A HISTOCHEMICAL INVESTIGATION OF SUCCINIC AND MALIC DEHYDROGENASES AND OF DPN- AND TPN-TRANSAMINASES IN EXPERIMENTAL MYOCARDIAL INFARCTION

E. F. Lushnikov

Laboratory of General Pathological Anatomy (Head, Corresponding Member AMN SSSR Professor A. I. Strukov), Institute of Normal and Pathological Physiology (Director, Active Member AMN SSSR Professor V. V. Parin) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 4, pp. 115-120, April, 1962
Original article submitted April 24, 1961

Biochemical investigations have shown [7, 8, 16] that the principal oxidation-reduction enzymes are situated in the mitochondria. Recent histochemical research using tetrazolium salts [4, 11, 13, 14] has largely confirmed that the oxidation-reduction enzymes and, in particular, the enzymes of the Krebs cycle are situated in the mitochondria.

The morphology of myocardial infarction has been studied experimentally by many workers using the ordinary histological or histochemical techniques [1, 2]. Only isolated papers, however, have been published [10, 12, 18] on the histochemical study of the oxidation-reduction enzymes in this disease. We accordingly decided that it would be of interest to investigate the activity of succinic and malic dehydrogenases and of DPN- and TPN-transaminases, which have a direct bearing on metabolism in the Krebs cycle.

EXPERIMENTAL METHOD

Experiments were conduted on 19 male rabbits weighing 1800-2500 g, one of which was used as a control. Myocardial infarction was produced by tying the anterior descending branch of the left coronary artery at the lower border of the left auricle through an incision in the midline of the chest. At various intervals after ligation of the coronary artery (from 10 minutes to 8 days) the animals were sacrificed by air embolism. The heart was at once extracted from the thorax and transverse histopographical sections were cut from it to a thickness of 30 μ in a cryostat. The sections were glued to dry glass slides without albumin.

Fig. 1. Uneven distribution of monoformazan granules in the cytoplasm of the muscle fibers outside the zone of ischemia. Reaction for succinic dehydrogenase. Neotetrazolium. Objective 20, eye-piece 10.

The activity of the above-mentioned enzymes was determined by the methods described in Pearse's textbook [14]. In the reaction for succinic dehydrogenase we used neotetrazolium (NT) and nitro-blue tetrazolium (nitro-BT), and in the other reactions nitro-BT. Control sections were heated and incubated in a solution without substrate, and sodium malonate was used to inhibit succinic dehydrogenase. Sections were also stained with hematocylin-eosin and toluidine blue.

EXPERIMENTAL RESULTS

During the investigation of the heart muscle of the control rabbit, and in the myocardial fibers outside the ischemic zone in the experimental animals, the activity of all the enzymes tested was shown by collections of small or large monoformazan granules of a dark purple color in the cytoplasm when NT was used, or of small dark blue diformazan granules when nitro-BT was used.

The distribution of the granules in the muscle fibers of the myocardium was uneven: some contained many granules, others fewer (Fig. 1); some fibers contained granules of monoformazan, others of diformazan (depending on the tetrazolium salt used). The reaction for succinic dehydrogenase showed the most marked activity. The blood vessels of the myocardium and the connective tissue cells of the annulus fibrosus of the heart gave a clearly positive reaction for all enzymes except succinic dehydrogenase, the activity of which was very weak.

Fig. 2. The three zones of enzyme activity in an infarct: 1) complete absence of formazan granules in the central part (bottom of the picture); 2) decreased number of granules (middle of the picture); 3) normal number of granules in intact muscle tissue at the periphery of the infarct (top, left). Reaction for succinic dehydrogenase. Objective 20, eye-piece 10.

Until 2 hours after ligation of the coronary artery no morphological changes or changes in enzyme activity could be found inside or outside the zone of ischemia. As other writers [11, 13] and we [3] have shown, the only change in this period is the disappearance of glycogen from the myocardium.

Two hours after the ligation of the coronary artery no morphological changes could be found. The reactions for enzymes, however, showed the first signs of disturbance of the coronary circulation, consisting of changes in the color of the monoformazan, which acquired a bluish hue; sometimes the unevenness in the distribution of the enzyme activity in the muscle fibers disappeared or the number of formazan granules in the fibers decreased. These changes were inconsistent, however, and ill-defined.

From 4 to 6 hours after ligation the first signs of infarction appeared in the sections stained with hematoxylin-eosin: edema of the stroma developed, and unevenness of staining of the muscle fibers with eosin, cloudy swelling of the fibers, small areas of extravasation, and infiltration of the stroma with leukocytes and lymphoid cells were observed. The myocardial stroma and the perivascular spaces began to show metachromasia when stained with toluidine blue. The enzyme reactions showed no unevenness in the distribution of granules of formazan in the myocardial fibers in the zone of ischemia, and all the granules were stained blue. Immediately next to the ligature fibers with lowered activity were observed, some without formazan granules.

