INTRACELLULAR BUFFERING AND OXYGEN TRANSPORT IN THE PACIFIC BLUE MARLIN (MAKAIRA NIGRICANS): ADAPTATIONS TO HIGH-SPEED SWIMMING[1]

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Blood obtained from Pacific blue marlin captured by hook and line displayed a pronounced acidosis by teleost standards (pH 7.22 ± 0.07 at 25 C), a high $PACO_2$ (17.3 ± 3.0 torr) and a low PAO_2 (9.7 ± 3.0 torr). Notwithstanding, these properties reflect a major anaerobic component contributing to the acid-base status of blood originating from muscle glycogenolysis during the capture period. This view is consistent with the finding that blood lactate concentration was linearly related to fight time. Marlin is exceptional in its ability to tolerate protons. The buffering capacities (β) of white and red skeletal muscles are 103.8 ± 5.2 slykes and 50.8 ± 3.9 slykes, respectively. The difference between the buffering capacities of the two muscle types likely is related to different rates of adenosine triphosphate (ATP) production and to the different fate of metabolic protons generated in each tissue. The in vitro nonbicarbonate buffering value of whole blood ($\Delta HCO_3^-/\Delta pH$) also is high when compared with that of other teleosts (β = 21.3 slykes) and represents an important adaptation for extending muscle performance in this species. The relationship between β and hemoglobin concentration is given by the equation $\beta = 1.82[Hb_4] + 3.1$. The Bohr coefficient, determined on whole blood following capture, is one of the largest ($\phi = -1.0$ over the pH range 6.95–7.60) reported for a vertebrate. We propose that having a large Bohr effect is an important strategy for overcoming the high buffering capacity of whole blood, thereby enhancing oxygen delivery to working muscle during high-speed aerobic swimming. It is concluded that the capability of the marlin for high-speed swimming is a result of specialized adaptations to both the O_2 transport and metabolic systems. Enhanced intracellular buffering in both these compartments (blood and skeletal muscle) enables marlin to achieve high work rates during both short-term burst activity and longer-term high-speed swimming required to capture prey.

INTRODUCTION

An important but often overlooked design constraint imposed on elite animal performers is that intracellular buffering must be scaled up to match proton production associated with high work rates (see Castellini and Somero 1981). The Pacific blue marlin (*Makaira nigricans*) is an excellent animal for investigating such a proposal because of its large body mass (50–500 kg) and extremely high anaerobic (proton-generating) potential. This latter feature is reflected by the high white:red muscle-mass ratio (~9:1) and the outstanding swimming and fighting capabilities of the fish; marlin have been estimated to swim at speeds of between 40 and 60 km hr^{-1} under natural conditions while pursuing prey such as skipjack tuna (*Katsuwonus pelamis*). With these data as background, we decided to investigate (1) the intracellular buffering of the different muscle types and of blood and (2) the implications of intracellular buffering on the pH sensitivity of the oxygen transport system. Because very little is known about the general physiology and biochemistry of marlin, we thought it important to compare the buffering capac-

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ity and O_2 transport measurements with some of the in vivo properties of whole blood in fish captured by hook and line following varying fight times.

MATERIAL AND METHODS

Pacific blue marlin (*Makaira nigricans*) were captured by hook and line by participants of the 25th Hawaiian International Billfish Tournament held during August 1983 at Kailua-Kona. The surface water temperature was 25 C. Blood was sampled from the dorsal and/or ventral aortae as soon as possible after boating the fish (usually within 1–2 min) via external puncture. Blood samples (25–50 ml) were placed on ice immediately and stored for periods between 30 min and 6 h prior to analysis. Muscle biopsies also were taken shortly after capture (15–45 min) and immediately frozen. Once captured, marlin were usually stored in long, sealed, insulated bags packed with ice.

