the fish is governed by the same physical laws which govern the diffusion of gases through any permeable membrane, it follows that as the carbon dioxide tension of the water, and hence of the blood, is increased,

there would be more carbon dioxide deposited in the swim-bladder.

The curve for hemoglobin shows a marked trend upward as the carbon dioxide tension of the water is increased. It has been shown (Powers and Shipe, 1932) that the red corpuscles of the blue cat, Ictalurus punctatus (Rafinesque), increase with an increase in the carbon dioxide tension of the water. What the stimulus is that calls forth an increase in the red blood cells or the hemoglobin of fish blood with an increase in the carbon dioxide of the water remains to be determined.

From an examination of Figure 1 alone it is evident that there is a tendency toward an increase in oxygen in the swim-bladder with an increase in carbon dioxide tension. This is not associated with the oxygen tension of the water, which decreases with an increase in carbon dioxide tension. (This will be clearer after an examination of Figure 2, based on oxygen increase in the water). Neither is the increase related to that of the carbon dioxide of the swim-bladder, which has been discussed. Evidently the increase in swim-bladder oxygen is due to the increase in carbon dioxide tension of the blood, because of the higher tensions of carbon dioxide in the water, or to the upward trend of the hemoglobin curve. None of the other tables nor figures, particularly Figure 5 (based on increase in hemoglobin) support the latter view. The explanation possibly lies in the shift to the right that occurs in all oxygen dissociation curves upon being exposed to higher carbon dioxide tensions at the same pressure of oxygen. This, however, does not appear to apply to the experiments under consideration for it seems that the

increase in carbon dioxide here is of such a degree that the blood would more tenaciously hold its oxygen, as has already been shown,(Page 19). The question of increased oxygen deposition in the swim-bladder at higher carbon dioxide tensions is discussed more fully elsewhere in this thesis,(Page 32).

TABLE II.

The same general arrangement is followed in this table as in the first except that the first and second columns have been reversed in position, and the table is arranged according to increase in oxygen left in solution when the fish died. Figure 2 is a graphic representation of this table, with the data averaged as has already been described.

The same relation noted under Table I between carbon dioxide tension of the water and oxygen content is evident in Figure 2; i.e., as the oxygen left in water increases, there is a decrease in the carbon dioxide tension of the water. This further supports the position that the increase in oxygen in solution is due to the inability of the blood to load fully as the isoelectric point is neared.

The relation between oxygen of the water and carbon dioxide tension is closely followed by per cent carbon dioxide in the swim-bladder. That is, with an increase in oxygen remaining in solution the carbon dioxide tension of the water decreases and so does the carbon dioxide of the swim-bladder. This again points to the conclusion reached in regard to this point under Table I, (Page 19).

Apparently no relation exists between oxygen of the swim-bladder and the oxygen of the water. Barcroft's (1928) oxygen dissociation curves of human blood exposed to varying carbon dioxide tensions show that, even with relatively high carbon dioxide tensions such as 20 mm., hemoglobin is 50% saturated at an oxygen partial pressure of 20 mm. and is 85% saturated at 40 mm. Powers and Hickman (1932) showed that at a carbon dioxide tension of 2% of an atmosphere (over twice that met with in any of these experiments) the blood of the yellow cat, Leptops olivaris (Rafinesque) is very nearly saturated with oxygen at 20 to 30 mm. oxygen pressure. The inference from these curves is that with blood normally very nearly saturated even at relatively low oxygen pressures, the tension of the gas in the blood, and hence its tendency to diffuse into the swim-bladder, will be more markedly affected by other factors (such as carbon dioxide tension or nature of the hemoglobin) than by the oxygen tension of the water.

Oxygen tension of the water does not have much effect on the hemoglobin of the blood of the blue bream, Lepomis pallidus, (Mitchill), an examination of Figure 2 will show. A slight tendency toward a decrease in hemoglobin with an increase in oxygen in solution is possibly evident. Such a relation would be in agreement with observations made on the effect of oxygen tension on the red corpuscles of the blue cat, Ictalurus punctatus, (Rafinesque), (Powers and Shipe, 1932).

TABLE III.

The arrangement of data in Table III is with reference to the increase in carbon dioxide of the swim-bladder. In the second and third columns of the table are listed per cent oxygen of the swim-bladder and carbon dioxide of the water. Oxygen content of the water and hemoglobin appear in the last two columns.

The relation between carbon dioxide tension of the water and the carbon dioxide content of the swim-bladder which was noted under Table I is evident here. That is, an increase in per cent carbon dioxide of the swim-bladder shows a definite increase in the carbon dioxide tension of the water. As was pointed out under Table I, it is the carbon dioxide content of the swim-bladder which follows the carbon dioxide tension of the water, because of the resulting changes in the carbon dioxide tension of the blood. However, the data of Table III, as presented graphically in Figure 3, lend other support to this position since they show that increasing carbon dioxide contents of the swim-bladder are directly associated with higher carbon dioxide tensions of the water in all cases except two. These two points are the last on the graph for carbon dioxide tension and represent only three actual experiments.

Similarly the relation pointed out between oxygen in the water and carbon dioxide of the swim-bladder, Figure 2, is further supported here, viz., that an increase in carbon dioxide of the swim-bladder is related to a decrease in oxygen of the water. These factors are not, however, correlated in themselves but appear to show a correlation since they are tied together by the factor of

carbon dioxide tension of the water. This directly bears upon the carbon dioxide of the blood. That is, an increase in carbon dioxide content of the swim-bladder may be looked for with an increase in carbon dioxide tension of the water; and this, bringing about a decrease in oxygen of the water at the end of the experiment apparently shows a decrease in oxygen of the water with an increase in swim-bladder carbon dioxide.

Inspection of Figure 3 shows a general upward trend in hemoglobin with an increase in carbon dioxide of the swim-bladder. This, as with the case of oxygen in the water, is not directly correlated with the carbon dioxide of the swim-bladder, but with the carbon dioxide tension of the water, and therefore the carbon dioxide tension of the blood. Apparently a fall in oxygen of the swim-bladder follows to some extent an increase in the carbon dioxide of the swim-bladder.

TABLE IV.

Table IV is arranged on the basis of increasing oxygen of the swim-bladder. Carbon dioxide of the swim-bladder appears in the second column, the rest of the arrangement being as in Table III. Figure 4 is a graphic representation of this table.

No distinct relations between oxygen of the swim-bladder and the other variables are evident in Figure 4, though an upward trend in carbon dioxide tension of the experimental water is indicated as the oxygen of the swim-bladder increases.

TABLE V.

The data of Table V are arranged according to increase in hemoglobin of the blood. Columns two and three give data on carbon dioxide tension and oxygen content of the water, with carbon dioxide and oxygen of the swim-bladder following in the last two columns in order.

A definite increase in carbon dioxide tension of the water with increase in hemoglobin is evident from Figure 5. This, however, as has been pointed out is because of the effect of carbon dioxide tension of the water on the carbon dioxide tension of the blood.

It may be seen from Figure 5 that no relation can be demonstrated between the oxygen content of the water and an increase in hemoglobin of the blood. An upward trend in carbon dioxide of the swim-bladder follows the increase in carbon dioxide tension of the water, and hence appears to follow the increase in hemoglobin.

A definite decline in the oxygen of the swim-bladder follows the increase in hemoglobin plotted as the base of Figure 5. This has been referred to under Table I. Both oxygen of the swim-bladder and hemoglobin appear to increase with an increase in the carbon dioxide tension of the water. In the discussion of Figure 4 it was pointed out that an increase in oxygen of the swim-bladder shows no corresponding increase in hemoglobin. It is evident, therefore, that hemoglobin and oxygen of the swim-bladder are not correlated, but that wherever they appear to be so it is because a third factor ties them together. This probably is carbon dioxide tension of the

blood.

Certain other data on the composition of the swim-bladder gas in normal fish as compared with the gas of the fish used in these experiments have not been presented in table form. Analyses on the gas of ten normal fish, that is, fish which were taken directly from the artificial pool at the Biology Building showed an average carbon dioxide content of 2.02% and an average oxygen content of 7.70%. Averages on the composition of the swim-bladder gas in 81 experimental fish show a carbon dioxide content of 2.07% and an oxygen content of 4.82%. Apparently the oxygen content is subject to greater variation under variations in the carbon dioxide tension of the water than is the carbon dioxide content of the swim-bladder.

CONCLUSIONS

The essential difference in the respiratory mechanism of gill breathers and lung breathers is that the former have an open system, whereas the lung breathers have more or less a closed system. Apparently there is no means whereby gill breathers may alter the gas tensions to which their gills are subjected (Hall, 1931). For this reason it would be expected that variations in the gaseous content of the water bathing the gills of an aquatic form would be reflected in pronounced physiological adjustments on the part of such an animal. Similar variations would be less likely to affect lung breathing animals, since their respiratory mechanism is such that the composition of the alveolar air can be maintained more nearly constant.

