Arch. Ryb. Pol.	Archives of Polish Fisheries	Vol. 7	Fasc. 2	329 - 342	1999

HISTOPATHOLOGICAL CHANGES IN JUVENILE CARP *Cyprinus carpio* (L.) CONTINOUSLY EXPOSED TO ALKALINE LEVELS OF pH, FROM HATCHING

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ABSTRACT. The fish were exposed to alkaline water of pH 10.2-10.3 for 21 days since hatching. Histological preparations showed pathological changes in epithelium, gills, esophagus, nasal cavities, eye, brain, heart, swim bladder, and liver compared with the control fish reared at pH 7.8-8.2.

Key words: CARP, pH, HISTOPATHOLOGICAL CHANGES

INTRODUCTION

Water pH is one of the most important environmental factors affecting fish. Excessive acidification as well as alkalisation are detrimental to fish development. According to EIFAC criteria (1971), water pH safe for fish ranges from 6.5 to 8.5.

High water pH very rarely occurs in nature. It is sometimes observed in reservoirs located on limestone substrate. In carp ponds temporary alkalisation may occur during hot summer, usually due to algal blooms, reaching sometime pH over 10.0 (Alabaster, Lloyd 1980). Industrial sewage may also cause water alkalisation. High pH (9.5-10) may result in high mortality and developmental disturbances in various fish species (Daye, Garside 1975, 1980 b, Jezierska 1988, Korwin-Kossakowski 1992).

The aim of the present study was to evaluate, using histological methods, the effect of alkaline pH on cell and tissue development in common carp juveniles.

MATERIAL AND METHODS

Newly hatched common carp larvae were used in the experiment. They were obtained from one pair of spawners in the hatchery of the Inland Fisheries Institute in Żabieniec. The fish were reared in aquaria of 20 dm^3 (three aquaria for each experimental group), at stocking density of 20 ind./dm^3. Water temperature was 20^oC,

pH 7.8-8.2 in the control, and 10.2-10.3 in the experimental groups. Final pH level was established in about 12 hours from the beginning of the experiment. Water pH in the tanks was maintained at constant level using slow inflow of water of appropriate pH. Any pH fluctuations were corrected using 1-% NaOH solution. Complete water exchange in the rearing aquaria took place about twice a day. Water was aerated, and DO saturation was maintained at 90% level. Chemical composition of water was stable during the experiment. Average values (in mg/dm^3) for control and experimental groups were respectively: $N\text{-}NH_4$ – 0.06, 0.02; $N\text{-}NO_2$ – 0.005-0.007, Ca^{2+} - 61.1, 3.0; Mg^{2+} - 6.7, 3.0; Cl^- - 8.86, 8.86.

The fish were fed *ad libitum* with live zooplankton. They were reared for 21 days, and then samples for histopathological examination were taken. The samples were fixed in Bouin-Holland solution, embedded in paraffin, and cut into slices of 6 μm thickness using a rotation microtome. Series of slices were treated according to standard histological procedure, and stained with hematoxylin and eosin according to Delafild. Histological preparations were made and photographed, using a light microscope, in the Department of Ichthyobiology and Fisheries of the Agricultural University (SGGW) in Warsaw. Pathological changes in cells and tissues were recorded and compared for experimental (reared at pH 10.3) and control (pH 7.8-8.2) fish.

RESULTS

EPITHELIUM

Excessive amounts of mucus were observed on body surface of fish reared at pH 10.3. Epithelium was most damaged at dorsal part of head, and partially peeled. Necrosis of mucous cells was also observed, and dysplasia of epithelial cells (Photo. 1 A). In the control fish mucous and epithelial cells did not show any anomalies (Photo. 1 B).

GILLS

Water pH 10.3 adversely affected structure and functioning of carp gills. The observed changes consisted of dilatation of branchial arteries and capillaries, lysis of erythrocytes, and decrease of their number were compared with the control. The walls of branchial arteries were thin and deformed (Photo. 2 A). Gill epithelium was separated from gill arches, some epithelial cells were necrotic and peeled off. Large amounts of mucus caused swelling of the epithelium which raised from the pillar cells (Photo. 3 A).

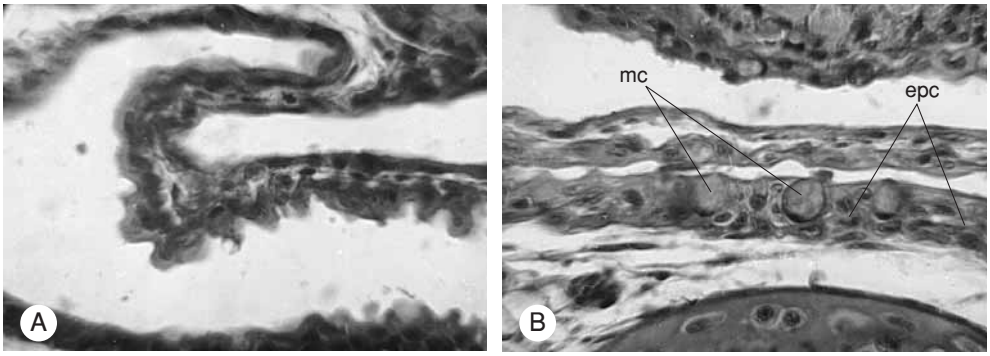

Photo. 1. Cross-section of body cover; hematoxylin, eosin x 1000; mc – mucus cells, epc – epithelial cells. A – pH 10.3; necrosis of mucus cells, dysplasia of epithelial cells. B – Control; correctly developed mucus and epithelial cells

