1968

Blood Physiology of a West Indian Frog, Leptodactylus fallax

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Recommended Citation

Gatten, Robert Edward, "Blood Physiology of a West Indian Frog, Leptodactylus fallax" (1968). *Dissertations, Theses, and Masters Projects.* William & Mary. Paper 1539624643.
https://dx.doi.org/doi:10.21220/s2-y5pt-5564

BLOOD PHYSIOLOGY OF A WEST INDIAN FROG, *LEPTODACTYLUS FALLAX*

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

By

Robert Edward Gatten, Jr.

1968

APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of
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ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Garnett R. Brooks, Jr., under whose guidance this investigation was conducted, for his suggestions and criticism throughout the study. The author also wishes to acknowledge the Smithsonian Institution for providing the opportunity to carry out this investigation on the island of Dominica, B.W.I. The author is indebted to Dr. Webster Van Winkle for advice regarding the statistical analysis of the data and for his careful reading of the manuscript, and to Dr. Robert E. L. Black for his suggestions upon reading of the manuscript. The author is grateful to Dr. Charlotte P. Mangum for her assistance in the absorption study and her constructive criticisms. The author also wishes to express his appreciation to Miss Carolyn Mohler for assistance in the electrophoretic study and to Mr. Arthur Conway for help in preparing the figures.

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ABSTRACT

The blood oxygen capacity of 25 male and 25 female *Leptodactylus fallax* was determined by the Grant modification of the Roughton and Scholander microgasometric syringe method during the summer of 1966 on the island of Dominica, British West Indies. Erythrocyte counts and hemoglobin content of the blood of 5 males and 5 females were determined during July of 1967 according to standard procedures on specimens which had been returned to the College of William and Mary and maintained in an animal room for one year.

Leptodactylus fallax has a mean blood oxygen capacity of 10.5 vol.%. There is no significant difference in blood oxygen capacity between populations living at 1,200 feet and those living at sea level. There is, however, a highly significant sexual difference in blood oxygen capacity with males having a higher value than females. The mean erythrocyte count is 672,000 cells per mm^3. Females have a significantly higher count than males. The mean hemoglobin content is 10.9 gm.%. Females have a higher hemoglobin content than males, but the difference is not statistically significant.

There is a significant increase in blood oxygen capacity with an increase in body weight, and a highly significant decrease in blood oxygen capacity per gram of body weight with an increase in body weight. Erythrocyte count increases slightly, but not significantly, with an increase in body weight, while hemoglobin content decreases slightly, but not significantly, with an increase in body weight.

Disc electrophoresis on polyacrylamide gels at pH 8.3 indicated that male and female blood have identical protein components. Determination of the wavelengths of maximum absorption of male and female oxyhemoglobin and carbon monoxyhemoglobin indicated that male and female hemoglobins were identical. It was concluded that male and female blood oxygen capacity must differ for reasons other than those revealed by these methods.

BLOOD PHYSIOLOGY OF A WEST INDIAN FROG,

LEPTODACTYLUS FALLAX

INTRODUCTION

Along the moisture gradient in nature there are found many widely different habitats ranging from purely aquatic to completely terrestrial. Amphibians have taken advantage of much of this wide range of habitats, and in adapting to a particular environment, each species has developed physiological capabilities suited to that environment. Previous research has indicated that among amphibians considerable interspecific variation exists with respect to blood oxygen capacity, erythrocyte count, and hemoglobin content, and that these factors are correlated with the habitat occupied by each species.

Blood oxygen capacity is a measure of how much oxygen can combine with a given volume of blood. It is usually expressed as volumes percent (vol. %) or the number of cubic centimeters of oxygen, corrected to standard temperature and pressure, which can combine with 100 ml of blood. Prosser and Brown (1961) tabulate values for several invertebrate and vertebrate species. Poikilothermic vertebrates generally have lower blood oxygen capacities than do homiotherms.

Redfield (1933) points out that the evolutionary transition from an aquatic to a terrestrial habitat was accompanied by changes in the method of aerating blood and in the conditions to which blood is exposed in respiratory organs and by an increase in activity which would require more effective circulation of oxygen. These increased demands for oxygen transport were met by an increase in red cell volume, in the amount of hemoglobin in erythrocytes, and in the speed and degree of oxygen exchange between blood and tissues (Redfield, 1933). These adaptations of the blood to the external and internal environment are reflected today within the class Amphibia, since terrestrial species generally exhibit higher blood oxygen capacities than do aquatic or semi-aquatic species.

The aquatic Congo eel, Amphiuma tridactyla, showed a mean blood oxygen capacity of 5.3 vol.% for seven specimens (Scott, 1931). In 1934 Wolvekamp and Lodewijks reported mean blood oxygen capacity values of 7.6 vol. % for nine specimens of the aquatic frog, Rana esculenta, and 12.0 vol. % for three specimens of the semi-terrestrial frog, R. temporaria (in Leftwich and Burke, 1964). McCutcheon (1936) published a mean blood oxygen capacity value of 10.2 vol. % for three adult bullfrogs, R. catesbeiana, and 7.8 vol. % for two tadpoles of the same species. Leftwich and Burke (1964) found that the aquatic frogs, R. clamitans and R. catesbeiana, had mean blood oxygen capacities of 6.0 and 6.9 vol. % for 43 and 52 specimens, respectively, while 50 individuals of the semi-terrestrial frog, R. pipiens, had a mean value of 10.3 vol. %. Eight specimens of the giant toad, Bufo paracnemis, exhibited a mean blood oxygen capacity of 14.8 vol. % (Johansen and Ditadi, 1966). For the class Amphibia, therefore, reported values for blood oxygen capacity range from 5.3 to 14.8 vol. % and show good correlation with habitat, being higher in terrestrial species than in aquatic species.

The oxygen capacity of blood is determined by many of its physical and chemical properties, two of which are the density of erythrocytes and the concentration of hemoglobin. Erythrocyte counts are expressed as the number of red blood cells per mm^3 of blood. Urodeles have low erythrocyte counts, ranging from 36,000 to 177,000/mm^3 (Foxon, 1964; Myers and Alexander, 1945), while ranid frogs have intermediate counts, ranging from 252,000 to 512,000/mm^3, and bufonids and hylids have high counts, ranging from 615,000 to 1,000,000/mm^3 (Hutchi~~n~~son and Szarski, 1965; Foxon, 1964). In general, there is good correlation of red cell number with habitat and with blood oxygen capacity among anurans, with terrestrial species having more erythrocytes per unit volume and greater blood oxygen capacity than aquatic species (Hutchi~~n~~son and Szarski, 1965; Leftwich and Burke, 1964).

Hemoglobin content of blood is expressed in grams percent (gm. %) or the number of grams of hemoglobin in 100 ml of blood. Few reports have been made concerning the hemoglobin content of urodele blood. Evidence from research on anuran blood, however, suggests that correlations exist between hemoglobin content and habitat and between hemoglobin content and blood oxygen capacity. Ranid frogs have blood with a hemoglobin content ranging from 6.5 to 9.9 gm. % and low blood oxygen capacities (Leftwich and Burke, 1964) while the more terrestrial bufonids and hylids have blood with a higher hemoglobin content, ranging from 8.7 to 11.3 gm. % and higher blood oxygen capacities (Goin and Jackson, 1965; Johansen and Ditadi, 1966; Stuart, 1951).

In the investigation reported here, the oxygen capacity, erythrocyte count, and hemoglobin content of the blood were determined for *Leptodactylus fallax*, a large frog endemic to the island of Dominica, British West Indies. It occurs along valleys and on mountain slopes from sea level to approximately 1,200 feet. By day it remains hidden under logs, rocks, or dense vegetation; at night it emerges to feed, mainly on insects. *L. fallax* is found only in moist regions of the island but is rarely found in water (Brooks, unpublished). Therefore, it can be termed a "semi-terrestrial" frog.

The major purpose of this investigation was to determine if the physiological properties of oxygen capacity, red cell count, and hemoglobin content of the blood of *Leptodactylus fallax* are of the same magnitude as in other semi-terrestrial and terrestrial anurans. In addition the correlations between blood oxygen capacity, erythrocyte count, hemoglobin content and the parameters of altitude, body weight, and sex were examined. During the course of the investigation it became clear that differences exist in oxygen capacity of the blood of males and females. Therefore, a third aspect of this investigation was to attempt to determine whether this difference reflected differences in electrophoretic properties or in absorption spectra of hemoglobins of males and females. It is of interest to note that no reports can be found in the literature concerning the respiratory properties of the blood of any member of the family Leptodactylidae. Since Gorman and Dessauer (1965) showed that populations of the iguanid lizard, *Anolis roquet*, living on different islands in the West Indies had hemoglobins which differed in electrophoretic pattern, it might be expected that *L. fallax* hemoglobin would have an electrophoretic pattern different from the pattern of hemoglobins from other species of *Leptodactylus* from the mainland of South America.

