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SUBCELLULAR LOCALIZATION OF TRANSAMINASE ENZYMES IN FISHES AND THEIR SIGNIFICANCE IN THE DETECTION OF WATER POLLUTION

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Summary

On the basis of our cytochemical experiments, it can be established that the method described for demonstration of transaminase in mammalian species is also applicable in the carp liver without modification.

The distribution of transaminase activity in different organs of carp (expressed as U/l) was the following: liver GOT and GPT 149 ± 55 and $311{,}2 \pm 6{,}8$ heart: $58{,}4 \pm 13$ and $4{,}4 \pm 1{,}5$; kidney: $25{,}6 \pm 6{,}8$ and $12{,}3 \pm 3{,}2$; gills: $59{,}7 \pm 8{,}5$ and $6{,}6 \pm 1{,}9$.

Paraquat caused very serious tissue damage in each investigated fishes, but the degree of if was depending on the species. The main target of paraquat is presumably the gills membrane with disturbing effect on oxidative biochemical processes depending on the specific O_2 demand of the fish species.

Introduction

In human diagnosis the detection of injuries of liver, kidney and muscle tissues by determination of transaminase activity in blood is a commonly used well proved method. Transaminases are located in cytoplasma and in mitochondria under normal conditions. The damages and lysis of the cells result an getting these enzymes in blood in relatively high quantities. This causes a rapid increase of enzyme activity „blood transaminase". The results of some laboratories drow the attantion to the possibility to indicate the tissue injuries in some fish species, caused by pollutions of their environment, by determination of changes in blood transaminase enzyme activity.

The aim of our work was to carry out comparative studies regarding the changes of blood transaminase activities after tissues damaging effects in fish species of different nourishment, i.e. in herbivorous, in carnivorous and in mixed nourishing species.

In our experiments paraquat was used as damaging agent, the effective substance of some commercial herbicides as GRAMOXON and GROMEX.

Materials and methods

Cammon carp, (*Cyprinus carpio* L.) silver carp *(Hypophthalmichtys molitrix V.)* and wells *(Silurus glanis L.)* of 350—400 g were obtained from Fisheries Research Institute in Szarvas and held for a minimum of 7 days before experimentation in 100 litre aquaria (5 fishes per aquarium) at a tempera-

ture of 20±1 °C. The length of the exposition to 1 and 10 ppm paraquat was 2 hours and at 100 ppm 10—15 minutes, because during this time all of the treated animals were died after the treatment. The detection of injuries of different tissues was carryed out by the determination of transaminase activity in blood.

Determination of GOT (glutamate-oxaloacetat transaminase) and GPT (glutamate-pyruvate-transaminase) activities:

Reaction mixture for GOT: 0.25 ml 0,1 M pH=7,4 phosphate buffer containing 0,1 M L-aspartat and 2 mMα-ketoglutarate +0,050 ml blood serum. (0.050 ml dest. water in blank).

Reaction mixture for GPT: 0.25 ml 0,1 M pH=7,4 phosphate buffer containing 0,2 M DL-alanin and 2 mMα-ketoglutarate +0.05 ml blood serum (0,05 ml dest. water in blank).

After 60 min. incubation (30 min. for GPT) at 37 °C to each samples 0,25 ml 1 mmol 2,4-dinitrophenyl-hydrazin was added and the mixture was incubated for 20 min. at 20 °C.

After the addition of 2,5 ml 0,4 M NaOH solution the extinction was measured at 540 nm.

Cytochemical demonstration of the glutamate oxalacetate transaminase

For cytochemical investigations carp liver was used. Fixation was carried out in modified KARNOVSKY-solution (1965) for 60 min., at 40 °C. After fixation, the tissue blocks were washed for two hours in a sucrose containing buffer solution (0.2 M imidazole pH 7.4), with several changes of solution at 4 °C. The last washing was performed at room temperature. Blocks were incubated according to LEE (1973) at room temperature for 30 min with continuous shaking. The final composition of the incubation medium was the following:

l-aspartic acid	20 mM
α-ketoglutaric acid	2—4 mM
Pb $(NO_3)_2$	6 mM
imidazol	50 mM
sucrose	0,25 mM

During incubation electron dense lead acetate precipitate was developed at places where GOT was present in cellular compartments. Two kinds of controls were used in our experiments: in the first case, α-katoglutaric acid was omitted from the incubation medium, in the second one, d-aspartic acid (the inactive isomere) was applied in the incubation medium.

