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Some Aspects of Myocardial Metabolism Outside the Zone of Experimental Myocardial Infarction

A. M. Vihert and N. M. Cherpachenko

Laboratory of Pathological Anatomy, The A. L. Myasnikov Institute of Cardiology, USSR Academy of Medical Sciences, Moscow, USSR

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Summary. In this experimental study the metabolic processes of unaffected myocardium (outside the necrotic and border-line zones) were examined by means of histochemical methods. In the unaffected part of the myocardium during the first 72 hours after the ligation of a coronary artery the aerobic production of energy decreased focally but was replaced by an increased metabolism of glycogen. By the 7–10 days, the role of glycolytic processes in the general formation of energy of cardial muscle gradually declined. These changes in the metabolism of the unaffected myocardium can be explained by a number of factors. The most important ones seem to be the hyperfunction of these regions of the heart and the increase of the catecholamine level in the blood.

Although much is known about the structural and biochemical changes in the muscular fibers of the myocardium in a disrupted blood circulation zone yet insufficient attention has been devoted to other parts of myocardium and only some aspects of this question were studied by other authors (Lushnikov, 1962; Glagoleva, Chechulin, 1967; Lichtenstein, 1967; Razumnaya, 1967; Severin, 1967; Strukov *et al.*, 1967; Gornak *et al.*, 1968; Chechulin *et al.*, 1967; Golubev, 1968; Danilova, 1969; Mihnev, 1965; Rees, Redding, 1969). Though the remaining parts of the myocardium continue to function and maintain the hemodynamics at a level which ensures the vitality of the organism, it iss upposed that they are not metabolically or functionally normal. It is very interesting to study the metabolic processes in these preserved muscular fibers when the heart accommodates to its new conditions of work.

An attempt is made in this work to describe the state of the important ways of forming energy in parts of the myocardium outside the ischemic and border zones. For this, purpose the activity of a number of oxidation-reduction enzymes were studied by means of histochemical methods, as well as changes in the glycogen content and the activity of some enzymes, taking part in its metabolism. This made it possible to judge about the dynamics and about the degree of aerobic and anaerobic ways in the formation of macroergetic phosphorous compounds, ensuring the contractive function of the myocardium as a whole.

Material and Methods

The experiments were carried out with male rabbits weighing 2.5–3 kg, in whom myocardium infarction was induced under nembutal anesthesia by ligating the descending branch of the left coronary artery. The animals were then sacrificed by decapitation in 1, 3, 6, 12 hours, 1, 2, 3, 5, 7, and 10 days after the operation. Altogether 112 test animals were used, out of which 22 went through a false operation and were used as control. Myocardial

infraction was diagnosed with ECG and visually at autopsy. A transverse segment of the myocardium was cut out through the whole heart, frozen by carbonic acid and cut up in a croystate at −20° C. The myocardium infarction was located in the anterior wall of the left ventrical, the preserved part of the left ventrical of the myocardium was studed in pasterior wall.

The activity of the oxidation-reduction enzymes of Kreb's cycle were determined histochemically: succinate dehydrogenase by the method of Nachlas *et al.* and malate dehydrogenase by Hess *et al.*, as well as the enzymes of the final chain of the electron transport (NAD-, NADF-diaphorases by the methods of Scarpelli et al. and cytochromeoxidase by the method of MOOG), lactate dehydrogenase (Hess and others).

The glycogen content was determined by the PAS reaction, as well as the activity of the enzymes of its metabolism: general phosphorylase by the method of Takeuchi, its active "a" and the inactive "b" forms (Godlewski) branching enzyme (Takeuchi) and UDPG-transferase (Takeuchi and Glenner). An electron microscopic method was used in a number of cases. The material was fixed in osmium, embeded into an epon and examined under a YEMB-100 microscope.

Experimental Data

Histochemical data show that one hour after the coronary arteries were ligated, the sections of muscular myocardium fibers which were out of the zone of disrupted blood circulation, when there was specific dehydrogenases, a fallout of formazan was observed, in the form of large blue, frequently incorrectly formed granules, which quite rarely fill the sarcoplasm (Fig. 1a, b). The closer to the ischemic area the greater the number of such fibers, can be found. An electron microscopic study showed significant changes in the muscular fibers of the mitochondria structure and the nature of their location in the sarcoplasma: in some of the preserved muscular fibers there also occurs a swelling of the mitochondria, clearing of the matrix and a decrease in the number of crysts in them. The affected mitochondria are situated in big clusters, and group close together (Fig. 1c). Apparently these changes are accompanied by increased permeability of the mitochondria, which makes it easier for tetrazole salts to penetrate them, and as a result of which granulated diformazan fells out in the mitochondria which differs from the normal. During this period of the experiment the myocardium is characterized by increased activity of one of the enzymes of the final chain of electron transport, namely, NAD-diaphorase.

Thus the histochemical and electron microscopic data show that, already one hour after the beginning of the experiment, the activity of the oxidation-reduction enzymes of Kreb's cycle in the preserved parts of the myocardium drops, while at the same time the activity of the NAD-diaphorase grows, as compared to the control.

