PROTECTIVE RESPONSE OF THE MYOCARDIUM TO DIPHTHERIA TOXIN

V. A. Frolov, G. Mall,
P. Rieger, H. Derks,
and Z. Antoni

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Pathological changes arising in the myocardium in response to toxic factors may often play a definite protective role [1]. From this point of view it was interesting to analyze morphological changes in the heart muscle in response to toxic factors of varied etiology, for pathological processes of this kind are characterized by penetration of the pathogenic factor from the blood stream into the tissue, and accordingly, definite changes in the vascular wall or perivascular zones may be expected to develop, and although the result of tissue damage, they may at the same time be capable of performing a protective function, preventing further infusion of the toxic principle.

EXPERIMENTAL METHOD

Experiments were carried out on 40 mature male Chinchilla rabbits weighing 2.5-3 kg. The animals were divided into eight groups (five rabbits in each group). Group 1 served as the control, and animals of the other three groups (2, 3, and 4) received a single intravenous injection of 1 MLD native diphtheria toxin, titrated beforehand on guinea pigs, thus creating a model of diphtheria intoxication. The rabbits of group 5 were given daily subcutaneous injections of oily solutions of retinol (10,000 IU/kg) and α-tocopherol (10 mg/kg) for 10 days. Vitamins A and E were used in these experiments because of a parallel study of the state of the lysomal membranes of the cardiomyocytes which was undertaken (in the above doses these vitamins stabilize lysosomal membranes). Animals of groups 6, 7, and 8, at the end of the vitamin injections, were given a single intravenous injection of 1 MLD of diphtheria toxin. The experimental animals were killed by thoracotomy under light hexobarbital anesthesia 24 and 72 h and 6 days after injection of the diphtheria toxin, and the hearts were removed and perfused with 2.5% glutaraldehyde solution. Pieces of the papillary muscles of the left and right ventricles were excised, postfixed in 1% buffered osmic acid solution (pH 7.2-7.4) in accordance with the usual methods, and embedded in Araldite. Pieces of myocardium of the control animals and of the rabbits receiving vitamins A and E were processed in the same way. Semithin (1-2 μ) and ultrathin (150-200 nm) sections were cut on an ultramicrotome (Reichert-Jung Ultracut). The semithin sections were stained with alkaline fuchsin and methylene blue by the method in [4] – histochemical reactions revealing various inter- and intracellular structures (especially collagen, which stains blue). Next, collagen was studied quantitatively in sections examined under the light microscope with an ocular fitted with a morphometric grid. Ultrathin sections were stained with lead hydroxide and uranyl acetate and examined in the Zeiss-9 electron microscope under a magnification of 3200-18,000. All the numerical data were subjected to statistical analysis by Student's test on a Commodore-64 personal computer. The significance of the difference between the means was determined at the $p \leq 0.05$ level. During correlation analysis, correlation was assessed as strong if the absolute value of the coefficient of correlation $r \geq 0.7$, as moderately strong when $r = 0.69$-0.3, and as weak when $r \leq 0.29$.

EXPERIMENTAL RESULTS

Small amounts of collagen were found in the myocardium of intact rabbits (Fig. 1). Large quantities of a substance giving the blue color characteristic of collagen were found in the

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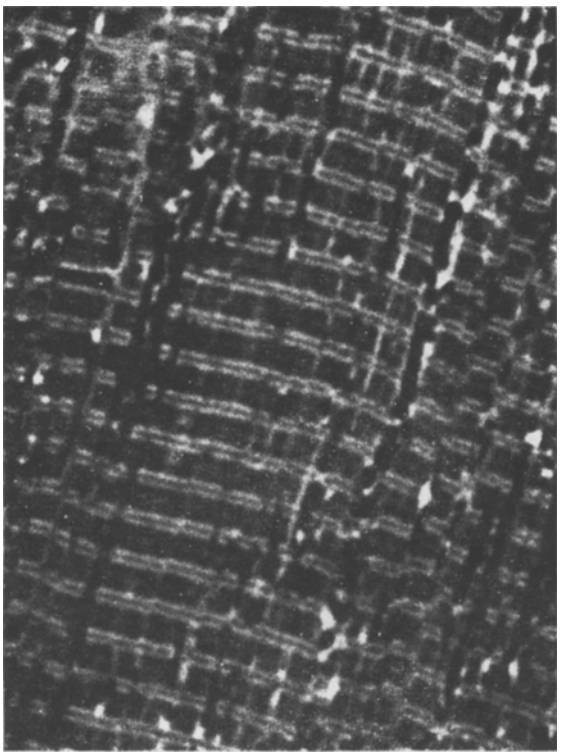

