Hazard, Edward Starr, III

ERYTHROCYTIC PHOSPHATES IN AMPHIBIANS

The University of Oklahoma PH.D. 1981

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __√__.

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages __✓__
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Other _____

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ERYTHROCYTIC PHOSPHATES IN AMPHIBIANS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

EDWARD STARR HAZARD III

NORMAN, OKLAHOMA

1981

ERYTHROCYTIC ORGANIC PHOSPHATES IN AMPHIBIANS

APPROVED BY

Victor H. Shoemaker

Howard Haines

Francis J. Sorlentino

Eddie Carol Smith

ACKNOWLEDGEMENTS

Educating a physiologist is a complex task requiring considerable effort from all involved. The realities of my graduate education have fallen far short of some of my expectations and far exceeded others. None of the principal people involved in my education, including myself, has come up to all of the ideals that I imagined some years ago. Nevertheless, my spirits have been buoyed up along the way by the metaphor of a tropical storm, slowly gathering strength before bursting on the populated coasts with a fury. The metaphor has a large number of amusing attributes but training a mind in Oklahoma and the gathering of a storm in the tropics seem to have a number of interesting parallels including the unpredictable impact each may have on the future.

I am indebted to my major professor, V.H. Hutchison, for leaving me alone for the most part and for listening to some of my ideas. I thank Drs. Howard Haines, Kirk Miller, Eddie Smith and Frank Sonleitner for their efforts and comments at various points during the writing of this dissertation. In additiion, my colleagues, Beth Braun, Dale Erskine and Andy Hughes reviewed early drafts. Brian Paulson and Robert Rawding provided fresh minds for proof-reading chores.

I must also express gratitude to my family for enduring long periods of silence while I pursued mysterious goals down here in Norman. They have listened and graciously acted as if they understood my strange utterances.

I have been given much advice and plenty of comments about the text of this dissertation. I have followed most but not absolutely all of the suggestions. Consequently, all sins of omission and commission remain exclusively my responsibility.

TABLE OF CONTENTS

LIST OF TABLES

PAPER I

PAPER II

LIST OF ILLUSTRATIONS

PAPER I

PAPER II

PAPER I

DISTRIBUTION OF ACID SOLUBLE PHOSPHATES IN THE ERYTHROCYTES OF SELECTED SPECIES OF AMPHIBIANS

This paper was prepared for
submission to the journal,
Comparative Biochemistry and Physiology

ABSTRACT

1. Enzymatic, colorimetric and /or ion-exchange chromatographic analyses of amphibian erythrocytic organic phosphates (EOPs) were performed to: 1) explore interspecific diversity, 2) determine the intraspecific effects of temperature, photoperiod and body-size, and 3) correlate interspecific EOP similarities with the behavioral and physiological capabilities.

2. The types and concentrations of EOPs ranged from reptilian to mammalian in character and responded weakly to temperature and photoperiod.

3. Inositol polyphosphate was detected in 5 species but never exceeded 5% of the total acid-soluble phosphate. 2,3-diphosphoglycerate (DPG) was the major EOP in 18 species and was present in all species examined. DPG:hemoglobin (Hb) molar ratios ranged from 0.07 to 1.9. Nucleoside triphosphate (NTP) was the major EOP in 12 species. NTP:Hb molar ratios ranged from 0.32 to 1.8.

4. Analysis of similarity among species based on body mass,[Hb], hematocrit, [NTP] and [DPG] revealed three clusters of amphibians. EOP:Hb molar ratios of 1.0 or larger in bufonids and Scaphiopus correlated with high dependence on aerobic activity

metabolism. EOP:Hb molar ratios less than one in ranids correlated with a dependence on anaerobic activity metabolism.

KEY WORDS

ERYTHROCYTIC ORGANIC PHOSPHATES, AMPHIBIA, 2,3-DIPHOSPHOGLYCERATE, ADENOSINE TRIPHOSPHATE, GUANOSINE TRIPHOSPHATE, ION-EXCHANGE CHROMATOGRAPHY, HEMATOCRIT, ANURA, URODELES

INTRODUCTION

Large concentrations of organic phosphate compounds occur in the erythrocytes of vertebrates (Greenwald, 1925; Rapoport & Guest, 1941; Johnson & Tate, 1969; Bartlett, 1970, 1976, 1978; Geoghegan & Poluhowich, 1974; Isaacks & Harkness, 1975; Isaacks et al., 1976, 1977, 1978; Borgese et al., 1978). These include adenosine triphosphate (ATP), 2,3-diphosphoglycerate (DPG), guanosine triphosphate (GTP) and several phosphorylated derivatives of inositol. The role of these compounds in regulating O_2 affinity of hemoglobin (Hb) became apparent after 1967 (Chanutin & Curnish, 1967; Benesch et al., 1968; Brewer et al., 1974). In mammals the major erythrocytic organic phosphate (EOP) is DPG. By binding and thus stabilizing the deoxy state of Hb, DPG reduces the affinity of Hb for oxygen. Under normoxic conditions this reduction in O_2 affinity can lead to an improved capacity for O_2 delivery to the tissues.

Rapid improvement of O_2 delivery can be achieved via increased ventilation or cardiac output; however, these physiological responses involve costly muscular effort. Alternatively, improved O_2 delivery can be achieved with higher

[Hb], but this anabolic process can not occur rapidly. Rapid (seconds to hours) increases in O_2 demand are probably met via increased ventilation or perfusion. Long term (days to weeks) changes in O_2 demand probably elicit increased [Hb] while during intermediate time periods (hours to days) altered O_2 affinity resulting from changes in EOP levels may be preferable. As modulators of O_2 affinity EOPs are one potentially labile parameter in the O_2 delivery process. The EOPs function in a similar fashion in most vertebrate red cells.

Amphibians are the only extant representatives of the stem groups which later gave rise to mammals and reptiles. By studying modern (i.e. highly specialized) amphibians, the evolutionary forces which shaped the primitive amphibians may be revealed as well as some of the physiological constraints within which the higher vertebrates later evolved (Gans, 1970; Romer, 1972; Packard, 1976). Amphibians of various sizes exist in a wide variety of temperate, tropical, aquatic and xeric habitats. These diverse environments have a substantial influence on metabolism and respiration. Amphibian respiration is quite varied; both bimodal (cutaneous and pulmonary) and trimodal (branchial, cutaneous and pulmonary) respiratory modes are known. The increased O_2 requirements resulting from elevated temperatures or activity are supplied almost entirely by increased pulmonary

ventilation rather than by increased branchial or cutaneous respiration (Gottlieb & Jackson, 1976; Miller & Hutchison, 1979; Miller and Hutchison, 1980). The energy expended per unit O_2 delivered in amphibians is likely to be higher than in mammals due to the positive pressure pulmonary ventilation and the incomplete separation of pulmocutaneous and systemic circulations (Gans, et al., 1969; Meyers & Felsher, 1976). Red blood cells survive 700-1400 days in some amphibians; thus, the erythropoietic activity of amphibians is not well developed compared to mammals (Altland & Brace, 1962). All amphibian red cells are nucleated, yet relatively little is known about the metabolism of such cells (Araki et al., 1971; Wood, 1971; Johansen & Lenfant, 1972; Coates, 1975b; Wood et al., 1975; Hazard & Hutchison, 1978; Garlick et al., 1979; Jokumsen and Weber, 1980). The unfavorable energetics of increased ventilation and perfusion and the slower response time of amphibian erythropoiesis suggest that these usual vertebrate reactions to hypoxia are less efficient in amphibians than in mammals and that altering EOP levels to improve O_2 delivery is likely.

This study was undertaken to determine the qualitative and quantitative features of amphibian EOPs and to monitor the responses of the EOPs to the environmentally pervasive factors of temperature and photoperiod. The effects of body size, geographic

origin and ontogenetic status on these EOPs were monitored. Twenty-two species of anurans and 8 of urodeles were selected as representative of the physiological and ecological diversity of amphibians. We hypothesized that due to their evolutionary position and because of their variety of respiratory mechanisms, habitat diversity and size range, amphibians would exhibit a qualitative and quantitative diversity of EOPs and hematological parameters (phenotypic characters affecting respiration). Phenotypic similarity within amphibians was assumed to reflect comparable respiratory strategies for coping with analogous evolutionary forces (Sneath & Sokal, 1973). Clustering of physiologically similar phenotypes has been a valuable technique in comparative vertebrate studies. Mammals, for example, have been classified into two categories based on their red cell [DPG] (Bunn et al., 1974; Kay, 1977). The low [DPG] group included the family Felidae and the ruminants; these mammals are all sprinters whose primary prey capture/predator avoidance strategy involves short, anaerobically-fueled dashes. The Hbs of these sprinters are refractory to DPG effects because of altered primary structures (Bunn et al., 1974; Taketa, 1974; Kay, 1977); and have a generally low O_2 affinity (high P_{50}). Mammals in the high [DPG] group possess DPG-sensitive Hbs and activity characterized by extended periods of aerobic work. Based on activity metabolism

and life styles, amphibians have also been divided into two groups (Seymour, 1973; Bennett & Licht, 1974; Bennett, 1978; Hutchison & Miller, 1979a). The group typified by bufonids, Scaphiopus sps. and Ambystoma tigrinum has a very low anaerobic scope (only 7-10 μmol ATP g^{-1} from lactate during exercise), cryptic coloration, pugnacious defensive behavior, noxious secretions, a very high aerobic scope, and they can remain active for relatively extended periods without fatigue (Miller & Hutchison, 1980). This "aerobic" group has high [Hb] and Hcts which support their high aerobic capacity. The second group is typified by the ranids and hylids. These animals utilize rapid saltatory escape movements fueled by anaerobic metabolism (17-23 μmol ATP g^{-1} from lactate during exercise); their relative heart mass, [Hb] and Hct are all lower than these same parameters from animals of the aerobic group (Hillman, 1976). High [EOP], [Hb], Hcts and low lactate accumulation are all characteristics of aerobic metabolism during activity.

MATERIALS AND METHODS

Animals were obtained from commercial suppliers, breeding aggregations in the vicinity of Norman, Oklahoma or colleagues. Within each species the animals were of mixed sexes and various body masses. Aquatic species were maintained in 20 l plastic aquaria with the water changed weekly. Semi-aquatic animals were maintained in small plastic boxes, partially filled with water and elevated at one end to allow the animals access to dry substrate. All animals were fed one or more of the following on a weekly basis; neonatal mice, crickets, earthworms, Tenebrio larvae, or "Frog brittle" (Nasco Biol. Supply Co.). Only animals in apparent good health were used. Upon arrival, animals were acclimatized to a photoperiod of LD 12:12 (photophase centered at 1200 CST) and $15^{\circ} \pm 2^{\circ}$ C in environmental chambers. Groups of animals were transferred to other conditions for further acclimation. Two photoperiods (LD 12:12 or LD 18:6) and four temperatures (5, 10, 15 or 25° C) were utilized. A minimum of one week was allowed for acclimation to new temperatures and two weeks for adjustments to new photoperiods. Blood was collected between 1200 and 1400 (CST) to preclude possible diel variations in EOP levels or hematological parameters (Hutchison & Hazard, 1981).

All anurans were double-pithed, weighed to the nearest 0.1g and placed onto ice-filled dissection trays covered with aluminum foil and plastic wrapping. Urodeles were placed into ice-water for anesthesia then double-pithed and placed onto iced dissection trays. Ventral incisions exposed the heart and excess body fluids were wiped away. The ventral aorta was severed and duplicate 20 µl blood samples taken for Hct and [Hb] determinations. The remainder of the blood was collected with cold heparinized Pasteur pipettes and transferred into graduated conical centrifuge tubes. The red cells were serially centrifuged at 5130 X g and resuspended three times in 5-6 volumes of cold 0.146 M NaCl or phosphate free amphibian Ringer's saline (ionic strength = 0.122). After the final centrifugation, the supernatant was discarded and six times the packed red cell volume of cold trichloroacetic acid (TCA, 0.57 M) was added and thoroughly mixed. The TCA-red cell mixture was allowed to remain on ice for 20 minutes, centrifuged and the clear supernatant retained for analysis. The duplicate Hct tubes were centrifuged, the Hcts measured and the [Hb] determined from these same Hct tubes as cyanomethemoglobin with human Hb as a standard (Hycel).

The red-cell extracts of the majority of species were analyzed by a combination of colorimetric and enzymatic assay procedures which required less than 3 hours to complete. Blanks

and known standards were analyzed concurrently. Total acid soluble phosphate concentration [P_{tot}] was determined colorimetrically (Ames & Dubin, 1960). Total nucleoside triphosphate [NTP] and [DPG] were determined enzymatically (Sigma UV-366 and 35 UV respectively).

The RBCs of several species were analyzed by ion-exchange chromatography methods modified from Bartlett (1959a-d,1976) and Isaacks et al. (1976). This procedure required approximately one week to complete. The TCA was removed from the TCA-RBC mixture by three liquid-liquid extractions with ether. The TCA-free solution was then neutralized with 5.0 N NH_4OH and placed onto an anion exchange resin (Bio-Rad 200-400 mesh, AG 1x8, formate form) packed into a 0.9x25 cm column. The column was eluted with a 1.0 l linear gradient of 500 ml each of distilled water and buffer (formic acid:ammonium formate, 3:2, pH 3.57, 5.0 N). Ultraviolet (UV) absorbance at 254 nm and total phosphate content were determined for each of 200, 3.8 ml fractions. Qualitative peak identification was made by comparison with standard samples applied to the column. Internal standards were not run with the red-cell extracts. Linear base line drift in the UV absorbance and interference with the phosphate analysis due to increasing concentrations of buffer, were corrected by fitting a linear equation to the baseline and subtracting the values of the drift

from either the UV or phosphate data. All solutions were made with glass distilled water; stock solutions of ammonium formate were filtered; and RBCs were washed in graduated conical NALGENE ® test tubes to eliminate iron complexes with GTP and to improve recovery of phosphate compounds.

Prior to statistical analysis, all Hct (%) values were converted by an arcsin transformation to angular variates. All data were tested for normality with the Shapiro-Wilk statistic. Linear regressions of several variables versus body mass, one way analysis of variance and covariance, with body mass as the covariate, were performed on intraspecific data for comparison of temperature and photoperiod effects. In all cases significance was assumed at the $P < 0.05$ level. Statistical analyses were performed via procedures documented in the Statistical Analysis System (Barr et al., 1979). Data from column chromatography were plotted on triangular coordinate systems (Batschelet, 1971) for comparisons between species and identification of clusters of species based on similarities in the relative proportions of the major EOPs (ATP, DPG and GTP) expressed as μmol of parent compound ml^{-1} RBC. These values were based on the phosphate equivalents of each compound assayed directly from ion-exchange column fractions. The concentrations of these compounds were summed to obtain a total for the major EOPs. The percentage

contribution of each compound was then plotted.

The determination of interspecific similarities based on the phenotypic characters of body mass (BM), [Hb], Hct, [DPG] and [NTP] values was performed by a series of clustering algorithms (NTSYS, Rohlf et al., 1981). The three dimensional "ball and stick" diagram (Fig. 5.) was drawn by the GRAFPAC plotting package (Rohlf, 1971). The NTSYS procedures are commonly employed for numerical taxonomic studies and are discussed in Sneath & Sokal (1973). The data matrix (rows, species; columns, variables) was standardized by rows to a mean of zero and standard deviation of one and then used to compute a distance matrix (a type of similarity matrix where small distances indicate high similarity). The distance matrix was used to produce a phenogram which depicts the similarities between the species. The cophenetic correlation coefficient comparing this phenogram to the original data matrix was calculated to quantify the distortion resulting from production of the phenogram from the distance matrix. Next the original data matrix was standardized by its columns and a second distance matrix computed. This variable standardized matrix was manipulated by a principle component analysis algorithm which extracted three vectors capable of explaining the majority of the variation present. These vectors provided the scales or principle axes for the three

dimensional "ball and stick" diagram. Each species was therefore represented by three variables and could be plotted as a point in the three dimensional space defined by the three principle axes. The distortion present in the " ball and stick" ordination method was revealed by the addition of the minimum spanning tree (MST). The MST connects the balls (species) in such a manner that the sum of the distances between the balls is a minimum while retaining the restriction that "travel" from any ball to any other is still possible. Distortion is revealed where balls (species) are closer together in three dimensional space than in the full hyperspace of the original five variables.

RESULTS

The slopes of linear regressions of BM versus the variables of [Hb], Hct, [DPG], [NTP] and [P_{tot}] were significantly different from zero in only a few species (Table 1.). In all cases the BM ranges were limited and therefore no attempt was made to fit the data to logarithmic equations.

Bufo cognatus from near Tucson, AZ showed significant differences from animals collected near Norman, OK for the variables of body mass, ($F_{(1,9)}$ = 7.295, P= 0.02); [DPG], ($F_{(1,8)}$ = 19.01, P=0.0024); [NTP] ($F_{(1,8)}$ = 5.79, P=0.0427); [P_{tot}], ($F_{(1,8)}$ = 41.94, P=0.0002); and Hct ($F_{(1,8)}$ = 6.34, P=0.0359). Since the animals used for the comparisons were tested in September (AZ) and May (OK), the differences between the groups may be due to a combination of seasonal and geographic factors.

Bufo boreas haliophilus, collected in Kern Co., CA at an elevation of 124 m, were maintained at 15 °C for 30 days in Norman, OK (304 m) to determine if the modest increase in altitude or lab maintenance affected the [EOPs]. Animals were examined on arrival and after 3, 4, 25 and 30 days in Norman. All of the variables decreased over the experimental period but none changed significantly.

Individuals of two anuran species (Bufo w. woodhousei and

Bufo b. haliophilus) and three urodeles (Ambystoma t. tigrinum, Necturus m. maculosis and Notopthalmus v. viridescens) were acclimated to a long (LD 16:8, 15°C) or a short (LD 12:12, 15°C) photoperiod. A. t. tigrinum had significantly ($F_{(1,9)}$ = 8.25, P=0.0239) higher Hct in the long (26.91%) than in the short photoperiod (20.82%). None of the other urodeles showed significant differences attributable to photoperiod for any other variables. Both B. boreas haliophilus and B. w. woodhousei showed significant decreases in [NTP] during the longer photoperiod (10.96 > 7.39 μmol gHb^{-1}, $F_{(2,26)}$ = 3.74, P=0.0374; 8.07 > 4.88 μmol gHb^{-1}, $F_{(1,7)}$ = 14.71, P=0.003 for B. boreas and B. woodhousei, respectively). The [DPG] was elevated (13.99 < 21.42 μmol gHb^{-1}, $F_{(2,26)}$ = 11.57, P=0.0004) during the longer photoperiod in B. boreas. Increased [DPG] balanced decreased [NTP] and left [P_{tot}] unchanged in B. boreas. The [P_{tot}] was reduced ($F_{(1,7)}$ = 39.12, P=0.0004) in B. woodhousei when the decrease in [NTP] was not compensated by changes in [DPG].

Acclimation to increasing temperatures resulted in increased [DPG] in Necturus ($F_{(2,15)}$ = 23.17, P=0.0001). The [DPG] was 2.86, 6.66 and 15.63 μmol gHb^{-1} at 5, 15 and 25°C, respectively. No variation with temperature was recorded for any other variable for any other urodele. Among anurans only B. woodhousei showed temperature induced Hct ($F_{(2,16)}$ = 4.88, P=0.0222, 23.81, 29.62

and 16.95 % at 5, 15 and 25 °C respectively) and [Hb] ($F_{(2,16)}$ = 5.08, P=0.0196, 6.72, 8.59 and 4.90 g dl^{-1} at 5, 15 and 25 °C, respectively). No variation attributable to temperature was observed for any other variable from any other anuran.

The purpose of the ion-exchange chromatography (Figs. 1 and 2) was to quantify the [ATP] and [GTP]; therefore no systematic effort was made to identify the early peaks (in fractions 1-20). Other phosphorylated intermediates such as glucose-6-phosphate may have been present, but not in sufficient concentrations to be detected from the small volumes of RBCs available.

