

Pathology of Red-Spot Disease in the Soft-Shelled Turtle (*Torionyx sinensis*)

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Abstract

This paper reports on the pathology of red-spot disease caused by *Aeromonas hydrophila* in the soft-shelled turtle, *Torionyx sinensis*.

Histopathological changes included hyperemia, hemorrhage and extensive degeneration of the liver, kidney and muscle. In diseased turtles, biochemical changes were characterized by the absence of activity of LDH subunit A in the liver and kidney. The mobility of LDH isozymes formed mainly by subunit A became greater, and the total protein content in muscle, liver and kidney decreased significantly.

Introduction

In China, the soft-shelled turtle, *Torionyx sinensis*, is a highly nutritious but expensive delicacy. Some believe that it possesses medicinal benefits. To meet the national and international demand for this aquatic

delicacy, turtle culture is being developed. However, disease problems have become a serious threat. For example, in the early spring of 1987 and 1988, in the Gungwin Aquatic Farm in the suburbs of Shanghai, red-spot disease broke out, killing about 20% of the turtle population.

The most noticeable clinical sign of the disease is red spots on the abdomen resulting from widespread hemorrhages, thus the term "red-spot disease." Information about the pathology and treatment of this condition is limited. In Japan, a similar disease caused by *Aeromonas* has been reported (Kawasaki 1983), but no detailed description was given. Sindermann (1977) briefly described the diagnosis of *Aeromonas*

disease in loggerhead turtles (*Caretta caretta*). Sun and Xiao (1988) proved that red-spot disease in soft-shelled turtles is caused by *Aeromonas hydrophila* and is basically identical with the diseases reported by Kawasaki (1983) and Sindermann (1977).

This study sought to examine the histopathological and biochemical changes associated with red-spot disease in turtles.

Materials and Methods

Turtle samples were collected from the Gungwin Aquatic Farm, Nanhui County, Shanghai City, China, in May 1988.

Tissue sections were prepared from six diseased adult turtles weighing 121-200 g and four healthy turtles weighing 130-190 g. Samples of liver, kidney and intestinal tissue were preserved in Bouin's solution, processed routinely for histology and sectioned at 5-6 μ thickness. Besides staining with hematoxylin and eosin (H & E), sections of liver tissue were also stained by the Gomori and Masson-Fontana methods (see Liu et al. 1983).

For the biochemical analyses, samples of liver, kidney and leg muscles without foci of infection were taken from live turtles after bleeding and preserved at -25°C.

Tissue samples (0.2-0.3 g) were homogenized in three times their volume of 0.03% nicotinamide-adenine dinucleotide (NAD). Homogenates of muscle and kidney were centrifuged at 15,000 rpm at 5°C for 20 minutes. The homogenate of liver was centrifuged twice to remove the fat; the supernatant was kept at 4°C and used for electrophoresis within 12 hours.

Electrophoresis was conducted using a LKB 2117 horizontal system using gels of 4.4% polyacrylamide, subjected for 10 minutes at 25 mA followed by about 2 hours at 275 V. The system was cooled to 5°C during electrophoresis using a LKB 2209 MultiTemp Thermostatic Circulator. After electrophoresis, the gels were subjected to histochemical staining following the method of Philipp et al. (1982), as modified by Cai and Huang (1986) and Cai (1988). The gels were scanned and integrated with a LKB UltroScan Laser Densitometer.

The absolute activity of isozymes or proteins is expressed as the absorbance of laser light by bands on the gel. The relative activity of isozymes or proteins is expressed as ratios under the scanning curve associated with individual bands relative to the total area under the scanned curve.

Results

Gross Signs of Disease

From April to mid-May, diseased turtles were observed climbing up the hillside of the grow-out pond. They did not feed, had slow reaction time and were easy to catch. After 2-3 days, these turtles died. In contrast, the healthy turtles were always active and alert. They remained in the water, were evasive and difficult to catch.

Externally, the abdomens of the diseased turtles were covered with many red spots; the mouth, tongue and nose were red, the pharynx swollen. The liver was red and the intestines were red and contained no residual food.

