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TEMPERATURE-INDUCED CHANGES IN BLOOD GAS EQUILIBRIA IN THE ALBACORE, *THUNNUS ALALUNGA*, A WARM-BODIED TUNA

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SUMMARY

Samples of unbuffered, whole blood from freshly-caught albacore (*Thunnus alalunga* Bonnaterre) were equilibrated at 5, 10, 15, 20, 25, 30 and 35 °C and at 0 and 1 % CO_2 for construction of oxygen dissociation curves. A strong Bohr effect (−1·17), a negligible Root effect, and a reverse temperature effect ($\Delta H = +1{\cdot}72$ for 0 % CO_2 and +0·26 for 1 % CO_2) characterized these hyperbolic (Hill's $n = 1{\cdot}1$) curves. The unusual reverse temperature effect was especially pronounced when blood was quickly warmed or cooled, simulating passage through the heat exchanging, countercurrent vascular rete system of this warm-bodied fish. A diagrammatic model of blood gas dynamics in the rete incorporating these *in vitro* data illustrates protection of arterial oxygen from premature haemoglobin dissociation and consequent loss to the venous circulation as blood warms in the rete. More conventional temperature effects on the carbon dioxide equilibria of albacore blood lower the P_{CO_2} of venous blood being cooled in the rete. This reduces the venous–arterial P_{CO_2} gradient, thereby minimizing the diffusion of CO_2 to arterial blood with resulting haemoglobin-oxygen dissociation *via* the strong Bohr effect. The temperature range (10–30 °C) over which the albacore haemoglobin-oxygen binding exhibits the reversed thermal effect closely matches the maximum thermal gradient (ambient water–core body temperature) typically present in this fish, suggesting that its highly specialized haemoglobin-oxygen dissociation characteristics evolved within – and now establishes thermal limits upon – the existing geographic distribution of this species.

INTRODUCTION

It is well established that tunas (family Scombridae) and mackerel sharks (Lamnidae) utilize countercurrent vascular retia mirabilia in conserving metabolic heat and

Key words: Haemoglobin, warm-bodied fish, albacore.

maintaining an elevated body temperature (Carey *et al.* 1971; Graham & Diener, 1978; Stevens & Neill, 1978). Although the brain and viscera are also warmed in some of these species, the highest temperatures always occur in and around the deeply positioned packs of red muscle (Carey & Teal, 1966, 1969*a*,*b*; Graham & Dickson, 1981). Retia are composed of dense bundles of arteries and veins and are situated in series with the major systemic arterial and venous circulation entering and exiting red muscle (Graham & Diener, 1978). Oxygenated blood that has just reached thermal equilibrium with ambient sea water in the gills enters the rete on the arterial side while warmed, deoxygenated, and carbon dioxide-laden blood enters on the venous end. In the rete, countercurrent flow and the high surface area contact between the two blood supplies facilitate the transfer of nearly all of the metabolic heat in the venous blood to arterial blood, thus conserving muscle temperature (Carey, 1973; Stevens & Neill, 1978; Carey, Teal & Kanwisher, 1981). After exiting the rete arterial blood continues to the red muscle capillary beds and cooled venous blood flows to the gills where carbon dioxide is excreted and oxygen is loaded.

This heat conservation system utilizes the blood as the heat transferance medium with conduction across the vessel walls of the rete. As a consequence, the blood warms and cools rapidly as it passes through the rete. However, it is known that temperature increases result in a drop in blood pH (Howell, Baumgardner, Bondi & Rahn, 1970) of approximately $-0{\cdot}012$ to $-0{\cdot}019$ pH units per °C, depending on the temperature (Reeves, 1976). Warming also decreases the solubility of oxygen and carbon dioxide in plasma. Together or separately, a temperature increase, a pH decrease, and a P_{CO_2} increase all characteristically reduce blood (haemoglobin) oxygen affinity of fish blood (see reviews by Randall, 1970; Wood & Lenfant, 1979).

Studies on blood gas equilibria in warm-bodied fish began with Rossi-Fanelli & Antonini's work (1960) on bluefin tuna (*Thunnus thynnus*) haemoglobin. They found a very small effect of temperature on oxygen affinity (apparent heat of oxygenation, $\Delta H = -1{\cdot}8$ kcal mol^{-1}), but performed their studies with crystalline haemoglobin in buffered solutions (Rossi-Fanelli & Antonini, 1960). Anderson, Olson & Gibson (1973) also noted small temperature effects for mako shark (*Isurus oxyrinchus*) and bigeye tuna (*Thunnus obesus*), although stripped haemoglobin haemolysate solutions were used. Graham (1973) proposed that the small temperature effects of warm-bodied fishes may have evolved to eliminate premature oxygen dissociation in the retia, but he included little data to support this hypothesis. Sharp (1975) constructed oxygen dissociation curves using erythrocytes of bigeye tuna, bluefin tuna, albacore (*Thunnus alalunga*), and yellowfin tuna (*Thunnus albacares*). However, Sharp suspended the cells in buffered glycerol solutions and many of the measurements were at higher temperatures (to 37 °C) and lower pH values (to 6·60) than are probably naturally experienced by these species. Carey & Gibson (1977) described a reverse temperature effect and crossing of oxygen dissociation curves constructed at 14 and 22 °C for bluefin tuna. However, buffered haemoglobin solutions were again used to arrive at these results. Carey & Gibson (1983) constructed oxygen dissociation curves from bluefin tuna whole blood at 5–35 °C. They found virtual temperature independence, but gave no pH data. However, they provide evidence for a reverse temperature effect with bluefin haemoglobin solutions between pH 7·0 and 8·5. Thus, a study using whole blood from freshly-caught specimens and employing appropriate

pH and temperature regimes was considered to be of particular value in advancing our understanding of temperature-related haemoglobin-oxygen dynamics in warm-bodied fishes.