Table 2 presents the same data as Figs. 1 and 2 plus additional hematological data and data from additional species. Both Scaphiopus and Rana berlandieri have higher [P_{tot}] at 15 °C than at 25° C. The [P_{tot}] of Rana berlandieri was elevated under LD 12:12 compared to LD 18:6; however, there was a large size difference between the animals. Ambystoma t. tigrinum decreased in size during ontogeny (P,Q,R). The [DPG], [Hb], Hct, [P_i] and [P_{tot}] were all larger in salamanders than axolotls while the [GTP] and [ATP] were highest during the transition. The erythrocytic [DPG] and [NTP] were higher and [P_i] lower in terrestrial than in aquatic A. tigrinum (Bartlett, 1976). Individuals of six species possessed DPG:Hb ratios greater than or equal to 0.7 (i.e. concentrations greater than 11 μmol gHb^{-1},

assuming a molecular weight for Hb of 64,500); these species were: Bufo americanus, Bufo cognatus, Pseudacris, Amphiuma, Cryptobranchus and Rana berlandieri. Amphiuma, Necturus and Scaphiopus showed ATP:Hb ratios > 0.7. In ten species neither ATP:Hb nor DPG:Hb molar ratios were greater than 0.7; these were: B. marinus, B. woodhousei, H. gratiosa, R. blairi, R. catesbeiana, Telmatobius, Xenopus and A. tigrinum. The [DPG] was greater than [ATP] in eleven species (all bufonids, R. blairi, R. berlandieri, Hyla, Pseudacris, Xenopus, Cryptobranchus and all ambystomids). The [ATP] was greater than [DPG] for only five species (Scaphiopus, Telmatobius, Amphiuma, Necturus and R. catesbeiana). Inorganic phosphate [P_i] in Table 2 averages 9.14 $\pm$ 0.84 % of the [P_{tot}] ($\bar{X}$= 5.75 $\pm$ 0.72 µmol gHb^{-1}. The mean concentration of adenosine diphosphate [ADP] was 0.66 $\pm$ 0.08 µmol gHb^{-1} (2.10 $\pm$ 0.21% of the [P_{tot}]). The NTP (ATP + GTP) pool contributed an average of 44.7 $\pm$ 3.77 % of the [P_{tot}]. The mean [DPG] was 8.04 $\pm$ 1.1 µmol gHB^{-1} (28.70 $\pm$ 3.49% of the [P_{tot}]. In both species of Hyla and in occasional individuals of Rana berlandieri and Telmatobious large UV peaks appeared after the elution of ATP. The identification of this peak was uncertain. This peak was assumed to be ATP and entered as such in Table 2. Uridine triphosphate elutes at this position in our system. There is also the possibility that these unknown UV peaks were an

ATP-Fe complex. In one case (R. berlandieri, 25°C, LD 12:12 Table 1) the UV peak appeared in an individual having considerable hemolysis and 25% methemoglobin. The [GTP] is low (11.08 ± 1.27% of the NTP pool; averaging 4.61 ± 0.71% of the [P_{tot}]) in all species except Amphiuma. The mean GTP:ATP ratio was 0.125 ± 0.018 for all species. Inositol polyphosphate (IPP) never contributed more than 5% of the [P_{tot}]. Putative IPP peaks appeared in some individuals of five species; Scaphiopus, R. blairi, Cryptobranchus, Ambystoma and Hyla versicolor. In four cases the EOPs were measured for the same individual by both chromatographic and enzymatic-colorimetric methods. These species were: R.berlandieri (chromatography measured 144% of the [P_{tot}] determined by the enzymatic tests), R. blairi (71%), Telmatobius (59%) and Necturus (128%); this gives an average agreement between the two methods of 98% on the same individuals.

The adenylate energy charge (Atkinson, 1971; [ATP]/([P_i] [AMP] [ADP]) and the phosphorylation potential (Lehninger, 1975 [ATP]/([P_i] [ADP]) are indications of the energy status of the cells. The adenylate charge (AEC) seldom deviates from the values of 0.85. The mean AEC value for Table 2 was 0.952 ± 0.008. The mean gamma was 15.8 ± 3.4 μM^{-1} (Table 2). The phosphorylation potential falls as low as 5.0 μM^{-1} during energy deficits. In most tissues the values for gamma range from 200 to 800 uM^{-1} .

Mean RBC values for gamma calculated from Isaacks et al. (1978) and Bartlett (1970) are 1.85 and 7.17 μM^{-1}, respectively. There is substantial relative error in the measurement of [AMP] with the system currently in use. Consequently, the values for gamma are more reliable and seem to correspond well with values from the literature.

The column chromatography data of Figs. 1 and 2 and Table 2 are graphically summarized in the triangular plot shown in Fig. 3. The phosphate complements of amphibian red cells range in type from nearly fish- or reptile-like to very nearly mammalian. Three groups are evident. The first appears near the top and includes Necturus (S) and Scaphiopus (H,I,J); the red cells of these species have very high [ATP] and are probably dependent upon oxidative phosphorylation to produce ATP. The second group appears in the lower right near the point representing man (Y). These species are all bufonids; their red cells have high percentages of P_{tot} as DPG. The third group is the largest and appears in the middle of the right of the plot. The [ATP] and [DPG] are roughly equal for species of this group and there is some overlap of bufonids with the ranids.

The data listed in Table 3 were collected entirely by enzymatic and colorimetric methods. For these data the DPG:Hb ratio was > 0.7 for all bufonids, ambystomids, Xenopus, Hyla

cinerea, Amphiuma, Desmognathus, Siren and Necturus (at 25°C). The [DPG] was greater than [NTP] in 31 of the 47 entries of Table 2. The NTP:Hb ratio was greater than 0.7 for Bufo canorus, Hyla cinerea, Pseudacris, Rana blairi, Scaphiopus, Siren, Necturus (at 5 and 15°C), Amphiuma and two of the ambystomids. The [NTP] was greater than [DPG] in 16 of the 47 entries. The EOP:Hb ratio was < 0.7 for both NTP and DPG in Hyla versicolor and all ranids. The phosphate equivalents of NTP and DPG exceeded the measured [P_{tot}] values in five cases (Table 3).

The material in Table 3 was analyzed by the NTSYS programs and is reproduced in summary form in Figs. 4 and 5. These graphical summaries are intended to condense the data of Table 3 and to cluster the species based on similarities in the quantitative variables associated with respiration in these animals. The phenogram (Fig. 4) was selected because it showed the highest cophenetic correlation coefficient (0.887) and therefore had the closest resemblance to the original data matrix. The NTSYS programs have assembled three groups in the phenogram of Fig. 4. The upper group contains most bufonids, Ambystoma and Desmognathus. The middle group contains a few bufonids (there was a similar overlap of bufonids in Fig. 3), ranids, Hyla, Notopthalmus and Siren. The third group is not as distinct but both Scaphiopus and Necturus appear together in this

group. Bufo mazatlanensis and Xenopus are isolated and Amphiuma is virtually alone. The placement of a species can be altered somewhat by temperature (e.g., B. woodhousei). These same groups are depicted in a three dimensional space in Fig. 5 which also shows the minimum spanning tree linking nearest neighbors.

Column chromatography (Table 2) was done on individuals; Table 3 represents the mean values from separate determinations on at least 5 individuals. There are 14 directly comparable sets of measurements in Tables 2 and 3. These are presented as the percentage of the colorimetric [P_{tot}] that was detected by the column chromatographic methods. These values were: B. cognatus (143%), B. marinus (50%), B. woodhousei (27%), Pseudacris (76%), R. berlandieri (122%), R. blairi-15°C (85%), R. blairi-25°C (57%), Scaphiopus-15°C (90%), Scaphiopus-25°C (72%), Telmatobious (59%), Xenopus (54%), A. tigrinum-15°C (85%) and Necturus-15°C (118%). The column chromatography (Table 2) averaged 80.0 ± 8.38% of the comparable [P_{tot}] determined with colorimetric techniques (Table 3).

DISCUSSION

The presence of DPG as the major EOP in bufonids suggested initially that DPG might be the major EOP for all terrestrial amphibians. However, the major EOP of the aquatic frog Xenopus is DPG and high [ATP] are found in both the aquatic salamander, Necturus and in the terrestrial spadefoot toad, Scaphiopus. Hence there is not a single EOP characteristic of terrestrial or aquatic amphibians. Consequently, the presence, absence and concentration of a particular EOP depends on factors other than habitat.

The responses of amphibian EOPs to the imposed stresses of temperature and altered photoperiod were not universal or substantial (Table 3, Fig. 4). The observed changes nevertheless suggest that amphibian EOPs are not static pools of phosphate but active metabolites responding to external environmental cues. Only one case of a substantial qualitative change in an EOP was observed; Necturus acclimated for one week to 25°C had elevated [DPG] compared to [DPG] in 5°C and 15°C acclimated mudpuppies (Table 3). In most cases, however, the components of the EOP pool appeared to rise and fall in concert. Consequently, the individual components of the EOP pool may not interact

selectively with particular Hb components.

Three clusters of similar species were generated by the graphical and mathematical clustering techniques (Figs. 3, 4 and 5). The animals in two of the three clusters possess EOP:Hb ratios > 1.0; the types of EOPs are quite different, however. Evidently the quantity of EOP produced is independent of the type compound and high EOP:Hb ratios can be produced by either glycolytic or oxidative phosphorylation mechanisms (e.g., bufonids and Scaphiopus respectively). We contend that these clusters are: (1) fundamentally distinct strategies for coping with hypoxia, (2) analogous to those of mammals (Bunn et al., 1974; Kay, 1977) and (3) congruent with similar classifications of amphibians based on activity metabolism (Bennett, 1980). Data for 9 species of bufonids, 3 ranids and one species each from the genera Scaphiopus and Xenopus (Table 4) show that high molar ratios of EOP:Hb are associated with high P_{50}, aerobic metabolism and rapid recovery of whole body lactate to resting levels after exercise to exhaustion. Low EOP:Hb ratios occur in species with low whole blood P_{50}, low anaerobic metabolism during activity and long periods for recovery of resting whole body lactate following activity. The respiratory properties of the whole blood and the erythrocytic metabolism function together to deliver O_2 at a rapid rate in species which utilize aerobic metabolism to sustain

activity. Different selection pressures appear to have shaped the O_2 delivery system of the anaerobic group. The position of the aquatic species in the clusters remains somewhat puzzling. The highly anaerobic Necturus associates with the highly aerobic Scaphiopus. The generalizations which appear to hold for terrestrial amphibians may not extend to aquatic forms (Hutchison & Miller, 1979a). Aquatic animals exhibit high EOP:Hb molar ratios (Table 2 and 3; Hazard & Hutchison, 1978), low buffer capacities (Lenfant & Johansen, 1967), and a reliance upon anaerobic metabolism for activity (Preslar & Hutchison, 1978; Hutchison & Miller, 1979a). A Hb which possesses a high affinity for an EOP would tend to remove the EOP from the cytosol and hence relieve end-product inhibition of the EOP's synthesis and lead to high levels of the EOP (Coates, 1975a). In general the Hb-EOP affinity is greater in the deoxy state of Hb. Aquatic animals exposed to hypoxic conditions would tend to have higher deoxyHb:oxyHb ratio and thus, elevated EOP concentrations. However, the primary problem of aquatic species is not O_2 delivery, but O_2 acquisition; the elevated EOP would aggravate the problem. The critical point is the degree of hypoxia experienced. At moderate hypoxia, the decrease in P_{50} resulting from elevated EOPs may nevertheless improve O_2 delivery and benefit the animal (Hazard & Hutchison, 1978). The precise

adaptive value of high EOP levels in aquatic amphibians remains conjectural. In the absence of high P_{CO_2} (Rahn, 1966), and given the usually very high O_2 affinity of their Hbs, the elevated EOPs of aquatic species may simply be a standard adaptation for achieving some degree of O_2 delivery. Xenopus represents a special case. These frogs are aquatic divers. Their metabolism is listed as "mixed" because they can tolerate extremely high whole body lactate concentrations, yet have a higher aerobic capacity than Rana (Hutchison & Miller, 1979b; Putnam, 1979; Hillman & Withers, 1981). In Xenopus the long recovery time for return of whole body lactate to resting levels may result from their apneic breathing pattern.

The EOPs of amphibians show a considerable qualitative and quantitative diversity; accordingly, amphibian RBCs can not be easily classified into aerobic or anaerobic types. There is considerable anaerobic metabolism in some RBCs as evidenced by substantial quantities of DPG (e.g., Bufo cognatus, Table 3). However, based on high [ATP], some RBCs seem to be almost entirely aerobic (e.g., Scaphiopus, Table 3). The relative activity and metabolite sensitivity of enzymes that control red cell metabolism in these two species must be quite different. Since DPG is produced anaerobically, the O_2 consumption in high DPG red cells may be lower than in the high ATP RBCs.

All of the clustering techniques reveal some overlap between bufonids and ranids. This overlap appears to blur the distinctions based on the relationship between EOP levels and the role of aerobic activity metabolism. The genus Bufo is one of the most widely distributed of amphibian genera. The relative contributions of aerobic and anaerobic metabolism may vary or the relative contribution of the cardiopulmonary system may be greater in some species than in others. In general, the high EOP levels in terrestrial amphibians are associated with a dependence on aerobic energy production for activity. High [EOP]s suggest a strategy for reduced O_2 affinity and enhanced O_2 delivery rather than a high affinity for O_2 acquisition, but do not a priori indicate that EOPs always play a role in modulation of O_2 affinity.

ACKNOWLEDGEMENTS; We thank Grant Bartlett, Dan Hough, Russell Isaacks and Scott Wood for technical advice, Roberta Allen for technical assistance and Jerry Black, Bayard Brattstrom, Martin Feder, Nixon Griffis, Julian Harrison, Loren Hill and Max Nickerson for animals. This work was supported by NIH Grant RO1 HL 18082 (to V.H.H.).

REFERENCES

Altland, P.D. & Brace, D.C. (1962) Red cell life span in the turtle and toad. Amer. J. Physiol. 203,1188-1195.

Ames, B.N. & Dubin, D.T. (1960) The role of polyamines in the neutralization of bacteriophage DNA. J. biol. Chem. 235, 769-775.

Araki, T., Kajita, A. & Shukuya, R. (1971) The effects of organic phosphates on the allosteric property of Rana catesbeiana hemoglobins. Biochem. biophys. Res. Commun. 43,1179-1185.

Atkinson, D.E. (1971) Adenine nucleotides as stoichiometric coupling agents in metabolism and as regulatory modifiers. The adenylate charge. In, Metabolic Pathways (Edited by Vogel, H.J.),pp. 1-21. Academic Press N.Y. Vol. V.

Barr, A.J., Goodnight, J.H., Sall, J.P., Blair, W.H. & Chilko, D.M. (1979) SAS User's Guide, 1979 Edition. SAS Institute Inc.

Bartlett, G.R. (1959a) Human red cell glycolytic intermediates. J. biol. Chem. 234,449-458.

Bartlett, G.R. (1959b) Methods for the isolation of glycolytic intermediates by column chromatography with ion exchange resins. J. biol. Chem. 234,459-465.

Bartlett, G.R. (1959c) Phosphorous assay in column

chromatography. J. biol. Chem. 234, 466-468.

Bartlett, G.R. (1959d) Colorimetric assay in column chromatography. J. biol. Chem. 234,469-471.

Bartlett, G.R. (1970) Patterns of phosphate compounds in red blood cells of man and animals. In Advances in experimental medicine and biology. Vol. 6. Red Cell Metabolism and Function. (Edited by Brewer, G.J.), pp. 245-256. Plenum Press N.Y.

Bartlett, G.R. (1976) Phosphate compounds in red cells of reptiles, amphibians and fish. Comp. Biochem. Physiol. 55A, 211-214.

Bartlett, G.R. (1978) Water soluble phosphates of fish red cells. Can. J. Zool. 56,870-877.

Batschelet, E. (1971) Introduction to Mathematics for Life Scientists. Springer-Verlag N.Y.

Benesch, R., Benesch, R.E. & Yu, C.I. (1968) Reciprocal binding of oxygen and diphosphoglycerate by human hemoglobin. Proc. natn. Acad. Sci. U.S.A. 59,526-532.

Bennett, A.F. (1978) Activity metabolism in lower vertebrates. A. Rev. Physiol. 400,447-469.

Bennett, A.F. (1980) The metabolic foundations of vertebrate behavior. Bioscience. 30,452-456.

Bennett, A.F. & Licht, P. (1974) Anaerobic metabolism during

activity in amphibians. Comp. Biochem. Physiol. 48A,319-327.

Borgese, T.A., Nagel, R.L., Roth, E., Murphy, D. & Harrington, J. (1978) Guanosine triphosphate (GTP), the major organic phosphate in the erythrocytes of the elasmobranch Mustelus canis (smooth dogfish). Comp. Biochem. Physiol. 60B,317-321.

Brewer, G.J., Oelshlegal Jr., F.J., Moore, L.G. & Noble, N.A. (1974) In vivo red cell glycolytic control and DPG-ATP levels. Ann. N.Y. Acad. Sci. 421,513-523.

Bunn, H.F., Seal, U.S. & Scott, A.F. (1974) The role of 2,3-diphosphoglycerate in mediating hemoglobin function of mammalian red cells. Ann. N.Y. Acad. Sci.241,498-512.

Chanutin, A. & Curnish, R.R. (1967) Effects of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. Arch. Biochem. Biophys. 121,96-102.

Coates, M.L. (1975a) Hemoglobin function in the vertebrates: an evolutionary model. J. mol. Evol. 6,285-307.

Coates, M.L. (1975b) Studies on the interaction of organic phosphates with haemoglobin in an amphibian (Bufo marinus), a reptile (Trachydosaurus rugosis) and man. Aust. J. biol. Sci. 28,367-378.

Gans, C. (1970) Respiration in early tetrapods-the frog is a red herring. Evolution 24,723-734.

Gans, C., de Jongh, H.J. & Farber, J. (1969) Bullfrog (Rana

catesbeiana) ventilation. How does the frog breathe? Science 163,1223-1225.

Garlick, R.L., Davis, B.J., Farmer, M., Fyhn, H.J., Fyhn, U.E.H., Noble, R.W., Powers, D.A., Riggs, A. & Weber, R.W. (1979) A fetal-maternal shift in the oxygen equilibrium of hemoglobin from the viviparous caecilian Typhlonectes compressicaudata. Comp. Biochem. Physiol. 62A,239-244.

Geoghegan, W.D. & Poluhowich, J.J. (1974) The major erythrocytic organic phosphates of the american eel Anguilla rostrata. Comp. Biochem. Physiol. 49B,281-290.

Gottlieb, G. & Jackson, D.C. (1976) Importance of pulmonary ventilation in respiratory control in the bullfrog. Amer. J. Physiol. 230,608-613.

Greenwald, I. (1925) A new type of phosphoric acid compound isolated from blood with some remarks on the effect of substitution on the rotation of l-glyceric acid. J. biol. Chem. 63,339-349.

Hazard, E.S. & Hutchison, V.H. (1978) Ontogenetic changes in erythrocytic organic phosphates in the bullfrog, Rana catesbeiana. J. exp. Zool. 206,109-118.

Hillman, S.S. (1976) Cardiovascular correlates of maximal oxygen consumption rates in anuran amphibians. J. comp. Physiol. 109,199-207.

Hillman, S.S. & Withers, P.C. (1981) Aerobic contributions to sustained activity metabolism in Xenopus laevis. Comp. Biochem. Physiol. 69A,605-606.

Hutchison, V.H. & Hazard, E.S. (1981) Diel variation of erythrocytic phosphates in Mus musculus acclimated to different photoperiod regimes. Comp. Biochem. Physiol. 70A,9-12.

Hutchison, V.H. & Miller, K. (1979a) Anaerobic capacity of amphibians. Comp. Biochem. Physiol. 63A,213-216.

Hutchison, V.H. & Miller, K. (1979b) Aerobic and anaerobic contributions to sustained activity in Xenopus laevis. Respir. Physiol. 38,93-103.

Isaacks, R.E. & Harkness, D.R. (1975) 2,3-diphosphoglycerate in erythrocytes of chick embryos. Science 189,393-394.

Isaacks, R.E., Harkness, D.R., Froeman, G.A. & Sussman, S.A. (1976) Studies on avian erythrocyte metabolism. I Procedure for separation and quantitation of the major phosphorylated metabolic intermediates by anion exchange chromatography. Comp. Biochem. Physiol. 53A,95-99.

Isaacks, R.E., Kim, H.D., Bartlett, G.R. & Harkness, D.R. (1977) Inositol pentaphosphate of a freshwater fish, Piraracu' (Arapaima gigas). Life Sci. 20,987-990.

Isaacks, R.E., Kim, H.D. & Harkness, D.R. (1978) Relationships

between phosphorylated metabolic intermediates and whole blood oxygen affinity in some air-breathing and water-breathing teleosts. Can. J. Zool. 56,887-890.

Johansen, K. & Lenfant, C. (1972) A comparative approach to the adaptability of O_2-Hb affinity. In Oxygen affinity of hemoglobin and red cell acid-base status. (Edited by Rorth, M. & Astrup, P.), pp. 750-780. Munksgaard, Copenhagen.

Johnson, L.F. & Tate, M.E. (1969) Structure of "phytic acids". Can. J. Chem. 47,63-73.

Jokumsen, A. & Weber, R.E. (1980) Haemoglobin-oxygen binding properties in the blood of Xenopus laevis, with special reference to the influences of aestivation and of temperature and salinity acclimation. J. exp. Biol. 86,19-37.

Kay, F.R. (1977) 2,3-diphosphoglycerate, blood oxygen dissociation and the biology of mammals. Comp. Biochem. Physiol. 57A,309-316.

Lehninger, A.L. (1975) Biochemistry. Worth Publishers Inc. N.Y.

Lenfant, C. & Johansen, K. (1967) Respiratory adaptations in selected amphibians. Respir. Physiol. 2,247-260.

