Histochemical response of alkaline phosphatase activity during the healing of cutaneous wounds in a cat-fish

A.K. Rai and A.K. Mittal

Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam (India), and Zoology Department, Banaras Hindu University, Varanasi-221005 (India), April 14, 1982

Summary. The activity of alkaline phosphatase in the various tissue components of the regenerating skin of a cat-fish has been studied. A marked increase in alkaline phosphatase activity in the cells of migrating epithelium has been correlated with their highly active state. High alkaline phosphatase in the basal cells after 2 days has been found to have played an important role in cell multiplication and differentiation. The functional significance of this enzyme is discussed in relation to the granulation tissue formation.

Considerable attention has been paid in recent years to investigating changes in alkaline phosphatase activity histochemically in fish skin[1-5]. In spite of the fact that alkaline phosphatase plays a significant role in various processes concerned with the regeneration and repair of different tissues[6-8], such studies in fish skin still remain almost unattempted. The present investigation is to elucidate the changes in alkaline phosphatase activity in the various cellular components of the regenerating skin of a fresh water cat-fish, *Heteropneustes fossilis* (Bloch) (Heteropneustidae, Pisces).

Materials and methods. Live specimens of *Heteropneustes fossilis* (approximately 16-18 cm in length) were collected from a local pond at Dibrugarh, Assam and were acclimatized to laboratory conditions before the experiment began. Incised wounds approximately 5 mm long and 2-3 mm deep, parallel to the longitudinal axis of the body, were made with a sharp scalpel blade between dorsal fin and the lateral line canal. Skin fragments each containing a wound were removed at various intervals and fixed in 10% neutral formalin at 4 °C for 18 h and frozen sections were cut at 15 µm with a freezing microtome. Alkaline phosphatase activity was visualized using the calcium-cobalt method[9] and the coupling azo-dye method[10]. Control sections were prepared by incubating the sections in the absence of the substrates or by incubating the sections after inactivation of the enzyme by 10-min treatment with boiling water[11].

Results. Following injury, the cells of migrating epidermis (fig. 1) give a very strong reaction for alkaline phosphatase activity until the epithelialization of the wound (fig. 2) by the end of 4 h. After this period the intensity of the reaction gradually declines in various cell types of the epidermis up to 20 h (table). After 24 h a gradual increase in the activity of this enzyme is observed in basal cells up to 6 days (fig. 3), but from the 7th day the activity of enzyme comes to the normal level. Moderate activity is observed up to 72 h in the polygonal cells of the middle layer but after this stage up to 20 days strong activity is shown by these cells. Polygonal cells and the giant cell of the epidermis also showed dramatic changes in their intensity of reaction (table) and by the end of 25 days various cell types in different layers of the epidermis showed the same pattern of enzyme distribution as was observed in normal epidermis. In subepidermal tissue, only blood cells occupying the wound gap showed a moderate reaction in the early hours after wounding. The newly formed granulation tissues, near the cut edges of the dermis, show very weak activity for the first 3 days and this activity intensifies between the 4th and 5th day. After this stage there is a gradual decline in the activity of the enzyme at the cut edges from the 6th day, and it finally disappears by the end of 8 days. The core of the granulation tissue, however, gives a weak reaction (fig. 4) from 8 to 12 days, but the enzyme disappears completely by the end of 25 days and returns to the normal condition. The blood capillaries invading the granulation tissue recorded very strong activity. The muscle bundles which do not give a positive reaction for alkaline phosphatase under normal conditions show no significant activity of the enzyme throughout the degenerative and regenerative phases of their repair.

Discussion. The most immediate enzymatic change noted just after the wound in the skin of *Heteropneustes fossilis* was a significant increase in the alkaline phosphatase in various cellular components of migrating epithelium up to the epithelialization. This increase in the enzyme activity may indicate the highly active state of the cells at this stage. Low alkaline phosphatase in the wound epithelium after epithelialization in the initial stages of healing has been reported in mammals[12], reptiles[13], and amphibians[14]. The present study in fish confirms this finding and indicates that the anabolic events in these cells have probably taken place at a very slow rate. The gradual increase in the amount of glycogen during this period further supports this view[15].