The main problem in studying tuna is that they range freely in habitats often at great distances from the closest laboratory having appropriate facilities and instrumentation to make the needed measurements. The first objective of the present study was to investigate the blood oxygen dissociation characteristics of albacore using fresh, unbuffered, whole blood over the range of internal and external temperatures appropriate for this species. This would include arterial and venous CO_2 tensions and the fast transitions in temperature that occur as blood traversed the rete. The second objective was to determine the thermal limits and optima for oxygen binding in albacore blood. This tested Graham's (1973) hypothesis and revealed how the albacore's blood gas transport mechanisms have evolved in response to both the range of ambient temperatures and dissolved oxygen conditions encountered by this species and to the gradient between its ambient and core body temperature which, although affected by many factors, remains primarily dependent upon fish activity.

MATERIALS AND METHODS

Albacore (body weight range = 5·720–10·325 kg) were captured by hook and line using trolled feathered jigs aboard the U.S. NOAA National Marine Fisheries Service R.V. *David Starr Jordan* during the first two of the three legs of the 1981 albacore physiology cruise. Using hydraulic fishing reels, fish were landed within 30 s of being hooked. A running seawater hose (1·2 cm i.d.) was immediately placed in the mouth of the fish as it rested upon the deck for artificial gill ventilation. Blood samples (44–94 ml) were immediately taken from the heart or a cutaneous vessel using heparinized, 20 ml polypropylene syringes and 20 gauge needles. Needles were removed and blood was immediately transferred to heparinized polypropylene vials. Clotting was negligible and blood was either used immediately or stored at 4 °C to be used within 12 h.

Aboard the research vessel, whole unbuffered albacore blood was equilibrated with gases in a pair of rotating, Hall-type glass tonometers (Hall, 1960). One tonometer had a continuous flow of humidified, deoxygenated gas (100 % N_2 or 99 % N_2 + 1 % CO_2), while the other had a continuous flow of humidified, oxygenated gas (100 % air or 99 % air + 1 % CO_2), respectively. Various mixtures of the blood from the two tonometers were taken in 1 ml glass syringes, each having a metal mixing bead, to simulate 'arterial' (0 % CO_2) and 'venous' (1 % CO_2) blood. Volumes of blood withdrawn from the tonometers, adjusted for the volume of the beads and the syringe and needle deadspaces, were selected to give 0, 20, 50, 80, 95 and 100 % oxygenated mixtures (= % saturation) after mixing in the syringe for 30 s. Oxygen tension (P_{O_2}) measurements from each mixture were made using a Radiometer PHM 71 Mk. 2e analyser and Radiometer E5046/D616 thermostatted P_{O_2} electrode assembly to construct blood oxygen dissociation curves (Edwards & Martin, 1966). In addition, pH measurements of the blood mixtures were made using a Radiometer G297/K497 thermostatted pH/reference electrode system wired to the PHM 71 Mk. 2e analyser. P_{O_2} and pH electrodes were 'two-point' calibrated with air/N_2 and an aneroid

barometer to ±0·1 Torr and Harleco precision buffers to ±0·001 pH units, respectively, immediately before each mixture measurement. Mean pH values for each fish at each P_{CO_2} level were calculated by initial conversion to $[H^+]$ values (Davenport, 1974). Blood oxygen capacity (Cb_{O_2}) was determined by direct measurements of oxygen content of the 100% saturation blood using a Lexington Instruments Lex-O_2-Con Model M analyser.

Temperatures (5–35 ± 0·5 °C) were maintained in the insulated tonometer water bath by opposition of a thermostatted Lauda Model K-2/R chiller and a 500 W immersion heater wired to an adjustable thermoregulator and relay (Versatherm) with vigorous circulation by a submersible pump. For construction of constant-temperature blood oxygen dissociation curves, another submersible pump continuously circulated water from the tonometry bath through the water jackets of the P_{O_2} and pH electrodes. Blood oxygen dissociation curves resulting from a rapid 20 °C temperature increase or decrease (simulating passage through the heat exchanging rete) were made possible by moving the water jackets' pump to a second insulated water bath kept 20·0 °C cooler or warmer than the tonometer bath. For these rapid temperature change measurements, mixed blood samples from the tonometers at 10 or 30 °C were injected into the electrode measurement chambers maintained at 30 or 10 °C, respectively, for P_{O_2} and pH measurements.

