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ARTICLE

# Giant Mitochondria as Possible Bioindicators of Environmental Injuries in Fish Liver

István BENEDECZKY and János NEMCSÓK

Department of Biochemistry, Attila József University, Szeged, Hungary

**The effect of hypoxia (80 pHg) and simultaneously applied paraquat (1,1'-dimethyl-4,4-bipyridynum dichloride) was investigated on carp liver using electron microscopic methods. The appearance of giant mitochondria was the most conspicuous alteration in the liver cells. Most of the giant mitochondria were elongated and rod-shaped, often arranged side by side forming clusters beside the nucleus. Crook-like and irregular forms also occured among giant mitochondria. The lenght of the giant mitochondria often was greater than the diameter of nucleus: namely 5-10 μm. The outer membrane of the giant mitochondria was well preserved, but inner membranes (cristae) were usually absent, and a high density matrix filled in the inner space of mitochondria. High power magnification often revealed a regular, filamentous paracristal arrangement in the dense material of the matrix. Swollen giant mitochondria with light matrix and tubular elements also occured in low number. Although fine structural characteristics of carp liver giant mitochondria are not specific for inducing agents (hypoxia + paraquat treatment) the appearance of altered giant mitochondria may be a useful signal for monitoring cell damaging enviromental xenobiotics.** (Pathology Oncology Research Vol 3, No 1, 20–25, 1997)

*Key words*: giant mitochondria, fish liver, herbicide (paraquat), electron microscopic cytopathology

## *Introduction*

Morgan-Hughes[1] discovery that some human muscle diseases are directly connected with mitochondrial deficiences provided a new impulse for mitochondrial research. Among mitochondrial alterations, "giant mitochondria" have been frequently found in the cells[2,3,4,5]. According to Tandler and Hoppel,[2] giant mitochondria may occur in different human diseases, in experimentally induced mitochondrial deficiency, in drug-treated animals and following after alcohol-abuse and hormon injections, as well. Most of these studies were carried out on human patients and different mammalian species, so data, on other animals are very rare. Since there do not seem to be systematic electron microscopic studies concerning fish tissues after treatment with different pesticides and herbicides, we performed several experiments to see the histopathological effects of these drugs. Among several chemical agents, only paraquat induced the formation of giant mitochondria (in carp liver, in combination with hypoxia). The fine structural characteristics of these giant mitochondria will be described in this paper.

## *Material and methods*

Male and female carp, with weights of 1200-1500 g were used. After anesthesia with MS 222, a cannula was introduced into the dorsal aorta. Three days after this operation the fish were put into a closed experimental box where pH, temperature and partial oxygen pressure were regulated. After adaptation for 3 days, the fish were divided into two groups. In the first group a 6h period of hypoxia was applied (80 pHg oxygen) daily for 5 days. In the second group, hypoxia was applied as above and on the first day 2.5 μg paraquat (Gramoxone with a PQ content of 25%) was injected via the cannula. For comparative purposes, a control group, not subjected to either treatment mode was also used.

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*Correspondence*: István BENEDECZKY, D.Sc., Otelló köz 7, H-2000 Szentendre, Hungary; Tel: (36) (26) 300 591