Miller, K. & Hutchison, V.H. (1979) Activity metabolism in the mudpuppy, Necturus maculosus. Physiol. Zool. 52,22-37.

Miller, K. & Hutchison, V.H. (1980) Aerobic and anaerobic scope for activity in the giant toad, Bufo marinus. Physiol. Zool.

53,170-175.

Meyers, R.S. & Felsher, J. (1976) Continuous recording and quantification of selective blood distribution in the amphibian Rana catesbeiana. Comp. Biochem. Physiol. 54A,359-363.

Packard, G.C. (1976) Devonian amphibians: did they excrete carbon dioxide via skin gills or lungs? Evolution 30,270-280.

Preslar III, A.J. & Hutchison, V.H. (1978) Energetics for activity in the salamander Amphiuma tridactylum. J. comp. Physiol. 128,139-146.

Putnam, R.W. (1979) The role of lactic acid accumulation in muscle fatigue of two species of anurans, Xenopus laevis and Rana pipiens. J. exp. Biol. 82,35-51.

Rahn, H. (1966) Aquatic gas exchange theory. Respir. Physiol. 1,1-12.

Rapoport, S. & Guest, G.M. (1941) Distribution of acid soluble phosphorous in the blood of various vertebrates. J. biol. Chem. 138,269-282.

Rohlf, F.J., Kishpaugh, J. & Kirk, D. (1981) NT-SYS, numerical taxonomy system of multivariate statistical programs. Tech. Rep. State Univ. of N.Y. at Stony Brook, N.Y. As implemented on the IBM 370/158 OS/VS2 at the Univ. of Oklahoma.

Rohlf, F.J. (1971) GRAFPAC, graphic output subroutine for the GE

635 Computer. Kansas Geol. Surv. Computer Contrib. No. 36. As implemented (1977) on the IBM 370/158 OS/VS2 at the Univ. of Oklahoma.

Romer, A.S. (1972) Skin breathing-primary or secondary? Respir. Physiol. 14,183-192.

Seymour, R.S. (1973) Physiological correlates of forced activity and burrowing in the spadefoot toad, Scaphiopus hammondii. Copeia 1973,103-115.

Sneath, P.H.A. & Sokal, R.R. (1973) Numerical Taxonomy. The Principles and Practice of Numerical Classification. W.H. Freeman and Co., San Franciso.

Taketa, F. (1974) Organic phosphates and hemoglobin structure-function relationships in the feline. Ann. N.Y. Acad. Sci. 241,524-537.

Wood, S.C. (1971) Effects of metabolism on blood respiratory properties and erythrocyte adenosine triphosphate level of the blood of the salamander Dicamptodon ensatus (Eschsholtz). Respir. Physiol. 12,53-65.

Wood, S.C., Weber, R.E., Maloiy, G.M.D. & Johansen, K. (1975) Oxygen uptake and blood respiratory properties of the caecilian Boulengerula taitanus. Respir. Physiol. 24,355-363.

TABLE HEADINGS

Table 1. Slopes and intercepts for the regression of the variables hemoglobin (Hb), hematocrit (Hct), nucleoside triphosphate (NTP) and total acid soluble phosphate (P_{tot}) versus body mass. The probability of obtaining a larger value for t while accepting the H_o is listed in the last column.

Table 2. Detailed data collected from ion-exchange chromatographic analyses of erythrocytic phosphates of amphibians. Species with letters in the ID column appear in Figs. 1,2 and 3. Units are body mass (BM,g), hematocrit (Hct,%), hemoglobin (Hb, g dl^{-1}), inorganic phosphate (P_i, µmol gHb^{-1}), adenosine diphosphate (ADP, µmol gHb^{-1}), adenosine triphosphate (ATP, µmol gHb^{-1}), 2,3-diphosphoglycerate (DPG, µmol gHb^{-1}), guanosine triphosphate (GTP, µmol gHb^{-1}) and inositol polyphosphate (IPP, µmol gHb^{-1}). Numbers in brackets (under P_{tot} only) represent values determined for the same individuals by enzymatic-colorimetric methods. Numbers in parenthesis under BM represent size ranges of the animals examined; under Hb and Hct the numbers in parenthesis are replications per animal and under the phosphate entries except P_{tot} the numbers in parenthesis give the percentage of P_{tot} contributed by the particular compound.

Under phosphorylation potential the numbers represent the addition of GTP to the calculation. Tabulated values represent the results of one chromatogram in most cases. Values in the BM, Hb and Hct columns are means $\pm$ 1.0 S.E.

Table 3. Detailed data collected from enzymatic-colorimetric analyses of erythrocytic organic phosphates of anuran and urodele amphibians. Numbers in the ID column represent the entries in Figs. 4 and 5. Abbreviations and units for the variables are identical with those in Table 2. Nucleoside triphosphate [NTP] is the sum of adenosine triphosphate and guanosine triphosphate. Numbers in parenthesis represent sample size and number of replications per animal, respectively.

Table 4. Summary of the relationships between erythrocytic organic phosphates, whole blood O_2 affinity, energetics for activity and recovery time at 15 ^{o}C for return of whole body lactate concentrations to resting levels following exercise to exhaustion. The molar ratios are dimensionless numbers. The P_{50} values are given in mm Hg P_{O_2}. Recovery times are listed in hours. The entries in the last two columns are taken from: Bennett, 1980; Hutchison & Miller, 1979; and Putnam, 1979.

TABLE 1.

SPECIES	VARIABLE	SLOPE	INTERCEPT	P
Bufo canorus	NTP	-0.76	23.19	0.0159
"	Ptot	-4.89	152.1	0.0293
Bufo marinus	Hb	0.037	0.265	0.014
Bufo valiceps	Hct	0.0047	0.2188	0.0117
"	Ptot	1.61	45.31	0.0246
Rana berlandieri forreri	Hb	0.053	1.104	0.0054
"	Hct	0.0023	0.039	0.0064
"	Ptot	-0.302	85.14	0.0251
Rana blairi	Hb	0.018	3.17	0.0100
"	Hct	0.0013	0.104	0.0238
"	Ptot	0.71	17.89	0.0251
Xenopus laevis	Hb	0.206	7.29	0.0393
"	Hct	0.0119	0.142	0.0014
Necturus m. maculosus	DPG	-0.203	23.56	0.019

TABLE 2.

ID	SPECIES	LOCALITY	ACCLIMATION C	ACCLIMATION L:D	BODY MASS (g)	Hb g dl^{-1}	Hct %	Pi	ADP	ATP	DPG	GTP	IPP	Ptot	GTP/ATP	PHOSPHORYLATION POTENTIAL (Γ)	% Ptot AS NTP + DPG
ANURANS																	
E	Bufo americanus charlesmithi	Oklahoma Cleveland Co.	25	12:12	21.9 -	6.93 (1,3)	23.07 (1,3)	1.98 (3.4)	0.30 (0.4)	4.65 (23.8)	19.84 (67.6)	0.57 (2.9)	0.00 (0)	58.71	0.122	60.1 (67.5)	94.3
A,B	Bufo cognatus	"	15	12:12	45.85 (44-47)	9.98 (2,3)	25.14 (2,3)	3.98 (7.9)	0.95 (3.8)	3.45 (20.6)	14.3 (57.0)	0.29 (1.8)	0.00 (0)	50.20	0.085	2.3 (2.5)	79.3
C	Bufo marinus	Commercial	25	12:12	474.8 -	4.61 (1,3)	11.76 (1,3)	3.39 (11.3)	0.18 (1.2)	3.69 (36.8)	6.16 (40.9)	0.35 (3.5)	0.00 (0)	30.10	0.096	15.4 (16.9)	81.2
D	Bufo w. woodhousei	Oklahoma Cleveland Co.	15	12:12	61.8 -	14.95 (1,3)	39.71 (1,3)	1.75 (7.8)	0.16 (1.4)	2.75 (36.7)	4.42 (39.3)	0.43 (5.8)	0.00 (0)	22.50	0.157	26.1 (30.2)	81.7
-	Hyla gratiosa	S.Carolina Berkeley Co.	15	12:12	14.0 -	4.49 (1,3)	18.18 (1,3)	15.2 (21.7)	1.38 (3.9)	5.39 (23.0)	6.39 (18.2)	0.79 (3.4)	0.00 (0)	70.20	0.146	1.0 (1.2)	44.6
-	Hyla versicolor	Oklahoma Cleveland Co.	25	12:12	3.84 (2.6-5.9)	4.63 (15,1)	13.65 (15,1)	0.98 (2.1)	0.35 (1.5)	5.80 (37.2)	9.25 (39.5)	0.20 (1.3)	0.00 (0)	46.80	0.034	49.9 (51.6)	78.0
F	Pseudacris s. streckeri	"	15	12:12	7.73 (6.2-9.6)	5.26 (4,2)	19.12 (4,2)	9.05 (14.6)	0.61 (2.0)	7.54 (36.5)	11.16 (36.1)	0.50 (2.4)	0.00 (0)	61.90	0.066	5.0 (5.3)	75.0
M	Rana berlandieri foreri	Sinaloa,Mexico Commercial	25	18:6	38.5 -	5.19 (1,3)	22.06 (1,3)	5.57 (10.1)	0.71 (2.6)	6.85 (37.4)	10.89 (39.6)	0.37 (2.0)	0.00 (0)	54.96	0.055	7.4 (7.8)	79.0
-	Rana berlandieri berlandieri	Commercial	25	12:12	54.1 -	7.56 (1,3)	26.35 (1,3)	6.87 (9.0)	1.31 (3.4)	9.01 (35.2)	13.89 (36.2)	1.51 (5.9)	0.00 (0)	76.71 (53.17)	0.168	3.5 (4.1)	77.4
K	Rana blairi	Oklahoma Cleveland Co.	15	12:12	40.5 -	7.43 (1,3)	21.47 (1,3)	1.78 (4.3)	0.36 (1.7)	3.53 (25.7)	5.50 (26.7)	0.32 (2.4)	0.00 (0)	41.26	0.092	15.9 (17.4)	54.7
L	Rana blairi	"	25	12:12	66.3 -	7.75 (1,3)	22.22 (1,3)	1.39 (4.3)	0.29 (1.8)	4.88 (45.8)	5.31 (33.2)	0.43 (4.0)	0.062 (1.2)	31.99 (45.04)	0.088	34.7 (37.8)	83.0
N	Rana catesbeiana	"	15	12:12	518.2 -	8.99 (1,3)	37.30 (1,3)	4.48 (10.1)	0.38 (1.7)	6.22 (42.1)	4.36 (19.7)	1.04 (7.0)	0.00 (0)	44.35	0.167	15.2 (17.7)	68.8
H	Scaphiopus bombifrons	"	15	12:12	41.67 -	15.00 (1,3)	41.67 (1,3)	6.95 (8.2)	0.68 (1.6)	21.00 (74.4)	0.03 (0.1)	2.38 (8.4)	0.528 (3.7)	84.73	0.113	12.3 (13.7)	82.9
I	Scaphiopus bombifrons	"	25	12:12	13.63 (10.4-18.6)	9.18 (3,2)	28.69 (3,2)	6.25 (9.02)	0.65 (1.9)	16.27 (70.0)	0.00 (0)	1.26 (5.0)	0.406 (3.5)	69.29	0.078	12.5 (16.5)	75.9
J	Scaphiopus bombifrons	"	-	-	9.48 (7.2-13.4)	9.46 (4,1)	33.22 (4,2)	7.02 (11.2)	0.73 (2.3)	11.45 (54.7)	0.00 (0)	0.53 (2.5)	0.00 (0)	62.82	0.046	7.8 (8.2)	57.2
V	Telmatobius culeus	Bolivia Lake Titicaca	10	12:12	120.1 -	6.73 (1,4)	25.58 (1,4)	5.21 (11.4)	0.14 (0.6)	9.05 (59.2)	4.64 (20.2)	0.20 (1.3)	0.00 (0)	45.88 (77.47)	0.022	46.8 (47.8)	80.7
G	Xenopus laevis	Commercial	25	12:12	49.2 -	14.62 (1,3)	44.78 (1,3)	4.26 (9.4)	0.78 (3.4)	5.06 (33.6)	7.58 (33.6)	0.80 (5.3)	0.00 (0)	45.18	0.157	4.7 (5.4)	72.5
URODELES																	
P	Ambystoma t. tigrinum (axolotl)	Kansas Reno, Co.	15	12:12	139.0 -	9.77 (1,1)	38.15 (1,1)	4.45 (9.6)	0.68 (2.9)	6.89 (44.7)	7.88 (34.1)	0.59 (3.8)	0.05 (0.5)	46.23	0.086	8.9 (9.7)	82.6
Q	Ambystoma t. tigrinum (transforming)	"	15	12:12	109.8 -	13.33 (1,1)	47.59 (1,1)	6.85 (10.8)	1.12 (3.5)	8.06 (38.0)	9.22 (29.0)	1.41 (6.6)	0.01 (0.1)	63.62	0.175	3.8 (4.4)	73.6
R	Ambystoma t. tigrinum (adult)	" "	15	12:12	33.58 -	10.10 (1,1)	38.81 (1,1)	6.42 (10.3)	0.76 (2.4)	4.34 (20.8)	9.26 (29.6)	0.63 (3.0)	0.503 (4.8)	62.56	0.145	3.4 (3.9)	53.4
T	Amphiuma tridactylum	Louisiana Commercial	25	12:12	- -	3.80 (1,1)	18.68 (1,2)	9.63 (5.8)	1.44 (1.7)	18.04 (32.4)	16.71 (20.0)	8.26 (14.8)	0.138 (0.5)	167.0	0.458	6.4 (9.3)	67.3
U	Cryptobranchus alleganiensis bishopi	Missouri Ozark, Co.	15	12:12	193.7 -	8.64 (2,1)	37.27 (2,1)	7.31 (10.1)	0.54 (1.5)	8.59 (35.7)	10.62 (29.4)	1.01 (4.2)	0.06 (0.5)	72.14	0.118	9.4 (10.5)	69.4
S	Necturus m. maculosus	Wisconsin	15	12:12	125.7 -	5.23 (1,2)	18.94 (1,2)	11.5 (7.8)	0.75 (1.0)	28.57 (57.8)	7.50 (10.1)	5.62 (11.4)	0.00 (0)	148.4 (119.2)	0.197	12.0 (14.4)	79.2

TABLE 3.

ID	SPECIES	LOCALITY (elevation-m)	ACCLIMATION °C	L:D	BODY MASS (g)	Hb g 100ml^{-1}	Hct %	DPG µmol gHb^{-1}	NTP µmol gHb^{-1}	P_{tot} µmol gHb^{-1}	% P_{tot} as DPG + NTP
ANURANS											
1	Bufo alvarius	Sonora, Mexico near Alamos	15	12:12	96.32±14.22 (50-156)	8.96±0.61 (6,1)	30.41 ±2.64 (6,2)	22.36±1.31 (6,3)	7.19±0.97 (6,3)	97.10±4.04 (6,3)	68.0
2	Bufo a. americanus	Wisconsin	15	12:12	16.53±1.05 (12-20)	7.99±1.08 (6,1)	26.88 ±3.11 (6,2)	17.10±1.66 (6,3)	5.97±0.60 (6,3)	70.86±6.06 (6,3)	73.5
3	Bufo boreas haliophilus	California, Kern Co.(124)	(on arrival)		49.00±6.93 (33-65)	9.59±0.72 (5,1)	37.02 ±2.74 (5,2)	18.10±0.70 (5,3)	10.56±1.33 (5,3)	75.71±5.52 (5,3)	89.7
3	"	"	15	12:12 (3 days)	40.2±7.31 (24-67)	9.00±0.60 (5,1)	30.96 ±1.93 (5,2)	13.58±2.26 (5,3)	10.74±1.37 (5,3)	87.66±13.10 (5,3)	67.7
3	"	"	15	12:12 (4 days)	37.56±2.26 (24-42)	8.26±0.50 (5,1)	29.68 ±1.37 (5,2)	14.19±0.95 (5,3)	9.18±0.46 (5,3)	84.81±2.96 (5,3)	65.9
3	"	"	15	12:12 (25 days)	34.20±2.89 (26-44)	8.85±1.11 (5,1)	31.22 ±4.13 (5,2)	-	10.41±0.47 (5,3)	67.97±2.28 (5,3)	-
3	"	"	15	12:12 (30 days)	42.44±1.65 (28-47)	6.17±0.95 (5,1)	24.21 ±3.68 (5,2)	14.18±1.10 (5,3)	10.86±1.35 (5,3)	74.55±5.49 (5,3)	81.7
4	"	"	15	16:8	35.94±7.61 (20-58)	9.17±0.84 (5,1)	33.47 ±3.04 (5,2)	21.40±1.58 (5,3)	7.18±0.55 (5,3)	71.68±4.73 (5,3)	89.8
5	Bufo canorus	California, Alpine Co.(2621)	(on arrival)		15.24±1.14 (11-17)	8.97±0.50 (5,1)	34.85 ±2.06 (5,2)	15.05±1.43 (5,3)	11.61±0.92 (5,3)	77.55±6.07 (5,3)	83.7
6	Bufo cognatus	Arizona Pima Co. (728)	15	12:12	43.32±0.95 (40-47)	9.27±0.56 (6,1)	34.47 ±1.90 (6,2)	11.90±0.51 (5,3)	8.00±0.55 (5,3)	84.90±3.06 (5,3)	56.3
6	"	Oklahoma Cleveland Co.(304)	15	12:12	31.72±4.61 (16-45)	7.67±1.13 (5,1)	26.52 ±3.47 (5,2)	16.62±1.38 (5,3)	4.86±0.49 (5,3)	35.10±4.43 (5,3)	136.2
7	Bufo marinus	Commercial	15	12:12	137.22±12.87 (114-188)	5.26±0.69 (5,1)	19.63 ±2.33 (5,2)	10.90±3.51 (5,3)	6.74±1.21 (5,3)	53.01±8.07 (5,3)	79.3
8	"	"	25	12:12	179.97±12.87 (158-213)	7.20±0.93 (5,1)	30.28 ±4.84 (5,2)	19.01±2.62 (5,3)	7.87±0.67 (5,3)	60.75±5.65 (5,3)	101.4
9	Bufo mazatlanensis	Sonora, Mexico near Alamos	15	12:12	48.65±5.60 (37-64)	10.27±0.85 (5,1)	43.52 ±2.85 (5,2)	22.59±1.35 (4,3)	8.84±0.78 (4,3)	97.60±2.65 (4,3)	73.5
10	Bufo valiceps	Louisiana	15	12:12	22.50±3.74 (16-37)	8.74±0.33 (5,1)	31.73 ±1.97 (5,2)	23.33±1.46 (5,3)	8.63±1.07 (5,3)	82.48±4.71 (5,3)	88.0
11	"	"	25	12:12	16.60±0.97 (13-19)	8.79±0.72 (5,1)	30.47 ±1.85 (5,2)	20.31±2.26 (5,3)	9.50±0.97 (5,3)	71.25±9.66 (5,3)	97.0
12	Bufo w. woodhousei	Oklahoma Cleveland Co.(304)	5	12:12	45.14±11.89 (20-79)	6.47±1.14 (5,1)	23.44 ±3.68 (5,2)	17.17±2.47 (5,3)	5.65±0.37 (5,3)	46.29±3.94 (5,3)	110.8
13	"	"	15	12:12	59.70±11.72 (35-98)	8.08±1.01 (5,1)	28.51 ±3.36 (5,2)	20.46±0.44 (5,3)	8.04±0.44 (5,3)	84.67±1.92 (5,3)	76.8
13	"	"	15	16:8	40.04±8.24 (21-70)	9.08±1.23 (5,1)	31.27 ±4.02 (5,2)	16.05±1.68 (5,3)	4.91±0.55 (5,3)	43.87±5.13 (5,3)	106.7
14	"	"	25	12:12	55.28±3.97 (44-64)	5.16±0.87 (5,1)	18.38 ±3.49 (5,2)	17.90±4.19 (5,3)	7.55±1.50 (5,3)	62.28±14.12 (5,3)	93.9
15	Hyla cinerea	Florida Alachua Co.	15	12:12	9.40±0.82 (7-12)	3.86±0.74 (5,1)	19.23 ±3.65 (5,2)	14.09±0.18 (5,3)	12.37±1.00 (5,3)	98.79±7.00 (5,3)	66.10
16	Hyla femoralis	"	15	12:12	3.23±0.22 (2.5-3.8)	4.50±0.48 (6,1)	19.79 ±2.67 (6,2)	10.76 (6,1)	10.58 (6,1)	77.04 (6,1)	69.1
17	Pseudacris s. streckeri	Oklahoma Cleveland Co.	15	12:12	4.24±0.28 (3.6-5.7)	3.67±0.3 (3,1)	13.42 ±1.05 (3,2)	21.43 (8,1)	17.28 (8,1)	80.95 (8,1)	94.7

