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Toxicology

**EFFECT OF DBS DETERGENT ON ACTIVITY OF SOME ENZYMES
OF YOUNG CARP (*CYPRINUS CARPIO* L.) BRAIN, GILLS AND SERUM**

**WPŁYW DETERGENTU DBS NA AKTYWNOŚĆ NIEKTÓRYCH ENZYMÓW MÓZGU,
SKRZELI I SUROWICY NARYBKU KARPIA (*CYPRINUS CARPIO* L.)**

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Effects of DBS (sodium dodecyl-benzene-sulphonate) on the activity of ATP-ases and cholinesterases of autumn young carp brain, gills, and serum were studied. On the second day of the brain ATP-ases activity was found to increase by ca 120%. On the twelfth day, the activity of these enzymes dropped by ca 30% as compared to the control. Blood serum cholinesterases exhibit a constant increase in their activity throughout the experimental period.

INTRODUCTION

Among many toxic substances consciously released by man into natural water reservoirs are the surface-active compounds called "detergents". These are mostly organic compounds, molecules of which have their hydrophilic or hydrophobic nature clearly marked. The compounds act mainly on the water surface; having reached the critical concentration, spherical aggregates (micelles) are formed in the water column (Niemi, 1974). Due to their property of decreasing the surface tension, detergents are destructive to biological membranes, the disintegration of which proceeds through lipids washout and release of enzymes associated with the membranes. Considering a negligible bacterial decomposition of detergents in natural water reservoirs and their common use they can be assumed to constitute a serious hazard to fishes and other aquatic organisms. The

Fig. 1. Natrium dodecyl-benzene-sulphonate (DBS)

present paper attempts to find an effect of a common anionic detergent, natrium dodecyl-benzene-sulphonate (DBS) (Fig. 1) upon the activity of ATP-ases and cholinesterases in brain, gills, and serum of young carp (*Cyprinus carpio* L.) kept in water to which the detergent was added.

MATERIAL AND METHODS

Young carp individuals caught in November 1976 at the State Fish Farm, Wdzydze, were subject to the experiment. The length and weight ranges of fishes studied were 8–16 cm and 15–50 g, respectively. Only the fishes devoid of any discernible pathologic lesions were tested. Over 12 days the fishes gradually acclimatized at $20 \pm 1^\circ\text{C}$ in filtered standing tap water aerated all the time, the oxygen content amounting to 7 mg O_2/l . The fishes were fed at 3-day intervals, thereafter were transported to identical 500-l tanks. No ammonia was detected in the water the pH of which was 8 ± 0.2 . Owing to the lack of any relationship between water hardness and detergent effect upon fish (Łukjanenko, 1974), the water was not tested for its hardness. The detergent was added in 2.5 mg/l concentrations. The sample and control were analyzed after 2, 6, and 12 days. Prior to killing a fish, a sample of its blood was withdrawn from the heart; after 10 min. at 0°C the blood taken was centrifuged for 10 min. at 3000 r.p.m. (Unipan microcentrifuge). In the serum thus obtained, the protein content was measured using the Lowry colorimetric method (Mejbaum-Katzenellenbogen, 1969), while the cholinesterases activity was determined by a colorimetric technique (Ellman et al., 1961) modified after Mahon et al. (1970) and Voss et al., (1970). The blood sample taken, the fish head was cut off and immediately transferred to dry ice to be kept at -20°C over a week. The tissue was homogenized in redistilled water, the volumetric tissue: water ratio being 1:10. The ATP-ases activity was measured colorimetrically (Abdel-Latif et al., 1967). The incubation temperature used in the enzymatic methods was 30°C .

The reagents used: acetylthiocholine Chemapol (Czechoslovakia), eserine, HClO_4 , Triton-X, DTNB, strophantine G BDH (England), Tris Fluka (Switzerland). The remaining reagents required by the techniques used were of analytical grade, produced by POCH (Poland). A "Specol" spectrum colorimeter was used to measure absorption changes.

RESULTS

The blood serum showed its protein content to be 4 g% which is indicative of a good condition of fish examined. No significant differences were revealed between blood serum protein contents in control and experimental carp. The ATP-ases and cholinesterases activities were determined in five and three individuals, respectively. Owing to sufficiently high 98% confidence limits obtained, no increase in the material studied was found necessary. Table 1 and 2 show mean activities of the enzymes tested.

Table 1

Specific activity of adenosine triphosphatase in control fish tissue homogenates (a mean of five measurements $\pm$ SD)

Na ⁺ , K ⁺ dependent ATP-ase		Mg ⁺⁺ dependent ATP-ase	
gills	brain	gills	brain
3.9 $\pm$ 1.94	18.85 $\pm$ 0.57	21.5 $\pm$ 0.74	36.1 $\pm$ 2.03
μ M P/mg protein/h			

Table 2

Specific activity of cholinesterases in control fish tissue homogenates (a mean of three measurements $\pm$ SD)

cholinoesterases		
gills	brain	serum
23.2 $\pm$ 3.7	440.5 $\pm$ 15.4	8.1 $\pm$ 1.9
μ M red. acetylthiocholine/mg protein/h		

Effects of the detergent upon the enzymatic activity of ATP-ases and cholinesterases are presented on Fig. 2 and Fig. 3, respectively.

As seen from Figs. 2 and 3, the activity of both brain ATP-ases increases as early as on the third day in the detergent-containing water, after which the activities gradually decrease, the differences in the Mg⁺⁺ – dependent ATP-ase activity being greater than those noted for the Na⁺K⁺ – dependent one. The gill ATP-ases activity alters to a small extent. The serum cholinesterases show their activity to grow with time, the brain cholinesterases activity undergoing small changes. In gills, the activity of cholinesterases declines below the level observed in the control fishes.

