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Fish toxicology

HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES OF MUSCLES, LIVER AND KIDNEYS OF BROWN BULLHEAD (*ICTALURUS NEBULOSUS*) AFTER EXPERIMENTAL CONTAMINATION WITH MERCURY

BADANIA HISTOLOGICZNE I ULTRASTRUKTURALNE MIĘŚNI, WĄTROBY I NEREK SUMIKÓW KARŁOWATYCH (*ICTALURUS NEBULOSUS*) PO DOŚWIADCZALNYM SKAŻENIU ZWIĄZKAMI RTĘCI

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Toxicity of mercuric compounds ($HgCl_2$ and CH_3HgCl) to fishes was determined basing an histological and ultrastructural studies of muscles, liver and kidney in brown bullhead exposed to solutions of these compounds. Histological observations revealed degenerative changes in liver and kidneys. Ultrastructural studies revealed the following changes: in muscles – disappearance of myofilaments and distension of the canals in the smooth sarcoplasmatic reticulum, in liver and kidneys – cytoplasm vacuolization increased number lysosomes and destruction of mitochondria:

INTRODUCTION

As results from the available literature, so far experimental studies on toxicity of mercuric compounds to fish took advantage of histological and ultrastructural observations only in case of gills (O'Connor et al., 1975; Lindahl et al., 1970; Olson et al., 1973; Wobeser, 1975). Changes in histological structure were described for kidneys

(Kendall, 1975; Wobeser, 1975), whereas no such observations were made for muscles and liver.

The aim of this work was to determine toxicity of mercuric compounds to fish basing on histological and ultrastructural observations of muscles, liver and kidneys.

MATERIAL AND METHODS

Brown bullhead was exposed to methyl mercury chloride (CH_3HgCl) and mercury chloride ($HgCl_2$). The first is the most toxic mercuric compound, whereas the latter is a component of industrial wastes. Toxicity of CH_3HgCl was determined after experimental establishment of the concentration of about 10 $mg \cdot kg^{-1}$, and within the range of 0.5–1.5 $mg \cdot kg^{-1}$ of total mercury in fish muscles. Concentrations of mercury of about 10 $mg \cdot kg^{-1}$ were found in muscles of fishes from highly contaminated waters, while concentrations of 0.5–1.5 $mg \cdot kg^{-1}$ constitute upper limit of suitability of fishes for human consumption in many countries.

Results of my previous studies (Studnicka, in print) revealed that concentration of mercury in muscles of fish from the Vistula River did not exceed 1.5 $mg \cdot kg^{-1}$. Hence, use of similar concentrations in the experiment might lead into conclusions on danger to fish health in the largest Polish river.

In order to obtain this concentration of mercury in fish muscles, a preliminary experiment was carried out, basing on the data from the literature (Calmari et Marchetti, 1973; Roales et Perlmutter, 1974; Rucker et Amend, 1969). Bullheads were immersed in water solutions of CH_3HgCl and $HgCl_2$, at the concentration of 0.15 mg $Hg.1^{-1}$ and temperature of 13 and 20°C. One litre of the solution was used for each 10 g of the fish weight. After many trials it appeared that 10 $mg \cdot kg^{-1}$ concentration of mercury in fish muscles was obtained only after 6 immersions, each of 1 h duration, at 20°C, and subsequent 3-week holding of the fish in clean, flowing water, at 11–13°C. Concentrations of 0.5–1.5 $mg \cdot kg^{-1}$ were obtained after 30 min. immersion of the fish, at 13°C, and their subsequent 3-week holding in clean, flowing water, at 11–13°C.

The experiment was carried out in glass, 100 l aquaria, equiped with an aeration device, and with regulated temperature of water. Brown bullheads were obtained from Miejskie Lake, near Ostrów Lubelski. They were divided into 4 groups: one control, and three experimental ones (I, II and III). Each group was composed of 20 fishes. Fishes were acclimated to aquaria conditions for 2 weeks. During this period temperature of water was gradually raised from 11 to 20°C in group I and II. Group III and the control were kept at 11–13°C. Tap water was used throughout the experiment; its physico-chemical parameters (data of the Municipal Laboratory of Drinking Water Supply System in Lublin) were as follows:

total hardness: 7.28 $mval.1^{-1}$ (20.0–20.4°N) alkalinity: 7.2 $mval.1^{-1}$ Fe: 0.03 $mg.1^{-1}$ sulphates: 14–15 $mg.1^{-1}$ chlorides: 10–11 $mg.1^{-1}$ Cl: 0.1 $mg.1^{-1}$, no more than 0.3 $mg.1^{-1}$ dry residue: 400–430 $mg.1^{-1}$ pH: 7.3; transparency: total

Control group

Fishes in this group were kept in clean, flowing water throughout the experiment.

Group I.

Basing on the results of the preliminary experiment, in order to obtain total mercury concentration in fish muscles on the level of 10 $mg \cdot kg^{-1}$, bullheads were immersed in CH_3HgCl solution of the concentration of 0.15 mg $Hg \cdot 1^{-1}$, and temperature of 20°C. They remained in the solution for 1 hour daily, for 6 consecutive days. Each time mercury solution was prepared immediately prior the fish immersion. After this treatment, the fishes were transferred to clean water of the same temperature. After the last, 6th exposition, the fishes were gradually adapted to lower temperatures of 11–13°C, and were kept in clean, flowing water of this temperature.

Group II.

Fishes in this group were exposed to mercury chloride ($HgCl_2$), conditions of the treatment being the same as in group I. The aim of this was to compare toxicity and accumulation of methyl mercury and inorganic mercury.

Group III.

Basing on the results of the preliminary experiment, in order to obtain mercury concentration in fish muscles of 0.5–1.5 $mg \cdot kg^{-1}$, fishes were immersed (only once) for 30 min. in the CH_3HgCl solution, at the concentration of 0.15 mg $Hg.1^{-1}$. Temperature of both the mercury solution and water in which fishes were kept was 11–13°C.

After the last exposure, fishes of all experimental groups were kept for 3 weeks in clean, flowing water. During this period mercury concentration in the muscles in group I and III reached the previsted levels. Total mercury content in tissues in the control fishes was determined before the experiment, and then again at the same time as for the experimental fishes.

After 3 weeks dorsal muscle, liver and kidney samples were taken from all fish groups. Total mercury content was determined, and sections of muscles and organs were taken for histological and ultrastructural studies.

Material for histological studies was preserved in neutralized formalin 1:9 and in the AFA liquid. Paraffin scraps 5–7 μ thick were stained with hematoxylin and eosine.

Tissue scraps for electrone microscopy were immersed for 2 hours in a 4% glutaric aldehyde with cacodyl buffer, at pH 7.2–7.4, then washed with the buffer only, and immersed for 2 hours in 1% osmium tetroxide with the same buffer. Dehydration was carried out in ethyl alcohol of increasing concentration, up to the absolute one. Tissue blocks were immersed in the Epon micture. Ultrascraps were prepared with ultra-microtone, Tesla BS 490, and stained with uranyl acetate and lead citrate. Observations were made and photographs taken from an electrone microscope Tesla BS 613.

Total mercury levels in fish tissues were determined with atomic absorption spectrophotometry, in lte Department of Pharmacology and Toxycology of the Veterinary Institute at Puławy. Electrone microscopic studies were made at the Medical Academy in Lublin.

Significance of defferences between mercury levels in the muscles and organs of the experimental and control fishes was determined with the Student's t test, takin 5% as the risk of error ($p < 0.05$).

RESULTS

Determination of total mercury levels in the tissues.

Concentrations of total mercury in the muscle tissue, liver and kidneys in the control group and the experimental groups are presented in Table 1.

Table 1

Average concentrations of total mercury in muscles, liver and kidneys of brown bullhead *(Ictalurus nebulosus)*; three weeks after the exposure to CH_3HgCl and $HgCl_2$ solutions at concentration of 0.15 mg $Hg.l^{-1}$ (values given in mg $Hg.kg^{-1}$)

Group	Immersion in solution at temp. of	Number and duration of immersions	muscles	liver	kidneys
Control	–	–	0.064	0.057	0.035
I	CH_3HgCl 20°C	6 x 1 hour	10.200	18.020	16.667
II	$HgCl_2$ 20°C	6 x 1 hour	0.917	1.823	6.979
III	CH_3HgCl 13°C	1 x 30 minutes	1.200	2.236	0.996

In all experimental fishes (group I, II and III) concentration of mercury in the muscles and organs was significantly higher ($p \leq 0.05$) than in the control fishes. Moreover, there were considerable differences in the accumulation of organic and inorganic mercury, after treatment with the same mercury concentrations and the same time of exposure (group I and II). In the tissues of fishes from group I, exposed to CH_3HgCl, accumulation of mercury was higher than in fishes from group II, exposed to $HgCl_2$ (in the muscles 11.1 times, in the liver 9.9 times, and in the kidneys 2.38 times higher). Treatment with CH_3HgCl resulted in the highest accumulation of mercury in fish liver whereas fishes exposed to $HgCl_2$ accumulated mercury mostly in the kidneys.