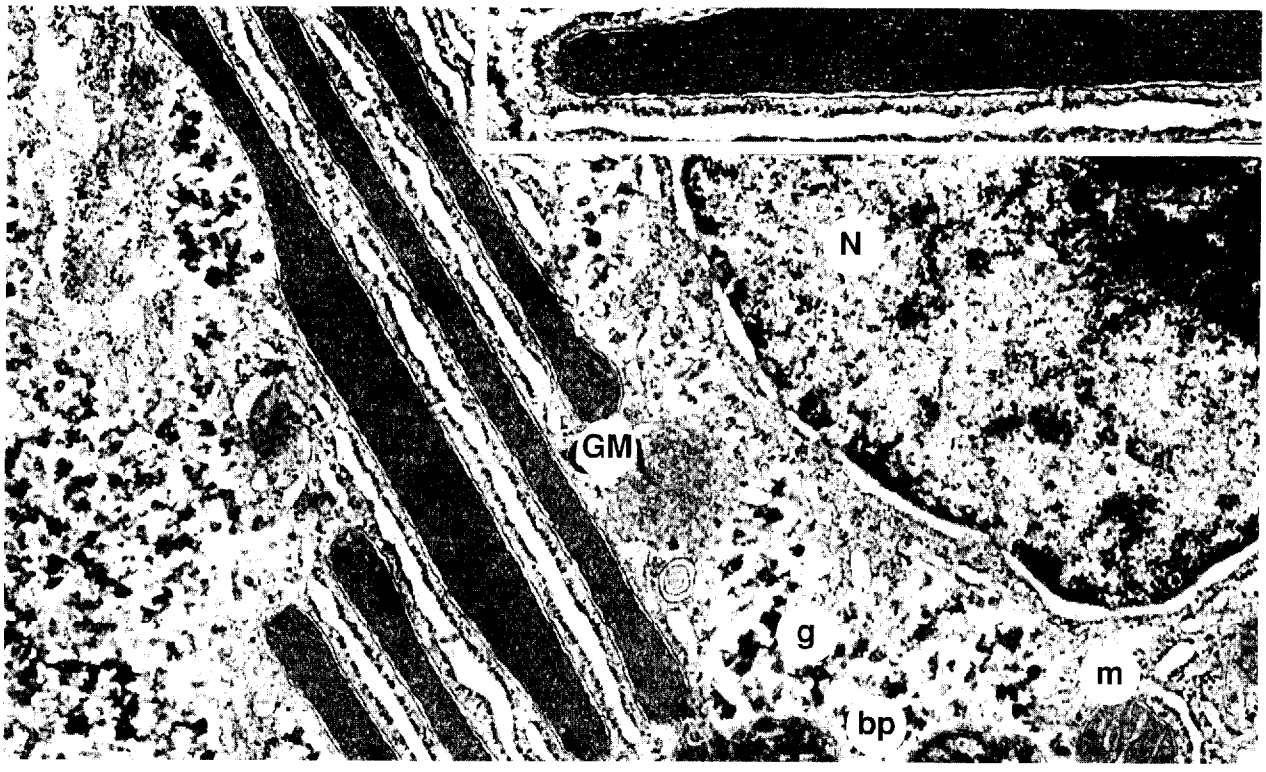


*Figure 1. Detail of a hepatocyte after hypoxia and paraquat treatment. N - nucleus, GM - giant mitochondria, m - mitochondria, g - glycogen, bp=bile pigment, Insert: paracrystals in the matrix of giant mitochondria ×15.000 and ×20.000.*

