SOME RESPIRATORY PROPERTIES OF THE BLOOD OF FOUR SPECIES OF ANTARCTIC FISHES

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Abstract-1. Fishes in Antarctic seas live at a temperature lower than that at which most other poikilotherms remain active.

2. Hematocrit, absolute oxygen capacity and oxygen equilibrium curves were determined for whole blood from each of four related species of Antarctic fishes. These parameters were related to the habits and activity of each species.

3. Temperature increase was found to have a marked effect on the affinity of the blood for oxygen. This effect was compared with data from other fishes and found to be extreme in the Antarctic species.

4. These fishes are known to be stenothermal and geographically restricted in distribution. It seems likely that the sensitivity of their oxygen transport systems to temperature increase could provide at least some of the physiological basis for this.

INTRODUCTION

The Low temperature of the Antarctic seas imposes on animals the necessity for certain adaptations, particularly in their metabolism and resistance to freezing. Some aspects of metabolism of nototheniid fishes have been studied, cf. Wohlschlag (1964). His work indicates that the Antarctic fishes are very stenothermal and that their metabolic rates are higher than would be expected at such low temperatures. He therefore considers them to be "cold adapted". In his various papers Wohlschlag has commented extensively on the nature of the environment in McMurdo Sound where the fishes in the present study were caught. Physicochemical conditions are more stable than in lower latitudes and data from Littlepage (1965) illustrate this. The mean water temperature is about $-1.9°$C, with a seasonal range from -1.4 to $-2.0°$C. Oxygen content is near saturation (about 8.0 ml/l at this temperature) and salinity is constant within narrow limits. For most of the year McMurdo Sound lies under an ice cover several feet in thickness and this results in reduced light penetration in summer when the sun is above the horizon.

Antarctic fishes live at temperatures lower than those at which most other poikilotherms remain active. In general, low temperatures increase the oxygen affinity of hemoglobin, an effect first investigated in fishes by Krogh & Leitch (1919). Subsequent studies on fishes by Kawamoto (1929), Dill et al. (1932) and Irving et al. (1941) indicate that the hemoglobin characteristic of the species is functional in the temperature range for that species. However, no studies appear to have been done at Antarctic temperatures, and since some Antarctic fishes have no respiratory pigment (Ruud, 1954, 1959), one object of the present study was to see if nototheniid hemoglobin is functional. The oxygen-hemoglobin equilibrium was investigated at environmental temperatures, and again at higher temperatures for comparison.

General comparisons between respiratory characteristics of blood and modes of life in different fishes have been made by many authors, notably Willmer (1934), Black (1940), Irving et al. (1941), Fish (1956) and Dubale (1959). In most cases these comparisons were made between fishes from different taxonomic groups, different environments, and having different habits. Apart from work on Salmonids, this is the first study of this nature to be made comparing several closely related fishes. The four species of *Trematomus* in the present study live under very similar environmental conditions and an attempt was made to relate differences in blood respiratory properties to different habits.

MATERIALS AND METHODS

The fishes used in this study were caught in 75-350 ft of water in McMurdo Sound (77° 51' S., 166° 38'E.), from October through December 1966. At this season the water temperature is about $-1.8°$C. *Trematomus bernacchii* (50-440g), *T. centronotus* (116210 g) and *T. hansoni* (90-200 g) were trapped on the bottom using methods described by Wohlschlag (1964). Specimens of *T. borchgrevinki* (40-112 g) were caught by hand line through holes in the ice. Specimens were identified from descriptions by Norman (1938). After collection, the fish were removed to the McMurdo Biological Laboratory where they were maintained in thermally controlled tanks until required.

Blood was removed from the common cardinal vein of smaller fish and by cannulation of the caudal artery in larger specimens. In several cases, a large nematode living in the dorsal aorta of *T. bernacchii* made this latter procedure difficult by obstructing the cannula. Solid heparin was used to prevent clotting. Attempts to store the chilled blood were unsuccessful because the plasma became opaque after half a day or so. Hence, all observations were made on freshly drawn blood.

Duplicate samples for cell volume determination were collected in heparinized capillary tubes and centrifuged in a standard microhematocrit centrifuge. Hematocrit determinations did not agree with those made on the same species by Kooyman (1963). However, he gave no indication of the type of centrifugation used and it was found early in the present study that the ordinary laboratory centrifuges were unsatisfactory.

