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# Oxygen peroxide enzymes in Antarctic fishes and some other vertebrates

## 1. Introduction

There are several reasons which make us decided to carry out certain experiments and to deal with oxygen metabolism enzymes in Antarctic vertebrates, and mainly fishes. Those are the following:

— low temperature of marine environment which may influence the enzyme activities,
— high degree of oxygen saturation in water environment, which may possibly stimulate peroxide metabolism enzymes,
— the occurence in Antarctica of fish species differing in erythrocyte number and hemoglobin content even up to their lack in white-blooded fishes, what is unusual and found only in Antarctica,
— as a result of low temperature of the environment the increased content of phospholipid polyunsaturated fatty acids within the cell membranes, especially of such stenotherms as fishes, consequence of which is the possibility of increased rate of lipid peroxidation in the presence of high oxygen saturation, and lastly,
— the presence of homoio- and poikilotherms in Antarctica make possible the comparison of the effect of environmental conditions.

## 2. Low temperature

The low temperature of Antarctic seas imposes on animals the necessity for certain adaptations, particularly in their metabolism and freezing resistance. According to the most recent studies glaciation of the Antarctic Continent began 20—30 mln years ago (Korotkevich 1972). The fauna of Antarctic waters is very old one and is considered that as a cold water fauna it corresponds in age to the deep water fauna (Zienkiewicz 1959). The permanency and the duration of environmental conditions in Antarctic waters lasted enough long time for the creation of the adaptative changes (see Rakusa-Suszczewski 1975, 1980, Arnaud 1977, for review). Therefore, Antarctic region is rich in endemic species, e.g. among fishes approx.

95% of species never cross so called Antarctic Convergence or Polar Front which is close to 50°S (Andrijašev 1967). Particularly high degree of endemism among fishes represent notothenioid families since as much as 87% of genera and 97% of species do not occur anywhere else except Antarctic waters. Such a high degree of endemism is believed to be a result of very stable conditions in this region and of its long lasting isolation. The physical characteristics of the environment are shown to be rather critical. At Mc Murdo Sound yearly mean temperature was estimated as −1.83°C at 100 m with an extreme range of 0.56°C and for the bottom waters close to 600 m as −1.89°C with an extreme range of only 0.07°C. The salinity of bottom waters averages 34.85‰ with the range of 0.24‰ and is comparable with the salinity of the other oceans (Knox 1970). Freezing conditions may last all year round in some regions of Antarctica. Survival of Antarctic marine fauna in an environment of permanent temperature close to −1.8°C or lower is achieved by high tolerance to supercooling and by maintaining an internal freezing point lower than that of sea water. The body fluids of Antarctic invertebrates are isoosmotic or slightly hyperosmotic so they achieve this on the basis of simple hyperosmotic regulation (Arnaud 1977). On the contrary the body fluids of teleosts are hypoosmotic so these fishes may freeze at temperature above the freezing point of the sea water (−1.9°C) (De Vries 1970). In marine fishes of temperate regions over 80% of the serum freezing point depression is due to sodium chloride. In Antarctic fishes the solutes responsible for the main part of the freezing point depression are not salts although these do have a higher concentration of sodium chloride as compared with fishes of temperate regions (Arnaud 1977). The presence of conjugated glycoproteins in the body fluids of Antarctic fishes is the way chosen by nature to lower their freezing point. Antifreeze glycoproteins are simple compounds being composed of three amino acid residue units i.e. two alanines and one threonine. N-acetylo-galactosamine and galactose are bound to each unit. The molecular weight ranges from 10,000 to 22,000 and depends on the number of units (Feeney 1974). The lowest value of serum freezing point, i.e. −2.07, was recorded for *Trematomus borchgrevinki* from Mc Murdo Sound. Antarctic fishes are strictly stenothermal and the limit of their resistance to higher temperatures can be only slightly modified. Acclimation to 2°C rises the freezing point of serum from −2.01°C to −1.68°C (De Vries 1970).

