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Histochemistry of sulfhydryls in acute myocardial infarction

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Summary. To study the changes in sulfhydryl and disulfide distribution in myocardial infarction we applied the fluorescent sulfhydryl reagent, monobromobimane to sections of myocardium from patients dying of infarction of 24 h to 7 days duration. Staining for both sulfhydryls and for disulfide after reduction of slides blocked with N-Ethyl maleimide showed that sulfhydryls were decreased in the infarcted areas. Disulfides were increased in the periphery of infarction but cells undergoing cytolysis showed loss of disulfide staining as well as sulfhydryl staining. The causes and implication of these changes are discussed.

Key words: Sulfhydryl compounds – Myocardial infarction

The problem of diagnosing early myocardial infarction and of defining the extent of myocardial infarction has been extensively studied and numerous techniques for making this assessment have been developed (Doerr 1970). In addition to its purely medical interest, the ability to establish infarction as a cause of death has serious legal implications and accurate knowledge of the pathologic process is essential as Doerr (1981) has pointed out in his review of the pathologic anatomy of sudden death due to heart disease.

Numerous approaches to the problem of defining the age and extent of myocardial infarctions are possible, but one that has been of particular interest to pathologists is the study of changes in histologic patterns. The techniques have varied from the evaluation of routinely stained sections for specific changes such as the "wavy" fibers described by Majno and Bouchardy (1972) to the examination of special stains. Recent investigators using special stains include Rajs (1979) and Sunni and colleagues (1984). Because of the difficulty in performing them, many such stains have not been widely used. We, therefore, present here our initial observations using an easily performed stain for sulfhydryl compounds which shows promise for fruitful application to the diagnosis of myocardial infarction.

Cellular sulfhydryl groups maintain the functional state of enzymes and protect the cell from reactive chemical species such as free radicals and peroxides. Glutathione, a tripeptide, is the major cytoplasmic non-protein sulfhydryl and is particularly important for protection against oxidant stress, both by direct reaction with toxic compounds and as a substrate for glutathione peroxidase (Hirayama et al. 1983; Moldeus and Jengstrom 1983).

The association of sulfhydryl compounds with heart disease includes the indirect evidence of Keshan disease, a congestive cardiomyopathy associated with selenium deficiency (Ge et al. 1983). This disease has been attributed to lack of glutathione peroxidase. More direct evidence is provided by the observation of Barts and colleagues (1979) that sulfhydryl concentration is decreased during ischemia and that of Guarnieri and co-workers (1980) who showed that the glutathione/glutathione disulfide ratio was decreased during reoxygenation of ischemic tissue and was associated with increasing myocardial damage. Galvin and Lefer (1978) have shown a salutary effect of sulfhydryl compounds in cardiogenic shock.

To study changes in sulfhydryl states in ischemic myocardium we have applied the monobromobimane stain of Gainer and Kosower to 18 autopsy cases of patients who died from acute myocardial infarction diagnosed by standard histologic criteria and compared the appearance to uninvolved myocardium from the same patient and from 6 patients dying without evidence of myocardial infarction.

Monobromobimane is one of a class of compounds synthesized by Kosower and Kosower (1979) as sulfhydryl reagents. The bimanes are characterized by the formation of a fluorescent compound by nucleophilic substitution of bromine by the sulfur of thiols. The parent bimane is not fluorescent but the mercaptan gives a distinctive blue fluorescence when excited with ultraviolet light. Gainer and Kosower adapted monobromabimane as a histochemical stain. In addition to direct staining of free thiols, they described a technique for staining disulfides by reduction with dithiothreitol after blocking of free thiols with N-ethyl maleimide (NEM). We utilized these techniques for staining the infarcted and uninfarcted myocardium in our cases.

Materials and methods

Autopsy cases of patients dying from acute myocardial infarction were identified from the files of The Ohio State University Department of Pathology. The complete autopsy protocols were evaluated, routine histologic slides of myocardium were reviewed and representative sections of infarcted and uninvolved myocardium were identified for each case. The age of infarction was estimated by the criteria of Mallory and White (1939). Eighteen cases were identified which showed acute infarction of 24 h to 7 days duration and these were used in the staining studies. Six cases of patients dying without clinical or histologic evidence of myocardial infarction were also studied. Slides were stained with monobromobimane for sulfhydryls and disulfides as described below.

