EXPERIMENTAL MYOCARDIAL INFARCTION IN ASSOCIATION WITH CERTAIN CARDIAC REVASCULARIZATION OPERATIONS IN LIGHT OF HISTOCHEMISTRY

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Of the methods for surgical treatment of chronic coronary insufficiency, recognition has been made of the operations for cardiac revascularization (bilateral transplantation of the internal mammary arteries, abdominalization of the heart, and the creation of aseptic exopericarditis). The corrective effect of this type of intervention on the circulatory organ in general and the coronaries in particular has been proven [1, 7]. However, the general biological role of organ revascularization operations has not been sufficiently studied. Only isolated works appear in the literature on this problem [6].

The morphology and histochemistry of experimental myocardial infarction has been studied in detail [3, 5, 8, 11, 14, 15, 22], but histochemical studies of experimental myocardial infarction incurred in association with surgical revascularization of the heart have not been carried out. The main changes in the zone of potential necrosis and in the paranecrotic region must be expected during the early phases of observation and are associated with energy material (glycogen); the regrouping of acid mucopolysaccharides occurs in the later phases. This served us as a basis for performing histochemical study of these substances in experiments on dogs in which myocardial infarction was produced after certain cardiac revascularization operations.

EXPERIMENTAL

A total of 29 experiments were performed, 13 of which were controls. Myocardial infarcts were produced in the experimental series by ligation of the main trunk of the descending branch of the left coronary artery in animals in which from 20-194 days previously bilateral transplantation of the internal mammary arteries had been performed, surgical aseptic exopericarditis created, or a combined procedure done. For controls we used dogs in which experimental myocardial infarction had been produced but no operation for cardiac revascularization performed. At different periods after production of the myocardial infarct (from several minutes to 102 days) the animals were sacrificed by intravenous injection of ether. To evaluate the data obtained in the experiment we also studied eight dogs after production of aseptic exopericarditis and four intact dogs.

For the morphological and histochemical investigations of various sections of the heart muscle, pieces were excised, fixed in 12% formalin solution, and embedded in paraffin by the usual method. To reveal substances which contain the 1, 2-glycol group, the periodic acid Schiff reaction [9] was carried out with the following controls: treatment of sections with α-amylase [9], acetylation (blockage of amino and hydroxyl groups) according to Lillie [6], blockage of aldehyde groups by hydroxylamine hydrochloride according to Culling [12], extraction of lipids in hot methanol-chloroform [4], weak acid hydrolysis according to Schmitz-Moormann [20]. Acid mucopolysaccharides were identified by staining the sections with Alcian blue, toluidine blue, and by the method of Mowry-Kiel [19]. The acid mucopolysaccharides were differentiated with the help of the following control experiments: blockage of carboxyl and sulfate groups (methylation) according to Fisher and Lillie [13], demethylation according to Spicer and Lillie [21], sulfuration according to Moore and Schoenberg [18], treatment of the sections with mucolytic enzymes

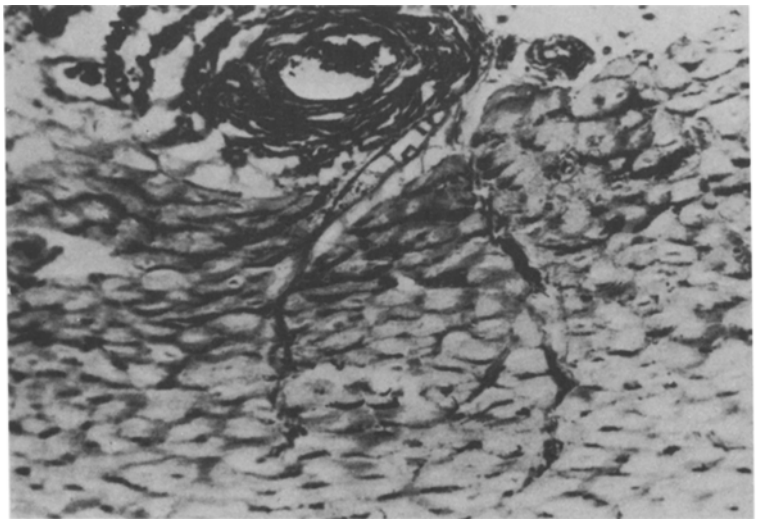

Fig. 1. Increase in glycogen content in cardiac muscle fibers around vessels after operation to create aseptic exopericarditis. Microphotograph. PAS-reaction. Objective 20 ×, ocular 7 ×.

