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Chapter 10

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Structural Lesions of Tissues and Cells Influenced by Environmental Pollution

Veterinary pathomorphology deals with lesions in the structure of tissues and cells in animals. This branch of science has always played a cognitive role. As in human medicine, its main purpose is to determine morphological changes, which makes it possible to determine the cause of deaths. This alone justifies the claim that pathomorphology is the basis for the progress of veterinary science.

It is noteworthy that as early as the 19^{th} century, post-mortal diagnosis of changes and diseases by macroscopic examination were joined by cell pathomorphology and in the 20^{th} century by clinical pathology, employing methods which enable diagnosis on live organisms, such as biopsies. Nowadays, live examination of tissues and organs morphology is common. This enables a clinician to start effective therapy and such an examination, when done in cases of doubtful or unfavourable prognosis, helps to make a decision on whether or not to perform euthanasia. Recently, histochemistry, immunopathology and molecular biology have been developing within the domain of pathomorphology. Examination techniques are being constantly improved which increases the diagnostic and prognostic capabilities (which is especially noticeable in oncology).

When using pathomorphological methods of examination, one should bear in mind that most long-term functional disorders are reflected in morphological changes. It is also important that each permanent morphological change entails functional changes. Based on these relationships, morphological diagnostics can determine:

- changes expressed in the transformations of cell structure,
- irregularities in cell arrangement,
- changes in the intercellular matrix.

It also allows for identification of the aetiological factor which has brought about the disease (e.g. bacteria, parasites). There are also cases when owing to a combination of microscopic method and chemical staining (so-called “histochemical technique”) it is possible to detect various substances in cells and tissues, to distinguish between them and to determine their levels. One example of such a procedure might be the staining of sections of organs by the PAS method according to McManus, which can be used to detect glycosaminoglycans.

These facts show that towards the end of the 19th century, pathomorphology was being used to demonstrate the effect of the environment on the health status of various animal species. Fish account for a large portion of such animals. Pathomorphological analysis is now becoming a useful tool in fish health assessment. The progress of changes, together with their distribution, determines the type of stimulus (e.g. a xenobiotic), its force and even the time of its effect. Such changes are determined in a microscopic examination, using hepatopancreas, and less frequently kidneys, spleen or gills. In the interpretation of such changes, it is important to indicate where the changes are situated. If their location is close to blood vessels, they may have been caused by environmental factors; if away from them, then the cause will be internal. It is noteworthy that retrogressive changes (degenerations of different kinds and necroses) found in hepatocytes of carp and situated close to blood vessels indicate the effect of environmental xenobiotics. Such changes in hepatopancreas are frequently accompanied by circulation disorders.

Pathomorphological analysis can be used to search effect of xenobiotics on animal organisms in natural environment. It is sensitive tool to search polluted ecosystems in this way. We used this kind of evaluation taking it under consideration to area situated close to PT presented in chapter 5. The PT was removed in 2004. A question was raised whether its negative effect on the environment still existed. The aim of this study was to compare the effect of the PT during the last years of its existence to its effect 3 years after it had been removed on carp fingerling living in a pond situated 100 meters away from the studied PT.

The study was performed in the period of 2002 – 2003 (before the PT removal) and in 2007 – 2008 (after its removal). During these periods, 10 carp fingerling (65 – 85 g) were caught each year (40 carp in total – group 1 fish caught in 2002 – 2003, group 2 – fish caught in 2007 - 2008) from the fish breeding pond located 100 m from the PT No. 1. The control group constituted the same number of fish obtained from pond No. 2 situated 2 km to the west of the PT: group 3 – fish caught in 2002 – 2003, group 4 – fish caught in 2007 – 2008.

Macroscopic examinations of 80 carp fish were carried out directly after the fish were caught and sacrificed. Hepatopancreas and kidney sections were used for histopathological examinations. The samples were taken from three different places of these organs and were fixed in 7% neutralized formalin. Paraffin sections (480) were stained with hematoxylin and eosin (HE). Moreover, the hepatopancreas samples were also stained with Sudan III using Lillie Ashburn’s method and by the PAS method according to McManus. The number of microscopic lesions observed in the examined organs was analyzed statistically. The Student *t*-test was used for the dependent samples to compare the mean values for the same groups in different years, and the mean values for different groups in the same years ("Statistica PL" software by StatSoft).

