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Serum Enzymes in Rainbow Trout as Tools in the Diagnosis of Water Quality

W. Wieser and S. Hinterleitner

Institut für Zoophysiologie, Universität Innsbruck, and Institut für Fischforschung, 6020 Innsbruck, Austria

The activities of serum enzymes are likely to reflect the metabolic state of fish in much the same way as they do in mammals (BOUCK et al. 1975). Thus it can be expected that toxic agents or factors which lead to chronic impairment of the animals' metabolism will cause changes, usually increases, of the activities of some serum enzymes. Many studies have been conducted in which fish were experimentally stressed and the ensuing changes in serum enzyme activities recorded (for salmonids see BELL 1968, McKIM et al. 1970, LOCKHART et al. 1973, RACICOT et al. 1975, SAUER and HAIDER 1977, 1979).

Some of these responses are likely to be of a more general nature, i.e. indicating the organism's answer to a situation of stress brought about by a general deterioration of water quality (MORGAN et al. 1973, OIKARI and SOIVIO 1977).

We have tested this idea further by measuring the activities of two transaminases, glutamic oxalacetate transaminase (GOT, E.C.2.6.1.1.) and glutamic pyruvate transaminase (GPT, E.C.2.6.1.8.) in the serum (S) of rainbow trout (Salmo gairdneri) caught at various distances from the outflow of a sewage plant into the river Inn near Innsbruck. The quality of the river water was also tested by measuring, at the points where the fish were caught, the concentration of NH_3, NO_3 and NO_2.

MATERIAL AND METHODS

Animals: Rainbow trout were caught by hook and line at various points downstream or upstream from where the sewage plant of the city of Hall in Tirol empties into the Inn. The fish were taken from the hook and put into large vessels with aerated river water where they quickly recovered. When 2 - 3 specimens had been collected they were taken to the laboratory, immediately killed and their blood sampled. From 1 - 3 hours

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elapsed between catching and blood sampling. No significant difference in SGOT and SGPT activity could be detected between samples of blood collected from fish right after catching and samples taken three hours later in the laboratory from fish caught at the same locality. These two enzymes do not appear to respond to the "angling stress" defined for cAMP, lactate and glucose by PERRIER et al. (1978). The weight of the fish also seemed to have no, or only negligible, effect on the enzyme activities.

Blood sampling: Approximately 20 µl of a 1% solution of EDTA were drawn into a 1 ml all-glass syringe. The heart of the fish was laid bare, the ventricle punctured with the needle of the syringe and the EDTA solution injected slowly into the blood stream. After a few seconds a sample of blood was withdrawn, transferred carefully into a conical glass centrifugation tube, and centrifuged at 4000 x g for 20 minutes. Strongly haemolyzed samples were discarded. However, slight haemolysis nearly always occurred. In these cases a correction factor was applied in the following way: SGOT and SGPT activities were determined in washed and lysed samples of the erythrocytes sedimented after the centrifugation step, and expressed in units per mg haemoglobin (Hb). The concentration of Hb in the precipitate was determined after haemolysis by the cyanmethaemoglobin method. By measuring the Hb content of a haemolyzed serum sample the enzyme activity due to lysis of erythrocytes could be determined and was subtracted from the total activity of the serum sample.

Measurement of enzyme activities: SGOT and SGPT activities were measured in the usual way by coupling the reaction to the oxidation of $NADH_2$ (BERGMEYER 1974). The change in extinction was followed at 340 nm in a spectrophotometer equipped with a thermostated cuvette. The optimum composition of the assay systems was the following:

$NADH_2$ 0.18 mM; aspartate or alanine 100 mM; α-ketoglutarate 6 mM; MDH 10 U/ml; LDH 4 U/ml; phosphate buffer pH 7.5; experimental temperature 20°C.