Measurements of the oxygen content of blood were made by the method described by Roughton & Scholander (1943) incorporating the modification for fish blood by Scholander & van Dam (1956). Further procedural modifications were made following the advice of Mr. Everett Douglas (personal communication, 1966). Oxygen capacity determinations were made on blood equilibrated to air in a temperature-controlled tonometer similar to that described by Finley et al. (1960). The water bath was held at −1.5°C for these determinations. Duplicate analyses usually agreed within 0.1 vol. per cent. If disagreement was more than 0.2 vol. per cent the readings were disregarded.

Oxygen equilibrium curves were obtained by the technique described by Lenfant & Johansen (1965) after Haab et al. (1960). Whole blood was used, and sometimes pooling from four to five fish was necessary, but this was kept to a minimum. The well-mixed sample was halved and one half equilibrated to air, the other half to helium in the tonometer at experimental temperature. This provided a sample of fully oxygenated and a sample of fully deoxygenated blood. Oxygen content in these samples was determined by the method described above. By mixing subsamples from each of these in different proportions, a series of mixtures was obtained over a range of known oxygen contents. The oxygen tensions (PO_2) of these mixtures was then measured by a Beckman Spinco Gas Analyser and a Beckman Macroelectrode. The electrode was calibrated at the temperature of the tonometer bath. Duplicate or triplicate measurements on the same sample were within 2 mm in the upper range of the 0-160 scale and within 1 mm over the 0-60 scale. Precautions were taken during the whole operation to keep the syringes and blood chilled at all times. All measurements were converted to standard temperature and pressure.

RESULTS
General respiratory properties of the blood
That the species *of Trematomus* in this study have a functional oxygen transporting pigment is beyond question. The dark venous blood was in sharp contrast to the brightly colored arterial blood. The oxygen affinities were seen to be well within the range of those from fishes in lower latitudes. Blood oxygen capacities and cell volumes are displayed in Table 1. It was found that a straight line relates these parameters in each species. From these items and a knowledge of the oxygen capacity of the plasma (0.8 vol. per cent), the oxygen capacity of 100 ml of cells was calculated and this is included in Table l. This allows comparison of the carrying capacity of the red cells themselves. These cell capacities are similar in the four species examined, but by comparison with data tabulated by Redfield (1933) and Prosser & Brown (1961) it is seen that these capacities are lower than those for fish in lower latitudes. This is in agreement with Kooyman's (1963) data *for T. borchgrevinki, T. bernacchii* and T. *centronotus,* in which the hemoglobin concentrations were lower than in temperate or tropical fishes, but similar to Arctic and some other Antarctic forms. The percentage of oxygen dissolved in the plasma at air saturation is also shown in Table 1. Because of the high oxygen solubility at low temperatures, the transport of oxygen by solution in the plasma cannot be ignored, and this may partly explain the observed lower hemoglobin concentrations in fishes from high latitudes.

TABLE 1-HEMATOCRIT, OXYGEN CAPACITY (OF CELLS AND WHOLE BLOOD) AND PERCENTAGE OF OXYGEN DISSOLVED IN THE PLASMA FOR FOUR SPECIES OF *Trematomus* AT −1.5ºC

Species	Hematocrit	Volumes per cent oxygen capacity		Plasma per cent contribution to absolute oxygen capacity
		Blood	Cells	
T. bernacchii	20.5%(14) [17-261	5.3 (10) 4.5-6.3	22.7	12.1
T. centronotus	22.0%(7) [17-24]	5.2(7) 4.2-5.5	21.0	12.4
T. hansoni	31.0% (3) [29-331	7.7(3) 7.1-8.1	22.9	7.3
T. borchgrevinki	30.0% (13) [26-32]	6.6(11) 6.1-7.0	20.3	8.5

Mean figures are given with the number of determinations in parentheses and the total range of determinations in brackets.

Species differences in oxygen equilibrium curves at environmental temperature can be compared in Figs. 1 and 2. The points shown are the average of data from five separate determinations in each case except for T. *hansoni,* in which only two determinations were made because of insufficient specimens. The tabulation of P_{5o} below expresses the total range of these determinations for each species. Only in *T. borchgrevinki* was a sigmoidal oxygen equilibrium curve apparent, the other three having the rectangular hyperbola frequently seen in fishes. P_{5o} values (in mmHg) are as follows:

T. bernacchii   8-5 (range 7-0-11-1)

T. centronotus   7-8 (range 5-8-11-6)

T. hansoni   10-8 (range 10-4-11-3)

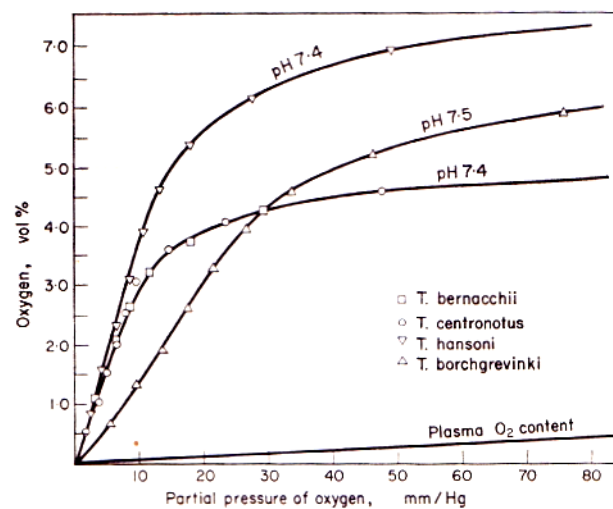*T. borchgrevinki*   21-5 (range 19-9-25-5)

Fig. 1. Oxygen equilibrium curves of whole blood from four species of *Trematomus* at 0.1 mm CO_2 tension and -1.5°C. Note the similarity of the loading and unloading abilities in the three benthic species.

Two methods of display have been used in Figs. 1 and 2 to illustrate more clearly certain differences and similarities among the pigments themselves and the blood as a whole in each species. Figure 1 permits comparison of the loading and unloading capabilities of the blood of the four species. The similarity of the three benthic fish in this respect is very striking. This similarity between *T. bernacchii* and *T. centronotus is* continued when their oxygen capacities are considered (Fig. 2). It is seen, however, that *T. hansoni* has a much higher oxygen capacity than the other benthic species. With respect to *T. borchgrevinki* there is little similarity in Zither case. It has a slightly sigmoidal curve, a lower oxygen affinity than the others, and an oxygen capacity in between that of *T. hansoni* and those of *T. bernacchii* and *T. centronotus.* These similarities and differences will be discussed later in elation to modes of life.
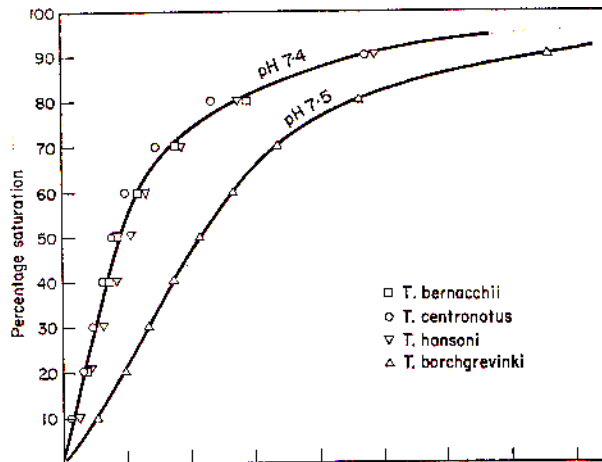
FIG. 2. Oxygen equilibrium curves of whole blood from four species of Trematomus at 0.1 mm CO_2 tension and $-1.5°C$, plotted to emphasize differences in oxygen capacity.

Effect of temperature

The magnitude of the effect of temperature on the oxygen-hemoglobin equilibrium was assessed in *T. bernacchii* and *T. borchgrevinki*. Curves were set up at $+4.5°C$ for comparison with those at $-1.5°C$ (Figs. 3 and 4). As in many other fishes it can be seen that the oxygen affinity of the blood is sensitive to temperature. Furthermore, the absolute oxygen capacity of the blood in *T. bernacchii* decreases considerably with temperature rise. At $+4.5°C$ it becomes only 70 per cent saturated even at atmospheric tensions. For a hematocrit of 20.5 per cent this means a reduction in oxygen capacity from 5.3 vol. per cent to 3.65 vol. per cent. This can be compared with the 15 per cent reduction in absolute oxygen capacity of blood of *Anguilla japonica* after a 25°C rise in temperature (Kawamoto, 1929), which is about one-eighth of the change per degree observed in *T. bernacchii*. This redaction in absolute oxygen capacity is not observed in *T. borchgrevinki* (Fig. 4) although the blood of this fish does suffer reduction in capacity up to at least 80 mm oxygen tension with the 6°C temperature increase. The magnitude of the effect of temperature on the oxygen equilibrium curve can be expressed as the change in P_{50} with temperature. This is plotted in Fig. 5 for comparison with similar data from other fishes. The sensitivity to temperature appears to be extreme in the two Antarctic forms.
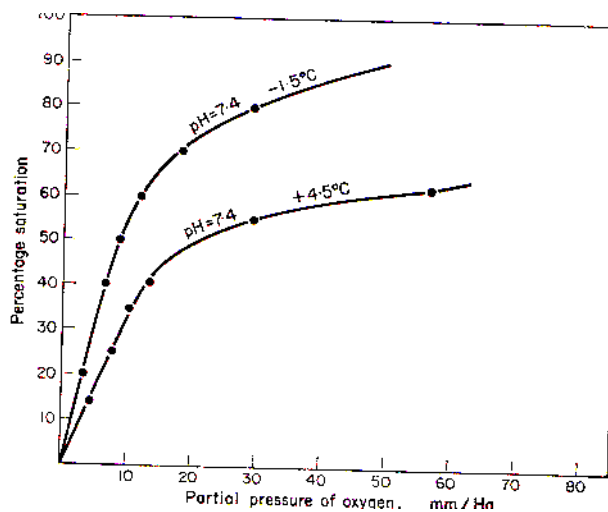