+ difference significant at $P \leq 0.02$, * difference insignificant statistically, control = 100%

Fig. 2. Effect of detergent on ATP-ases activity

Fig. 3. Effect of detergent on cholinesterases activity

DISCUSSION

A comparison between the results obtained here and data reported in the literature is difficult to be made as all the papers refer to partly purified enzymes, thereby higher activities per protein content of the sample are shown (Uitida, 1969; Koch et al., 1971).

As seen from the studies reported here, the detergent used (DBS) does influence the activity of brain ATP-ases as well as it affects the activity of gill and blood serum cholinesterases. The latter as the enzymes associated with cellular membranes can be taken as indicators of the detergent penetration into the fish organism. These enzymes show an increased activity at low detergent concentrations; by destroying the membranes, detergents release the enzymes making easier their contact with a substrate. An increase in the gill cholinesterases activity recorded on the sixth day and a decrease observed after 12 days can evidence the detergent penetration into the gill tissues followed by a partial destruction thereof. Fish breathing is rendered more difficult, thus a fish ability to withstand a physical effort can be significantly decreased (Węgrzynowicz et al., 1975). The blood serum cholinesterases activity increase is presumably due to a rise in an acetylcholinesterase level derived from injured gills and erythrocytes as well as to an increase in activity of free nonspecific cholinesterase quantitatively prevailing in the serum. Owing to the lack of any pseudocholinesterase-specific substrate, an effect of activity of this enzyme on the results obtained is hard to determine.

Interesting is a high increase in the ATP-ases activity observed on the second day of fish exposure to the detergent. ATP-ases are very susceptible to the presence of catecholamines which, when released beyond neurons due to a stress, may increase the enzymatic activity by ca 200%. This results from the neuron back resorption of catecholamines, the process being mainly controlled by ATP-ases. Resorbed catecholamines are subsequently decomposed within a neuron by monoamino oxidase a high activity of which is found in fish brains (Mazeaud, 1974). The process plays a significant part in controlling the catecholamine level in brain.

The results obtained point to the detergent as a stress-causing agent within the initial period of keeping fishes in the detergent-containing water. Later on, a toxic action is observed resulting from a direct exposure of fish tissues to the detergent or its metabolites. Metabolites of some toxic compounds, being formed as a result of detoxication proceeding in liver, are often more toxic than the compounds concerned themselves (Przeździecki, 1976).

It seems obvious in the light of the results obtained that the detergent can significantly alter the activity of fish enzymes, although explanation of this effect is difficult to offer without further studies.

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WPŁYW DETERGENTU DBS NA AKTYWNOŚĆ NIEKTÓRYCH ENZYMÓW MÓZGU, SKRZELI I SUROWICY NARYBKU KARPIA (*CYPRINUS CARPIO* L.)

Streszczenie

Niniejsza praca przedstawia wyniki badań nad wpływem dawki 2,5 mg SA/l detergentu dodecylbenzeno-sulfonianu sodowego (DBS) na aktywność ATP-azy i cholinesteraz mózgu, skrzeli oraz surowicy jesiennego narybku karpia (*Cyprinus carpio* L.). Oznaczenia aktywności enzymatycznej oraz zawartości białka przeprowadzono w oparciu o metody kolorymetryczne. Stwierdzono wzrost aktywności Na^+, K^+ zależnej ATP-azy w mózgu o 100% zaś Mg^{++} zależnej ATP-azy o 140% w stosunku do grupy kontrolnej już w drugim dniu przebywania ryb w wodzie z detergentem. Po dwunastu dniach doświadczenia aktywność tych enzymów była o ok. 30% niższa niż w grupie ryb kontrolnych. ATP-azy skrzeli są mało wrażliwe na działanie detergentu, podobnie jak acetylcholinoesteraza mózgu. W skrzelach cholinesterazy wykazują niewielkie zmiany aktywności.

Sukcesywny wzrost aktywności cholinesteraz stwierdzono w surowicy, w której w dwunastym dniu doświadczenia aktywność była wyższa o ok. 72% niż w grupie kontrolnej. Na podstawie uzyskanych rezultatów trudno wyjaśnić zadowalająco mechanizm wpływu detergentu DBS na zbadane enzymy.

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ВЛИЯНИЕ ДЕТЕРГЕНТА ДБС НА АКТИВНОСТЬ НЕКОТОРЫХ ФЕРМЕНТОВ
МОЗГА, ЖАБЕР И СЫВОРОТКИ МОЛОДИ КАРПА (*Cyprinus carpio* L.)

Р е з ю м е

Настоящая работа представляет собой результаты исследований касавшиеся влияния дозы 2,5 мг АВ/л детергента додецилбензилсульфата натрия (ДБС) на активность АТФ-азы и холинэстеразы мозга, жабер и сыворотки у осенней молоди карпа (*Cyprinus carpio* L.)

Определения активности ферментов и содержания белка проводили колориметрическим методом. Установлено увеличение активности Na^+ , K^+ , зависимой АТФ-азы мозга на 100% и Mg^{++} зависимой АТФ-азы на 140% по сравнению с контрольной группой уже на второй день пребывания рыбы в воде с детергентом. Спустя 12 дней опыта активность этих ферментов являлась на около 30% меньшей чем в контрольной группе. Отзывчивость АТФ-азы жабер и ацетилхолинэстеразы мозга на действие детергента являлись небольшими. Холинэстеразы жабер проявляли небольшие изменения активности.

Последовательное увеличение активности холинэстераз установлено в сыворотке, для которой на 12 день опыта активность являлась большей на около 72% чем в контрольной группе. На основании полученных результатов невозможно является удовлетворительно выяснить механизм влияния детергента ДБС на исследованные ферменты.

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