At the same time there is a notable intensification of enzymic activity of the anaerobic cycle in part of the muscular fibers: general phosphorylase (Fig. 2a, b), UDPG-transerase (Fig. 3a, b) and branching enzyme, which is an evidence of the increased capacity of the tissue, to synthesize branched polysaccharides (glycogen) with the participation of both enzyme ways. Despite this, however, the content of glycogen in the myocardium during this period, is significantly lower as compared to control (Fig. 4a, b).

Three hours after the ligation of the coronary artery the activity of the oxidation-reduction enzymes remains low in the preserved section of the muscular fibers of the myocardium, since while tracing succinate and malate dehydro-

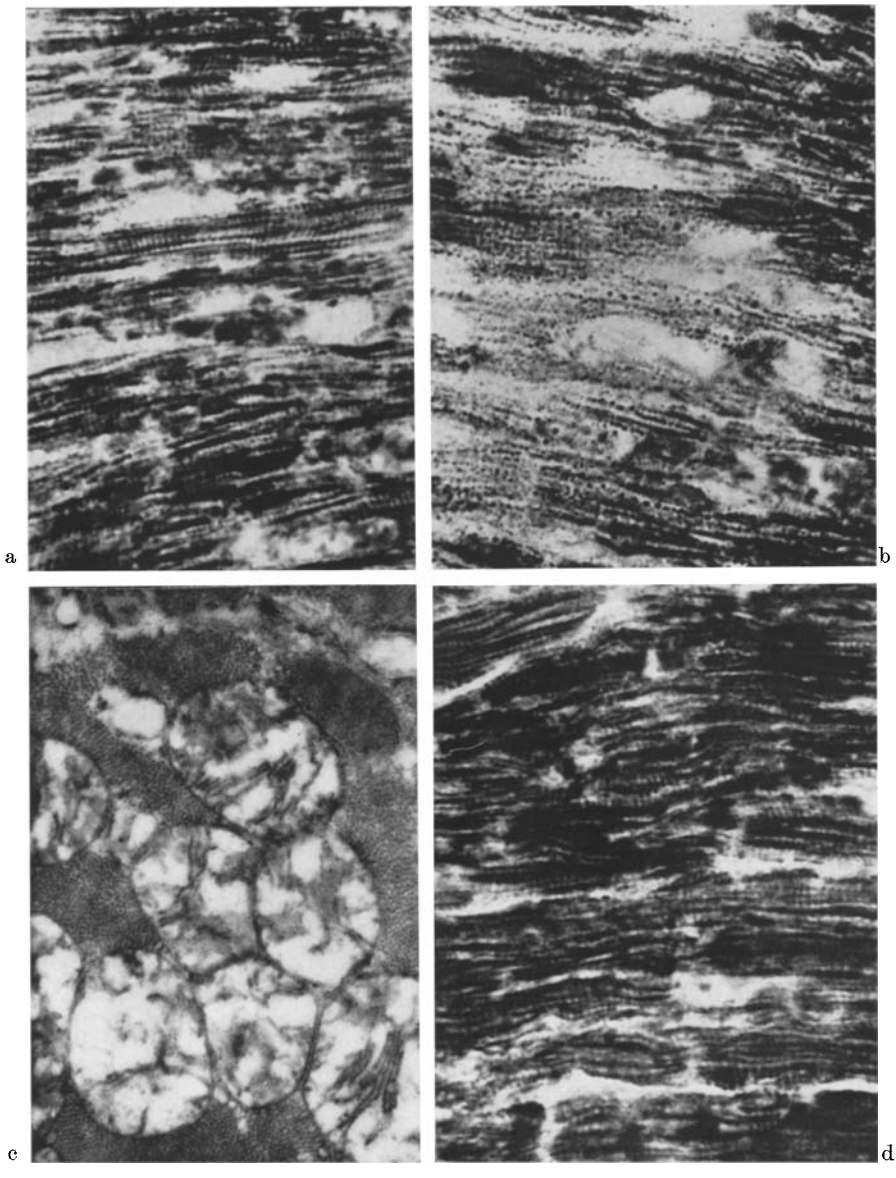


Fig. 1. a Succinate-dehydrogenase activity in the myocardium of a control rabbit. ×600. b The fall-out of granulated formazane in some myocardial fibers observed far away from the necrotic zone. One hour after the operation. ×600. c The swelling of the mitochondria clearing, of their metrix and a decrease in the number of crysts. Electronmicroscopy. ×15600. d The increase of the activity of succinate-dehydrogenase in myocardial fibers ten days after the operation. ×600

genases granulated formazan continue to fall out. The activity of enzymes of the final chain of electron transport drops during this period to normal level and lower. Simultaneously there is a decrease in the general phosphorylase activity,