MEASUREMENT OF BUFFERING CAPACITY

To determine nonbicarbonate blood buffering–capacity values (β), 5-ml blood samples were placed into 25-ml round-bottom rotating flasks that were immersed in a constant-temperature bath (25 C) as described by Seymour, Dobson, and Baldwin (1981). Blood was equilibrated with humidified gas mixtures of CO_2 diluted with air according to the method of Perry et al. (1985) to produce a range of P_{CO_2} between ~0 and 15 torr. Following 15–30-min equilibration, 1-ml samples of blood were analyzed for pH, total CO_2 content (C_{CO_2}), and hemoglobin concentration ([Hb_4]). pH measurements were made with a Radiometer PHM-71 digital acid-base analyzer and associated "micro" pH electrode at 25 C. C_{CO_2} was determined according to the method of Cameron (1971). [Hb_4] was determined using a hand-held hemoglobinometer. P_{CO_2} and bicarbonate concentration ([HCO_3^-]) were calculated using a reorganization of the Henderson-Hasselbalch equation. pK′ values of carbonic acid were obtained from Severinghause, Stupfel, and Bradley (1956), and the solubility coefficient of CO_2 (αCO_2) was obtained from Albers (1970). Separate buffer curves for each [Hb_4] were plotted (N = 8) and β values ($dHCO_3^-/dpH$) determined. Finally, a curve relating buffering capacity to [Hb_4] was constructed.

In vitro red- and white-muscle buffering capacities of marlin and trout were determined via muscle biopsies according to the method of Bate-Smith (1938) as described in detail by Castellini and Somero (1981). One modification was the use of a metabolic inhibitor (iodoacetate) in the homogenization medium comprising 145 mM KCl, 10 mM NaCl, and 5 mM iodoacetic acid (pH = 7.0 at 25 C). The unit of buffering capacity (β) is the slyke, which is defined as micromoles of base required to alter the homogenate pH by one pH unit per gram wet weight of tissue between pH 6.0 and pH 7.0.

BLOOD METABOLITES

Blood lactate concentration was determined by conventional methods described in Bergmeyer (1974).

OXYGEN AFFINITY OF BLOOD: THE BOHR EFFECT

The P_{50} (expressed in torr) was determined using the mixing technique as described by Edwards and Martin (1966). Blood tonometry was performed in a specially designed apparatus (Seymour et al. 1981). P_{O_2} was determined using a Radiometer PHM-71 acid-base analyzer and associated O_2 electrode at 25 C. Log P_{50} was expressed as a function of whole-blood pH. Red blood cell pH (RBC) was determined using the fast freeze–thaw technique (see Zeidler and Kim 1977).

RESULTS

Respiratory and acid-base properties of marlin blood following exhaustive exercise and angling stress are summarized in table 1. Since it is not possible to determine blood acid-base and respiratory properties of freely swimming marlin in a preexercise state, we must compare our measurements with those well-documented values for other teleost fishes (see Jones and Randall 1978). Assuming a preexercise blood pH value of ~7.80 at 25 C, marlin blood at the time of capture is, by teleost standards, highly acidic (7.22 ± 0.07). Accompanying this reduction in pH is an increased P_{ACO_2} (17.3 ± 3.0 torr) and a decreased P_{AO_2} (9.7 ± 3.0 torr) compared to expected preexercise values of 2–4 torr and 100–110 torr,

TABLE 1

RESPIRATORY AND ACID-BASE PROPERTIES OF THE BLOOD OF PACIFIC BLUE MARLIN (*Makaira nigricans*) AT THE TIME OF CAPTURE

Property	Mean ± SE (*N*)
Weight (kg)	94.5 ± 5.4 (29)
Hb_4 (g/100 ml)	10.4 ± .4 (29)
Hct (%)	43.1 ± 1.7 (26)
Arterial blood pH	7.22 ± .07 (13)
Red cell pH	7.04 ± .06 (13)
PAO_2 (torr)	9.7 ± 3.0 (8)
$PACO_2$ (torr)	17.3 ± 3.0 (8)
C_{CO_2} (mM)	9.5 ± .1 (16)
Lactate (mM)	12.8 ± 2.1 (15)
Buffering value (whole blood)	−21.3 slykes, $[Hb_4]$ = 10 g/100 ml, Hct = 40%
Bohr coefficient (ϕ, whole blood)	−1.0 over the pH range 6.95–7.60 (9)

respectively (Randall, Perry, and Heming 1982). Two other respiratory parameters, blood hemoglobin concentration and hematocrit, also are high (10.4 ± 0.4 and 43.2 ± 1.7, respectively) compared with typical teleostean values following exercise (Yamamoto, Itazawa, and Kobayashi 1980; Wood and Perry 1985). Blood lactate increased in a linear relationship with fight time (fig. 1). All other measured and calculated variables (PAO_2, $PACO_2$, C_{CO_2}, Hct, and $[Hb_4]$) showed no significant correlation with fight time.