Twelve hours after ligation the edema and metachromasia of the myocardial stroma were more advanced, the infiltration with leukocytes and dystrophic changes in the muscle fibers increased, and here and there their nuclei had broken up. At this time the number of fibers in which the enzymes were inactive was now considerable, as shown by the absence of formazan granules. Next to them were muscle fibers with diminished enzyme activity (decreased number of formazan granules), and beyond these were fibers with uniform enzyme activity. Reactions for all the enzymes revealed formazan granules in the lymphoid and epitheloid cells and in the leukocytes in the zone of necrosis. The number of granules in one cell varied from 2-3 to 6-8, and they were arranged around the nucleus.

Twenty-four hours after ligation the zone of necrosis was clearly visible after staining with hematoxylin-eosin. The edema and metachormasia of the stroma were well defined. The interstitial tissue in the zone of necrosis contained many leukocytes, lymphoid cells, and a smaller number of large fibroblasts. The cytoplasm of the muscle fibers stained intensively with eosin, and had undergone granular degeneration. Enzyme activity was completely absent in the fibers of the central part of the infarct, and at its periphery muscle fibers could be seen with a reduced number of formazan granules. However, the enzyme activity of individual muscle fibers was preserved or actually increased. Around the zone of increased enzyme activity a third zone was observed in which the myocardial fibers contained intense blue formazan granules. No unevenness was observed in the distribution of enzyme activity (Fig. 2).

After 2 to 4 days only "fragments" of dying muscle fibers could be seen at the periphery of the zone of necrosis; some of these contained solitary formazan granules, whereas others were characterized by normal or increased enzyme activity. Evidence of organization of the infarct was in the forefront at this period. The border between the infarct and the preserved fibers was clearly seen, and there were fewer myocardial fibers with diminished enzyme activity than in the earlier stages. The activity of all the enzymes was clearly seen in the connective tissue cells, although the succinic dehydrogenase activity was weaker.

After 6 to 8 days many fibroblasts and fibrocytes could be seen in the zone of necrosis. The "fragments" of muscle fibers were now hardly visible, and an even distribution of intensely blue formazan granules was observed in the preserved muscle fibers around the infarct, while some fibers showed increase enzyme activity (Fig. 3). The enzyme activity in the connective tissue cells remained fairly high, but it was less in the fibrocytes than in the fibroblasts. The enzyme activity in the myocardium outside the zone of ischemia was unevenly distributed, as in the earlier stages.

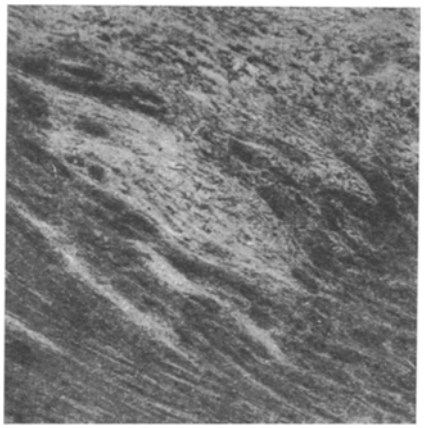

Fig. 3. The clear border between the organizing infarct (left) and the preserved myocardial fibers, characterized by a more intensive TMN-transaminase reaction than the muscle fibers further from the infarct (right). A reaction is present in the connective tissue cells inside the organizing infarct. Nitro-blue tetrazolium. Objective 20, eye-piece 10.

Our results showing the reduction or disappearance of succinic and malic dehydrogenase activity in the zone of ischemia after ligation of the coronary artery agree with those obtained by other writers [10, 12, 18]. Simultaneously the activity of the DPN- and TPN-transaminases also disappeared.

The irregular distribution of enzyme activity in the various myocardial fibers outside the zone of ischemia which we observed was also found by other workers. Some [5] regard this phenomenon as the result of differences in the functional activity of the muscle fibers, others [17] ascribe the lowered enzyme activity to the conducting system of the heart. Presumably this uneven distribution of enzyme activity depends on both factors mentioned above.

In our experiments the initial sign of disturbance of the coronary circulation after ligation of the coronary artery was a decrease, followed by disappearance of the activity of all the enzymes studied, which took place before the first morphological signs of necrosis were observed.

In our opinion three zones of enzyme activity may be distinguished in the process of formation and development of an infarct in the period between 12 hours and 2-4 days after ligation: 1) a zone of complete absence of activity (absence of formazan granules in the muscle fibers); 2) a zone of diminished enzyme activity (a decrease in the number of formazan granules); 3) a zone of preserved or increased activity (the normal number of formazan granules and disappearance of unevenness of distribution of enzyme activity).

In our experimental conditions the first two zones did not always develop at the same time after application of the ligature, probably on account of individual differences in the blood supply to the heart in these animals. It must be emphasized that these zones were not always of constant size: sometimes a small zone of total absence of enzyme activity (corresponding to the zone of visible necrosis when strained with hematoxylin-eosin) was accompanied by a larger zone of diminished enzyme activity, although the opposite was more commonly true.