In lung breathing animals the alveolar carbon dioxide partial pressure is determined by the rate of carbon dioxide production and the depth and rate of inspiration; and is independent of the barometric pressure of carbon dioxide, since the air is essentially a vacuum for this gas. This is of prime importance in a consideration of mountain sickness among lung breathing forms. Thus Barcroft has shown (1928) that acapnia is not the cause of the deficiency of carbon dioxide nor the increased alkalinity of the blood met with at high altitudes.

The pH of the blood of a given individual appears to be very constant, although in different individuals the variation may be from 7.25 to 7.45 (Van Slyke, 1921). The consistency of this reaction is due to the ability of the blood as a physiological-physico-chemical

system to maintain the proper $H_2CO_3/BHCO_3$ ratio. At the pH of the blood this ratio in a solution buffered with sodium bicarbonate is 1/20. At the carbon dioxide partial pressure of alveolar air, 40 mm., 3 volumes per cent of carbon dioxide will be dissolved. Thus to maintain the proper pH of 7.35, the ratio of acid to salt will be 3/60.

It is now generally recognized that the essential cause of mountain sickness is a diminution in the oxygen in the air inspired (Haldane, 1927). With a decrease in the alveolar oxygen partial pressure the alkali reserve of the blood is too high, as compared to the pressure of oxygen. Respiration is augmented and carbon dioxide blown off to excess. To maintain the proper acid-alkali ratio under this condition the amount of alkali in the blood must be diminished, and this accordingly happens with a passage of alkali out of the blood. The factor through which oxygen and alkali interact is unknown, and at one time the term "respiratory X" was applied to it by Yandell Henderson. He has since stated, though, that the use of,"...such a term as 'respiratory X' is merely a confession of ignorance"(1925). The essential point of interest in this discussion is that a lowered oxygen partial pressure induces a lowering in the alkali of the blood in lung breathing forms. There is no reason to deny that such is probably true of fish.

When applying principles which have been accepted in regard to human blood to other bloods, it must be kept in mind that the hemoglobins of different forms of life show marked differences in their dissociation curves over the same ranges of oxygen partial pressure (Barcroft, 1928, and Powers and Hickman, 1932). Figures on the blood of the scup given Barcroft by Hall and Gray (Barcroft, 1934),"...showed a

variation in the hydrogen ion concentration of the blood between cH of $3x10^{-8}$ and of $2.5x10^{-7}$, i.e., a variation not of 22 per cent but of 800 per cent." This variation, expressed in terms of pH, means a normal range of 6.60 to 7.52, as compared with a range of 7.25 to 7.45 in man.

In these experiments fish have been exposed to various carbon dioxide tensions for periods ranging from 48 to 72 hours on the average. The highest tensions met with have been slightly less than 6 mm. mercury, or about .7% of an atmosphere; while the lowest tensions at the end of the experiments have been about 1.3 mm. mercury, or .2% of an atmosphere. Throughout the period of each experiment the oxygen content of the water constantly decreased, and at the end was reduced to .4 to .5 ml. per liter on the average.

It has been shown that the carbon dioxide tension of the water regulates the carbon dioxide content of the blood of the blue cat, Ictalurus punctatus, and the German carp, Cyprinus carpio (Powers, Hopkins, and Hickman, 1932). The exact relation existing between carbon dioxide content of the blood and carbon dioxide tension is not known, either for blood or any other buffered solution. Presumably there is an increase in the tension with an increase in the total carbon dioxide content. Enough is known, though, to justify the general conclusion that with an increase in the carbon dioxide tension of the water there will be an increase in the carbon dioxide tension of the blood of the fish.

With an increase in the carbon dioxide tension of the experimental water it is apparent that there is an increase in the hemoglobin of the blood. This is in agreement with the findings of Powers and Shipe on the blood of the blue cat. What the stimulus is in this case is

not known, though it may be suggested that the increased carbon dioxide tension of the blood acts as a chemical stimulus to the endothelial cells of the red marrow. It is suggested by Evans (Starling's Physiology, 1933) that the stimulus normally is a chemical one emanating from the liver. On the other hand, the immediate increase in hemoglobin might be due to other factors than an increased production. It is well known that at high altitudes there occurs an immediate increase in the red corpuscles through contraction of the spleen. It has also been shown by Barcroft (1928) that a deficiency of hemoglobin or of oxygen causes the spleen to contract, thus increasing the number of erythrocytes in the blood.

With an increase in the carbon dioxide tension the ability of the fish to extract oxygen was apparently increased. This is not what one would have expected on the basis of knowledge of the physiology of respiration in general. Hickman (1929) found that the ability of goldfish to obtain oxygen decreases as the carbon dioxide tension of the water increases. However, the tensions employed by Hickman were several times greater than those met with in these experiments. At the carbon dioxide tensions met with in this work the blood was doubtless close to the isoelectric point, but slightly on the acid side. As the carbon dioxide tension of the water increased, the carbon dioxide content of the blood would increase (Powers, Hopkins, and Hickman, 1932). However, in a buffered solution the acidity would not increase with an increase in carbon dioxide if more alkali reserve were available; so that a shift to the acid side of the isoelectric point cannot be explained purely on the basis of increased carbon dioxide tension of the experimental water. The

question thus arises as to what other factors might play a part in this increase in the acidity of the blood. A possible explanation is to be found in the decreasing oxygen tension of the water. It is known that with a decrease in oxygen tension the alkali reserve of the blood of man decreases (Y. Henderson, 1925): should a similar change occur in fish blood, with a decreasing oxygen tension the increasing carbon dioxide tensions of the water would cause a rise in hydrogen ion concentration of the blood which would not be compensated for by the buffer action of additional alkali. Thus the pH of the blood would be shifted to the acid side.

It has been pointed out by Clark (1925) that, "... the isoelectric point of an amphoteric electrolyte is a point at or near which there should tend to occur maximal or minimal properties of its solution." Therefore, on either side of the isoelectric point the capacity of the blood to load with oxygen would be increased. It appears that on the acid side this effect is soon overcome by the increasing acidity of the blood as slightly higher tensions of carbon dioxide are met with, thus preventing the normal loading of oxygen. That is, with higher carbon dioxide tensions of the water the actual loading of hemoglobin is prevented by diffusion of carbon dioxide from the water into the blood, rather than in the reverse direction as it should. Thus, if the blood in the gills is subjected to higher carbon dioxide than in the tissues, the blood would not load but would tend to unload oxygen. Apparently in these experiments we are dealing with fish whose blood is increasing in oxygen carrying capacity with slight increases in carbon dioxide tension. This would account for the fact that as far as these

experiments were carried, and increased carbon dioxide tension of the water increased the ability of the fish to extract oxygen. Had the tensions been greatly increased, it would have been expected that the ability of the fish to obtain oxygen would have been reduced as was found by Hickman (1929).

The increased carbon dioxide tension of the water would be expected to increase the carbon dioxide tension of the blood as has been pointed out. Thus the diffusion pressure of carbon dioxide would be increased; and it would be expected, as was found in these experiments, that the carbon dioxide of the swim-bladder would increase.

Oxygen is deposited into the swim-bladder of the fish by the mechanism of the rete mirabile as described by Powers (1932). With an increase in the carbon dioxide tension of the blood the carbon dioxide tension of the gas gland would be increased. As this tension in the rete mirabile increases it tends to drive oxygen out of solution. Furthermore, in these experiments the carbon dioxide tension increases in the blood were of such a definitely small degree that the efficiency of the hemoglobin to load oxygen was increased. In this manner the actual oxygen content of the blood probably was greater. As the blood reaches the rete mirabile the exchange of gases would take place as described by Powers (1932), with the result that the oxygen tension would be still more increased. Thus it seems that all factors herein considered would tend to increase oxygen deposition in the swim-bladder.

No particular relation exists between oxygen of the water and oxygen of the swim-bladder. This is in agreement with the facts known in regard to the oxygen dissociation curves for human hemoglobin

(Barcroft, 1928) and the dissociation curve for the blood of the yellow cat (Powers and Hickman, 1932). The curves given by Powers and Hickman show that the hemoglobin is very nearly saturated at low oxygen tensions, even in the presence of carbon dioxide tensions much higher than normal. The mechanism of oxygen deposition into the swim-bladder does not depend upon a blood that is saturated, since the oxygen tension can be and is raised by the exchange of carbon dioxide between the gas gland capillaries; and therefore it would be expected that even with a blood not fully saturated a continuous deposition of oxygen would take place.