Ammonia, nitrate and nitrite: Water samples were taken at the localities where fish were caught. NH_3 and the sum of NO_3 and NO_2 were determined by means of a Cenco autoanalyzer; ammonia by an indophend-blue method; nitrate and nitrite after cadmium reduction by the Griess-Ilosvay method (see ALLEN et al. 1974)

TABLE 1

The activities of GOT and GPT in the serum of rainbow trouts from the Inn, caught at various distances (m) from the sewage plant inflow of the city of Hall in Tirol (- = upstream; + = downstream). Total ammonia and NO_x concentrations at the angling sites are also given. Collecting period: 12.13.1978-06.15.1979.

fish no.	weight (g)	SGOT (mU/ml)	SGPT (mU/ml)	Σ NH_3 (µg/l)	NO_3+NO_2 (µg/l)	distance from inflow (m)
1	300	100	12.5			
2	690	147	12.8	60	910	
3	477	204	13.4			-300
4	163	330	-	60	988	
5	196	195	-			
6	224	200	-			
7	446	524	30.9	68	1112	- 20
8	249	564	53.7	7260	2255	- 10
9	325	1290	80.6	11000	2064	0
10	964	1532	107.5	10560	1870	+ 29
11	320	866	-	10780	1958	+ 58
12	245	887	-	10560	2035	+ 87
13	520	1048	53.7			
14	119	913	67.2	11110	2200	+116
15	710	443	20.8	643	930	+249
16	936	255	32.2	490	890	+379
17	370	248	-	450	900	+404
18	614	188	20.2			
19	2700	125	18.7	475	870	+424
20	561	160	-	450	905	+444

RESULTS

The data summarized in Table 1 show clearly that SGOT and SGPT activities are a function of distance from the sewage plant inflow and are correlated with the nitrogen content of the river water. The specimens caught within the plume of the sewage inflow had very high values of SGOT activity, but their behaviour as well as the appearance of liver and gills did not differ from those of the fish caught further upstream in less polluted water.

During the period of study pH values varied between 7.5 and 8.4, and water temperature ranged from 3.7 to 10 °C.

DISCUSSION

We know little about the range of serum enzyme activities in natural populations of Salmo gairdneri, but from the literature it can be inferred that "normal" values of SGOT activity lie between 150 and 250 mU/ml (SAUER and HAIDER 1977, 1979). This is the range found in trouts collected 300 m upstream and 400 - 500 m downstream of the sewage inflow which suggests that whatever detrimental factors are added to the Inn by the sewage plant they are sufficiently diluted within a short distance along the length of the river. Total NH_3 concentrations at the two most distant collecting sites upstream and downstream respectively differed by a factor of nearly ten. However, this difference is not reflected in the SGOT activities of the fish caught at these two sites. The large ammonia levels measured around the inflow of the sewage plant prove that the latter has little capacity of removing dissolved organic material from the water. EIFAC (1970) suggested that a concentration of 0.025 mg unionized NH_3 . l^{-1} may be the maximum which can be tolerated by fish (rainbow trout) for a long period. Within the ranges of pH and temperature measured in the river this amount of unionized ammonia is contained in the following concentrations of total (NH_3 + NH_4^+) ammonia (in mg . l^{-1}):

temperature °C	pH value 7.5	8.0	8.5
5	6.3	2.0	0.65
10	4.3	1.37	0.45

Thus according to pH and temperature conditions a concentration of 12 mg . l^{-1}, measured around the sewage plant inflow, exceeds the maximum "safe" threshold approximately 2 to 20 times. Still, adult rainbow trouts were regularly caught within the zone of maximum ammonia concentration along the shore of the river (Table 1 and additional information by Mr.Quintern). These fish have SGOT and SGPT levels comparable to those found in fish experimentally stressed with high doses of toxic agents. For example, BELL (1968) poisoned Pacific salmon with a lethal dose of 450 mg . kg^{-1} bromobenzene which caused GOT activity to increase within hours from 250 to 900 - 1600 mU . l^{-1}.

The fact that rainbow trouts with elevated SGOT activities occur with some regularity in what by all accounts appears to be extremely polluted water suggests the following:

1) Contrary to experimental situations rainbow trouts in large natural bodies of water can tolerate very high local concentrations of pollutants, either by moving in and out of the zone of maximum toxicity or by adaptation (SCHULZE-WIEHENBRAUCK 1976).

2) The high concentration of serum enzymes in the specimens caught near the sewage inflow indicate that these fish were not just occasional visitors but must have spent considerable time in the polluted area.

3) The fish appeared healthy enough, thus in rainbow trout strongly elevated SGOT and SGPT levels may indicate changes of metabolism, mainly in the liver, but need not necessarily signal the sublethal condition suggested by the short-term experiments of BELL (1968), THURSTON and RUSSO (1978) and others.

In conclusion it can be stated that the levels of SGOT and SGPT activity in S.gairdneri appear to be good indicators of certain types of organic water pollution, at least within a range characterized by total ammonia concentrations from approximately 0.5 to 12 mg . l^{-1}.

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