TABLE 3. continued

ID	SPECIES	LOCALITY (elevation)	ACCLIMATION °C	ACCLIMATION L:D	BODY MASS (g)	Hb g 100ml^{-1}	Hct (%)	DPG µmol gHb^{-1}	NTP µmol gHb^{-1}	Ptot µmol gHb^{-1}	% P_{tot} as DPG + NTP
ANURANS continued											
18	Rana berlandieri foreri	Sinaloa, Mexico Commercial	15	12:12	74.39±13.44 (27-135)	5.03±0.85 (9,1)	20.77 ±3.70 (9,2)	4.43±0.50 (9,3)	10.72±0.54 (9,3)	62.65±4.78 (9,3)	65.5
19	Rana blairi	Oklahoma Cleveland Co.	15	12:12	51.88±11.74 (28-81)	3.51±0.66 (5,1)	14.60 ±2.43 (5,2)	8.73±2.98 (5,3)	9.38±2.18 (5,3)	49.02±12.36 (5,3)	45.6
20	"	"	25	12:12	45.75±5.34 (36-61)	4.73±0.87 (4,1)	19.44 ±3.24 (4,2)	6.61±0.39 (4,3)	11.26±1.83 (4,3)	56.56±4.12 (4,3)	83.10
21	Rana catesbeiana	"	15	12:12	47.34±6.31 (36-68)	4.92±0.39 (5,1)	21.77 ±2.63 (5,2)	2.88±0.83 (5,3)	10.47±1.03 (5,3)	-	-
22	"	"	25	12:12	53.60±4.34 (39-66)	5.58±0.22 (5,1)	28.10 ±1.04 (5,2)	2.55±0.45 (5,3)	9.45 ±1.31 (5,3)	66.02±5.01 (5,3)	50.7
23	Scaphiopus bombifrons	"	15	12:12	9.10±1.59 (6-13)	8.65±1.05 (4,1)	33.42 ±4.69 (4,2)	1.06±0.49 (4,3)	21.16±1.51 (4,3)	93.82±10.66 (4,3)	69.9
23	"	"	25	12:12	13.63±2.52 (10-19)	18.36±0.81 (3,1)	29.42 ±0.46 (3,2)	0.0 (3,1)	12.12 (3,1)	47.85 (3,1)	76.0
24	Scaphiopus holbrooki hurteri	"	15	12:12	10.4	7.98 (1,1)	31.34 (1,1)	0.0 (1,1)	17.62 (1,1)	70.85 (1,1)	74.6
-	Telmatobius culeus	Bolivia Lake Titicaca(3000)	10	12:12	120.1	6.78±0.10 (1,4)	25.58 ±0.71 (1,4)	5.83±0.48 (1,3)	17.70±0.59 (1,3)	77.47±1.14 (1,3)	83.6
25	Xenopus laevis	Commercial	25	12:12	28.00±3.20 (19-37)	13.06±0.74 (5,1)	47.45 ±3.84 (5,2)	13.57±0.85	8.52±0.69	84.02±6.59	62.7
URODELES											
26	Ambystoma maculatum	Arkansas	15	12:12	-	7.98 (1,1)	26.52 (1,1)	15,12 (1,1)	7.50 (1,1)	61.53 (1,1)	85.7
27	Ambystoma t. tigrinum	Commercial	5	12:12	34.68±3.35 (28-46)	6.48±1.17 (5,1)	29.44 ±5.36 (5,2)	19.01±1.75 (5,3)	10.42±0.85 (5,3)	70.20±5.95 (5,3)	98.7
28	"	"	15	12:12	34.48±2.95 (25-42)	5.59±0.65 (5,1)	23.01 ±2.50 (5,2)	13.85±1.39 (5,3)	10.52±0.90 (5,3)	73.73±7.48 (5,3)	80.4
28	"	"	15	16:8	35.98±3.56 (26-48)	5.84±0.42 (5,1)	27.02 ±1.27 (5,2)	17.57±3.58 (5,3)	12.04±1.24 (5,3)	67.62±7.80 (5,3)	105.4
29	"	"	25	12:12	38.20±3.66 (29-50)	6.78±0.91 (5,1)	31.63 ±4.04 (5,2)	15.74±2.56 (5,3)	13.11±0.96 (5,3)	87.72±4.22 (5,3)	80.7
30	Amphiuma tridactylum	Louisiana	25	12:12	511.20±38.75 (431-626)	5.06±0.85 (5,1)	24.65 ±3.51 (5,2)	29.22±2.93 (5,3)	27.60±1.72 (5,3)	197.68±13.45 (5,3)	71.4
31	Desmognathus brimleyorum	Arkansas-	15	12:12	-	6.86±0.67 (6,1)	36.97 ±3.29 (6,2)	21.39 (6,1)	10.12 (6,1)	93.91 (6,1)	77.9
33	Necturus m. maculosis	Wisconsin	5	12:12	82.18±7.36 (54-94)	4.80±0.58 (5,1)	23.37 ±2.12 (5,2)	2.54±0.57 (5,3)	26.50±1.65 (5,3)	103.44±12.31 (5,3)	81.8
34	"	"	15	12:12	75.84±4.37 (61-87)	4.42±0.50 (5,1)	24.30 ±1.34 (5,2)	6.14±0.26 (5,3)	28.03±1.69 (5,3)	125.84±3.60 (5,3)	76.6
34	"	"	15	16:8	84.88±10.79 (64-116)	4.37±0.63 (4,1)	22.47 ±2.39 (4,2)	6.19±2.21 (4,3)	27.50±2.45 (4,3)	169.12±34.14 (4,3)	56.1
35	"	"	25	12:12	64.5±.02 (53-77)	4.32±0.47 (5,1)	25.27 ±3.26 (5,2)	16.29±1.66 (5,3)	28.21±2.41 (5,3)	132.32±23.37 (5,3)	88.6
32	Notophthalmus v. viridescens	Pennsylvannia	15	12:12	2.20±0.15 (1.6-2.7)	3.16±0.36 (5,1)	20.77 ±2.27 (5,2)	3.55 (7,1)	5.55 (7,1)	37.71 (7,1)	63.0
32	"	"	15	16:8	1.96±0.07 (1.7-2.2)	3.38±0.40 (7,1)	24.22 ±5.48 (7,2)	5.15 (7,1)	5.50 (7,1)	56.89 (7,1)	47.1
36	Siren lacertina	Florida	20	12:12	-	4.26±0.25 (3,1)	24.03 ±4.48 (3,2)	12.08±1.74 (3,3)	16.82±4.50 (3,3)	127.10±22.15 (3,3)	58.7

TABLE 4.

Genus (no. species)	DPG (molar ratio)	NTP (molar ratio)	P_{50} 2% CO_2	Energetics for activity	Recovery time @ 15 °C
Bufo (9)	1.15	0.53	35.0	aerobic	2.5
Rana (9)	0.33	0.65	26.0	anaerobic	6.0
Scaphiopus (1)	0.07	1.36	—	aerobic	3.0
Xenopus	0.88	0.55	34.0	"mixed"	9.0

FIGURE CAPTIONS

Figure 1. Ion-exchange chromatograms of six species of anuran amphibians. The abscissa in each plate is the fraction number (3.8 ml per fraction) collected from a linear 0.0-5.0 N (3:2 formic acid:ammonium formate buffer, pH 3.57) gradient (Isaacks et al., 1976). The ordinates show micromoles phosphate (as PO_4) per ml RBC (solid line) or UV absorbance 254 nm (dashed line). Peaks are identified as inorganic phosphate (P_i), adenosine diphosphate (ADP), 2,3-diphosphophosphate (DPG), guanosine triphosphate (GTP) and inositol polyphosphate (IPP).

Figure 2. Ion-exchange chromatograms of four species of urodeles and two anuran species. Symbols and manner of presentation are identical with Fig. 1.

Figure 3. The percentages of the erythrocytic organic phosphates ATP, DPG and GTP plotted on a trilinear chart (Batschelet, 1971). Each point represents the sum of three percentages which equal 100%. All of the points appear in Table 2 except points O,W,X,Y and Z which were taken from the literature (O, R. catesbeiana tadpoles, Bartlett, 1976; W, Anguilla

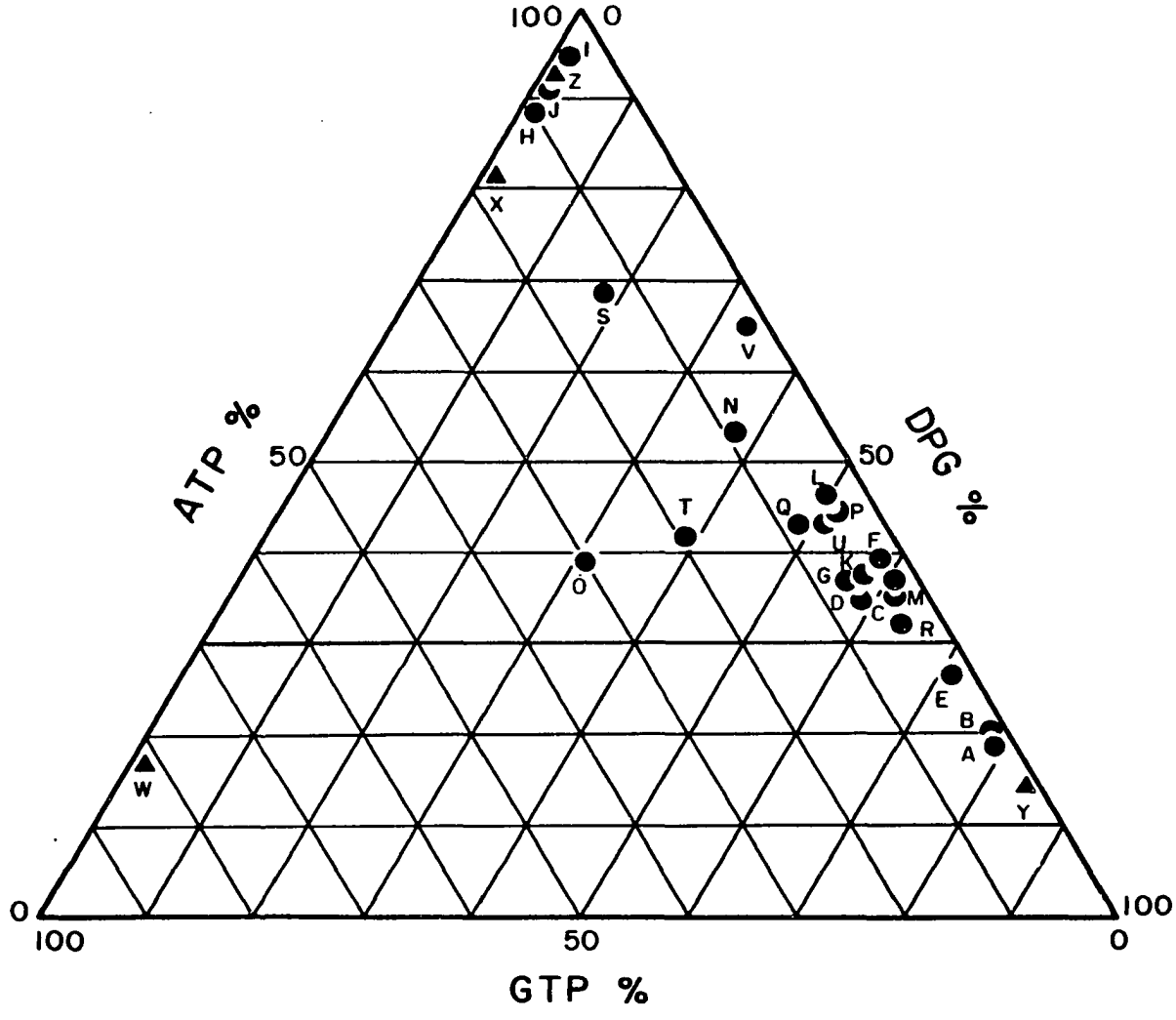
rostrata, Geoghegan and Poluhowich, 1974; X, Scomber japonicus, Bartlett, 1978; Y, man, Bartlett, 1971; Z, Crotophytus collaris, Hughes and Hutchison, in prep).

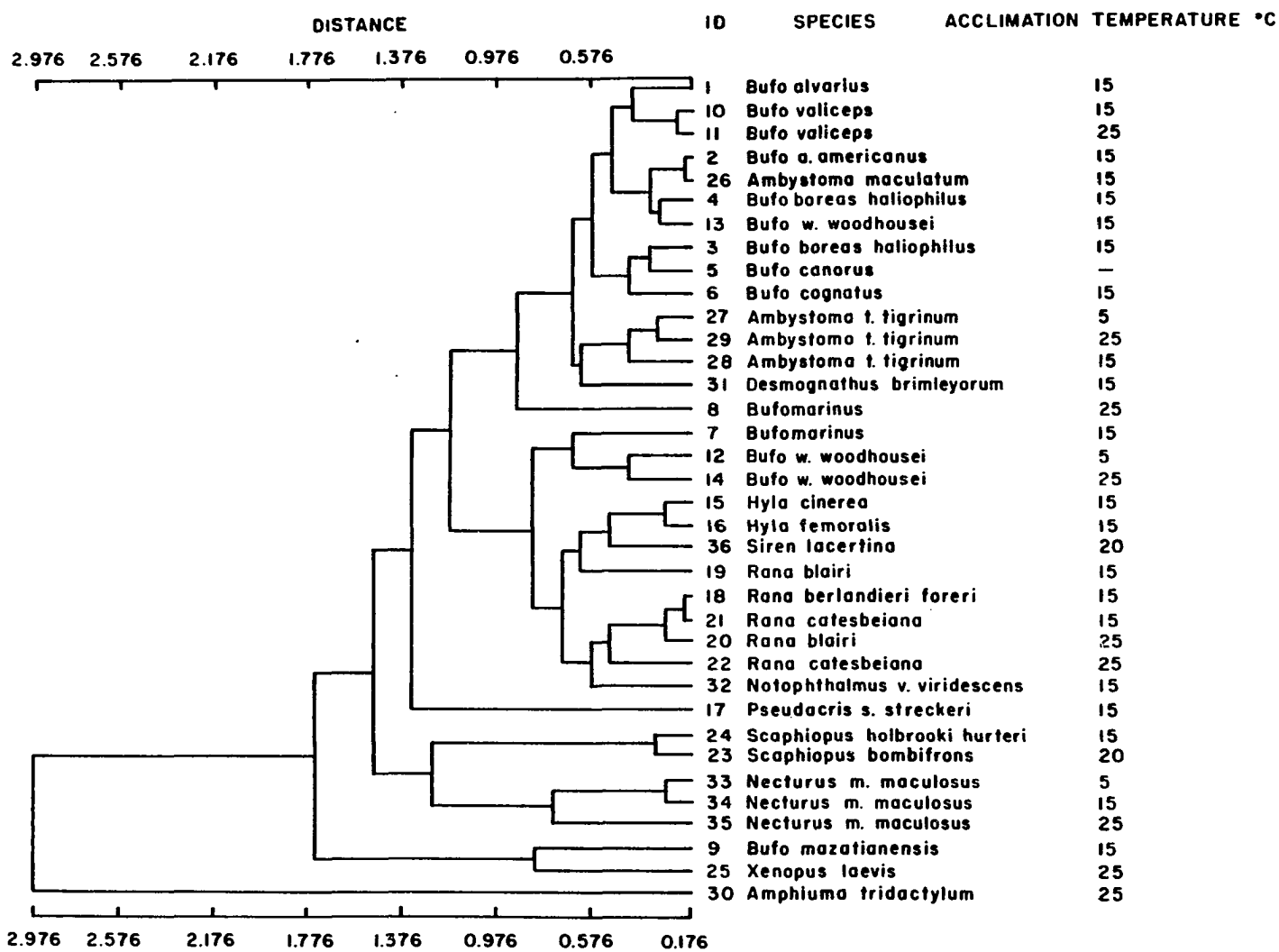
Figure 4. Distance phenograms based on the unweighted pair group arithmetic average clustering (UPGMA) method for the five parameters listed in Table 3 and Fig. 5. The ID numbers are identical with entries in Table 3 and Fig. 5.

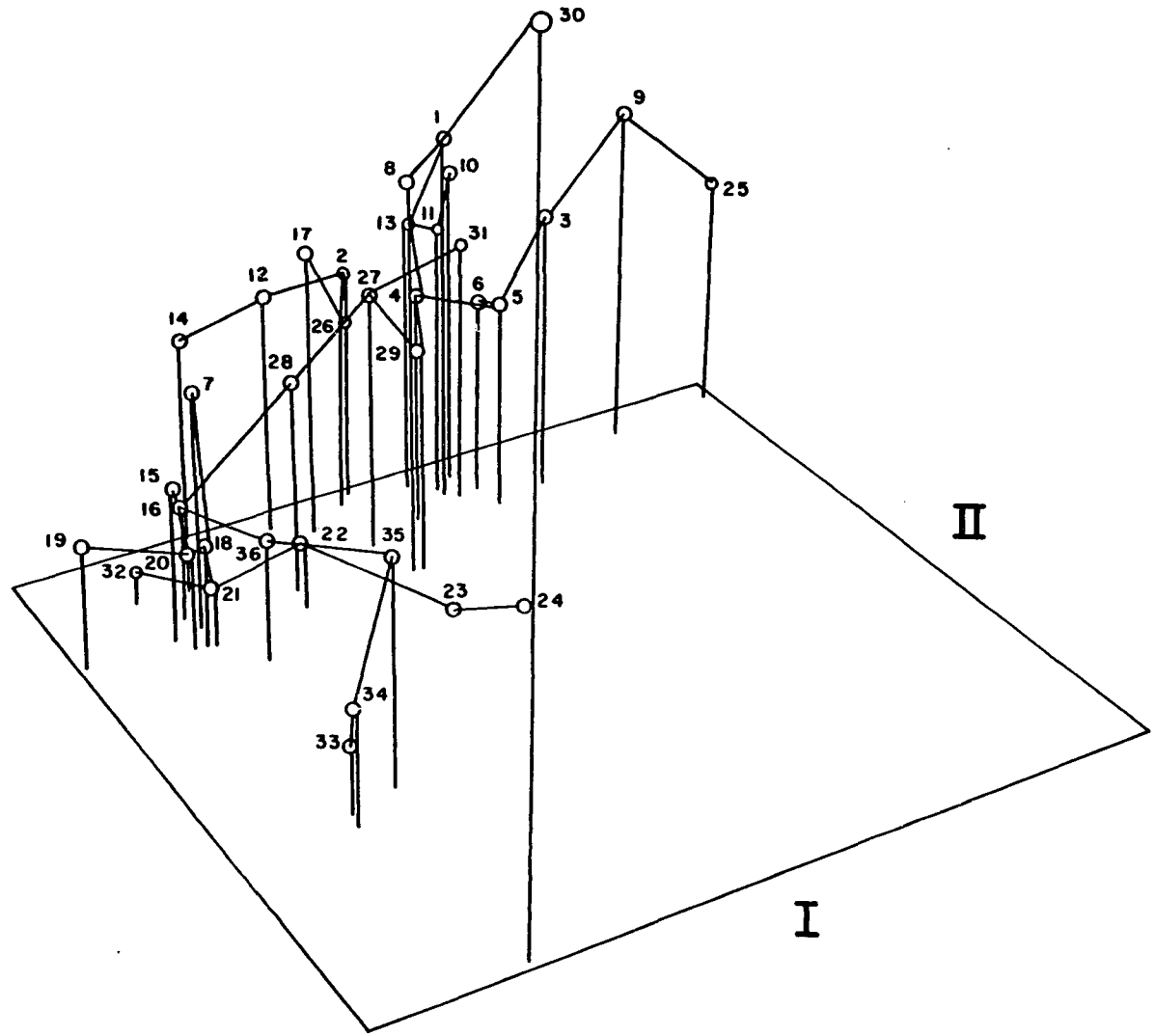
Figure 5. Three-dimensional projection of the entries of Table 3 onto the first three principal components derived from a matrix of distances among the parameters listed in Table 3. The ID numbers are identical with those in Table 3 and Fig. 4. Axes I and II are labeled, axis III is represented as height. The shortest minimally connected network (MST) is projected onto the principal component space to connect nearest neighbors.

