THE MORPHOLOGY OF THE EARLY STAGES OF EXPERIMENTAL MYOCARDIAL INFARCT IN ATHEROSCLEROSIS

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Coronary insufficiency and myocardial infarction represent the central problem of the pathology of the cardiovascular system. Although by now through numerous experimental, clinical, and morphological investigations, something has been learnt of the origin and course of myocaridal infarction, many problems of pathogenesis and morphology remain obscure.

First of all there is no solution yet to the basic problem of the relationship between the organic and functional changes. Some authors [2, 14] attribute the condition primarily to atherosclerosis and coronary thrombosis, while others [11, 12, 21] find the cause in disturbances of the coronary circulation associated with abnormalitites of the intra- and extracardial reflexes [3, 13, 18]. The latter view has been confirmed, in particular, by investigations in which application of various stimuli has revealed an abnormal response of coronary vessels when atherosclerotic changes are present [1, 11, 24]; the same investigations also showed that dystrophic processes and focal myocardial infarcts developed as a consequence of increased work performed by the heart [4, 5, 7]. A. I. Strukov [22] and his co-workers considers that the principal conditions responsible for the development of a myocardial infarct are: 1) atherosclerosis of the coronary arteries; 2) acute obstruction of the coronary arteries induced by their spasm, or by thrombosis; 3) an increased load placed upon the myocardium and a disturbance of general circulatory conditions.

Insufficient work has been done on the problem of the part played by disturbance of the autonomic control of the heart, and of its significance in the development of myocardial infarct. It is particularly important to study experimentally the development of myocaridal infarct in conditions when the reactivity of the organism is impaired as a result of the preceding atherosclerosis, especially because the two conditions are very frequently encountered in man. We have used histochemical methods to make a combined study of the functional, structural and metabolic disturbances at the very earliest stages of the development of an experimental myocardial infarct developed during atherosclerosis. We here report the morphological part of this study.

METHOD

Acute experiments were carried out on rabbits under 1 - 1.5 g/kg urethane anesthesia. Pressure in the aorta was recorded by a mercury and a spring manometer, and tracheal respiration by a Marey's capsule. To investigate the lability of the peripheral and central ends of the vagus, this nerve was cut on the left side, and brought into contact with electrodes. Synchronous electrocardiogram records were made from the three standard and one thoracic leads. The descending anterior branch of the left coronary artery was ligatured at the level of the lower edge of the left auricular appendage, access being gained through an incision in midline. All recordings were made before the ligature was applied, and then afterwards at intervals of 5, 10, 15, 30, 60, 120 and 180 minutes. After the experiment, the animal was killed by bleeding. Altogether, 44 rabbits were investigated, and of these the first group consisting of 23 animals was atherosclerotic, and the second group of 21 animals was not.

Atherosclerosis was induced by the classical method of N. N. Anichkov and S. S. Khalatov, by giving daily injections for 100 days of 0.2 g cholesterol per kg weight as a 10% solution in vegetable oil. In the first group, the

coronary artery was ligatured in 13 of the rabbits, and in the second group in 15; 10 rabbits of the first group and 6 of the second served as controls in which the coronary artery was not ligatured.

The aorta and heart were taken for histological examination. The material was fixed in acetone at 4°, in Carnoy's fluid, or in 10% neutral formalin. The transverse sections of the heart and the longitudinal sections of the aorta were embedded in paraffin or in celloidin by the usual method. Sections to be stained for fat were cut on the freezing microtome. The following stains and histochemical reactions were used: hematoxylin-eosin, van Gieson's picrofuchsin, Heidenhain's azocarmine, orcein, Tibor-Papp's silver impregnation, Sudan III, toluidine blue, Shabadash's PAS reaction, Feulgen's reaction for DNA, Brachet's method for RNA, and Selye's stain for revealing "fuchsin degeneration" of muscle fibers.

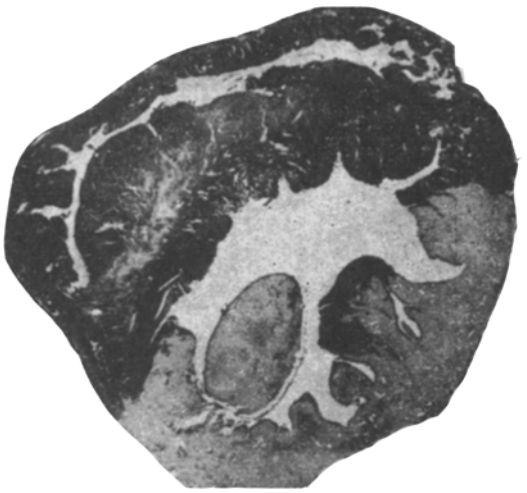

Fig. 1. Localized disappearance of glycogen from an ischemic zone (antero-lateral and posterior walls of the left ventricle, except for the part of the posterior papillary muscle) two hours after ligature of the coronary artery of a rabbit free from atherosclerosis. PAS reaction. Loupe.

