

Preliminary evalution of δ -aminolevulinic acid
dehydratase in blood of lesser spotted dogfish
(*Scyliorhinus canicula* L.) from the
middle Adriatic

Preliminarni rezultati aktivnosti dehidrateze δ -aminolevulinske
kiseline u krvi mačke bljedice (*Scyliorhinus canicula* L.)
iz srednjeg Jadrana

Mladen Tudor

Institute of Oceanography and Fisheries, Split

INTRODUCTION

The activity of δ -aminolevulinic acid dehydratase (ALA-D) catalyzes the formation of one molecule of porphobilinogen from two molecules of δ -aminolevulinic acid.

ALA-D contains highly active tiol groups which easily bind heavy metals and inhibit enzyme activity (Shemin, 1976). It is well known that the activity of ALA-D in blood of humans is particularly depressed by the presence of lead in the blood (Bonsignore et al., 1956; Kuhnert et al., 1977). ALA-D activity is also depressed in the erythrocytes of fish under the influence of lead (Jackim, 1973; Hodson, 1976; Hodson et al., 1977). It was also shown that lead inhibited ALA-D in the erythrocytes of lesser spotted dogfish (*Scyliorhinus canicula* L.) (Beritić, et al., 1980, Tudor, 1980).

This paper is a preliminary evaluation of ALA-D activity in blood of *Scyliorhinus canicula* L. from the middle Adriatic. It may be an useful parameter for the biological monitoring of pollution by lead.

MATERIALS AND METHODS

Lesser spotted dogfish specimens were trawled from the Split channel (43°26'N; 16°13'E). Alive fish were acclimated to aquarium condition for four weeks.

Blood of fish was collected by cardinal puncture and Na-heparin used as anticoagulants (Hattingh, 1975).

ALA-D activity in blood of *S. canicula* was determined after Berlin and Schaller (1974) at 37°C. The amount of porphobilinogen produced in the incubation of enzymes was measured at 555 nm using Pay Unicam SP 600 spectrophotometer. Hematocrit was determined by microhematocrit Janetzki TH 11 centrifuge.

In ten repeated analyses the coefficient of variation of the method was 2.28%.

ALA-D activity is expressed as μmol porphobilinogen/minute/1 erythrocytes.

RESULTS

Effects of temperature and time of incubation on ALA-D activity was examined at 25 and 37°C. Enzyme activity was constant over 180 minutes (Fig. 1). However, ALA-D activity at 37° was about twice that at 25°C. ALA-D activity and hematocrit were examined at 10 males and 10 females of *Scyliorhinus canicula*.

Ranges, means, standard deviation, standard error and variability coefficient of ALA-D activity, as well as hematocrit and weight of tested fish are given in Table 1.

Table 1. ALA-D activity ($\mu\text{molPBG}/\text{min}/1$ erythrocytes), hematocrit (%) and weight of *Scyliorhinus canicula* L. from the middle Adriatic

	ALA-D (U/l)		Hem. (%)		Weight (g)	
	♂ 10	♀ 10	♂ 10	♀ 10	♂ 10	♀ 10
Range	17,4—57,8	11,1—41,9	12,9—25,7	15,6—25,9	170—265	166—305
$\bar{x}$	33.7	27.6	20.6	22.2	214	210
s	12.7	10.8	3.8	3.7	36	39
Sx	4.0	3.4	1.2	1.2	11	12
CV %	37.8	39.3	18.4	16.4	17	18

Event hough mean ALA-D activity in *Scyliorhinus canicula* females was somewhat lower than that in males the difference between them was not statistically significant ($t = 1.15$; $P > 0.05$). No statistically significant difference was found in mean hematocrit value ($t = 0.96$; $P > 0.05$) between examined males and females, either.

Regression relationship between ALA-D activity and fish weight log are given in Fig. 2. Negative correlation $r = -0.658$ between these two parameters was statistically significant at $P < 0.01$.