A representative in vitro buffer curve for marlin blood and the relationship between buffering capacity and hemoglobin concentration is shown in figure 2 and summarized in table 1. The buffering capacity of whole blood is 21.3 slykes for a hemoglobin concentration of 10 g/100 ml (~40% Hct). The relationship between β and hemoglobin concentration is $\beta = 1.82\ [Hb_4] + 3.1$ at 25 C. The in vitro buffering capacity of marlin white muscle is 103.8 ± 5.2 slykes, whereas that of red muscle is twofold higher than the respective muscle types for rainbow trout (*Salmo gairdneri*) (table 2).

The effect of pH on the oxygen-binding properties of marlin whole blood is shown in figure 3. The Bohr coefficient is −1.0 over the pH range 6.95–7.6 and over the intraerythrocytic pH range of 6.5–7.2 at 25 C (table 1).

DISCUSSION

PHYSIOLOGICAL AND METABOLIC STATUS OF MARLIN BLOOD DURING FORCED EXHAUSTIVE EXERCISE

At the time of capture the Pacific blue marlin thrashes violently, leaping out of the water with a ferocity and majestic power that is unsurpassed by any other sport fish inhabiting the oceans. Indeed, the ability of marlin to perform both short-term explosive and longer-term steady-state swimming is no better illustrated than by their impressive fight times, ranging from a few minutes to many hours (25th Hawaiian International Billfish Tournament) and by their natural ability to pursue and capture prey such as skipjack tuna.

It is important to emphasize that those marlin that were successfully boated represent a highly specific physiologic and metabolic state: that of extreme exhaustion. Although the blood gas and acid-base properties presented in table 1 support this proposal, we cannot at present distinguish between what fraction of the change in blood gases and pH is due to exercise per se during the angling period and what fraction is due to inadequate gill ventilation on capture just prior to blood sampling. Using preexercise values of blood pH and $PACO_2$ for other active teleosts (pH of 7.8 at 25 C and $PACO_2$ of 3 torr; see Randall et al. 1982), we predict a total CO_2 (C_{CO_2}) for marlin blood of ~8.0 mM. Given an in

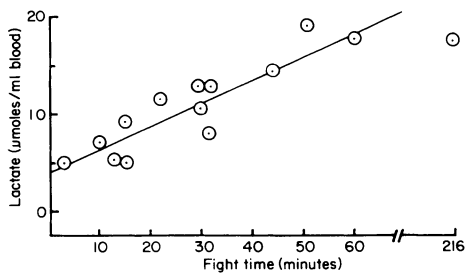


FIG. 1.—Relationship between blood [lactate] and fight time for the Pacific blue marlin (*Makaira nigricans*). Each point represents an individual fish, and each determination was measured in duplicate. [lactate] = 0.24. Fight time + 3.93; correlation coefficient = 0.90.

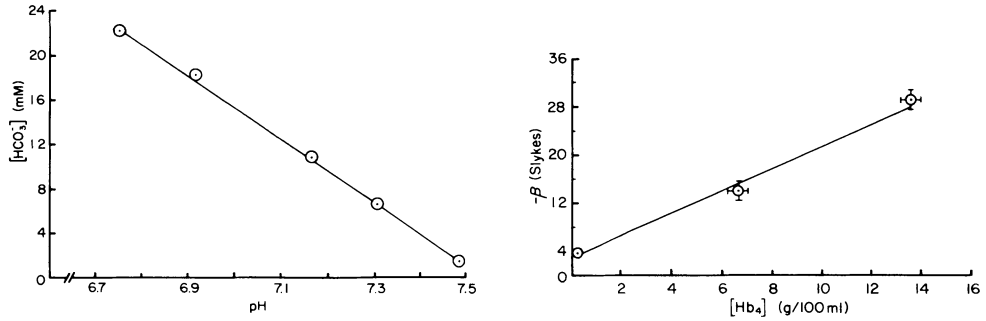


FIG. 2.—*A*, A representative in vitro buffer curve for Pacific blue marlin blood at 25 C; and *B*, the relationship between blood buffering value and hemoglobin concentration at 25 C ($N = 7$). $\beta = 1.82\ [Hb_4] + 3.10$; correlation coefficient = .995.