## 3. High oxygen saturation

The low temperature of Antarctic seas enhances the capability of water to absorb oxygen. This depends mainly on temperature and only slightly on the salinity. In the surface layer south of Antarctic Convergence

an oxygen content of more than 7.5 ml up to 9.0 ml per liter that is 80 to 100% saturation is generally found (Rakusa-Suszczewski 1972) and it decreases gradually until it reaches half of that value at the depth of 400 m (Ostapoff 1965, Chłapowski and Grelowski 1978). The observations in particular regions of Antarctica indicate even extensive supersaturation in the surface layers generally down to 25 m (Ostapoff 1965). The data quoted by Knox (1970) for the deep shelf benthic waters of the Mc Murdo Sound (depth over 500 m) show that even there the oxygen saturation amounts to about 70%. Whereas Andriashev (1977) has noticed that endemic notothenioid fishes of the Antarctic live in fact in well areated cold circumantarctic waters in contrast to the more widely distributed fish families occuring on the continental slope where they live in a bit warmer water with an oxygen content decreased to some 50%. Therefore one can say that even rather high oxygen needs can be satisfied in the Antarctic marine environment. In contrast the oxygenation of temperate freshwater reservoirs is extremly variable within wide range depending on the actual temperature, water transparency and the presence of plants.

## 4. Erythrocytes and hemoglobin

In such conditions of water environment as described above almost 160 fish species belonging to about 40 families live in Antarctic seas (Andriashev 1970). Among them 97 species are of benthic type (Jakubowski et al. 1969) and superfamily *Notothenioidea* predominates. Superfamily consists of four families: *Nototheniidae, Harpagiferidae, Bathydraconidae* and *Channichthyidae*. It is a well known fact that among Antarctic fishes there exists a general trend to reduce the number of erythrocytes and hemoglobin content in the blood. The average erythrocyte number for teleost fishes of temperate regions ranges from 1 to 2 mln, (max. 3—4 mln) for such fishes like mackerel, carp or tuna. The amount of hemoglobin ranges from 6 to 12 g per 100 ml, max. 18 g/100 ml in tuna (Everson and Ralph 1968, Jakubowski 1971). Among Antarctic fishes the representatives of the family *Nototheniidae* possess the highest number of erythrocytes ranging from 0.4 to 0.8 mln per $mm^3$ and also the highest content of hemoglobin amounting to 5—6 g per 100 ml (Everson and Ralph 1968). The representatives of the family *Harpagiferidae* were never examined in this respect so far. Among species belonging to the family *Bathydraconidae* in *Parachaenichthys charcoti*, the level of hemoglobin and the number of erythrocytes were found similar to that in *Nototheniidae*; however the other bathydraconid *Parachaenichthys georgianus*, was notable in having

very low hemoglobin content and low erythrocyte count in which it was closer to channichthyids (Everson and Ralph 1968). The lowest rate of hemoglobin production is observed in *Channichthyidae*, white-blooded family characteristic for Antarctic region (Hureau 1966). Some erythrocytes have been observed in their blood however they do not represent more than 30% of solid elements of the blood (in other teleosts approx. 99%). They are more or less modified, fragile, colorless and apparently devoid of hemoglobin (Hureau 1966). There have been many speculations concerning the correlative compensations which may offset the absence of oxygen-carrying compound in the blood of channichthyids. Until now some possible compensations are well documented. As a result of the lack of erythrocytes and hemoglobin the blood oxygen capacity is very low amounting to only 0.7% of the blood volume as compared with that of sea water. The average oxygen capacity of other Antarctic fishes constitutes 6—8% of the blood volume and in the case of fishes of temperate region it constitutes 5—17% of blood volume (Jakubowski 1971). Anyway those fishes are able to maintain a generally low metabolic rate probably as a result of low constant temperature (Hemmingsen and Douglas 1970). They do not use much energy in the process of preying on other fishes and krill. In general, channichthyids represent a sit and wait type of predators. Direct estimations of respiratory metabolism showed its very low level ranging from 17 to 28 ml $O_2$ per kg per h (Hemmingsen and Douglas 1972). In other fishes of this region, e.g. in nototheniids, the value of respiratory metabolism ranges from 30 to 60 ml $O_2$ per kg per h and for fishes of temperate regions ranges from 100 to 300 ml $O_2$ per kg per h (Jakubowski 1971). Partial oxygen pressure of the blood of white-blooded fishes is slightly higher than that of red-blooded ones ranging from 80 to 90 mmHg (Hemmingsen and Douglas 1970). It was found also that the blood of *Channichthyidea* can constitute up to 9% of the body weight against about 2.5% in other Antarctic fishes (Hemmingsen and Douglas 1972, Twelves 1972). An increase of cardiac output as a result of bigger heart being 3 to 5 times as large as in other fishes and beating at similar frequency is belived to be another compensation (Holeton 1970). Next may be an increased blood flow through the gills and the tissue due to very low viscosity amounting to 3 centistokes at 0 C (Holeton 1970). Surely the low viscosity is a result of the lack of erythrocytes and low concentration of proteins i.e. only 10—20% of that obtained for red-blooded fishes (Suzuki 1980). And the last considered compensation may be an increased vascularization of the skin and fins with an increased cutaneous respiration (Jakubowski et al. 1969). All above supports the earlier hypothesis that oxygen is transported by the blood of the white-blooded fishes as a physical solution in plasma.