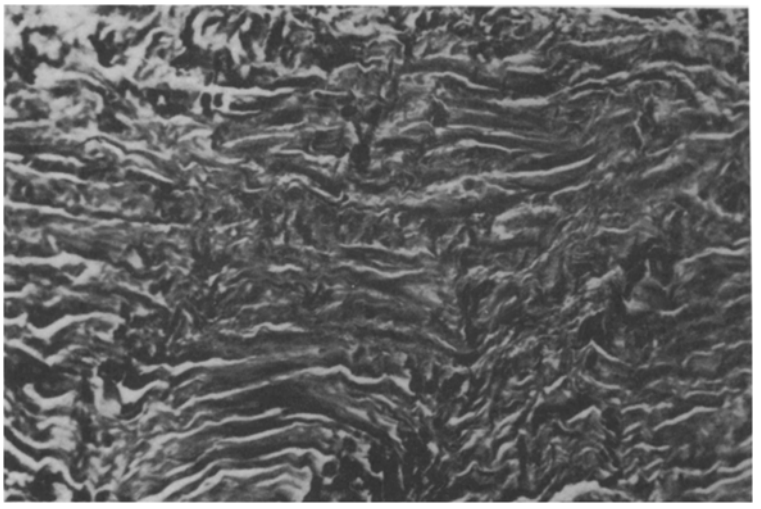

Fig. 2. Experimental myocardial infarction. Avascular coarse fibrous hyalinized scar. Microphotograph. Van Gieson stain. Objective 20 ×, ocular 7 ×.

(testicular and bacterial hyaluronidase) according to the schema carried out under the guidance of Pearse [9]. Besides the histochemical methods of treating the sections they were stained with hematoxylin-eosin, with Van Gieson's mixture with resorcin-fuchsin for elastic tissue, and were impregnated with Gomori's stain.

RESULTS

Upon investigation of the heart of intact animals it was found that glycogen (as granules of a dark-violet color in the periodic acid Schiff reaction) is more or less evenly distributed in the muscle fiber. In different muscles the fibers are noted to be unequally PAS-positive in staining, which is explained by the different functional activity of different groups of muscle fibers [10]. The glycogen appears in the sarcoplasm and in the myofibrils. The main

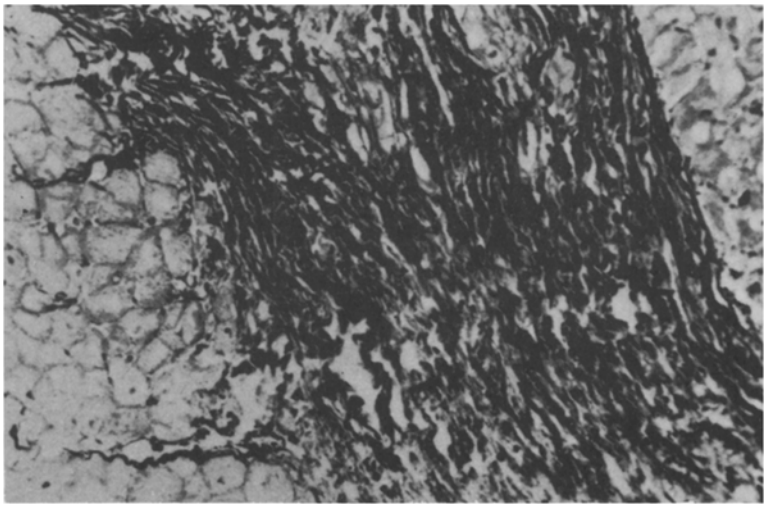

Fig. 3. Experimental myocardial infarction after previously performed operation to create aseptic exopericarditis. Finely fibrillar scar without signs of hyalinization. Microphotograph. Van Gieson stain. Objective 20 x, ocular 7 x.

interstitial substance of the intermuscular layers and vessels stains metachromatically with toluidine blue, a deep blue color with Alcian blue, and gives a positive Mowry-Kiel reaction. Carrying out the reactions in the controls it was possible to establish that the main substance in these structures contains acid mucopolysaccharides, mainly hyaluronic acid and chondroitin sulfate (mainly chondroitinsulfuric acid A and C).

Histochemical study of the heart in animals which previously had undergone an operation for aseptic exopericarditis revealed a distribution of glycogen and mucopolysaccharides in the above enumerated structures. This distribution was identical to the normal. In muscle fibers which lay near vessels which, after this intervention, underwent changes of considerable reorganization, an increased glycogen content was noted (Fig. 1).