SUMMARY

1. The hemoglobin of the blood of the blue bream, Lepomis pallidus (Mitchill), increases as the carbon dioxide tension of the water increases.

2. The experiments described do not indicate any relation between oxygen content of the water and hemoglobin content of the blood.

3. When exposed to increasing carbon dioxide tensions ranging from .17 to .74% of an atmosphere the ability of the blue bream to extract oxygen increases. It is suggested that this is due to changes which occur around the isoelectric point of fish blood, and is directly associated with the ability of the hemoglobin to load oxygen.

4. The per cent oxygen of the swim-bladder increases with an increase in the carbon dioxide tension of the water.

5. The per cent oxygen of the swim-bladder is not related to the oxygen content of the water at the end of the experiments.

6. The per cent carbon dioxide of the swim-bladder increases as the carbon dioxide tension of the water increases.

ACKNOWLEDGEMENTS

I wish to thank Professor E. B. Powers, Head of the Department of Zoology of the University of Tennessee, for assistance and suggestions given during the course of this work; and the Department of Zoology for the laboratory facilities which have been placed at my disposal.

Miss Lula Mae Shipe of the Department of Zoology performed all gas analyses, and for these I am indebted to her.

Fish for the experimental work were provided by the State Game Department. My sincere thanks go to Mr. Gordon Powers of the Game Department for his many courtesies.

TABLE NO. I. DATA ARRANGED ACCORDING TO INCREASE IN CO_2 TENSION OF WATER.

Exp.	CO_2 Tension H_2O, mm. Hg.	O_2 Water; ml. per L.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
87	.254	4.528	5.18	1.00	2.49
89	1.306	.686	8.04	.76	2.08
88	1.370	.551	7.38	1.24	3.81
82	1.435	.753	8.43	.92	1.15
84	1.504	.558	10.23	2.65	1.10
85	1.576	.406	8.78	3.14	1.04
76	1.576	.271	10.15	.56	.60
81	1.576	.737	9.46	--	--
(16	1.651	.473	5.35	1.83	3.73
(17	1.651	.473	6.64	--	--
90	1.651	.542	7.14	2.95	1.31
93	1.691	.609	6.89	1.17	2.93
(0	1.731	.136	6.94	--	--
(1	1.731	.136	8.60	--	--
(2	1.731	.252	--	1.40	6.27
(3	1.731	.252	5.82	--	--
39	1.731	4.648	11.94	3.35	2.32
(12	1.772	.231	5.07	--	--
(13	1.772	.231	5.74	4.70	2.88
83	1.814	.548	9.02	--	--

TABLE NO. I. (CONTINUED)

Exp.	CO_2 Tension H_2O, mm. Hg.	O_2 Water; ml. per L.	Hemoglobin, grs. per 100 cc. blood	Swim-bladder gases % CO_2	% O_2
(4	1.901	.113	11.82	1.62	4.74
((5	1.901	.113	8.05	2.86	1.47
75	1.945	.582	9.60	1.02	1.68
(28	2.039	.510	--	1.75	4.97
((29	2.039	.510	3.99	2.00	4.65
86	2.039	.429	11.48	--	--
91	2.087	.458	9.36	.77	2.98
(24	2.136	.529	7.01	1.17	13.63
((25	2.136	.529	6.08	1.87	11.00
92	2.136	.482	5.26	.63	2.63
77	2.292	.463	6.82	.42	5.93
78	2.346	.445	10.36	3.00	1.88
(20	2.458	.438	6.20	0.00	.91
((21	2.458	.438	6.01	1.84	11.24
72	2.458	.810	9.50	1.31	6.12
73	2.576	.583	11.76	.59	3.65
(14	2.576	.360	6.34	1.41	13.80
((15	2.576	.360	5.24	2.12	13.75
33	2.576	.565	11.27	1.40	1.21
74	2.637	.396	13.33	--	--

TABLE NO. I. (CONTINUED)

Exp.	CO_2 Tension H_2O, mm. Hg.	O_2 Water; ml. per L.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
80	2.637	.581	10.20	1.20	3.02
34	2.763	1.113	6.01	1.24	5.94
51	2.764	.224	--	3.37	4.45
(18	2.828	.271	7.83	0.00	.13
(19	2.828	.271	4.18	2.46	.12
(22	2.828	.431	6.77	1.54	3.46
(23	2.828	.431	7.75	2.74	9.02
30	2.896	.360	9.08	2.86	3.30
31	2.896	.268	8.89	1.27	3.38
32	2.965	.297	7.52	1.16	3.04
35	3.036	.513	10.52	--	--
52	3.108	.414	11.57	2.88	3.73
54	3.108	.396	11.38	1.38	8.55
56	3.108	.360	--	1.86	2.71
79	3.107	.346	10.20	2.89	1.07
(26	3.201	.711	6.60	2.38	7.66
(27	3.201	.711	5.85	--	--
44	3.333	.338	10.90	1.00	2.00
45	3.493	.439	12.07	1.95	4.35
42	3.493	.269	9.84	--	--

TABLE NO. I. (CONTINUED)

Exp.	CO_2 Tension H_2O, mm. Hg.	O_2 Water; ml. per L.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
53	3.494	.408	9.51	1.16	6.70
59	3.494	.324	8.04	3.10	3.30
(10	3.579	.141	6.51	3.62	2.01
(11	3.579	.141	8.05	2.01	5.37
37	3.663	.300	8.02	4.17	3.44
41	3.663	.497	12.23	1.38	7.10
(6	3.747	.235	5.28	.81	13.37
(7	3.747	.235	8.84	3.00	7.43
(8	3.747	.168	8.90	1.70	--
(9	3.747	.168	8.31	1.02	12.31
55	3.748	.523	12.23	2.31	19.80
58	3.748	.404	10.52	1.56	.37
36	3.836	.256	8.78	1.51	2.10
46	3.836	.313	11.88	1.30	3.96
38	3.926	.506	10.11	.50	2.82
40	3.926	.299	6.61	.86	1.72
47	3.926	.345	12.41	13.22	2.06
49	3.926	.403	8.50	2.77	7.24
60	3.928	.407	--	2.90	2.39
43	4.020	.330	8.72	1.12	2.79

TABLE NO. I. (CONTINUED)

Exp.	CO_2 Tension H_2O, mm. Hg.	O_2 Water; ml. per L.	Hemoglobin, grs. per 100 cc. blood	Swim-bladder gases % CO_2	% O_2
57	4.022	.224	---	3.56	2.43
48	4.217	.279	11.16	3.20	8.99
50	4.414	.326	6.68	1.28	4.81
61	4.416	.382	13.20	1.94	4.27
64	4.416	.243	11.71	4.37	4.58
66	4.416	.283	8.42	1.01	12.01
68	4.416	.390	---	3.23	4.85
65	4.627	.277	12.05	1.90	6.43
63	4.627	.400	11.57	1.59	5.89
71	4.737	.427	---	3.88	2.86
70	4.964	.427	8.15	2.60	5.92
69	5.326	.325	---	3.85	4.08
62	5.579	.409	9.71	2.81	2.97
67	5.579	.387	10.00	.97	10.35

TABLE NO. II. DATA ARRANGED ACCORDING TO INCREASE IN OXYGEN CONTENT OF WATER

Exp.	O_2 Water; ml. per L.	CO_2 Tension H_2O, mm. Hg.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
(4	.113	1.901	11.82	1.62	4.74
(5	.113	1.901	8.05	2.86	1.47
(0	.136	1.731	6.94	---	---
(1	.136	1.731	8.60	---	---
(10	.141	3.579	6.51	3.62	2.01
(11	.141	3.579	8.05	2.01	5.37
(8	.168	3.747	8.90	1.70	---
(9	.168	3.747	8.31	1.02	12.31
51	.224	2.764	---	3.37	4.45
57	.224	4.022	---	3.56	2.43
(12	.231	1.772	5.07	---	---
(13	.231	1.772	5.74	4.70	2.88
(6	.235	3.747	5.28	.81	13.37
(7	.235	3.747	8.84	3.00	7.43
64	.243	4.416	11.71	4.37	4.58
(2	.252	1.731	---	1.40	6.27
(3	.252	1.731	5.82	---	---
36	.256	3.836	8.78	1.51	2.10
31	.268	2.896	8.89	1.27	3.38
42	.269	3.493	9.84	---	---

TABLE NO. II. (CONTINUED)