The staining method follows that of Gainer and Kosower (1980). Deparafinized slides which had been rehydrated were immersed in the 0.5 mM monobromobimane solution for

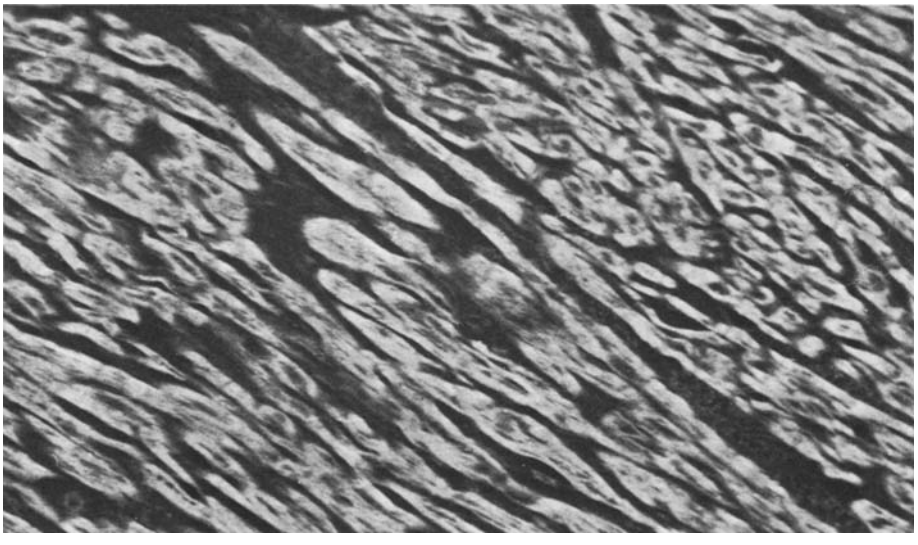

Fig. 1. Uninfarcted myocardium directly stained with monobromobimane for sulfhydryls. (Ultraviolet excitation epi-illuminated fluorescence, original magnification 100 ×)

20 min at room temperature, rinsed in phosphate buffered saline (pH 7.4) and coverslipped using an aqueous nonfluorescent mounting medium. To block the sulfhydryl groups the slides were immersed in 240 mM NEM in PBS at 37° C for 10 min. The slides were then washed in PBS and immersed in 50 mM DTT/1 mM EDTA for 5 min. The slides were then washed and stained for thiols as above.

The slides were examined using a Nikon Optiphot photomicroscope with a mercury source providing epi-illumination through a U.V. excitation filter. The excitation wavelengths for the bimanes are from 370–385 nm.; the emission wavelengths are from 477–484 nm. Kodak Panex film was used for the photomicrographs.

Results

The uninfarcted hearts and the uninvolved portions of infarcted myocardium showed the staining of myocardial cells shown in Fig. 1. The cells showed diffuse staining of the cytoplasm with visible cross-striations which were somewhat obscured by the cytoplasmic staining. Blocking with NEM abolished staining almost completely. Reduction with DTT after NEM blocking restores a small amount of myocyte while accentuating the fibrous tissue elements which are rich in disulfides.

In all cases of myocardial infarction, the infarcted myocardium showed a decrease in staining of the infarcted myocytes (Fig. 2). Areas of advanced myocytolysis showed nearly complete loss of staining while the surrounding areas showed decreased staining with accentuation of the cross-striations due to loss of cytoplasmic staining. Loss of staining was not uniform in areas of infarction. This was particularly evident in the subendocardial regions where there was often preservation of a layer three to six cells thick immediately under the endocardium (Fig. 2).