In fishes from group III one immersion in CH_3HgCl solution (at 13°C) resulted in similar total mercury levels in the muscles and liver as those observed in fishes from group II, after 6 immersions in $HgCl_2$ solution at 20°C.

No symptoms of fish poisoning were observed.

Histological studies.

Muscles.

No pathological changes were noted as regards structure of the muscle tissue in the control group and in the three experimental groups of fishes. Muscle fibres had proper structure and striations were clearly visible, both in the control fishes and in groups I, II and III.

LIVER

Control group.

Livers in fishes from the control group had proper structure, with the trabecular cell arrangement. Single phagocyte cells filled with brownish-yellow haemosiderin, the so-called siderocytes, were observed in the liver parenchyma. Blood vessels were filled with moderate amount of blood (Fig. 1).

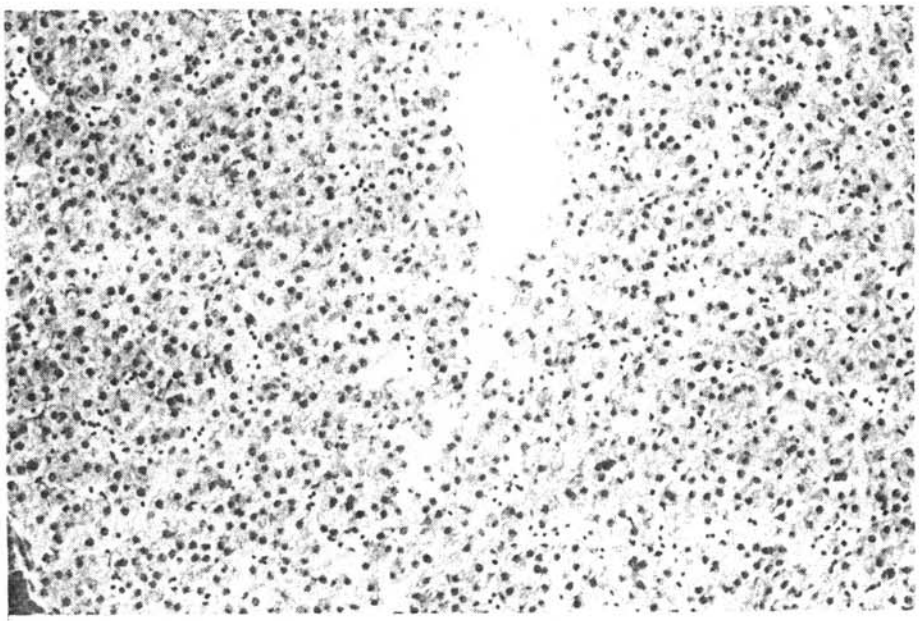

Fig. 1. Control group. Liver. Magn. about 250 x.

Group I.

Compared to the control group, livers in fishes from group I were noticeably congested. Close to the middle veins, and sometimes also in other part, liver cells had loose structure. Sometimes symptoms of steatosis were noted, as well as destruction caused by necrosis and cell desintegration. Necrotic zones were characterized by increased number of siderocytes and extracellular deposits of bile pigments (Fig. 2).

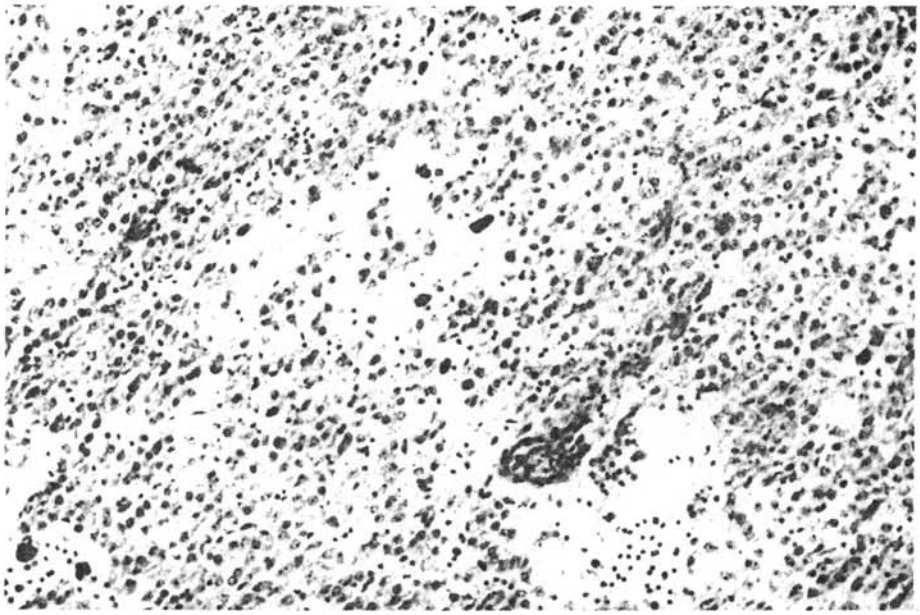

Fig. 2. Loosening of the structure, steatosis and necrosis of liver cells. Magn. about 250 x

Group II.

Slight congestion of liver was observed in fishes in this group. Steatosis and necrosis were less noticeable than in group I. In two instances changes were of the character of steatotic degeneration, with pronounced symptoms of the jaundice.

Group III.

In 50% of fishes in this group liver had proper structure and did not differ from the control group. In the remaining fishes similar changes were observed as in group I, viz. symptoms of steatosis, especially in the vicinity of middle veins and vessels of the portal zone of lobules. Amount of haemosiderin in the parenchymal cells was not increased, nor was the amount of bile in the bile ducts.

KIDNEYS

Control group.

As regards structure of the kidneys, in most cases round tubules were observed, their sections being of different size. They weree paved with cells containing homogenous plasma and follicular, oval nucleus (Kendal et Hawkins, 1975). Tubule insides were usually empty. Intertubular tissue showed symptoms of proper haematopoietic activity. Single siderocytes were observed among numerous red and white corpuscles. Renal space around portal veins was creviced, of moderate size (Fig. 3).

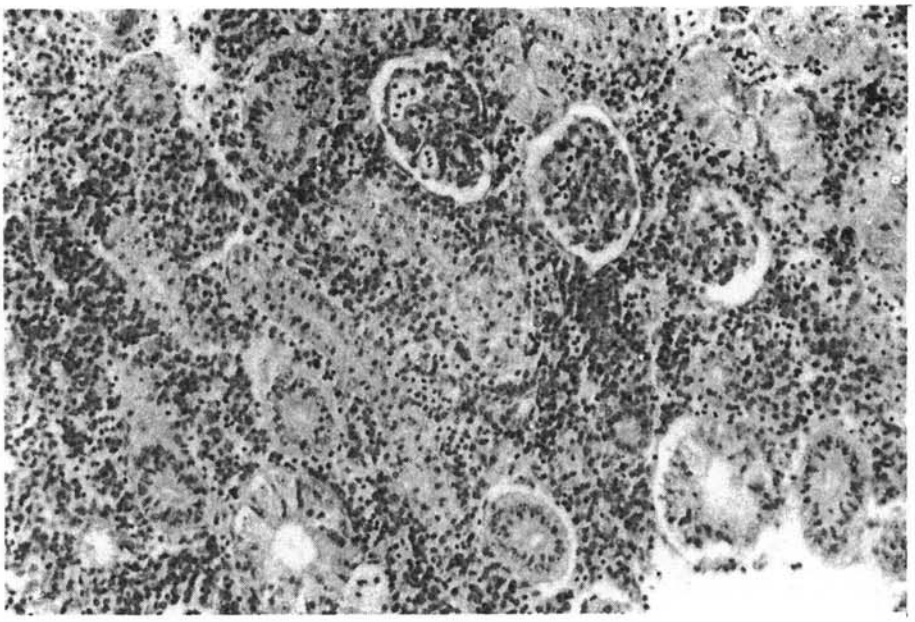

Fig. 3. Control group. Kidneys. Magn. about 250 x

Group I.

Changes were noted as regards the nephrons and renal tubules. Nephrons were sometimes contracted, so that renal space distened. Renal tubules, especially the proximal ones (of the I order) showed partial or even total obliteration; epithelial cells were noticeably swollen, and parenchymal degeneration was observed, with empty spaces appearing in the plasma, resembling vacuoles. In singular tubules degenerative changes consisted of contraction of cell nuclei and flaking off of the upper part of the cells, down to tubule inside. The intertubular tissue was essentially similar to the control group, but siderocytes were more numerous. Blood supply was more intensive than in thy control group, and vessels were more abundantly filled with blood corpuscles (Fig. 4).

