A FLUORESCENCE-MICROSCOPY STUDY OF THE EARLY STAGES OF EXPERIMENTAL MYOCARDIAL INFARCT

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After ligation of the coronary artery there is a disturbance of the oxidative processes of the muscle fibers in the ischemic zone [8,9,22,29,31,32]. However there is a certain lack of correspondence between the time at which the metabolism of the mitochondria is affected, which can be found by histological methods, and the occurrence of changes in the cytoplasm as revealed by the normal histological methods or by histochemical tests (not including the test for glycogen).

It has been shown that functional changes in the condition of the cells are rapidly and accurately indicated by the kind of fluorescence [1,7,11-16,29].

We have used fluorescence microscopy to study the early stages of myocardial infarction produced in various ways.

EXPERIMENTAL METHOD

The condition was induced in rabbits by ligation of the descending branch of the left coronary artery. Altogether we used 87 animals. The first group comprised 43 rabbits (of which 10 were controls) in which only the coronary artery was ligated; the second set consisted of 21 rabbits (6 controls) in which the coronary artery was ligated and the vagus stimulated; in the third group of 23 rabbits (10 controls) the coronary artery was ligated and the vagus stimulated after the induction of alimentary atherosclerosis.

The hearts were fixed in acetone or in Bouin, embedded in paraffin, sectioned, cleared in xylol, and then fluorochromed by solutions of dyes diluted 1:10,000. We used the following fluorochromes: acridine orange, coryphosphine,rhodamine 6 Zh, primuline, and pyronine B. The dyes were diluted in buffer solutions (Mikhaélis and Zerensen's phosphate buffer, Gomori's tris-buffer, Walpole's acetate) at pH 4.5, 5.0, 7.0, and 8.0. The sections were incubated in freshly prepared dyes for 6 minutes, washed with distilled water, and then placed in hydroquinone solution [19]. Also we selectively prepared permanent preparations by Arutyunov's method [3]. All the work was done on the MUF-3M Soviet microscope built for observation of fluorescence in light incident from above and passing through the objective, and also by transmitted light. We used light filters SS-8 and SS-4, FS-1-2, and FS-2-4, as well as a yellow ocular light filter 2N.

The histochemical and histological methods have been described previously [9,10,11].

EXPERIMENTAL RESULTS

The myocardium of the control and experimental animals outside the ischemic zone gave various fluorescent pictures according to the fluorochrome and the pH employed. Of the true fluorochromes, at various pH values very definite pictures were obtained with acridine orange and coryphosphine at pH 5.0 and 7.0.

Muscle fibers fluorochromed with acridine orange at pH 5.0 showed up as dark green, and at pH 7.0 the color changed to orange-red. At pH 5.0 the nuclei of the muscle fibers were an intense orange, and at pH 7.0 the color was reddish-orange. The connective tissue of the stroma of the myocardium and vessels gave off light of various colors from bright green to grass-green. The walls of the vessels showed up clearly owing to the bright yellowish-greenish color of the fluorescence of the elastic coat. The erythrocytes did not fluoresce.

TABLE. Changes in the Myocardium in the Ischemic Zone After Ligation of the Coronary Artery

Conditions of experiment	Morphological changes of the muscle fibers	Times of observation								
		10 min	30 min	1 hr	2 hrs	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs
Ligation of the coronary artery	Disappearance of glycogen	−−+	−++	+++	Complete disappearance					
	Disappearance of activity of enzymes	−−−	−−−	−−−	−−−	−−+	−++	+++	+++	+++
	Change of fluorescence of cytoplasm	−−−	−−−	−−−	−−−	−−+	−++	+++	+++	+++
	Dystrophy	−−−	−−−	−−−	−−−	−−−	−−+	−++	+++	+++
	Necrosis	−−−	−−−	−−−	−−−	−−−	−−−	−−+	−++	+++
Ligation of the coronary artery and stimulation of the vagus	Disappearance of glycogen	−−+	−++	+++	+++	+++				
	Disappearance of activity of enzymes	Not investigated								
	Change of fluorescence of cytoplasm	−−−	−−−	−−−	−−+	−++				
	Dystrophy	−−−	−−−	−−−	−−+	−++	No investigation made			
	Necrosis	−−−	−−−	−−−	−−−	−−−				
Ligation of the coronary artery and stimulation of the vagus in rabbits with atherosclerosis	Disappearance of glycogen	−−+	−++	+++	+++	+++				
	Disappearance of activity of enzymes	Not investigated								
	Change of fluorescence of cytoplasm	−−−	−−−	−−+	−++	+++	No investigation made			
	Dystrophy	−−−	−−−	−−+	−−+	−++				
	Necrosis	−−−	−−−	−−−	−−−	−−−				

Indications. −−− not found; −−+ weakly shown; −++ definitely shown; +++ well shown.