## 5. Unsaturated fatty acids

The last problem to consider is a relatively high content of fat in fish tissues, especially of polyunsaturated fatty acids what is a well known fact (Eschmeyer and Philips 1965, Malins et al. 1965, Love 1970, Roche et al. 1983). Polyunsaturated fatty acids are particularly abundant in biological membranes and their oxidation constitutes an obvious threat to the integrity of these structures. It was reported recently that relative rate of peroxidation was increasing with increasing number of double bonds (Dirks et al. 1982). In general a decrease in temperature results in an increase of the unsaturation degree of fatty acids (Hazel and Sellner 1980) (Table I). As far as fishes are concerned a few experiments with

Table I

Ratio of saturated to unsaturated fatty acids of phosphoglicerides isolated from synaptosomal membranes of different fish species (after Cossins and Prosser 1978)

| Phosphogliceride | Arctic[1] sculpin | Gold fish[2] | | Desert[3] pupfish |
|---|---|---|---|---|
| | 0°C | 5°C | 25°C | 34°C |
| Choline | 0.593 | 0.659 | 0.817 | 0.990 |
| Ethanolamine | 0.260 | 0.340 | 0.506 | 0.568 |
| Serine/inositol | 0.477 | 0.459 | 0.633 | 0.616 |

[1] *Phobetor tricuspis*
[2] *Carassius carassius*
[3] *Cyprinodon macularius*

animals kept at different temperatures indicate that there is a tendency in certain species to increase polyunsaturated fatty acids of 22 carbons, mainly $C_{22:6}$ and to decrease palmitic and stearic acids at lower temperature. Although there are no available data on fatty acid composition in Antarctic fishes an attempt to compare fatty acid composition of animals living in cold and warm environment has been made (Cossins and Prosser 1978, Sellner and Hazel 1982). Arctic sculpin (*Phobetor tricuspis*) and desert pupfish, (*Cyprinodon macularius*, a hot-spring fish) were examined in respect to phosphoglyceride fatty acid content in synaptosomal membranes (Table II). For comparison the ratios of saturated to unsaturated fatty acids in several fishes living in or acclimated to different temperatures are presented in Table I.

## 6. Peroxide metabolism enzymes

The enzymes we dealt with constitute the part of cellular defense system against active forms of oxygen which are generated in aerobic

Table II

Comparison of fatty acid composition of choline phosphoglicerides isolated from synaptosomal membranes of the Arctic sculpin and desert pupfish (after Cossins and Prosser 1978)

| Fatty acid | Choline phosphoglicerides | |
|---|---|---|
| | Arctic sculpin[2] | Desert pupfish[3] |
| 16:0 | **31.08**[1] | **33.14** |
| 16:1 | 6.37 | 5.61 |
| 18:0 | **4.09** | **12.26** |
| 18:1 | 19.77 | 27.36 |
| 18:2 | 0.93 | 11.76 |
| 18:3 | 0.34 | 0.64 |
| 20:4 | 1.32 | 1.63 |
| 20:5 | 4.57 | 0.29 |
| 22:5 | 0.28 | 0.90 |
| 22:6 | 25.78 | 6.97 |
| Others | 5.19 | 9.29 |
| Saturated | 36.85 | 49.29 |
| Monounsaturated | 26.69 | 34.47 |
| Polyunsaturated | 35.41 | 15.31 |
| Unidentified | 0.77 | 0.78 |

[1] weight %,
[2] *Phobetor tricuspis.*
[3] *Cyprinodon macularius*

organisms during the chain of molecular oxygen reductions. Those enzymes which defend mainly against lipid peroxidation present in every cell of the organism are as follows: superoxide dismutase catalyzing the scavenging of superoxide radicals and catalase and peroxidase eliminating hydrogen peroxide from the surrouding environment. Superoxide dismutase and catalase were estimated in the livers of different vertebrates inhabiting Antarctica. The livers for the study were collected in the region of King George Island, South Shetlands, during two Polish Expeditions in 1980 and 1981 to Polish Antarctic "Arctowski" Station. The following Antarctic animal species were used in this study:

mammals — *Lobodon carcinophagus*
— *Hydrurga leptonyx*
birds — *Pygoscelis adeliae*
— *Pygoscelis papua*
— *Pygoscelis antarctica*
red-blooded fishes — *Notothenia gibberifrons*
— *Notothenia coriiceps neglecta*
— *Notothenia nybelini*
white-blooded fishes — *Chaenocephalus aceratus*
— *Pseudochaenichthys georgianus*