Bohr effect was calculated by $\Delta \log P_{50}/\Delta pH$ and Root effect was calculated from % loss in Cb_{O_2} (Hayden, Cech & Bridges, 1975). The apparent heat of oxygenation (ΔH) was calculated using a form of the Van't Hoff equation (Wood & Lenfant, 1979). Packed cell volumes (haematocrits) were determined numerous times on blood samples from each fish to monitor changes as blood was added to tonometers for each curve and to ascertain visually the presence of significant haemolysis. Almost all of the plasma had a pale straw colour and no blood was used which had more than a very slight pink tinge in plasma colour, indicating minimal haemolysis. Blood samples from each fish were also fixed for lactic acid determinations (BMC Single Vial Lactate Kit).

Albacore blood gas equilibria measurements were continued at the Scripps Institution of Oceanography, Physiological Research Laboratory using whole blood from legs two and three of the cruise. Available instrumentation at this laboratory allowed us to measure blood CO_2 tensions (Radiometer PHM71 Mk. 2e analyser with E5036 electrode) and CO_2 concentrations (Capni-Con, Cameron Scientific Instruments, Port Aransas, Texas) on blood mixtures from the same tonometers in addition to the other measurements made at sea. In addition, whole blood CO_2 dissociation curves were constructed at 10 and 30 °C from the blood of one albacore in similar fashion to the blood oxygen dissociation curves described above. In this case, however, blood was equilibrated with N_2 and CO_2 in the paired tonometers. (P_{O_2} = 0 Torr, 1 Torr = 133·322 Pa). Blood P_{CO_2} and pH was measured with the Radiometer systems and CO_2 contents of each mixture were measured with the Capni-Con. Plasma CO_2 contents were calculated using the Henderson-Hasselbalch equation (Davenport, 1974) with constants published by Reeves (1976). These laboratory determinations were made on blood within 24 h post-sampling, except for the CO_2 dissociation curves which were made 3 days post-sampling. However, the blood was stored at 4 °C in polypropylene syringes or vials having large air spaces for the

metabolic oxygen requirements of these aerobic, nucleated erythrocytes (Eddy, 1977). The air in these containers was renewed and gently mixed with the blood every 4–12 h.

RESULTS

The morphological and physiological characteristics of the five shipboard-sampled albacore are shown in Table 1. Generally the blood oxygen dissociation curves of albacore were quite consistent among fish tested, hyperbolic in shape, and unusually affected by temperature. Curves at 25 °C and 0 % CO_2 were constructed for all five albacore sampled at sea and showed a mean ± S.D. (range) of 8·3 ± 1·5 (6·5–9·9) Torr for the P_{50}, 26·6 ± 2·5 (23·3–29·0) Torr for the P_{80} and 56·7 ± 5·6 (53·7–65·0) Torr for the P_{95}. The variation in P_{50} seemed to stem primarily from small differences in blood pH measured for individual fish (Table 1). The curves were strongly hyperbolic with a Hill's coefficient (Wyman, 1948) of 1·11 and showed a *reverse* temperature effect (Fig. 1). In contrast to almost all haemoglobins previously studied, oxygen affinity *increases* with increasing temperature (Figs 1–3). The overall calculated apparent heat of oxygenation (ΔH) was +1·72 for 0 % CO_2 and +0·26 for 1 % CO_2.

A temperature increase accentuated the reverse temperature effect. Whereas the mean P_{95} of 'arterial' blood (0 % CO_2) was 93·3 Torr at 10 °C and 47·0 Torr at 30 °C, it was only 36·3 Torr when rapidly heated from 10 to 30 °C (Fig. 3).

In contrast to the atypical temperature effects on the albacore haemoglobin(s), the pH/CO_2 effects were more conventional. The mean Bohr effect for all shipboard data (10–35 °C) was −1·17. Using only P_{50} and pH data measured from the same fish at both CO_2 tensions, the mean Bohr effect was −1·31 (25–35 °C). A small mean Root effect of −4 % was calculated for all shipboard albacore over 10–35 °C. A small gain (+5 %) in mean Cb_{O_2} was calculated for the fish in which Cb_{O_2} was measured at both P_{CO_2} levels over 25–35 °C.

To visualize the blood gas dynamics across the heat exchanger, a diagrammatic model has been fitted with available *in vitro* data (Fig. 4). Because of the lack of *in vivo* blood gas data on settled-down fish, 'arterial' blood is assumed to be at 95 %

Table 1. *Morphological and physiological characteristics of shipboard-sampled albacore*

Fish No.	Fork length (cm)	Live weight (kg)	Blood P_{50} at 25 °C and 0 % CO_2 (Torr)	$\bar{x}$ Haematocrit (%)	Blood oxygen capacity at 25 °C and 0 % CO_2 (ml dl^{-1})	$\bar{x}$ Blood pH at 25 °C and 0 % CO_2	Blood lactic acid concentration (mg dl^{-1})
1	77·5	8·333	9·8	56·9	22·9	7·68	57·67
2	66·5	5·720	6·5	47·7	19·0	7·61	46·88
3	78·5	9·250	8·1	52·6	22·1	7·76	57·80
4	76·0	8·630	7·1	56·1	22·6	7·66	44·37
5	82·5	10·325	9·9	51·5	22·6	7·76	43·88
$\bar{x}$	76·2	8·450	8·3	53·0	21·8	7·69	50·12
S.D.	5·9	1·705	1·5	3·7	1·6	0·06	7·04