RESULTS

The morphological picture of atherosclerosis of the aorta and coronary arteries of rabbits fed with cholesterol agreed with previous descriptions [6, 9, 19]. In the aorta and in the arteries within the myocardium atherosclerotic plaques at various stages of development were found. Lipoid infiltration had occurred in the aortal plaques and in the walls of the branches of the coronary arteries within the myocardium; quite frequently they were found also in the annulus fibrosus of the heart and in the stroma of the myocardium, and in four cases small droplets of fat were found in the myofibrils. Collagen, elastic, and argyrophil fibers, and acid and neutral mucopolysaccharides were found in the plaques. The chief subepicardial branches of the coronary arteries were not involved in the process.

Small regions of perivascular cardiosclerosis were always present in the left ventricle. The argyrophil fibers of the stroma were thickened. In two of the rabbits, necrosis of the muscle fibers and indications of organization were revealed in the papillary muscle of the left ventricle. In three cases, arterio-arterial anastomoses were found in the myocardium.

Changes of the myocardium after ligature of the coronary artery affected the whole of it, but chiefly the left ventricle.

The first indication of a disturbed coronary circulation was the disappearance of glycogen from the muscle fibers, as has been previously reported [28, 29, 33, 34]. In the control animals, glycogen appeared as dark violet granules more or less evenly distributed along the muscle fiber but only 10 minutes after arrest of the coronary circulation they had disappeared, and in experiments on the first group (with atherosclerosis) they did not disappear in all parts of the left ventricle, whereas in the second group (without atherosclerosis) their distribution was localized to correspond with the ischemic zone. As the period of ischemia was extended, the amount of glycogen decreased, and after $1\frac{1}{2}$ - 2 hours it remained only in the endocardium of the first group, whereas in the second group, the amount of glycogen outside the ischemic zone was only slightly below normal (Fig. 1). The disappearance of glycogen from various animals of one group during ischemia did not always occur at the same time, nor to the same extent; the reason was probably the different blood supply to the heart of different animals. Even in the zone from which glycogen had disappeared, sometimes muscle fibers were found which contained it, particularly in the regions round the veins. We failed to observe any PAS-positive material in the ischemic zone which was not removed by amylase.

It is known [8] that cardiac contraction depends on the interaction of proteins of the actomyosin complex with energy-rich phosphate compounds, and the reduction of the latter occurs during biological oxidation and anaerobically. The normal heart always maintains the amount of glycogen at a constant level [23, 27], and it is only anoxia which causes it to fall [27, 30, 31]. Also, in hypoxia there is a disturbance of adenosintriphosphoric acid and phosphocreatinine metabolism [16, 17, 31].

In our experiments the glycogen loss from the two groups of animals was different, probably on account of the condition of the vessels. In the group with atherosclerosis, the diffuse disappearance of glycogen may be due to mobilization associated with the damage and serving to preserve the heart; in the second group the unchanged