Histopathological Changes

Liver. The liver contained hemorrhaged blood. Its central vein was hyperemic. Liver cells were swollen, reacting negatively to Gomori's stain, while reacting positively to the Masson-Fontana stain, indicating that the black dots in the liver were black pigments. These black pigments were numerous in the livers of heavily infected turtles (Fig. 1).

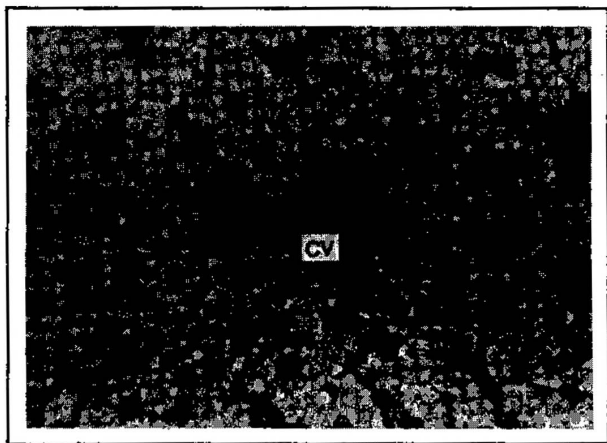


Fig. 1. Cloudy swelling of hepatic cells (arrow). Blood congestion in central vein of hepatic lobule (cv). Black pigments (large arrow). H & E stain x 1008.

Kidneys. The kidneys showed signs of extensive degeneration. The glomerulus was atrophied and the epithelial cells of the renal tubules were cloudy and swollen. The cytoplasm of some cells exhibited granulation. In the tubules, H & E staining demonstrated red protein. The blood vessels were expanded, hyperanemic and even hemorrhaged. Lymphoid cells had infiltrated the urinary interstice (Figs. 2 and 3).

Lung and intestine. The lungs contained hemorrhaged blood. The intestine was hyperemic.

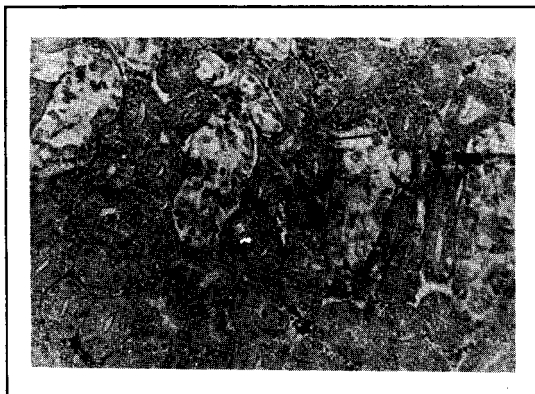


Fig. 2. Granular degeneration of epithelial cells of renal tubules. Some demonstrate partial degeneration (large arrow), but nuclei remain intact. Lymphoid cell infiltrating the urinary interstitium (arrow). H & E x 1008.



Fig. 3. Kidney showing degeneration. Some cells have been sloughed off into the lumen of the tubules (arrows). Glomeruli are swollen and tubule lumen are expanded. H & E x 504.

Biochemical Changes

Lactate dehydrogenase (LDH, 1.1.1.27). In the liver, LDH showed five bands: A4, A3B1, A2B2, A1B3 and B4. In turtles with red-spot disease, the relative activities of the isozymes were decreased (Fig. 4-1), with the A4 and A3B1 bands disappearing. In both diseased and healthy turtles, the A2B2 isozyme had the highest relative activity, but as the disease progressed, its relative activity decreased by 8-13% (Fig. 4-1 and Table 1).

In the kidneys, LDH of healthy turtles also showed five bands and the effects of the disease on the isozyme pattern were similar (Fig. 4-2 and Table 2).

In the muscles, the A4 isozyme was the most active enzyme in both diseased and healthy animals, but in diseased turtles, the activity of the A4 isozyme decreased by 9% (Fig. 4-3). In diseased turtles, the mobilities (the distances of bands from the

origin) of A4, A3B1 and A2B2 became greater, for example, the mobility of A4 was 37% greater in diseased than in the healthy turtles (Table 3).