Fig. 3. *T. bernacchii.* Effect on the oxygen equilibrium curve (at 0.1 mm CO_2) of a 6°C rise in temperature. Each curve is the mean of five separate determinations, and the groups of data showed no overlap.
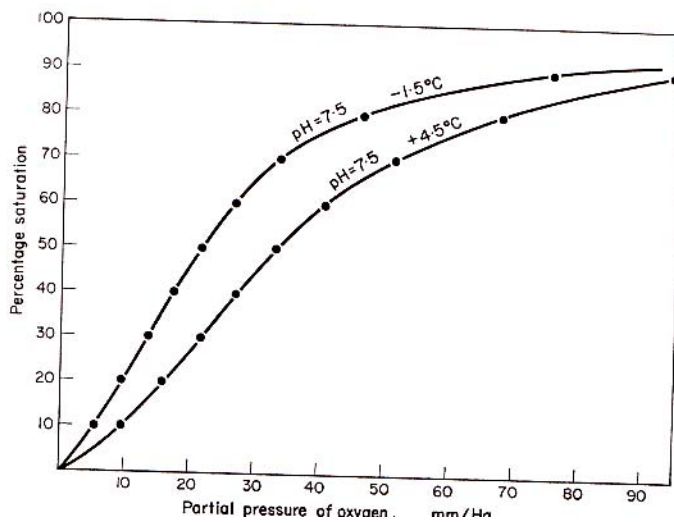
Fig. 4. *T. borchgrevinki.* Effect on the oxygen equilibrium curve (at 0.1 mm CO_2) of a 6°C rise in temperature. Each curve is the mean of five separate determinations, and the groups of data showed no overlap.
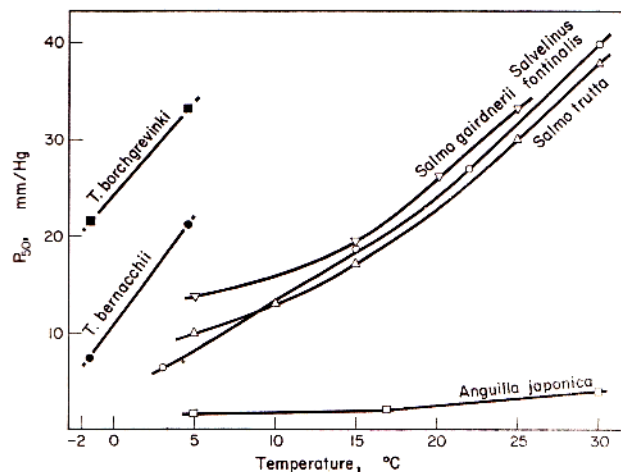

Fig. 5. *T. bernacchii* and *T. borchgrevinki.* Magnitude of change in P_{50} with temperature compared with that in trout (Irving et al., 1941*)* and *Anguilla japonica* (Kawamoto, 1929).