MATERIALS AND METHODS

A total of 60 specimens of Leptodactylus fallax were used in this study. They were caught by hand at night with the aid of a flashlight during June, July, and August of 1966 at three different locations on the island of Dominica, British West Indies. Fifty of these specimens (45 from Hillsborough Estate and Mannet's Gutter, elevation 50-400 feet; 5 from Springfield Estate, elevation 1,200 feet) were used in the determination of blood oxygen capacity within one week after their capture during the summer of 1966. These animals were maintained until used in moist cloth bags. Ten specimens (all from Hillsborough Estate) were returned to Williamsburg and used in the determination of erythrocyte density and hemoglobin content during the summer of 1967. These ten animals were also used as blood sources for the electrophoretic and absorption studies in 1968. They were maintained from 1966 to 1968 at 70-80°F in a diurnally lighted room in seven-foot diameter corrugated metal enclosures containing sterilized soil, wooden boards for shelter, and pans of water. Live crickets were supplied for food.

At the time of testing the following information was recorded: body weight to the nearest 0.1 gram, snout-vent length in ml, and sex as determined by the presence of black thumb pads on males and by dissection and gonadal examination in questionable cases. Each specimen used for blood oxygen capacity determination was doubly-pithed, the coelom and pericardium were slit open, and blood was removed from the ventricle by means of a No. 27 needle attached to a heparinized, one-cc. syringe. Between tests blood was stored in the syringe which was capped and immersed in ice water. Those specimens used for erythrocyte and hemoglobin determinations could not be sacrificed; therefore, a small incision was made in the skin of the venter and blood was removed from the ventral abdominal vein by means of a No. 27 needle attached to a heparinized, one-cc. syringe. Blood for electrophoresis and absorption studies was obtained in a similar syringe by forcing the needle through the skin and body wall just posterior to the sternum and directly into the heart.

Blood oxygen capacity was determined by the microgasometric syringe technique as described by Roughton and Scholander (1943) and modified by Grant (1947). Three determinations were made on each of the 50 specimens. Erythrocyte counts were made on ten specimens using the method of Levedahl and Barber (1963). Two determinations from the same pipette were made for each animal. Hemoglobin determinations were made by the unopette-colorimeter method (Gerarde, 1965). Single measurements were made on each of the ten specimens.

In order to compare the electrophoretic pattern of male and female hemoglobin, blood was obtained on three occasions from one male and one female, with no frog being used twice. Blood was centrifuged at 1, 000 g's to precipitate cells from plasma. Plasma was then drawn off and the red cells washed three times with 1% saline. Erythrocytes were lysed by the addition of a volume of distilled water equivalent to three times the volume of the packed cells. Cell membranes were separated from the hemoglobin solution by centrifugation and the hemoglobin solution was drawn off. Two hemoglobin solutions, one from a male and the other from a female, were then diluted to approximately the same concentration with distilled water by visual comparison. To each hemoglobin solution was added enough sodium chloride and sucrose to produce a 0. 05 M sodium chloride and 30% sucrose solution. Electrophoresis was carried out at pH 8. 3 in a buffer of tris boric acid in disodium EDTA. Two hundredths of a milliliter of male or female solution were placed on each of two chambers of a Buchler Polyanalyst Disc Electrophoresis Apparatus containing a 5% polyacrylamide gel (Richards and Coll, 1965). The same was done for a solution containing a mixture of the two solutions, since if a difference between male and female hemoglobin existed, such a mixture might produce a different electrophoretic pattern than either male or female hemoglobin alone. A current of 6 milliamps and 200 volts per tube was applied for one and one-half hours. The gels were hardened in 1.0 M acetic acid for 10 minutes and stained in 1% napthol blue black in 7% acetic acid for one hour. Since this stain is not specific for hemoglobin, the electrophoretic patterns produced must be interpreted as those of total erythrocyte protein, excluding cell membrane protein. Excess stain was removed by rinsing the gels in distilled water.

Blood from one male and one female was used for determination of the wavelengths of maximum absorption. Distilled water was added to lyse the erythrocytes, and cell membranes were separated from the hemoglobin solution by centrifugation. Wavelengths of maximum absorption of male and female oxyhemoglobin were determined with a Hartridge Reversion Spectroscope. Carbon monoxide was then bubbled through the hemoglobin solutions to convert oxyhemoglobin to carbon monoxyhemoglobin. Conversion was revealed by a change in the color of the solution from red to purple. Wavelengths of maximum absorption for male and female carbon monoxyhemoglobin were then determined in the same manner.

Standard statistical techniques were used to analyze blood oxygen capacity, erythrocyte count, and hemoglobin content data (Steel and Torrie, 1960). The regression of these three responses on body weight indicated that body weight had a significant effect on blood oxygen capacity but not on red cell count or hemoglobin content. Therefore, blood oxygen capacity values were adjusted for differences in body weight before further analysis. Student's "t" test was used to evaluate differences in oxygen capacity between high and low elevation populations and between males and females. Differences in erythrocyte count and hemoglobin content between males and females were also analyzed using Student's "t" test, as was the difference in regression coefficients between males and females for blood oxygen capacity regressed on body weight. The same method was used to determine the probability that regression coefficients were equal to zero. Differences were considered "significant" at the 5% probability level and "highly significant" at the 1% level. Analysis of variance techniques were used on the blood oxygen capacity data to determine the relative importance of the factors responsible for the observed variation.

RESULTS

Blood oxygen capacity values, erythrocyte counts, and hemoglobin concentrations are presented in Table 1. There is a significant increase in blood oxygen capacity with an increase in body weight (Figure 1 and Table 2), and a highly significant decrease in blood oxygen capacity per gram of body weight with an increase in body weight (Figure 2 and Table 2). Elevation differences do not contribute significantly (F=1. 95) to variation in blood oxygen capacity while sexual differences do (F=1, 270).

There is a slight, but not statistically significant, increase in red cell count (Figure 3 and Table 2) and decrease in hemoglobin content (Figure 4 and Table 2) with an increase in body weight. Females have a significantly higher red cell count than males, whereas there is no statistically significant sexual difference in hemoglobin content (Table 1).

The electrophoretic patterns of male, female, and a mixture of male and female blood cell proteins were identical under the conditions used (Figure 5). Each blood sample was separated into two protein components, the major component having the greater electrophoretic mobility. The wave lengths of maximum absorption of male and female oxyhemoglobin are also very nearly identical (Table 3). The same is true of male and female hemoglobin when it is bound to carbon monoxide (Table 3).

FIGURE 1

The relation of blood oxygen capacity in volumes percent to body weight in grams in *Leptodactylus fallax*. Regression line fitted by the method of least squares.