In control animals for 20-30 min after the ligation of the main trunk of the left coronary artery the amount of glycogen in the muscle fibers in the zone of ischemia significantly decreased. However, after 60 min in other groups of muscle fibers in the ischemic zone the glycogen content was maintained. Glycogen was also found in the paranecrotic area and along the vessels. By the sixth to eighth hour after disruption of the coronary circulation rather marked signs of hemodynamic injury were observed (hyperemia, stasis of blood, perivascular extravasation, eosinophilia of muscle fibers, orthochromasia of fibers with toluidine blue staining). At this time the PAS-positive reaction as shown in the control studies was explained by the appearance of compounds which contained neutral mucopolysaccharides, lipoproteins, and, probably, sialic acid. The identical reactions were also observed in the edema fluid. After 24 h, besides the intensification of the hemodynamic disruption and the marked dystrophy (eosinophilia of the cytoplasm, disappearance of the cross striations, and karyolysis of groups of muscle fibers), an increase in PAS-positive staining was found. By the second to third day pronounced centers of γ-metachromasia were observed in the infarction zone upon staining with toluidine blue, and this increased with time.

Control experiments permitted us to establish that this reaction is explained by the accumulation in centers of necrosis of hyaluronic acid and chondroitin sulfate type C. By the fourth day, in the ground substance of the connective tissue are found regrouped acid mucopolysaccharides with an increase in chondroitin sulfate B, which coincides in time with the fibroblast reaction and the appearance of an increased number of bundles of argyrophilic fibers. In the later period of observation (6 to 12 days), together with the collagenization of the argyrophilic fibers an increased content of chondroitin sulfate B is found in the ground substance, and compounds containing the 1,2-glycol grouping are found in the newly organized collagen bundles. By the thirtieth to sixtieth days the quantity of acid mucopolysaccharides and PAS-positive material in the scar zone has decreased. At this time elastic fibrils appear. By the third month massive avascular, coarsely fibrillar scars have formed with characteristics of hyalinization (Fig. 2).

Histochemical and histological study of the heart in animals in which myocardial infarction was produced in association with a previous Fieski operation gave results similar to those noted above. In the case of ligation of the coronary artery in animals which had previously undergone a procedure to create aseptic exopericarditis or the latter procedure in combination with the Fieski operation, the histochemical method permitted the finding of essential differences from the changes produced after experimental infarction in the control animals. In the first minutes after ligation of the artery no morphological nor histochemical changes in the cardiac muscle were observed. At 30 min the ischemic zone could not be determined. The distribution and content of glyogen in the muscle fibers was similar to normal. At $3\frac{1}{2}$ h in the zone of the ligated coronary artery there appeared groups of muscle fibers, the glycogen content of which was markedly decreased; at the same time fibers were found with increased glycogen content. This might be particularly clearly seen around vessels, the number of which was considerably increased in pericarditis. At a later period the muscle fibers which lacked glycogen underwent dystrophy and necrobiosis. On the second day signs of fibrillary disruption might be seen with the appearance of necrotic fibers of acid mucopolysaccharides in the stroma. These had previously been PAS-positive, evidently at the expense of the neutral mucopolysaccharides. By the tenth to fifteenth day bundles of connective tissue strands had formed, between which were found unchanged groups of muscle fibers. After 2 to 3 months well vascularized scars had developed, consisting of thin argyrophilic collagen and elastic fibers which did not show hyalinization (Fig. 3).

The presence of high-caloric energy material in the cytoplasm of the muscle fibers (material which appeared to be glycogen) characterizes the functional state of these cells to a known degree. As the most labile energy material, glycogen rather rapidly disappears from tissue structures which are in a hemodynamically injured state. Similar data have been obtained by numerous authors [2, 11, 17]. The quantitative content of glycogen in the protoplasm of cellular elements is in definite proportion to the blood flow to the organ. Intensification of the latter is accompanied by the accumulation or synthesis within the muscle tissue (a question which requires further study) of an increased amount of polysaccharide and is limited in known degree by the potential capacity of the vascular system. Marked hemodynamic interruption is accompanied by the mobilization of the intracytoplasmic energy resources, mainly glycogen or by aerobic or anaerobic glycogenolysis.

The accumulation of acid mucopolysaccharides in the stroma of muscle fibers which have expended their glycogen evidently characterizes the irreversibility of the dystrophic processes and is explained by the depolymerization of the ground substance. This occurs initially at the disruption and injury of the cell membrane and is accompanied by the outflow of myocardial stroma and the accumulation of mucopolysaccharides in the ground substance of the connective tissue. Further regrouping of the latter with an increase in the sulfated acid mucopolysaccharides is linked with the process of collagen formation. These processes, however, are determined in considerable degree by the type of ischemia in the zone of infarction.

Surgical revascularization of the heart, which gives a marked hemodynamic effect, in the experimental myocardial infarct decreases the degree of ischemia in the muscle fibers, permits the intensification of metabolism, decreases the zone of necrobiosis, and prevents the development of a coarsely fibrous scar. To intervention of this type, we first added an operation to create an aseptic exopericarditis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