The role of alkaline phosphatase in cell division and its high level in differentiating tissues have been reported[4,5,13,14,16-18]. A fairly high level of alkaline phosphatase

Distribution and relative activities of alkaline phosphatase in the basal cells, polygonal cells and giant cells at different phases of the epidermal repair

Time after wounding	Epidermis Basal cells	Polygonal cells Middle layer	Polygonal cells Outermost layer	Giant cells
15 min	+ + +	+ + +	+ + +	+
30 min	+ + +	+ + +	+ + +	+
45 min	+ + +	+ + +	+ + +	+
1 h	+ + +	+ + +	+ + +	+
2 h	+ + +	+ + +	+ + +	+
3 h	+ + +	+ + +	+ + +	+
4 h	+ + +	+ + +	+ + +	+
6 h	+	+	+	+
8 h	−	−	−	−
8 h	±	±	±	−
10 h	±	±	−	−
13 h	±	±	*	−
16 h	±	±	*	−
18 h	±	±	*	−
20 h	±	±	*	−
24 h	+ ±	+	*	−
2 days	+ +	+	*	−
3 days	+ + +	+	±	+
4 days	+ + +	+	±	±
5 days	+ + +	+ +	±	+ +
7 days	+ +	+ +	±	+
9 days	+ +	+ +	±	+
11 days	+ +	+ +	±	+
14 days	+ +	+ +	±	+
20 days	+ +	+ +	±	+
25 days	+ +	+ + +	±	+

Symbols: *very weak or almost negative; − negative reaction; ± weak; + moderate; + + strong; + + + very strong reaction.