vitro nonbicarbonate blood buffering value of 21.3 slykes (see fig. 2), a pure respiratory acidosis required to bring about the measured blood pH of 7.22 would correspond to a blood C_{CO_2} of ~20 mM. That the measured blood C_{CO_2} was much lower (table 1) than this empirical value of 20 mM is indicative of base deficit and suggests that a major component of the blood acidosis was of metabolic origin or, alternatively, that a significant fraction of total blood HCO_3^- was transferred to intracellular or other extracellular compartments (including the environment). The direct relationship between blood lactate and fight time (fig. 1) also is suggestive of an important metabolic component to the blood acidosis. This relationship demonstrates activation of anaerobic glycogenolysis during the initial stages of the fight, with the accompanying slow release of lactate from muscle into the blood as fight time increases. Furthermore, the low pH of arterial blood in fish boated during the first 20 min is consistent with the view that anaerobic glycolysis was activated. Whereas H^+ are known to be effluxed more rapidly than lactate from white muscle into the blood of some active fish (Piiper, Meyer, and Drees 1972; Turner,

TABLE 2

BUFFERING CAPACITIES OF RED AND WHITE SKELETAL MUSCLE OF THE PACIFIC BLUE MARLIN (*Makaira nigricans*) AND THE RAINBOW TROUT (*Salmo gairdneri*)

Species and Muscle Type	Mean ± SE Buffering Capacity (N)[a]
Marlin:	
White	103.8 ± 5.12 (5)
Red	50.8 ± 3.9 (5)
Trout:	
White	59.7 ± .8 (5)
Red	31.6 ± 1.1 (5)

[a] Buffering capacity (β) is expressed in slykes and defined as micromoles of base (NaOH) required to titrate the pH of 1 g (wet weight) of muscle by 1 pH unit at 25 C.

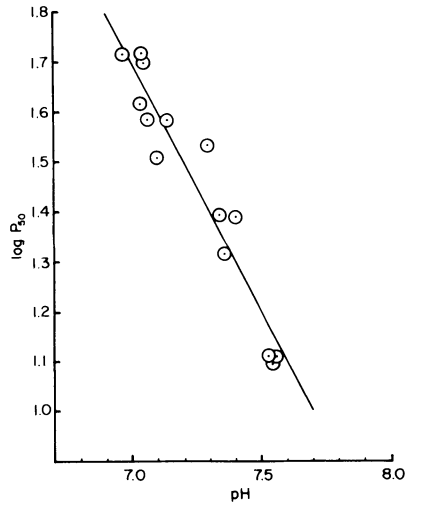


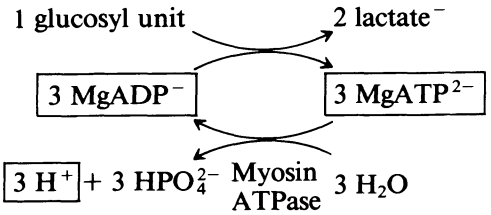
FIG. 3.—CO_2-Bohr effect of whole blood for the Pacific blue marlin at 25 C. Each determination was done in duplicate, and blood was obtained from nine fish weighing between 45 and 90 kg. The Bohr coefficient, $\phi = \Delta\log P_{50}/\Delta pH$ is −1.0 over the pH range 6.97–7.60. Linear regression analysis revealed a correlation coefficient of .96.

Wood, and Clark 1983; Wood, Turner, and Graham 1983; Perry et al. 1985), the reverse is known to occur in more sluggish species (Wood, McMahon, and McDonald 1977; Wood and Perry 1985; C. M. Wood and M. S. Graham, unpublished data; C. L. Milligan and A. P. Farrell, personal communication).

BUFFERING CAPACITIES OF RED AND WHITE MUSCLE: WHY ARE THEY DIFFERENT?

On the basis of the high in vitro nonbicarbonate buffering capacity of marlin white muscle (table 2), we place this species in the same category as the tunas (*Thunnus* sp.) and mackerel (*Auxis thazard*), which are high-speed "cruisers" with a high anaerobic potential (Castellini and Somero 1981). The following discussion extends these observations by considering intracellular buffering in the different muscle types as well as in the blood and attempts to suggest how these properties might be functionally related to oxygen transport.