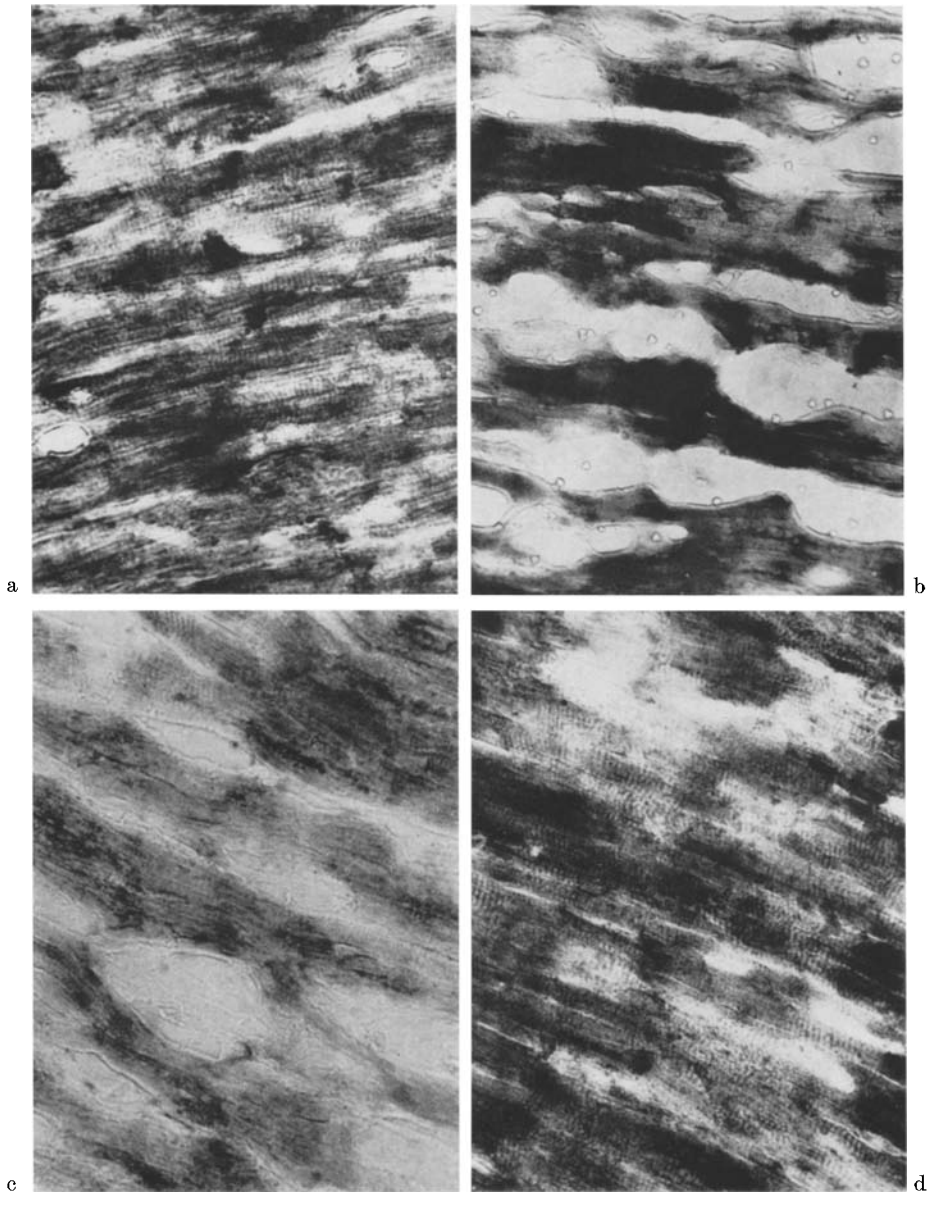


Fig. 2a–d. Phosphorylase in the myocardium of rabbits. ×250. a Control animal. b An increase in the activity of the enzyme in some myocardial fibers outside the necrotic zone. One hour after the operation. c A decrease in the enzyme activity three hours after the ligation of the coronary artery. d High activity of the enzyme ten days after the operation

as compared to control (Fig. 2a c), and an increase in of the active form of phosphorylase (Fig. 5a, b) in decreased general phosphorylase activity. The activity of the UDPG-transferase and branching enzyme remains slightly higher (Fig. 6a,

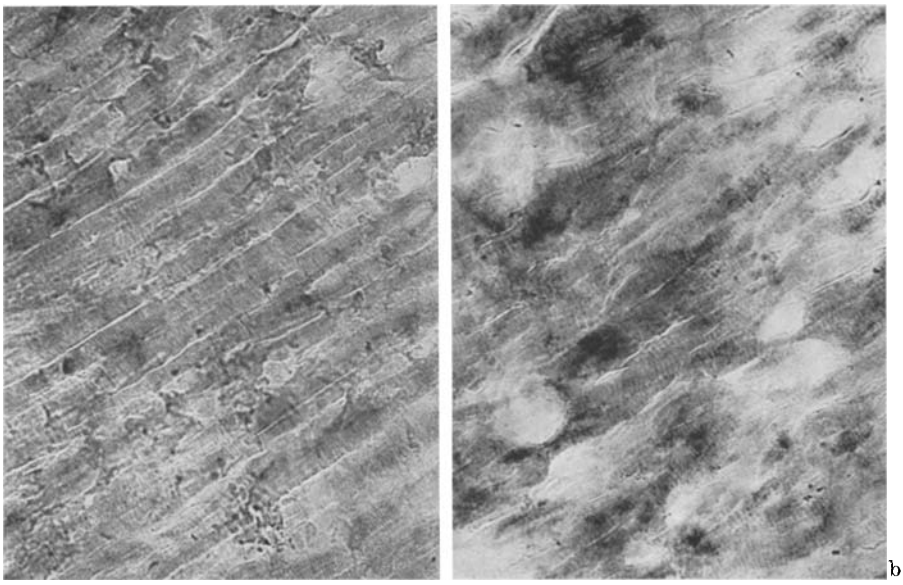


Fig. 3a and b. UDPG-transferase activity in the myocardium. ×200. a Control animals. b Increased activity in some muscular fibers. One hour after the operation

b) which might reflect the increased capacity of the myocardium to synthetise glycogen through UDPG. The quantity of glycogenremains unequally decreased.