Group II.

Changes of the degenerative character and swelling of the tubular epithelial cells were mostly noticed in this group of fishes. Tubule light was smaller, and sometimes there was no passage at all. However, parenchymal degeneration and other degenerative changes in the tubular epithelium were less pronounced than in the fishes from group I. Nephrons were not changed compared with the control fishes. Siderocytes in the intertubular tissue were more numerous than in the control group, but intensity of blood supply was similar.

Fig. 4. Group I – kidneys. Enlargement of urinary space of the coils, necrosis of the epithelium in single tubules of the I order, vacuolization of the cytoplasm. Magn. about 250 x

Group III.

Changes in this group were restricted to a slight increase of nephron cavity size, and some parenchymal degeneration of the tubular epithelium. Number of siderocytes and blood supply were similar as in the control group.

ULTRASTRUCTURAL STUDIES

Muscles.

Control group.

Fibres of the skeletal muscles had regular sarcomers, with pronounced striations. Ultrastructure of myofibres consisted of delicate myofilaments. In the intermyofibre spaces broad canals of smooth sarcoplasmatic reticulum were noticeable (Fig. 5).

Group I.

Compared with the control, ultrastructure of muscle fibres in fishes of this group was characterized by considerable changes, consisting of enlarged canals of the smooth sarcoplasmatic reticulum. Spaces between bundles of myofibres were also enlarged. Partial or total disappearance of myofilaments was observed in some sarcomers (Fig. 6).

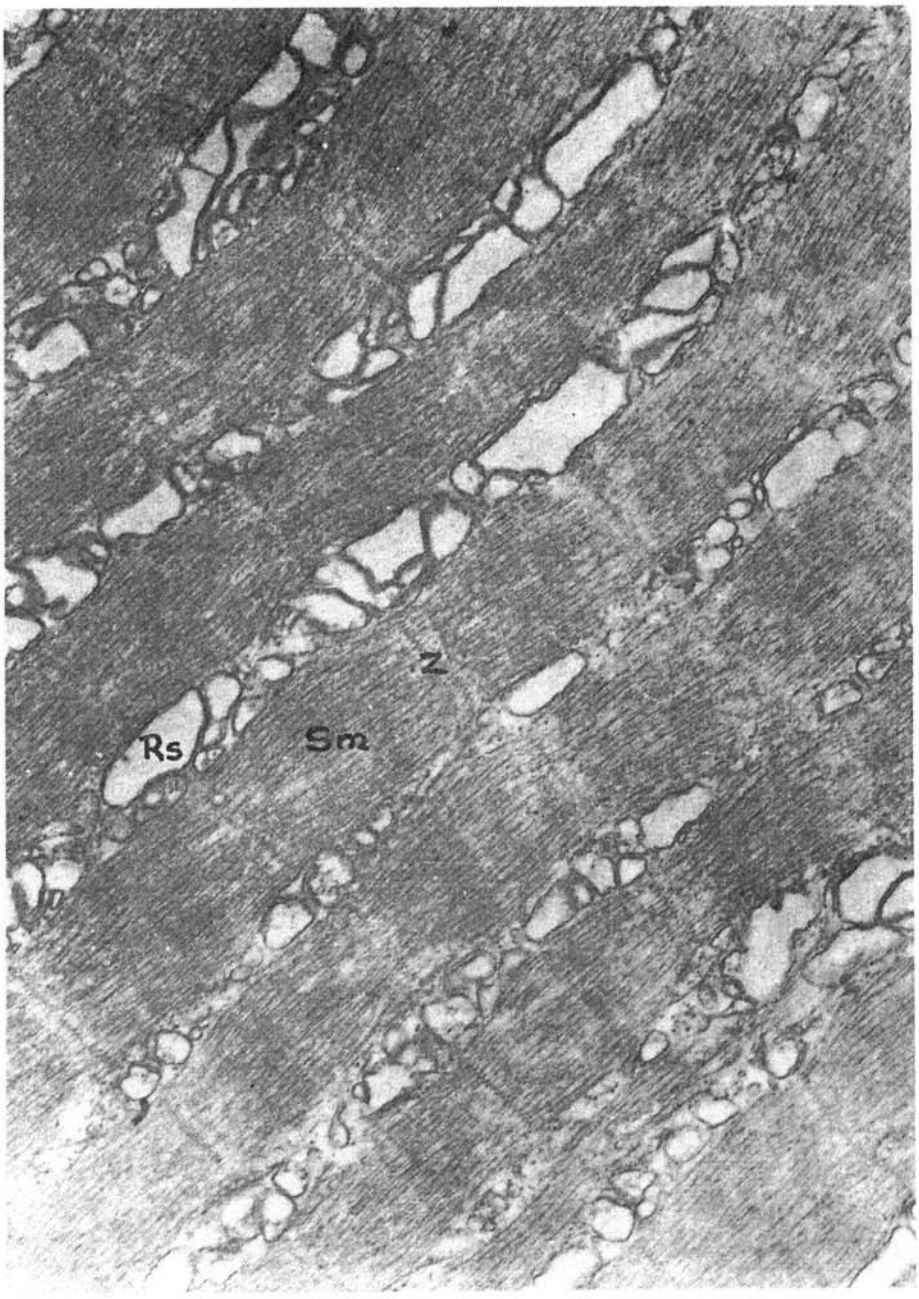

Fig. 5. Control group. Ultrastructure of the skeletal muscle fibre. Magn. about 25000 x.
Sm – sarcomer, Rs – sarcoplasmatie reticulum, Z – Z-strip

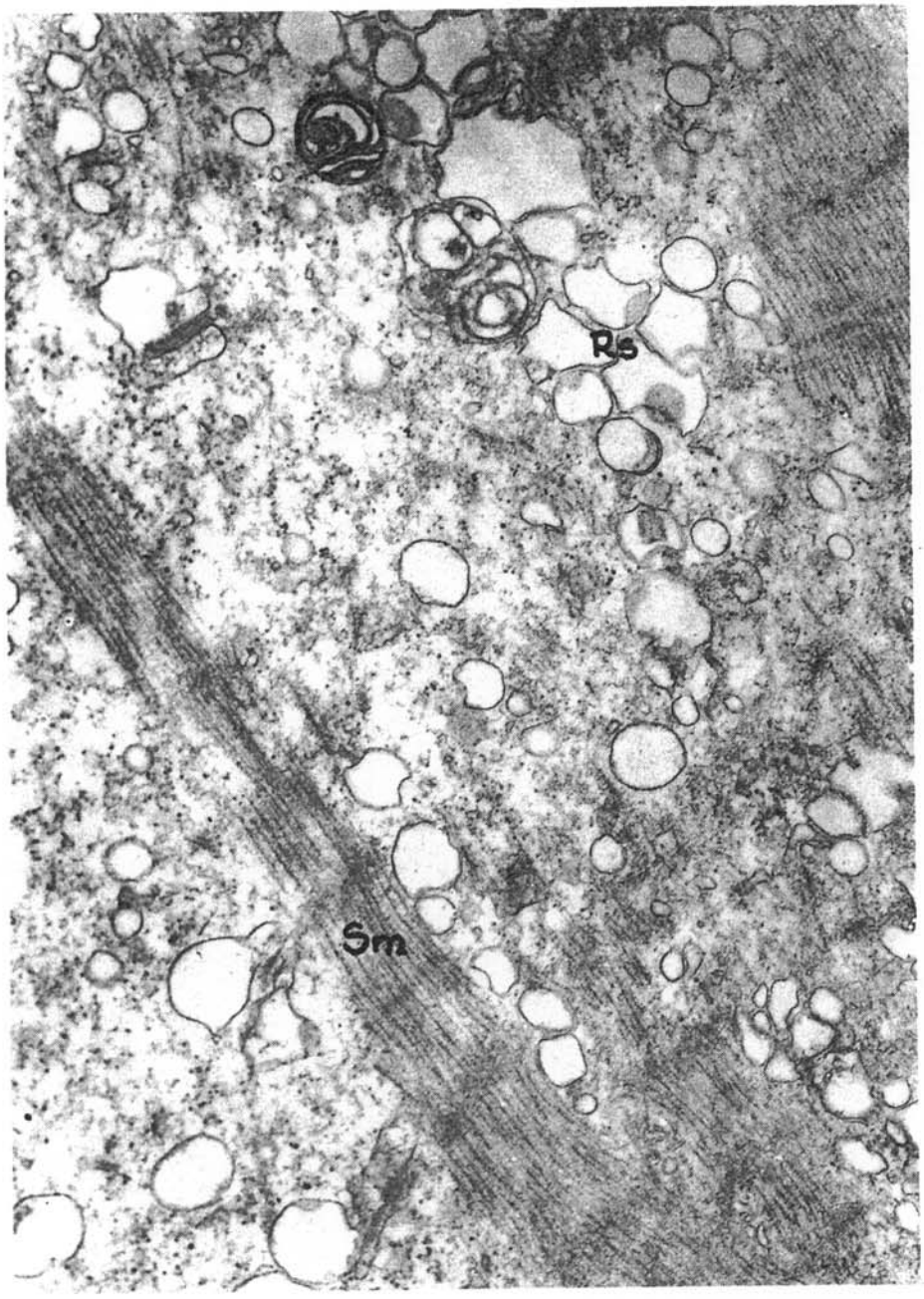

Fig. 6. Group I. Ultrastructural changes in muscle fibres: enlargement of the canals of the smooth sarcoplasmatic reticulum and interfibre spaces, disapperance of myofilaments, total destruction of the sarcomers. Magn. about 25000 x. Sm – sarcomer, Rs – sarcoplasmatic reticulum