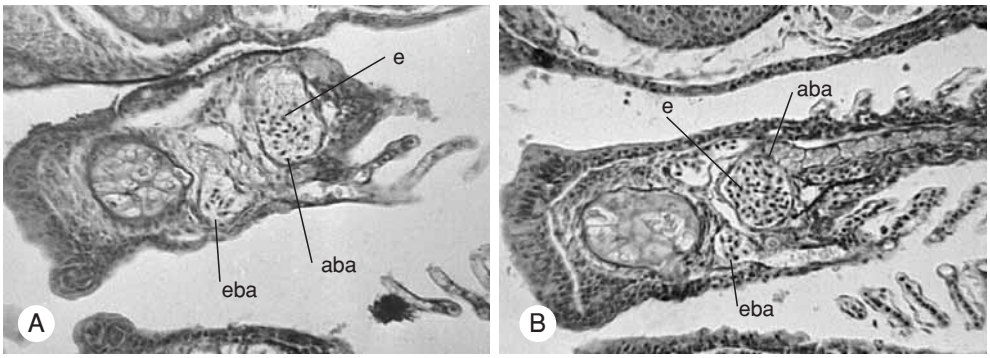

Photo. 2. Cross-section of gill arch; hematoxylin, eosin x 400; aba – afferent branchial artery, eba – efferent branchial artery, e – erythrocytes. A - pH 10.3; branchial arteries dilated, lysis of erythrocytes, thin arterial walls of irregular shape. B – Control

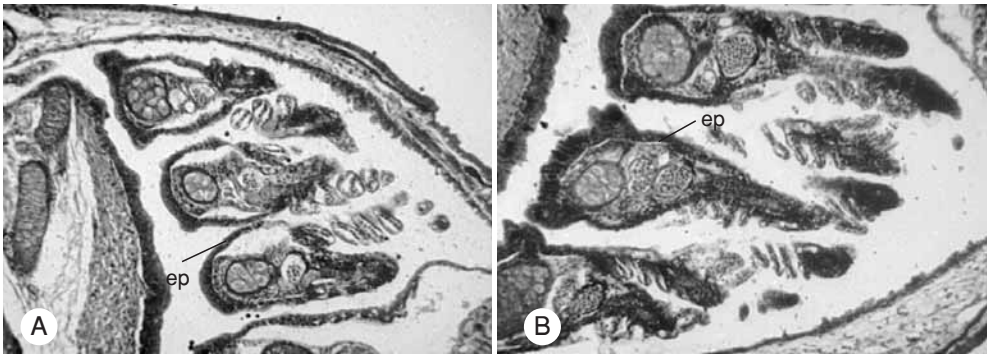

Photo. 3. Cross-section of gills; hematoxylin, eosin x 200; ep-epithelium. A – pH 10.3; Epithelium separated from gill arch, gill lamellae swollen. B – Control

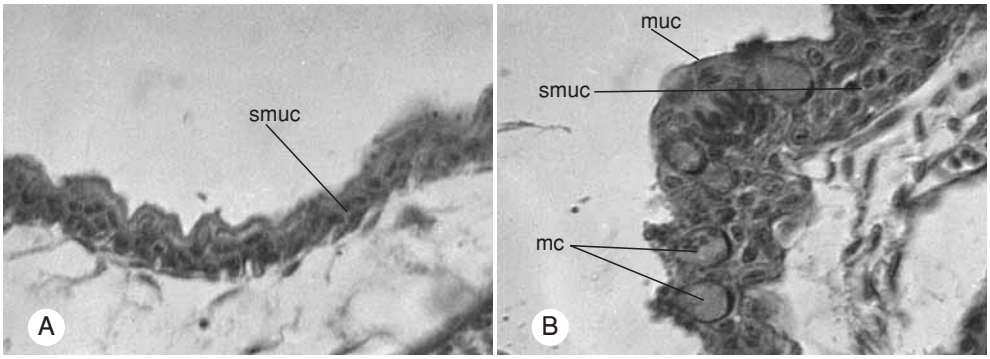

Photo. 4. Cross-section of esophagus epithelium; hematoxylin, eosin x 1000; mc – mucus cells, smus – submucosa, muc – mucosa. A – pH 10.3; mucus cells lacking, submucosa visible. B – Control