Exp.	O_2 Water; ml. per L.	CO_2 Tension H_2O, mm. Hg.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
76	.271	1.576	10.15	.56	.60
(18	.271	2.828	7.83	0	.13
(19	.271	2.828	4.18	2.46	.12
65	.277	4.627	12.05	1.90	6.43
48	.279	4.217	11.16	3.20	8.99
66	.283	4.416	8.42	1.01	12.01
32	.297	2.965	7.52	1.16	3.04
40	.299	3.926	6.61	.86	1.72
37	.300	3.663	8.02	4.17	3.44
46	.313	3.836	11.88	1.30	3.96
59	.324	3.494	8.04	3.10	3.30
69	.325	5.326	---	3.85	4.08
50	.326	4.414	6.68	1.28	4.81
43	.330	4.020	8.72	1.12	2.79
44	.338	3.333	10.90	1.0	2.00
47	.345	3.926	12.41	13.22	2.06
79	.346	3.107	10.20	2.89	1.07
(14	.360	2.576	6.34	1.41	13.80
(15	.360	2.576	5.24	2.12	1.21
30	.360	2.896	9.08	2.86	3.30

TABLE NO. II. (CONTINUED)

Exp.	O_2 Water ml. per L.	CO_2 Tension H_2O, mm. Hg.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
56	.360	3.108	---	1.86	2.71
61	.362	4.416	13.20	1.94	4.27
67	.387	5.579	10.00	.97	10.35
68	.390	4.416	---	3.23	4.85
54	.396	3.108	11.38	1.38	8.55
74	.396	2.637	13.33	---	---
63	.400	4.627	11.57	1.59	5.89
49	.403	3.926	8.50	2.77	7.24
58	.404	3.748	10.52	1.56	.37
85	.406	1.576	8.78	3.14	1.04
60	.407	3.928	---	2.90	2.39
53	.408	3.494	9.51	1.16	6.70
62	.409	5.579	9.71	2.81	2.97
52	.414	3.108	11.57	2.88	3.73
70	.427	4.964	8.15	2.60	5.92
86	.429	2.039	11.48	---	---
(22	.431	2.828	6.77	1.54	3.46
(23	.431	2.828	7.75	2.74	9.02
(20	.438	2.458	6.20	.00	..91
(21	.438	2.458	6.01	1.84	11.24

TABLE NO. II. (CONTINUED)

Exp.	O_2 Water ml. per L.	CO_2 Tension H_2O, mm. Hg.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
45	.439	3.493	12.07	1.95	4.35
78	.445	2.346	10.36	3.00	1.88
91	.458	2.087	9.36	.77	2.98
77	.463	2.292	6.82	.42	5.93
(16	.473	1.651	5.35	1.83	3.73
(17	.473	1.651	6.64	---	---
92	.482	2.136	5.26	.63	2.63
41	.497	3.663	12.23	1.38	7.10
38	.506	3.926	10.11	.50	2.82
(28	.510	2.039	---	1.75	4.97
(29	.510	2.039	3.99	2.00	4.65
35	.513	3.036	10.52	---	---
55	.523	3.748	12.23	2.31	19.80
(24	.529	2.136	7.01	1.17	13.63
(25	.529	2.136	6.08	1.87	11.00
90	.542	1.651	7.14	2.95	1.31
83	.548	1.814	9.02	---	---
88	.551	1.370	7.38	1.24	3.81
84	.558	1.504	10.23	2.65	1.10
33	.565	2.576	11.27	1.40	1.21

TABLE NO. II. (CONTINUED)

Exp.	O_2 Water ml. per L.	CO_2 Tension H_2O, mm. Hg.	Hemoglobin, grs. per 100 cc.blood	Swim-bladder gases % CO_2	% O_2
80	.581	2.637	10.20	1.20	3.02
75	.582	1.945	9.60	1.02	1.68
73	.583	2.576	11.76	.59	3.65
93	.609	1.691	6.89	1.17	2.93
89	.686	1.306	8.04	.76	2.08
(26	.711	3.201	6.60	2.38	7.66
(27	.711	3.201	5.85	---	---
81	.737	1.576	9.46	---	---
82	.753	1.435	8.43	.92	1.15
72	.810	2.458	9.50	1.31	6.12
34	1.113	2.763	6.01	1.24	5.94
87	4.528	.254	5.18	1.00	2.49
39	4.648	1.731	11.94	3.35	2.32

TABLE NO. III. DATA ARRANGED ACCORDING TO PERCENT INCREASE IN CARBON DIOXIDE OF SWIM-BLADDER

Exp.	Swim-bladder gases % CO_2	% O_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc. blood
20	00	.91	2.458	.438	6.20
18	00	.13	2.828	.271	7.83
77	.42	5.93	2.292	.463	6.82
38	.50	2.82	3.926	.506	10.11
76	.56	.60	1.576	.271	10.15
73	.59	3.65	2.576	.583	11.76
92	.63	2.63	2.136	.482	5.26
89	.76	2.08	1.306	.686	8.04
91	.77	2.98	2.087	.458	9.36
6	.81	13.37	3.747	.235	5.28
40	.86	1.72	3.926	.299	6.61
82	.92	1.15	1.435	.753	8.43
67	.97	10.35	5.579	.387	10.00
44	1.00	2.00	3.333	.338	10.90
87	1.00	2.49	.254	4.528	5.18
66	1.01	12.01	4.416	.283	8.42
9	1.02	12.31	3.747	.168	8.31
75	1.02	1.68	1.945	.582	9.60
43	1.12	2.79	4.020	.330	8.72
32	1.16	3.04	2.965	.297	7.52
53	1.16	6.70	3.494	.408	9.51

TABLE NO. III. (CONTINUED)

Exp.	Swim-bladder gases % CO_2	% O_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc. blood
24	1.17	13.63	2.136	.529	7.01
93	1.17	2.93	1.691	.609	6.89
80	1.20	3.02	2.637	.581	10.20
34	1.24	5.94	2.763	1.113	6.01
88	1.24	3.81	1.370	.551	7.38
31	1.27	3.38	2.896	.268	8.89
50	1.28	4.81	4.414	.326	6.68
46	1.30	3.96	3.836	.313	11.88
72	1.31	6.12	2.458	.810	9.50
41	1.38	7.10	3.663	.497	12.23
54	1.38	8.55	3.108	.396	11.38
2	1.40	6.27	1.731	.252	---
33	1.40	1.21	2.576	.565	11.72
14	1.41	13.80	2.576	.360	6.34
36	1.51	2.10	3.836	.256	8.78
22	1.54	3.46	2.828	.431	6.77
58	1.56	.37	3.748	.404	10.52
63	1.59	5.89	4.627	.400	11.57
4	1.62	4.74	1.901	.113	11.82
8	1.70	---	3.747	.168	8.90

TABLE NO. III. (CONTINUED)

Exp.	Swim-bladder gases % CO_2	% O_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc. blood
28	1.75	4.97	2.039	.510	---
16	1.83	3.73	1.651	.473	5.35
21	1.84	11.24	2.458	.438	6.01
56	1.86	2.71	3.108	.360	---
25	1.87	11.00	2.136	.529	6.08
65	1.90	6.43	4.627	.277	12.05
61	1.94	4.27	4.416	.362	13.20
45	1.95	4.35	3.493	.439	12.07
29	2.00	4.65	2.039	.510	3.99
11	2.01	5.37	3.579	.141	8.05
15	2.12	13.75	2.576	.360	5.24
55	2.31	19.80	3.748	.523	12.23
26	2.38	7.66	3.201	.711	6.60
19	2.46	.12	2.828	.271	4.18
70	2.60	5.92	4.964	.427	8.15
84	2.65	1.10	1.504	.558	10.23
23	2.74	9.02	2.828	.431	7.75
49	2.77	7.24	3.926	.403	8.50
62	2.81	2.97	5.579	.409	9.71
5	2.86	1.47	1.901	.113	8.05
30	2.86	3.30	2.896	.360	9.08

TABLE NO. III. (CONTINUED)

Exp.	Swim-bladder gases % CO_2	% O_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc.blood
52	2.88	3.73	3.108	.414	11.57
79	2.89	1.07	3.107	.346	10.20
60	2.90	2.39	3.928	.407	---
90	2.95	1.31	1.651	.542	7.14
7	3.00	7.43	3.747	.235	8.84
78	3.00	1.88	2.346	.445	10.36
59	3.10	3.30	3.494	.324	8.04
85	3.14	1.04	1.576	.406	8.78
48	3.20	8.99	4.217	.279	11.16
68	3.23	4.85	4.416	.390	---
39	3.35	2.32	1.731	4.648	11.94
51	3.37	4.45	2.764	.224	---
57	3.56	2.43	4.022	.224	---
10	3.62	2.01	3.579	.141	6.51
69	3.85	4.08	5.326	.325	---
71	3.88	2.86	4.737	.427	---
37	4.17	3.44	3.663	.300	8.02
64	4.37	4.58	4.416	.243	11.71
13	4.70	2.88	1.772	.231	5.74
47	13.22	2.06	3.926	.345	12.41