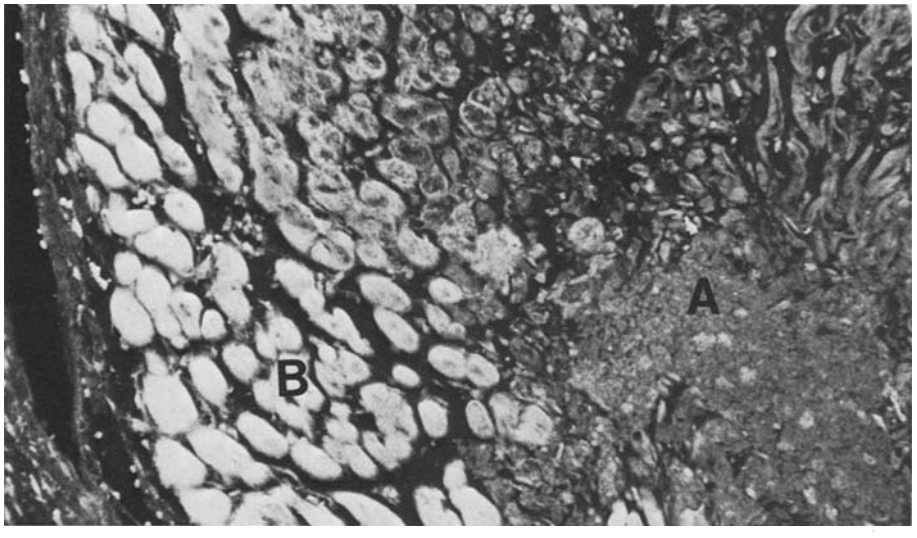

Fig. 2. Myocardial infarction directly stained with monobromobimane for sulfhydryls. Note loss of staining in areas of necrosis **A** and retention of staining in the subendocardial areas **B**. (Ultraviolet excitation epi-illuminated fluorescence, original magnification 100 ×)

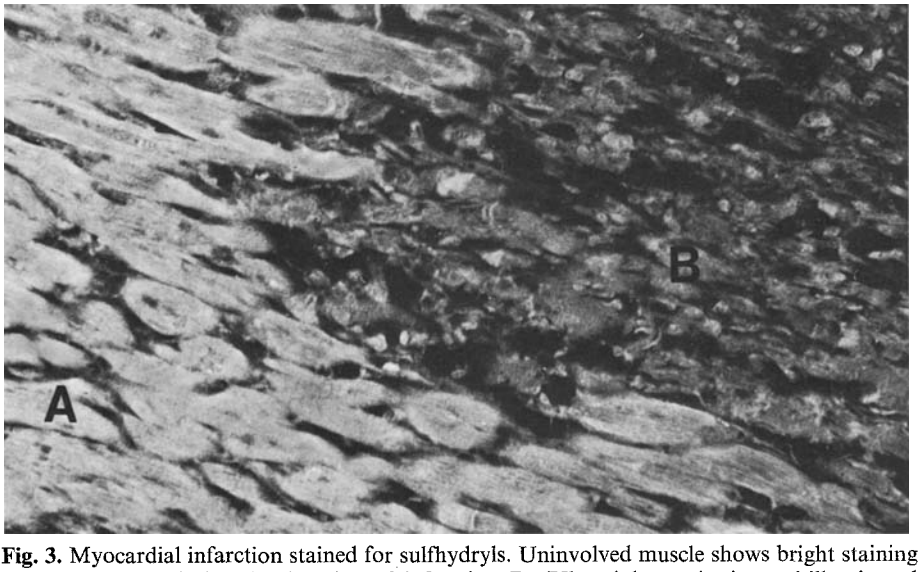

Fig. 3. Myocardial infarction stained for sulfhydryls. Uninvolved muscle shows bright staining **A** while staining is lost in the area of infarction **B**. (Ultraviolet excitation epi-illuminated fluorescence, original magnification 200 ×)

When infarcted sections were stained for disulfides, in addition to the usual acentuation of the fibrous tissue, variable staining of the infarcted area was seen. Total loss was persistent in the necrotic areas, while the better preserved fibres often showed complete recovery of staining. Uninvolved fibers in the area had no significant staining for disulfide (Figs. 3 and 4).