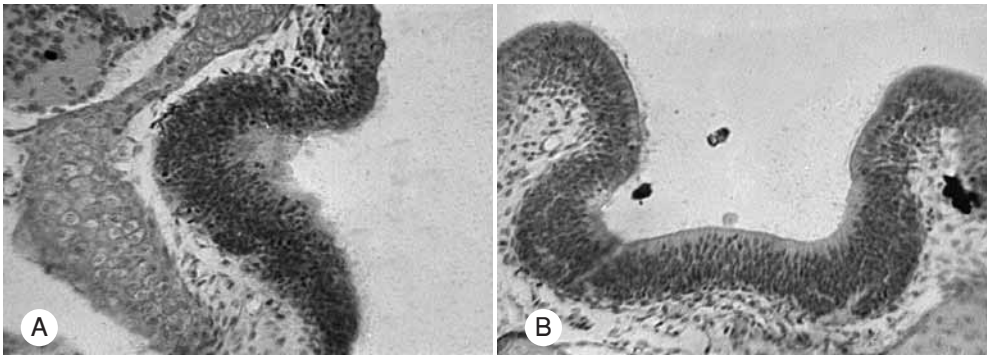

Photo. 5. Cross-section of nasal cavity; hematoxylin, eosin x 400. A – pH 10.3; mucus accumulation, cellular dysplasia, nuclear pyknosis. B – Control

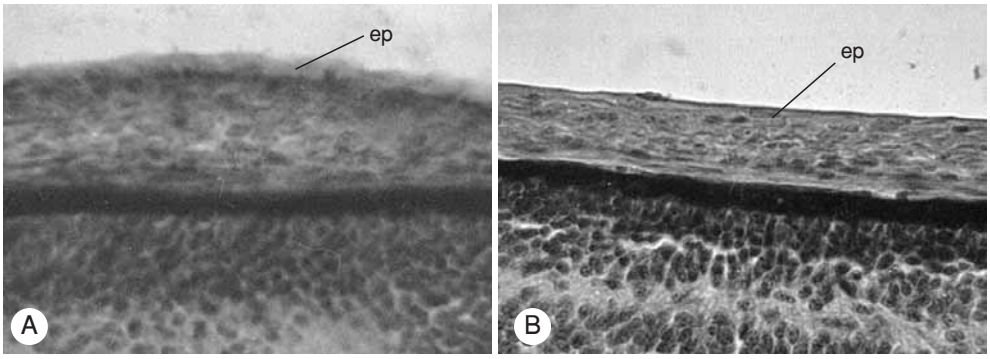

Photo. 6. Cross-section of eye ball; hematoxylin, eosin x 1000; ep- epithelium. A – pH 10.3; epithelium partly necrotic and layered, peeling. B – Control; smooth epithelium, no pathological changes

In the control fish (pH 7.8-8.2) epithelium was connected with gill arches, and lamellae epithelium surrounded pillar cells. Branchial arteries were regular in shape and filled with numerous erythrocytes (Photo. 2 B). No excessive mucus secretion occurred (Photo. 3 B).

ESOPHAGUS MUCOSA

In fish reared in alkaline water (pH 10.3) no mucus cells were observed in the esophagus as mucosa was completely destroyed. Only submucosa was visible (Photo. 4 A). In the control fish mucosa of the esophagus was well developed, with numerous mucus cells (Photo. 4 B).

NASAL CAVITY

Nasal cavities of fish from alkaline water were filled with mucus. Olfactory epithelium showed cell dysplasia and pyknotic nuclei. The latter were dark and condensed (Photo. 5 A). Epithelial cells were more numerous compared with the control. Nasal cavities of control fish showed normal number of epithelial cells; no excessive mucus secretion occurred (Photo. 5 B).

EYE

Alkaline pH caused also some anomalies in fish eyeball structure. In 21-day old carp the lens epithelium was partially necrotic and delaminated. Partial anaplasia and peeling of epithelial cells also occurred (Photo. 6 A). Differentiation of retina was inhibited: external limiting membrane was lacking, and number of chromatophores was reduced (Photo. 7 A).

Control fish did not show any pathological changes in eyeball structure. Lens epithelium was smooth (Photo. 6 B), and retina had correctly developed layers (Photo. 7 B).

BRAIN

Brain of 21-day old carps from alkaline water was metaplastic and smaller than in the control. Cellular anaplasia and nuclear pyknosis was observed in the diencephalon, accompanied by brain necrosis. Nuclei of the neurocytes were hypertrophic (Photo. 8 A). In the control fish no pathological changes were observed. The two brain hemispheres were properly developed, with unchanged neurocytes (Photo. 8 B). No nuclear pyknosis took place (Photo. 12).