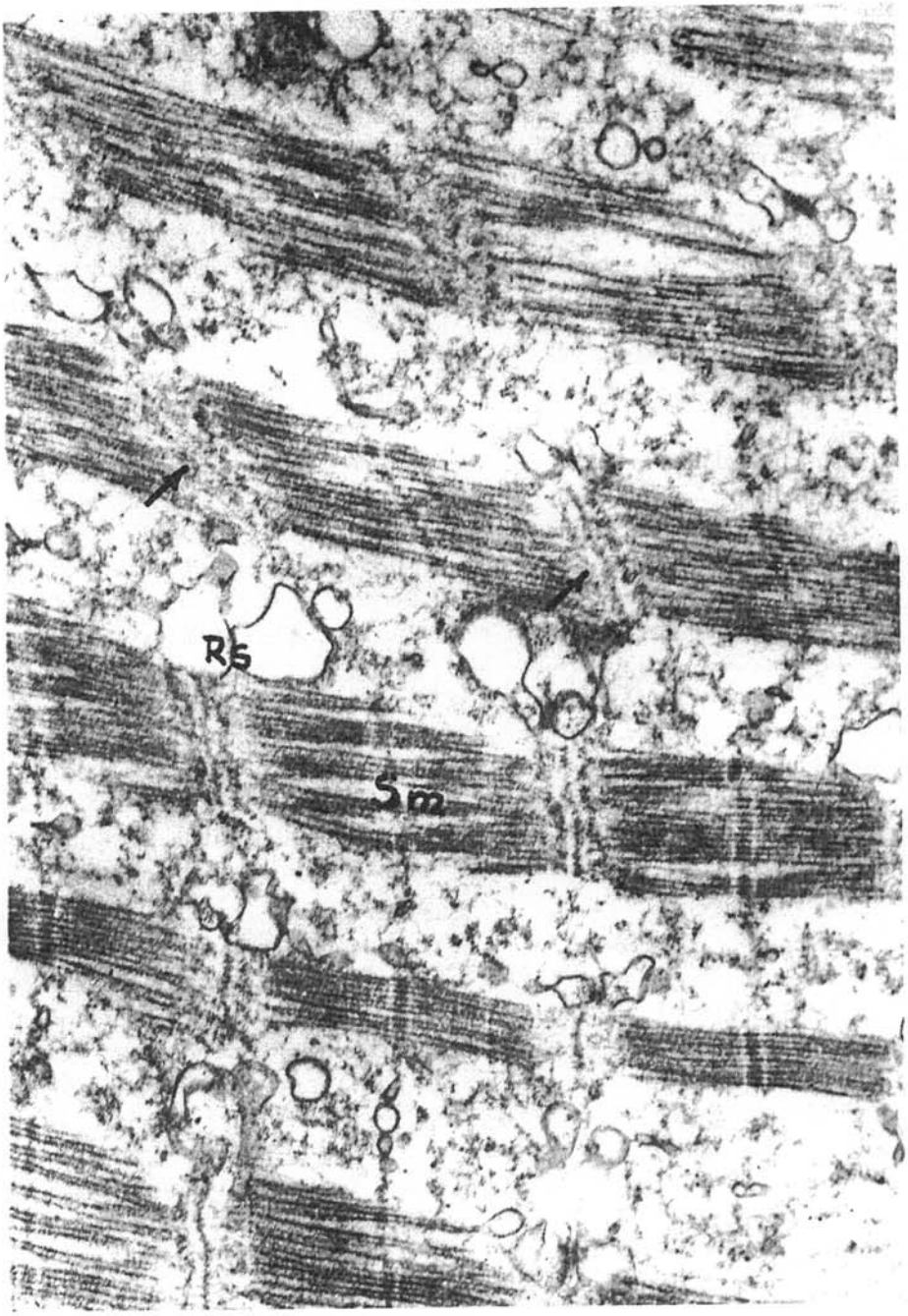

Fig. 7. Group II. Ultrastructural changes in muscle fibres: enlargement of the interfibre spaces, disturbances in the myofilament pattern (arrows). Magn. about 25000 x. Sm – sarcomer, Rs – sarcoplasmatic reticulum

Group II.

Canals of the smooth sarcoplasmatic reticulum were enlarged, and so were interfibre spaces, but to a much smaller extent than in group I. On both sides of the Z strip disturbances in the myofilament structure were observed. In these places myofilaments were irregular, and sometimes totally disappeared (Fig. 7).

Group III.

No changes were noted in the muscle ultrastructure in this group compared with the control. Only canals of the smooth sarcoplasmatic reticulum were slightly enlarged.

LIVER

Control group.

Ultrastructure of liver cells in fishes from this group did not differ from the usually observed in teleost fishes (Berlin, 1967; Byczkowska-Smyk, 1965, 1968; Hinton et al., 1972, 1975, 1976; Kendall et Hawkins, 1975; Langaste et Kärner, 1970, 1976; Weiss, 1972, 1974; Yamamoto, 1962, 1965). Hepatocytes were oval or multiangular, with visible edges. Intercellular spaces were irregular. Bile ducts and Disse's spaces were normal. All organella usually present in liver cells were visible in the hepatocytes. Nuclei of the hepatocytes were circular or irregular, with typical grain structure and normal membranes. Smooth intraplasmatic reticulum was noticeable in the cytoplasm. Canals of the grain intraplasmatic reticulum were regularly arranged. Numerous mitochondria, most frequently round-shaped, rarely oval, were regularly distributed in the cytoplasm. They had typical internal structure, with matrix of moderate electrone density. Lysosomes of various sizes contained substance of low electrone density. Within large lysosomes filamentous structure was observed. Peroxysomes were present in the cytoplasm (Fig. 8).

Group I.

Ultrastructure of liver cells in this group of fishes differed from that in the control group. In most cases structure of the mitochondria in cells only slightly differed from the mitochondrial pattern in the control cells. Lighter centers were observed in the mitochondrial matrix. In some mitochondria external membrane was destroyed. Canals of the granular intraplasmatic reticulum were frequently surrounded by rings of mitochondria. Sometimes they were fragmentated. Smooth intraplasmatic reticulum was considerably enlarged. Numerous vacuoles were observed in the cytoplasm, as well as myelin structures and free collagen fibres. Numerous bodies with single membrane were very frequent; most probably they corresponded to secondary lysosomes. Fibre structure of the lysosomes was hardly visible. Frequently the lysosome membrane disappeared. In some cases remnants of these organella were found close to empty spaces in the cytoplasm (Fig. 9, 10).