After incubation a postincubation rinse was done in l-aspartic acid and sucrose containing buffer solution for 30 min.

Postfixation was carried out in 2% OsO_4 for 2 hrs and blocks were embedded into Spurr's medium (SPURR, 1969).

Results and discussion

The distribution of transaminase activity (expressed as U/l) in different organs of the carps are the following: liver GOT and GPT: 149±55 and 31,2±4,5; heart: 58,4±13 and 4,4±1,5; kidney: 25,6±6,8 and 12,3±3,2. The relative high GOT and GPT activities (59,7±8,5 and 6,6±1,9) in gills can reflect, that the increase of activity in blood serum might be due to the damage of gills as well.

The GOT activity in blood serum is differing according to the species (carp 15,8± ±1,7; silver carp 12,3±1,0; wells 20±4,2). The GPT activity of carp and silver carp is about the same (2,9±0,6 and 2,7±0,4) and in wells is the highest (4,9±0,6). The blood serum transaminase activity of the three investigated fish species after 1 and 10 ppm paraquat treatment changed differently. The order of increase of GOT was the following: silver carp > carp < wells (Fig. 2.a, b, c).

The GPT activity in the three species was more significant after the paraquat treatment.

The order of increase of GPT activity was: carp<wells<silver carp. There was no significant changes after the 100 ppm paraquat treatment regarding the three investigated fish species.

The reason of it might be due to the rapid fish dying after the treatment because paraquat could not cause tissue damaging effect yet within such a short time.

The increase of transaminase activity after 1 and 10 ppm paraquat reflecting the serious damage of tissues. The slight increase of GOT activity of wells over the control, refers to the fact that the tissue damage is not so significant comparing to the other two species.

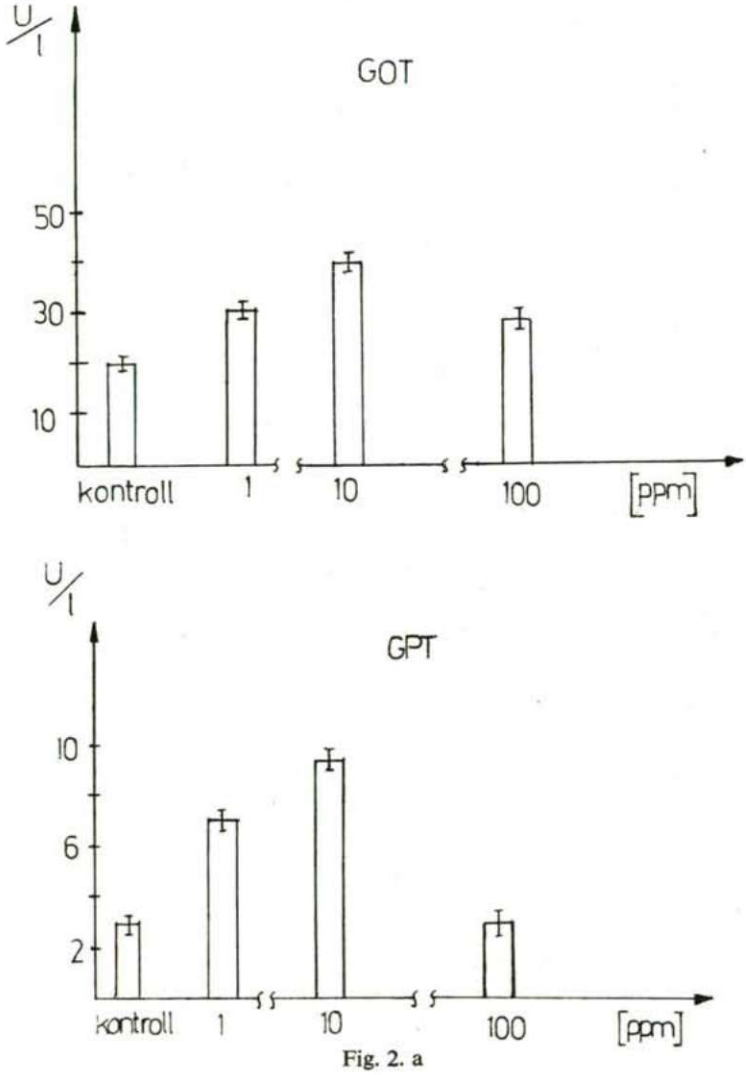


Fig. 2. a

To decide the organ specific damage of paraquat the electronmicroscopic investigations of liver, gills and kidney are in progress in our laboratory.