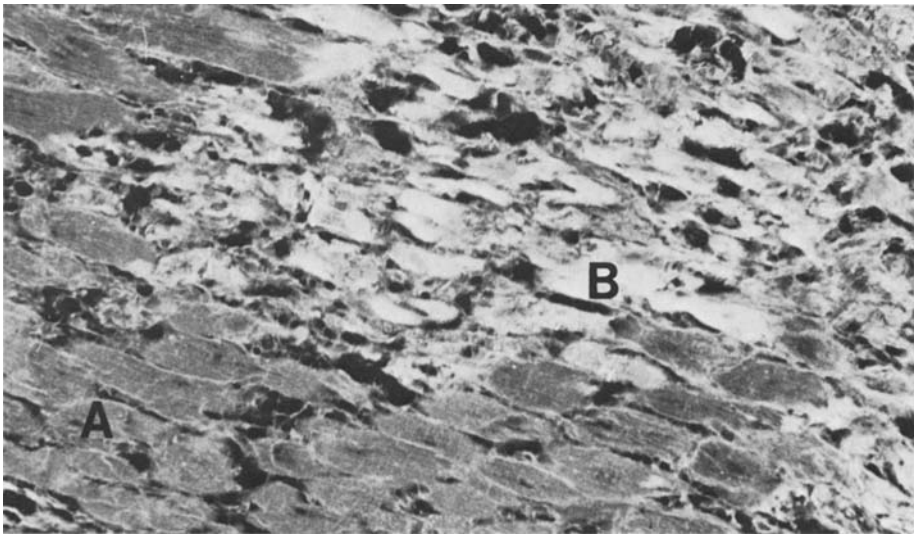

Fig. 4. Section from the same areas as shown in Fig. 3 stained for disulfides. Note the loss of staining of the muscle **A** and partial restoration of staining in the infarcted area **B**. (Ultraviolet excitation, epi-illuminated fluroescence original magnification 200 ×)

Discussion

The most likely cause of the decrease in thiols in ischemic cells is loss of ability to produce NADPH which is necessary for glutathione reductase. The oxidation of glutathione would result in the loss of a reducing substance to maintain protein sulfhydryls in a reduced state. The depletion of glutathione would be speeded up by the presence of free radicals which could react directly with thiols or produce lipid peroxides which would react with glutathione through the mechanism of glutathione peroxidase. The presence of such free radicals in ischemic myocardium is indicated by the studies of Rao et al. (1983), and McCord (1985) has reviewed the evidence for the production of oxygen free radicals in ischemic tissue and discussed the mechanisms for the production of these radicals. The lack of disulfide staining in necrotic myocardium suggests that with severe damage the sulfhydryls react in a manner which results in a product other than disulfides, probably an oxygenated acid of sulfur. The better preserved cells which show disulfide staining suggest that enzymatic reactions which favor disulfide formation were taking place. These observations may indicate that the staining for disulfides in ischemically damaged myocardial cells is an indication of the severity of the injury but this theory remains to be proven.

A further pattern of staining of interest is the preservation of subendocardial staining in certain cases. The extremely limited extent of such preservation (3–6 cells thick) may be explained by the limited oxygenation of this area by intraventricular blood. The sparing of this area supports the conclusion that the loss of sulfhydryl staining is the result of ischemia.

Although the majority of changes in sulfhydryl in infarcted cardiac myocytes can be attributed to direct anoxic damage, other factors may add

to this loss. Most important of these is the possibility of oxidation by peroxides and other active species produced by activated neutrophils (Sagone et al. 1984). It is difficult to determine the importance of such a mechanism in infarction, but studies of primary inflammatory disorders such as myocarditis may clarify this issue.

Readily discernible differences in myocardial sulfhydryls and disulfides have been described in acute myocardial infarction. This staining technique allows easy distinction of damaged tissue in acute infarction. The time course of these changes has not been determined but it is our opinion that these chemical changes will serve as useful markers for the age, extent and severity of myocardial ischemic damage, as well as further elucidating the chemical damage caused by ischemia.

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