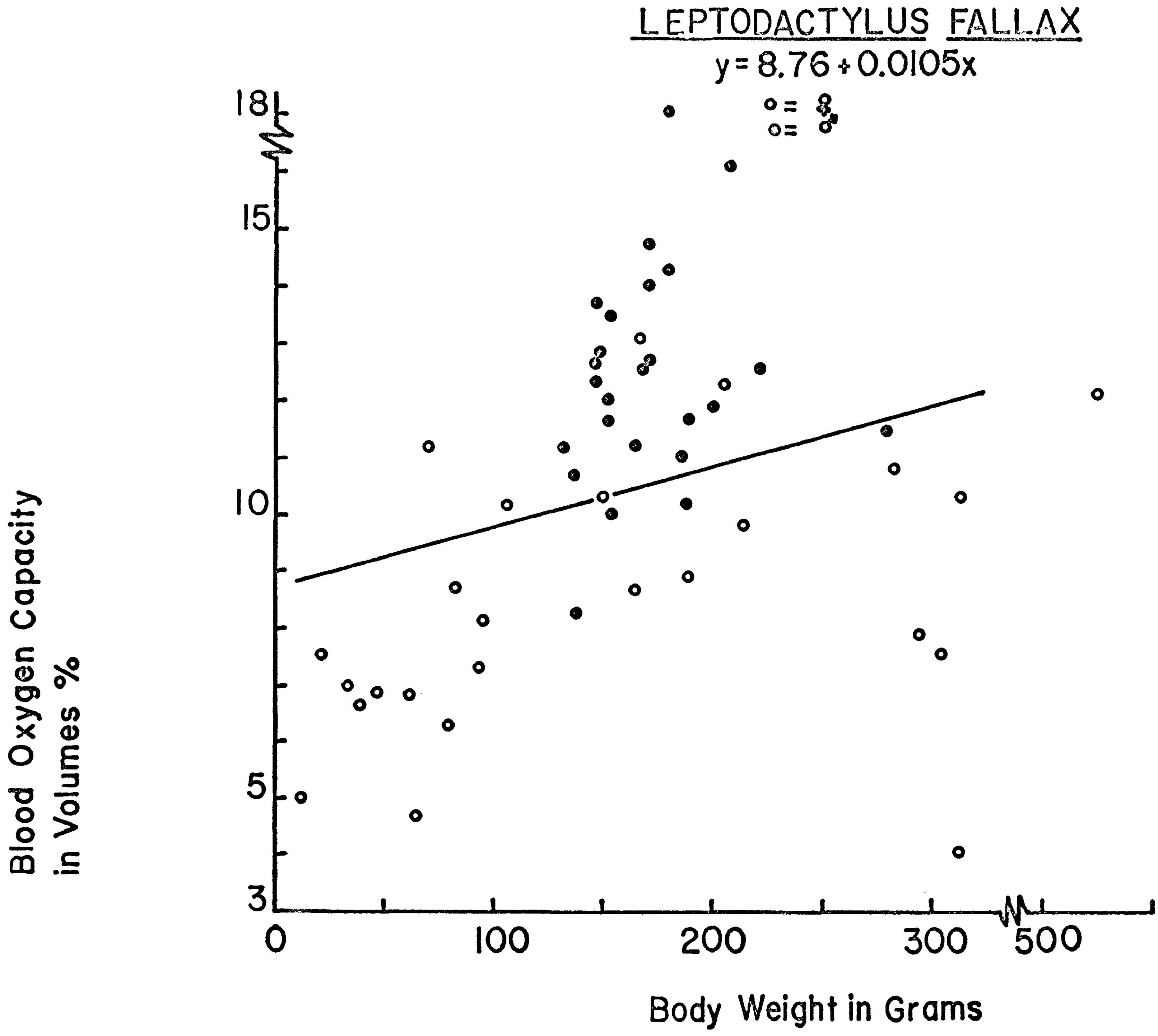

LEPTODACTYLUS FALLAX
y = 8.76 + 0.0105x
● = ♀
○ = ♂
Blood Oxygen Capacity in Volumes %
18
15
10
5
3
0
100
200
300
500
Body Weight in Grams

FIGURE 2

The relation of blood oxygen capacity in volumes percent per gram of body weight to body weight in grams in *Leptodactylus fallax*. Regression line fitted by the method of least squares.

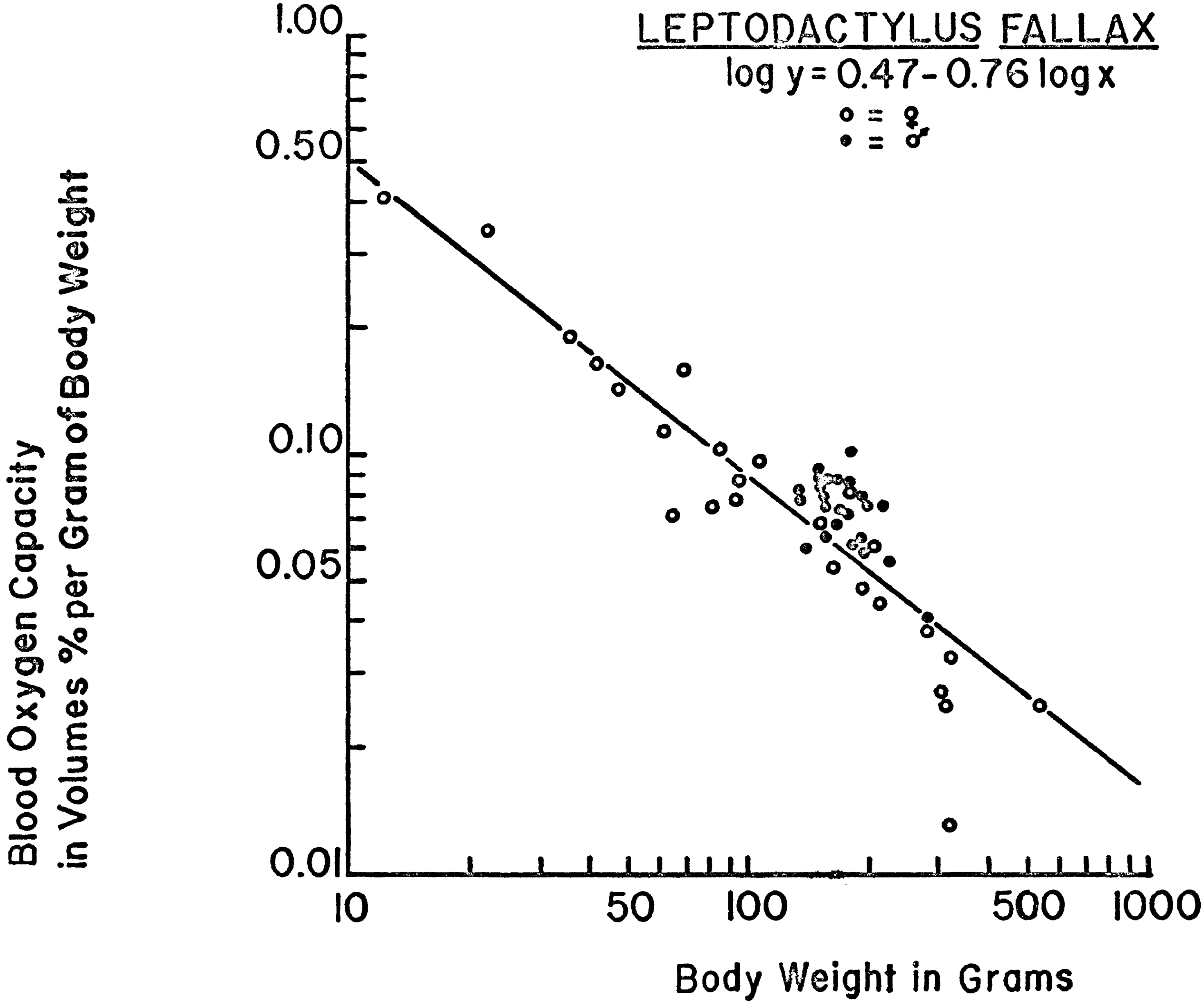

LEPTODACTYLUS FALLAX
log y = 0.47 - 0.76 log x
○ = ♀
● = ♂
Blood Oxygen Capacity in Volumes % per Gram of Body Weight
1.00
0.50
0.10
0.05
0.01
10
50
100
500
1000
Body Weight in Grams

FIGURE 3

The relation of erythrocyte count in cells per $mm^3 \times 10^{-4}$ to body weight in grams in *Leptodactylus fallax*. Regression line fitted by the method of least squares.