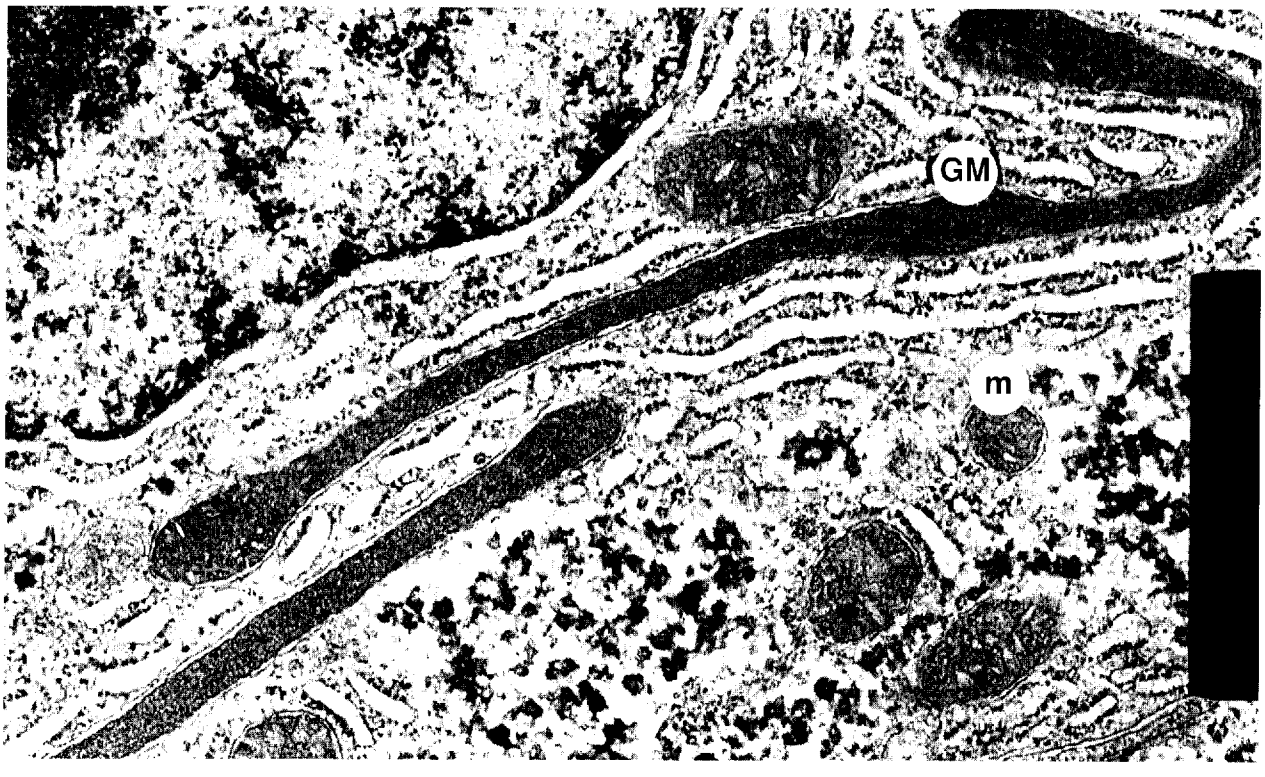


*Figure 2. The same as Fig.1. Note a crook-like giant mitochondria (GM) beside normal mitochondria (m). ×20.000.*

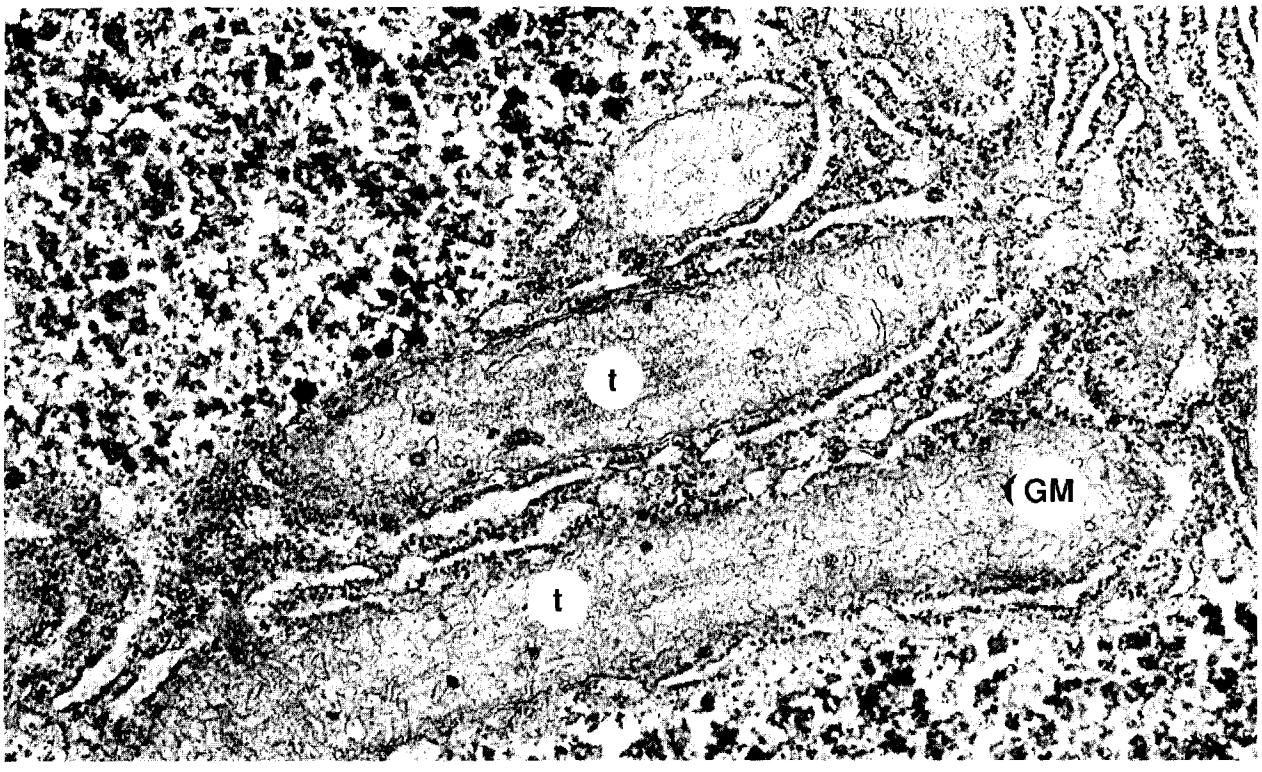


*Figure 3. Giant mitochondria (GM) with electron transparent matrix, and tubules (t), in a hepatocyte, after hypoxia and paraquat treatment. ×20.000.*