Esterases (Est, 3.1.1.1). Both healthy and diseased turtles showed only one band of Est in liver and kidneys, but the absolute activity decreased by 50-60% in diseased turtles (Fig. 5).

Protein. In healthy turtles, protein in the muscles showed four main bands. In diseased turtles, two fast-moving bands overlapped, so that only three peaks could be seen on scanning (Fig. 6-1). In diseased turtles, protein content decreased by 39% from the normal.

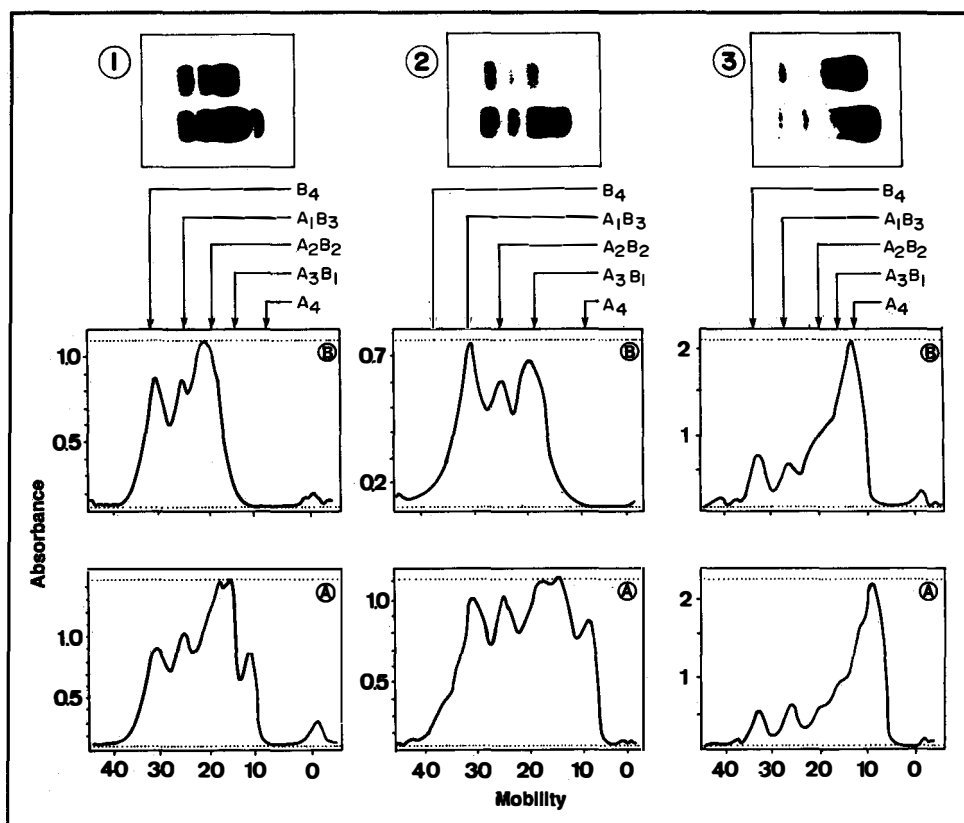


Fig. 4. Zymograms and densitometer scans of LDH from healthy (A) and diseased (B) turtles: (1) liver, (2) kidney, (3) muscle. Dashed lines represent upper and lower limits of absorbance.

Table 1. Relative activity of LDH in livers of healthy and diseased turtles (%). Mean values $\pm$ S.D.

	Band				
	A4	A3B1	A2B2	A1B3	B4
Healthy turtle (n = 4)	14.4 $\pm$ 0.2	25.4 $\pm$ 0.3	26.4 $\pm$ 0.7	17.6 $\pm$ 0.5	16.0 $\pm$ 0.3
Diseased turtle (n = 6)	0	0	39.1 $\pm$ 1.6	30.2 $\pm$ 1.9	30.5 $\pm$ 3.1