The experiments were carried out on the livers which were frozen just after sampling, and stored at −20°C during transport, and at −70°C before every other series of experiments. The activity of superoxide dismutase was measured by the adrenaline method of Misra and Fridovich (1972). Catalase activity was measured by the method of Beers and Sizer (1952). The results obtained demonstrate certain variations of superoxide dismutase activity in Antarctic vertebrates (Table III). Statistically significant differences

Table III

Superoxide dismutase activity in livers of different Antarctic vertebrates and some freshwater fish species (after Witas et al. 1984)

| Animal | n | $\bar{x}$ | ±SD |
|---|---|---|---|
| **Mammals** | | | |
| *Lobodon carcinophagus* | 13 | 2542.5 | 212.3 |
| *Hydrurga leptonyx* | 13 | 2434.9 | 147.3 |
| **Birds** | | | |
| *Pygoscelis adeliae* | 13 | 1714.5 | 205.9 |
| *Pygoscelis papua* | 12 | 1694.1 | 166.6 |
| *Pygoscelis antarctica* | 12 | 1628.9 | 118.8 |
| **Red-blooded fishes** | | | |
| *Notothenia gibberifrons* | 10 | 1600.4 | 69.2 |
| *Notothenia coriiceps neglecta* | 11 | 1707.5 | 118.7 |
| *Notothenia nybelini* | 4 | 1671.3 | 101.4 |
| **White-blooded fishes** | | | |
| *Chaenocephalus aceratus* | 14 | 263.1 | 28.8 |
| *Pseudochaenichthys georgianus* | 14 | 274.6 | 33.1 |
| **Freshwater fishes** | | | |
| *Cyprinus carpio* | 5 | 694.1 | 46.0 |
| *Tinca tinca* | 5 | 679.3 | 59.2 |
| *Carassius carassius* | 5 | 669.1 | 88.0 |

Enzyme activity expressed in units per gram of wet tissue

in enzyme activity can be seen between mammals as one group — the highest value, birds and red-blooded fishes as a second group and white-blooded fish as a third group with the lowest enzyme activity i.e. 7-fold lower comparing with red-blooded. For comparison, superoxide dismutase activities in livers of freshwater fishes such as carp, tench and crucian carp are shown also in the table III. The value of the enzyme activity is in between the values obtained for Antarctic red-blooded and white-blooded fishes. Next table (Table IV) presents catalase activities which were similar or lower in livers of mammals comparing with birds whereas several times lower values were obtained for red-blooded and white-blooded fishes. Enzyme activities in freshwater fish livers were similar in all species examined and were approximately doubled in value as compared with red- and white-blooded Antarctic fishes. Results obtained for Antarctic mammals and birds are of the same order of magnitude and in pretty good

Table IV

Catalase activity in livers of different Antarctic vertebrates and some freshwater fish species (after Witas et al. 1984)

| Animal | n | $\bar{x}$ | ±SD |
|---|---|---|---|
| **Mammals** | | | |
| *Lobodon carcinophagus* | 16 | 4.17 | 0.30 |
| *Hydrurga leptonyx* | 16 | 7.08 | 1.11 |
| **Birds** | | | |
| *Pygoscelis adeliae* | 16 | 7.49 | 0.72 |
| *Pygoscelis papua* | 14 | 6.28 | 0.49 |
| *Pygoscelis antarctica* | 15 | 7.45 | 0.53 |
| **Red-blooded fishes** | | | |
| *Notothenia gibberifrons* | 13 | 0.71 | 0.10 |
| *Notothenia coriiceps neglecta* | 14 | 0.65 | 0.07 |
| *Notothenia nybelini* | 4 | 1.22 | 0.43 |
| **White-blooded fishes** | | | |
| *Chaenocephalus aceratus* | 15 | 0.72 | 0.10 |
| *Pseudochaenichthys georgianus* | 14 | 0.76 | 0.04 |
| **Freshwater fishes** | | | |
| *Cyprinus carpio* | 5 | 1.73 | 0.19 |
| *Tinca tinca* | 5 | 1.36 | 0.28 |
| *Carassius carassius* | 5 | 1.38 | 0.25 |