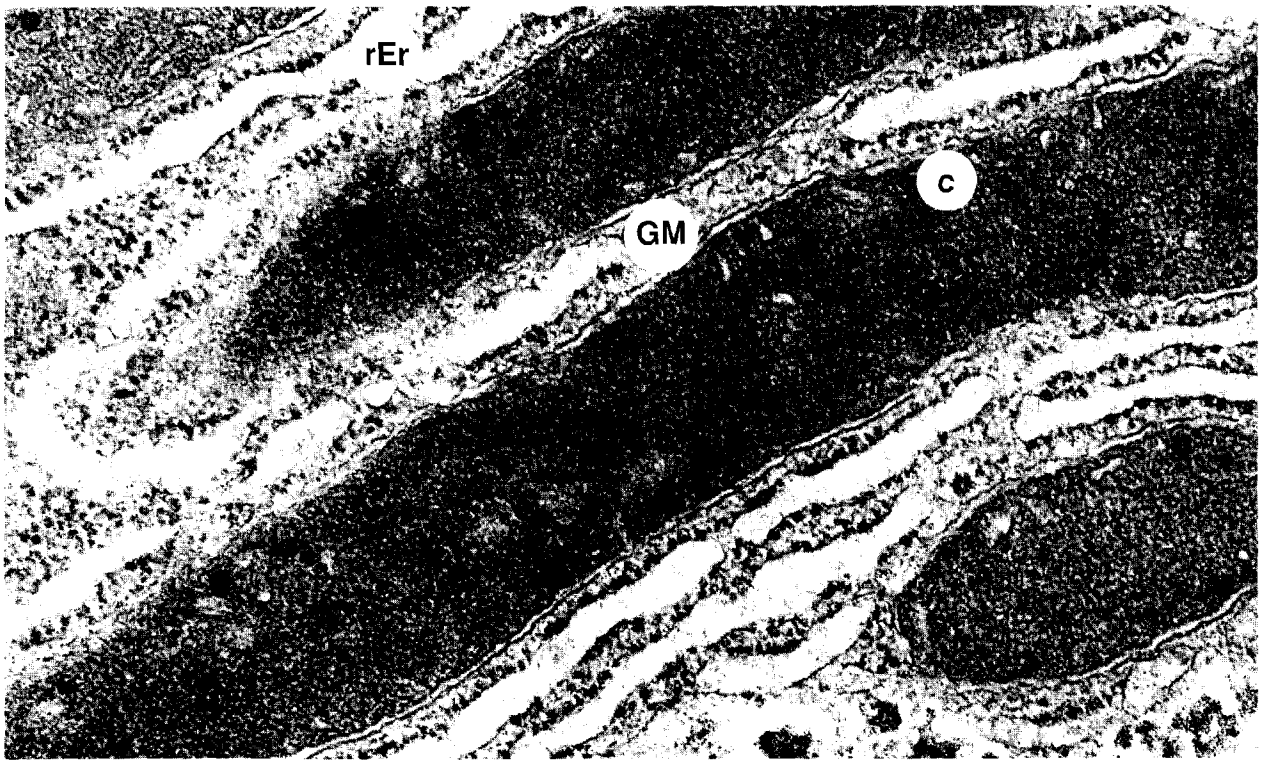


*Figure 4. Giant mitochondria (GM) with dense matrix and remnants vesicular cristae. (c). rEr - closely attached rough surfaced endoplasmic reticulum. ×40.000.*

On the fifth day after treatment, animals were sacrificed and small pieces (about 1 $mm^3$) were excised from the liver and kidney. Specimens were fixed in a cold fixative containing 4% paraformaldehyde and 2.5% glutaraldehyde for 24 h. Osmium tetroxyde fixation was performed for a further 2h. After the dehydration procedures, samples were embedded in Durcupan ACM resin. The ultra-thin sections were contrasted with lead and examined by electron microscope.