The buffering capacity of marlin white muscle is twofold higher than that of red muscle (table 2), and this relationship has been reported for a number of fish species (Heisler and Neumann 1980; Abe 1981; Castellini and Somero 1981; Abe et al. 1985). The significant difference between fiber type and buffering capacity becomes apparent when comparing the rates of ATP production in each tissue and the fate of protons generated under working conditions. Marlin white muscle is predominately a fast-twitch glycolytic muscle that can support power outputs equivalent to ~51 μmoles of ATP/g wet wt muscle per minute at 25 C, a value approximately five times the maximum anaerobic power output of red muscle (Johnston and Salamonski 1984). Since buffering capacity of vertebrate and some invertebrate muscles can be related to the anaerobic potential (see Somero and Castellini 1981; Morris and Baldwin 1984), it is important to understand why this should be so. There are at least two potential sources of protons in working muscle: first, those protons generated during anaerobic glycolysis (anaerobic production) and, second, those protons generated via CO_2 hydration (aerobic production). Because fish white muscle has relatively few mitochondria and tissue capillaries compared to red muscle (Love 1970; Johnston 1981*a*, 1981*b*), the major source of protons in this tissue is the coupling of ATP hydrolysis with glycogenolysis according to the following scheme (see Gevers 1977; Zilva 1978; Hochachka and Mommsen 1983):



For this discussion a point of emphasis is that protons generated during anaerobic glycolysis do not arrive from the dissociation of lactic acid as often reported in the literature since the end product of anaerobic glycolysis is the lactate anion and not the free acid (see Gevers 1977). At pH 7.4 glycogen fermentation in fact consumes 0.4 H^+ per glucosyl unit (Hochachka and Mommsen 1983), which in simplified terms means that during burst work the number of protons generated per mole of substrate fermented in white muscle is trivial compared to the amount generated by coupling with ATP hydrolysis over the physiological pH range. Once the buffering capacity of white muscle is surpassed, a net acidification occurs and intramuscular pH falls. In red muscle the situation is quite different because under strictly aerobic conditions there is no net production of protons, since their release rate in ATP hydrolysis is matched by H^+ consumption during oxidative phosphorylation in the mitochondria (Krebs, Woods, and Alberti 1975). Because red muscle is predominately aerobic, the major source of protons during high work rates is the hydration of metabolically produced CO_2 according to the following reaction: $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$. For these reasons it is not surprising that the buffering capacity of red muscle of different fish species is lower than that of white muscle (see Abe 1981). That marlin red muscle has a higher buffering capacity than red muscle of trout (table 2) supports the proposal that compared to a less active species like the rainbow trout (*Salmo*

gairdneri), this particular species has "tuned up" both the aerobic and anaerobic potential for ATP generation for high muscle work rates.

RELATIONSHIP BETWEEN BLOOD BUFFERING AND O_2 TRANSPORT

The high buffering capacity of marlin blood (21.2 slykes) compared to blood of other marine and freshwater teleosts (see Perry et al. 1985) is an important strategy of this species to extend muscle performance. However, a consequence of scaling up blood buffering capacity is the need to increase the pH sensitivity of hemoglobin for releasing oxygen, otherwise O_2 delivery to the tissues during exercise would be compromised. Thus, our interpretation of the high Bohr coefficient ($\phi = -1.0$ over the pH range 7.0–7.7 at 25 C) is that it facilitates the delivery of oxygen from the blood to the aerobic muscles for a small change in blood pH during exercise. This strategy together with blood-flow adjustment (Randall and Daxboeck 1982; Neumann, Holeton, and Heisler 1983) represents an important mechanism for coupling oxygen supply to oxygen demand. The relationship between blood buffering and O_2 transport has been often overlooked in investigations dealing with comparative aspects of gas transport among the vertebrates.

Note added in proof.— The recent study by R. M. G. Wells and P. S. Davie, "Oxygen binding by the blood and hematological effects of capture stress in two big gamefish: mako shark and striped marlin" (Comp. Biochem. Physiol. 81A:643–646, 1985), similarly shows a high whole blood Bohr coefficient of −0.75 for the closely related striped marlin.

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