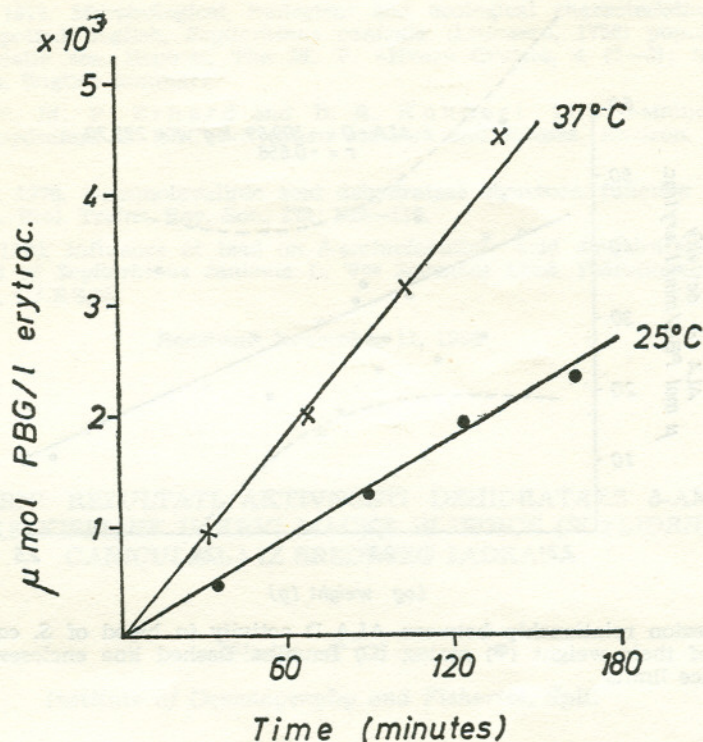


Fig. 1. Concentration of produced porphobilinogen as affected by the incubation time of enzyme and substrate at 25°C and 37°C temperature

Weight of examined specimens and their hematocrit were not interdependent (males $r = 0.07$; females $r = -0.13$).

As shown by our results ALA-D activity of tested *S. canicula* specimens is likely to be dependent on the growth of fish, that is their age. After Jardas (1979) age composition of examined male and female *S. canicula* shows mainly specimens of the third year of age.

It has already been suggested (Tudor, 1980) that relative (%) ALA-D activity of individual *S. canicula* specimens and log of intraperitoneal lead level (ppm) are negatively correlated with the regression coefficient 29.8. Hodson et al. (1982) found that the inhibition of *Salmo gairdneri* due to the lead added to the water was independent of fish weight.

Owing to the very pronounced variability of ALA-D activity the biological monitoring of *S. canicula* should take care of taking the fish belonging to the same age groups.

CONCLUSION

Negatively correlated ALA-D activity of *S. canicula* blood and log of fish weight are indicative of the fact that enzyme activity is likely to be dependent on fish age.

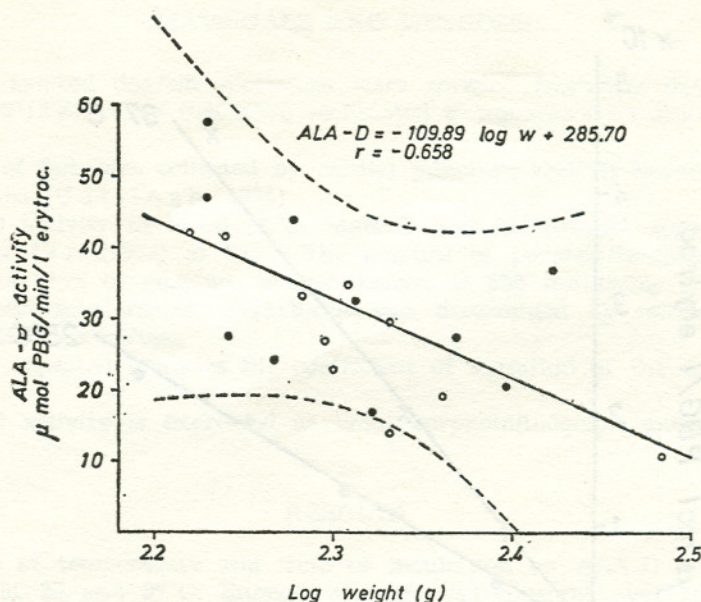


Fig. 2. Regression relationship between ALA-D activity in blood of *S. canicula* and log of their weight (●) males, (○) females. Dashed line encloses 95% confidence limits