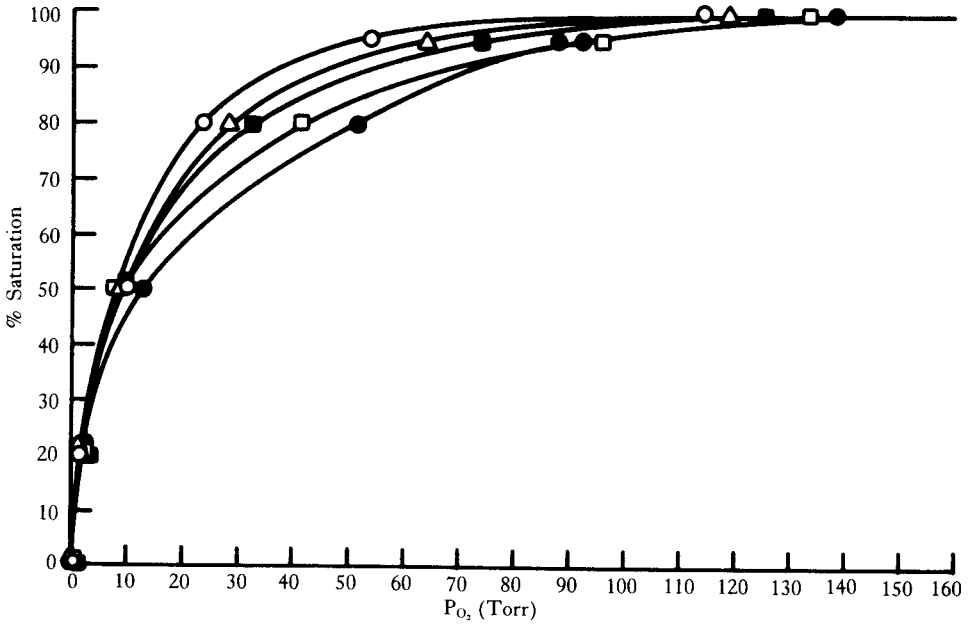

Fig. 1. Whole blood oxygen dissociation curves from albacore (*Thunnus alalunga*) no. 1, equilibrated at 0 % CO_2 and 5 °C (●), 10 °C (□), 15 °C (■), 20 °C (△) and 25 °C (○).

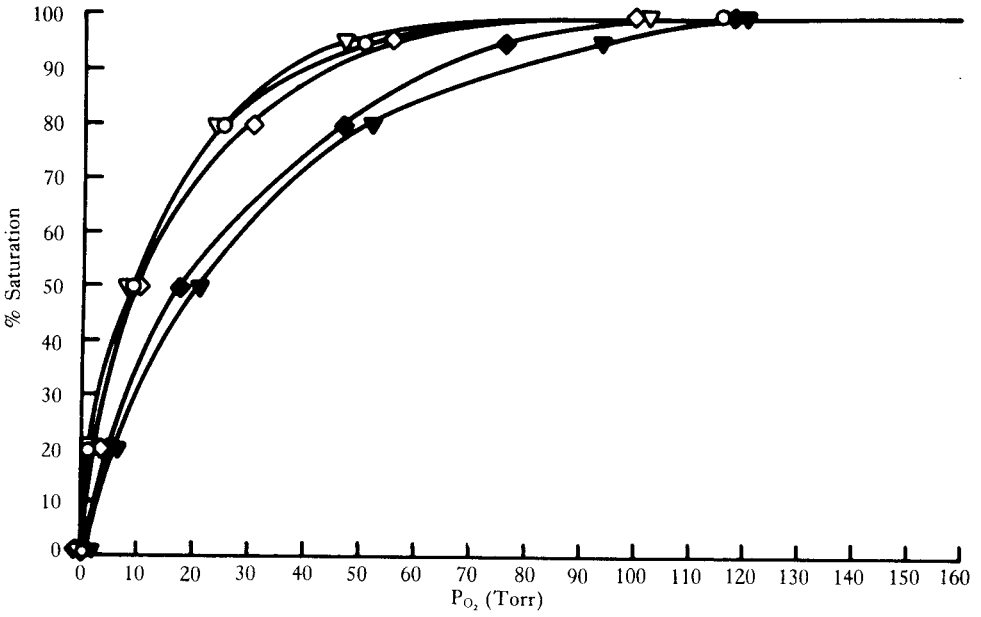

Fig. 2. Whole blood oxygen dissociation curves from albacore (*Thunnus alalunga*) no. 3, equilibrated at 0 % CO_2 and 25 °C (○), 30 °C (▽), and 35 °C (◇) and at 1 % CO_2 and 30 °C (▼) and 35 °C (◆).