Six hours after the coronary artery is ligated a significant focal decrease in the activity of the oxidation-reduction enzymes under investigation can be observed in the preserved tissue of the myocardium. In studying general phosphorylase and phosphorylase "a" we can observe a vividly expressed fluctuation of their activity—from complete absence of reaction in some muscular fibers to activity which is higher than in the control, in others. However, there are more fibers lacking these enzymes in the myocardium of rabbits with infarction than in those of the control animals. Just as in rabbits with a 3 hour infarction, there is a notable focal activity of UDPG-transferase and the branching enzyme.

This distribution of enzymes remains up to the 3rd day of the experiment. The quantity of glycogen in the myocardium increases unevenly after 6–12 hours (Fig. 4a, c) and then drops again by the 24th hour, to a level lower than the control.

Beginning with the 3rd day of the experiment there is a noticeable further change in the activity of aerobic and glycolitic enzyme activity in the preserved myocardial tissue. No deposits of granulated formazan in the muscular fibers is seen the intensity of histochemical reactions demonstrating of oxidation-reduction enzymes approaches that of normal, and in 7–10 days time gets higher (Fig. 1a, d). Simultaneously a growth of activity is observed of the branching enzyme and general phosphorylase at first up to the control level and then even higher (Fig. 2a, d), moreover the share of the phosphorylase "a" drops and by the 7–10th day of the experiment approaches the normal level. The activity of UDPG-transferase, beginning with the 3rd day of the experiment, starts dropping

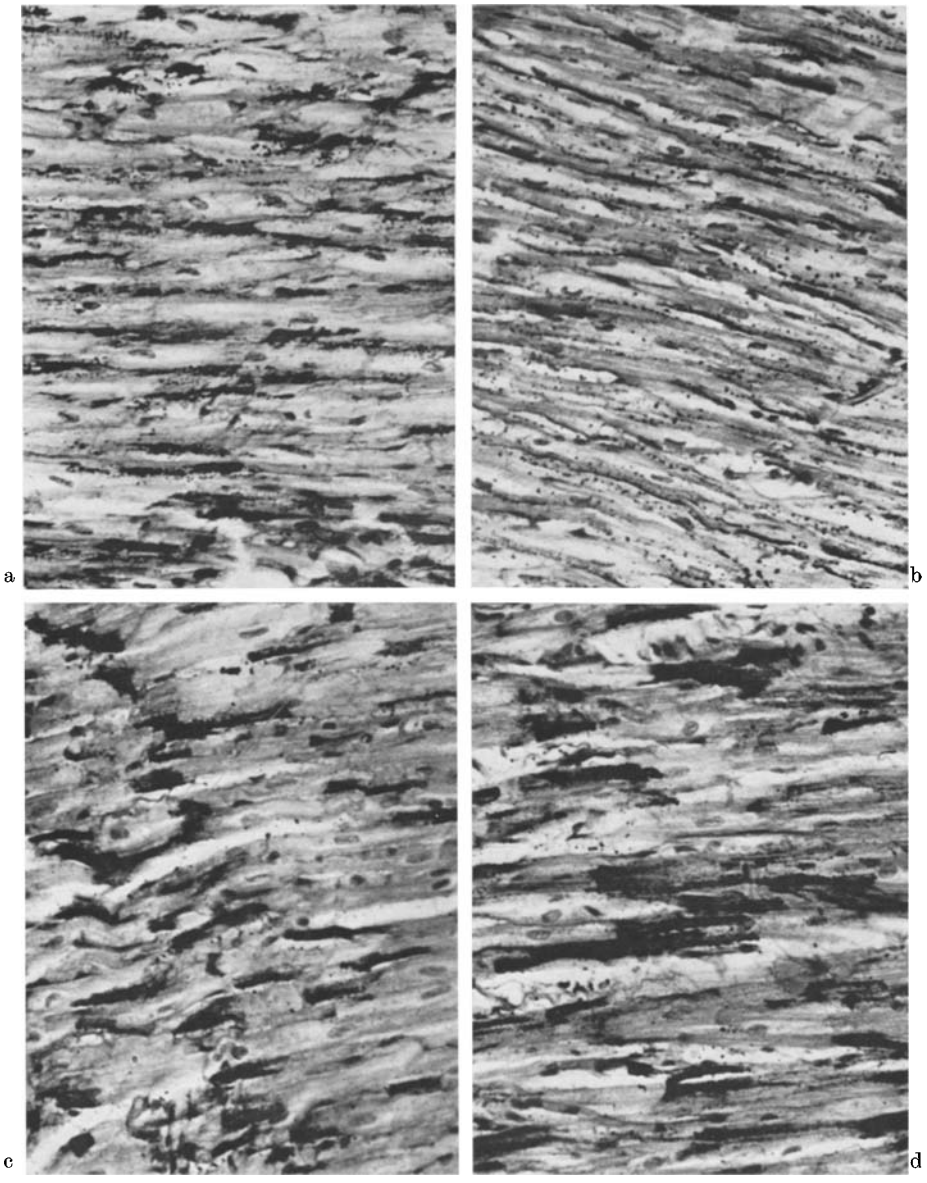


Fig. 4a–d. Glycogen in the myocardium of the rabbits. ×200. a Control animals. b The decrease in the amount of glycogen in muscular fibers outside the affected zone. c A big amount of glycogen twelve hours after the operation. d Increased amount of glycogen in the preserved part of myocardium ten days after the operation

and reaches normal by the 10th day. Gradually the amount of glyogen in the majority of fibers reaches normal amount and by the 10th day becomes higher (Fig. 4a, d).