Many references are to be found in the literature that paraquat exerts its effect as an electron donor, transforms molecular oxygen to active radicals, which are responsible for the membrane destruction (BLOCK, 1979; STANCLIFFE and PIRIE, 1971). In clinical examination was observed lung membrane damage due to the paraquat exposure, which caused difficult breathing (CLARK et al., 1966, FISHER et al., 1971).

On the basis of our experiment the detection of transaminase activity in blood serum is a suitable tool for the detection of tissue damage. The transaminase activity

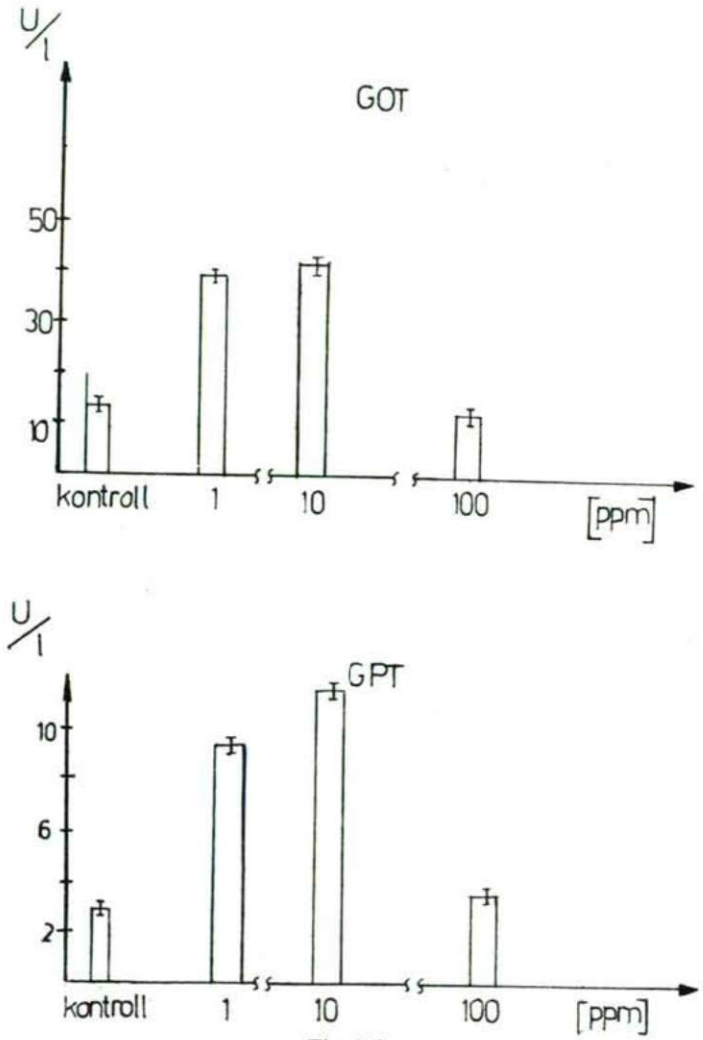