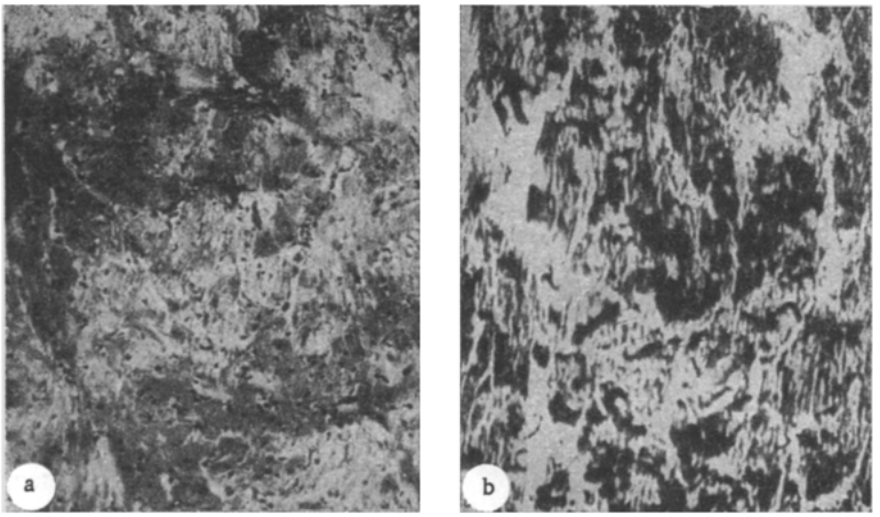

Fig. 2. "Picrinophilla" of some of the fibers of the myocardium in the ischemic zone 1½ hours after ligature of the coronary artery (a). Stain picrofuchsin. Objective 20 ×, ocular 7 ×. "Fuchsinophilia" of groups of muscle fibers in the ischemic zone 3 hours after ligature of the coronary artery (b). Selye's stain. Objective 20 ×, ocular 7 ×.

vessels supplied the necessary amounts of oxygen and substrates for oxidation, and in this case the disappearance of glycogen occurred chiefly in the ischemic zone.

The early myocardial changes which followed ligature of the coronary artery also involved vascular disturbances in all parts of the heart, and they were more extensive in the group with atherosclerosis than in the other group. Signs of stasis, perivascular edema, and extravasates were visible 20 - 30 minutes after the beginning of the experiment, and they progressed as the ischemia continued. At the end of the experiment, small accumulations of lymphoid infiltrates could be seen around the small vessels and capillaries, and leucocytes could be seen to have left the blood stream. The stroma had acquired some small degree of metachromasia, which was most marked around the blood vessels.

It was very difficult to determine the early necrotic changes of the muscle fibers. Electron microscopical studies [25, 26] have shown that during the first minutes of ischemia there is a change in the mitochondria and endoplasmatic reticulum of the muscle fibers, as well as an acute focal necrosis.

In our experiments, even during the first hour after the ligature of the coronary artery, abnormalities in the staining of the muscle fibers with eosin and picrofuchsin were observed. In the rabbits with atherosclerosis, in all parts of the left ventricle (and less frequently of the right) zones of fibers which stained more strongly were observed, and the section acquired a spotted appearance (Fig. 2). In rabbits without atherosclerosis these changes were observed principally in the ischemic zone. The dystrophic processes were shown best by the stain described by Selye [32], when the affinity of the protoplasm of the muscle fibers for fuchsin was shown. One or two hours after circulation in the myocardium had ceased, fuchsinophil "muscular tubules" were clearly seen lying chiefly in the ischemic zone (Fig. 2). According to Selye, the "fuchsinophil degeneration" disappears when the muscle fiber starts to disintegrate. In the experiments in which the coronary arteries were not ligatured, again zones in the myocardium were found in which the fibers were unevenly stained and showed an affinity for fuchsin, but they were isolated, and not confined to any one area. In the atherosclerotic rabbits these effects were more clearly shown. Evidently, the staining differences indicate a condition of "paranecrosis" of the muscle fibers, as has been described previously [15]. What is most probably a proof, is the increased staining of the protoplasm by pyronine, and the reaction for RNA which depends on the liberation of phosphorus during the disintegration of the muscle fibers [35].

The nuclear changes in the dystrophic muscle fibers are shown pyknosis, or vacuolization with loss of chromatin.

In our experiments the changes in the myocardium after ligature of the coronary artery and division and stimulation of the vagus occurred earlier and were more widespread [10, 20] than was found to be thus by other authors who did not divide or stimulate the vagus [5]. The explanation is probably that the normal neurotropic influences on the metabolic processes of the myocardium were disturbed.

SUMMARY

The following operations were performed in rabbits with and without atherosclerosis. At one operation, the left vagus was ligatured and the left anterior descending coronary artery divided. The first sign of a disturbance of the coronary circulation was that glycogen disappeared diffusely from the myocardium of rabbits of the atherosclerotic group, while from those which were free from atherosclerosis it disappeared from certain foci. Vascular disturbances and myocardial dystrophy appeared early in the atherosclerotic rabbits, and were more pronounced than in the operated controls. Protein dystrophy of cardiac muscle is revealed by the distinctive reaction to staining by Selye's method. Disappearance of glycogen from the ischemic zone, and fuchsinophilia of the muscle fibers in this zone constitute a morphological test for the early stages of myocardial infarction.