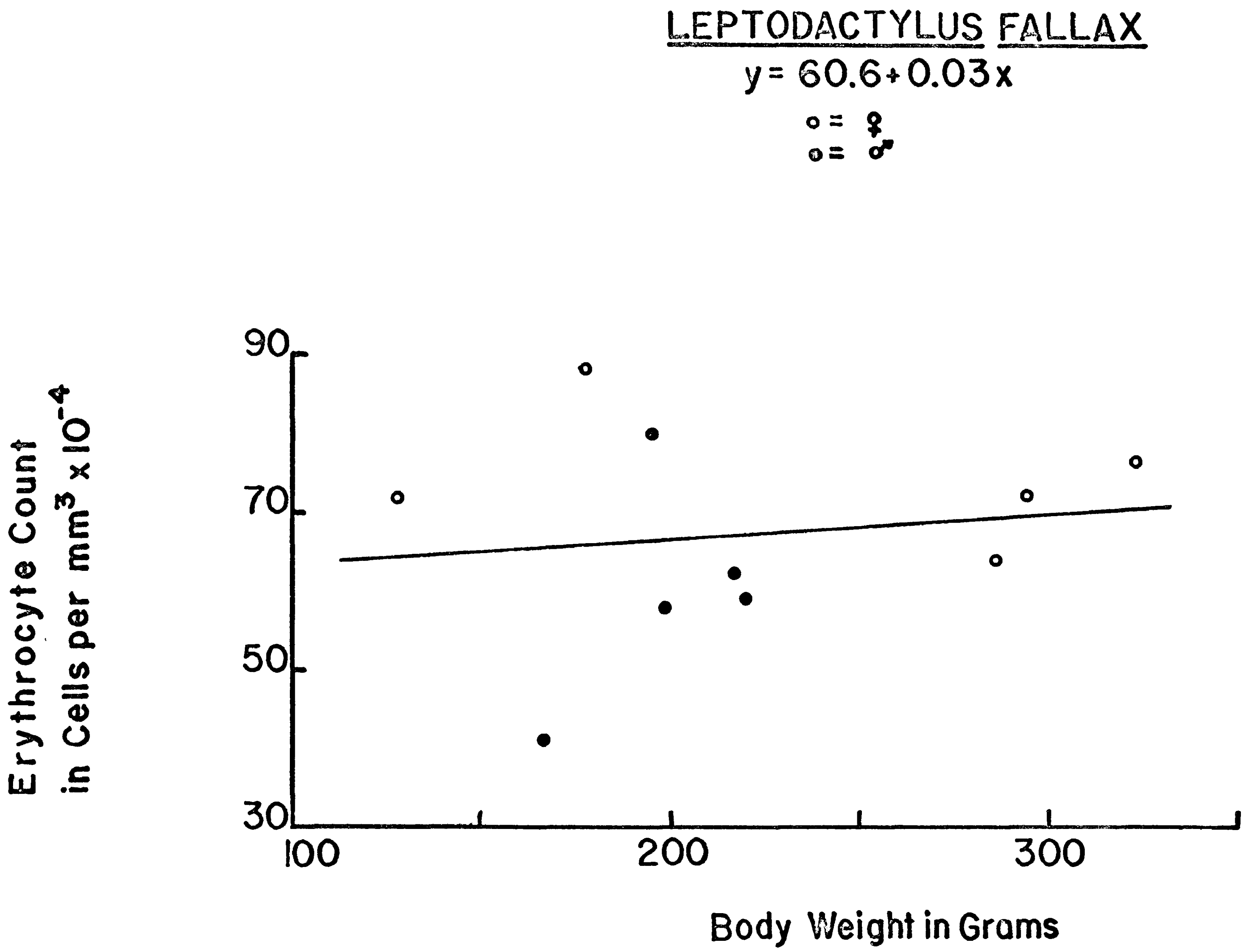

LEPTODACTYLUS FALLAX
y= 60.6+0.03x
○ = ♀
● = ♂
90
70
50
30
100
200
300
Erythrocyte Count in Cells per mm^3 x 10^{-4}
Body Weight in Grams

FIGURE 4

The relation of hemoglobin
content in grams percent
to body weight in grams in
Leptodactylus fallax.
Regression line fitted by
the method of least squares.

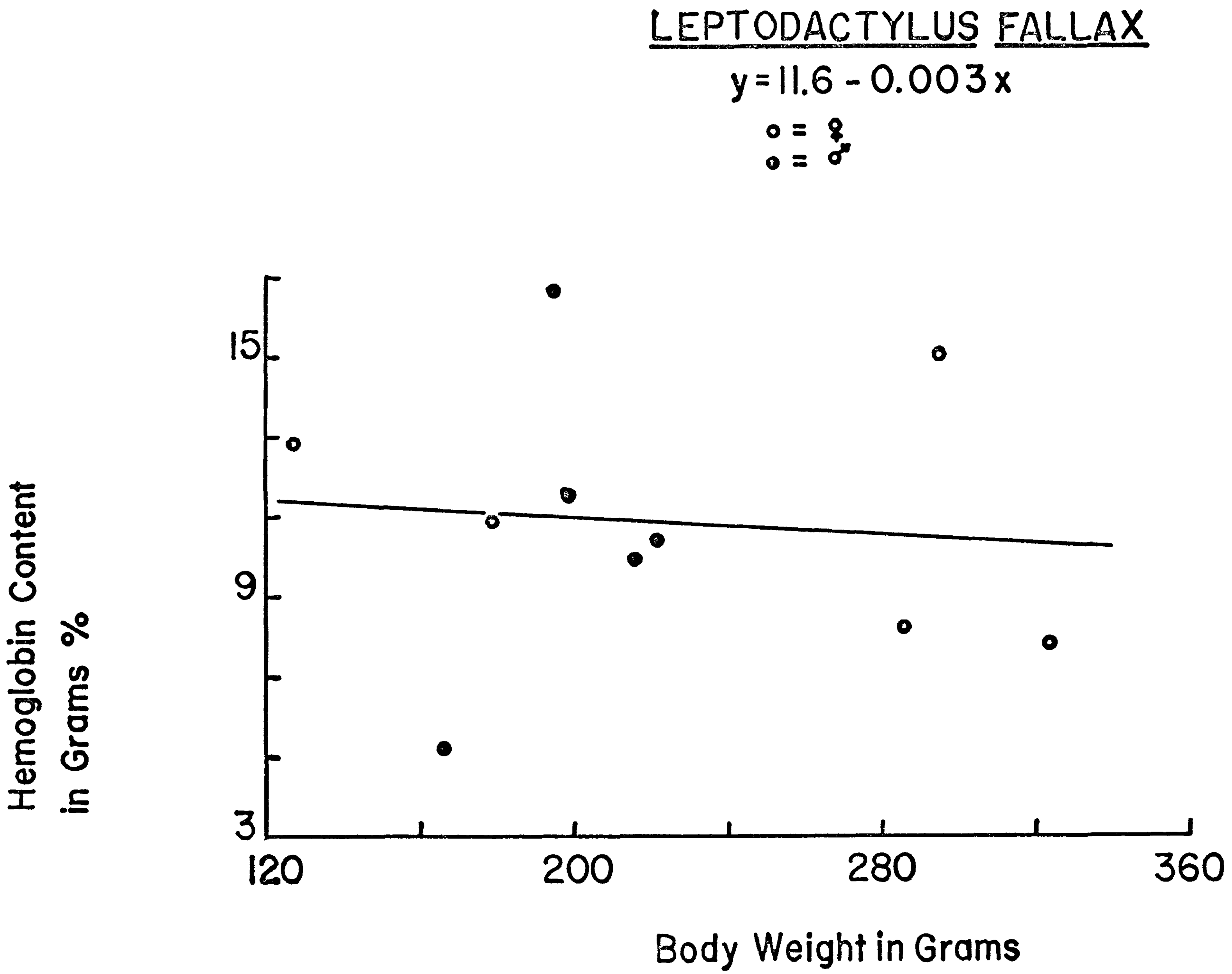
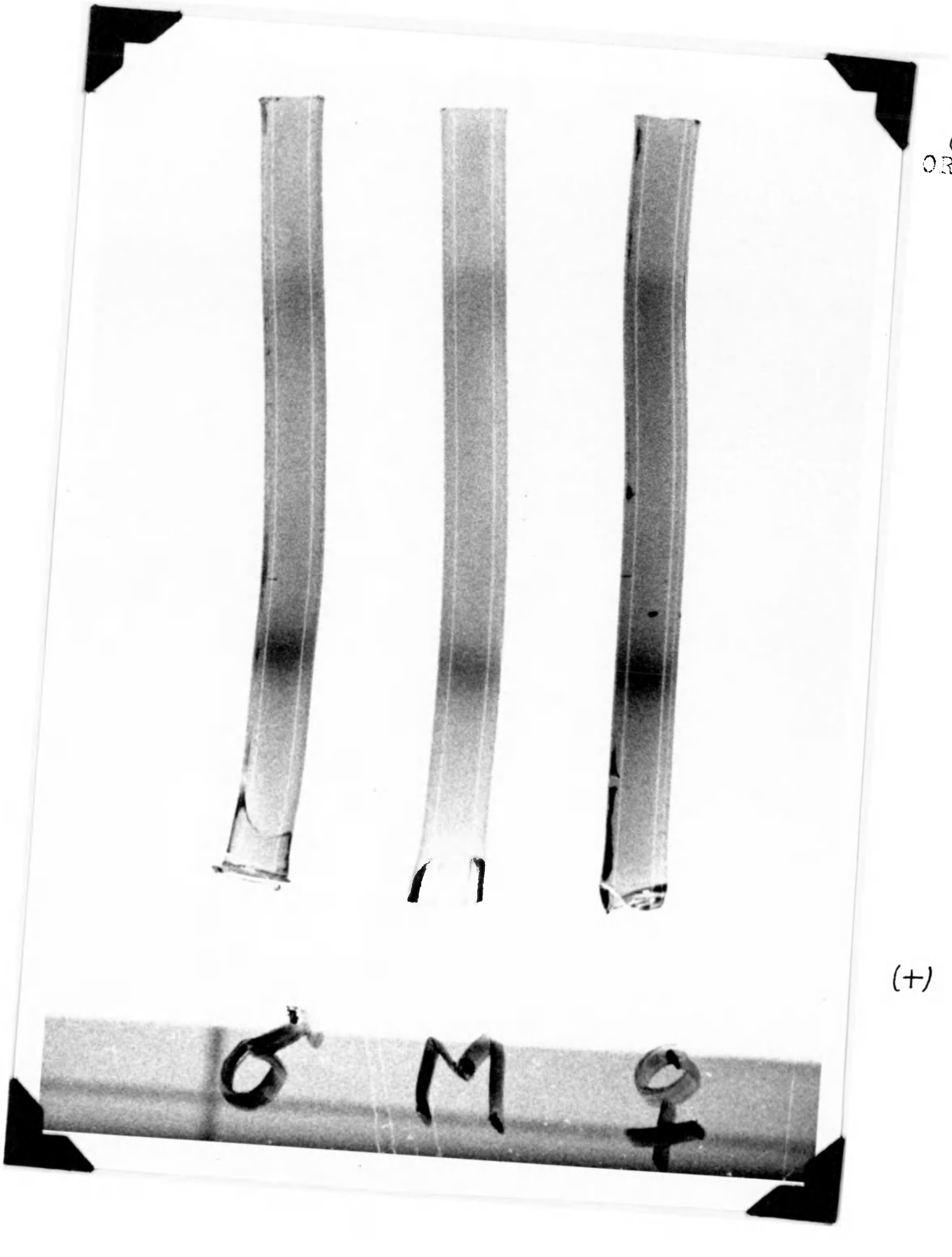