MICROMOLES PHOSPHATE ML⁻¹ RBC (——)
Bufo cognatus
Bufo marinus
Bufo M. woodhousei
Rana blairi
Rana catesbeiana
Xenopus laevis
Pi
ADP
ATP
DPG
GTP
IPP
FRACTION NUMBER
ABSORBANCE AT 254 NM (– – –)

MICROMOLES PHOSPHATE ML⁻¹ RBC (——)
Ambystoma t. tigrinum
Amphiuma tridactylum
Cryptobranchus alleganiensis bishopi
Necturus m. maculosis
Pseudobranchus striatus
Siren lacertina
Pi
ADP
ATP
DPG
GTP
IPP
FRACTION NUMBER
ABSORBANCE AT 254 NM (- - -)


100
0
ATP %
50
DPG %
50
0
100
100
50
0
GTP %
I
Z
J
H
X
S
V
N
T
O
L
Q
P
U
F
K
G
D
C
M
R
E
B
A
W
Y


DISTANCE
2.976 2.576 2.176 1.776 1.376 0.976 0.576
0.176
ID SPECIES ACCLIMATION TEMPERATURE °C
1 Bufo alvarius 15
10 Bufo valiceps 15
11 Bufo valiceps 25
2 Bufo a. americanus 15
26 Ambystoma maculatum 15
4 Bufo boreas haliophilus 15
13 Bufo w. woodhousei 15
3 Bufo boreas haliophilus 15
5 Bufo canorus —
6 Bufo cognatus 15
27 Ambystoma t. tigrinum 5
29 Ambystoma t. tigrinum 25
28 Ambystoma t. tigrinum 15
31 Desmognathus brimleyorum 15
8 Bufomarinus 25
7 Bufomarinus 15
12 Bufo w. woodhousei 5
14 Bufo w. woodhousei 25
15 Hyla cinerea 15
16 Hyla femoralis 15
36 Siren lacertina 20
19 Rana blairi 15
18 Rana berlandieri foreri 15
21 Rana catesbeiana 15
20 Rana blairi 25
22 Rana catesbeiana 25
32 Notophthalmus v. viridescens 15
17 Pseudacris s. streckeri 15
24 Scaphiopus holbrooki hurteri 15
23 Scaphiopus bombifrons 20
33 Necturus m. maculosus 5
34 Necturus m. maculosus 15
35 Necturus m. maculosus 25
9 Bufo mazatlanensis 15
25 Xenopus laevis 25
30 Amphiuma tridactylum 25


30
1
9
8
10
25
13
11
3
31
17
2
12
27
4
6
5
14
26
29
28
7
15
16
19
18
22
35
36
32
20
21
23
24
34
33
II
I

PAPER II

THE RESPONSES OF ERYTHROCYTIC ORGANIC PHOSPHATES TO EXERCISE, TEMPERATURE AND ALTITUDE IN THE ANURAN *XENOPUS LAEVIS*

This paper was prepared for
submission to the journal,
Respiration Physiology

Abstract

This study is an assessment of the physiological role of erythrocytic organic phosphates (EOPs) of the anuran, Xenopus laevis in gas delivery. Manipulation of O_2 transport requirements was accomplished by forced exercise, altered acclimation and simulated altitude. Both short and long term experiments were performed and equal numbers of males and females were included for each experiment. Sex differences were not observed for any variable. Daily 20 min. exercise for 1 and 6 weeks had no effect on the levels of 2,3-diphosphoglycerate, the major EOP of Xenopus. Acclimation temperature significantly altered pH and EOP levels but did not alter the overall oxygenation enthalpy of blood (-5.7 kcal M^{-1}). The changes in pH and EOP levels acted to reduce the effect of temperature on the P_{50} of blood. A simulated altitude of 6096 m was not a significant hypoxic stress. Significant elevations of [DPG] (30%) and P_{50} (23%) occurred after 4 days at 9144m. Whole blood pH may control long term changes in EOP concentrations while short term control may be due to a combination of pH and other factors. The observed changes in EOP concentrations are consistent with the strongly developed diving physiology of Xenopus.

KEY WORDS

Xenopus laevis, anura, 2,3-diphosphoglycerate, hypoxia, temperature, exercise, oxygen delivery, altitude, amphibians

INTRODUCTION

The O_2 affinity of blood is the result of genetic factors altering the hemoglobin (Hb) molecule, the blood temperature and pH, as well as the concentrations of erythrocytic organic phosphates (EOPs) (Greaney et al., 1980; Bunn et al., 1977). These EOPs are produced by the red cell metabolism; factors affecting this metabolism can therefore alter the O_2 affinity of Hb and change the P_{O_2} gradient and the rate of O_2 delivery (Brewer et al., 1974).

The bulk of the information about erythrocyte metabolism is based on the enucleated mammalian red cell with the human red cell as the principal model. The metabolism of the nucleated vertebrate red cell is less well known (Araki et al., 1971; Gillen and Riggs, 1971; Geoghegan and Poluhowich, 1974; Bartlett, 1976; Isaacks et al., 1977; Borgese et al., 1978). Amphibian EOP profiles range in type from those resembling fishes and reptiles to those of mammals (Hazard, 1981). A detailed study of one anuran species was initiated to assess the physiological role of EOPs in amphibians. The African clawed frog, Xenopus laevis, is an unusual anuran in many respects but was chosen for several reasons: (1.) The major EOP is 2,3-diphosphoglycerate (DPG); therefore direct comparisons could be made between Xenopus RBCs

and the more familiar mammalian RBC whose major EOP is also DPG. (2.) Xenopus has quite high [Hb] and hematocrits (Hcts) for an amphibian (Hillman, 1976). (3.) These frogs do not possess the multiple Hb types which provide wide functional capacity in fish (Maclean and Jurd, 1971; Powers, 1972; Brunori, 1975). (4.) The sexual dimorphism of these frogs allows convenient examination for sex differences in hematological parameters and EOP concentrations (Harris, 1972; Parker et al., 1972)

We determined the responses of the [EOPs] to both long- and short-term challenges to the O_2 delivery capacity achieved by: (1.) an active increase in tissue demand for O_2 due to forced exercise, (2.) a passive alteration of O_2 consumption by exposure to different temperatures, and (3.) a restriction of O_2 supply by simulation of high altitude.

MATERIALS AND METHODS

Statistics

Experiments were designed for analysis by a model I two-way analysis of covariance (ANCOVA). The fixed factors were: sex (m and f) and treatment (control and experimental). Body mass was chosen as the covariate to provide a statistical control for the generally smaller size of males and because body mass (BM) is associated with significant differences in oxygen affinity, Bohr effect and DPG sensitivity of hemoglobin (Schmidt-Nielsen and Larimer, 1958; Riggs, 1960; Riggs, 1976). All Hct, methemoglobin (METHB) and percentage change (delta) in BM (ΔBM) values were arcsin transformed to angular variates to assure normality prior to statistical analyses (Barr et al., 1979). Comparison of regression lines was done according to Snedecor and Cochran (1980). In all cases significance was assumed at the $P < 0.05$ level.

Animal Maintenance

Equal numbers of males and females were selected from laboratory stocks and maintained separately in 76 l aquaria at a density of about one animal per 4 l. The water was not aerated but was changed weekly after each feeding ("Frog Brittle", NASCO Biol. Supply Co.). The aquaria were kept in large environmental chambers with a constant photoperiod (LD 12:12 centered at 1200 CST) and a temperature of $25 \pm 2^{\circ}C$.

Animal Selection

Experimental animals were selected in the following manner. Two males of equal mass ($\pm$ 1.0g) were chosen and one placed into each of two plastic boxes containing 3.0 l of water. Two females of equal mass ($\pm$1.0g) were selected and one placed into each of the plastic boxes. The females and males were as closely matched in mass as possible. The male-female pairs were then randomly assigned to experimental or control status and returned to the appropriate environmental chambers.

Exercise

Both experimental and control groups were removed from the environmental chamber and transported to the laboratory. The boxes of the experimental and control animals were handled approximately equally. Large jars were placed into the centers of two circular plastic containers (15" diameter, 5" deep) to create perimeter raceways. Water from a tap was run into a T-tube and directed into the chambers so that it flowed clockwise in one chamber and counter-clockwise in the other. No effort was made to determine the flow rate; the water temperature was adjusted to 25 $\pm$ 3^{o}C. Animals were placed into each of the chambers for 10 minutes and forced to swim into the current by manual or electrical stimulation. The animals were allowed access to air at all times but were not allowed to drift in the current or hang onto the sides for longer than 30 seconds. This process was

continued daily for seven consecutive days in the short-term experiment. In the long-term experiment the animals were exercised 10 minutes per day, five days per week for two weeks and then 20 minutes per day, five days per week for another four weeks. Animals were fed on the middle day of their exercise week during the short-term experiments and weekly during the long-term experiments to prevent excessive depletion of energy reserves.

All frogs were exercised between 0800 and 1200; short-term animals were pithed approximately one hour after their last exercise. Long-term animals were allowed to recover for 24 hours after their last exercise. The short- and long-term exercise experiments were performed in September and February-March, respectively. All pH and P_{50} measurements were made at 25°C.

Temperature

Six sets of five male-female pairs were used. The animals at 15 °C and 25° C were maintained at these temperatures for six months or longer. Short term exposure to 5 and 30 °C was accomplished by transfer of the animals from 25 °C to the respective temperatures for one week (5S and 30S). Long term exposure to 5 and 30°C (5L and 30L) was achieved by altering the environmental chamber temperature 1° C per week until the final temperature was reached; frogs were maintained at the final temperature for at least one month prior to analysis. The pH and P_{50} of blood from each animal was determined at 15, 25 and 35° C.

The blood was kept in gas-tight syringes until the pH measurements were made. The temperature-induced pH changes are therefore closed system constant CO_2 heating curves (Cameron, 1978).

Altitude

Altitudes of 6096 m (20,000 feet, 0.46 atmosphere, P_{O_2} ca. 70 mm Hg, 9.33 kPa) and 9144 m (30,000 feet, 0.30 atmosphere, P_{O_2} ca. 40 mm Hg, 5.33 kPa) were simulated in a cylindrical (18" by 6') plexiglas hypobaric chamber (Vista Scientific, Ivyland PA.). A vacuum pump reduced the pressure within the chamber while a constant inflow of air (425 l min^{-1}) prevented anoxia. The particular sequence of exposures to 6096 m was chosen to reveal the time course of any changes in blood parameters and the order of the experiments was selected to bracket the time of the first significant response in EOP levels. Animals were exposed to 9144 m for only 4 days. Control pairs were placed on a shelf next to the hypobaric chamber as physically close to the experimental pairs as possible. All pH and P_{50} measurements were made at 25°C.

Blood Analysis

Within 10 minutes of the end of an experiment both experimental and control pairs were returned to the laboratory, double-pithed, wiped, weighed to the nearest 0.1 g and placed onto a plastic and foil covered ice-filled dissection tray. An alternate method involved double-pithing the frogs prior to

transport to the lab. These animals were therefore chilled within one minute of their removal from experimental conditions. This latter method ameliorated to some extent the extreme acidosis encountered with the former method. The ventral chest was opened to expose the irridescent pericardium and the latter cut open to expose the heart. A heparinized 1.0 cc tuberculin syringe with a 25 gauge needle was inserted into the ventricle and 0.1-0.5 ml of blood withdrawn. Samples of blood from the syringe were used to fill three 20 and one 100 microliter (μl) capillary tubes. The syringe was then placed onto ice. The 20 μl tubes were emptied to the calibration lines (the excess was used to form a blood smear), centrifuged at 5130 X g for five minutes and the Hcts measured. One of these tubes was used to determine total [Hb] as cyanomethemoglobin (based on human Hb as a standard) while the other two were used to determine the percentage of methemoglobin (METHB, Evelyn and Malloy, 1938; Dubowski, 1960). The 100 μl tube provided plasma for determination of glucose (Sigma 15-UV) and lactate (Boeringer-Mannheim Lactate-UV). The remaining blood in the syringe was used for pH determination (Radiometer G297-G2 electrode calibrated to NBS-type standards S1500 and S1510; the electrode was recalibrated at every measurement temperature), red blood cell count (RBCCT), mean cell volume (MCV), mean cellular Hb (MCH), mean corpuscular Hb concentration (MCHC), [Hb], Hct (the latter six variables were measured with a model ZBI Coulter

Counter equipped with a P_{64} Size distribution analyzer, an X-Y plotter, a Hemoglobinometer, and a MHR MCV/HCT/RBC computer) and P_{50} (Aminco Hem-O-Scan). After capillary tubes were filled, the ventral aorta was severed and an additional 0.3-0.7 ml of blood collected and prepared for analysis of organic phosphates as described in Hazard (1981). Ion-exchange chromatography (IEC) was performed for one male and one female from each experimental group (Isaacks et al., 1976; Hazard, 1981).

After blood collection, the egg masses of the females were dissected free and weighed. The eggs were termed "mature" if the majority possessed grey and white hemispheres. No further effort was made to determine the maturity of the females. Males were dissected to confirm their sex but no record was kept of the presence of pigment patches on the forelegs (these indicate sexual maturity, Thompson and Franks, 1978). The liver and a mass of femoral tissue from the altitude exposed animals were dissected and frozen for later analysis of glycogen (Hutchison et al., 1977) and lactate (Boeringer-Mannheim, Lactate-UV).

Oxygen Affinity

The Hem-O-Scan was operated at the slowest programmable O_2 delivery rate. Pairs of gases (2% CO_2 in N_2 and 2% CO_2 in air) were either calibration or primary standard grade ($\pm$0.2% and $\pm$0.02%, respectively). The accurate and reproducible determination of P_{50} for high affinity respiratory pigments

requires significant modification of the Hem-O-Scan (Lapennas et al., 1980; Arp and Childress, 1981). However, the performance of the unmodified instrument was judged adequate for use with Xenopus whole blood since the P_{50} was greater than 20 mm Hg at all measurement temperatures. Most O_2 binding curves (OBCs) were determined at 25°C (the reference acclimation temperature) although additional curves were determined at 5, 15, and 35°C.

RESULTS

The major EOP of Xenopus was DPG (fig. 1). The mean [DPG] was 18.09 ± 1.47 μmol gHb^{-1} (45.9 ± 1.57% of the total acid soluble phosphate [P_{tot}]) as determined by IEC (table 1). The IEC results were not compared for sexual or treatment effects due to the small sample sizes. Simultaneous enzymatic-colorimetric (EC) analyses were performed for 15 of the 18 individuals. The IEC results as a percentage of the EC results were: 104.5, 82.7 and 108.7 % for [DPG], [nucleoside triphosphate] (=NTP i.e. the sum of adenosine triphosphate (ATP) and guanosine triphosphate (GTP)), and [P_{tot}] respectively. The mean inorganic phosphate $[P_i]_{IEC}$ was 6.27 ± 0.81 μmol gHb^{-1} (7.63 ± 0.63% of [P_{tot}]). The mean concentration of adenosine diphosphate $[ADP]_{IEC}$ was 0.620 ± 0.144 μmol gHb^{-1} (1.65 ± 0.36% of [P_{tot}]). The mean $[ATP]_{IEC}$ was 5.11 ± 0.326 μmol gHb^{-1} (20.24 ± 1.03 % [P_{tot}]). The mean $[GTP]_{IEC}$ was 1.20 ± 0.114 μmol gHb^{-1} (4.88 ± 0.38% [P_{tot}]). The [GTP] was significantly correlated with BM (r= 0.63, P=0.0005, n=18). The mean GTP:ATP ratio was 0.230 ± 0.013. The mean adenylate charge or gamma(Γ) ([ATP] /([P_i] [ADP])) was 10.88 ± 3.07 uM^{-1} (Lehninger, 1975). Gamma was significantly correlated with pH (r=0.53, P=0.03, n=18). The coefficient of variation for ADP was quite high (95%) as was the coefficient of variation for gamma (119.9%).

Exercise

After one week of daily exercise there were no significant differences between experimental and control groups for any EOPs or hematological parameters. The experimental group (males did not differ from females) lost a larger percentage of initial BM than the control group ($F_{(1,19)}$ =19.15, P=0.005). Whole blood P_{50} was significantly correlated with pH and NTP (r=-0.49 and n=20 for both variables). The least square regression lines were: P_{50} =-2.28 [NTP] + 51.02 and P_{50} =-9.86 pH + 7.805. Large animals had lower blood pH (pH= -0.0137(BM) + 7.805) than small animals.

After six weeks of exercise, there were still no significant differences between experimental and control groups for any EOPs or hematological parameters. The experimentals again lost higher= percentages of initial BM than controls ($F_{(1,19)}$ =10.44, P=0.0056). Whole blood P_{50} was again significantly correlated with pH (P_{50} = -21.98(pH) + 196.6, r=0.79) but not with any other parameter. Large animals also had a lower pH (pH= -0.0058(BM) + 7.532, r=0.45, n=20).

The short and long term exercise data sets were pooled and reanalyzed. A higher BM loss in male animals was detected as were sex differences for [P_{tot}], [NTP] and [DPG] (table 2). There were significant differences attributable to treatment effects in this pooled group. However, with the exception of percentage BM loss (larger for the long term group) there were no differences

between experimental and control groups (table 3). All possible comparisons between groups were calculated, but to insure overall protection against type I error, only these pre-planned comparisons are listed (Barr et al., 1979).

Temperature

With the exception of BM ($F_{(1,59)}$ =13.41, P=0.0006) there were no differences between the sexes in these experiments (table 4). All measured parameters showed significant variation attributable to acclimation temperature. The RBCCT, MCV, MCHC, Hct and [Hb] all showed regular increases with temperature. The percentage of METHB was highest in the 30S group; the METHB was negatively correlated with whole blood pH (r=-0.32, P=0.022, n=52) whose lowest value was also in the 30S group. The [DPG] decreased from a high in the 5L group to a low in the 30S group (fig. 3). The 5S frogs were little changed from the 25 group while the 30L group had a large increase in [DPG]. The decreases in [DPG] (5L to 30S) roughly paralleled the decrease in pH while the increase in [DPG] at 30L accompanied an elevation in whole blood pH. The H^+ changed a maximum of 400% with increasing temperature; the [DPG] decreased about 50% over this same range but the P_{50} varied only 28%. The [NTP] and [P_{tot}] were quite variable but without apparent trends. The [NTP] (measured by U-V absorbance of thin-layer chromatograms which would not detect DPG) changed little with acclimation to temperature, aestivation

or osmotic stress and ATP only raised the P_{50} of Hb by 2.7 mm Hg over the molar ratio range of 0.0-3.0 (mole ATP/mole Hb) in Xenopus (Jokumsen and Weber, 1980). The BM tended to be larger in the colder acclimation groups (table 4). The transition from 25°C to 5°C for one week in the 5S group resulted in an average weight gain of about 16%; there was no significant weight loss after one week at 30°C.

Whole blood pH was significantly different at all measurement temperatures for all acclimation regimes (fig. 3). The slopes of the lines for the pooled male and female values were not significantly different ($F_{(11,32)}$ =0.2947 $0.50<P<0.25$) although the elevations of the lines were ($F_{(11,143)}$ =72087.0, $P<0.0001$). For the 5S and the 15°C animals, the males and females had different elevations ($F_{(1,33)}$= 13.58; $F_{(1,21)}$= 6.996 respectively) but not different slopes (table 5).

The temperature sensitivity of whole blood was determined as the overall oxygenation enthalpy (ΔH) calculated from van't Hoff plots (Powers et al., 1979). The ΔH value was calculated for all 60 animals but was not correlated with any other variable. The P_{50} values were then grouped by acclimation treatment and sex and the 12 van't Hoff equations compared; there were no significant differences between slopes ($F_{(11,54)}$ =1.13) or elevations ($F_{(11,165)}$ =0.33). The pooled regression equation was $Ln(P_{50}) = -2872.6(1/T^{o}K) + 13.12$; the ΔH was therefore -5.7 kcal mol^{-1}.

ALTITUDE

The first experiment at 6096 m lasted 24 hours; the [DPG] in experimental frogs was elevated over control levels but there were no differences in P_{50}. The next experiment was 4 days at altitude since the increase in [DPG] seemed more likely to result in an increase in the P_{50} after the longer exposure; the results were negative. This experiment was followed by the two day, then the half-day experiments and finally the 28 day experiment; all three of these latter experiments gave negative results for all variables.

The control animals for the 12 hour exposure to 6096 m had highly elevated ($F_{(1,15)}$ = 7.15, P=0.0174) concentrations of DPG (22.7 $\pm$ 1.60 μmol gHb^{-1} vs 16.7 $\pm$ 1.60 μmol gHb^{-1} for the altitude exposed animals). The altitude exposed animals had [DPG] comparable to those of control animals from other altitude experiments. None of the other measured variables was significantly different between the two groups.

After 24 hours at 6096 m, the [DPG] was significantly ($F_{(1,15)}$ =8.83, P=0.0086) elevated in the altitude animals (altitude= 21.0 $\pm$ 1.03 μmol gHb^{-1}; control= 16.8 $\pm$ 0.94 μmol gHb^{-1}).The [DPG] was also higher ($F_{(1,17)}$= 5.88, P=0.0268) in males (20.7 $\pm$ 0.99 μmol gHb^{-1}) than in females (17.2 $\pm$ 1.00 μmol gHb^{-1}). Plasma lactate of altitude animals (165.3 $\pm$ 17.6 mg%) was elevated ($F_{(1,15)}$= 5.95, P=0.0277) above control (104.4 $\pm$ 17.6

mg%) animals in the 4 day altitude experiment. The MCH was higher ($F_{(1,15)}$= 6.28, P=0.0242) in females (142.1 $\pm$ 3.4 pg $cell^{-1}$) than in males (128.2 $\pm$ 3.4 pg $cell^{-1}$) for the 28 day group. In three out of the five 6096 m experiments, the BMs of the males and females were not significantly different. There were no significant differences between treatment groups for any other measured variable from any other 6096 m experiments.