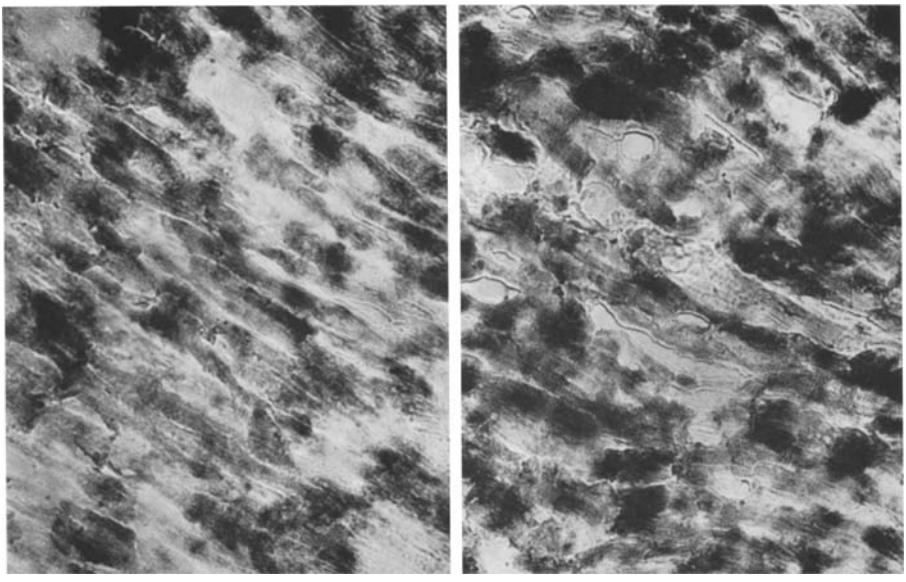
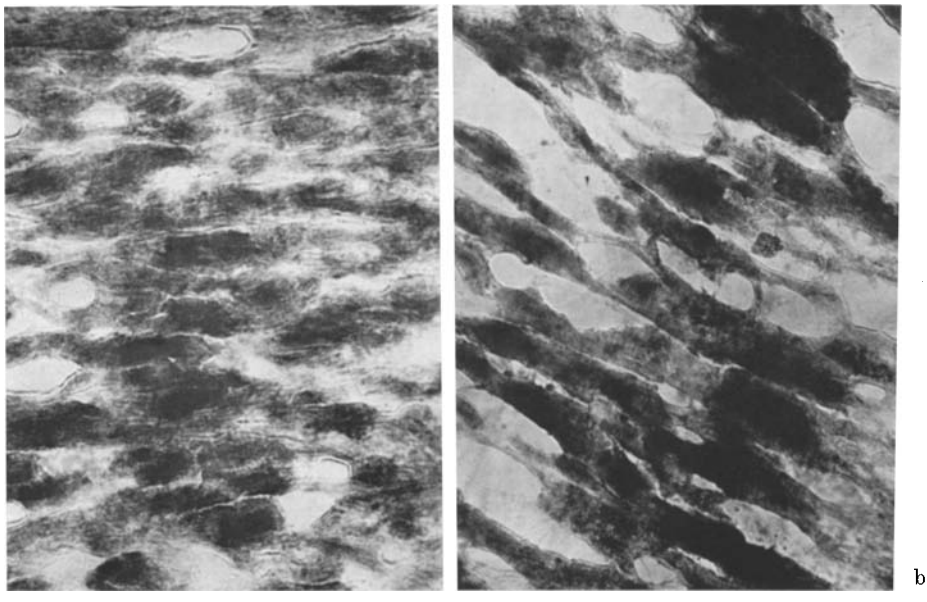


Fig. 5a and b. Phosphorylase "a" in the myocardium of the experimental animals. ×200. a Control animals. b Increased activity of the enzymes outside the zone of disrupted blood circulation. Three hours after the operation

The activity of the lactate dehydrogenase in the muscular fibers outside the zone of disturbed blood circulation was low during 7 days (Fig. 7a, b) and reached the normal level only by the 10th day of the experiment, which, apparently can be taken as evidence of low oxidation of lactic acid and its accumulation in the first 7 day of the experiment.