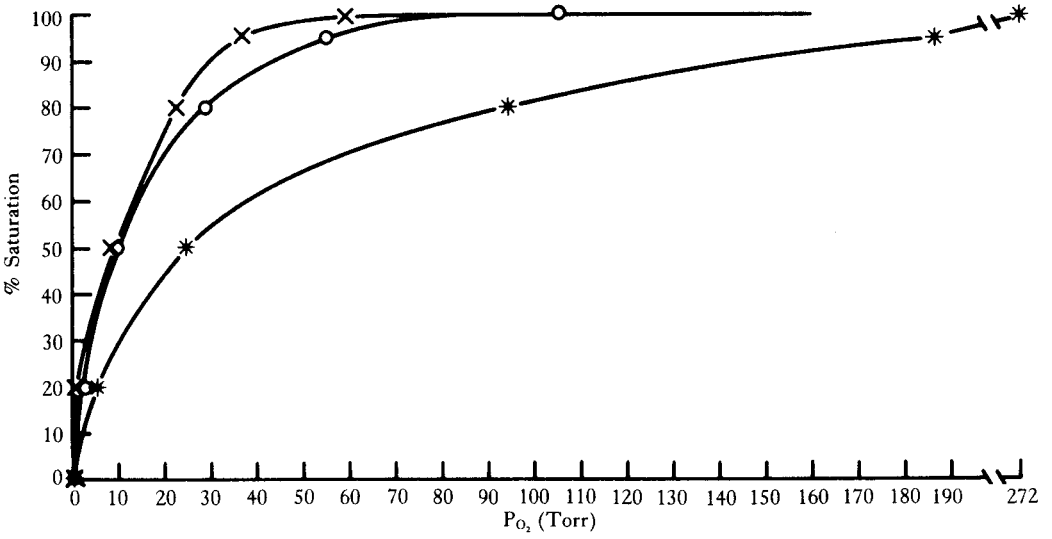

Fig. 3. Whole blood oxygen dissociation curves from albacore (*Thunnus alalunga*) no. 5, equilibrated at 0 % CO_2 and 25 °C (○), and at 10 °C and then quickly warmed to 30 °C (×), and at 1 % CO_2 and 30 °C and then quickly cooled to 10 °C (*).

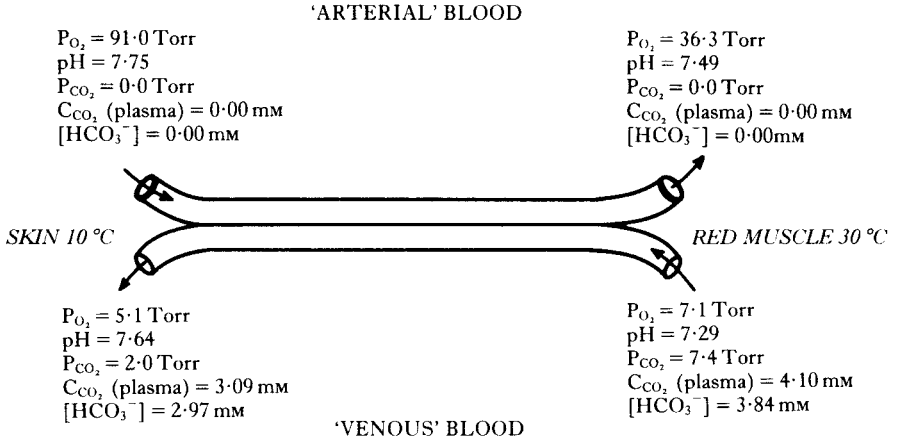

Fig. 4. Diagrammatic model of blood gas changes occurring along a single artery (upper) and adjacent vein (lower) within a heat exchanging rete in albacore (*Thunnus alalunga*) using appropriate *in vitro* data from blood oxygen dissociation curves (see text).

saturation with respect to air and at 0 Torr P_{CO_2}. Similarly, the 'venous' blood is assumed to be at 20 % saturation and 7·6 Torr P_{CO_2}. To examine the near-maximal effects of warming (Sharp & Vlymen, 1978; Graham & Dickson, 1981) a 20 °C temperature gradient was assumed with the cutaneous vessel blood at 10 °C and the red muscular blood at 30 °C. For blood flowing into the rete at each end, *in vitro* data for albacore blood equilibrated at its respective temperature was used, while the appropriate fast temperature change data was used for outflowing blood.

Notable characteristics of this model include the inverse relationships of pH with P_{CO_2} and with temperature (Fig. 4). Blood pH decreases with increasing P_{CO_2} because of the resulting shift in the bicarbonate buffer equilibrium which dissociates more carbonic acid into hydrogen and bicarbonate ions (Albers, 1970). The relationship between temperature (T) and albacore blood pH is represented by the least-squares equations:

$$\hat{pH} = 8{\cdot}08 - 0{\cdot}016T$$
$$\hat{pH} = 7{\cdot}78 - 0{\cdot}016T$$

for 0 Torr P_{CO_2} and 7·6 Torr P_{CO_2}, respectively (Fig. 5).