REFERENCES

- Beritić, T., J. Zibar-Šikić, D. Prpić-Majić and M. Tudor 1980. Some morphological and biochemical hematological parameters of abnormal lead absorption in fish. — Lead in marine environment, 263—270. Branica M. and Z. Konrad (eds.) Pergamon, Oxford (UK).
- Berlin, A. and K. H. Schaler. 1974. European standardized method for the determination of δ -aminolevulinic acid dehydratase activity in blood. Z. Klin. Chem. Klin. Biochem., 12: 389—390.
- Bonsignore, D., P. Callissano e C. Cartasegna, 1965. Un semplice metodo per la determinazione della δ -amino-levulinico deidratasi nel sangue. Med. Lavoro, 56: 199—205.
- Hattingh, J. 1975. Heparin and ethylenediamine tetra-acetate as anticoagulants for fish blood. Pflügers. Arch. 355: 317—352.
- Hodson, P. V. 1976. δ -aminolevulinic acid dehydratase activity of fish blood as an indicator of harmful exposure to lead. J. Fish. Res. Board Can., 33: 268—271.
- Hodson, P. V., B. R. Blunt, D. J. Spry and K. Austen. 1977. Evaluation of erythrocyte δ -aminolevulinic acid dehydratase activity as a short-term indicator in fish of a harmful exposure to lead. J. Fish. Res. Board Can., 34: 501—508.
- Hodson, P. V., D. G. Dixon, D. J. Spry, D. M. Whittle and J. B. Sprague. 1982. Effect of growth rate and size of fish on rate of intoxication by waterborne lead. Can. J. Fish. Aquat. Sci., 39: 1243—1251.
- Jackim, E. 1973. Influence of lead and other metals on fish δ -aminolevulinic acid dehydratase activity. J. Fish. Res. Board Can., 30: 560—562.

- Jardas, I. 1979. Morphological, biological and ecological characteristics of the lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758) population in the Adriatic sea. Reports, The M. V. »Hvar« Cruises, 4 (2—3): 104 p. In Croatian, English summary.
- Kuhnert, P. M., P. Erhard and B. R. Kuhnert. 1977. δ -aminolevulinic acid dehydratase in RBC's of urban mothers and fetuses. Environ. Res., 14: 73—80.
- Shemin, D. 1976. 5-aminolevulinic acid dehydratase structure, function and mechanism. Phil. Trans. Roy. Soc., 273: 109—115.
- Tudor, M. 1980. Influence of lead on δ -aminolevulinic acid dehydratase activity in blood of *Scyliorhinus canicula* L. Ves. Journées Étud. Pollutions, 757—760. Cagliari, C.I.E.S.M.

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PRELIMINARNI REZULTATI AKTIVNOSTI DEHIDRATAZE δ -AMINOLEVULINSKE KISELINE U KRVI MAČKE BLJEDICE (*SCYLIORHINUS CANICULA* L.) IZ SREDNJEG JADRANA

Mladen Tudor

Institute of Oceanography and Fisheries, Split

KRATKI SADRŽAJ

Aktivnost dehidrataze δ -aminolevulinske kiseline (D-DALK) u krvi mačke bljedice u negativnoj je korelaciji sa težinom riba. Prema ovim rezultatima aktivnost D-DALK vjerovatno je povezana sa starošću ispitanih primjeraka *Scyliorhinus canicula*.