In the macroscopic examination only insignificant changes were found in group 2 carps. There were three cases hyperaemia of the gills, two cases of parenchymatous degeneration of the hepatopancreas and one liver hyperaemia observed.

However, microscopic examination of the HE stained hepatopancreas sections revealed morphological discrepancies from the norm in fish representing all four groups (Table 1). In groups 1 and 2, the retrogressive lesions dominated. Parenchymatous degeneration was present in over half of the carps of these groups (Table 1, Figures 1, 2). In the majority of cases it concerned the hepatocytes situated close to blood vessels. These areas were relatively small, they covered about a dozen parenchymatous degeneration hepatocytes and were scarcely visible over the entire visual field. They were of limited character and were sometimes accompanied by necrosis (Figures 3, 4). This lesion did not affect more than a few areas of up to a dozen cells. The described lesions almost never concerned three sections originating from one fish; they were found most often in a single preparation.

Almost all fish from group 1 and 2 characterized by present of small lipids vacuoles in hepatocytes (Figures 1, 2, 5). It was physiological lesion (steatosis simplex). Circulatory disturbances were present in 30 % of group 1 carps. They were reflected in the form of a small hyperaemia and limited extravasations of blood in the form of petechias in three cases (Figures 1, 5). These changes in group 2 fish were more explicit and concerned almost half of the fish (Table 1, Figures 3, 4).

Inflammatory lesions were sporadic in both group 1 and 2 carps (Table 1). Small, often limited infiltrations of mononuclear cells were observed (Figure 3). In the majority of cases they were situated in the vicinity of blood vessels. Such changes were slightly more common in group 2 carps (Figure 2).

Progressive lesions were also rarely determined. In five cases they were expressed by hyperplasia of stellate cells and in three cases by hyperplasia of connective tissue (Figure 2). Such changes were dominant in the hepatopancreas of the fish caught in the vicinity of the PT before and after its removal.

Single melanomacrophages or their centers were stated occasionally in hepatopancreas of fish groups 1 and 2 (Figure 4).

Relatively few morphological lesions were observed in the hepatopancreas preparations originating from the control carps from pond No. 2 (group 3 and 4) (Table 1, Figure 6). A similar distribution of changes in respect to their number and intensity was found for the particular years of study. Retrogressive lesions and circulatory disturbances were observed rarely and other changes were scarce. These changes were clearly limited and frequently they were observed only in one of every three sections examined.

Steatosis simplex or rarer steatosis degenerativa were found in the Sudan III-stained hepatopancreas sections of the fish from all four groups.

Relatively uneven distribution of glycosaminoglycans was observed in group 1 and 2 hepatopancreas sections stained by PAS method according to McManus. Frequently, large clusters of hepatocytes had low levels of these substances; their trace amounts were found in their cytoplasm. PAS+ grain-like structures clearly varied in size also occurred, which varied from fine to large grains.

In contrast to the above image, the control carp hepatopancreas was abundant with glycosaminoglycans. In the majority of cases, the hepatocyte cytoplasm was intensively purple which indicates the presence of the above substances.

Table 1

Microscopic changes in the hepatopancreas of carps bred in a pond situated in the vicinity of a PT and in a control one in 2002 – 2003 and 2007 – 2008

Type of lesion	The number of lesions in the hepatopancreas of carps bred in:							
	pond No. 1 (situated in the vicinity of a PT) in				pond No. 2 (control pond) in			
	2002	2003	2007	2008	2002	2003	2007	2008
	Group 1		Group 2		Group 3		Group 4	
regressive	5	7^{a}	8^{b}	8^{c}	2	3^{a}	2^{b}	3^{c}
circulatory perturbations	3	3	6^{d}	4	4	3	3^{d}	2
inflammation	2	1	3	2	1	2	3	1
progressive	1	3	2	2	0	1	1	0
Total in each year	11^{ef}	14^{g}	19^{efg}	16^{h}	7^{eh}	9^{gc}	9^{gf}	6^{h}
Total	before PT removal		after PT removal		16^{i}		14^{j}	
	25^{i}		35^{j}					
Total in examination period	60^{k}				30^{k}			

aa – pairs of the same letters indicate results which differ statistically at $p < 0.05$