Fig. 1. Semithin section through papillary muscle of right ventricle of an intact rabbit (482 ×).

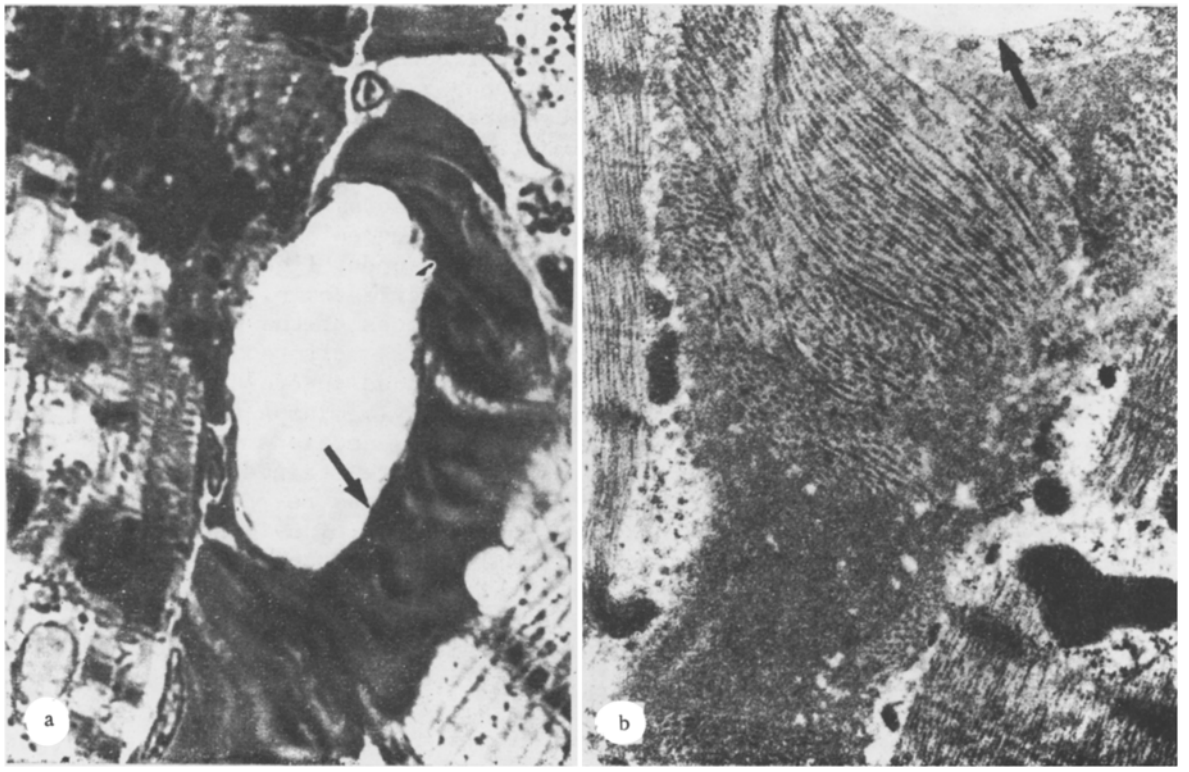

Fig. 2. Right ventricular myocardium (papillary muscle) of a rabbit 24 h after injection of diptheria toxin. a) Semithin section: structureless "cut" around a blood vessel (arrow), hypercontraction bands of muscle fibers (482 ×); b) ultrathin section: collagen fibrils in amorphous mass around the capillary (arrow). 6200 ×.

myocardium 24 h after injection of the diphtheria toxin (Fig. 2); it was located between the muscle fibers and it was particularly plentiful around the blood vessels, forming distinctive "cuffs." These phenomena were much more marked in the right ventricle than in the left. After 72 h this substance was less plentiful, but after 6 days that amount increased again.