### *Results*

*Carp liver after hypoxia and paraquat treatment*

Most of the giant mitochondria were found in a parallel arrangement clustered in the neighbourhood of the nucleus (*Fig.1*) although such clusters occur also in the vicinity of the plasma membrane. Solitary giant mitochondria were quite rare in the liver cells. A great majority of the giant mitochondria preserved a rod-shaped form (*Figs.1-5*) in the hepatocytes, however crook shaped (*Fig.2*) and irregular forms (*Fig.5*) were also found in low number. In addition to giant mitochondria, "normal" mitochondria also occurred in the liver cells in a significant number (*Figs.1-2*). Lenght of giant mitochondria often was higher than the diameter of the nucleus, namely: 5-10 μm.

The outer membrane of giant mitochondria was usually well preserved, but inner membranes (cristae) were absent or fragmented, and a high density matrix filled the inner space of the mitochondria (*Figs.1,2,4,5*). High power magnification often revealed a regular, filamentous paracristal deposit in the matrix of giant mitochondria (*Figs.1, 1 insert, 4,5*). Swollen giant mitochondria, with an electron transparent matrix also occurred in low number in a few hepatocytes (*Fig.3*). More of less cristae, and parallely arranged tubular elements were recognized in the matrix of these "light" giant mitochondria.

Noteworthy, that long tubules of closely attached rough surfaced endoplasmic reticulum have been always observed around giant mitochondria in the hepatocytes (*Fig.4.*). At the same time however, we could not detect giant mitochondria in the control and only hypoxia treated carp liver.

*Wild carp studies (Lake Balaton, Hungary)*

Liver cells (*Fig.6.*) showed several cytopathological alterations in their cytoplasm.

Glycogen-poor areas, collapsed Golgi apparatus, myelin figures and demaged mitochondria were frequently seen in the cells. Most of the mitochondria were swollen and fragmanted cristae occurred in the light mitochondrial matrix.

### *Discussion*

Oceans, lakes and jungles are the most stable selfregulatory exosystems. These have become dangerously unstable in the last decades as a consequence of the accumulation and spread of anthropogenic chemical pollutants. Water soluble xenobiotics reach rivers and lakes, bind to epithelial cells of fish by bronchial, oral and dermal absorption, and penetrate into the tissues trans - and paracellularly.[6] Chemicals from epithelial cells can diffuse into the blood because cell membranes are permeable to most of xenobiotics.[7] Blood circulation transports the xenobiotics into the organs where they may become concentrated.[8] Accumulated chemicals can act as toxic agents and, in combination with unfavourable environmental circumstances (high temperature, low oxygen content, eutrophication), lead to catastrophic fish kills in many cases.[9] In Hungary, 20-30 severe fish kills occur annually as a consequence of water pollution.[10,11] Classical toxicological, as well scientist applied histopathological, biochemical and physiological methods have been used to determine the "reasons" for these fish kills. In the course of biochemical studies[12,13,14,15,16,17,18] it has been established that all pesticides cause organospecific tissue necrosis as demonstrated by increased level of lactate dehydrogenase activity in blood plasma.[19] Paraquat significantly increased transaminase and lactate dehydrogenase activity in the sera.[19] All these data suggest that paraquat demages the liver and kidney, reduces $O_2$ uptake by injuring gill epithelium and can cause functional defects in the nervous- and other tissues inhibiting acetylcholin esterase activity.[19]

Our tandem electron microscopic studies have shown that paraquat alone did not produce significant cytopathological effects in the liver. However extensive degenerative alterations were found in the exocrine pancreas.[11] Hypoxia (80 pHg) and simultaneously applied paraquat caused several cytopathological alterations also in the liver[20] but the appearance of giant mitochondria was the most conspicuous phenomenon in the parenchyma cells. What is the reason and significance of this structural alteration? A large amount of data suggests that formation of giant mitochondria can be induced with many different chemical substances[2] therefore "structural changes in mitochondria are not specific markers for underlysing biochemical defects".[21]

However, it is known [19,22] that paraquat

1. decreases the NADH concentration in the cells and mitochondria, inhibits the synthesis of fatty acids and compound lipids;