After ligation of the coronary artery, in the myocardium of the experimental animals of the various groups, under the miocroscope the fluorescence observed was uniform in time, intensity, and extent (see the table). The table shows the results of histochemical studies made on these animals previously [8,9,10].

In animals of the first group, in the ischemic zone the first change in the colour of the fluorescence was found three hours after ligation of the coronary artery. At first the changes were scarcely noticeable but they progressed with the duration of the ischemia; at pH 5.0 the cytoplasm began to fluoresce an intense green, and at pH 7.0 a golden green color (acridine orange) developed. After six hours from the start of the ischemia, and still more after 12 hours, a definite boundary could be seen between the unchanged muscle fibers and those in the ischemic zone (Fig. 1). In the formed infarct the cytoplasm of the muscle fibers fluoresced pale green at pH 5.0 and an intense orange-green at pH 7.0. Between the necrotic muscle fibers there were a varying number of leucocytes, whose cytoplasm fluoresced a ruby-red color.

Fig. 1. Bright fluorescence of muscle fibers in the ischemic zone (top of picture) with gradual reduction in the preserved parts of the myocardium (bottom of picture). Time of ischemia 12 hours. Acridine orange. Magnification 60 x.

In the animals of the second group the first changes in the color of the fluorescence were observed two hours after the start of the experiment. The changes in the color of the muscle fibers corresponded to the changes already described, and were restricted to the ischemic region.

In the myocardium of the animals of the third group changes in the color of the fluorescence were observed one hour after the start of the experiment. However, the results were different from the previous two experiments in that the change of fluorescence toward an intense green at pH 5.0 and a golden-green at pH 7.0 was not restricted to the ischemic zone. Outside the zone supplied by the ligated artery (right ventricle and side and rear wall of the left ventricle) some of the muscle fibers also changed their fluorescence, though the change was not as marked as in the ischemic region. By the end of the experiment (three hours) we could be sure we had detected a difference in the fluorescence of the cytoplasm of the preserved and of the altered muscle fibers (Fig. 2)

As can be seen from the results presented fluorescence microscopy can be used on a heart fixed in acetone or Carnoy's fluid. To fluorochrome sections cut in paraffin the most useful agents are acridine orange and coryphosphine at pH 5.0 or 7.0.

The change of color and intensity of the fluorescence of the muscle fibers in the ischemic zone caused by ligation of the coronary artery varied in the different groups in both their time of occurrence and extent.

In 1940 Gollwitzer-Meier and Kroetz [26] found that on stimulation of the vagus there was an increased oxygen supply to the heart because the reduced demands made on it more than compensated for the reduced coronary supply. M. E. Raiskina and Z. T. Samoilova [21] found that stimulation of the cardiac nerve brings into play a number of mechanisms regulating the work of the heart and makes it relatively independent of the coronary flow. E. B. Novikova and M. G. Udel'nov [20] showed that the response of the coronary vessels to stimulation of the vagus depends upon the duration of the stimulus, because direct stimulation of the nerve causes a dilatation of the vessels which is followed immediately by a compensatory restriction. Morphological studies [7,10,25] have shown that ligation of the coronary artery combined with vagal stimulation leads to more extensive myocardial damage than occurs without stimulation of this nerve.

Therefore, the degree of damage to the myocardium after ligation of the coronary artery depends upon the relationship between the load on the heart and its blood supply.

The mechanism involved in the change of fluorescence of the tissues is to a large extent unexplained, but we may make certain suggestions, bearing in mind that in the ischemic zone the complex oxidation-reduction processes are disturbed. Naturally, in a discussion of the results we take into account that fixation of the myocardium in

acetone causes denaturation of the proteins and alters both the sorption of the dye and the physicochemical properties of the muscle fibers.

In many investigations [2,17,18] it has been shown that when various stimuli act on the cell. in all cases the cytoplasm undergoes a corresponding series of complex changes. We may mention a shift of the cytoplasm toward greater acidity, the loss from the cells of various substances (potassium ions, phosphates, creatine), the activation of many chemical groups, a reduction in the degree of dispersion of the colloids, etc. These "paranecrotic" changes are due to reversible alterations in the proteins, alterations which closely resemble the intial phases of the denaturation of proteins.