TABLE NO. IV. DATA ARRANGED ACCORDING TO PERCENT INCREASE IN OXYGEN IN SWIM-BLADDER

Exp.	Swim-bladder gases % O_2	% CO_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc.blood
19	.12	2.46	2.828	.271	4.18
18	.13	00	2.828	.271	7.83
58	.37	1.56	3.748	.404	10.52
76	.60	.56	1.576	.271	10.15
20	.91	00	2.458	.438	6.20
85	1.04	3.14	1.576	.406	8.78
79	1.07	2.89	3.107	.346	10.20
84	1.10	2.65	1.504	.558	10.23
82	1.15	.92	1.435	.753	8.42
33	1.21	1.40	2.576	.565	11.27
90	1.31	2.95	1.651	.542	7.14
5	1.47	2.86	1.901	.113	8.05
75	1.68	1.02	1.945	.582	9.60
40	1.72	.86	3.926	.299	6.61
78	1.88	3.00	2.346	.445	10.36
44	2.00	1.0	3.333	.338	10.90
10	2.01	3.62	3.579	.141	6.51
47	2.06	13.22	3.926	.345	12.41
89	2.08	.76	1.306	.686	8.04
36	2.10	1.51	3.836	.256	8.78
60	2.39	2.90	3.928	.407	---

TABLE NO. IV. (CONTINUED)

Exp.	Swim-bladder gases % O_2	% CO_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc. blood
39	2.32	3.35	1.731	4.648	11.94
57	2.43	3.56	4.022	.224	---
87	2.49	1.00	.254	4.528	5.18
92	2.63	.63	2.136	.482	5.26
56	2.71	1.86	3.108	.360	---
43	2.79	1.12	4.020	.330	8.72
38	2.82	.50	3.926	.506	10.11
71	2.86	3.88	4.737	.427	---
13	2.88	4.70	1.772	.231	5.74
93	2.93	1.17	1.691	.609	6.89
62	2.97	2.81	5.579	.409	9.71
91	2.98	.77	2.087	.458	9.36
80	3.02	1.20	2.637	.581	10.20
32	3.04	1.16	2.965	.297	7.52
59	3.30	3.10	3.494	.324	8.04
30	3.30	2.86	2.896	.360	9.08
31	3.38	1.27	2.896	.268	8.89
37	3.44	4.17	3.663	.300	8.02
22	3.46	1.54	2.828	.431	6.77
73	3.65	.59	2.576	.583	11.76

TABLE NO. IV. (CONTINUED)

Exp.	Swim-bladder gases % O_2	% CO_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc.blood
16	3.73	1.83	1.651	.473	5.35
52	3.73	2.88	3.108	.414	11.57
88	3.81	1.24	1.370	.551	7.38
46	3.96	1.30	3.836	.313	11.88
69	4.08	3.85	5.326	.325	---
61	4.27	1.94	4.416	.362	13.20
45	4.35	1.95	3.493	.439	12.07
51	4.45	3.37	2.764	.224	---
64	4.58	4.37	4.416	.243	11.71
29	4.65	2.00	2.039	.510	3.99
4	4.74	1.62	1.901	.113	11.82
50	4.81	1.28	4.414	.326	6.68
68	4.85	3.23	4.416	.390	---
28	4.97	1.75	2.039	.510	---
11	5.37	2.01	3.579	.141	8.05
63	5.89	1.59	4.627	.400	11.57
70	5.92	2.60	4.964	.427	8.15
77	5.93	.42	2.292	.463	6.82
34	5.94	1.24	2.763	1.113	6.01
72	6.12	1.31	2.458	.810	9.50

TABLE NO. IV. (CONTINUED)

Exp.	Swim-bladder gases % O_2	% CO_2	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Hemoglobin, grs. per 100 cc.blood
2	6.27	1.40	1.731	.252	---
65	6.43	1.90	4.627	.277	12.05
53	6.70	1.16	3.494	.408	9.51
41	7.10	1.38	3.663	.497	12.23
49	7.24	2.77	3.926	.403	8.50
7	7.43	3.00	3.747	.235	8.84
26	7.66	2.38	3.201	.711	6.60
54	8.55	1.38	3.108	.396	11.38
48	8.99	3.20	4.217	.279	11.16
23	9.02	2.74	2.828	.431	7.75
67	10.35	.95	5.579	.387	10.00
25	11.00	1.87	2.136	.529	6.08
21	11.24	1.84	2.458	.438	6.01
66	12.01	1.01	4.416	.283	8.42
9	12.31	1.02	3.747	.168	8.31
6	13.37	.81	3.747	.235	5.28
24	13.63	1.17	2.136	.529	7.01
15	13.75	2.12	2.576	.360	5.24
14	13.80	1.41	2.576	.360	6.34
55	19.80	2.31	3.748	.523	12.23

TABLE NO. V. DATA ARRANGED ACCORDING TO INCREASE IN HEMOGLOBIN IN BLOOD

Exp.	Hemoglobin, grs. per 100 cc.blood	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Swim-bladder gases % CO_2	% O_2
29	3.99	2.039	.510	2.00	4.65
19	4.18	2.828	.271	2.46	.12
12	5.07	1.772	.231	---	---
87	5.18	.254	4.528	1.00	2.49
15	5.24	2.576	.360	2.12	13.75
92	5.26	2.136	.482	.63	2.63
6	5.28	3.747	.235	.81	13.37
16	5.35	1.651	.473	1.83	3.73
13	5.74	1.772	.231	4.70	2.88
3	5.82	1.731	.252	---	---
27	5.85	3.201	.711	---	---
21	6.01	2.458	.438	1.84	11.24
34	6.01	2.763	1.113	1.24	5.94
25	6.08	2.136	.529	1.87	11.00
20	6.20	2.458	.438	0.00	.91
14	6.34	2.576	.360	1.41	13.80
10	6.51	3.579	.141	3.62	2.01
26	6.60	3.201	.711	2.38	7.66
40	6.61	3.926	.299	.86	1.72
17	6.64	1.651	.473	---	---

TABLE NO. V. (CONTINUED)

Exp.	Hemoglobin, grs. per 100 cc.blood	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Swim-bladder gases % CO_2	% O_2
50	6.68	4.414	.326	1.28	4.81
22	6.77	2.828	.431	1.54	3.46
77	6.82	2.292	.463	.42	5.93
93	6.89	1.691	.609	1.17	2.93
0	6.94	1.731	.136	---	---
24	7.01	2.136	.529	1.17	13.63
90	7.14	1.651	.542	2.95	1.31
88	7.38	1.370	.551	1.24	3.81
32	7.52	2.965	.297	1.16	3.04
23	7.75	2.828	.431	2.74	9.02
18	7.83	2.828	.271	0.00	.13
37	8.02	3.663	.300	4.17	3.44
59	8.04	3.494	.324	3.10	3.30
89	8.04	1.306	.686	.76	2.08
5	8.05	1.901	.113	2.86	1.47
11	8.05	3.579	.141	2.01	5.37
70	8.15	4.964	.427	2.60	5.92
9	8.31	3.747	.168	1.02	12.31
66	8.42	4.416	.283	1.01	12.01
82	8.43	1.435	.753	.92	1.15

TABLE NO. V. (CONTINUED)

Exp.	Hemoglobin, grs. per 100 cc. blood	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Swim-bladder gases % CO_2	% O_2
49	8.50	3.926	.403	2.77	7.24
1	8.60	1.731	.136	---	---
43	8.72	4.020	.330	1.12	2.79
36	8.78	3.836	.256	1.51	2.10
85	8.78	1.576	.406	3.14	1.04
7	8.84	3.747	.235	3.00	7.43
31	8.89	2.896	.268	1.27	3.38
8	8.90	3.747	.168	1.70	---
83	9.02	1.814	.548	---	---
30	9.08	2.896	.360	2.86	3.30
91	9.36	2.087	.458	.77	2.98
81	9.46	1.576	.737	---	---
72	9.50	2.458	.810	1.31	6.12
53	9.51	3.494	.408	1.15	6.69
75	9.60	1.945	.582	1.02	1.68
62	9.71	5.579	.409	2.81	2.97
42	9.84	3.493	.269	---	---
67	10.00	5.579	.387	.97	10.35
38	10.11	3.926	.506	.50	2.82
76	10.15	1.576	.271	.56	.60

TABLE NO. V. (CONTINUED)