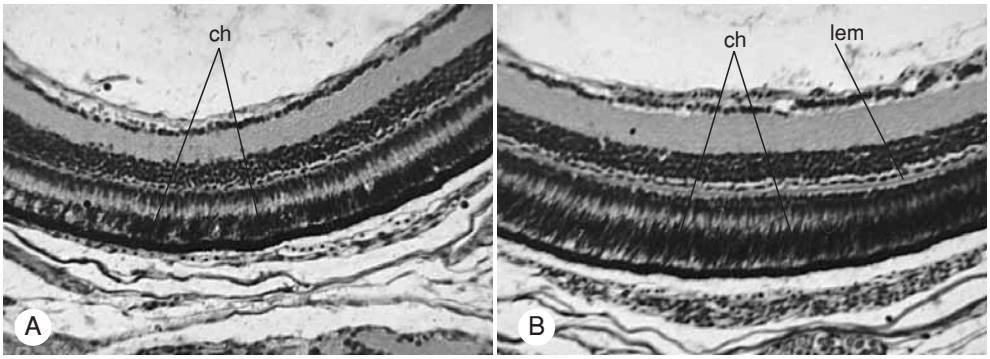

Photo. 7. Cross-section of eye ball; hematoxylin, eosin x 400; lem – limiting external membrane, ch – chromatophores. A – pH 10.3; limiting external membrane lacking, reduced number of chromatophores. B – Control; limiting external membrane well developed, correct number of chromatophores

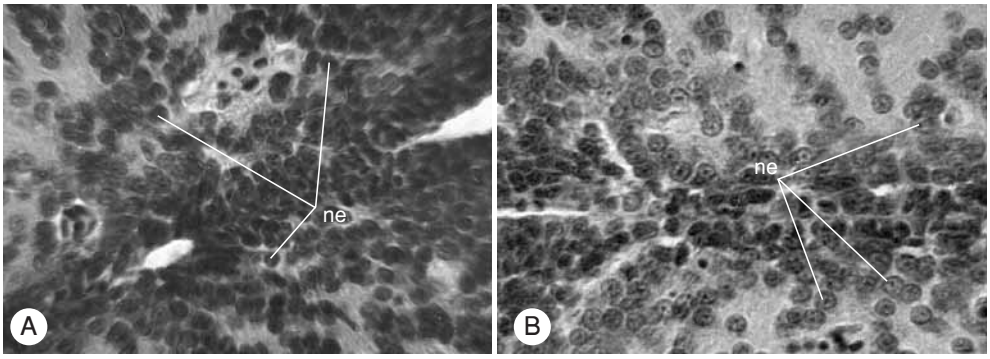

Photo. 8. Cross-section of brain; hematoxylin, eosin x 1000; ne – neurocytes. A – pH 10.3; hypertrophy of neurocytes nuclei. B – Control

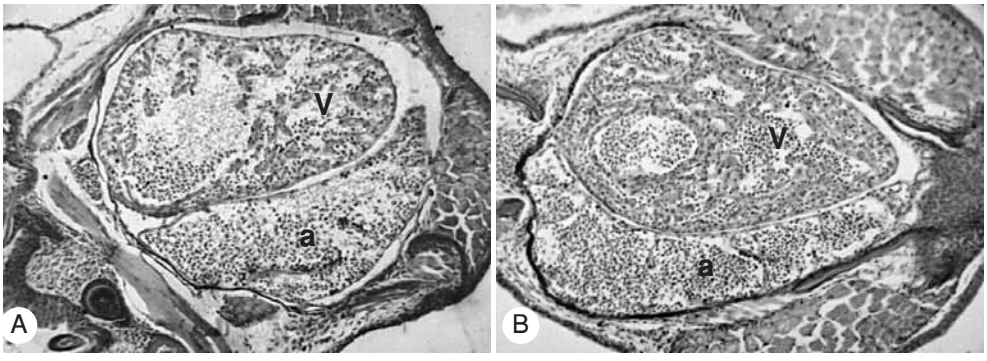

Photo. 9. Cross-section of heart; hematoxylin, eosin x 200; v – ventricle, a –atrium. A – pH 10.3; thin walls of ventricle and atrium, abnormal shape, separation from surrounding tissues, circumcardiac transudate. B – Control; heart walls adhering to the surrounding tissues, no leakage or malformations

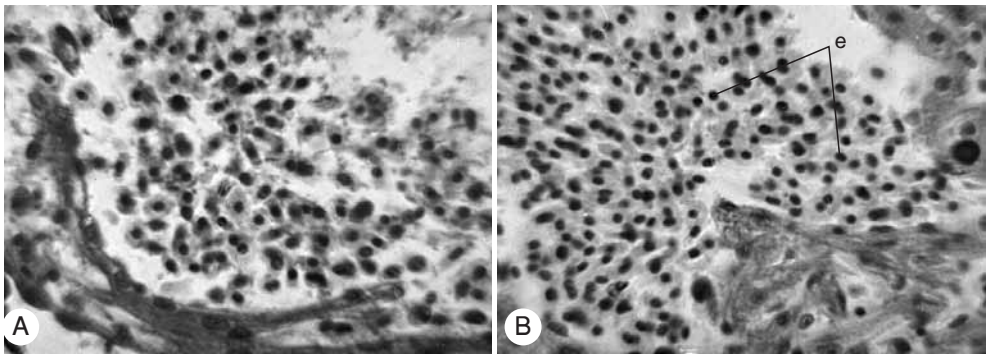

Photo. 10. Cross-section of heart ventricle; hematoxylin, eosin x 1000; e – erythrocytes. A – pH 10.3; erythrocyte nuclei hypertrophic. B – Control; erythrocytes well developed