The following variables from the 6096 m experiments showed significant correlations with BM: muscle glycogen (MGY); [13.68 (BM) + 62.59= MGY, r= 0.53, n=84], liver lactate (LL); [-0.745(BM) + 99.65= LL, r=-0.32, n= 83], [Hb] [0.0709(BM) + 9.21=[Hb], r=0.31, n=108] and Hct [0.00254(BM) + 0.3025=Hct, r=0.32, n=108].

Exposure of animals to 9144 m resulted in elevations of [DPG], P_{50} (the correlation between P_{50} and [DPG] was 0.54, P=0.013, n=20) and [P_{tot}] (approximately 50% contributed by DPG, table 6). No other variables showed significant differences attributable to altitude exposure.

DISCUSSION

The reason for rigorously including equal numbers of both males and females in each experiment was to determine whether the different reproductive strategies of the macrogametic sex and the microgametic sex would affect their respective blood physiology. Because of their necessarily larger energy commitment to gamete production, females might show a reduced scope for physiological adjustment; the results are not conclusive. Sex differences do reappear often enough to warrant further investigation, however. Some sex differences in small frogs could be due to differences in maturity since small females (less than 20g) were more likely to be immature than the males. Although these experiments were adequate to challenge the O_2 delivery system they were not necessarily appropriate to elicit different responses in males and females.

Throughout the experiments the pH of the ventricular blood of both experimental and control animals was low, although there were never differences between experimental and control animals. These extreme pHs may result from the handling associated with double-pithing. The BM of all control and experimental females was positively correlated with pH under these conditions (pH= 0.00674 (BM) + 6.94, r=0.358, n=85). The BM/pH relationship was not significant for males. Under the most careful conditions, the double-pithing led to excursions of 0.1-0.3 pH units below that

expected for amphibians (Reeves, 1972). Xenopus are extremely skittish and the actual acid-base status of these animals cannot be approached by the methods used. Alteration of human erythrocytic [DPG] was not detectable until approximately three hours after in vitro pH changes (Bellingham et al., 1971). The pH rise at the end of one week at 5°C (5S) was about 0.5 pH unit above the blood pH of the 25°C frogs (both measured at 25°C). During this week the [DPG] only increased about 5%. The observed pH excursions therefore seem less likely to have resulted in spurious EOP concentrations during the 1-10 minutes prior to the cooling of the animals.

EXERCISE

Both short- and long-term experimental animals lost higher percentages of BM than controls, indicating that the exercise regimens did increase metabolic demand for O_2 in the experimental groups and therefore met the design goals of the experiment. That none of the measured parameters consistently showed differences between experimental and control groups means that both the short and the prolonged exercise regimens were well within the physiological scope of the O_2 delivery system. Analysis of the activity of the control enzyme citrate synthetase in muscles of the male Xenopus trained for six weeks also revealed no differences between experimental and control animals (Miller and Camilliere, 1981). The blood and muscles of Xenopus are therefore

fully capable of maintaining aerobic metabolism for extended periods without modification. In contrast, the muscles of Rana pipiens show increased activities of citrate synthetase as a result of treadmill training (Cummings, 1979). The differences between the short- and long-term groups indicate that the longer period was in general the more stressful experiment. However, since the experiments were conducted approximately 5 months apart the possibility of seasonal differences must be considered. All such explanations appear to require either a complex entrainment of animals to some non-apparent exogenous factor in an otherwise standardized environment or the long term persistence of genetically acquired endogenous rhythms in these laboratory raised animals. There is a distinct 28 day sinusoidal pattern in mean endurance performance of male Xenopus (Miller and Camilliere, 1981). If the EOP concentrations do affect the exercise performance in Xenopus, then analysis at the end (March) of the long-term exercise prototcol could conceivably have fallen on an EOP "high" relative to the September EOP "low" for the short-term exercise group. The mechanism controlling such a cycle is not apparent.

The BM loss of the long-term exercised males was larger than that in females and may occur whenever the combination of handling and experimental conditions combine to produce a higher level of stress in males. Sex differences in $[P_{tot}]$, [DPG] and

[NTP] were also significant. The precise origin of these differences is not evident.

This general lack of response to exercise in Xenopus EOPs is not particularly unusual. Responses in human athletes to exercise include increases (Eaton et al., 1969; Böning et al., 1975; Boswart et al., 1980), decreases (Dempsey et al., 1971) or no changes in [DPG] (Bonner et al., 1975;Shappel et al., 1971). In race horses, training decreased [DPG] (Lykkeboe et al., 1977). The effect of EOP levels on work performance in trained animals depends on many factors including the type of training and the individual variations in cardiovascular capacity associated with health and age.

TEMPERATURE

Amphibians acutely exposed to temperatures frequently gain weight (Schmidt-Nielson and Forster, 1954; Hutchison, 1961). In view of this, the weight gains of the 5S animals are not unusual. The joints of these animals were swollen, their skin was edematous and their abdomens distended. The lack of unusual changes in Hct, [Hb], RBCCT and MCV, however, do not indicate that the extracellular fluid was particularly dilute. The increases of [Hb], Hct, RBCCT, MCV and MCHC are the expected responses to the increased metabolic rates which accompany higher temperatures in ectotherms. The 30S animals at once exhibited the highest METHB, the lowest pH and [DPG]. These phenomena are

related; the formation of METHB is frequently encountered at high temperatures in in vitro experiments with Hb solutions (Sullivan, 1974). The overall effects of temperature on Xenopus blood are comparable (fig. 3) but the relative metabolic and respiratory acidosis is different in the various acclimation groups. Presumably, at low temperatures Xenopus' $\dot{V}_{O_2}$ would be lower and less CO_2 and lactate would be produced (although reduced lactate was not observed in rainbow trout; Randall and Cameron, 1973; Conners et al., 1978). At 5 C in Xenopus gas exchange would be entirely aquatic-cutaneous, P_{CO_2} would be quite low and the blood alkaline (Rahn, 1966; Mackenzie and Jackson, 1977). The low temperature alkalosis is the result of the physico-chemical properties of blood (Howell et al., 1970; Reeves, 1972) coupled with the purely aquatic respiration and temperature induced Q_{10} effects on metabolism. The overall rate of glycolysis in human RBCs is much higher at alkaline pH and [DPG] increases as well. In addition to enhancement of glycolysis, alkaline pH activates the anabolic DPG-mutase enzyme (Rose, 1968,1973), inhibits the catabolic DPG-phosphatase enzyme (Rose and Liebowitz, 1970) and leads to elevated [DPG]. Since the effects of pH and [DPG] on P_{50} are opposite, the red cell metabolism tends to stabilize the variation in the P_{50} (Lenfant, 1974). High extracellular pH increases [DPG] and leads to a decreased buffering capacity and an increased Bohr effect within the human red cell (Duhm, 1976;

Wranne et al., 1972). The increase in [DPG] which accompanies the elevated pH in Xenopus may indicate that pH has a long-term controlling effect on EOP concentrations. The relative alkalinity at the lower temperatures is comparable in spite of the altered pH. The mechanism for the elevation of the EOPs in Xenopus therefore must remain speculative. Jokumsen and Weber (1980) report that the concentration of urea increased with decreasing temperature; the effects of urea on Xenopus RBC metabolism are unknown. Based on the observed EOP profiles and the EOP changes with temperature, the indications are that in spite of its nucleus (usually associated with aerobic metabolism, Greaney and Powers, 1978) the Xenopus RBC is quite similar to its mammalian homolog.

The van't Hoff estimates of the dH of Xenopus blood measured under constant P_{CO_2} (various pHs at 15, 25 and 35°C) are about 2 kcal mol^{-1} smaller than the oxygenation enthalpy reported by Jokumsen and Weber (1980) for Xenopus blood at the constant pH of 7.6 at 10 and 25°C. Since the relative acidity of pH 7.6 at 10 C is much greater than that at 25°C, a part of the dH that Jokumsen and Weber observed might be due to effects associated with the liberation of Bohr protons. Jokumsen and Weber(1980) report a ΔH of -6.3 kcal mol^{-1} when comparing blood P_{50} measured at the acclimation temperatures of the donor frogs. In any case the temperature sensitivity of Xenopus is rather low, and places Xenopus into the strategy I category of Wood (1980). Values for

Rana esculenta are -9.7 (5°C acclimated) and -9.8 (20°C acclimated) kcal mol^{-1} (Gahlenbeck and Bartels, 1968). In Bufo marinus the ΔH is -7.4 kcal mol^{-1} (Hall, 1966). The cold acclimation process can produce a blood of lower O_2 affinity than that of warm acclimated animals when both bloods are compared at the same temperature (strategy II of Wood's terminology). This is interpreted as reducing the temperature sensitivity in vivo below the apparent in vitro values (e.g. Grigg, 1969). In this study that trend was not observed (table 4). The P_{50} values for the 5L, 15 and 25°C acclimated animals are not significantly different at any measurement temperature; between 5 and 25°C there is a substantial compensation of whole blood O_2 affinity in spite of the differences in pH and long-term acclimated Xenopus have one OBC over this range.

ALTITUDE

The experiments at 9144 m indicate that the EOPs can change in response to hypoxia and that the 9144 m altitude represents such a stress. In the absence of significant differences in blood pH between altitude and control animals the mechanism for the elevation of the [DPG] is not evident. Apparently some factor(s) other than pH can significantly alter the EOP levels in Xenopus. It appeared that the EOP-induced P_{50} responses in Xenopus exposed to 9144 m were maladaptive since their Hb would not reach 50% 02 saturation at the P_{O_2}s of 9144 m. The data were therefore

subjected to a Turek analysis (Turek et al., 1973). The analysis was first used for mammalian systems and assumes that the optimum OBC is the one which results in the highest mixed venous P_{O_2} ($P\bar{v}_{O_2}$) for any given venous O_2 content ($C\bar{v}_{O_2}$). The highest $P\bar{v}_{O_2}$ would give the steepest diffusion gradient to the tissues and leave the tissues in the most highly oxygenated state. The analysis was performed with the actual OBCs of three females. The mean control P_{50} was 40 mm Hg; the control female used for the analysis had a P_{50} of 39 mm Hg. The mean altitude P_{50} was 49 mm Hg; the two altitude females had P_{50}s which bracketed this mean (i.e. 46 and 54.5 mm Hg). The OBCs and the associated [DPG] are shown in fig. 4. Five arterial P_{O_2}s were chosen: 100, 80, 70, 60 and 40 mm Hg. The mean systemic arterial Pa_{O_2} is 80.4 $\pm$ 11.5 (maximum values 110 mm Hg, Emilio and Shelton, 1972). A $Ca_{O_2}-C\bar{v}_{O_2}$ difference of 25% of capacity was chosen; measured values are 1.9 to 9.8 vol% (10-56% of the O_2 capacity, Hillman, 1978). From the Pa_{O_2} and the OBC a Ca_{O_2} was determined and the a-v difference was subtracted to obtain a $C\bar{v}_{O_2}$; the latter along with the OBC gave the $P\bar{v}_{O_2}$ by inspection. Families of such curves are shown in fig. 5, one for each Pa_{O_2}. If animals can obtain Pa_{O_2} levels as high as 100 mm Hg, then the rightward shift in P_{50} (mediated by elevated [DPG]) can lead to a higher $P\bar{v}_{O_2}$ which is advantageous

according to the assumptions of the analysis. At 70 mm Hg Pa_{O_2} (the P_{O_2} @ 6096 m), a right-shifted OBC fails to achieve much benefit in $P\bar{v}_{O_2}$. This may explain why the animals at 6096 m did not right-shift their OBCs. Apparently, 6096 m is not a severe hypoxic stress for Xenopus. By the time the Pa_{O_2} drops to 40 mm Hg (P_{O_2} @ 9144 m ca, 40 mm Hg) the right-shifted OBC yields a much lower $P\bar{v}_{O_2}$ and hence at this P_{O_2} the frogs would be better served by a left-shifted OBC. The right-shifted curves indicate, according to the Turek approach, that the response was , in fact, maladaptive. Pulmonary ventilation in Xenopus is both intermittent and irregular (Emilio and Shelton, 1972). Therefore, depending on the duration of their dives, the actual P_{O_2} of systemic blood may fall precipitously and there is no defense of any particular $P\bar{v}_{O_2}$. The respiratory system of Xenopus has undoubtedly been selected to tolerate periods of apnea followed more or less regularly by return to normoxic conditions at the surface. Return to the surface allows the animals to achieve P_{O_2}s of 80-100 mm Hg and under these circumstances the right-shifted curves would yield the most favorable $P\bar{v}_{O_2}$ and provide the steepest P_{O_2} gradients to return the tissues to aerobic metabolism. The response of the animals at 9144 m may be the "programmed" response of a respiratory system which regularly encounters low $P\bar{v}_{O_2}$ during dives, but eventually regains access to atmospheric O_2. The right-shifted OBC of Xenopus at 9144 m is

an inappropriate response for maintenance of high tissue P_{O_2}s but it may allow larger $Ca_{O_2}-C\bar{v}_{O_2}$ differences and thus aid the "metering out" of pulmonary O_2 stores during prolonged dives. Large a-v differences would allow maintenance of $\dot{V}_{O_2}$ in spite of the bradycardia induced reduction in cardiac output. Since the ultimate O_2 consumption occurs in the mitochondria where P_{O_2}s as low as 1 to 5 mm Hg maintain O_2 consumption, dive-related low $P\bar{v}_{O_2}$s would probably not stop aerobic respiration in critical areas.

Xenopus exhibit all the standard features of a diver. They have large [Hb] and Hcts and a strongly developed diving physiology which includes blood flow redistribution and bradycardia. Their anaerobic capacity and their ability to tolerate lactic acid build up are also typical of divers (Anderson, 1966; Hutchison and Miller, 1979). The capability of the blood to deliver O_2 to the tissues was not affected by either short- or long-term exercise or by the altitude of 6096 m. Long-term temperature acclimation results in substantial changes in EOP levels which may be due in part to the altered pH which accompanies the temperature changes. Elevation of EOPs also occurred in the absence of pH changes, i.e. after altitude exposure, indicating that there are other factors controlling [EOP]s. The EOPs of Xenopus therefore do represent a physiologically labile parameter in the O_2 delivery process. The

observed changes in EOP levels also provide some insight into the factors which affect the metabolism of Xenopus RBCs and indicate that their metabolism is similar to that of the mammalian RBC. Whether Xenopus RBC metabolism is passively controlled via pH or actively controlled via other means is not apparent from these studies.

Acknowledgements

We thank A. Hughes and K. Miller for valuable discussions. This study was supported in part by an NIH Grant RO1 HL 18082 (to V.H.H.).

REFERENCES

Anderson, H.T. (1966). Physiological adaptations in diving vertebrates. Physiol. Rev. 46:212-243.

Araki, T., A. Kajita and R. Shukuya (1971). The effect of organic phosphates on the allosteric property of Rana catesbeiana hemoglobins. Biochem. biophys. Res. Comm. 43:1179-1185.

Arp, A.J. and J.J. Childress (1981). Blood function in the hydrothermal vent vestimentiferan tube worm. Science 217: 342-344.

Barr, A.J., J.H. Goodnight, J.P. Sall, W.H. Blair and D.M. Chilko (1979). SAS User's Guide, 1979 Edition. SAS Institute Inc. Raleigh, N.C. 495pp.

Bartlett, G.R. (1976). Phosphate compounds in red cells of reptiles, amphibians and fish. Comp. Biochem. Physiol. 55A:211-214.

Bellingham, A.J., J.C. Detter and C. Lenfant (1971). Regulatory mechanism of hemoglobin oxygen affinity in acidosis and alkalosis. J. Clin. Inves. 50:700-706.

Böning, D., U. Schweigart, U. Tibes and B. Hemmer (1975). Influences of exercise and endurance training on the oxygen dissociation curve of blood under in vivo and in vitro conditions. Europ. J. Appl. Physiol. 34:1-10.

Bonner, H.W., C.A. Tate and C.K. Buffington (1975). Changes in

erythrocyte 2,3-DPG in women following short term maximal exercise. Europ. J. Appl. Physiol. 34:227-232.

Böswart, J., I. Kuta, Z. Lisy and P. Kostiuk (1980). 2,3-diphosphoglycerate during exercise. Europ. J. Appl. Physiol. 43:193-199.

Borgese, T.A., R.L. Nagel, E. Roth, D. Murphy and J. Harrington (1978). Guanosine triphosphate (GTP): the major organic phosphate in the erythrocytes of the elasmobranch Mustelus canis (smooth dogfish). Comp. Biochem. Physiol. 60B:317-321.

Bunn, H.F., B.G. Forget and H.M. Ranney (1977). Human Hemoglobins. W.B. Saunders Co. Philadelphia PA. 432pp.

Brewer, G.J., F.J. Oelshlegel Jr., L.G. Moore and N.A. Noble (1974). In vivo red cell glycolysis control and DPG-ATP levels. Ann. N.Y. Acad. Sci. 241:513-523.

Brunori, M. (1975). Molecular adaptation to physiological requirements: The hemoglobin system of trout. In: Current topics of cellular regulation. eds. B.L. Horecker and E.R. Stadtman. Academic Press N.Y. pp 1-39.

Cameron, J.N. (1978). Regulation of blood pH in teleost fish. Respir. Physiol. 33:129-144.

Conners, T.J., M.J. Schneider, R.G. Genoway and S.A. Barraclough (1978). Effect of acclimation temperature on plasma levels of glucose and lactate in rainbow trout Salmo gairdneri. J. Exp. Zool. 206:443-449.

Cummings, J.W. (1979). Physiological and biochemical adaptation to training in Rana pipiens. J. Comp. Physiol. B 134:345-350.

Dempsey, J.A., J. Rodriguez, N.T. Shadidi, W.B. Reddan and L. MacDougall (1971). Muscular exercise, 2,3-DPG and oxy hemoglobin affinity. Int. Z. Angew Physiol. 30:34-39.

Dubowski, K.M. (1960). Measurement of hemoglobin derivatives. In: Hemoglobin: Its precursors and metabolites. eds. F.W. Sunderman and F.W. Sunderman Jr. Lippincott, Philadelphia, pp. 49-60.

Duhm, J. (1976). Influence of 2,3-diphosphoglycerate on the buffering properties of human blood. Role of the red cell membrane. Pflügers Arch. 363:61-67.

Eaton, J.W., J. Faulkner and G.J. Brewer (1969). Response of the human red cell to muscular activity. Proc. Soc. Exp. Biol. Med. 132:886-887.

Emilio, M.G. and G. Shelton (1972). Factors affecting blood flow to the lungs in the amphibian, Xenopus laevis. J. Exp. Biol. 56:67-77.

Evelyn, K.A. and H.T. Malloy (1938). Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. J. Biol. Chem. 125:655-662.

Gahlenbeck, H. and H. Bartels (1968). Tempertur adaptation der saurestoff-affinitat des blutes von Rana esculenta L. Z.

Vergl. Physiol. 59:232-240.

Geoghegan, W.D. and J.J. Poluhowich (1974). The major erythrocytic organic phosphates of the american eel, Anguilla rostrata. Comp. Biochem. Physiol. 49B:281-290.

Gillen, R.G. and A. Riggs (1971). The hemoglobins of a freshwater teleost, Cichlasoma cyanguttatum (Baird and Guard). I. The effects of phosphorylated organic compounds upon the oxygen equilibria. Comp. Biochem. Physiol. 38B:585-595.

Greaney,G.S., M.K. Hobish and D.A. Powers (1980). The effects of temperature and pH on the binding of ATP to carp (Cyprinus carpio) deoxyhemoglobin (Hb I). J. Biol. Chem. 255:455-453.

Greaney, G.S. and D.A. Powers (1978). Allosteric modifiers of fish hemoglobins: in vitro and in vivo studies studies of the effect of ambient oxygen and pH on erythrocytic ATP concentrations. J. Exp. Zool. 203:339-350.

Grigg, G.C. (1969). Temperature-induced changes in the oxygen equilibrium curve of the blood of the brown bullhead, Ictalurus nebulosis. Comp. Biochem. Physiol. 28:1203-1223.

Hall, F.G. (1966). Hemoglobin properties in the toad. J. Cell and Comp. Physiol. 68:69-74.

Harris, J.A. (1972). Seasonal variation in some hematological characteristics of Rana pipiens. Comp. Biochem. Physiol. 43A:975-989.

Hazard, E.S. (1981). Distribution of acid-soluble erythrocytic organic phosphates from selected species of amphibians. Ph.D. Dissert. Univ. Oklahoma.

Hillman, S.S. (1976). Cardiovascular correlates of maximal oxygen consumption rates in anuran amphibians. J. Comp. Physiol. B 109:199-207.

Hillman, S.S. (1978). The roles of oxygen delivery and electrolyte levels in the dehydrational death of Xenopus laevis. J. Comp. Physiol. B 128:169-175.