In the kidney preparations of carps caught in pond No. 1, the retrogressive and progressive lesions were relatively most frequent among the observed morphological discrepancies from the norm (Table 2). These lesions less frequently occurred in the kidney preparations obtained from group 3 and 4 fish (Table 2). In both cases, parenchymatous degeneration of renal tubule epithelium was most frequent (Figures 7 - 11. The analysis of the sections revealed lumen narrowing in the tubules with epithelial swelling. Sometimes, hyaline deposits were present in the tubular lumen and the tubular cells looked as if they had undergone granular degeneration. Necrosis of tubular epithelial cells were observed sporadically in fish of groups 1 and 2 (Figure 10). These retrogressive lesions were sometimes accompanied by hyperaemia, and petechia and vibices were observed in two cases in group 2 fish (Figures 8, 9).

Hepatopancreas proved to be an organ which is more susceptible to the formation of morphological changes than the kidney. In the hepatopancreas of fish bred in the pond situated in the vicinity of a TP, the number of morphological changes was 35% higher than the number of such changes found in the kidneys. Moreover, the number of the mentioned disturbances in the hepatopancreas of a control group fish (3 and 4) was similar to the number of changes recorded in the kidneys of the same fish. Single melanomacrophages or their centers were more often stated in hepatopancreas than in kidneys. It can be concluded that the hepatopancreas of carps is more susceptible to structural damages formed as the result of environmental factors. This fact was also confirmed by the results obtained from the carps caught in 2004 and in 2006 within the same are. The same dependence was also found in grey herons and black-striped field mouse (*Apodemus agrarius,* Pallas 1771) living in the vicinity of the PT. Other authors (Depledge, Fossi 1994; Schramm et al. 1998; Schweiger et al. 1997; Triebskorn et al. 1997) have also pointed out the particular susceptibility of hepatopancreas to xenobiotics in varied animals. The positive role of biomarkers was also underlined in the literature.

Table 2

The number of lesions in the kidneys of carps bred in a pond situated in the vicinity of a PT and in control one in 2002 – 2003 and 2007 – 2008

Type of lesion	The number of lesions in the kidneys of carps bred in:							
	pond No 1 (situated in the vicinity of a PT) in				pond No 1 (situated 2 km from PT) in			
	2002	2003	2007	2008	2002	2003	2007	2008
	Group 1		Group 2		Group 3		Group 4	
regressive	3	5	6	4	2	4	3	3
circulatory perturbations	1	3	5	3	1	2	3	1
inflammation	0	1	0	1	0	1	1	0
progressive	2	2	2	1	1	2	2	2
Total in each year	6^{a}	11^{ab}	13^{ab}	9	4^{c}	9^{c}	7	6
Total	before PT removal		after PT removal		13^{d}		13^{de}	
	17^{d}		22^{de}					
Total in examination period	39^{f}				26^{f}			

aa – pairs of the same letters indicate results which differ statistically at $p < 0.05$

Among the morphological lesions occurring in the studied organs, the retrogressive changes were the most frequent followed by the circulatory disturbances, especially in group 1 and 2 carps. Other changes were observed considerably less often. Similar tendencies were also observed in the previous studies on animals populating the vicinity of the PT. Parenchymatous degeneration was dominant among the retrogressive lesions and hyperemia was dominant among the circulatory disturbances. Within the range of the discrepancies from the norm in group 1 and 2 fish, adaptation changes were found next to the destructive changes.

The variety of the number of morphological lesions in the studied organs and their intensity is significant. These values were statistically significantly greater in the carps caught in the pond in the vicinity of the PT in comparison to the control fish. The number of all the morphological changes in the hepatopancreas was twice as many in the group 1 and 2 carps than in the group 3 and 4 carps.

The described variety of the morphological lesions and their character and intensity indicates that they could have been caused by the natural environment in which the fish lived. It is therefore justified that the pesticide dump may have had an effect on the development of these changes.