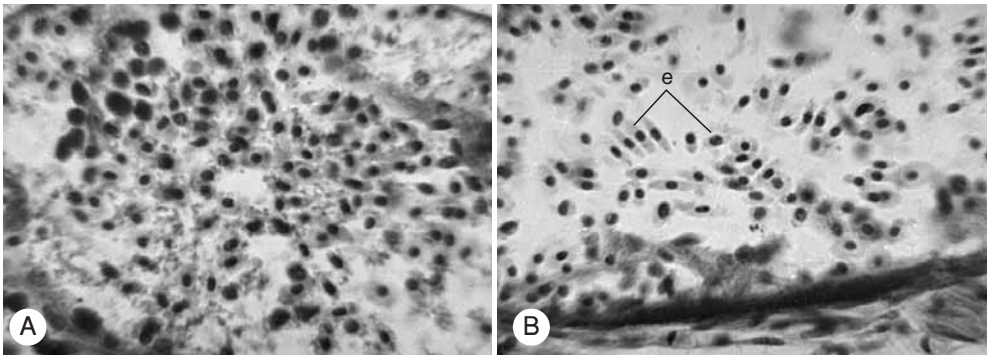

Photo. 11. Cross-section of heart atrium; hematoxylin, eosin x 1000; e – erythrocytes. A – pH 10.3; erythrocyte nuclei hypertrophic. B – Control; erythrocytes well developed

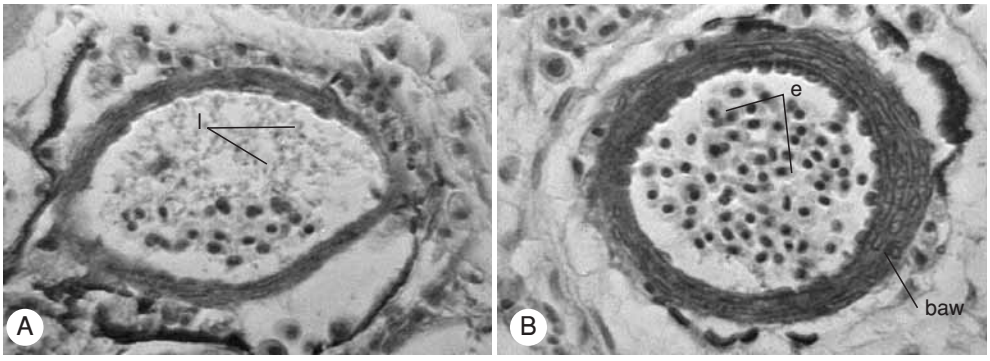

Photo. 12. Cross-section of arterial bulb; hematoxylin, eosin x 1000; e – erythrocytes, baw - arterial bulb walls, l – erythrocyte lysis. A – pH 10.3; thin and deformed arterial bulb walls, erythrocyte lysis, circumcardiac transudate. B – Control; thick, multilayered arterial bulb walls, well developed erythrocytes

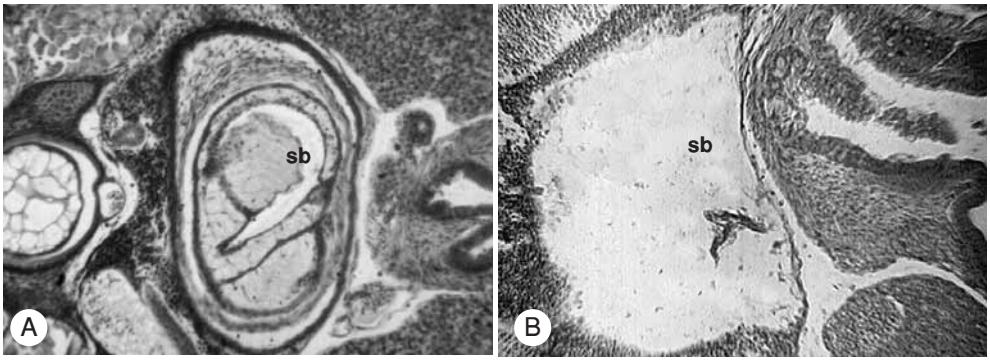

Photo. 13. Cross-section of anterior chamber of swim bladder; hematoxylin, eosin x 200; sb – swim bladder. A – pH 10.3; thick, irregular shape, mucus accumulation. B – Control