LITERATURE CITED

1. S. V. Andreev, Recovery of Human Cardiac Activity After Death. [in Russian] Moscow (1955).
2. N. N. Anichkov, A. V. Val'ter, K. G. Volkova and others. Transactions of the All Union Conference of Pathologists and Anatomists. Moscow (1956), p. 246.
3. G. N. Aronova, Byull. éksper. biol. i med., No. 4 (1953), p. 20.
4. S. S. Vail', Functional morphology of Disturbances of Cardiac Activity. [in Russian] Leningrad (1960).
5. S. A. Vinogradov, Experimental Myocardial Infarct and the Influence of Certain Conditions of the Organism on its Development. Dissertation for Doctorate. Moscow (1957).
6. K. G. Volkova, Arkh. biol. nauk, Vol. 30, No. 3 (1930), p. 269.
7. M. A. Zakhar'evskaya, In book: Collected Works in Honor of the 60th Birthday of N. N. Anichkov. Leningrad (1946), p. 62.
8. B. I. Zbarskii, I. I. Ivanov and S. R. Mardashev, Biological Chemistry. [in Russian] Moscow (1960).
9. N. N. Kipshidze, In book: Atherosclerosis and Coronary Insufficiency. Moscow (1956), p. 90.
10. A. V. Kuz'mina-Prigradova, Byull. éksper. biol. i med., No. 9 (1956), p. 67.
11. G. F. Lang, Hypertensive Disease [in Russian] Leningrad (1950).
12. B. A. Lapin, Transactions of the All-Union Conference of Pathologists and Anatomists. Moscow (1956), p. 263.
13. A. V. Lebedinskii, V. I. Medvedev and I. A. Peimer, The Significance of Spasm of the Coronary Arteries in the Pathogenesis of Coronary Insufficiency. Leningrad (1953).
14. A. L. Myasnikov, Hypertensive Disease. [in Russian] Moscow (1954).
15. D. N. Nasonov and V. Ya. Aleksandrov, The Reaction of Living Matter of External Action. The denaturation theory of damage and stimulation. [in Russian] Moscow–Leningrad (1940).
16. M. E. Raiskina, Byull. éksper. biol. i med., No. 12 (1957), p. 62.
17. M. E. Raiskina, Transactions of the 14th All-Union Congress of Therapeutists. Moscow (1958), p. 161.
18. N. P. Simanovskii, The Problem of the Influence of Stimulation of the Sensory Nerves on Poisoning and Nutrition of the Heart. Dissertation. St. Petersburg (1881).
19. T. A. Sinitsyna, In book: Atherosclerosis and Myocardial Infarct. [in Russian] Moscow (1959), p. 26.
20. A. I. Smirnov and A. I. Shumilina, Klin. med., No. 2 (1955), p. 62.
21. A. V. Smol'yannikov, Klin. med., No. 7 (1956), p. 40.
22. A. I. Strukov, (Editor). Problems of the Morphology and Pathogenesis of Infarction. Moscow (1959).
23. D. Ferdman and P. Dvornikova, Byull. éksper. biol. i med., Vol. 5, No. 1 (1938). p. 87.
23a. R. Tsanev, In book: Symposium on Connective Tissue. [in Russian] Moscow (1960). p. 84.
24. Yu. S. Chechulin, Arkh. pat., No. 4 (1958), p. 40.
25. R. E. Bryant, W. A. Thomas and R. M. O'Neal, Circulat. Res., Vol. 6 (1958), p. 699.
26. J. Caulfield and B. Klionsky, Am. J. Path., Vol. 35 (1959), p. 489.
27. G. Evans, J. Physiol., Vol. 82, London (1934), p. 468.
28. S. P. Kent and M. Diseker, Lab. Invest., Vol. 4 (1955), p. 398.
29. A. W. Merrick and D. K. Meyer, Am. J. Physiol., Vol. 177 (1954), p. 441.
30. H. Schumann, Ergebn. inn. Med. Kinderheilk., Bd. 62, S. 869 (1942).
31. H. Selje, The Chemical Prevention of Cardiac Necroses. New York (1958).

32. R. Tennant, D. M. Grayzel and F. A. Sutherland, et al., Am. Heart J., Vol. 12 (1936), p. 168.
33. B. Wittels, L. Reiner and H. Frank, Arch. Path., Vol. 68 (1959) , p. 501.
34. H. O. Yokoyama, R. B. Jennings and G. F. Clabaugh, et al., Ibid., Vol. 59 (1955), p. 347.

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.