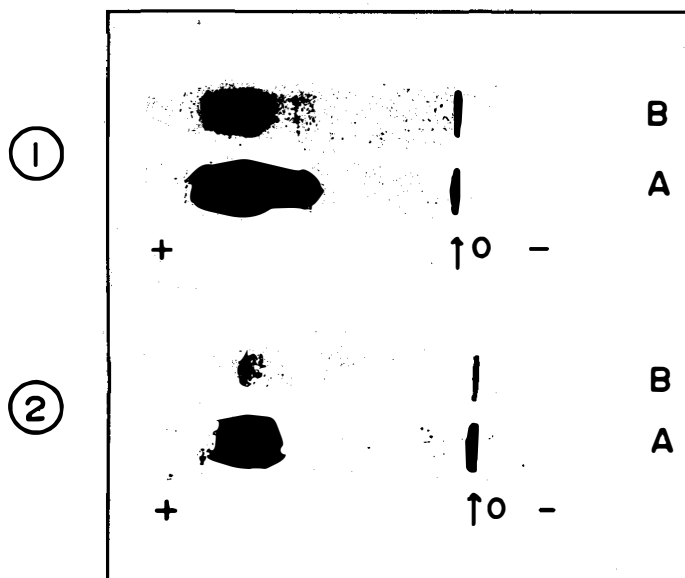
The liver protein of diseased turtles showed four or five major bands which moved to the anode as usual, but also one band which moved to the cathode, and the activity of this band decreased similarly (Fig. 6-2). Protein in the kidneys of diseased turtles decreased also.

Table 2. Relative activity of LDH in kidneys of healthy and diseased turtles (%). Mean values $\pm$ S.D.

	Band				
	A4	A3B1	A2B2	A1B3	B4
Healthy turtle (n = 4)	16.1 $\pm$ 0.4	22.1 $\pm$ 0.8	22.1 $\pm$ 0.4	20.0 $\pm$ 0.7	19.5 $\pm$ 0.1
Diseased turtle (n = 6)	0	0	33.4 $\pm$ 2.7	28.3 $\pm$ 2.8	38.7 $\pm$ 4.4

Table 3. Changes in mobilities of LDH in muscles of healthy and diseased turtles (%). Mean values $\pm$ S.D.

	Band				
	A4	A3B1	A2B2	A1B3	B4
Healthy turtle (n = 4)	32.6 $\pm$ 0.2	47.8 $\pm$ 0.3	59.3 $\pm$ 0.2	81.4 $\pm$ 0.2	100
Diseased turtle (n = 6)	44.7 $\pm$ 0.4	58.8 $\pm$ 0.5	65.9 $\pm$ 0.3	81.4 $\pm$ 0.2	100
Diseased/healthy	1.37	1.23	1.11	1.00	1.00