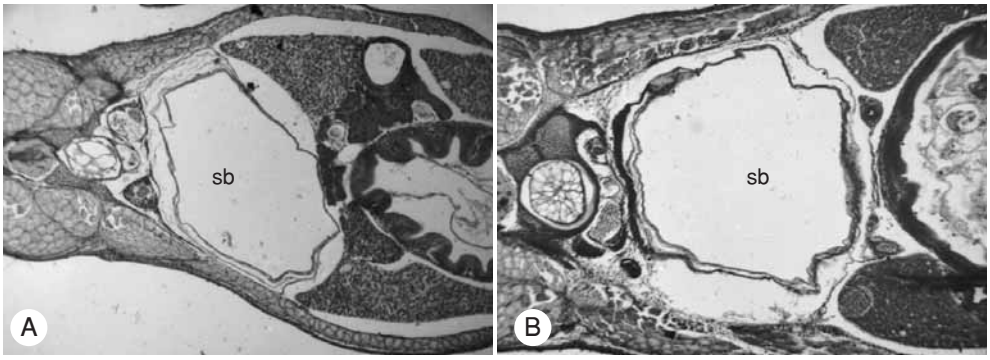

Photo. 14. Cross-section of posterior chamber of swim bladder; hematoxylin, eosin x 100; sb – swim bladder. A – pH 10.3; lesser gas content, deformed, thin walls. B – Control; double walls, regular shape

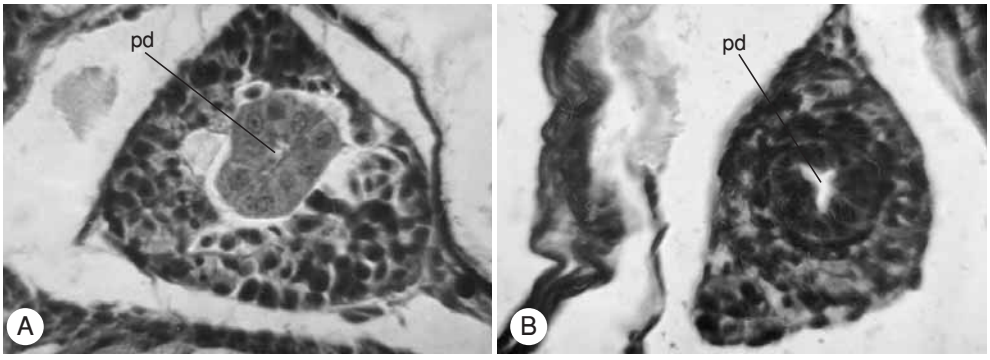

Photo. 15. Cross-section of pneumatic duct; hematoxylin, eosin x 1000; pd – pneumatic duct. A – pH 10.3; pneumatic duct partly occluded. B – Control; pneumatic duct open

HEART

In fish from alkaline water (pH 10.3) circumcardiac transudate was observed, indicating permeability of blood vessels. Walls of ventricle, atrium, and pericardium were considerably thinner than in the control fish. Circumpericardiac transudate resulted in separation of ventricle and atrium walls from the surrounding tissues (Photo. 9 A). Some erythrocytes in the ventricle and atrium were partially lysed, and some showed nuclear hypertrophy (Photo. 10 A, 11 A). Normally developed blood cells were less frequent compared with the control. Arterial bulb showed abnormal wall shape and lower number of erythrocytes. Partial lysis and denaturation of dead red blood cells occurred. Walls of arterial bulb were separated from the surrounding tissues, and infiltration of blood serum to the body cavity was noted (Photo. 12 A)

Heart of control fish was well developed. Ventricle and atrium were filled with unchanged healthy erythrocytes (Photo. 10 B, 11 B). Heart walls adjoined the surrounding tissues, no leakage or malformation were observed (Photo. 9 B). Arterial bulb was round and had thick multilayered walls. It was filled with well-developed red blood cells. No blood serum infiltration to the body cavity was noted (Photo. 12 B).

SWIM BLADDER

The swim bladder of fish reared in alkaline water considerably differed from that observed in control fish. Anterior chamber of the bladder was not filled with gas. It was thick and irregularly shaped, inflamed inside, showing excessive mucus secretion (Photo. 13 A). Posterior chamber was abnormally shaped, filled with little gas, and had thinner walls than in the control fish (Photo. 14 A). Pneumatic duct showed narrow lumen and was occluded in some parts (Photo. 15 A).

Swim bladders of control fish had two chambers, both filled with gas and regularly shaped. Anterior chamber was entirely filled, and situated between kidneys and intestines (Photo. 13 B). Posterior chamber had double walls of regular thickness (Photo. 14 B). Thin-walled pneumatic duct was open, with wide lumen (Photo. 15 B).

INTESTINE

Considerable differences of intestine size were observed between experimental (Photo. 16 A) and control (Photo. 16 B) fish. Intestine of the fish reared in alkaline water contained also less food, and this resulted in its more pronounced undulation . No differences in cellular structure were observed between the groups.