Exp.	Hemoglobin, grs. per 100 cc.blood	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Swim-bladder gases % CO_2	% O_2
79	10.20	3.107	.346	2.89	1.07
80	10.20	2.637	.581	1.20	3.02
84	10.23	1.504	.558	2.65	1.10
78	10.36	2.346	.445	3.00	1.88
35	10.52	3.036	.513	---	---
58	10.52	3.748	.404	1.56	.37
44	10.90	3.333	.338	1.00	2.00
48	11.16	4.217	.279	3.20	8.99
33	11.27	2.576	.565	1.40	1.21
54	11.38	3.108	.396	1.38	8.55
86	11.48	2.039	.429	---	---
52	11.57	3.108	.414	2.88	3.73
63	11.57	4.627	.400	1.59	5.89
64	11.71	4.416	.243	4.37	4.58
73	11.76	2.576	.583	.59	3.65
4	11.82	1.901	.113	1.62	4.74
46	11.88	3.836	.313	1.29	3.96
39	11.94	1.731	4.648	3.35	2.32
65	12.05	4.627	.277	1.90	6.43
45	12.07	3.493	.439	1.95	4.35

TABLE NO. V. (CONTINUED)

Exp.	Hemoglobin, grs. per 100 cc.blood	CO_2 Tension H_2O, mm. Hg.	O_2 Water ml. per L.	Swim-bladder gases % CO_2	% O_2
41	12.23	3.663	.497	1.38	7.10
55	12.23	3.748	.523	2.31	19.79
47	12.41	3.926	.345	13.22	2.06
61	13.20	4.416	.362	1.94	4.27
74	13.33	2.637	.396	---	---

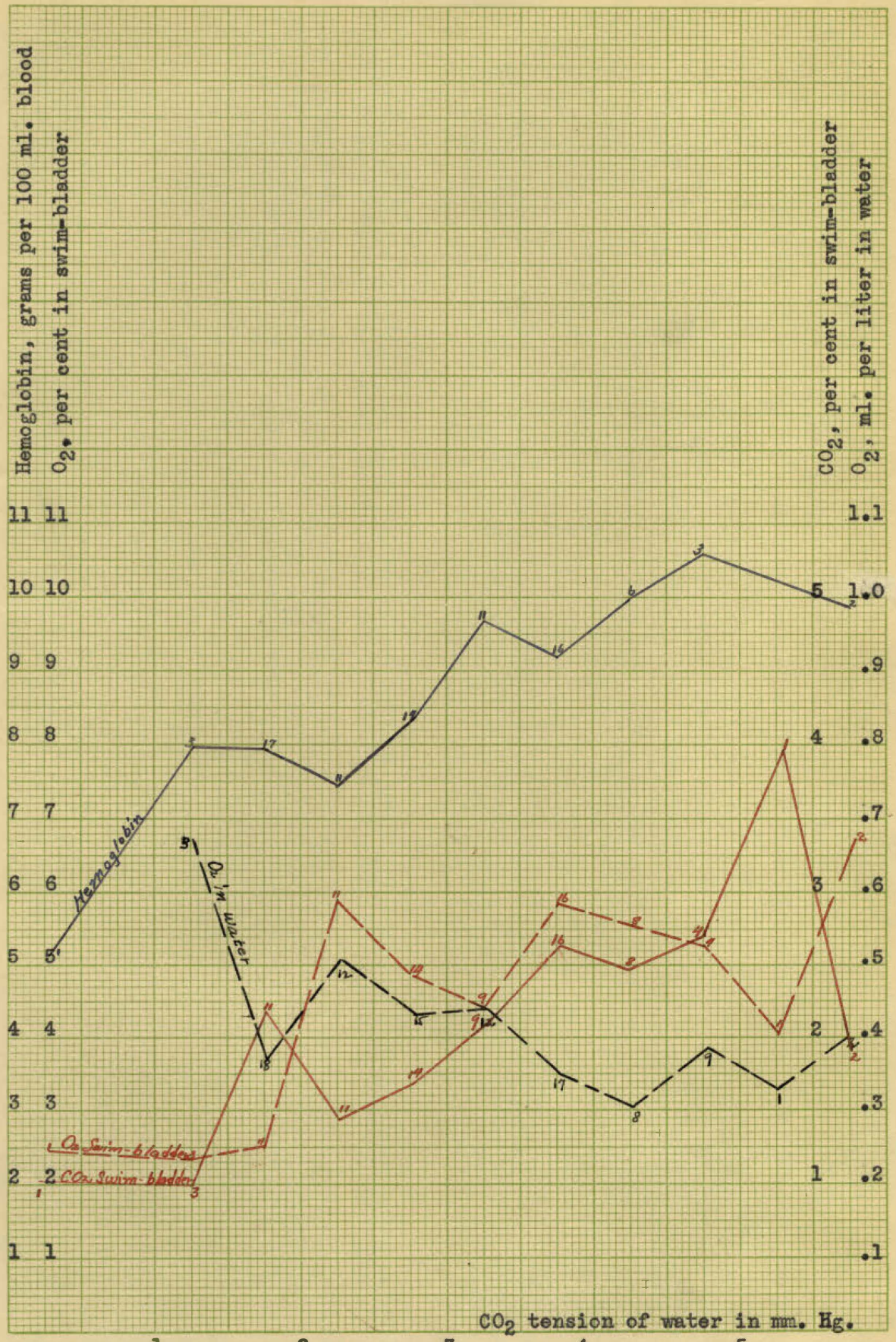

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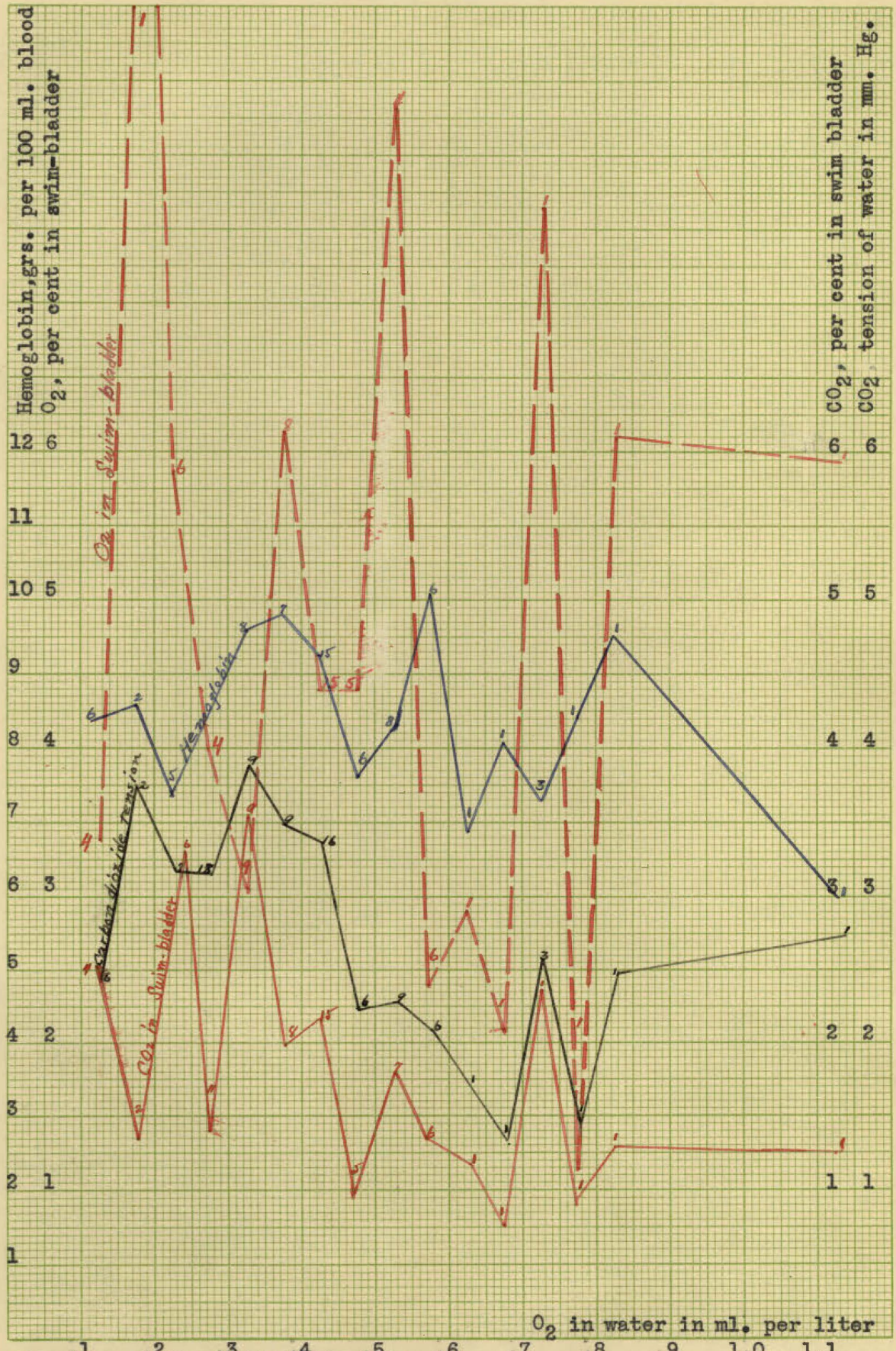
Figure 1. Data in Table I.