LEPTODACTYLUS FALLAX
y = 11.6 - 0.003 x
○ = ♀
● = ♂
Hemoglobin Content in Grams %
15
9
3
120
200
280
360
Body Weight in Grams

FIGURE 5

Electrophoretic patterns of male (♂), female (♀), and a mixture (M) of male and female blood cell proteins of *Leptodactylus fallax*. Gels were stained with napthol blue black.



(−)
ORIGIN

(+)

TABLE 1

BLOOD OXYGEN CAPACITY, ERYTHROCYTE COUNT, AND HEMOGLOBIN CONTENT IN *LEPTODACTYLUS FALLAX*

	B. O. C.			R. B. C.			Hb.		
	n	$\bar{x}$	S. E.	n	$\bar{x}$	S. E.	n	$\bar{x}$	S. E.
Total Sample	50	10.5	0.4	10	672,000	42,000	10	10.9	1.1
High Elevation	5	12.5	0.6	-	-				
Low Elevation	45	10.3	0.5	-	-				
Probability that difference is zero[a]	-	$P > 0.05$						-	
Males	25	12.5	0.4	5	600,000	39,000	5	10.8	1.8
Females	25	8.5	0.5	5	744,000	39,000	5	11.1	1.3
Probability that difference is zero[a]	-	$P < 0.01$			$P < 0.05$		-	$P > 0.05$	

[a] Blood oxygen capacity values adjusted for differences in body weight before application of "t" test.

B. O. C. = Blood oxygen capacity in volumes percent
R. B. C. = Erythrocyte count in cells per mm^3
Hb. = Hemoglobin content in grams percent
n = Sample size
$\bar{x}$ = Sample mean
S. E. = Standard error of the mean

TABLE 2

RELATIONSHIP BETWEEN BODY WEIGHT AND BLOOD OXYGEN CAPACITY, ERYTHROCYTE COUNT, AND HEMOGLOBIN CONTENT IN *LEPTODACTYLUS FALLAX*

Regression Equation	Correlation Coefficient	Probability that Slope is zero
Males		
$B.O.C. = 10.5 + 0.0114\ Wt.$	0.18	$P>0.05$
Females		
$B.O.C. = 7.14 + 0.0087\ Wt.$	0.44	$P<0.05$
Total Sample		
$B.O.C. = 8.76 + 0.0105\ Wt.$	0.32	$P<0.05$
$\text{Log}\left[B.O.C./Wt.\right] = 0.47 - 0.76\ \text{Log}\ Wt.$	-0.77	$P<0.01$
$R.B.C. = 60.6 + 0.03\ Wt.$	0.14	$P>0.05$
$Hb. = 11.6 - 0.003\ Wt.$	-0.06	$P>0.05$

B.O.C. = Blood oxygen capacity in volumes percent

Wt. = Body weight in grams

R.B.C. = Erythrocyte count in cells per $mm^3 \times 10^{-4}$

Hb. = Hemoglobin content in grams percent

TABLE 3

WAVELENGTHS (mμ) OF MAXIMUM ABSORPTION OF OXYHEMOGLOBIN AND CARBON MONOXYHEMOGLOBIN OF MALE AND FEMALE *LEPTODACTYLUS FALLAX*

	Oxyhemoglobin	Carbon Monoxyhemoglobin
Male	576.4, 540.0	571.4, 535.6
Female	577.8, 539.1	571.4, 535.8

DISCUSSION

The variation in blood oxygen capacity within each vertebrate class is greater than the variation among classes. Blood oxygen capacity values range from 4.9 to 19.8 vol. % among fishes (Prosser and Brown, 1961); from 5.3 to 14.8 vol. % among amphibians (Scott, 1931; Johansen and Ditadi, 1966); from 5.9 to 12.6 vol. % among reptiles (Payne and Burke, 1964; Dawson and Poulson, 1962); from 10.5 to 20.0 vol. % among birds (Prosser and Brown, 1961); and from 13.1 to 29.3 vol. % among mammals (Prosser and Brown, 1961; Larimer, 1959).

Correlation of blood oxygen capacity with habitat and activity has been demonstrated for fishes, reptiles, and mammals. Fish which inhabit sluggish, acid waters have blood oxygen capacity values which are significantly higher than the values from fish occupying flowing waters; and active fish have higher oxygen capacities than sluggish ones (Prosser and Brown, 1961). Species of fish which inhabit cold waters, where metabolism would be low, have lower blood oxygen capacities than those inhabiting warmer waters, where metabolism would be greater (Scholander and van Dam, 1957). However, Burke and Woolcott (1957) showed that although the black crappie (*Pomoxis nigromaculatus*) and the bluegill (*Lepomis macrochirus*) are in the same family and occupy the same habitat, their blood oxygen capacities are significantly different. Whether there is a difference in metabolic rate or activity is not known. Species of lizards which live at high elevations do not exhibit significantly different oxygen capacity values than those from lower elevations even though there is a large drop in ambient oxygen pressure (Dawson and Poulson, 1962). The active,aquatic turtle, Chrysemys picta, has a higher blood oxygen capacity than the more sluggish,terrestrial box turtle, Terrapene carolina (Payne and Burke, 1964).

The correlation between habitat and blood oxygen capacity is clearly evident within the class Amphibia. As mentioned in the Introduction, aquatic amphibians have lower blood oxygen capacities than terrestrial or arboreal forms. The terrestrial habitat may be related to an increased metabolic rate in anurans since Cronheim showed that semi-terrestrial frogs have a higher rate of oxygen consumption than aquatic frogs (in Leftwich and Burke, 1964). Terrestrial salamanders of the genus Ambystoma have higher rates of oxygen consumption than those from an aquatic environment (Helff, 1927). Thus, the high blood oxygen capacity of terrestrial amphibians would appear to assist in maintaining a high metabolic rate.

The blood oxygen capacity of the semi-terrestrial Leptodactylus fallax, 10.5 vol. %, is of the same magnitude as the values for other semi-terrestrial and terrestrial anurans. Therefore, it may be concluded that blood oxygen capacity reflects habitat as it does in other amphibians.