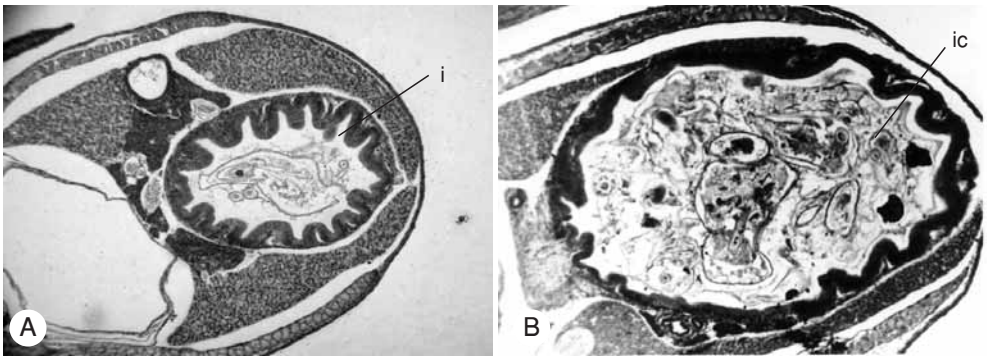

Photo. 16. Cross-section of intestine; hematoxylin, eosin x 100; i – intestine, ic – intestine content. A – pH 10.3; smaller intestine size. B – Control; intestine filled with food, correct size

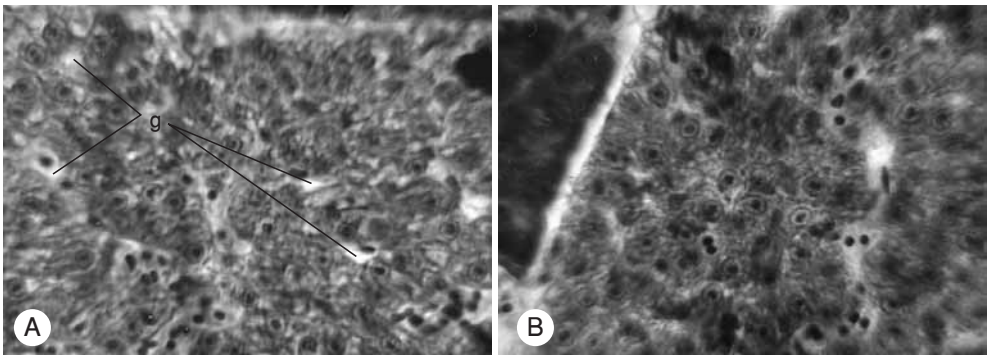

Photo. 17. Cross-section of liver; hematoxylin, eosin x 1000; g – glycogen. A – pH 10.3; higher glycogen content in the hepatocytes. B - Control

LIVER

Liver of carp juveniles from alkaline water contained more glycogen (Photo. 17 A) compared with control individuals (Photo. 17 B). Vessel and sinus leakage was also observed, as well as dysplastic erythrocytes in hepatic veins. In the control fish no anomalies in liver structure occurred.

DISCUSSION

Extreme water pH, high as well as low, may cause similar physiological disturbances and anatomo - pathological changes in fish. Changes observed in body surface of fish reared in alkaline water were a typical reaction to inappropriate pH values, and comprised excessive secretion of mucus. Similar reaction of epithelium was ob-

served in fish by Daye and Garside (1976, 1980 b). These authors observed extensive necrosis, peeling of epidermis and exposure of inner skin at pH over 10.8.

Gills and their epithelium are specific indicators of water pollution. Gill epithelium damage, cell necrosis, and dysplasia have been considered as a common reaction to strong water pollution (Brandt 1936) and high pH (Lloyd 1960). Large amounts of mucus in gills of fish exposed to alkaline stress were observed by Daye and Garside (1976), and Jezierska (1988). Similar reaction was also noted in case of acidic stress (Vaala, Mitchell 1970, Ultsch, Gros 1979).

Malformation during gill development, and excessive mucus secretion caused by high water pH, result in gas and ion exchange disturbances. According to Ultsch and Gros (1979), mucus is impermeable for oxygen, so its excessive secretion in gills or in other parts of fish body may considerably reduce oxygen uptake. According to Laurent et al. (1995), supersecretion of mucus and increased number of mucus cells play a protective role against environmental conditions in fish continuously exposed to water pH of 10 (Magadi Lake, Kenya). These changes are reversible after fish transfer to water of neutral pH. Thus, the reaction detrimental for respiration and ionic exchange is actually a protective response.

Daye and Garside (1976) observed necrosis and peeling of lamellar epithelium, followed by exposure of pillar cells in brook trout. Daye and Garside (1980 a) noted also dilatation of branchial arteries, erythropenia, and hemolysis in Atlantic salmon fry exposed to acidic water.