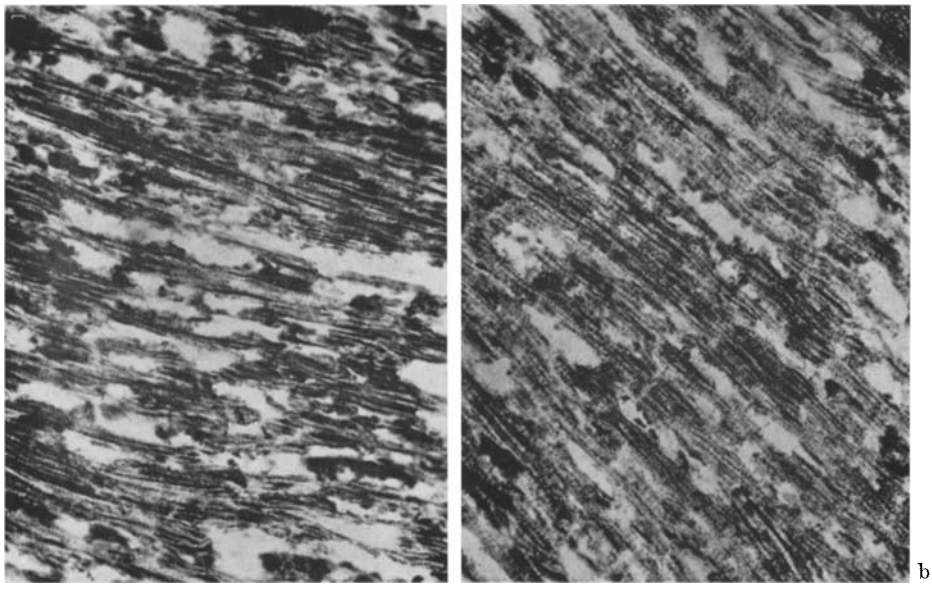
Thus it can be considered that at the early stages of the experimental myocardial infarction (up to the 3rd day) there is a focal decrease in oxidation-reduction enzyme activity in the preserved myocardium, i.e. a decrease of aerobic formation of energy. This process is of a reversible nature, since by the 7–10 day of the experiment, the activity of these enzymes comes back to normal and then gets even higher.

As far as the anaerobic ways of energy formation are concerned, there is a marked low phosphorylase activity during the first three days (with the exception of the first 60 minutes) and the share of phosphorylase "a" increasing in the general phosphorylase activity, which can be seen as an indirect evidence of a more intense use of glycogen. Approximately by the 3rd day of the experiment the phosphorylase activity increases and remains high to the end of the experiment, and the content of "a" form drops to approximately the control level. The capacity of the tissue to synthesize glycogen from UDPG in the first hours and days after ligation of the coronary artery somewhat increases, and from the 3rd of the 10th day of the experiment it gradually drops to the control level. No direct dependence was noted between the activity of the enzymes, taking part in the glycogen synthesis and the amount of this polysaccharide in the muscular fibers.



a b

Fig. 6a and b. Branching enzyme. ×200. a Control animals. b Increased activity of the enzyme in some myocardial fibers in the intact part of the myocardium. Three hours after the operation



a b

Fig. 7a and b. Lactate dehydrogenase in the myocardium. ×200. a Control animals. b Low activity of the enzyme during the first days after the operation

Discussion

The changes in the anaerobic and aerobic capacity of energy formation observed in the preserved parts of the myocardium during experimental infarction are due to a number of factors, two of which, apparently, are the most important.

The *first factor* is the acute and sharply increased physical strain on the unimpaired section of the myocardium, which requires increased formation and utilization of energetic substances. This leads to the mobilisation of all the internal energetic resources in the myocardium, in particular, to the increase in the activity of the oxidation-reduction enzymes, which was reflected in an increase in the NAD-diaphorase activity, in our experiments, one hour after the ligation of the coronary artery. Certain increases in aerobic metabolism in the non-ischemized sections of the heart when the coronary artery was occluded was also observed by Kaltenbach *et al.* (1960), Hecht *et al.* (1961). This activation, however, does not last long. The compensatory hyperfunction, which was demonstrated in the work of Lukomski *et al.* (1967) and Dauksha (1969) and in our previous experiments lead to the rapid exhaustion of the capacity of the enzymic systems of the myocardium, which is shown by the changes in the ultrastructure of the mitochondria and a focal decrease in the activity of the oxidation-reduction enzymes, observed during the first hours and days of the experiment.

The reduction of the aerobic energy-formation entails the activation of the anaerobic processes, in particular, extensive glycogenolysis. During this period, the glycogen deposits are activly used and was reflected in the drop of the amount of glycogen in myocardium fibers as early as one hour after the operation. Simultaneously we can also suspect a disruption of the processes of glycogen synthesis, since there is a drop in general phosphorylase activity in the myocardial tissue during the first 72 hours, accompanied by its increased capacity to synthesize glycogen from UDPG. At the same time it is difficult to explain the relatively high content of glycogen in a big number of the preserved muscular fibers during the 3 to 12 hours after the ligation of the coronary artery. As has already been noted we were unable to discover any strict parallelism between the amount of glycogen and the activity of phosphorylase, UDPG-transferase and the branching enzyme in the first days of the experiment. This is apparently explained by the fact that the histochemical reactions cannot directly reflect the state of the enzyme systems of the tissue in vivo. They can only show the maximal potential capacity of enzymes to synthesise, since they function in an artificially created medium (Godlewski 1963; Schulze *et al.*,1969).