Published by The H. Cole Co., Columbus, Ohio. No. 290 G.

Figure 2. Data in Table II.

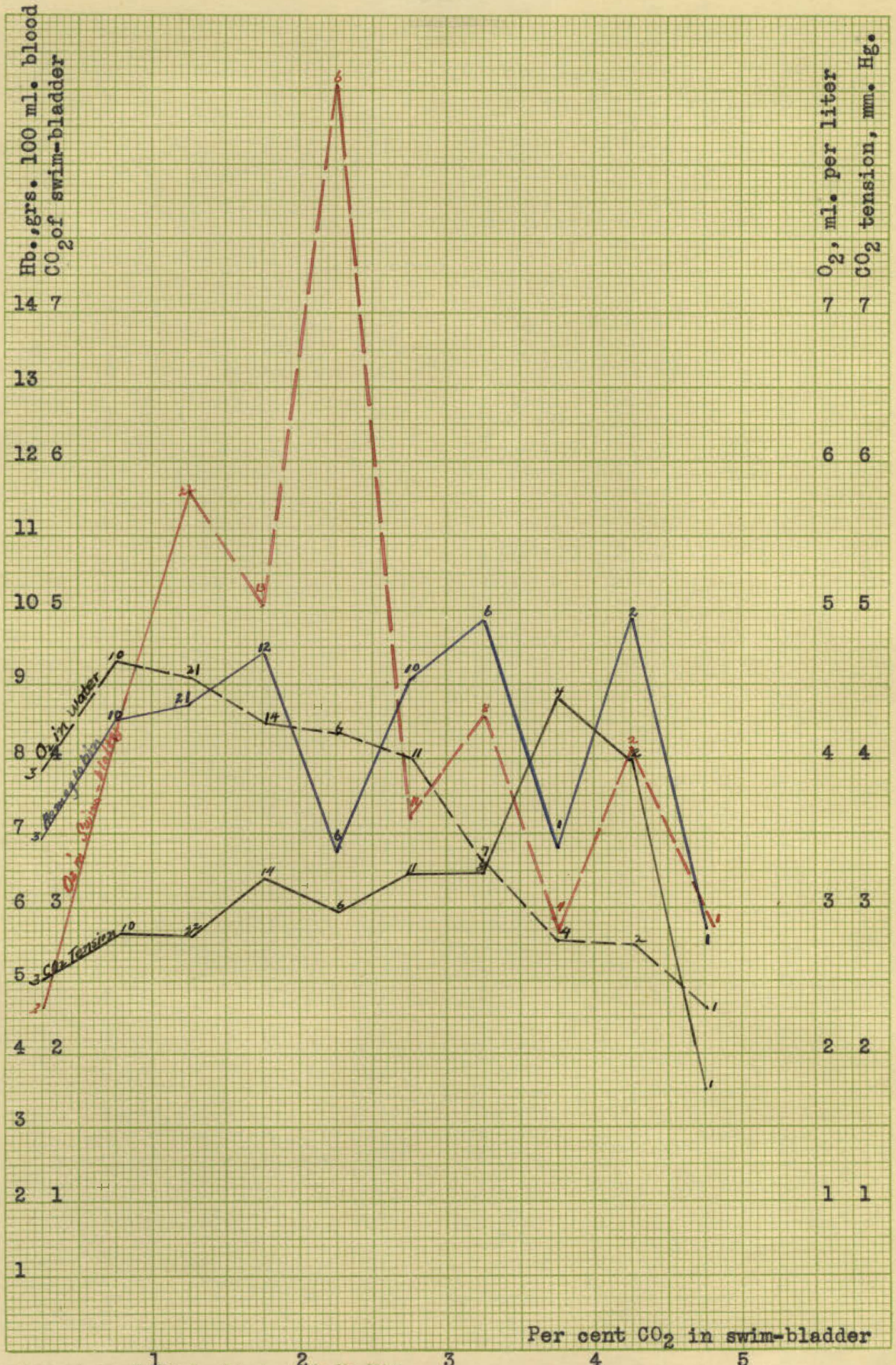


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Figure 3. Data in Table III.

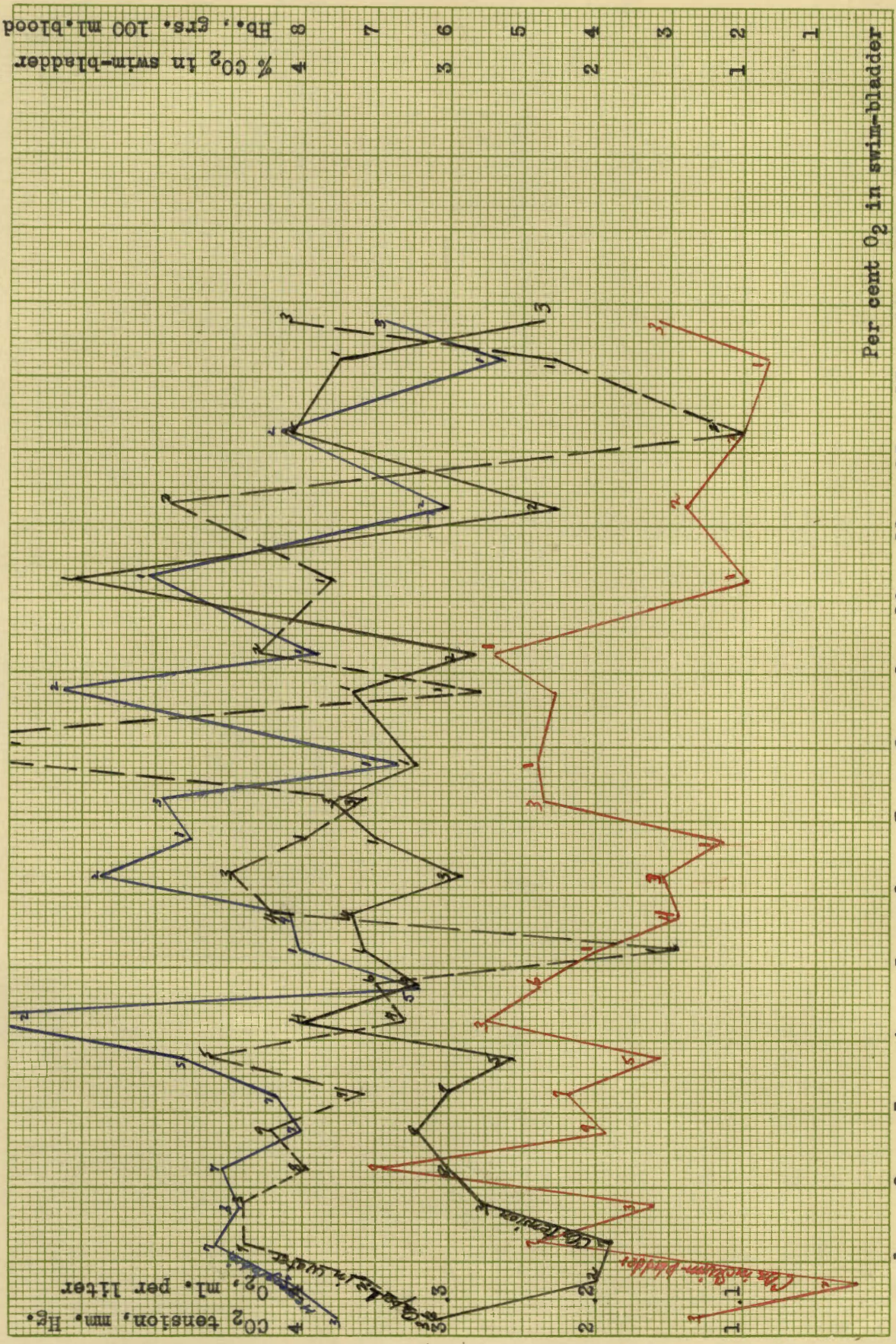

Figure 4. Data in Table IV.