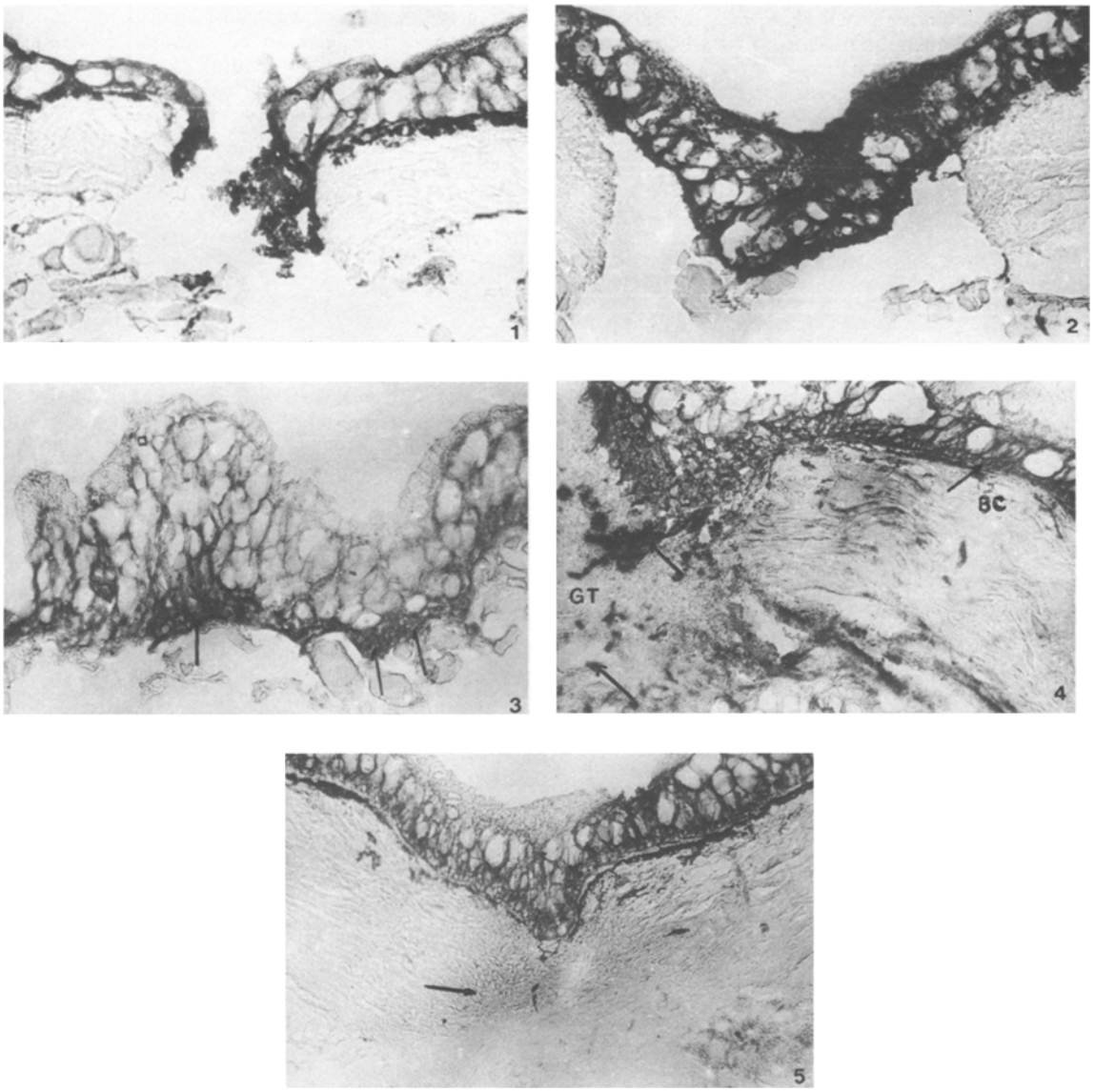

Figure 1. A view of the wound showing the very strong reaction for alkaline phosphatase activity in the migrating epidermal cells (frozen section, coupling azo-dye technique for alkaline phosphatase) (after 15 min) 160:1.

Figure 2. A photomicrograph showing a very strong reaction for alkaline phosphatase activity in the cells of the epidermis epithelializing the wound (frozen section, coupling azo-dye technique for alkaline phosphatase) (after 4 h) 160:1.

Figure 3. A view of the part of the epidermis showing an increase in the activity of alkaline phosphatase in the basal cells (marked by arrows) (frozen section, coupling azo-dye technique for alkaline phosphatase) (after 24 h) 160:1.

Figure 4. A view of part of the granulation tissue, dermis and epidermis showing moderate reactions for alkaline phosphatase activity in granulation tissue, strong reaction at the cut edges of the dermis and subcutis and a very strong reaction in the blood capillaries (marked by arrows). Note very strong reaction in basal cells of the epidermis (frozen section, coupling azo-dye technique for alkaline phosphatase) (after 5 days) 160:1.

Figure 5. A photomicrograph showing alkaline phosphatase activity in the epidermis covering the wound gap almost similar to that of the normal tissue. Note weak reaction for the enzyme activity at the core of the granulation tissue (frozen sections, coupling azo-dye technique for alkaline phosphatase) (after 7 days) 100:1.

activity in the basal cells between 2 and 6 days showed that this enzyme plays a significant role in cellular division and differentiation. The highly proliferative activity of the basal cells and the appearance of developing giant cells[19] reemphasize this assumption.

The present study shows a striking parallel between the distribution of alkaline phosphatase activity and that of acid mucopolysaccharides[20]. This enzyme is believed to be involved in the synthesis of mucopolysaccharides[3,21]. Further, the mucopolysaccharides synthesized may be utilized in the formation of connective tissue.

Although it was generally believed that alkaline phosphatase activity is associated with collagen fiber synthesis[22], later investigators have not been able to confirm this[23].

Recently it has been suggested that the activity of this enzyme has some function in phagocytosis and degradation of collagen[24]. The same authors also speculated that alkaline phosphatase acts in conjunction with collagenase in collagen breakdown and is probably involved in the removal of some phosphate groups associated with the intact collagen before collagenase acts to disrupt a peptide bond, or, as alkaline phosphatase is associated with calcium transport, it is involved in the provision of calcium ions, necessary for collagenase activity[25]. The demonstration of alkaline phosphatase activity in relation to collagen degradation rather than to collagen synthesis in the connective tissue[24] does not seem to be compatible with the long-standing suggestion that alkaline phosphatase is related to collagen synthesis, unless of course it is involved in both synthesis and degradation. In any case, rapid synthesis of collagen and collagen degradation often go side by side. The gradual disappearance of alkaline phosphatase with the maturation of the collagen fibers in *Heteropneustes fossilis* may indicate that synthesis and degradation of collagen have ceased with the completion of the dermal repair.