Fig. 2. b

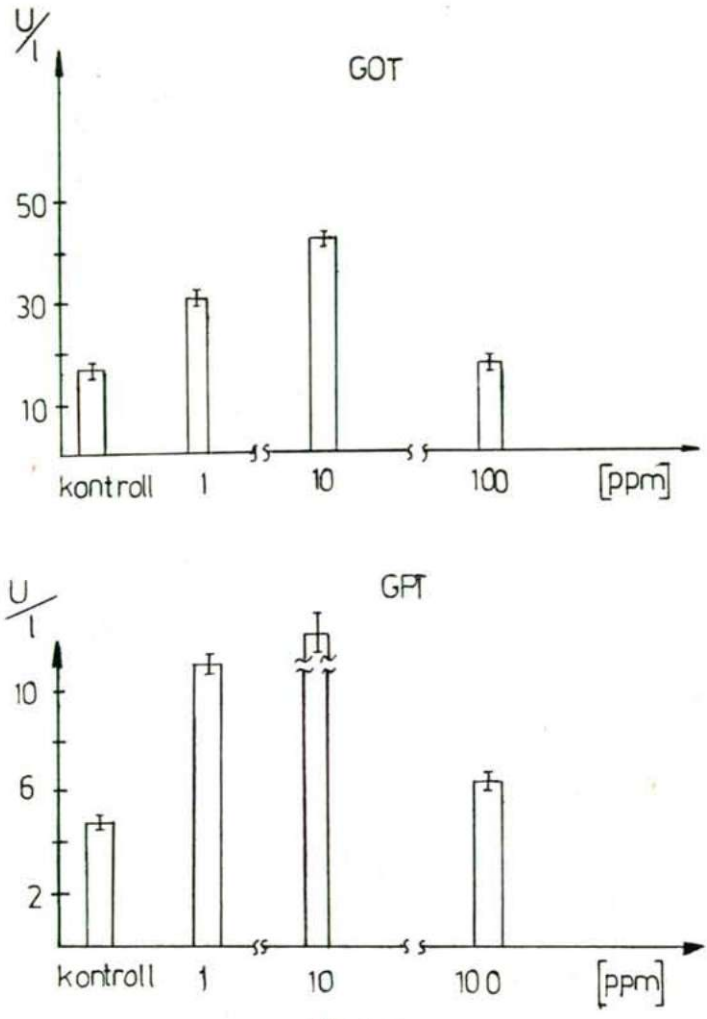
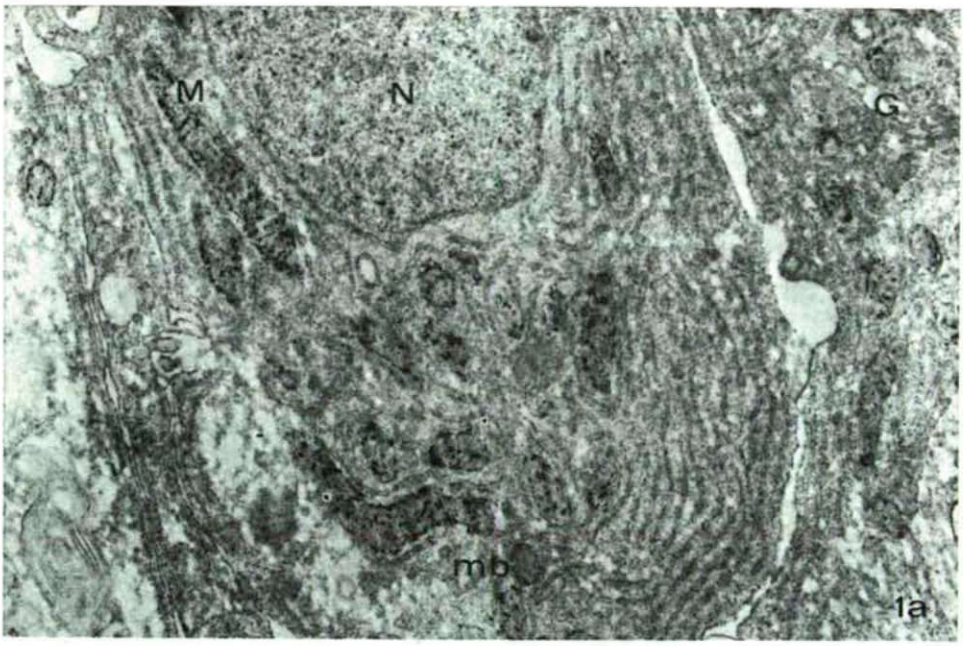


Fig. 2. c

Fig. 2a., b., c. The effect of 1, 10 and 100 ppm paraquat on the serum GOT and GPT activity of carp, (a), silver carp (b) and wells (c). Water temperature 20±1 °C. The given values are the average of 3—8 fishes (±S.D.) expressed in percent of the controls. Exposition time 2 hours, except at 100 ppm paraquat, where 10—15 min.

is depending on the species and on the different organs. These are supported by the findings of other authors (APPOLONIA and ANDERSON, 1980; KRISTOFFERSON et al., 1974).