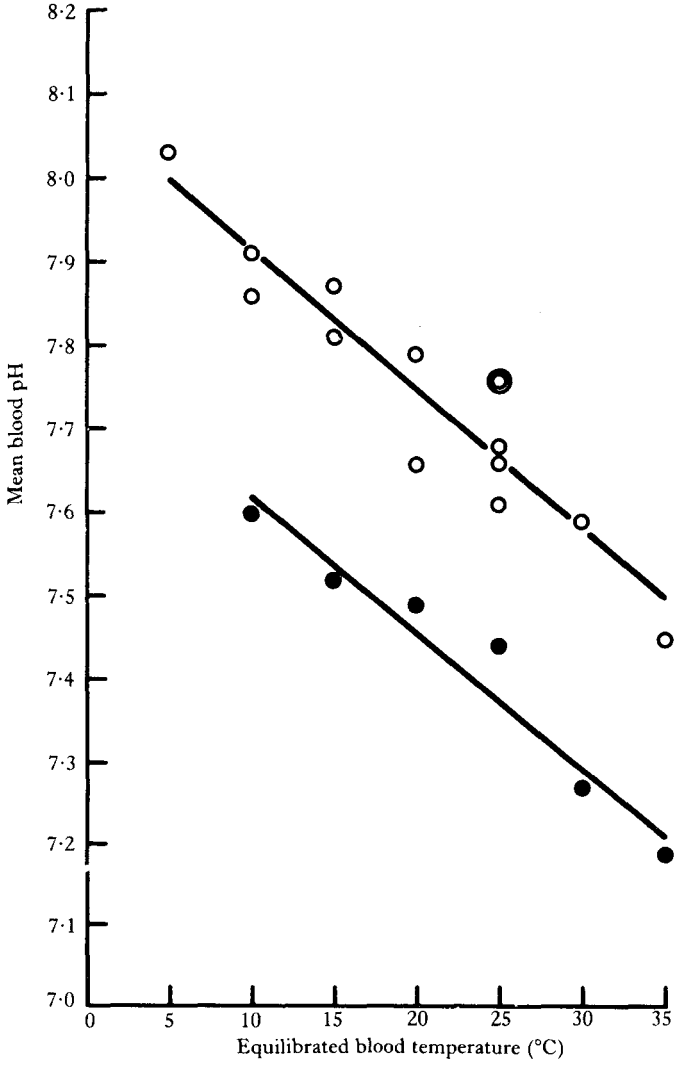

Fig. 5. Mean, unbuffered whole blood pH values of albacore (*Thunnus alalunga*) as a function of equilibrium temperature. Blood equilibrated with 1% CO_2 (●); blood equilibrated with 0% CO_2 (○). Heavy circle shows two identical data points.

The diagrammatic model gives insight into the importance of the reverse temperature effect. The P_{O_2} of arterial blood, declines as the blood warms in the rete, a function of the greater haemoglobin oxygen affinity at 30 °C (Figs 2, 3). Thus, rather than a temperature-induced premature oxygen dissociation in the rete the albacore haemoglobin(s) actually increases its oxygen binding, reducing the P_{O_2} gradient between 'arterial' and 'venous' blood and limiting oxygen diffusion from arterial to venous blood along the length of the retial vessels. On the venous side of the rete, P_{O_2} stays approximately constant with cooling because the reverse temperature effect is much reduced at the lower percent saturation (Figs 1–3). However, the P_{CO_2} is considerably reduced with cooling in the rete because the albacore carbon dioxide dissociation curves show a greater CO_2 affinity (i.e. more carbaminohaemoglobin) with cooling (Fig. 6). The resulting lower P_{CO_2} gradient would reduce CO_2 diffusion from venous to arterial blood and prevent premature oxygen dissociation in the rete by the Bohr effect. The calculated CO_2 content in the plasma and bicarbonate ion concentration change little with cooling (Fig. 4).

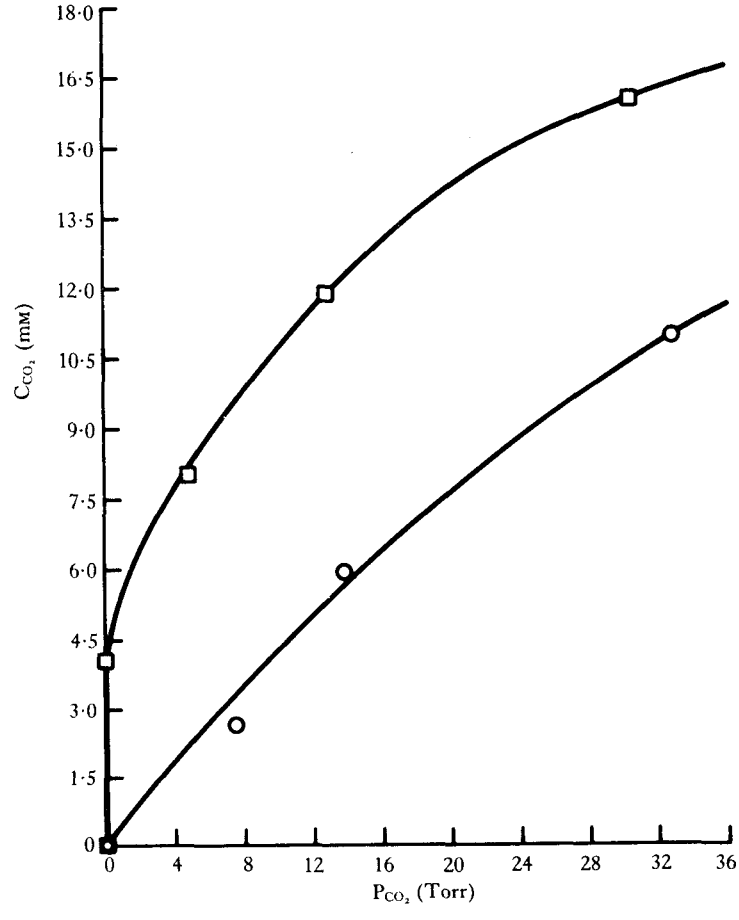

Fig. 6. Whole blood carbon dioxide dissociation curves of albacore (*Thunnus alalunga*) equilibrated at 0 % O_2 and 10 °C (□) and 30 °C (○).