It is also important to mention that the present study described a wide-range observations from four years with a three-year interval. In this period (in 2004), the PT was removed and the study also involved carps bred 3 and 4 years after its removal. After the removal of the PT, the morphological lesions were found to intensify in the carps took to examination in this period. Their number increased statistically significantly in both the hepatopancreas and in the kidneys in comparison to the fish studied before the removal of the PT, i.e. in 2002 and 2003. At that time, the number of changes found in the hepatopancreas of the fish caught in the pond situated in the vicinity of the PT was over twice as high as in the control fish.

The increase in the intensity of the microscopic lesions in the studied organs of the carps 3 and 4 years after the removal of the PT could be explained by a secondary contamination of the environment, which occurred at the time of digging the xenobiotics from underground. This activity temporarily increased the concentration of DDT, its metabolites and heavy metals in the already polluted environment. Carps bred in the pond situated in the contaminated area responded to the increase in the intensity of morphological clesions, especially in their hepatopancreas, which proved to be a good biomarker. Based on the study, it can also be concluded that the three-year period after the removal of the PT was too short to eliminate the effect of this contamination source from the natural environment which existed there for 30 years.

It is significant that both in the mesenteric fat and the muscular fat greater levels of DDT and its metabolites were found in the carps caught in the pond situated in the vicinity of the PT than in the carps obtained from the pond located approx. 2 km away from the source of those xenobiotics.

On the base of our pathomorphological examination we can conclude that:

1. The pathomorphological microscopic examination of the carp hepatopancreas, and to a smaller extent of the kidneys, served as a good tool in the investigation of natural environment pollution sources.
2. A three-year period after the removal of the studied pesticide dump is too short of a period for the elimination of the effect of environmental xenobiotics on the development of morphological changes in farmed carp within the studied area.

Acknowledgements

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HEPATOPANCREAS – CARPS FROM THE POND No. 1

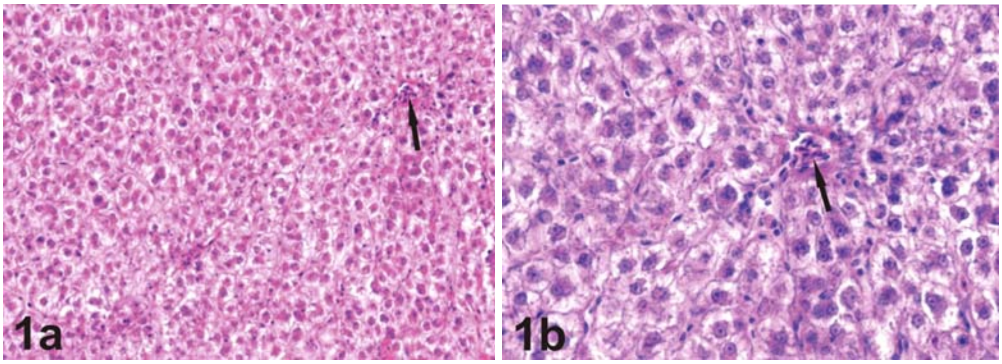


Figure 1. Carp caught in 2003. Parenchymatous degeneration, steatosis simplex (small lipids vacuoles in hepatocytes) and hyperaemia (arrow). HE staining, a. magn. 180 x. b. magn. 320 x.