2. injures the cell membrane by lipid peroxidation which results in the loss of cell integrity;

3. uncouples mitochondrial electron transfer arresting energy supply to cells.[19,22]

The above mentioned effects of paraquat may lead to the formation of giant mitochondria in liver cells after hypoxia

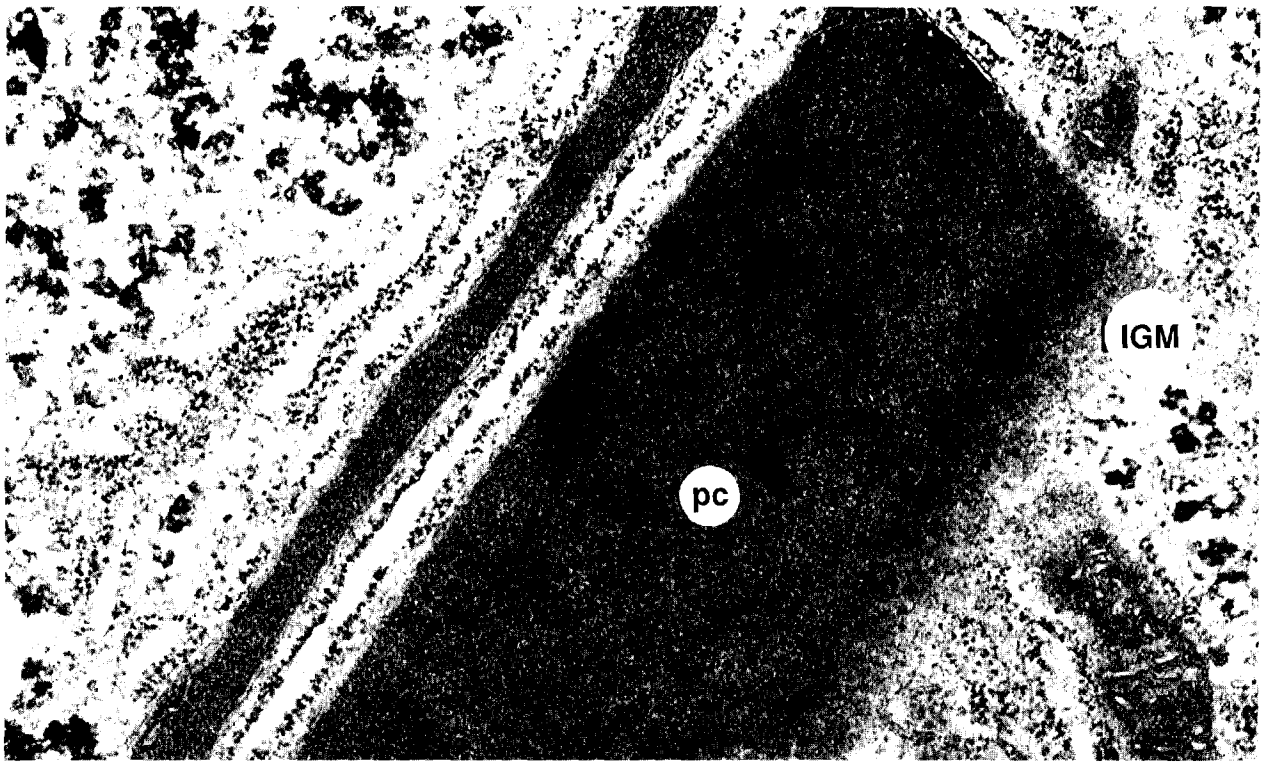


*Figure 5. Rarely, irregular giant mitochondria (IGM) also occured in the hepatocytes, after hypoxia and paraquat treatment. Paracrystals (pc) are accumulated in the dense mitochondrial matrix. ×20.000.*