Howell, B.J., F.W. Baumgardner, K. Bondi and H. Rahn (1970). Acid-base balance in cold-blooded vertebrates as a function of body temperature. Amer. J. Physiol. 218:600-606.

Hutchison, V.H. (1961). Critical thermal maxima in salamanders. Physiol. Zool. 34:92-125.

Hutchison, V.H. and K. Miller (1979). Aerobic and anaerobic contributions to sustained activity in Xenopus laevis. Respir. Physiol. 38:93-103.

Hutchison, V.H., L.D. Turney and R.K. Gratz (1977). Aerobic and anaerobic metabolism during activity in the salamander Ambystoma tigrinum. Physiol. Zool. 50:189-202.

Isaacks, R.E., D.R. Harkness, G.A. Froeman and S.A. Sussman (1976). Studies on avian erythrocyte metabolism. I. Procedure for separation and quantitation of the major phosphorylated metabolic intermediates by anion-exchange chromatography.

Comp. Biochem. Physiol. 53A:95-99.

Isaacks,R.E., H.D. Kim, G.R. Bartlett and D.R. Harkness (1977). Inositol pentaphosphate in erythrocytes of a fresh water fish, Piraracu' (Arapaima gigas). Life Sci. 20:987-990.

Jokumsen, A. and R.E. Weber (1980). Hemoglobin-oxygen binding properties in the blood of Xenopus laevis, with special reference to the influences of aestivation and of temperature and salinity acclimation. J. Exp. Biol. 86:19-37.

Lapennas, G.N., J.M. Colacino and J. Bonaventura (1980). Errors caused by diffusion lag in continuous hemoglobin-oxygen binding curves. The Physiologist 23:25.

Lehninger, A.L. (1975). Biochemistry. Worth Publishers Inc. N.Y.

Lenfant, C. (1974). Effects of acid-base status on oxygen transport by whole blood. In: Carbon dioxide and metabolic regulation. eds. G. Nahas and K.E. Schaefer. Springer-Verlag. N.Y. pp. 127-131.

Lykkeboe, G., H. Schougaard and K. Johansen (1977). Training and exercise change respiratory properties of blood in race horses. Respir. Physiol. 29:315-325.

Mackenzie, J.A. and D.C. Jackson (1977). The effect of temperature on cutaneous CO_2 loss and conductance in the bullfrog. Respir. Physiol. 30:1-10.

Maclean, N. and R.D. Jurd (1971). The haemoglobins of healthy and anaemic Xenopus laevis. J. Cell. Sci. 9:509-528.

Miller, K. and J.J. Camilliere (1981). Physical training improves swimming performance of the african clawed frog, Xenopus laevis. Herpetologica 37:1-10.

Parker, J.P., G.J. Beirne, J.N. Desai, P.C. Raich and N.T. Shahidi (1972). Androgen-induced increase in red-cell 2,3-diphosphoglycerate. New Eng. J. Med. 287:381-383.

Powers, D.A. (1972). Hemoglobin adaptation for fast and slow water habitats in sympatric catastomid fishes. Science 177:360-362.

Powers, D.A., J.P. Martin, R.L. Garlick, H.J. Fyhn and U.E.H. Fyhn (1979). The effect of temperature on the oxygen equilibria of fish hemoglobins in relation to environmental thermal variability. Comp. Biochem. Physiol. 62A:87-94.

Rahn, H. (1966). Aquatic gas exchange theory. Respir. Physiol. 1:1-12.

Randall, D.J. and J.N. Cameron (1973). Respiratory control of arterial pH as temperature changes in rainbow trout Salmo gairdneri. Amer. J. Physiol. 225:997-1002.

Reeves, R.B. (1972). An imidazole alphastat hypothesis for vertebrate acid-base regulation, tissue carbon dioxide content and body temperature in bullfrogs. Respir. Physiol. 14:219-236.

Riggs, A. (1960). Nature and significance of the Bohr effect in mammalian hemoglobins. J. Gen. Physiol. 43:737-752.

Riggs, A. (1976). Factors in the evolution of hemoglobin function. Fed. Proc. 35: 2115-2118.

Rose, Z.B. (1968). The purification and properties of diphosphoglycerate mutase from human erythrocytes. J. Biol. Chem. 243:4810-4820.

Rose, Z.B. (1973). Effects of salts and pH on the rate of erythrocyte diphosphoglycerate mutase. Arch. Biochem. Biophys. 158:903-910.

Rose, Z. B. and J. Liebowitz (1970). 2,3-diphosphoglycerate phosphatase from human erythrocytes. J. Biol. Chem. 245:3232-3241.

Schmidt-Nielsen, B. and R. P. Forster (1954). The effect of dehydration and low temperature on renal function in the bullfrog. J. cell and Comp. Physiol. 44:233-246.

Schmidt-Nielson, K. and J. P. Larimer (1958). Oxygen dissociation curves of mammalian blood in relation to body size. Amer. J. physiol. 195:424-428.

Shappel, S. D., J. A. Murray, A. J. Bellingham, R. D. Woodson, J.C. Detter and C. Lenfant (1971). Adaptation to exercise: the role of hemoglobin affinity for oxygen and 2,3-diphosphoglycerate. J. Appl. Physiol. 30:827-832.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods, 7th Ed. The Iowa State University Press. Ames.

Sullivan, B. (1974). Amphibian hemoglobins. In: M. Florkin and

B.T. Scheer eds. Chemical Zoology Vol XI: Amphibia and Reptilia. Academic Press N. Y. pp. 77-122.

Thompson, D.E. and R.L.Franks (1978). Xenopus care and culture. Carolina Biological Supply Burlington, N. C.

Turek, Z., F. Kreuzer and L.J.C. Hoofd (1973). Advantage or disadvantage of a decrease of blood oxygen affinity for tissue oxygen supply at hypoxia. A theoretical study comparing man and rat. Pflügers Arch. 342:185-197.

Wood, S.C. (1980). Adaptation of red cell function to hypoxia and temperature in ectothermic vertebrates. Amer. Zool. 20:163-172.

Wranne, B., R.D. Woodson and J. C. Detter (1972). Bohr effect: interaction between H^+, CO_2, and 2,3-DPG in fresh and stored blood. J. Appl. Physiol. 32:749-754.

TABLE HEADINGS

Table 1. Ion-exchange chromatography of erythrocytic phosphates from Xenopus laevis.

Table 2. Comparison of male and female Xenopus laevis for change in body mass (ΔBM), total acid soluble phosphate (P_{tot}), nucleoside triphosphate (NTP), 2,3-diphosphoglycerate (DPG) and total body mass (BM) after exercise.

Table 3. Comparison of exercised and control Xenopus laevis for: change in body mass (ΔBM), total acid soluble phosphate (P_{tot}), nucleoside triphosphate (NTP), 2,3-diphosphoglycerate (DPG), hemoglobin (Hb) and mean cell volume (MCV).

Table 4. Variation of erythrocytic organic phosphates and hematological parameters with acclimation temperature in Xenopus laevis.

Table 5. Anaerobic changes in whole blood pH with increasing temperature in Xenopus laevis.

Table 6. Two-way analysis of covariance of factors affecting blood O_2 transport in Xenopus laevis under control conditions and after 4 days at the simulated altitude of 9144 m.

TABLE I.
Ion-exchange chromatography of erythrocytic phosphates
from Xenopus laevis

EXPERIMENT†	SEX	BODY MASS (g)	Hb (g dl^{-1})	Hct (%)	Pi	ADP	ATP††	DPG††	GTP	GTP/ATP	I	Ptot††
					µmol glb^{-1}					mol/mol	mH^{-1}	µmol glb^{-1}
1 wk exercise	f	35.5	13.3	36.55	1.63	0.14	1.41 (2.42*)	5.73 (4.89)	0.31	0.22	6.3	21.68 (21.90)
1 wk exercise	m	28.1	13.3	31.77	2.37	0.17	2.19 (2.75*)	8.31 (5.75)	0.55	0.25	5.6	31.59 (25.26)
6 wk exercise	f	40.0	12.0	35.66	0.71	0.08	1.03 (3.02*)	2.54 (5.26)	0.22	0.21	18.2	11.18 (24.78)
6 wk exercise	m	27.4	10.5	33.83	1.71	0.70	1.33 (2.02*)	5.07 (4.97)	0.27	0.21	1.12	21.66 (20.14)
1 wk 5°C (5S)	f	66.7	10.8	39.62	1.84	0.40	2.14 (2.62*)	6.30 (5.22)	0.68	0.32	1.37	28.15 (23.70)
1 wk 5°C (5S)	m	49.4	11.6	37.99	3.10	0.14	1.50 (2.25*)	5.38 (5.15)	0.44	0.30	3.42	22.57 (21.39)
4 wk 5°C (5L)	f	43.4	8.98	27.94	1.58	0.14	1.74 (2.85*)	6.04 (8.84)	0.53	0.31	7.6	26.35 (32.57)
4 wk 5°C (5L)	m	36.2	12.2	32.35	1.74	0.04	2.73 (2.81*)	10.2 (11.05)	0.62	0.23	40.7	37.09 (31.24)
6 mo 15°C	f	52.1	10.5	33.75	3.78	0.03	2.05 (2.49*)	9.59 (6.96)	0.42	0.21	17.4	40.2 (31.53)
6 mo 15°C	f	56.4	13.8	45.30	4.66	0.62	2.19 (2.42*)	8.99 (6.04)	0.53	0.24	0.76	51.73 (30.96)
6 mo 15°C	m	40.0	11.7	33.09	1.88	0.12	1.41 (1.63*)	5.86 (5.85)	0.23	0.16	6.5	24.78 (24.79)
6 mo 25°C	f	49.2	14.6	44.78	1.39	0.18	1.65 (-)	3.48 (-)	0.26	0.16	6.7	14.75 (-)
6 mo 25°C	m	31.2	14.1	44.12	0.88	0.11	1.04 (1.31*)	4.165 (4.17)	0.28	0.27	10.6	15.06 (15.08)
1 wk 30°C(30S)	f	40.6	10.8	32.84	1.39	0.24	2.02 (2.70*)	4.14 (3.20)	0.55	0.27	6.0	21.81 (18.37)
1 wk 30°C(30S)	m	38.9	15.3	49.75	1.50	0.23	1.90 (2.74*)	4.28 (3.65)	0.38	0.20	5.4	20.42 (19.07)
4 wk 30°C(30L)	f	33.5	10.9	34.56	1.06	0.04	1.98 (3.39*)	5.50 (5.61)	0.50	0.25	46.6	22.90 (24.15)
2 days @ 20,000 feet	f	19.6	8.46	30.88	2.35	0.10	1.01 (-)	- (-)	0.1046	0.10	4.5	- (-)
2 days @	m	22.5	7.10	25.94	2.48	0.11	1.17 (-)	4.20 (-)	0.20	0.17	4.5	18.57 (-)

TABLE 2.

COMPARISON OF MALE AND FEMALE *XENOPUS LAEVIS* FOR CHANGE IN BODY MASS (ΔBM), TOTAL ACID SOLUBLE PHOSPHATE (Ptot), NUCLEOSIDE TRIPHOSPHATE (NTP), 2,3-DIPHOSPHOGLYCERATE (DPG), AND TOTAL BODY MASS (BM) AFTER EXERCISE

VARIABLE	UNITS	MALE	FEMALE	df	F	P
ΔBM	%	-10.5±0.04	-7.1±0.04	1,31	4.41	0.0439
Ptot	μmol gHb^{-1}	73.23±1.95	66.63±1.95	1,31	5.17	0.0301
NTP	μmol gHb^{-1}	8.65±0.25	7.84±0.25	1,31	4.82	0.0358
DPG	μmol gHb^{-1}	15.57±0.72	13.28±0.72	1,31	4.94	0.0337
BM	g	30.1±2.07	36.3±3.47	1,31	8.03	0.0079

Values are $\bar{X}$ ± 1.0 S.E.

TABLE 3.

COMPARISON OF EXERCISED AND CONTROL XENOPUS LAEVIS FOR: CHANGE IN BODY MASS (ΔBM), TOTAL ACID SOLUBLE PHOSPHATE (Ptot), NUCLEOSIDE TRIPHOSPHATE (NTP),2,3-DIPHOSPHOGLYCERATE (DPG), HEMOGLOBIN (Hb), AND MEAN CELL VOLUME (MCV)

		SHORT TERM		LONG TERM		COMPARISONS†			
		CONTROL	EXERCISE	CONTROL	EXERCISE				
VARIABLE	UNITS	1	2	3	4	1-2	3-4	1-3	2-4
ΔBM	%	-3.68±0.07	-7.01±0.07	-7.88±0.07	-19.08±0.08	0.0543	0.0001	0.0223	0.0001
Ptot	μmol gHb^{-1}	58.5±2.74	81.9±2.61	62.6±2.60	76.8±2.80	0.2815	0.1896	0.0001	0.0010
NTP	μmol gHb^{-1}	7.92±0.35	8.48±0.33	7.57±0.33	9.01±0.36	0.4730	0.2859	0.2156	0.0068
DPG	μmol gHb^{-1}	10.95±1.01	17.73±0.97	13.23±0.96	16.00±1.04	0.1105	0.2307	0.0001	0.0642
Hb	g dl^{-1}	13.9±0.57	11.4±0.54	14.3±0.54	11.6±0.58	0.5822	0.2128	0.0032	0.0020
MCV	μ^3	431.4±7.54	412.3±7.18	438.0±7.15	399.0±7.72	0.5244	0.2128	0.0778	0.0010

Values are $\bar{X}$ ± S.E.
† P value for t-tests comparing least-square means adjusted for differences in final body weight.

TABLE 4.

VARIATION OF ERYTHROCYTIC ORGANIC PHOSPHATES AND HEMATOLOGICAL PARAMETERS WITH ACCLIMATION TEMPERATURE IN XENOPUS LAEVIS

VARIABLE	UNITS	ACCLIMATION TEMPERATURE °C								
		5L	5S	15	25	30S	30L	df	F	P
RBCCT	$x10^6$ mm^{-3}	0.754±0.045	0.897±0.04	-	-	1.05±0.043	0.657±0.076	5,46	7.89	0.0001
MCV	μ^3	362.8±9.95	388.2±9.21	-	-	392.6±9.92	414.1±15.32	5,47	3.11	0.0194
MCH	μμg	147.7±7.72	153.3±5.92	-	-	131.2±6.46	172.5±10.27	5,44	3.94	0.0064
METHB	%	0.34±0.16	0.18±0.15	0.43±0.15	0.65±0.15	4.3±0.16	0.08±0.35	5,59	2.48	0.0449
Hb	g dl^{-1}	10.0±0.65	12.2±0.62	11.6±0.63	12.7±0.62	14.0±0.65	9.6±0.95	5,59	5.38	0.0005
Hct	%	28.6±0.05	37.9±0.05	34.5±0.05	38.5±0.05	44.3±0.05	31.0±0.11	5,59	6.39	0.0001
DPG	μmol gHb^{-1}	25.2±1.14	15.8±1.09	18.4±1.10	15.5±1.09	12.5±1.14	15.5±1.67	5,59	14.6	0.0001
NTP	μmol gHb^{-1}	10.01±0.45	7.02±0.43	7.38±0.43	6.58±0.43	8.07±0.45	9.74±0.66	5,59	8.59	0.0001
Ptot	μmol gHb^{-1}	96.16±3.48	64.21±3.31	85.88±3.34	60.17±3.33	59.73±3.48	78.35±5.07	5,59	19.3	0.0001
BM	g	38.9±3.4	44.0±4.8	41.3±3.8	38.5±1.6	39.4±2.7	27.0±3.2	5,59	3.58	0.0078
pH @15°C		7.97±0.04	7.65±0.04	7.55±0.06	7.28±0.05	7.22±0.04	7.72±0.06	5,59	41.2	0.0001
pH @25°C		7.76±0.04	7.53±0.04	7.43±0.05	7.14±0.05	7.09±0.04	7.58±0.06	5,59	36.4	0.0001
pH @35°C		7.64±0.04	7.42±0.04	7.29±0.04	7.00±0.05	6.95±0.04	7.40±0.06	5,59	42.9	0.0001
P_{50}@15°C	mm Hg	23.8±1.18	23.3±1.13	24.9±1.14	23.8±1.13	25.2±1.18	20.2±1.73	5,59	1.35	0.2601
P_{50}@25°C	mm Hg	34.4±1.41	29.0±1.34	34.1±1.35	34.8±1.35	35.7±1.41	29.6±2.06	5,59	4.01	0.0041
P_{50}@35°C	mm Hg	42.8±1.77	40.7±1.86	47.8±1.70	46.8±1.69	51.9±1.77	38.0±2.58	5,59	6.98	0.0001

Values are $\bar{X}$ ± 1.0 S.E.
Red blood cell count (RBCCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), methemoglobin (METHB), Hemoglobin (Hb), hematocrit (Hct), 2,3-diphosphoglycerate (DPG), nucleoside triphosphate (NTP), Total acid soluble phosphate (Ptot)

TABLE 5.

ANAEROBIC CHANGES IN WHOLE BLOOD pH WITH INCREASING TEMPERATURE IN XENOPUS LAEVIS

ACCLIMATION	SEX	n	SLOPE	INTERCEPT	r†
5L	M	5	-0.0164	8.188	-0.91
5L	F	5	-0.0169	8.224	-0.93
5S	M	6	-0.0128	7.184	-0.72
5S	F	6	-0.0103	7.855	-0.59
15	M	6	-0.0123	7.657	-0.74
15	F	5	-0.0132	7.881	-0.72
25	M	2	-0.0135	7.449	-0.86
25	F	2	-0.0153	7.549	-0.60
30S	M	5	-0.0138	7.407	-0.58
30S	F	5	-0.0133	7.441	-0.63
30L	M	3	-0.0158	7.987	-0.82
30L	F	3	-0.0177	8.018	-0.95
Water††	-	-	-0.0176	7.448	-

† Pearson product-moment correlation coefficient
†† Howell et al., 1970

TABLE 6.

TWO-WAY ANALYSIS OF COVARIANCE† OF FACTORS AFFECTING BLOOD O_2 TRANSPORT IN *XENOPUS LAEVIS* UNDER CONTROL CONDITIONS AND AFTER 4 DAYS AT THE SIMULATED ALTITUDE OF 9144 m

		SEX					TREATMENT				
VARIABLE	UNITS	MALE	FEMALE	df	F	P	ALTITUDE	CONTROL	df	F	P
P_{50}	mm Hg	47.3±1.22	42.6±1.22	1,15	5.96	0.0275	48.8±1.07	41.1±1.07	1,15	25.62	0.0001
pH	-	6.94±0.05	7.07±0.05	1,15	3.08	0.1000	7.03±0.04	6.98±0.04	1,15	0.82	0.38
NTP	µmol gHb^{-1}	7.24±0.42	6.24±0.42	1,15	2.23	0.1558	6.70±0.37	6.78±0.37	1,15	0.02	0.87
Ptot	µmol gHb^{-1}	93.2±4.5	77.0±4.5	1,15	5.17	0.0381	95.0±4.00	75.3±4.00	1.15	12.18	0.0033
DPG	µmol gHb^{-1}	19.6±1.33	18.1±1.33	1,15	0.46	0.51	21.7±1.17	16.0±1.17	1,15	11.93	0.0033

Values are $\bar{X}$ 1.0 S.E.
† post-experimental body mass was used as the covariate
Nucleoside triphosphate (NTP), total acid soluble phosphate (Ptot), 2,3-diphosphoglycerate (DPG)

FIGURE CAPTIONS

Figure 1. Ion-exchange chromatogram of a female Xenopus laevis acclimated to 15°C and a photoperiod of 12L:12D. The equivalents of inorganic phosphate (P_i) per ml RBC (solid line) and the ultraviolet absorbance at 254 nm (dashed line) are shown on separate ordinate axes; the abscissa represents the number of 3.8 ml fractions collected from a 0.9x25 cm column of Bio-Rad 200-400 mesh, AG 1x8, formate form anion exchange resin beads. The chromatogram was developed with a linear 0-5.0 N, one l gradient composed of 500 ml buffer (formic acid:ammonium formate, 3:2, pH 3.57) and 500 ml of glass distilled water. The peaks for oxidized nicotinamide dinucleotide (NAD^+), P_i, adenosine diphosphate (ADP), adenosine triphosphate (ATP), 2,3-diphosphoglycerate (DPG) and guanosine triphosphate (GTP) are labeled.