DISCUSSION

In general, these albacore data reflect the high blood oxygen capacity (Table 1) and unique temperature characteristics one might expect in a highly aerobic, fast swimming, and warm-bodied tuna (Graham & Laurs, 1982). Haematocrit values closely matched the previous measurements of Alexander, Laurs, McIntosh & Russell (1980) and blood pH levels (Table 1) were in the predicted range of the curves of Howell *et al.* (1970). The most striking finding in the present study concerns the reverse temperature effect of albacore haemoglobin-oxygen binding in fresh, whole blood samples. This effect is especially dramatic with fast temperature changes. Sharp (1975) previously described a hyperbolic oxygen dissociation curve for albacore blood but failed to find the reverse temperature phenomenon, probably because of the high temperatures (25 and 37 °C) he used. Data from the present study show that the reverse temperature effect is diminished and 'reverts' to the more typical form between 30 and 35 °C. Collett & O'Gower (1972) described a reverse temperature effect in buffered haemolysate haemoglobin solutions from three species of arcid clams. Normal, decreased oxygen affinities were displayed by these haemoglobins with increasing temperatures up to 20–25 °C, but affinities increased at higher temperatures. Collett & O'Gower attributed this phenomenon to part of these species' physiological adaptations to intermittent respiratory stress in warm, marine environments. Carey & Gibson (1977, 1983) imply an increased respiratory efficiency in the bluefin tuna heat exchanger because there is a reverse temperature effect. Since the reverse temperature phenomena described by Collett & O'Gower (1972) and by Carey & Gibson (1977, 1983) both arose from experiments using haemoglobin solutions, we conclude that this effect has a haemoglobin molecular basis in the present study with albacore whole blood.

Potential experimental drawbacks of our study merit comment. One concerning our fast temperature change blood gas values concerns the time course of the temperature change. Injection of blood mixtures equilibrated at one temperature into an electrode sample chamber thermostatted at the second temperature probably does not exactly mimic the changes in blood temperature that takes place in the seconds required for blood to traverse the rete *in vivo*. Moreover, at 30 and 10 °C, it takes approximately 1·5 and 3·5 min, respectively, for the P_{O_2} electrode and meter to come to a stable reading. As *in vivo*, oxygen cannot escape from the electrode chamber and thus the 'instantaneous' P_{O_2} values may actually be more extreme, i.e. lower with quick warming and higher with quick cooling, based on comparisons with temperature-equilibrated curves (Figs 1–3). This seems particularly likely in view of the respiration of these nucleated red blood cells which would deplete oxygen during the time needed to obtain stable readings. The especially prolonged time course of the 30 to 10 °C switch would actually have a minimal effect on the functional relationships shown in the diagrammatic model (Fig. 4) due to the vastly reduced reverse temperature effects at 20 % saturation (Figs 1–3).

Assumptions concerning the model also deserve comment. A 20 °C temperature shift across the rete is probably maximal (Sharp & Vlymen, 1978; Graham & Dickson, 1981), and this large shift was desired to elucidate the relevant blood gas dynamics. Graham & Dickson (1981) measured red muscle temperatures between 28 and 29 °C

in an exercising albacore situated in water at 13 °C in a shipboard tank. Laurs, Dotson, Dizon & Jemison (1980) found that acoustically-tagged albacore remained in water between 9 and 14 °C. Also, the 10–30 °C range lies wholly within and roughly defines the thermal zone of the reverse temperature effect (Figs 1, 2). Blood entering the white muscle would obviously be warmed less than that entering the red muscle through the rete (Graham & Dickson, 1981). Also, a 20 °C gradient between the ambient water and the entire red muscle mass would not be expected at all times. P_{O_2} and pH conditions during a <20 °C temperature shift could be crudely estimated from the curves shown in Figs 1, 2 and 5. The percent saturation (95 % for 'arterial' and 20 % for 'venous') and P_{CO_2} levels (0 Torr for 'arterial' and 7·6 Torr for 'venous') seem reasonable for fish of high routine activity levels (Root, 1931; Stevens & Randall, 1967; Cameron, 1971; Holeton, Pawson & Shelton, 1982).

It is logical that the fast temperature change data be used for both the arterial and venous blood flowing from the rete due to the probable short residence time of blood in this structure. It also seems reasonable that 10 °C equilibrium data be used to model the arterial flow. Free-swimming albacore occur in water of this temperature (Laurs *et al.* 1980). Also, arterial blood reaches thermal equilibrium with ambient water in the gills and does not gain heat *en route* to the rete (Carey *et al.* 1971; Stevens & Neill, 1978). On the venous side, all regions in the red muscle would not be expected to be at 30 °C, although this temperature may reflect the average (Graham & Dickson, 1981). Thus, most CO_2 would be added to venous blood at about 30 °C (Fig. 4).