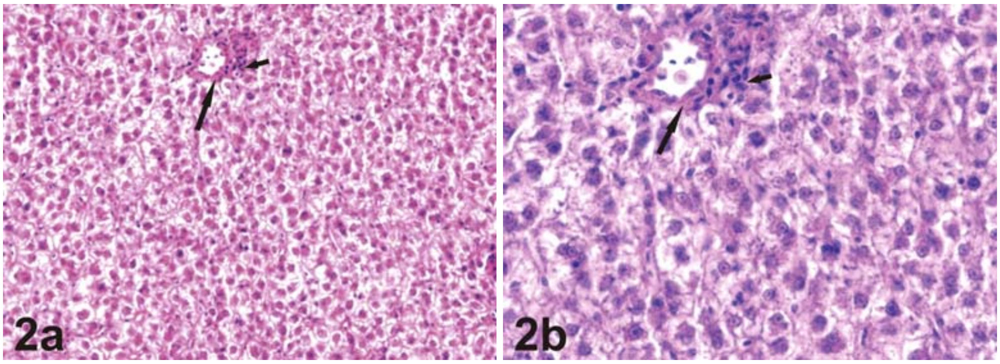


Figure 2. Carp caught in 2007. Hyperplasia of connective tissue of blood vessel (long arrow) with infiltration of histiolymphoid cells (short arrow), parenchymatous degeneration, steatosis simplex (small lipids vacuoles in hepatocytes). HE staining, a. magn. 170 x. b. magn. 320 x.

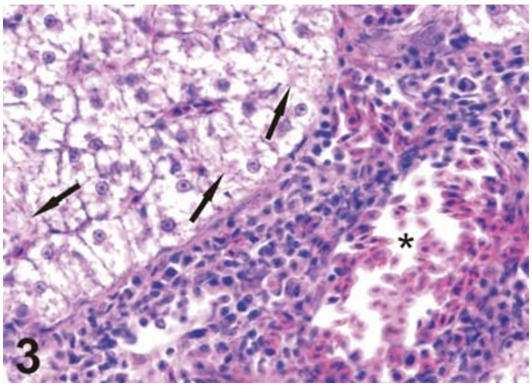
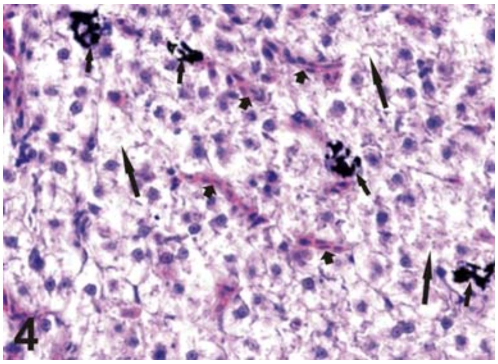


Figure 3. Carp caught in 2008. Steatosis degenerative with necrosis of hepatocytes (arrows), hyperaemia (asterisk) with extravasations, infiltration of histiolymphocytic cells. HE stain., magn. 320 x.



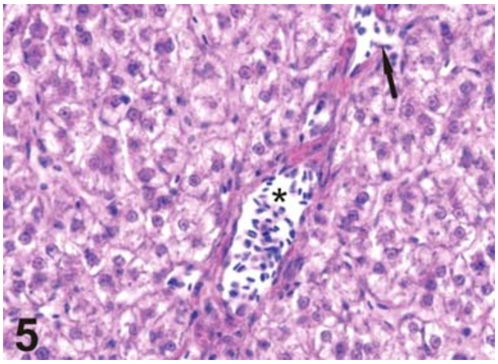


Figure 4. Carp caught in 2007. Steatosis degenerative with necrosis of hepatocytes (long arrows), vibices (heads of arrows), centers of melanomacrophages (short arrows). HE stain., magn. 320 x.

Figure 5. Carp caught in 2002. Steatosis simplex (lipids vacuoles in hepatocytes), hyperaemia (asterisk) with damage of blood vessel (arrow). HE stain., magn. 320 x.

HEPATOPANCREAS – CARPS FROM THE POND No. 2

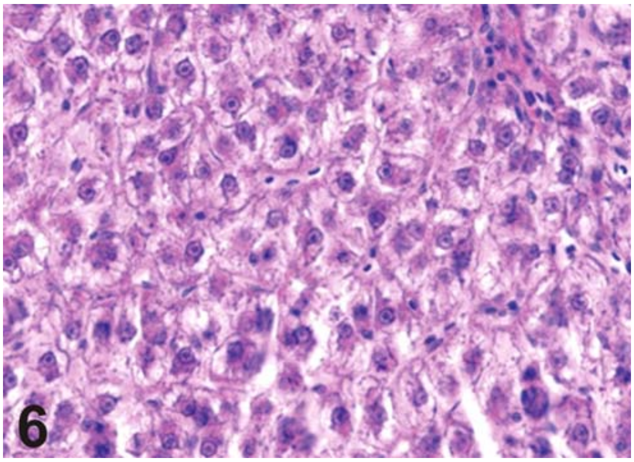
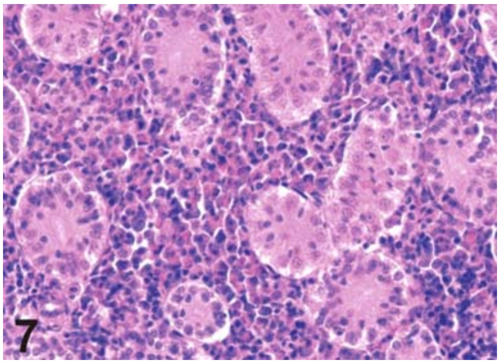