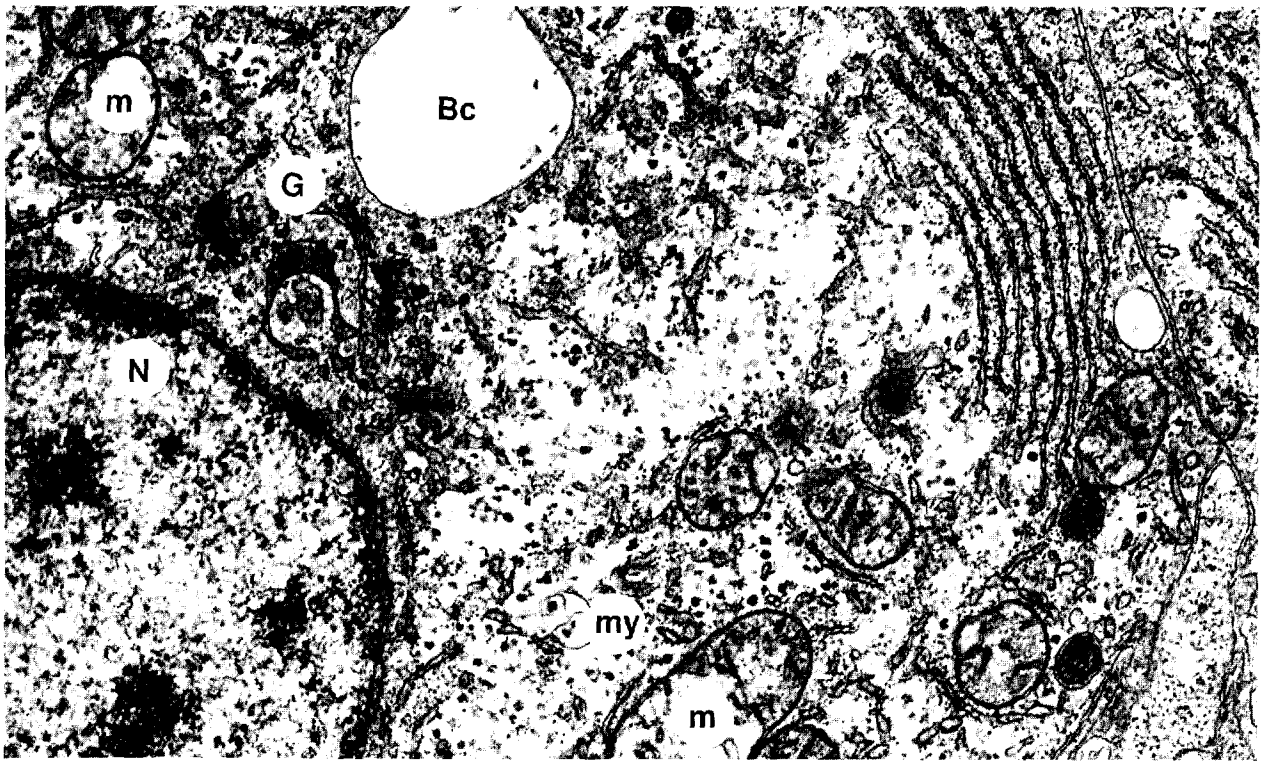


*Figure 6. Cytoplasm detail, from an untreated carp liver cell. N=nucleus. Most of mitochondria (m), are damaged. Myelin figure (my), collapsed Golgi apparatus (G) can be seen in the cells. Bc= bile canalicules. ×15.000.*

and paraquat treatment. Considering this conclusion, we may suppose that the appearance of giant mitochondria may represent those biochemical alterations in the cells which are closely related to the decrease in NADH concentration, membrane injuries by lipid peroxidation and uncoupling of mitochondrial electron transfer. Assuming that giant mitochondria represent a sensitive cellular, the presence of cell demaging agents in the aquatic environment several questions await for further research. First, among these is to clarify the exact mechanism of the formation of giant mitochondria.

Our present observation, that formation of giant mitochondria can be induced by certain chemical substances, supports the "old" theory: some drugs (as paraquat) inhibit division of mitochondria. At the same time, protein synthesis seems to be unaltered, thus the increased amount of protein simply distends the outer mitochondrial membrane. The protein nature of inner mitochondrial cristalloids was proven long time ago.[23] A recently published human case report[4] indicates that the disorders of mitochondrial oxidative phosphorylation and morphology; i.e. giant mitochondria, accompany cardiomyopathy. This suggests that studies of giant mitochondria can help in the identification of human and animal diseases as well. Our unpublished data[24] on cytopathological alterations occurring in the livers of randlomly caught carps indicate the importance of systematic histopathological studies on fish in polluted freshwater lakes such as Lake Balaton, Hungary.

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