Reduced oxygen consumption in fish exposed to alkaline water was observed by Murthy et al. (1981); it resulted from difficulties in oxygen uptake by gills and disturbances in oxygen transport by blood. Similar changes were observed by Jezierska (1988) in fish exposed to alkaline environment, and by Korwin-Kossakowski and Jezierska (1985) in acidic water. Damage or covering with mucus of gill epithelium and chloride cells may also result in impaired ion exchange. Muniz and Leivestad (1980) reported that disturbances in ionic regulation, especially loss of sodium, were a direct cause of fish mortality.

Summarising, changes in gills impair fish respiration. Hypoxic stress response, such as release of erythrocytes from the spleen and increase of their volume, may partly compensate these disturbances. However, high number of damaged erythrocytes was observed in fish subjected to alkaline stress (Jezierska 1988). According to Daye and Garside (1980 a), not only swelling, but also damage of blood cells, followed by hemolysis, occurred in fish after long-term exposure to acidic water.

Daye and Garside (1976) observed pathological changes in nasal cavities of brook trout developing in water of pH under 4.8 or over 9.5: excessive mucus, dark condensed nuclei, and cellular vacuolisation. These authors noted also that highly alkaline water caused less disturbances than acidic conditions.

Eyeball damage in brook trout and Atlantic salmon was reported by Daye and Garside (1976, 1980 a). The changes consisted of anaplasia of lens filaments, peeling of eyeball epithelium, and dispersion of iris chromatophores. In the present study, also limiting external membrane of retina was lacking, and number of chromatophores was reduced.

Alkalisation and acidification of water may adversely affect fish brain. Daye and Garside (1980 b) observed smaller brains and abnormal metaplasia in Atlantic salmon larvae incubated at pH 9 and 9.5. They also reported considerable nuclear pyknosis, and necrosis of all brain regions in salmon larvae at pH under 5 (Daye and Garside 1980 a).

Histological analysis of heart of common carp juveniles from pH 10.3 confirmed the results obtained by Daye and Garside (1980 b) for Atlantic salmon reared in pH over 9.5. They consisted of irreversible changes in ventricle, atrium, and blood cells. Other disturbances, such as pericardial haemorrhages, thinner atrium and ventricle walls, and coagulation of dead erythrocytes, were observed in salmon larvae at pH 9.5 (Daye and Garside 1980 b), and in pike-perch at pH under 6 (Ostaszewska and Wojda 1997).

Differences in shape and thickness of arterial bulb walls reported in the present study for common carp juveniles at pH 10.3 were not described by other authors.

Swim bladder and pneumatic duct are lined with mucosa (Harder 1975). Excessive mucus secretion may cause obstruction of the duct, making swim bladder filling impossible. This results in irreversible changes of bladder structure (Korwin-Kossakowski 1988). Beside excessive mucus secretion, pneumatic duct may be also occluded due to damage of other cells. Doroshev and Cornacchia (1979) observed in striped bass that after some critical period filling of swim bladder becomes no more possible. If it fails to fill within normal time (5-7 days), inner epithelium degenerates, and the bladder cannot be filled. In the studied carps, additionally to occluded pneumatic duct, changes in swim bladder chambers occurred. Anterior chamber was entirely filled with mucus, and the walls of posterior chamber were deformed.

No data on the changes in size or structure of fish intestine caused by alkaline or acidic water pH were found in the literature. Witschi and Ziebell (1979) observed re-

duction of food uptake by the fish living in strongly alkaline water. Alkaline environment reduced also juvenile carp growth (Jezierska 1988, Korwin-Kossakowski 1992).

Changes of glycogen content in hepatocytes were observed by Daye and Garside (1980 a) in Atlantic salmon reared at pH under 4.

Water pH 10.3 is near lethal level for juvenile carp. Survival of the fish on 17 day of rearing was 63% (versus 92% in the control), the larvae developed but grew slower compared with the control (Korwin-Kossakowski 1991, 1992). The results of acute toxicity tests showed that carps acclimated at pH 10.3 died earlier than those acclimated at pH 9.4, 9.7, and the control.

The results of histological studies confirm that water pH 10.2-10.3, although not lethal, causes considerable pathological changes adversely affecting fish growth and development.

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STRESZCZENIE

ZMIANY HISTOPATOLOGICZNE U MŁODOCIANEGO KARPIA *Cyprinus carpio* (L.) ROZWIJAJĄCEGO SIĘ OD WYKLUCIA W WODZIE O ODCZYNIE ALKALICZNYM

Odczyn środowiska wodnego, zarówno kwaśny jak i alkaliczny, wpływa istotnie na śmiertelność, wzrost i rozwój larw karpia. Przeprowadzono hodowlę młodocianego karpia w wodzie alkalicznej o odczynie pH 10,2 – 10,3, rozpoczętą po wykluciu i trwającą 21 dni. Wykonane po tym okresie preparaty histologiczne pozwoliły stwierdzić zmiany histopatologiczne w nabłonku, skrzelach, przełyku, zagłębieniu nosowym, oku, mózgu, sercu, pęcherzu pławnym i wątrobie, w porównaniu do ryb z grupy kontrolnej hodowanej w wodzie o odczynie pH 7.8 - 8.2.

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