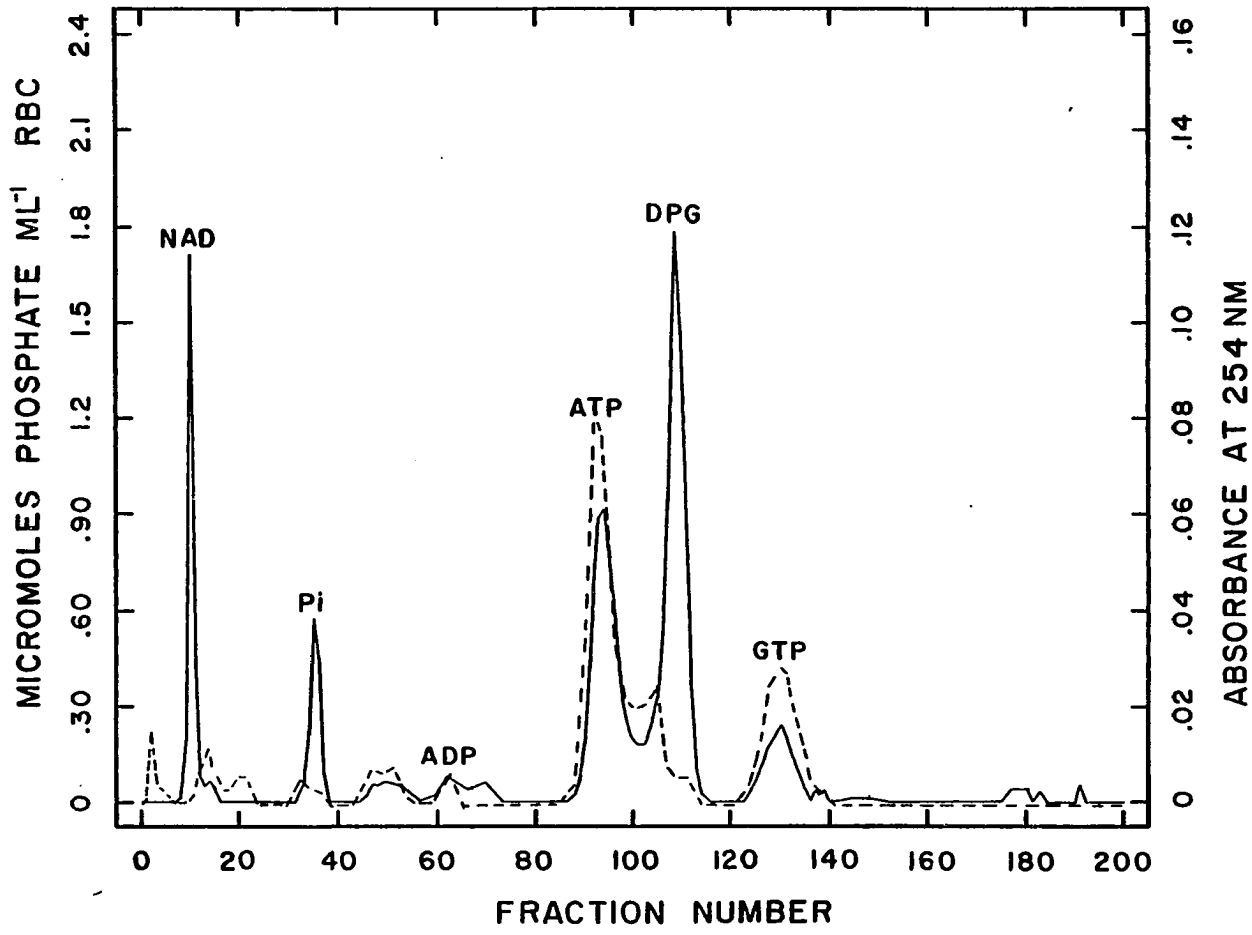
Figure 2. The effect of acclimation temperature on the concentration of 2,3-diphosphoglycerate (DPG) in Xenopus laevis RBCs. Both female (circles) and males (squares) are plotted. The symbols are positioned at the means; vertical bars span two standard errors. The solid symbols at 5 and 30°C represent animals placed at those temperatures for only one week. The remainder of the animals were acclimated at the respective

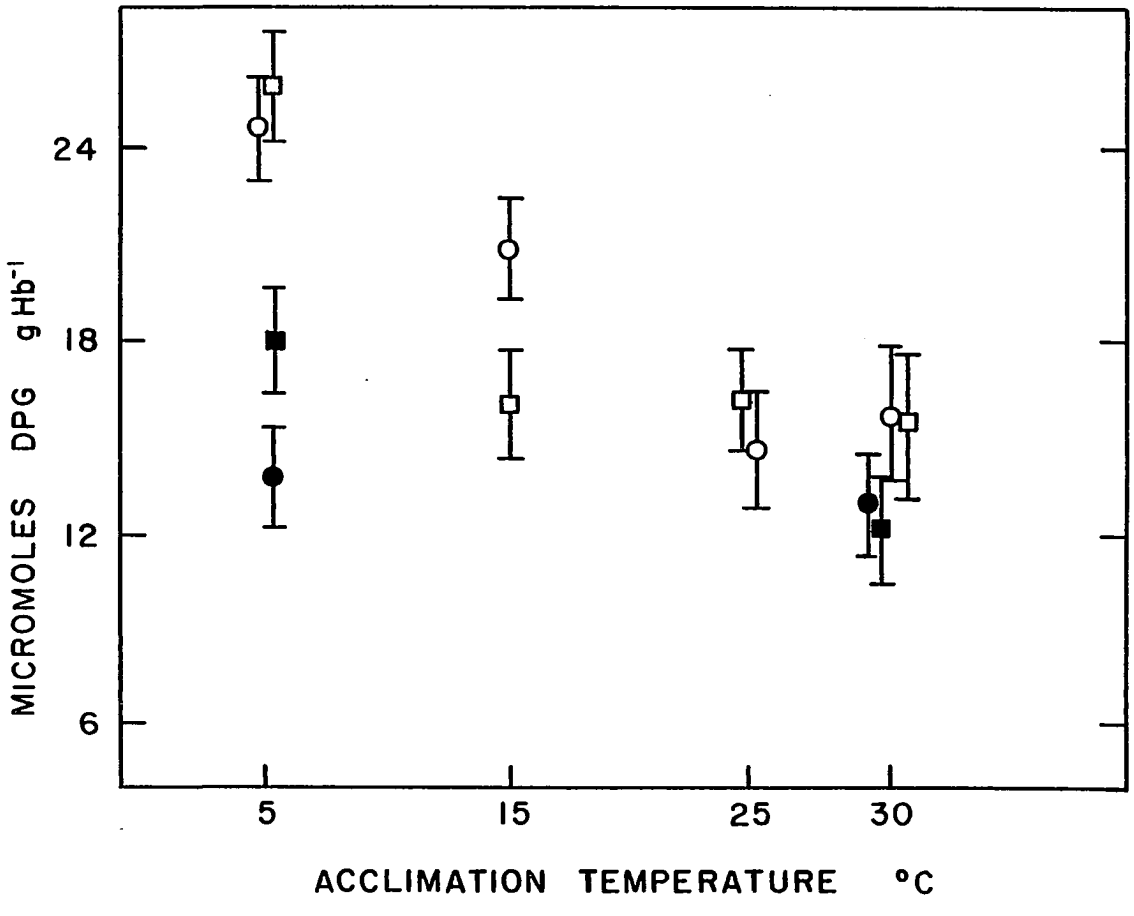
temperatures for at least 30 days.

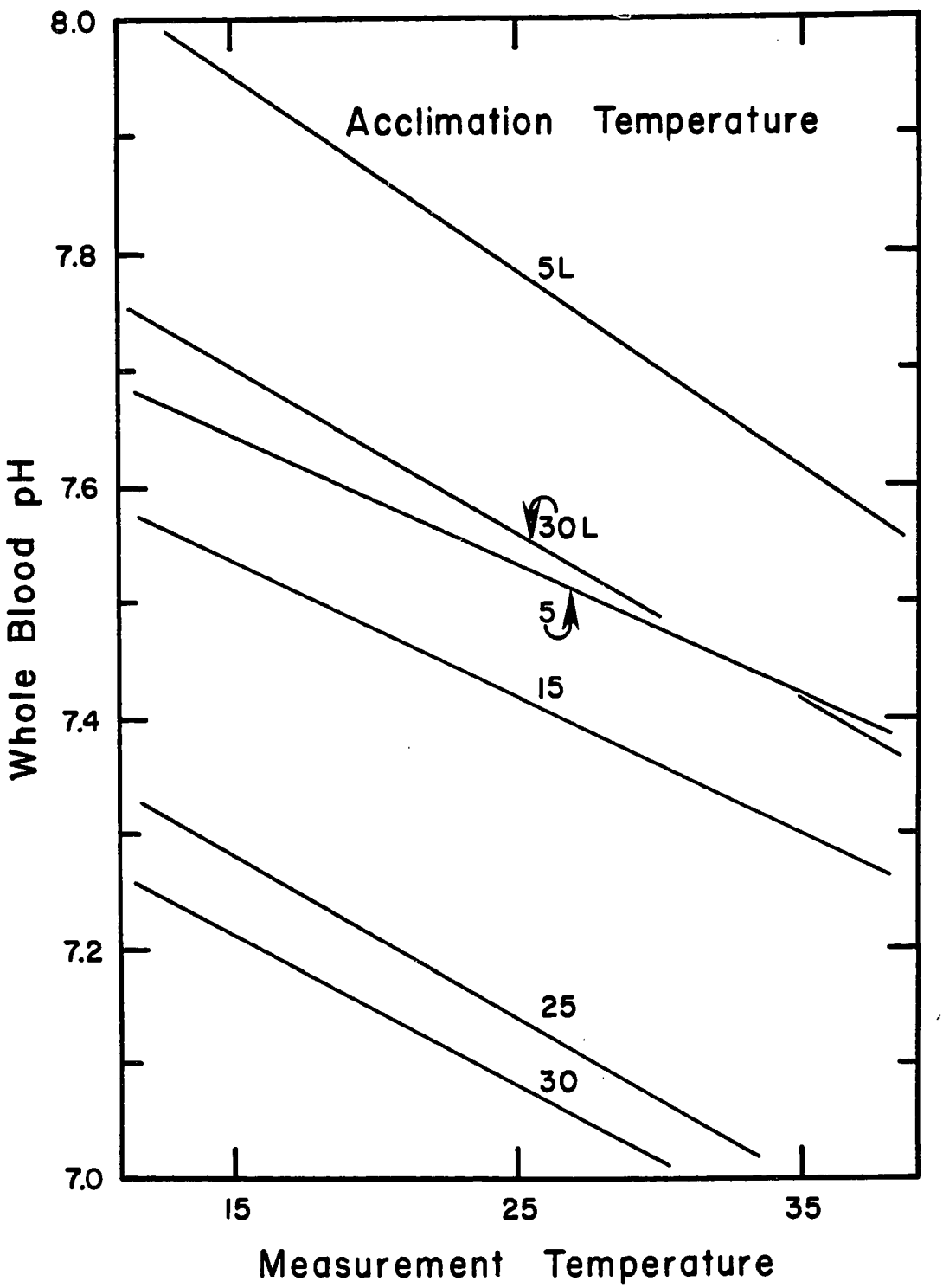
Figure 3. Comparison of pH for closed system constant CO_2, *in vitro* warming curves for whole blood from *Xenopus laevis* acclimated to the indicated temperatures. Whole blood pH was measured at 15,25 and 35°C. Long-term (four weeks) exposures to 5 and 30°C are labeled 5L 30L, respectively. Short-term (one week only) exposures to 5 and 30°C are labeled 5 and 30. The 15 and 25°C had been acclimated for at least 6 months.

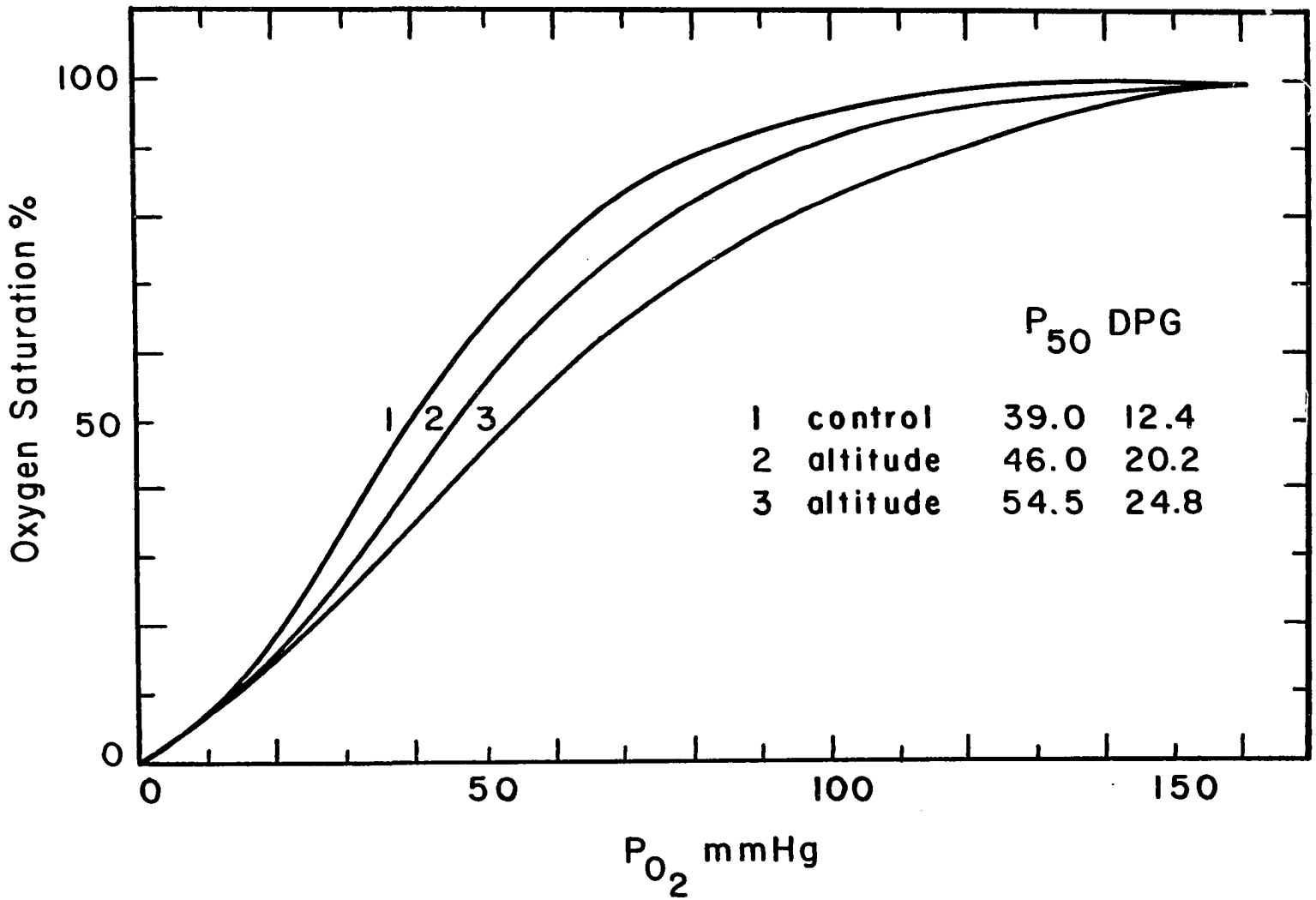
Figure 4. Oxygen binding curves (OBCs) of three female *Xenopus laevis* from the simulated altitude experiments. The P_{50} (mm Hg), and the 2,3-diphosphoglycerate (DPG) concentration (μmol gHb^{-1}) for each individual are shown. Animal 1 was a control; animals 2 and 3 had been exposed to 9144 m for four days.

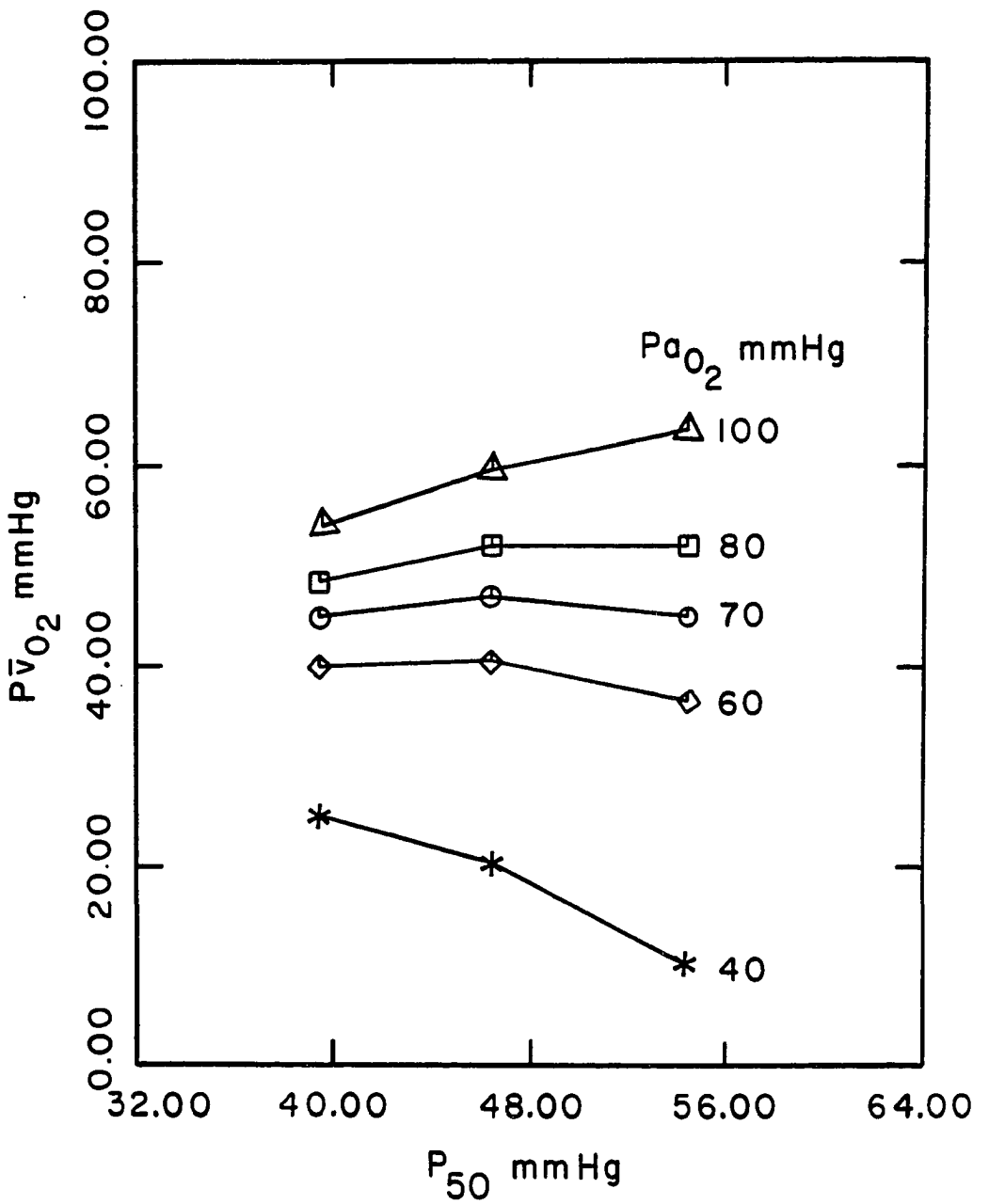
Figure 5. Graphic summary of the Turek analysis based on the OBCs from figure 4. The final mixed venous P_{O_2} ($P\bar{v}_{O_2}$, mm Hg) is shown on the ordinate; the abscissa represents the P_{50}s (mm Hg) of the OBCs from figure 4. The family of lines was generated for arterial P_{O_2}s (Pa_{O_2}, mm Hg) of: 100, 80, 70 (ca. P_{O_2} at 6096 m), 60 and 40 (approximately the P_{O_2} at 9144 m). Increasing the P_{50} with Pa_{O_2}s in the 80-100 mm Hg range leads to little or no change in $P\bar{v}_{O_2}$ while with Pa_{O_2}s as low as 40 mm Hg, the right-shifted OBC yields a lower $P\bar{v}_{O_2}$ than the left-shifted OBC.


MICROMOLES PHOSPHATE ML⁻¹ RBC
ABSORBANCE AT 254 NM
FRACTION NUMBER
NAD
Pi
ADP
ATP
DPG
GTP
0
.30
.60
.90
1.2
1.5
1.8
2.1
2.4
.02
.04
.06
.08
.10
.12
.14
.16
0
20
40
60
80
100
120
140
160
180
200


MICROMOLES DPG g Hb⁻¹
24
18
12
6
5
15
25
30
ACCLIMATION TEMPERATURE °C


8.0
7.8
7.6
7.4
7.2
7.0
Whole Blood pH
Acclimation Temperature
5L
30L
5
15
25
30
15
25
35
Measurement Temperature


100
50
0
Oxygen Saturation %
0
50
100
150
P_{O_2} mmHg
1 2 3
P_{50} DPG
1 control 39.0 12.4
2 altitude 46.0 20.2
3 altitude 54.5 24.8


100.00
80.00
60.00
40.00
20.00
0.00
$P\bar{v}_{O_2}$ mmHg
Pa_{O_2} mmHg
100
80
70
60
40
32.00
40.00
48.00
56.00
64.00
P_{50} mmHg