Assuming, then, that the diagrammatic model gives us a reasonable idea of the *in vivo* retial blood gas dynamics, what does this mean to the albacore? On the arterial side, it means that more oxygen is bound by haemoglobin rather than released prematurely in the rete. The greater haemoglobin-oxygen affinity has two favourable aspects. The first is that the plasma P_{O_2} is lowered in the arteries, which lowers the arterio-venous P_{O_2} gradient and minimizes the diffusion potential for oxygen to the venous blood, ensuring that it reaches the muscle capillaries. The second is that some of the oxygen is removed from the plasma, thereby opening some solubility 'space' for the inert gases (principally, nitrogen) to stay dissolved. Using gas solubility table (Weiss, 1970) calculations, a 1 % salt solution (simulating plasma) that is 95 % air-saturated at 10 °C will increase in total gas saturation to 140 % (1·4 atm = 141 855 Pa) when heated to 30 °C. The P_{O_2} reduction in albacore blood concomitant with this warming would limit this total gas saturation to 119 %. Using the reasoning of Kiesow (1974), this excess P_{N_2} may be the mechanism for the higher oxygen affinity of the haemoglobin upon rapid warming. However, human erythrocytes and 50–100 atm P_{N_2} were utilized in Kiesow's study. Obviously, nitrogen gas bubbles would severely impair capillary circulation (Hochachka & Somero, 1973). Although a total gas saturation of 119 % is capable of supporting bubble formation (Bouck, 1980), a combination of hydrostatic (depth) and blood pressure may satisfactorily ameliorate this condition (Bouck, 1980). Indeed, the prevention of gas bubble formation in the blood may be one limitation on the maximum temperature differential across the rete.

On the venous side, the P_{CO_2} is reduced with cooling, decreasing the P_{CO_2} arterio-venous gradient and thereby minimizing the potential for CO_2 diffusion into retial arteries. The strong Bohr shift calculated for albacore indicates that oxygen would dissociate from the haemoglobin in the rete if significant quantities of CO_2 were

introduced into the arteries. The albacore Bohr effect (−1·17 overall) compares with the continually active, but not warm-bodied Atlantic mackerel, *Scomber scombrus* at −1·2 (Hall & McCutcheon, 1938). Both of these display more dramatic Bohr effects than rainbow trout, *Salmo gairdneri* at −0·57 (Eddy, 1971). The small Root shift of albacore befits a species with a relatively small swimbladder (Riggs, 1970)

Blood lactic acid concentrations of the albacore were not high when compared with other species. For example Holeton *et al.* (1982) measured mean lactate concentrations of >80 mg dl^{-1} in feather-jig-caught Atlantic mackerel. Also, Soivio, Nikinmaa, Nyholm & Westman (1981) measured mean lactate concentrations approximating 68 mg dl^{-1} (arterial) and 81 mg dl^{-1} (venous) in resting rainbow trout. The lack of a significant correlation ($r = 0{\cdot}25$, $P >> 0{\cdot}05$) between albacore blood lactate concentrations and mean blood pH, measured at the same temperature and P_{CO_2} conditions, also indicates a minimal effect of lactate on blood gas relationships in the present study (Table 1).

This study reveals that temperature has a reversed effect on the haemoglobin-oxygen dynamics of albacore blood. Our analysis of this effect in relation to the structure and function of a heat-exchanging rete suggests that it is an adaptation which enables the albacore to optimize the physiological consequences of rapid changes in blood temperature for red muscle respiratory gas transport. The close correspondence between the optimal thermal range (10–30 °C) for the reversed effect and the typical gradient present between ambient (sea water) and core (red muscle) temperatures for albacore is noteworthy and additionally suggests that the oxygen transport mechanism of this species has become, through natural selection, precisely poised to operate between environmental and deep body temperatures for the purpose of sustaining aerobic muscle metabolism. This further implies that geographical (by temperature) distribution limits of albacore may in turn be set by its thermal optimum for oxygen transport. Of course, even within its normal zoogeographic range, an albacore's water–red muscle thermal gradient is not constant and could become altered either by changes in red muscle metabolism (i.e. swimming velocity), shifts in ambient temperature, or both (Graham & Dickson, 1981). However, a large sustained shift in the gradient seems unlikely. This is because albacore swimming in nature spend most of their time in a narrow range of temperatures (9–12 °C, Laurs *et al.* 1980) and the scope for metabolic heat production by albacore red muscle is constrained within limits. These limits are set at the low end by the minimum swimming velocity needed for an albacore to maintain hydrostatic equilibrium (Dotson, 1976; Magnuson, 1978), and at the high end by the capacity of the cardiovascular system to sustain aerobic metabolism (heat production) in active red muscle (Graham, 1983). On the other hand albacore migrating into the eastern Pacific Ocean may encounter layers of 9–12 °C water in which the oxygen tensions reflect 60 % air saturation (Laurs & Lynn, 1977) which would reduce haemoglobin saturation to some extent (Figs 1–3). If this requires an albacore to change depth to shallower (warmer), more oxygenated water an acclimatory shift may occur in its optimal thermal gradient for oxygen transport. Alternatively this species could compensate for a decreased ambient oxygen supply by increasing its blood oxygen affinity.

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