**Fig. 5. Zymograms of Est from healthy (A) and diseased (B) turtles: (1) liver, (2) kidney.**

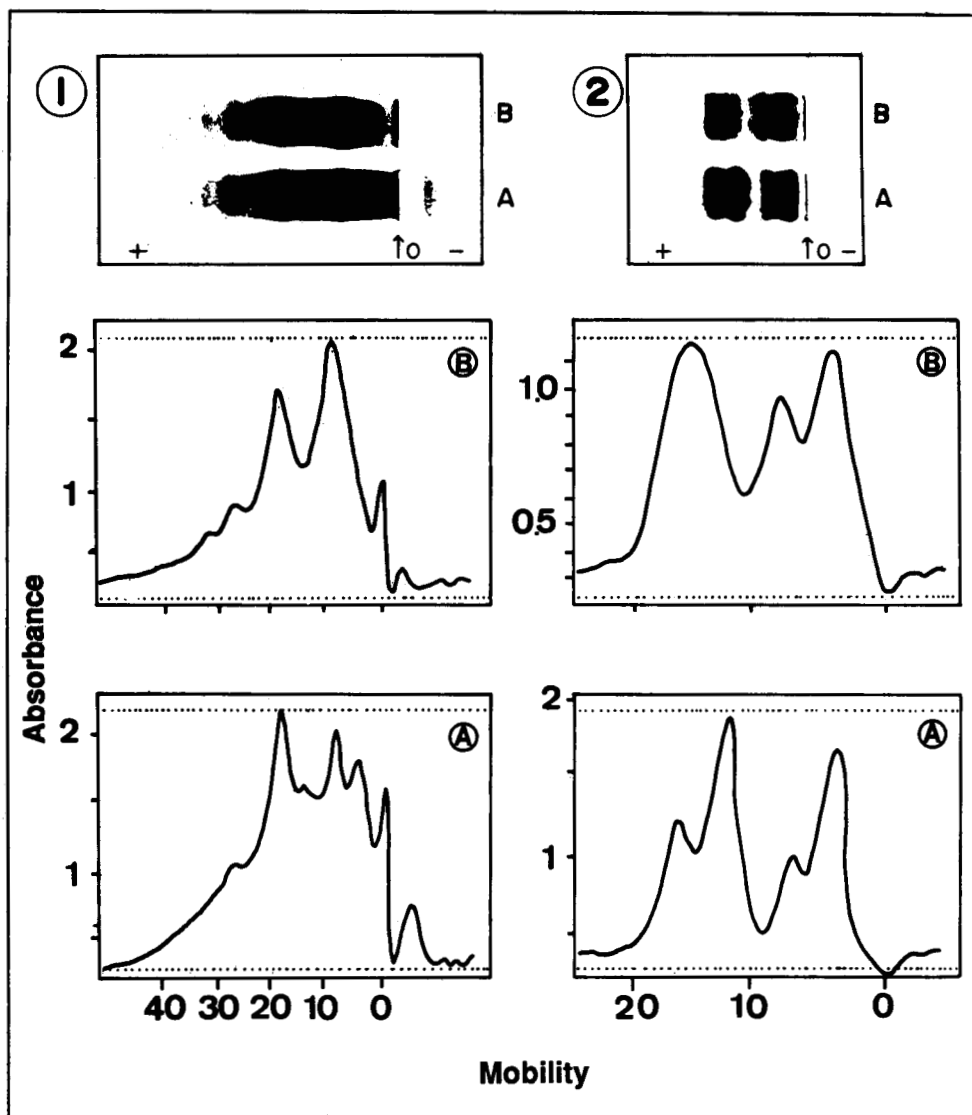


Fig. 6. Electrophoretograms and densitometer scans of protein from healthy (A) and diseased (B) turtles: (1) muscle, (2) liver. Dashed lines represent upper and lower limits of absorbance.

Discussion

Aeromonas spp. are common inhabitants of aquatic environments and may be facultatively pathogenic to animals living under conditions of environmental stress. After about six months hibernation in ponds with low-quality water, the turtles were weakened, providing an opportunity for invasion by *Aeromonas*.

Swelling and hemorrhage are the most common degenerative changes observed in diseased turtles. The dark pigments exist in the liver of both healthy and diseased turtles; but in diseased turtles, the pigments were more numerous.

The loss of activity of some isozymes and the decrease of protein content in diseased turtles represents a great biochemical change.

The suggested functional significance of the difference in LDH isozymes is that subunit A is principally involved in the conversion of pyruvate to lactate (i.e., anaerobic glycolysis), while subunit B is principally involved in the conversion of lactate to pyruvate (i.e., gluconeogenesis and aerobic metabolism) (Everse and Kaplan 1973). The near depletion of LDH subunit A in the liver and kidneys of diseased turtles may indicate a loss of function of anaerobic respiration. The corresponding behavior is that the turtles leave the water and lie on the ground.

During aerobic respiration, subunit B plays an important role. The situation is different in teleosts. In Nile tilapia (*Oreochromis niloticus*) with dermal-ulcer disease, the missing subunit is not A, but B (Cai and Huang 1986). The reason for this difference may be that fish depend on their gills to breathe, and when they lose this capacity due to weakness brought on by disease, the maintenance of anaerobic respiration corresponds to subunit A. Based on the above analysis, it is possible to infer that the tissue specificity and physical function of LDH in reptiles and teleosts may be quite different; but this needs rigorous proof.

In diseased turtles, the mobility of isozymes formed mainly by subunit A was greater. This phenomenon may result from a change in the electrical charge and/or size of molecule. It is clearly an interesting phenomenon worthy of future study.

Acknowledgements

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