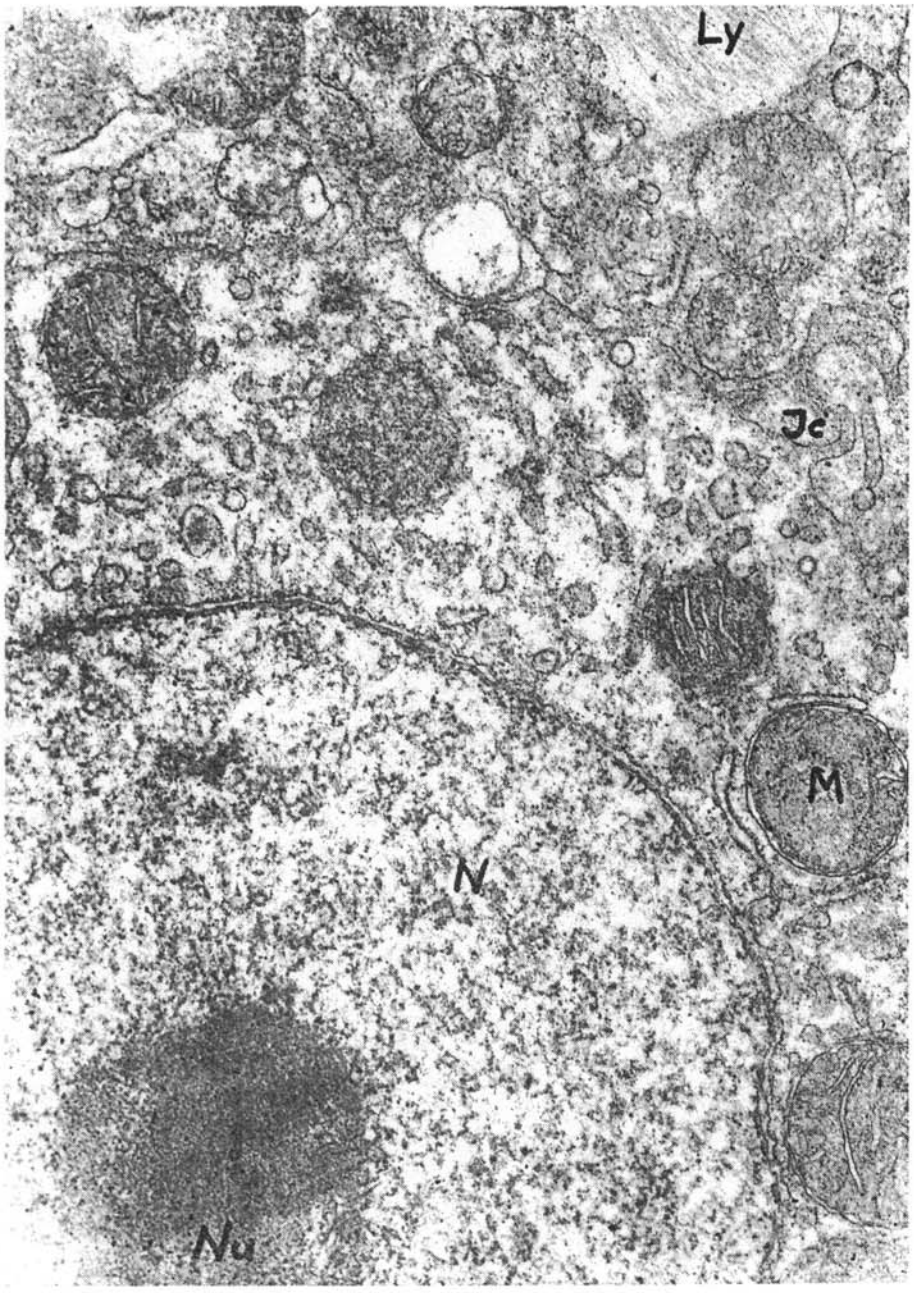

Fig. 8. Control group. Normal ultrastructure of liver cells. Magn. about 25000 x. N – nucleus, Nu – nucleolus, M – mitochondrium, Ly – lysosome, Ie – intracellular bile duct

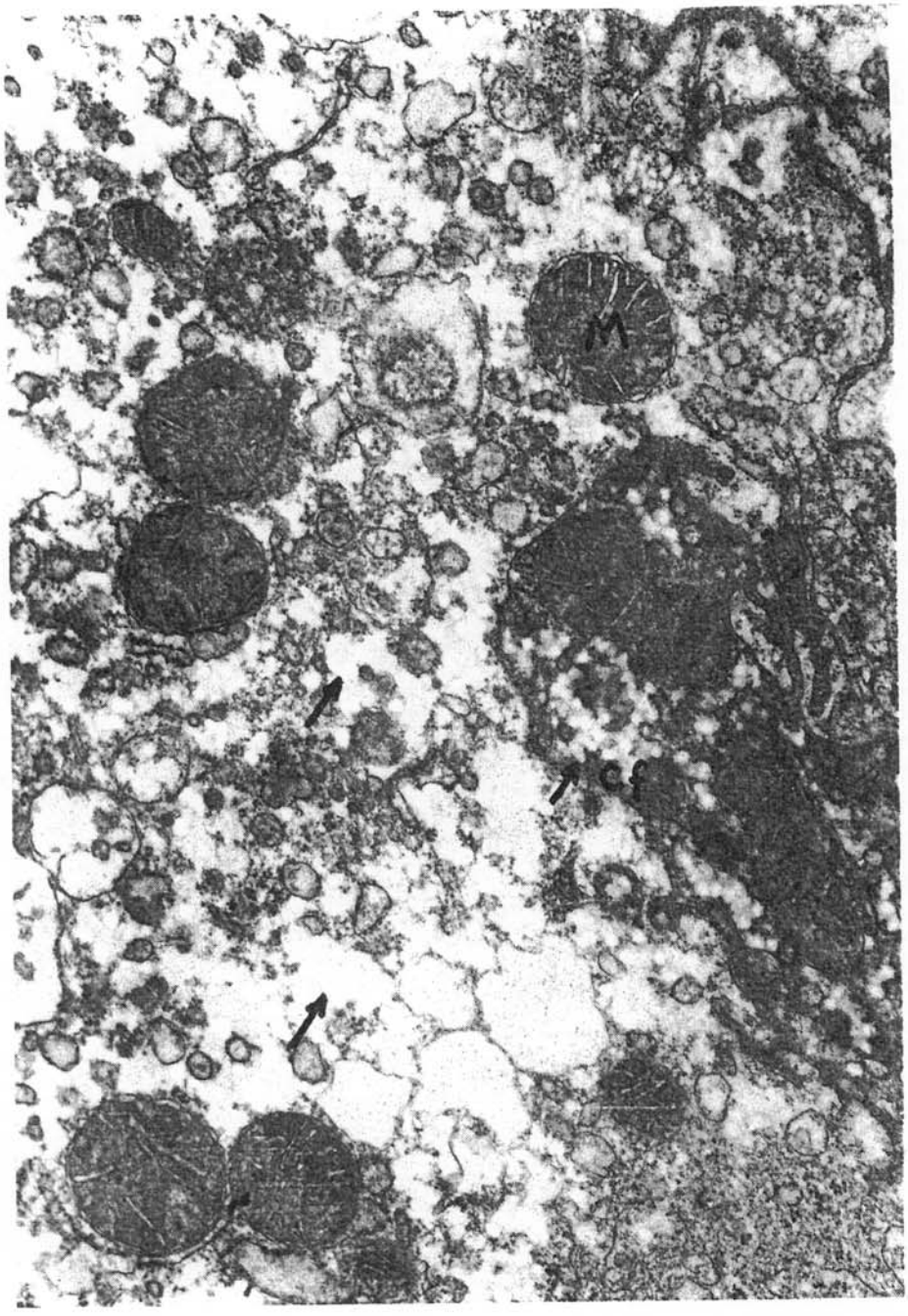

Fig. 9. Group I – liver. Vacuolization of the cytoplasm, free collagen fibres (arrows). Magn. about 25000 x. Cf – collagen fibre, M – mitochondrium