1 Mittal, A.K., and Banerjee, T.,K., Can. J. Zool. *53* (1975a) 833.
2 Mittal, A.K., and Banerjee, T.K., Can. J. Zool. *53* (1975b) 844.
3 Banerjee, T.K., Agarwal, S.K., Rai, A.K., and Mittal, A.K., Mikroskopie *32* (1976) 294.
4 Mittal, A.K., Rai, A.K., and Banerjee, T.K., Cell molec. Biol. *22* (1977) 227.
5 Saxena, M., and Kulshrestha, S.K., Experientia *37* (1981) 190.
6 Danielli, J.F., Fell, H.B., and Kodicek, E., Br. J. exp. Path. *26* (1945) 367.
7 Pilo, B., Asnani, M.V., and Shah, R.V., J. Anim. Morph. Physiol. *19* (1972) 205.
8 Ten Cate, A.R., J. Anat. *112* (1972) 401.
9 Gomori, G., Microscopic histochemistry: Principles and practice, University of Chicago Press, Chicago/Illinois 1952.
10 Pearse, A.G.E., Histochemistry, theoretical and applied, vol. 1, p. 1. Churchill Ltd, London 1968.
11 Barka, T., and Anderson, P.J., Histochemistry theory, practice and bibliography. Harper and Row, New York 1965.
12 Fell, H.B., and Danielli, J.F., Br. J. exp. Path. *24* (1943) 196.
13 Shah, R.V., and Chakko, T.V., J. Anim. Morph. Physiol. *14* (1967) 69.
14 Preda, V., Cristea, S., and Cracium, M., Morf. norm. Patol., Buc. *7* (1962) 305.
15 Rai, A.K., Ph. D. thesis, Banaras Hindu University, 1977.
16 Bevelander, G., and Johnson, P.L., J. cell. comp. Physiol. *26* (1945) 25.
17 Karczmar, A.G., and Berg, G.G., J. exp. Zool. *117* (1951) 139.
18 Schmidt, J., and Weary, M., J. exp. Zool. *150* (1962) 69.
19 Mittal, A.K., Rai, A.K., and Banerjee, T.K., Z. Mikrosk-anat. Forsch. Leipzig *2* (1978) 270.
20 Mittal, A.,K., Rai, A.K., and Banerjee, T.K., Mikroskopie *35* (1976) 265.
21 Kroon, D.B., Acta anat. *15* (1952) 317.
22 Bradfield, J.R.G., Q. J. microsc. Sci. *92* (1951) 87.
23 Gold, N.I., and Gould, B.S., Archs Biochem. *33* (1951) 155.
24 Ten Cate, A.R., and Syrbu, S., J. Anat. *117* (1974) 351.
25 Fullmer, H.M., and Lazarus, G.S., J. Histochem. Cytochem. *17* (1969) 793.

0014-4754/83/050520-03$1.50+0.20/0

Retention of cadmium in a freshwater fish, *Channa punctatus*

P.H. Shah[1] and M.S. Dubale

Zoology Department, University School of Sciences, Gujarat University, Ahmedabad-380009 (India), August 25, 1981

Summary. The study revealed a significant retention of cadmium in the liver and kidney of *Channa punctatus* exposed to various concentrations of cadmium. A linear relationship was observed between the period of exposure, the cadmium level in the medium and its retention in the target organs except for a slight decline in the quantity of cadmium in the liver when exposed to 0.01 ppm concentration.

Cadmium is known to enter the bodies of animals from the environment through the respiratory and gastrointestinal tracts[2]. The major sites of retention seem to be the liver and kidney[3]. A good deal of information is available on the absorption, distribution and excretion of cadmium in mammals, especially rats; however, not much is known about the absorption of cadmium in aquatic animals, including edible fishes. A few reports can be cited, for example that of Smith et al.[4] who studied acute effects of cadmium on the fish *Ictalurus punctatus*, and reported a higher accumulation of cadmium in the kidney than in the liver. Recently Banerjee et al.[5] recorded a slightly higher quantity of cadmium in the liver than in the kidney of *Tilapia mossumbica* and *Clarius batrachus* exposed to a very low dosage of cadmium chloride for 4 weeks.

Pandya[6] carried out estimations of cadmium in the drinking water from 14 different centers at Ahmedabad and found cadmium concentrations ranging between 0.001 and 0.004 ppm with an average concentration of 0.002 ppm. Patel[7] recorded cadmium concentrations between 0.001 and 0.007 ppm with an average value of 0.003 ppm in 30 samples collected from the river Sabarmati.

During the present investigation an air-breathing fish, *Channa punctatus*, was released in water containing different concentrations of cadmium to see whether this metal is retained in the target organs selected here viz., the liver and kidney and investigate the correlation between the concentration of cadmium in the medium, its retention in the body organs and the exposure period.

Materials and methods. Acquisition, care, maintenance and cadmium treatment of *Channa punctatus* have been described earlier[8]. The concentrations of cadmium and the time intervals at which the fishes were sacrificed were as follows:

Cadmium concentrations	Animals sacrificed at the end of
a) 0.01 ppm	1, 7, 14, 21, 28 and 35 days
b) 0.03 ppm	1. 7, 14, 21, 28 and 35 days
c) 0.05 ppm	1, 7, 14, 21, 28 and 35 days