Removal of contaminating hemoglobin from peroxidase in traumatic skin lesions

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Summary. A method is described for the removal of contaminating hemoglobin from the peroxidase enzyme in traumatic skin lesions. The procedure is based on hemoglobin precipitation in a combination of ammonium sulfate half-saturation, and chloroform shaking of the cetyltrimethylammoniumbromide extract. The procedure as such somewhat increases the activity of the peroxidase extract if the extract contains no hemoglobin. On the other hand, the peroxidase activity of the extract decreases as the amount of precipitating hemoglobin increases. On average, about 90% of the peroxidase activity persists after hemoglobin precipitation if the hemoglobin concentration in the extract does not exceed 25 mg/100 ml. In experimental incision wounds, the peroxidase activities obtained with this procedure were the same as when enzyme determinations were done without the removal of hemoglobin or slightly higher. In addition, the amount of peroxidase activity in the wounds was estimated, based on the granulocytes of the contaminating blood.

Key word: Peroxidase activity, removal of hemoglobin

Introduction

It has recently been shown that there is a rapid and remarkable increase in peroxidase activity in traumatic skin lesions during the first post-traumatic day, beginning in experimental lesions at 30 min after injury. This peroxidase increase, based presumably on the inflammatory reaction, was demonstrable in both experimental and autopsy specimens taken several days postmortem [1, 2]. Measurements of peroxidase activity might be useful for forensic medical purposes in estimation of the vitality and of the time of infliction of lesions. Before this assumption can be confirmed much more work is needed; in particular a large autopsy material must be collected and methods of eliminating the analytical errors that contaminating blood in the specimens can cause must be developed. These methodological errors are based (1) on the pseudoperoxidase activity of hemoglobin, (2) on the inhibition of myeloperoxidase activity by hemoglobin in the assay reaction, and (3) on the myeloperoxidase activity located in the leukocytes of the contaminating blood [3, 4]. Particularly when the activity levels in the specimens are very low, the contaminating blood may cause significant errors in the analytical results. Most of the inhibition by hemoglobin can be avoided by diluting the extracts made from the specimens, and the pseudoperoxidase activity as such is rather low [3]. If, however, the peroxidase could be purified of the contaminating hemoglobin, both the pseudoperoxidase effect and the inhibition could be avoided.

The present paper describes a simple, rapid method for removing the contaminating hemoglobin from the peroxidase in extracts of traumatic skin lesions, and attempts further, a rough estimate of the amount of myeloperoxidase derived from the granulocytes in the contaminating blood.

Materials and methods

To test the hemoglobin removal procedure mentioned in the Introduction, experimental incision wounds in rats were used. Incision wounds 5 cm long and perforating the skin were made on the right side of the dorsal skin. After different periods of vital time, zones about 1 mm thick were removed from the edges of wounds as specimens. Similar incision wounds of the skin were also made 1 min post mortem; in this case the specimens were taken 1 h later. In all cases, control specimens of normal skin were taken from opposite side of the dorsal skin of the rats. All the specimens were stored at −70°C for from some hours to several days or, if analyzed directly, first frozen. Part of each specimen was used for the peroxidase measurements and part for the hemin determinations.

Myeloperoxidase extraction and the removal of hemoglobin were accomplished as follows: the stored specimens were thawed to room temperature, minced with scissors in a 0.5% solution of cetyltrimethylammoniumbromide (Merck), and homogenized thoroughly at room