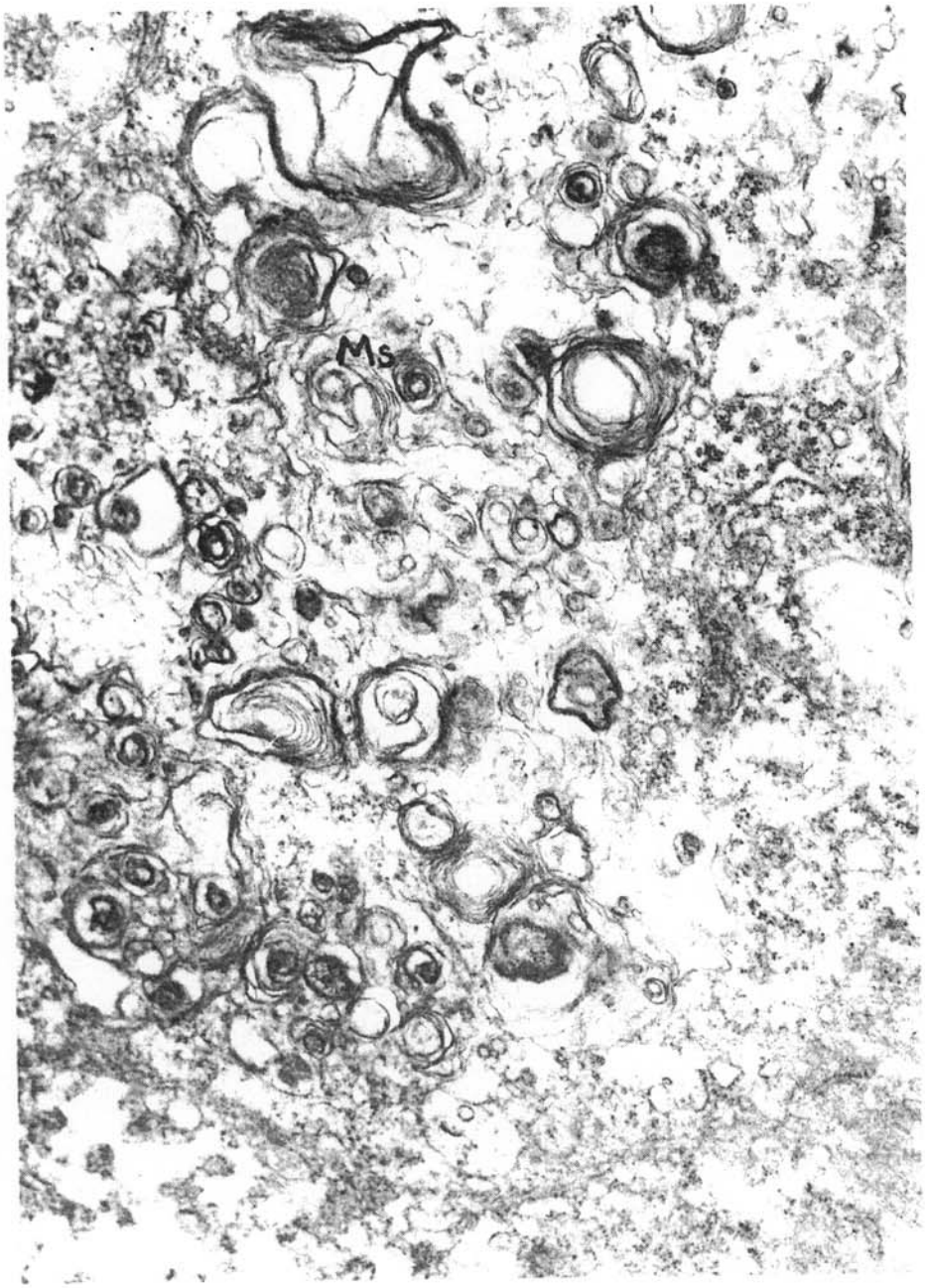

Fig. 10. Group I, liver. Myelin structures in the cytoplasm. Magn. about 25000 x. Ms – myelin structures

Group II.

Changes in the liver cells were essentially similar to those in group I, but not so pronounced. Nore should be made of considerably lower number of lysosomes than in grup I.

Group III.

Changes in the liver ultrastructure were similar to those in groups I and II, but, their extent was significantly smaller. Smoth intraplasmatic reticulum was enlarged, and vacuolization of the cytoplasm was observed. There were lighter spaces in the matrix of some mitochondria. In most hepatocytes no changes were observed in the granular intraplasmatic reticulum, which was less abundant than in group I and II. Lysosomes were more numerous, large, with noticeable symptoms of digestion.

KIDNEYS

Control group.

Cells of the coiled tubule of the I order lied upon flat, distinct basal membrane of a homogenous structure. Their cell membranes at the basal side were visibly concave, protruding into the cytoplasm, with characteristic arrangement along the mitochondria, whereas they were smooth and flat at all other sides. In these cells, surfaces directed towards the tubule interior had many villi, regularly arranged. At the pole end of the cells pinocytar vescicles of various sizes were observed, together with large vacuoles. Mitochondria were elongated, rarely oval or circular, of typical structure. Fibre or membrane structures were noted in the lysosomes. Round or irregular nuclei were centrally located in the cells (Fig. 11).

Group I.

Compared with the control group, number of lysosomes in the epithelial cells in the coiled tubule of the I order was much greater. Some mitochondria in these cells were destroyed, as reflected by light spaces in the matrix and broken external membranes. It seems that cell membranes at the cell base were less concave (Fig. 12, 13).

Group II.

Abundance of lysosomes in the epithelial cells of the coiled tubule of the I order was much higher. Also number of vacuoles in the cytoplasm increased, this being due to digestion by the lysosomes. Changes in the mitochondria were less pronounced than in group I. Slight changes were noted only in the mitochondrial matrix, in form of scarce lighter spaces. Internal membranes of the mitochondria were unchanged. As regards other cell elements, no changes were observed in their ultrastructure compared with the control group.

Group III.

Increased numbers of vacuoles were observed in the cytoplasm of cells of the coiled

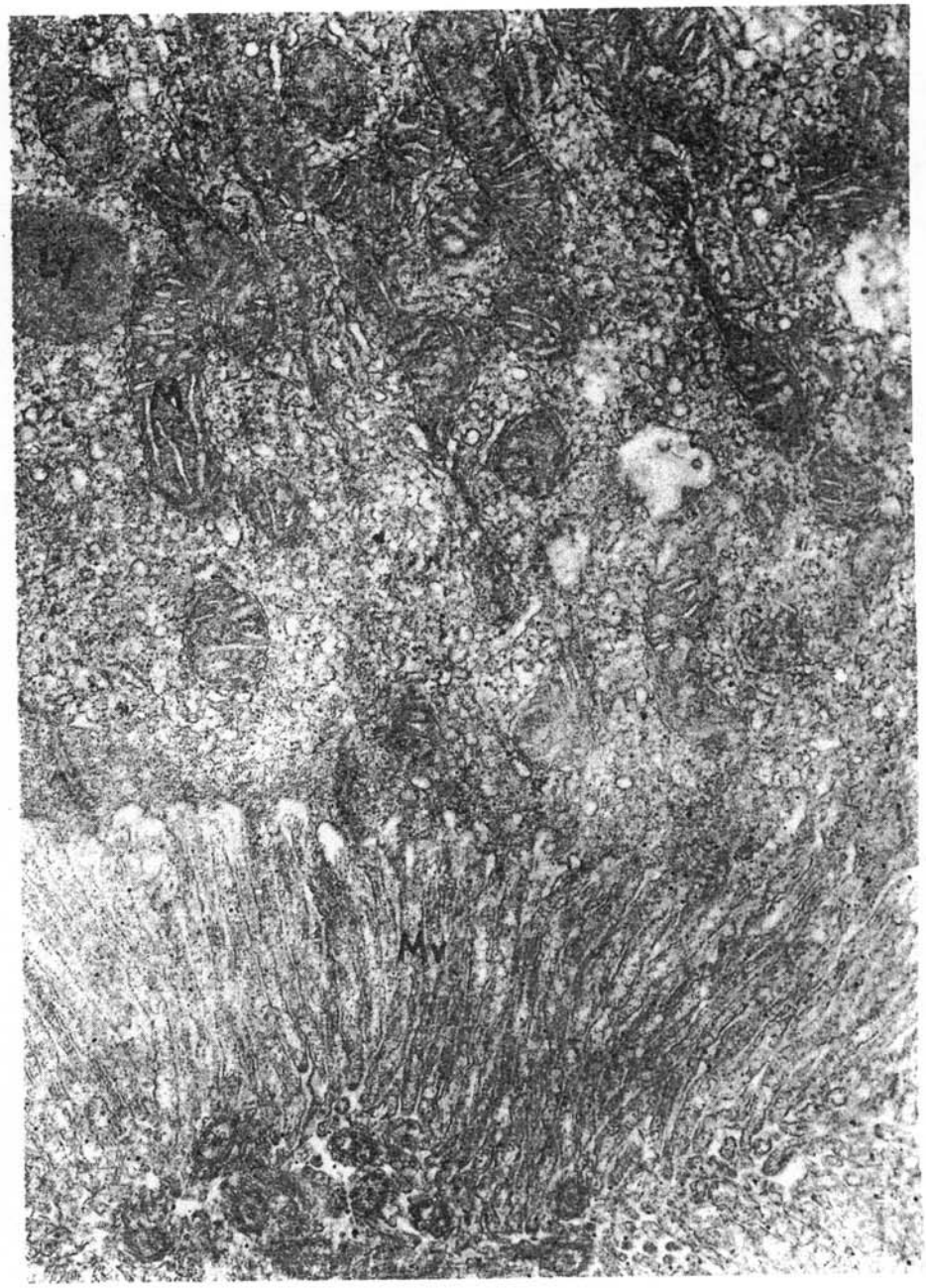

Fig. 11. Control group – kidneys. Coiled tubule of the I order. Magn. about 25000 x. M – mitochondrium, Ly – lysosone, Mu – microvilli