Figure 6. Carp caught in 2002. Steatosis simplex (small lipids vacuoles in hepatocytes). HE stain., magn. 340 x.

KIDNEY – CARPS FROM THE POND No. 1



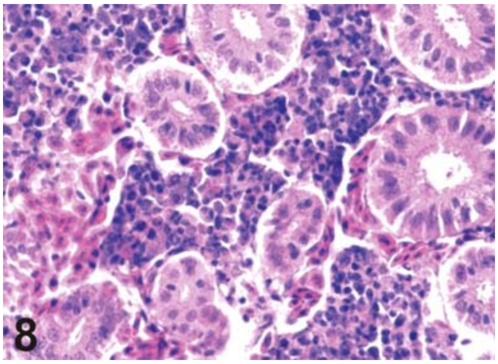
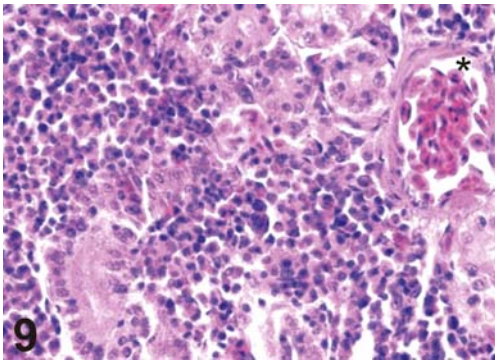


Figure 7. Carp caught in 2002. Parenchymatous degeneration of the tubular epithelial cells. HE stain., magn. 320 x.

Figure 8. Carp caught in 2007. Parenchymatous degeneration of the tubular epithelial cells and extravasations surrounding tubules. HE stain., magn. 370 x.



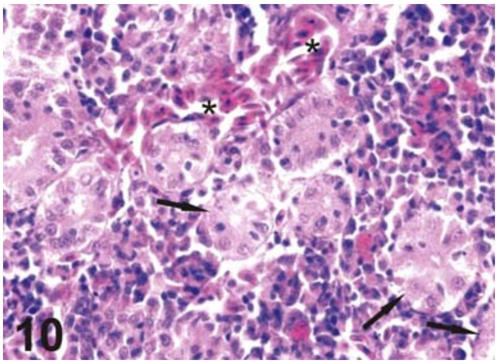


Figure 9. Carp caught in 2003. Parenchymatous degeneration of the tubular epithelial cells and hyperaemia (asterisk) with petechia. HE stain., magn. 370 x.

Figure 10. Carp caught in 2007. Parenchymatous degeneration of the tubular epithelial cells with necrosis of the single cells (arrows) and wide extravasation close to tubules (asterisks). HE stain., magn. 330 x.

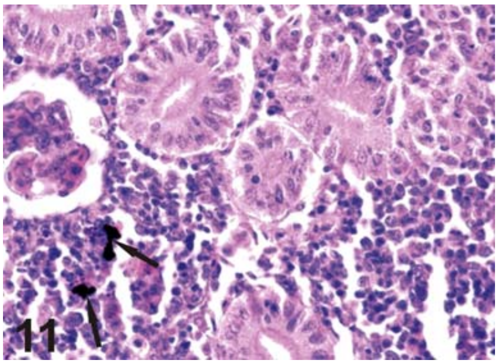


Figure 11. Carp caught in 2008. Parenchymatous degeneration of the tubular epithelial cells and centers of melanomacrophages (arrows). HE stain., magn. 320 x.