The *second factor*, cauzing changes in the aerobic and anaerobic processes in the myocardium, is the increase of the catecholamine level in the blood, caused by the ligation of the coronary arteries. Increased secretion of catecholamines was noted both in experimental conditions and in the clinic among patients with myocardial infarction (Wollenberger, Schahab, 1968; Gazes *et al.*, 1959; Richardson *et al.*, 1960; Richardson, 1966; Starcich, 1966). It is known that catecholimines sharply increase the requirement of the myocardium in oxygen (Raab, 1960, 1963). Our observations suggest that the focal decrease in the activity of the oxidation-reduction enzymes of the myocardium and the disruption of the ultra-

structure of the mitochondria is connected with this. Similar changes were found when big doses of catecholmines were administered to rats (Ferrans *et al.*, 1964).

It is known also that catecholamines stimulate glycolitic processes in the myocardium through the activation of the transformation of inactive phosphorylase "b" into its active form "a", which, in turn, takes part in the synthesise and decay of glycogen (Wollenberger, Schahab, 1968; Sutherland, Cori, 1951; Haugaard, Hess, 1966; Krebs *et al.*, 1966; Helmreich, Cori, 1966; Sutherland, Robinson, 1966; Sutherland, 1951, Entman *et al.*, 1969; Krause, Wollenberger, 1965). The influence of catecholamines on the glycogenolysis seems to explain the smaller content of glycogen and the enzymic activity of its metabolism, wich was seen during the first days of our experiment. A similar drop in the activity of phosphorylase in the myocardium, was discovered histochomically by Bajusz, Raab (1966) when animals were administered catecholamines.

It is likely that a *combination of these two factors*, namely, the increased physical strain on the preserved section of the myocardium during the first days after the operation, and a high level of catecholamines cause the above mentioned changes, in the aerobic and anaerobic processes in the myocardium, which, possibly, is one of the reasons of the cardial insufficiency in the first few days of the myocardial infarction (Bing *et al.*, 1956). It is quite possible, however, that with time, some other factors may be discover.

The above named changes are mainly of a reversible nature, later on part of the preserved muscular fibers undergo hypertrophy (Norman, Coers, 1960), the amount of energy-forming and contracting structures increases, in them, which leads to the establishment of metabolism on a higher energetic level and decreases the strain per unit of mass of the miocardium. Besides, us some experiments revealded the level of catecholamines in the blood returns to normal 3 days after the coronary arteries are ligated (Richardson, 1966). Some muscular fibers, however, with pronounced changes of mitochondria and with a sharp fall in enzymic activity undergo necrosis.

Thus due data obtained show that at early stages of experimental myocardial infarction a focal decrease in aerobic formation of energy in the preserved part of the left ventrical takes place, which is compensated by an increase of glycogen metabolism. With the healing of the infrarction, the role of the glycolitic processes in energy formation gradually decreases.

References

Bajusz, E., Raab, W.: Early metabolic aberrations through which epinephrine elicit myocardial necrosis. In: Prevention of ishemic heart disease. Principles and practice. Comp. and ed. by W. Raab, p. 21–30 (1966).

Bing, R. I, Castellanos, A., Gradel, E., Lupton, C., Siegel, A.: Experimental myocardial infarction: Circulatory, biochemical, and pathologic changes. Amer. J. med. Sci. **232**, 533–554 (1956).

Chechulin, U. S., Bondarenko, M. E., Alekseeva, L. M.: Characteristics of protein content in the cardial muscle in an experimental myocardial infarction [Russ.]. The First all-Union conference of CSRL of Medical Institutes. USSR (p. 118–121) 1967.

Chechulin, U. S., Glagoleva, W. W., Saakjan, I. R.: Changes in the oxidative phosphorilation and in the structure of mitochondria in an experimental myocardial infarction [Russ.]. The first all-Union conference CSRL of Medical Institutes. USSR (p. 141–144) (1967).

Danilova, K. M.: Metabolic disturbances in myocardial infarction [Russ.]. Arch. Path. **4**, 27–33 (1969).

Dauksha, K. K.: The Morphology of the contracting function of myocardium in patients with myocardial infarction [Russ.]. Proceedings of the first conference of pathologist of Belorussia (p. 166–168) 1970.

Entman, M. L., Levey, G. S., Epstein, S. E.: Mechanism of action of epinephrine and glucagon on the canine heart. Evidence for increase in sarcotubular calcium stores mediated by cyclic-3,5-AMP. Circulat. Res. **25**, 429–438 (1969).

Ferrans, V. Y., Hibbs, R. G., Black, W. C., Wellbaecher, D. G.: Isoproterenol-induced myocardial necrosis. A histochemical and electron microscopic study. Amer. Heart. J. **68**, 71–90 (1964).

Gazes, P. C., Richardson, I. A., Woods, E. F.: Plasma cathecholamine concentrations in myocardial infarction and angina pectoris. Circulation **19**, 657 (1959).

Glagoleva, W. W., Chechulin, U. S.: The influence of disturbed coronary blood circulation on the ultrastructure of myocardium [Russ.]. Proceedings of the Fourth All-Union Congress of Pathologists. Kishinev, 1965. Moscow, 1967 (p. 157–161).

Godlewski, H. G.: Are active and inactive phosphorylases histochemically distinguishable? J. Histochem. Cytochem. **2**, 108–112 (1963).

Golubev, A. M.: The mechanism of electron transport in the redox chain in experimental myocardial infarction [Russ.]. Arch. Pathol. **3**, 39–43 (1968).