Enzyme activity expressed in units per gram of wet tissue

agreement with the results obtained for temperate region homoiotherm livers (Matkovics et al. 1977). Lower activity of catalase seems to be understandable as compensated by higher activity of superoxide dismutase in Antarctic red-blooded fishes. The simplest conclusion drawn out on the basis of the obtained results is that superoxide dismutase may be the main enzyme of fish liver responsible for protection of the cells against uncontrolled oxidation processes. But there are some other problems that seems to be at least disputable. Above discussed results are presented in table V. Those are: erythrocyte count, hemoglobin content, respiratory metabolism, blood oxygen-carrying capacity, supposed ratio of saturated to unsaturated fatty acids, superoxide dismutase and catalase. Along first four horizontal lines all values are decreasing in the direction from temperate freshwater fishes through marine cold-water red-blooded to white-blooded ones. Fifth line i.e. the ratio of saturated to unsaturated fatty acids give rather supposed values which do not represent tested species but they represent fishes from the environment of different temperature. Next two lines give superoxide dismutase and catalase activities. Comparing vertically all those data one can confront red-blooded fishes from different temperatures of environment accompained by different oxygen saturation — two left lines. Looking for the explanation of higher superoxide dismutase activity in marine Antarctic than in freshwater red-blooded fishes one should take into

account at least three possible agents which may be responsible for such relatively high enzyme activity. Those re: mentioned above lower catalase activity, supposed higher content of polyunsaturated fatty acids in membrane phospholipids and direct induction of the enzyme by molecular oxygen as it was found in bacterial systems. The mechanism is not clear but there was found also an increase of up to 50% in the concentration of cytochrom C and cytochrom oxidase during cold acclimation. On the other hand

Table V

Comparison of some physiological and biochemical parameters obtained for temperate freshwater and marine cold water red- and white-blooded fishes

| | Freshwater fishes | Red-blooded fishes | White-blooded fishes |
|---|---|---|---|
| | +4/+25°C | −2/+2°C | |
| Erythrocyte number per $mm^3$ | 1—2 mln | 0.4—0.8 mln | trace |
| Hemoglobin content g per 100 ml | 6—12 | 5—6 | trace |
| Blood oxygen capacity ml $O_2$ per 100 ml | 5—17 | 6—8 | 0.7 |
| Respiratory metabolism ml $O_2$ per kg per h | 100—300 | 30—60 | 17—28 |
| Saturated FFA / Unsaturated FFA | 0.7—0.8 | 0.5* | ? |
| Superoxide dismutase U per g wet tissue | 600—700 | 1600—1800 | 250—300 |
| Catalase U per g wet tissue | 1.4—1.7 | 0.6—0.7 | 0.7 |

* Value obtained for Arctic aculpin living in 0°C

there are no available data on freshwater fish superoxide dismutase content during wintertime. Looking at the table one can compare fishes from the same environment namely the same temperature and oxygen saturation but differing in respect to hemoglobin content — right side of the table. Very low value of oxygen capacity and the low level of respiratory metabolism in white-blooded fishes on one hand and on the other the low activity of superoxide dismutase suggest some correlation comparing with red-blooded ones in the same respects. Most of authors dealing with white-blooded fishes suggest that white-bloodness or hereditary anemia may have appeared during evolution processes due to the high oxygenation of the environment (Jakubowski 1971). But there are some authors (Arnaud 1977) who consider above hypothesis as doubtful because some channichthyid species live however in waters poor in oxygen. In any case the energetic costs of white-bloodness is much higher than usual among fishes. White-

-blooded fishes utilize approximately 30% of total energy for cardiac work that is five times more than others (Hemmingsen et al. 1972). On the basis of obtained results one can consider the following hypothesis: the lack of hemoglobin or hemoglobin-like oxygen transporting system results in decreased threat to the organism arising due to the active oxygen forms, mainly oxygen free radicals. This may be reflected in low activity of oxygen peroxide enzymes (Witas et al. 1984). The above hypothesis can be considered as an attempt in explaining the advantages of white-bloodness which are not clearly understood until now. Nevertheless it can be considered also the hypothesis that hemoglobin is involved in the regulation of superoxide dismutase level in the tissues.

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