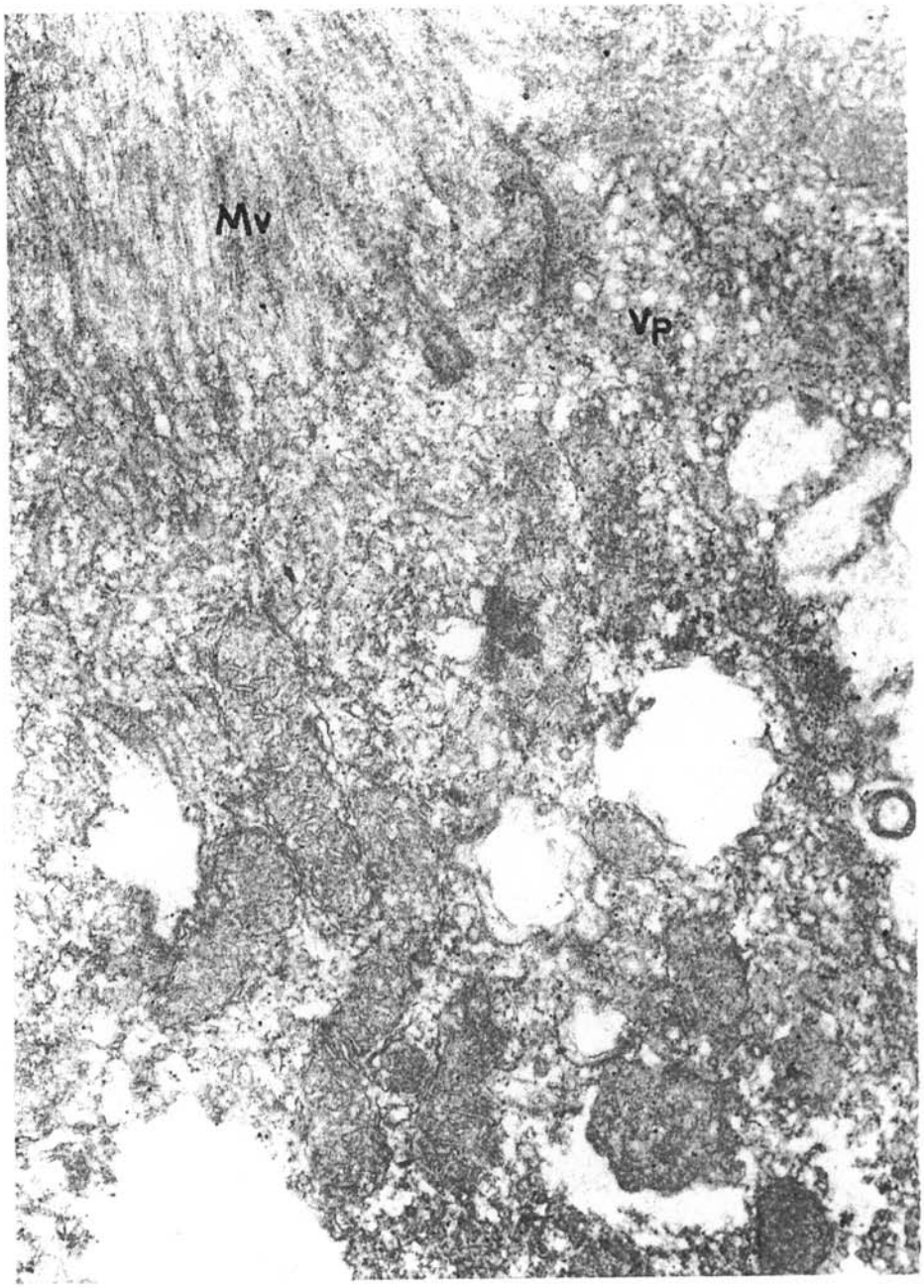

Fig. 12. Group I – kidneys. Coiled tubule of the I order. Pole part of the epithelial cell. Vacuolization of the cytoplasm, increased number of pinoytar vescicles. Magn. about 25000 x.
Vp – pinocytat vesicle, Mv – microvilli

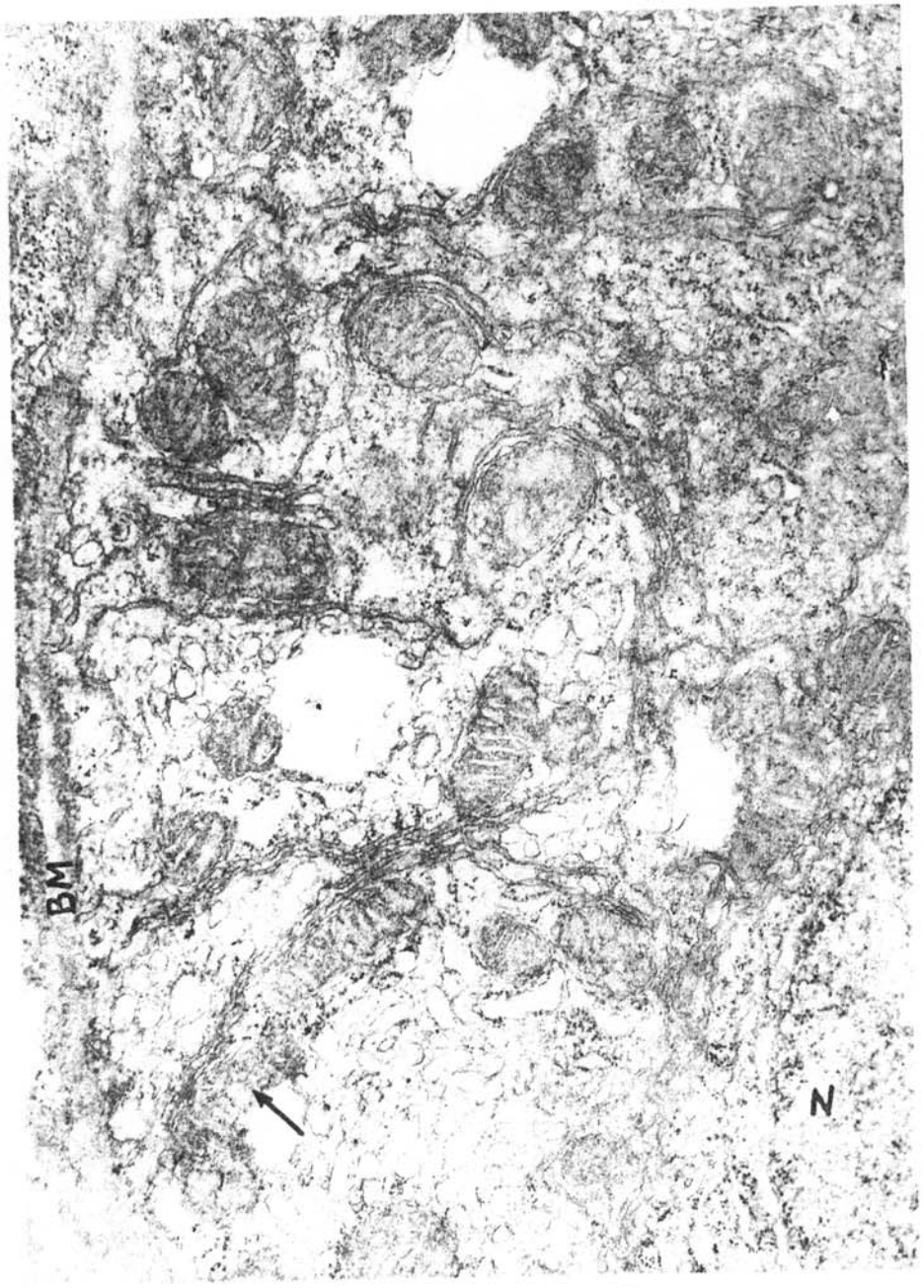

Fig. 13. Group I – kidneys. Coiled tubule of the I order. Basal part of epithelial cell. Cell membrane less convex, destroyed mitochondrium (arrow), vacuolization of the cytoplasm. Magn. about 25000 x. N – nucleus, Bm – basal membrane

tubule of the I order. It seemed that cell membranes were more concave than in group I and II. No other ultrastructural changes were noted.

DISCUSSION

Immersion of fishes from group I in a solution of methyl mercury chloride (CH_3HgCl) resulted in considerable accumulation of total mercury in the liver, kidneys and muscles (Tab. 1), but no clinical symptoms of fish poisoning were observed. These results agree with the literature data. Trouts fed feeds containing methyl mercury chloride accumulated 10 $mg \cdot kg^{-1}$ of total mercury in the muscles, although no clinical symptoms were noted (Wobeser, 1975).