At the time of formation of a morphologically distinct myocardial infarct (from 2 to 4 days after ligation of the coronary artery), the zone of diminished enzyme activity became less obvious and the border between the infarct and the preserved myocardial fibers became sharper. There were then two instead of three zones: 1) a zone of total absence of enzyme activity and 2) a zone of preserved or increased enzyme activity. The intermediate zone was reduced in size and disappeared, perhaps on account of individual differences in the blood supply of the heart and to the presence of a collateral circulation. In some of the little damaged muscle fibers, with an adequate supply of oxygen and of oxidation substrates, the enzyme activity was restored to its original level, but when these were inadequate the mitochondria died and the zone of necrosis was enlarged. Evenness in the distribution or increased activity of the enzymes in the fibers situated close to the infarct were probably the result of the stimula-

tion of metabolism in this zone, probably compensatory in character. An increase in the function of an organ or of its individual parts, or even of individual cells, evidently must lead to an increased intensity of metabolism in the mitochondria. It must be emphasized that compensatory phenomena on the part of submicroscopic structures may be observed in various pathological states [4].

The loss of evenness of the distribution of enzyme activity in the myocardial fibers next to the area of necrosis is not, of course, the only form of compensation. Some workers [6, 9], for instance, observed a 2-3 fold increase in the coronary blood flow after blocking of the enzyme systems with cyanides.

In our experiments, in the initial stages of the formation of the infarct, the cells of the newly formed connective tissue, mainly young fibroblasts, were distinguished by the comparatively low activity of the enzymes we were studying. When the processes of organization of the infarct became stronger, many formazan granules were observed in the fibroblasts. This was evidently due to the fact that oxidation and reduction are fairly highly developed in maturing fibroblasts. As the granulation tissue became more mature, however, the number of formazan granules again decreased, and the enzyme activity in the fibrocytes was low.

These suggestions must be verified in the future by means of a wide variety of histochemical methods.

SUMMARY

Myocardial infarction was provoked in rabbits by ligature of the descending branch of the left coronary artery; dehydrogenase of the succinic and malic acids, DPN and TPN diaphorases were investigated by histochemical methods. In 2-4 days after placing the ligature the first signs of reduction of the enzyme activity appeared in the zone of ischemia, which increased with the progress of the latter. With the formation of the infarct the activity of the mentioned enzymes manifested itself in the connective tissue cells, increasing with their maturation. During the process of formation and development of myocardial infarction (up to 2 days) three zones could be distinguished: the zone of complete disappearance of the enzyme activity, the zone of their decreased activity and the zone of retained or increased activity; at a later date (after 2-4 days) only 2 zones of the enzyme activity were distinguishable.

LITERATURE CITED

1. S. A. Vinogradov, Experimental Myocardial Infarction and the Effect of Certain States of the Organism on its Development. Doctorate dissertation [in Russian], Moscow (1957).
2. B. A. Lapin, Features of the Circulation in the Heart in Angina Pectoris, The Mechanism of Development of a Myocardial Infarct. Candidate dissertation [in Russian], Moscow (1952).
3. E. F. Lushnikov, In: Abstracts of Proceedings of the Sixth Conference of Junior Scientists of the Institute of Normal and Pathological Physiology [in Russian], Moscow (1960), p. 46.
4. V. V. Portugalov, In: Plastic and Repair Processes [in Russian], Moscow (1959), p. 202.
5. N. T. Raikhlin and A. B. Linnik, In: Plastic and Repair Processes [in Russian], Moscow (1959), p. 216.
6. J. E. Eckenhoff, J. H. Hafkenschiel, et al., Am. J. Physiol. (1947), Vol. 148, p. 582.
7. M. Fuld and M. H. Paul, Proc. Soc. exp. Biol. (N.Y.)(1952), Vol. 79, p. 355.
8. D. E. Green, et al., J. biol. Chem. (1948), Vol. 172, p. 389.
9. H. D. Green and R. Wegria, Am. J. Physiol. (1942), Vol. 135, p. 271.
10. R. B. Jennings, et al., Arch. Path. (1957), Vol. 64, p. 10.
11. H. A. Malaty and G. H. Bourne, Nature (1953), Vol. 171, p. 295.
12. L. Neoral, et al., Zbl. allg. Path path. Anat. (1959), Bd. 99, S. 239.
13. A. G. E. Pearse and D. G. Scarpelli, Nature (1958), Vol. 181, p. 702.
14. A. G. E. Pearse, Histochemistry; Theoretical and Applied, London (1960).
15. H. O. Yokoyama, et al., Arch. Path (1958), Vol. 59, p. 347.
16. G. Plaut and K. Plaut, J. Biol. Chem. (1952), Vol. 199, No. 1, p. 141.
17. T. H. Schiebler, Klin. Wschr. (1956), Bd. 34, No. 7-8, S. 181.
18. M. Wachstein and E. Meisel, Am. J. Pathl. (1955), Vol. 31, No. 2, p. 353.