Gornak, K. A., Lushnikov, E. F., Chernjishova, G. W.: The metabolism in the heart in the experimental moycardial infarction. [Russ.]. Proceedings of the First Moscow Medical Institue **63**, (p. 128–141) 1968.

Haugaard, N., Hess, M.: The influence of catecholamines of heart function and phosphorylase activity. Pharmacol. Rev. **18**, 197–203 (1966).

Hecht, A., Korb, G., David, H.: Vergleichende histochemische, fluorescenzmikroskopische und electronen-optische Untersuchungen zur Frühdiagnose des Herzinfarktes der Ratte. Virchows Arch. path. Anat. **334**, 267–284 (1961).

Helmreich, E., Cori, C. F.: The activation of glycolisis in frog sartorius muscle by epinephrine. Pharmacol. Rev. **18**, 189–196 (1966).

Kaltenbach, Y. P., Jennings, R. B.: Metabolism of ischemic cardiac musle. Circulat. Res. 8, 207–213 (1960).

Krause, E., Wollenberger, A.: Über die Aktivierung der Phosphorylase und die Glykolyserate im akut anoxischen Hundeherzen. Biochem. Z. **342**, 171–189 (1965).

Krebs, E. G., Lange, R. I., Kemp, R. G., Riley, W. D.: Activation of skeletal muscle phosphorylase. Pharmacol. Rev. **18**, 163–171 (1966).

Lichtenstein, I. E.: Carbohydrate metabolism in myocardial infarction [Russ.]. Proceedings of the Kiev Scientific Conference on Cardial vascular pathology (p. 121–123) 1967.

Lukomsky, P. E., Meerson, F. Z., Soloviev, W. W., Shenderov, S. M., Markovskaya, G. I., Zharov, E. I., Mdinaradze, U. S., Arshakuni, R. O., Taraeva, N. G.: Disturbances of the contractile function of the heart in myocardial infarction and the therapeutic use of cofactors of synthesis and precursors of nucleic acids [Russ.]. Cardiol. **1**, 3–10 (1967).

Lushnikov, E. F.: Experimental histochemical investigation of myocardial infarction [Russ.]. Arch. Path. **1**, 55–62 (1962).

Michnev, A. L.: Clinico-biochemical alterations in acute myocardial infarction. [Russ.]. Cardiol. **4**, 11–19 (1965).

Norman, T. D., Coers, C. R.: Cardiac hypertrophy after coronary artery ligation in rats. Arch. Path. **69**, 181–184 (1960).

Raab, W.: Key position of catecholamines in functional and degenerative cardiovascular pathology. Amer. J. Cardiol. **5**, 571–578 (1960).

— Neurogenic multifocal destruction of myocardial tissue. Rev. Canad. Biol. **22**, 217 (1963).

Rasumnaya, N. M.: Breathing and oxidative phosphorilation in the mitochondria of cardial muscle in experimental myocardial infarction [Russ.]. Proceedings of the Kiev Scientific Conference on Cardial vascular pathology (p. 177–179) 1967.

Richardson, I. A.: Plasma catecholamines in angia pectoris and myocardial infarction. In: Prevention of ischemic heart disease. Comp. and ed. Raab W., p. 96–102 (1966).

Richardson, I. A., Woods, E. F., Bagwell, E. E.: Circulating epinephrine and norepinephrine in coronary occlusion. Amer. J. Cardiol. **5**, 613–618 (1960).

Schulze, W., Krause, E., Wollenberger, A.: Über die unterschiedlichen Aussagen der histochemischen und quantitativ-biochemischen Bestimmung der Phosphorylase-Aktivität im Herzmuskel nach Coronararterienverschluß und nach künstlich erzeugtem Glykogenmangel. Acta histochem.. (Jena) **32**, 270–280 (1969).

Severin, S. E.: The main trends of research in biochemistry of the myocardium [Russ.]. Cardiol. **11**, 22–30 (1967).

Starcich, R.: Plasma catecholamines and urinary vanillyl mandelic acids in clinical ischemic heart desease. In: Prevention of ischemic heart desease. Comp. and ed. W. Raab, p. 103–111 (1966).

Strukov, A. I., Lushnikov, E. F., Gornak, K. A.: Histochemistry of myocardial infarction. [Russ.] (1967).

Sutherland, E. W.: The effect of the hyperglycemic factor and epinephrine on liver and muscle phosphorylase. In: Phosphorus metabolism, ed. by W. D. McElroy and B. Glass, vol. **1**, p. 53+61 (1951).

— Cori, C. F.: Effect of hyperglycemic-glycogenolytic factors and epinephrine on liver phosphorylase. J. biol. Chem. **188**, 531–543 (1951).

— Robison, G. A.: The role of cyclic-3,5-AMP in responses to catecholamines and other hormones. Pharmacol. Rev. **18**, 145–161 (1966).

Wollenberger, A., Shahab, L.: Neurohumoral regulation of anaerobic formation of energy in the ischemic myocardium [Russ.]. Cardiol. **11**, 5–15 (1968).

Dr. A. M. Vihert
Laboratory of Pathological Anatomy
The A. L. Myasnikov Institute of Cardiology
USSR Academy of Medical Sciences
Moscow, USSR