In fishes from group II, exposed to mercury chloride ($HgCl_2$) in similar conditions as in group I, content of mercury in the muscles and liver was ten times lower (Tab. 1). Similarly as in group I, no symptoms of poisoning were noted. The results on the accumulation of organic (group I) and inorganic (group II) mercury agree with those obtained by other authors, who also found that methyl mercuric compounds were accumulated at higher rates than inorganic mercury (Hannerz, 1968; Kramer et Neidhart al., 1975; Middangh et Rose, 1974, Zanini et al., 1974, 1975).

Mercury accumulated in fish muscles did not result in any changes perceptible with histological methods in all fish groups under study (I, II, III). On the other hand, ultrastructural observations revealed perceptible changes. Most intensive changes were observed in fishes from group I, exposed to methyl mercury chloride. They were reflected by an enlargement of the canals in the smooth sarcoplasmatic reticulum and spaces between myofibre bundles, a disappearance of myofilaments and a destruction of some sarcomers. Similar changes were noted in fishes from group II, exposed to mercury chloride, but they were less extensive. Apart from these changes, some disturbances in the myofilament arrangement on both sides of the Z strip were observed in fishes from group II. In fishes from group III changes were limited to local enlargement of the canals of the smooth sarcoplasmatic reticulum and spaces between myofibres. These changes suggest that mercury concentration of about 1 $mg \cdot kg^{-1}$ (group II and III) in fish muscles result in some disturbances in the contraction activity. This is supported by changes observed in smooth sarcoplasmatic reticulum, which is responsible for accumulation and liberation of Ca^{+} ions during muscle contraction. Destruction of some myofibres might also disturb the transmission process. Similar mercury levels in fish muscles in group II and III (Tab. 1) were obtained after fish exposure to different mercury compounds and in different conditions. Consequently, range and character of changes induced in fish muscles were also different. It may be assumed that intensity and type of changes induced by mercury depended on many factors, such as type of the compound used, duration of exposure, mercury concentration, and other physico-chemical and biological factors which were not taken into account in the present study.

Histological studies of fish liver and kidneys revealed changes in all experimental groups of fishes, although their range and intensity were different. Congestion and steatosis of hepatocytes were observed, frequently leading into necrosis. Most intensive changes were observed in group I, less intensive in group II, while in group III changes were restricted to degenerative steatosis, and this was observed in 50% of fishes only.

Ultrastructural picture of hepatocytes suggests, however, toxic effect of mercury in all experimental groups of fishes. In all groups vacuolization of the cytoplasm was the most frequently observed symptom of this effect. Some changes were also noted in the granular intraplasmatic reticulum. It is possible that these changes were due to the affinity of mercury for sulfhydryl groups of protein. Similar changes of the granular intraplasmatic reticulum were observed in mice exposed to mercury chloride (Djaczenko, 1968). Other changes observed in the ultrastructure of hepatocytes were: destruction of the mitochondria, presence of myelin structures, increased number of secondary lysosomes, enlargement and proliferation of smooth intraplasmatic reticulum. Changes within mitochondria and vacuolization of the cytoplasm suggest parenchymal dimness in the cells, and result from toxic effect of mercury, which disturbed cell respiration and functioning of hepatic cells responsible for protein biosynthesis. Considerable extent of these changes in reflected by a destruction of the mitochondrial membranes; such changes being usually irreversible. Appearance of many secondary lysosomes, with symptoms of advanced autolysis, must lead to cell destruction. As results from the literature, similar ultrastructural changes were observed in mouse and cat hepatocytes under the effect of methyl mercury (Chang et Yamaguchi, 1974; Pekkanen et Lindberg, 1972). Presence of myelin structures, which are formed when protein – lipid balance is disturbed, also points to advanced degenerative changes. Degenerative changes in liver cells of fishes from group I were additionally characterized by the presence of free collagen fibres in the hepatocyte cytoplasm and in the intercellular spaces. Hence, process of fibrosis extended over the whole organ. In pathologic states of the liver, intracellular collagen fibres are usually surrounded by vacuole membrane. There were, however, some papers on the presence of free collagen in human hepatocytes in cases of chronic liver diseases (Gerlach et al., 1969; Gmelin et al., 1973; Groniowski et Walski 1975). Occurrance of free collagen was never fully explained, but it might result from disturbances in protein synthesis. This is also suggested by the presence of monosomic ribosomes in the hepatocyte cytoplasm in the experimental fishes, and total lack of the polysomes.

Histological changes in fish kidneys, consisting of parenchymatic degeneration of the epithelial cells of the proximal tubules, and symptoms of necrosis, suggest toxic effect of mercuric compounds. These changes were observed in all experimental groups of fishes, but they were most pronounced in group I. This fact points to high toxicity of methyl mercury chloride. Electrone microscopy showed that changes in the epithelial cells of the coiled tubule of the I order involved vacuoles, lysosomes and mitochondria in all experimental fishes, but they were least noticeable in group III. Note should be made of

increased number of secondary lysosomes in group I and II compared to the control. Increased number of vacuoles, resulting from digestive action of the lysosomes, leads to cell necrosis. In fishes from group II changes in the mitochondria consisted of the occurrance of lighter spaces in the matrix, but the mitochondrial membranes remained unbroken. On the other hand, in group I of fishes, mitochondrial membranes were sometimes destroyed. Similarly as in case of liver, changes observed in the epithelium of the uriniferous tubules might disturb cell respiration.

Scarce data on histological changes in fishes under the effect of methyl mercury chloride suggest that in case of *Ictalurus punctatus* intraperitoneal injection of this compound results in peeling off of the epithelium of proximal tubule in kidneys (Kendall, 1975). In rainbow trout 15-week feeding with methyl mercury chloride resulted only in slight swelling of the epithelial cells in Browman's capsules (Kendall, 1975).

Basing on the results obtained in course of the present study it can be stated that mercury levels found in the muscles of fish originating from mercury contaminated waters (about 10 $mg \cdot kg^{-1}$) and from the Vistula (up to 1.58 $mg \cdot kg^{-1}$), defined as the highest permissible for consumption (0.5–1.5 mg·kg), were toxic to fishes. Toxicity of mercury can be defined from changes in fish organs (liver, kidneys) observed during histological and ultrastructural studies. On the other hand, changes in the muscles can be defined during ultrastructural studies only.

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M. STUDNICKA

BADANIA HISTOLOGICZNE I ULTRASTRUKTURALNE MIĘŚNI, WĄTROBY I NEREK SUMIKÓW KARŁOWATYCH (*ICTALURUS NEBULOSUS*) PO DOŚWIADCZALNYM SKAŻENIU ZWIĄZKAMI RTĘCI

STRESZCZENIE

Sumiki karłowate (Ictalurus nebulosus) kąpano w roztworach wodnych CH_3HgCl i $HgCl_2$, a następnie przetrzymywano przez okres 3 tygodni w czystej wodzie bieżącej. Po tym czasie pobierano skrawki mięśni, wątroby i nerek do badań histologicznych i ultrastrukturalnych.

W budowie tkanki mięśniowej histologicznie nie stwierdzono zmian, natomiast w obrazie ultrastrukturalnym obserwowano poszerzenie kanałów gładkiej siatki sarkoplazmatycznej, zanik częściowy lub całkowity miofilamentów, ponadto u ryb, na które działano $HgCl_2$ wystąpiły zaburzenia w układzie miofilamentów po obu stronach prążka Z.

W wątrobie i nerkach badaniem histologicznym stwierdzono zmiany zwyrodnieniowe, a w ultrastrukturze komórek tych narządów obserwowano wakuolizację cytoplazmy, zwiększoną ilość lizosomów wtórnych oraz uszkodzenia mitochondrii.

М. Студницка

ГИСТОЛОГИЧЕСКИЕ И УЛЬТРАСТРУКТУРНЫЕ ИССЛЕДОВАНИЯ МЫШЦ, ПЕЧЕНИ И ПОЧЕК СОМИКА ICTALURUS NEBULOSUS ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОГО ЗАРАЖЕНИЯ СОЕДИНЕНИЯМИ РТУТИ

Резюме

Сомиков (Ictalurus nebulosus) погружали в водяных растворах CH_3HgCl и $HgCl_2$ а потом в течение 3 недель выдерживали в чистой проточной воде. По истечении этого срока изымали кусочки мышц, печени и почек для гистологических и ультраструктурных исследований. Гистологически не обнаружили изменений в строении мышечной ткани, но в ультраструктурной картине наблюдали расширение каналов гладкой эндоплазматической сети, частичное или полное исчезновение миофиламентов, кроме того у рыб подвергнутых действию $HgCl_2$ проявились нарушения в структуре миофиламентов с обеих сторон полосы Z. В печени и почках гистологическими исследованиями нашли дегенеративные изменения, а в ультраструктуре клеток этих органов наблюдали вакуолизацию цитоплазмы, увеличенное количество вторичных лизосомов а также повреждения митохондрии.

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