Because terrestrial and semi-terrestrial anurans have higher rates of oxygen consumption and can utilize their skin as a respiratory surface to a lesser extent than can aquatic species, terrestrial frogs require more efficient oxygen transport than aquatic frogs (Krogh, 1904, in Foxon, 1964). The work of Czopek (1965b) indicated that, in general, terrestrial and arboreal anurans have a smaller percentage of their total respiratory capillary length in their skin than do aquatic frogs. Also, tests of survival of submergence in well aerated water indicate that aquatic anurans can use their skin for respiratory exchange to a much greater extent than can terrestrial species (Czopek, 1965a; Hutchi~~n~~son and Dady, 1964; Hutchi~~n~~son and Whitford, 1966). Apparently the higher blood oxygen capacity of terrestrial frogs does not compensate for the lack of respiratory capillaries in the skin in such experiments. Although *L. fallax* is a semi-terrestrial frog, the tropical environment in which it lives has a relatively high moisture content (Brooks, unpublished). Whether the skin is well supplied with capillaries which would allow substantial cutaneous respiration has not been investigated.

Variation of blood oxygen capacity with changes in altitude within a species has not been shown for any poikilothermic vertebrate. Since the highest elevation at which Leptodactylus fallax has been found is 1,200 feet, decrease in ambient oxygen level would not be great enough to require increased oxygen transport efficiency of the blood.

The tendency for blood oxygen capacity to increase with an increase in body weight among individuals of the same species has been shown for fish (Burke and Woolcott, 1957), frogs (Leftwich, 1958), turtles (Payne, 1957, in Leftwich, 1958), and mammals (Burke, 1953, 1957). None of these studies include regression equations expressing the relationship between body weight and blood oxygen capacity. The increase in blood oxygen capacity with an increase in weight which occurs in Leptodactylus fallax, therefore, cannot be compared statistically with increases found in other organisms. It is interesting to note that when blood oxygen capacity is regressed on weight separately for each sex (Table 2), the slope of the regression line is greater for males than for females by a highly significant ($P<0.01$) margin. However, the male correlation coefficient is low, 0.18, and the slope is not significantly different from zero ($P>0.05$), while the female correlation coefficient is somewhat higher, 0.44, and the female regression coefficient is significantly ($P<0.05$) different from zero. Therefore, it may be concluded that in those males which were examined, body weight does not have a significant influence on blood oxygen capacity, while the opposite is true for females. However, it must be borne in mind that the males which were tested ranged in weight from 133.6 to 280.3 grams, while females had a weight range of 11.8 to 529.3 grams. Such a statement may not apply to the total population where the weight range for males is certainly larger.

There is an increase in oxygen consumption per unit time per individual with an increase in body weight among animals (Zeuthen, 1953). Studies on amphibians in which oxygen consumption is expressed as an increasing function of body weight include those of Norris *et. al.* (1963) and Whitford and Hutchinson (1967) on salamanders, and of Davison (1955) on frogs. The increase in blood oxygen capacity which occurs with an increase in body weight in vertebrates, therefore, parallels the increase in metabolism with an increase in body weight. Such an increase in blood oxygen capacity might be due to an increase in the erythrocyte number, in the hemoglobin concentration, or in the oxygen affinity of hemoglobin, as will be discussed below.

A much more useful and biologically meaningful presentation of the relationship between body weight and blood oxygen capacity can be obtained by examining the change in blood oxygen capacity in vol. % per gram of body weight with an increase in body weight. A decrease in blood oxygen capacity per gram of body weight with an increase in body weight has been reported for many representatives of all vertebrate classes, and is the case with *Leptodactylus fallax*. Burke (1966) presents log—log regression equations, correlation coefficients, and graphs of this relationship for data gathered by himself and other workers on several species of vertebrates. The regression coefficients for the three ranid frogs examined by Leftwich and Burke ranged from -0.69 to -0.81. The slope for the regression line calculated for *L. fallax*, -0.76, falls within this range. The correlation coefficient, which measures the strength of association between the two factors, is -0.77, somewhat less than the values found for the three species of frogs, for fishes, birds, and mammals. However, it is of the same magnitude as those coefficients found for two species of turtles (Burke, 1966).

The relationship between blood oxygen capacity per unit of body weight and body weight can be compared with the relationships between metabolic rate per unit weight and body weight and between blood volume per unit weight and body weight. There is a decrease in oxygen consumption per gram of body weight with an increase in body weight within and among several species of anurans (Davison, 1955; Pettus and Spencer, 1964). A similar trend within and among species has been observed in urodeles (Evans, 1939; Helff, 1927; Norris *et. al.*, 1963; Vernberg, 1952, 1955). The decrease in metabolism per unit body weight with an increase in body weight has been attributed to a relative increase in tissues with low oxygen consumption, such as connective tissue, and to a decrease in the surface to volume ratio which would cause decreased cutaneous respiration per unit of body weight in large amphibians (Prosser and Brown, 1961; Szarski, 1964). Blood volume studies on frogs (Prosser and Weinstein, 1950), turtles (Hutton, 1961), birds (Bond and Gilbert, 1958), and mammals (Burke, 1954, 1957) indicate that as body weight increases, blood volume per unit of body weight decreases. Therefore, the change in blood oxygen capacity which occurs with a change in body weight parallels the change in metabolism and blood volume. Since oxygen consumption and blood volume were not measured for _L. fallax_, no correlation between these factors and blood oxygen capacity can be made. No studies known to the author have involved the determination of oxygen consumption, blood volume, and blood oxygen capacity on the same series of animals of one species. An investigation of this type would clarify the relationship among these three factors.

Sexual differences in blood oxygen capacity were not found in fish (Burke and Woolcott, 1957), turtles (Payne and Burke, 1964), or rats (Burke, 1957). Dawson and Poulson (1962) failed to mention if any difference in blood oxygen capacity between male and female lizards existed. Leftwich (1958) found that there was no significant sexual difference in blood oxygen capacity in _Rana pipiens_, even though the average weight of females was 12.1 grams greater than the average weight of males. However, he found significant sexual differences in blood oxygen capacity for _R. clamitans_ and _R. catesbeiana_. The basis for this sexual dimorphism is obscured by the fact that the average weights for females and males of the two species differed by 23.8 grams and 65.6 grams, respectively. Since larger animals have higher blood oxygen capacities, the cause for the higher average values in females may be their higher average weights. For _Leptodactylus fallax_, analysis of variance using blood oxygen capacity values adjusted for differences in body weight showed that sex was a highly significant source of variation (F=1, 270). The basis for such a difference will be discussed below where sexual differences in erythrocyte count and hemoglobin concentration are mentioned. Noble (1954) states that male frogs have higher metabolic rates than females during the breeding season; therefore, oxygen demands would be greater in male frogs. Thus, higher blood oxygen capacity in males would have a physiologically adaptive value during this time of the year.

It is interesting that males, whose weight averaged 171.7 grams, had a higher blood oxygen capacity than females, whose mean weight was 157.9 grams. This situation would be expected since, as shown above, larger animals generally have greater blood oxygen capacities. However, when the mean blood oxygen capacities for males and females are divided by their respective mean weights, male blood oxygen capacity per gram of body weight is 0.073, while the corresponding value for females is 0.054. Since it has been shown above that larger animals in general have lower blood oxygen capacity per unit weight than smaller animals, it appears that male blood oxygen capacity per unit weight is greater than would normally be expected.

There is also considerable variation in erythrocyte number among vertebrates. Homiotherms generally have a greater and less variable red blood cell count than poikilotherms. Hutchinson and Szarski (1965) point out that the regulation of erythrocyte number by feedback mechanisms is much less precise in poikilotherms than in homiotherms because of their fluctuating body temperature and metabolism. Thus erythrocyte counts should not be regarded as a reliable indicator of physiological condition unless large, uniform samples are taken. Since only 10 specimens of *L. fallax* were used for erythrocyte counts and since the animals used had been maintained in an unnatural environment for a period of 11 months, the determinations may not represent the true red cell density of this species in its natural habitat. Also, since blood oxygen capacity was not measured in these 10 animals, this response cannot be plotted as a function of erythrocyte count. The same is true for the hemoglobin concentration determinations.

As mentioned in the Introduction, urodeles generally have lower red cell counts than ranid frogs, which in turn have lower counts than bufonids and hylids. Since there is an inverse relationship between erythrocyte number and size, urodeles have larger red cells than anurans (Hartman and Lessler, 1964). High red cell count and small size lead to greater efficiency in respiratory exchange and may allow higher metabolism (Vernberg, 1955).