Paraquat caused very serious tissue damage in each investigated fishes, but the degree of it was depending on the species. The main target of paraquat is presumably the gills-membrane, with disturbing effect on oxidative biochemical processes depending on the specific O_2 demand of the fish species.



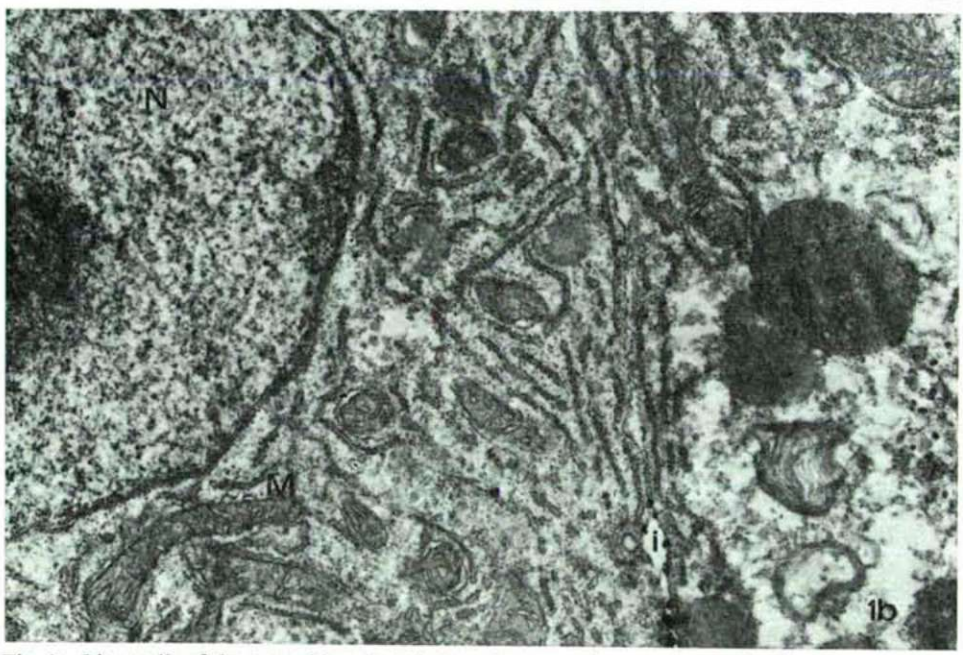


Fig. 1a. Liver cells of the carp. Abundant electron dense reaction product indicating the presence o enzyme — glutamate oxalacetate transaminase — can be seen in mitochondria (M) and multivesicular bodies (mb). Note fine granulted aspecific deposits also in the nucleus (N). G=Golgi apparatus. X 18.000.

Fig. 1b. Substrate free control. Note the lack of the reaction product in mitochondria (M) and other cell organelles in the liver cell of the carp. Rough granules in the interstitium (i) are aspecific accumulations of the lead. N=Nucleus. X 22.000.

On the basis of our cytochemical experiments, it can be established that the method described for demonstration of transaminase in mammalian species (LEE, 1973) is also applicable in the carp liver without any modification. The electron dense lead deposits — indicating the presence of the enzyme, glutamate oxalacetate transaminase — were accumulated in the mitochondria and multivesicular bodies (Fig. la). Other cell organelles, as for example Golgi apparatus were free from the reaction product but in the nucleus small aspecific granules were usually seen. Such kind of aspecific led accumulation used to be common also in other cytochemical procedures where lead ions were applied as capture agent (GÖMÖRI, 1950; NOVIKOFF, 1951). As regard the control tissues (Fig. 1b), both mitochondria and other cell organelles were free from reaction product, however, discontinuous granular deposits may occur in the intercellular spaces of liver cells as well as in the bile canaliculi.

Although LEE (1973) has been described the presence of transaminase in the cisternae of nuclear envelope in the rat liver, we were not able to demonstrate it in the case of liver cells of the carp.

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