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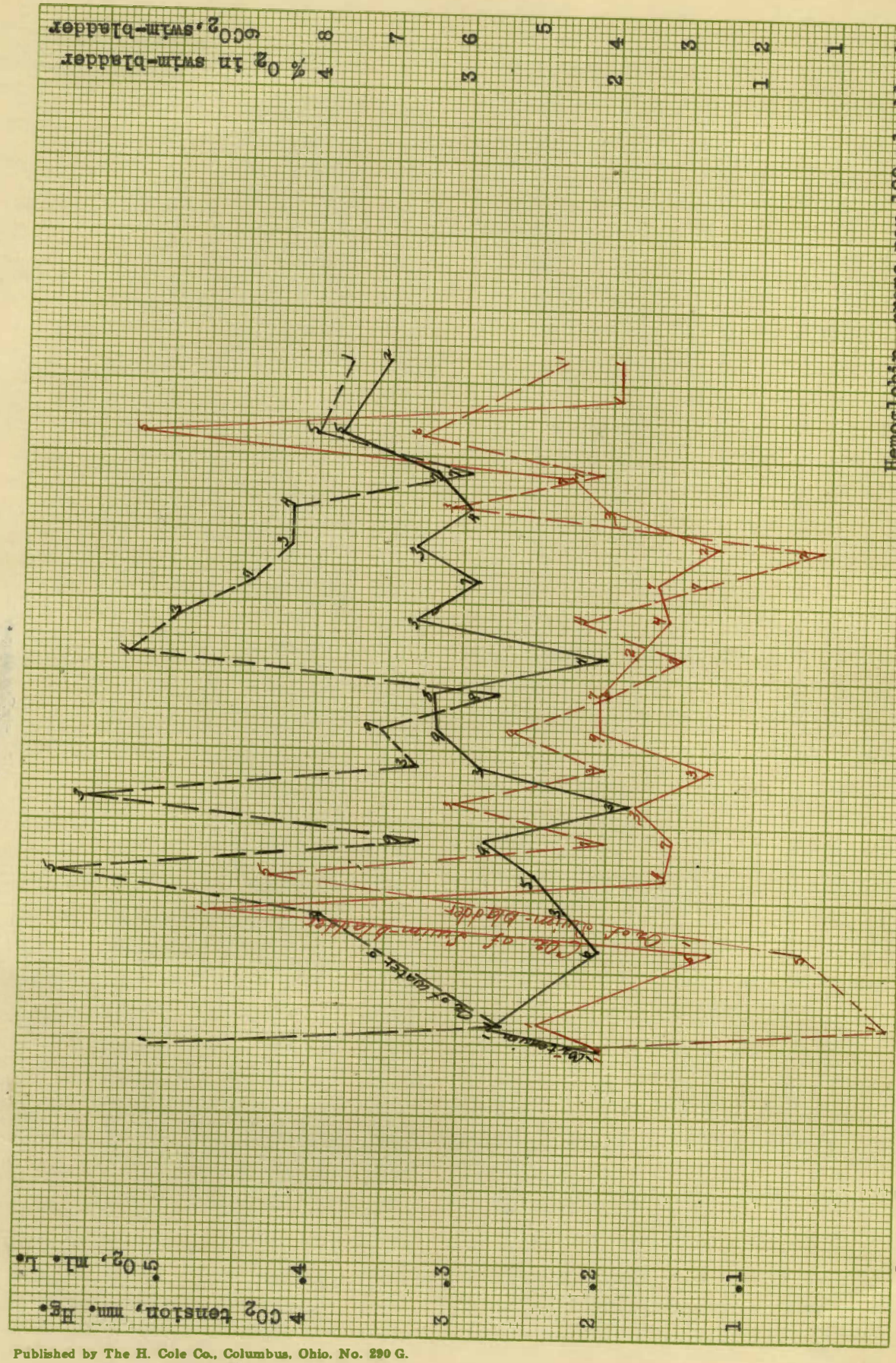

Figure 5. Data in Table V.

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BIBLIOGRAPHY

Barcroft, J. 1928. The Respiratory Function of the Blood.

1934. The Architecture of Physiological Function.

Brock, James M. 1934. A Determination of Volume Per Cents of Carbon Dioxide in Sodium Carbonate and Potassium Carbonate. Thesis.

Carpenter, Thorne M. 1915. A Comparison of Methods for Determining the Respiratory Exchange of Man. Carnegie Institution of Washington Publication No. 216.*

Clark, W. M. 1925. The Determination of Hydrogen Ions.

Haldane, J. S. 1920. Methods of Gas Analysis.

1927. Acclimatisation to High Altitudes. Physiol. Rev., 7; 263-385.

Hall, F. G. 1931. The Respiration of Puffer Fish. Biol. Bull., 61; 623-631.*

Henderson, Y. 1925. Physiological Regulation of the Acid-Base Balance of the Blood and Some Related Functions. Physiol. Rev., 5; 131-161.

Hickman, T. 1929. Effect of Carbon Dioxide Tension upon Goldfish. Thesis.

McClendon, F. F. 1922. The Standardization of a New Colorimetric Method for the Determination of Hydrogen Ion Concentrations, CO_2 Tensions and O_2 Content of Sea Water, of Animals, and of CO_2 of Air, with a Summary of Similar Data on Bicarbonate Solutions in General. Jour. Biol. Chem., 30; 265-288.*

Mitchell, P. H. 1932. A Text-Book of General Physiology.

Newcomer, H. S. 1919. Absorption Spectra of Acid Hematin, Oxyhemoglobin, and Carbon Monoxide Hemoglobin. A New Hemoglobinometer. Jour. Biol. Chem., 37; 465-496.

Powers, E. B. 1930. The Relation between pH and Aquatic Animals. The American Naturalist, 64; 342-366

1932. The Relation of Respiration of Fishes to Environment. I. Introduction. Ecol. Mon.,2; 387-389

1932. The Relation of Respiration of Fishes to Environment. X. Mechanism of the Deposition of Gases into the Swim-Bladder. Ecol. Mon. 2; 443-465.

Powers, E. B.,and J. D. Bond. 1928. Further Notes on the Colorimetric Method for Field Determination of the Carbon Dioxide Tension of Natural Waters. Ecology, 9; 364-366.

Powers, E. B., and T. A. Hickman. 1932. The Relation of Respiration of Fishes to Environment. VI. Oxygen and Carbon Dioxide Dissociation Curves of Whole Blood. Ecol. Mon., 2; 421-430.

Powers, E. B., F. G. Hopkins, and T. A. Hickman. 1932. The Relation of Respiration of Fishes to Environment. IV. Relation of Carbon Dioxide and Oxygen Contents of the Blood to the Carbon Dioxide and Oxygen Tensions of the Environmental Water. Ecol. Mon., 2; 396-413.

Powers, E. B.,and L. M. Shipe. 1932. The Relation of Respiration of Fishes to Environment. V. Effect of the Oxygen and Carbon Dioxide Tensions of the Water upon

the Number of Red Corpuscles in the Blood of the Blue Cat, Ictalurus punctatus (Rafinesque). Ecol.Mon., 2; 414-420.

Rideal, S., and C. G. Stewart. 1901. Determination of Dissolved Oxygen in Waters in Presence of Nitrites and of Organic Matter. Analyst, 26; 141.*

Standard Methods for the Examination of Water and Sewage, 1933. American Public Health Association.

Starling, E. H. 1933. Principles of Human Physiology. Sixth Edition, Edited and Revised by C. Lovatt Evans.

Van Slyke, D. D. 1921. The Carbon Dioxide Carriers of the Blood. Physiol. Rev., 1; 141-176.

Van Slyke, D. D.,and J. M. Neill. 1924. The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement. IV. Calculation. Jour. Biol. Chem., 61; 538-543.

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*Cited indirectly.

APPENDIX I.

Table of factors for converting carbon dioxide partial pressure readings in mm. mercury to volumes per cent carbon dioxide, when: S = 10 ml., gas volume = .5 ml., and i (reabsorption factor for carbon dioxide) = 1.03.

Temperature, Degrees Centigrade	Factor	Log of factor
20°	.015586	8.19278 -10
20.5°	.015487	8.18996 -10
21°	.015389	8.18721 -10
21.5°	.015344	8.18594 -10
22°	.015299	8.18466 -10
22.5°	.015235	8.18284 -10
23°	.015172	8.18104 -10
23.5°	.015110	8.17926 -10
24°	.015048	8.17749 -10
24.5°	.014988	8.17574 -10
25°	.014928	8.17400 -10
25.5°	.014870	8.17231 -10
26°	.014813	8.17066 -10
26.5°	.014763	8.16917 -10
27°	.014713	8.16739 -10
27.5°	.014663	8.16622 -10
28°	.014613	8.16474 -10
28.5°	.014555	8.16301 -10
29°	.014498	8.16132 -10
29.5°	.014447	8.15978 -10
30°	.014397	8.15828 -10