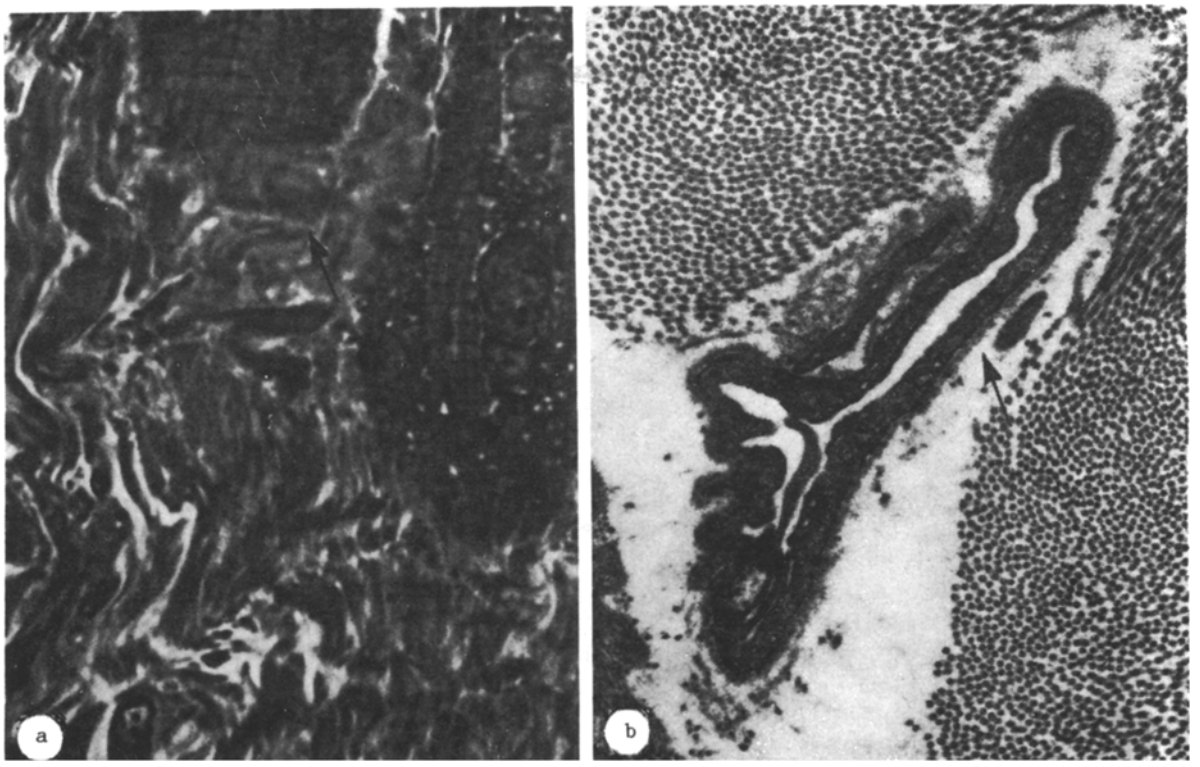

Fig. 3. Right ventricular myocardium of a rabbit (papillary muscle) 24 h after injection of diphtheria toxin preceded by injection of vitamins A and E. a) Semithin section: large quantity of amorphous substance, areas of conversion of muscle fibers into it (arrow). 482 ×; b) ultrathin section: capillary (arrow) surrounded by collagen fibers. 6200 ×.