Terrestrial and arboreal anurans have a relatively high number of red blood cells per cubic millimeter: *Bufo americanus*, 658,000; *B. marinus*, 817,000; *Hyla versicolor*, 615,250 (Hutchi~~n~~son and Szarski, 1965; Hall, 1966). Semi-terrestrial frogs have intermediate counts: *Rana temporaria*, 400,000; *R. pipiens*, 379,000 (Foxon, 1964; Leftwich and Burke, 1964). Aquatic ranids have relatively low counts: *R. clamitans*, 320,000; *R. catesbeiana*, 280,000 (Leftwich and Burke, 1964). A similar trend is absent in the order Urodela: the blood of terrestrial salamanders is not significantly different from that of aquatic species (Andrew, 1965). *Leptodactylus fallax* has an average erythrocyte count of 672,000 which is in the same range as that of other terrestrial anurans. The relatively high red cell count found here may partially explain the relatively high blood oxygen capacity of this species.

Both Roofe (1961) and Noble (1954) state that aquatic amphibians have lower erythrocyte counts than terrestrial amphibians because of the dilution of the blood of aquatic forms. Whether or not this is actually the case among anurans is not known and should be experimentally investigated.

In *L. fallax* there is no significant (P>0.05) increase of red blood cell count with an increase in weight, as shown in Figure 3. The correlation coefficient for this relationship is very low, 0.14, indicating that there is little association between weight and erythrocyte count. Therefore, the increase in blood oxygen capacity with an increase in body weight cannot be attributed to changes in erythrocyte number. Lack of effect of body weight on red cell number was also found in *Ambystoma tigrinum* (Roofe, 1961) and in *Bufo melanosticus* (Church, 1961). Leftwich's data (1958) indicate that the same is true for *Rana pipiens*.

With regard to sex, erythrocyte counts are generally higher in male amphibians than in females; such is the case in *Ambystoma tigrinum* (Roofe, 1961), *Rana temporaria* (Foxon, 1964), and *R. pipiens* (Leftwich, 1958). Noble (1954) states that male frogs have higher red cell counts than females, but gives no supporting data. Conflicting data is reported by Foxon (1964) who cites the work of Kaplan who found that *R. pipiens* females had higher red cell counts than males. However, absence of sexual differences in red cell count has been reported for several amphibians (Hutchinson and Szarski, 1965; Foxon, 1964). Conflicting reports of sexual differences in erythrocyte counts have been made for turtles (Altland and Thompson, 1958; Gaumer and Goodnight, 1957). The difference in red cell count between males and females of L. *fallax* has statistical significance ($P < 0.05$), with females having a higher count than males. However, this difference has little biological significance, since hemoglobin concentrations of male and female blood are approximately the same, as will be discussed below, and since males have a higher blood oxygen capacity than females. Whether there is a sexual difference in metabolic rate or erythrocyte size is not known.

Hemoglobin concentration of vertebrate blood varies over a wide range, with homiotherms generally having higher values than poikilotherms (Prosser and Brown, 1961). Goin and Jackson (1965) point out that the wide range of hemoglobin values among cold-blooded vertebrates, both inter- and intra-specifically, is to be expected since hemoglobin concentration is dependent on many variables such as ontogeny, activity, altitude, and temperature, each of which fluctuates widely. However, there is a general trend among poikilotherms for blood hemoglobin concentration to be correlated with habitat and activity. Fishes living in cold waters have lower metabolism and hemoglobin concentration than those living in warmer waters, and active species have higher hemoglobin content than sluggish ones (Scholander and van Dam, 1957). The active, aquatic snapping turtle, *Chelydra serpentina*, has a higher hemoglobin content and a more sigmoid oxygen equilibrium curve than the less active, terrestrial *Terrapene carolina* (Gaumer and Goodnight, 1957). The opposite trend is found in anurans where terrestrial species are generally more active than aquatic forms (Cronheim, 1927, in Leftwich and Burke, 1964). The aquatic *Rana clamitans* and *R. catesbeiana* both have a hemoglobin content of 6.5 gm. %, while the semi-terrestrial *R. pipiens* exhibited a value of 9.9 gm. % (Leftwich and Burke, 1964). Four species of *Bufo* had values ranging from 10.2 to 12.8 gm. % (Goin and Jackson, 1965; Hall, 1966; Johansen and Ditadi, 1966). *Hyla septentrionalis* and *H. cinerea* had hemoglobin values of 9.4 and 11.3 gm. %, respectively (Goin and Jackson, 1965). *Bufo marinus*, which inhabits lowland areas up to 1,500 meters, had blood with 8.66 gm. % hemoglobin, while *B. bocourti*, which is found in upland areas to 3,600 meters, had a value of 10.57 gm. % (Stuart, 1951). Stuart (1951) cites Alder and Huber who found that the hemoglobin values of a lowland bufonid, hylid, and microhylid of Europe ranged from 7.5 to 13.5 gm. %, while the alpine toad, *Bombinator pachypus*, had values of from 14.3 to 15.5 gm. %. The hemoglobin content determined for the blood of *L. fallax*, 10.9 gm. %, is in the same range as those found for other terrestrial anurans, and may contribute to the relatively high blood oxygen capacity of this species.

A highly significant increase in hemoglobin concentration with an increase in body weight was found in Bufo bocourti but not in B. marinus (Stuart, 1951). Hemoglobin concentration increases with body weight in Bufo melanosticus (Church, 1961), but not in Ambystoma tigrinum (Roofe, 1961). Leftwich's data (1958) indicates that there is a definite increase in hemoglobin concentration with an increase in body weight in Rana pipiens, R. clamitans, and R. catesbeiana. However, statistical analyses which would indicate the significance of such an increase were not presented. There is essentially no change in hemoglobin concentration with increasing body weight in Leptodactylus fallax, as indicated in Figure 4. Also, the correlation coefficient is quite low, -0.06, indicating a weak association between body weight and hemoglobin concentration. Therefore, as an animal increases in weight, the increase in blood oxygen capacity may be due to an increase in the oxygen affinity of the hemoglobin, rather than to an increase in hemoglobin concentration. Such an increase in oxygen affinity with an increase in body weight has been shown in mammals by Foreman (1954) and Schmidt-Nielsen and Larimer (1958).

Significant differences in hemoglobin content of the blood of males and females have been shown to be absent in R. pipiens, R. catesbeiana (Leftwich, 1958), Bufo bocourti (Stuart, 1951) and Ambystoma tigrinum (Roofe, 1961). Sexual dimorphism in hemoglobin concentration has been reported for R. clamitans, although statistical interpretation of this difference was not furnished (Leftwich, 1958). Significant sexual differences in hemoglobin content are absent in turtles (Gaumer and Goodnight, 1957; Altland and Thompson, 1958) but present in adult humans (Haden, 1940). The difference in hemoglobin content between males and females of L. fallax is not statistically significant ($P>0.05$). Therefore, the difference in blood oxygen capacity between sexes must be due to some other factor, as will be discussed below.

Foreman (1954) and Schmidt-Nielsen and Larimer (1958) show that in mammals oxygen affinity of the blood increases with increasing body weight. Since males of L. fallax, on the average, weigh more than females, and since males and females have approximately the same amount of hemoglobin, it is possible that the greater oxygen capacity of male blood is due to a greater oxygen affinity of male hemoglobin. The basis for such a difference in blood oxygen affinity is not immediately clear.

It is well known that hemoglobin produced by different organs at different stages in the life history of anurans have different molecular structures and thus different oxygen capacities (Hamada and Shukuya, 1966; McCutcheon, 1936; Trader and Frieden, 1966). Since hemoglobins with different structures have different oxygen affinities, structural differences between male and female hemoglobins might be the cause of the different oxygen capacities, even though no sexual difference in hemoglobin concentration occurs. Therefore, a preliminary investigation was undertaken to determine if male and female hemoglobins of *L. fallax* had dissimilar structures.

Electrophoresis of the hemoglobin of several species of anurans has indicated that the number of components and their relative mobilities vary considerably, and that these electrophoretic patterns can be useful in taxonomic studies (Dessauer *et al.*, 1957; Rodnan and Ebaugh, 1957). Toads of the genus *Bufo* may exhibit one or two hemoglobin components (Fox *et al.*, 1961; Guttman, 1967). The electrophoretic patterns of hemoglobin of tadpoles and adults are dissimilar in both *Rana catesbeiana* (Hamada *et al.*, 1964) and *R. grylio* (Bennett and Frieden, 1962). Three of the five species of South American *Leptodactylus* studied by Bertini and Rathe (1962) have two hemoglobin components, the other two having one and three components, respectively. Since paper electrophoresis was used in these studies on Leptodactylids, their electrophoretic patterns cannot be compared quantitatively with the pattern of *L. fallax* blood reported here. No other studies on *Leptodactylus* hemoglobin are known to the author.

