DYNAMICS OF ALKALINE PHOSPHATASE ACTIVITY DURING HEALING OF ADRENALIN

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Nonspecific alkaline phosphatase activity was studied by the simultaneous azo-coupling histochemical method in the myocardium of rats at different times of healing of adrenalin-induced lesions of the muscle cells (from 3 h to 38 days). The results ruled out the direct participation of alkaline phosphatase in the synthesis of acid mucopolysaccharides and in the formation of collagen fibers in the granulation tissue. The characteristic localization of the fibroblasts, giving a positive reaction for alkaline phosphatase, suggested that the enzyme plays a role in the transport and metabolic function of fibroblasts at the sites of greatest mechanical load.

Little information can be found in the contemporary literature on enzymic aspects of connective tissue metabolism during healing of injured organs. Considering the principle of organ-specificity of the connective tissue, the study of this problem could yield interesting results.

EXPERIMENTAL METHOD

Incomplete regeneration of the myocardium was obtained by the use of adrenalin-induced lesions as a model, for all stages of mobilization of connective-tissue cells in their resorptive and productive functions can be studied. Attention was concentrated on nonspecific alkaline phosphatase (AP) activity, which in fibroblasts is associated with collagen synthesis [1-3].

Noninbred albino rats were used. After intramuscular injection of 1:1000 adrenalin solution in repeated small doses up to a total of 0.2 ml/100 g body weight the rats were sacrificed at intervals during the experiment from 3 h to 38 days. Activity of the enzyme was determined in frozen sections by the simultaneous azo-coupling method with AC-TP-phosphate as substrate and diazotized p-rosaniline as the azo dye, and counterstaining of the nuclei with hematoxylin. Paraffin sections obtained from the other half of the heart were stained with toluidine blue at pH 5.6, with picrofushsin by Van Gieson's method, and with Schiff's reagent by McManus' method with an amylase control.

EXPERIMENTAL RESULTS

A considerable decrease in the intensity of staining for alkaline phosphatase, or even its total disappearance, was observed in the capillary endothelium throughout the myocardium 3 h after the injection of adrenalin (Fig. 1a, b). This decrease in enzyme activity continued until the 5th day, with a return to normal by the 8th day of the experiment. The same result was found in the zone of injury to the myocardial cells, the only difference being that in the endothelium of capillaries passing through areas of newly formed connective tissue this reaction was particularly strong by the 8th day (Fig. 1c). Consequently, the formation and viability of the connective tissue require high alkaline phosphatase activity in the endothelium of the capillaries passing through it.

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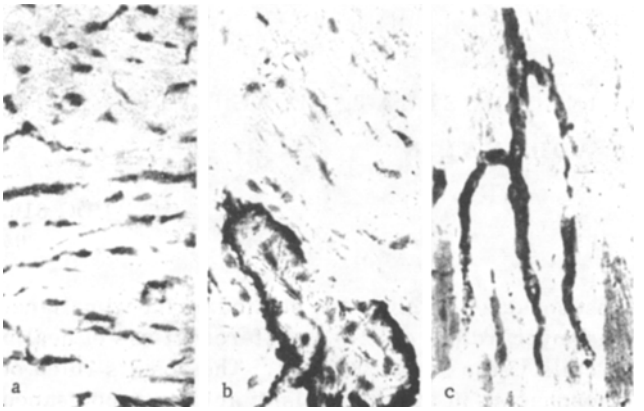

Fig. 1

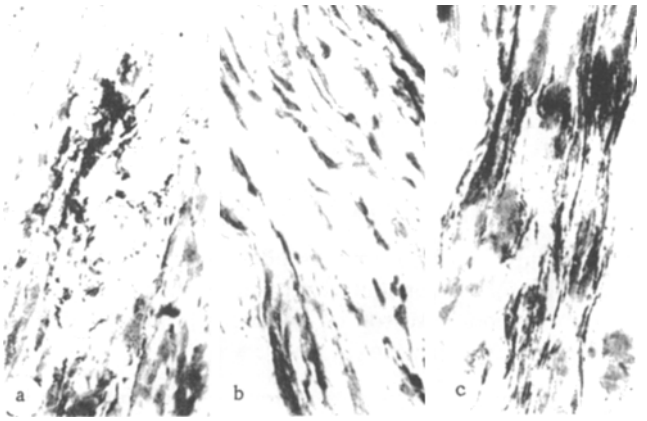

Fig. 2

Fig. 1. Alkaline phosphatase activity in capillaries of rat myocardium (280 ×): a) high activity in control; b) decrease in activity 12 h after beginning of experiment. High activity can be seen in adventitia of vessel; c) high activity in capillaries of scar on 8th day of experiment.

Fig. 2. Alkaline phosphatase activity in fibroblasts: a) high activity in adventitial cells migrating into zone of injury 48 h after beginning of experiment, 140×; b) enzyme activity in fibroblasts of scar is localized in cytoplasm and processes of cells. Collagen fibers are unstained, 280 ×; c) high activity in fibroblasts and their processes near "stamps" of muscle fibers, 140 ×.

The reaction for phosphatase was always absent in proliferating fibroblasts in a focus of regeneration, which were clearly visible 48 h after the beginning of the experiment. The first very weak signs of AP activity were not observed until the 6th-8th day of the experiment, in solitary fibroblasts, as a diffuse pink staining of the cytoplasm. Subsequently the AP-positive fibroblasts became more numerous, their enzyme activity increased, and the pattern of the reaction became "felt-like" with deposition of the dye mainly localized around the stumps of the muscle fibers, which had lost their cross-striation. This distribution of the enzyme in the fibroblasts distinguished them from the endothelial cells. At later stages fibroblasts with long processes, rendered clearly visible by the brightness of their reaction for alkaline phosphatase,

lay in close contact with the newly formed collagen fibers, giving an appearance like that of tendons, so that the continuity of the muscle fibers was restored (Fig. 2b, c).

The fibroblasts of the connective tissue which normally accompanies large arterial branches behaved rather differently. Here, in the control there was a strongly positive reaction for AP in the adventitial cells forming a ring around the vessel. In these areas, if foci of necrosis were adjacent, migration of AP-positive cells could be seen into the zone of necrosis 48 h after the beginning of the experiment, and vessels with no cells containing AP could be seen in this area (Fig. 2a). The intensity of γ-metachromatic staining, a characteristic feature of acid mucopolysaccharides, was highest 2 days after the beginning of the experiment, and fell rapidly until the 5th-8th day.

By the 5th day, when AP activity was still not well defined in the fibroblasts, thin collagen fibers uniformly distributed in the granulations were already observed in material stained with picrofuchsin and Schiff's reagent. By the 8th day their number had increased considerably.

The results thus showed that the dynamics of alkaline phosphatase, characterizing the connective-tissue response of the myocardium, with respect to the times of its appearance provides no evidence for the participation of AP either in the production of acid mucopolysaccharides or in the synthesis of collagen protein by fibroblasts.

The localization of AP-positive fibroblasts was initially strictly with the stumps of the muscle fibers, but later it spread between the collagen fibers in which enzyme activity remained until complete maturation of the scar. These facts suggest rather that AP plays a role in the transport and metabolic functions of fibroblasts, as Romanul and Bannister [5] have shown for endothelial cells. This enzyme evidently plays an essential part in maintaining the stability of the collagen fibers where the mechanical load is greatest. This hypothesis corresponds to the normal localization of the enzyme in the adventitial cells of the blood vessels which are always accompanied by collagen fibers. It is these connective tissue bands together with the arteries which have to support the greatest mechanical load in the phase of diastole [4].

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