In the animals receiving preliminary injections of retinol and α-tocopherol, very large quantities of this substance were observed in the myocardium 24 h after injection of the diphtheria toxin (Fig. 3); it was distributed not only as well-developed bands between the muscular layers or in the form of perivascular cuffs, but also, as was shown histochemically, directly in the muscle fibers, which can be interpreted as transformation of muscle tissue into this substance.

The results of quantitative analysis of the semithin sections are given in Table 1. Clearly they confirm the trend noted above in the formation of collagen-like substance in the myocardium in diphtheria intoxication.

Correlation analysis showed that in diphtheria intoxication there was no significant correlation between the amounts of this substance in the right and left ventricles ($r = +0.46$). In diphtheria intoxication, induced after preliminary injection of vitamins, correlation was present and was strong ($r = +0.97$). It can thus be postulated that the additional toxic action of these vitamins on the myocardium (and the doses of vitamins A and E which we used were undoubtedly not physiological) intensifies this pathological process in the left ventricle, bringing it up to the level observed in the right ventricle.

In previous investigations [2, 3, 5] the writers observed similar phenomena in the myocardium on animals exposed to the toxic effects of ethanol and drugs. Meanwhile in pathological processes in the myocardium not based on toxic damage [3, 6], the features described above were not found, or were observed only in the final stage of the process.

Comparison of the results of the present investigation with data in the literature shows that the formation of this substance in heart muscle is characteristic of pathological processes in which the toxic agent penetrates into myocardial tissue, and it is a particularly clear general rule that this substance is distributed in the form of perivascular "cuffs." It can accordingly be postulated that in this case we are dealing with a unique type of protective response formed on the basis of myocardial damage, but preventing further penetration of the toxic agent from the blood stream into the tissues. However, the formation of large quantities of this substance must undoubtedly reduce the elasticity of the heart muscle and

TABLE 1. Formation (in vol. %) of Collagen-like Material in Left and Right Ventricular Myocardium of Rabbits after Injection of Diphtheria Toxin Alone and Preceded by Injection of Large Doses of Retinol and α-Tocopherol

Experimental conditions	Time of investigation			
	background	24 h	72 h	6 days
Diphtheria intoxication				
Left ventricle	3,8±0,36	5,77±0,45	3,27±0,34	6,03±0,45
Right ventricle	6,95±0,42	13,77±0,69	5,67±0,44	5,73±0,45
Diphtheria intoxication preceded by injection of vitamins A and E:				
Left ventricle	6,77±0,48	12,13±0,65	2,87±0,31	6,27±0,46*
Right ventricle	6,28±0,45	16,77±0,76	3,9±0,37	7,97±0,52

Legend. Asterisk indicates that mean values within the group do not differ significantly from background values.

also, consequently, its contractile properties. Moreover, these perivascular "cuffs" prevent transport of oxygen and nutrients into the myocardium. This protective reaction thus begins to acquire a pathogenetic character.

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CYTOPHOTOMETRIC STUDY OF MYELOPEROXIDASE IN BLOOD NEUTROPHILS AND WOUND EXUDATE DURING EXPERIMENTAL WOUND HEALING

L. A. Mamedov, A. V. Nikolaev, V. V. Zakharov, A. B. Shekhter, and Yu. R. Khrust

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Myeloperoxidase (MP) is the main component of the most important bactericidal system of neutrophilic granulocytes [6, 9]. MP activity is found in virtually all cells [5]. Its content in neutrophils varies depending on the pathological state [5, 7], including in many surgical diseases [2]. However, the dynamics of the changes in MP activity during wound healing has not yet been explained, although the treatment of wounds remains an urgent problem in modern surgery [4].

This paper describes a cytophotometric investigation of the content of the end product of the cytochemical reaction for MP in the blood neutrophils and wound exudate during healing of full-thickness aseptic and infected skin wounds in rats.

I. M. Sechenov First Moscow Medical Institute. M. V. Lomonosov Moscow University.

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