Effect of pH

    The effect of carbon dioxide on the affinity of the blood for oxygen was not Investigated. However, over a range of pH from 7.1 to 7.6, P_{50} was modified for *T. bernacchii* and *T. borchgrevinki* as shown in Fig. 6. The magnitude of the Bohr effe^{c}t has been found to vary considerably in different fishes, and it can be expressed as the change in P_{50} per unit change in pH, that is, $\Delta \log P_{50}/\Delta$ pH. Values for *T. bernacchii* and *T. borchgrevinki* are −0.70 and −0.35, respectively, indicating that pH has a moderate effect on the oxygen affinity compared with values tabulated by Prosser & Brown (1961).
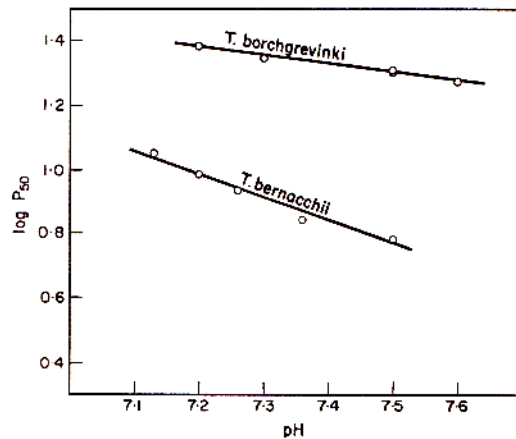
Fig. 6. T. *bernacchii* and T. *borchgrevinki*. Effect of pH on log P$_{50}$.

DISCUSSION

Krogh & Leitch (1919) were the first to appreciate the effect of temperature on the oxygen-hemoglobin equilibrium in fishes. Unaware of the diversity of hemoglobins with different properties, they thought that at low temperatures the high affinity of hemoglobin for oxygen would prevent unloading at the tissues, and that the Bohr effect was an adaptation to counteract this. In the Antarctic fishes, however, the positions of the oxygen equilibrium curves are in the same general range as in fishes from lower latitudes at their respective physiological temperatures. Oxygen transport mechanisms in the Antarctic fishes in this study are, however, very sensitive to temperature. This was particularly noticeable in *T. bernacchii,* where a 6°C temperature rise reduced the absolute oxygen capacity of the blood by 30 per cent. In T. *borchgrevinki* there was a large shift in P$_{50}$ with temperature and a large drop in oxygen capacity in the probable physiological range. These fishes are known to be stenothermal (Wohlschlag, 1964), and it seems likely that the sensitivity of their oxygen transport systems to temperature could provide at least some of the physiological explanation for this.

Because of the inaccessibility of the environment under the ice, knowledge of the behaviour of the fishes is limited and has been gained mainly by indirect methods. *T. borchgrevinki is* a pelagic fish and feeds on plankton. The other three species were caught at the bottom and are probably sluggish, a conclusion which is reinforced by considerations of their body form. It has been seen that the three benthic fishes have similar oxygen equilibrium curves, but T. *hansoni* has a much higher oxygen capacity. Redfield (1933) and Prosser & Brown (1961) draw attention to a general relationship between the oxygen capacity of the blood and the activity of the species, and it seems probable that *T. hansoni* is rather more active than the other two benthic species. In support of this, it was observed that *T. hansoni* was more active in aquaria than *T. bernacchii* or *T. centronotus.* The pelagic *T. borchgrevinki* also has a high oxygen capacity and it is probably an active fish ranging about within water masses rich in plankton. The three benthic fishes have hyperbolic equilibrium curves which are very similar (Fig. 1), so similar in fact that only one curve was drawn for the three. *T. borchgrevinki,* however, has a sigmoid curve and a much lower oxygen affinity. The affinity of the blood for oxygen determines its effectiveness not only in absorbing oxygen at the respiratory surface, but also in discharging it at the tissues. A fish like *T. borchgrevinki* (with a moderately low oxygen affinity, a sigmoid curve and a high oxygen capacity), living in a well-oxygenated environment, is probably able to unload more oxygen per unit blood flow and at a higher tension in the tissues than the benthic species. This would benefit an active fish. *T. hansoni,* on the other hand, probably unloads more oxygen than *T. bernacchii* and *T. centronotus,* but at an oxygen tension similar to theirs.

The four species of fishes investigated represent three levels of adaptation which appear to correlate with levels of activity in a fairly uniform environment. The Three benthic fishes have a high oxygen affinity, the most active one adapting to this higher activity with a greater oxygen capacity. The active pelagic *T. borchgrevinki* has a higher oxygen content than the two sluggish benthic fishes and it has the potential to unload this at a higher oxygen tension in the tissues.

Wohlschlag (1964) considered that the endemic Antarctic fishes (such as *Trematomus*) were "cold-adapted" and stenothermal compared with some fishes which lived in Antarctic waters but were not restricted to them (e.g. Zoarcidae). The cold-adapted fishes had higher metabolic rates at Antarctic temperatures the more widespread forms. The present observations on Antarctic fishes correlate well with these views. The cold-adapted fishes are seen to have oxygen transport systems well adapted to low temperatures only.

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