No mention of sexual differences in electrophoretic pattern was made by any of these authors. Hutchi~~n~~son (personal communication) knows of no such sexual differences in amphibian or reptilian hemoglobin electrophoretic patterns. The fact that the electrophoretic patterns of the proteins of erythrocytes of male and female Leptodactylus fallax were identical is an argument against sexual dimorphism in hemoglobin structure (Dessauer, 1966).

The absorption spectrum for L. fallax oxyhemoglobin has very nearly the same maxima as those found for tadpoles and adults of the bullfrog (Bennett and Frieden, 1962), and even for the turtle, Pseudemys scripta (Ramirez and Dessauer, 1957). The absorption bands for the carbon monoxide form of L. fallax hemoglobin correspond very closely to those found in man (Prosser and Brown, 1961). The differences in wavelengths of maximum absorption between males and females for both forms of hemoglobin are not great enough to justify the conclusion that there is a significant difference in hemoglobin type between the sexes.

If it is assumed that anuran blood binds 1.34 ml of oxygen per gram of hemoglobin, the blood of Leptodactylus fallax should have an oxygen capacity of 14.6 vol. % (Hall, 1966). However, since the observed oxygen capacity is 10.5 vol. %, approximately 30% of the hemoglobin present is not transporting oxygen. Blood of snakes, dogs, and humans contains methemoglobin, an oxidized form of hemoglobin not capable of combining reversibly with oxygen (Prado, 1946). In fact, all 23 specimens of the snake, Bothrops jararaca which were examined by Prado (1946) had methemoglobin in their blood, varying in percentage of total hemoglobin from 6.0 to 28.0% and averaging 17.0%. Methemoglobin may comprise 5 to 90% of the total hemoglobin of turtles (Sullivan and Riggs, 1964). However, since methemoglobin has a wavelength of maximum absorption of 635 mμ (Hawk *et al.*, 1954), and since an absorption band at that wavelength was not seen in the absorption spectrum of *L. fallax* hemoglobin, it is probable that methemoglobin is not present and is therefore not responsible for the inactivity of some of the hemoglobin of *L. fallax*. Using the same method of calculation, it appears that 13% of the hemoglobin is inactive (non-oxygen binding) in males and 43% in females. The molecular basis for this sexual dimorphism in the oxygen-binding ability of hemoglobin is not known. However, it is possible that such a difference may be genetically controlled since sexual differences in erythrocyte proteins determined by sex chromosomes are well known (Ohno *et al.*, 1965).

In 1935, Hall reported that chick hemoglobin has a greater affinity for oxygen than does adult chicken hemoglobin, and that there are two distinct hemoglobin types, one occuring early in incubation and the other replacing it during hatching. Huisman and van Veen (1964) found that these changes are accompanied by a decrease in the phosphate content of red cells and that lower phosphate levels decrease the ability of adult chicken hemoglobin to bind oxygen. Huisman *et al.* (1964), however, found that there were two components in adult hemoglobin and that while these hemoglobins had identical absorption spectra in the oxy- and carbon monoxy- states and identical molecular weights, they differed in their oxygen affinity, heme-heme interaction, and physiological properties when treated with identical salt solutions. Thus, it appears that the intracellular concentration of inorganic ions can affect the oxygen affinity of the blood of adult chickens. Whether a difference in ion concentration or in oxygen affinity exists between the blood of males and females of _L. fallax_ is not known, and might be investigated profitably. If such a difference exists, it might explain the difference in blood oxygen capacity between males and females.

SUMMARY

1. The blood oxygen capacity of 25 male and 25 female *Leptodactylus fallax*, a frog endemic to the island of Dominica, British West Indies, was determined by the microgasometric syringe method.
2. Erythrocyte counts of 5 males and 5 females were made using standard techniques. Hemoglobin concentrations of the blood of 5 males and 5 females were determined by the unopette-colorimeter method, using a Spectronic 20 Colorimeter.
3. *Leptodactylus fallax* has a mean blood oxygen capacity of 10.5 vol. %. There is no significant difference in blood oxygen capacity between populations living at 1,200 feet and at sea level. Males and females have mean values of 12.5 and 8.5 vol. %, respectively, the difference between sexes being statistically significant after blood oxygen capacity values are adjusted for differences in body weight.
4. The mean erythrocyte count is 672,000 cells per mm^3. Females have a significantly higher count (744,000 cells per mm^3) than males (600,000 cells per mm^3). The mean hemoglobin content is 10.9 gm. %, with no significant sexual difference being present.
5. There is a significant increase in blood oxygen capacity and a highly significant decrease in blood oxygen capacity per gram of body weight with an increase in body weight. These trends accompany

the tendency for blood volume per gram of body weight and metabolism per gram of body weight to decrease with an increase in body weight. There is no significant change in erythrocyte count or hemoglobin content with an increase in body weight.

6. The electrophoretic patterns at pH 8.3 of male, female, and of a mixture of male and female blood cell proteins are identical. Each blood sample was separated into two components, the major component having the greater mobility.
7. Examination of the absorption spectra of male and female blood indicates that there is no significant sexual difference in the wavelengths of maximum absorption when hemoglobin is combined with oxygen or with carbon monoxide.
8. It was concluded that the sexual difference in blood oxygen capacity is due to some factor not revealed by the methods employed.

APPENDIX A

Sex	Body Weight in grams	Snout-vent Length in Mm	Location[a]	Blood Oxygen Capacity in Vol. %
F	11.8	48	H	4.9
F	22.2	67	H	7.5
F	36.6	81	H	7.0
F	41.5	91	H	6.7
F	47.5	93	H	6.9
F	61.0	89	H	6.8
F	64.0	101	H	4.7
F	68.0	95	H	11.2
F	81.5	103	H	6.3
F	84.7	101	M	8.7
F	93.4	106	M	7.3
F	93.9	107	H	8.1
F	106.7	112	H	10.2
M	133.6	117	S	11.2
M	137.5	121	H	10.7
M	137.5	121	H	8.2
M	147.2	116	H	12.4
F	147.5	119	H	10.3
M	148.0	122	M	12.7
M	148.7	124	H	13.7
M	148.8	130	H	12.8
M	151.5	125	H	12.0
M	152.1	122	M	11.7
M	152.3	121	M	10.0
M	156.7	126	H	13.6
M	164.9	126	H	11.3
F	165.0	122	H	8.7
F	165.6	126	H	13.1
M	167.1	129	M	12.6
M	171.4	119	M	14.7
M	171.5	130	H	12.7
M	172.7	131	M	14.0
M	178.6	127	H	18.3
M	180.0	126	S	14.3
M	183.6	121	H	11.0

APPENDIX A--Continued

Sex	Body Weight in grams	Snout-vent Length in Mm	Location[a]	Blood Oxygen Capacity in Vol. %
M	186.1	132	M	11.6
M	186.6	126	H	10.2
F	190.8	125	H	8.9
M	200.7	139	H	11.9
F	204.5	137	H	12.3
M	211.6	133	M	16.1
F	216.2	135	H	9.8
M	223.0	134	S	12.5
M	280.3	141	S	11.4
F	283.6	151	M	10.7
F	297.5	153	M	7.9
F	308.1	156	M	7.6
F	312.6	151	H	4.0
F	313.9	147	H	10.3
F	529.3	169	S	13.1
Mean	164.8	121		10.5

[a] H: Hillsborough Estate, elevation 50-100 feet above sea level
M: Mannet's Gutter, elevation 200-400 feet above sea level
S: Springfield Estate, elevation 1,200 feet above sea level

APPENDIX B

Sex	Body Weight in grams	Snout-vent Length in Mm	Erythrocyte Count[a]	Hemoglobin Content[b]
F	127.7	111	72	12.6
M	166.0	112	41	5.1
F	178.0	120	88	10.9
M	195.3	124	80	16.6
M	198.3	126	58	11.6
M	216.7	133	62	10.1
M	220.8	133	59	10.4
F	285.9	139	64	8.5
F	294.7	145	72	15.3
F	323.3	146	76	8.2
Mean	220.7	129	67	10.9

[a] In cells per $mm^3 \times 10^{-4}$

[b] In gm. %

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