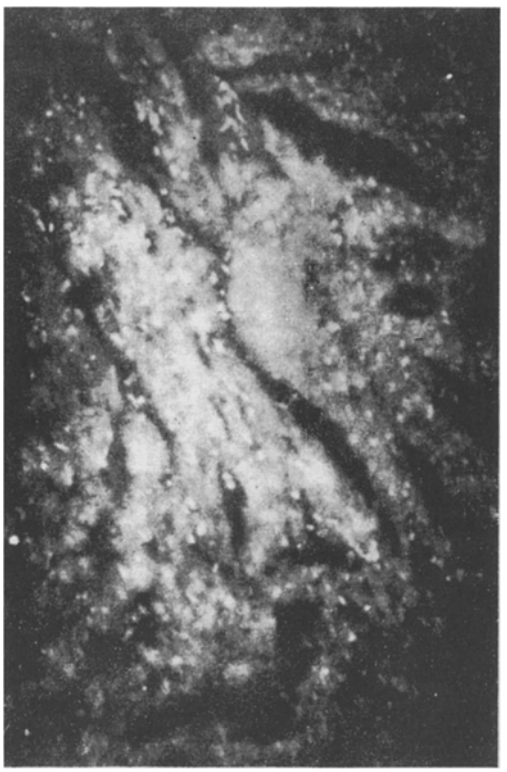

Fig. 2. Regional change of fluorescence of dystrophic muscle fibers outside the ischemic zone (center of picture). Time of ischemia three hours. Acridine orange. Magnification 60 x .

The extent to which the cytoplasm is stained depends upon the properties both of the dye and of the cytoplasm; in each separate case either chemical or physical properties may be chiefly concerned [23]. Fluorochroming of the tissues is also a complex physicochemical process, but we lack information on the nature and means of relating the fluorochromes to the various chemical and structural components of the cytoplasm [12,16].

After ligation of the coronary artery, the biological oxidation processes in the muscle fibers of the ischemic zone are disturbed. The morphological and biochemical indications of this disturbance are the disappearance of glycogen and the accumulation of lactic acid [5,8,10, 31,32], a fall in the level of ATP and creatine phosphate, and an increase in the amount of inorganic phosphate [22, 28,30]; there is also a reduction in the number of potassium ions and an increase of sodium ions [24,27], a change in the activity of the oxidation-reduction enzymes [8,9,29-33], etc. Hypoxia and ischemia also alter the protein content of cardiac muscle [4].

Therefore in the ischemic zone complex changes occur during the disturbance of the physicochemical condition of the muscle fibers, and the result is shown by the color and intensity of the fluorescence. The accumulation in this zone of acid and unoxidized products of metabolism leads to a reduction of pH (increased acidity). But because in an acid medium protein dissociates as a base and becomes charged positively, no connection is formed between it and the cations of the alkaline fluorochromes, with the result that the muscle fibers fluoresce a greenish color. Outside the ischemic zone the proteins of the actomyosin complex, which have many negative valences, combine with the fluorochrome to give an orange-red fluorescence.

According to the observations of M. N. Meisel' [12] the color changes of the cells at death (fluorochroming with acridine orange) depend to a large extent on the death "mechanism". Meisel' claims that acid fluorochromes show a far more definite difference between living and dead cells (stained vitally). In our experiments we obtained worse results with acid than with alkaline fluorochromes, probably on account of the characteristics of the material used.

The problem of the changes of the nucleoproteins in the ischemic zone is interesting. It has been shown [15] that acridine orange, aurophosphine and coryphosphine (all used in the same concentration) give a bright green fluorescence with DNA and its compounds, and a bright red fluorescence with RNA and with its products loosely bound to protein. In this report it was also shown that the more complex compounds of the nucleoproteins, particularly their combination with lipids, does not combine so easily with the fluorochromes of the acridine orange series.

Probably in ischemia the complex ribonucleoproteins break down in the cytoplasm of the muscle fibers, with the result that the phosphate residues are liberated. Apparently, these phosphate groups combine with pyronine in Brachet's reaction and result in the appearance of a pyroninophil cytoplasm in the muscle fibers in the ischemic zone, a condition which is not prevented by ribonuclease [8].

Fluorochroming tissues is a complex physiocochemical process, and we may suppose that the disturbance of the metabolic processes in the mitochondria is associated with a simultaneous change in the structure of the polypeptides, polysaccharides, and polynucleotides of the cytoplasm of the muscle fiber. The fluorescence microscopy method is the most sensitive of all we have used, and by this means these changes may be perceived earlier, whereas certain histochemical methods, particularly histological processes, show considerable damage to the structural units of the cytoplasm. The results of our experiments are in agreement with previous studies [29].

A study by means of fluorescence microscopy combined with new histological methods of investigation of tissues and organs may be used for the diagnosis of ischemia and of the early phases of experimental myocardial infarction.

Because the method is practical and rapid it may be applied in pathology for the determination of the early stages of myocardial infarction in man.

SUMMARY

Fluorescence microscopy was used for the study of early changes occurring in the myocardium after ligation of the coronary artery of rabbits; atherosclerosis was induced in some of the animals, and the effect of vagal stimulation was also investigated. It was found that in conjunction with recent histochemical methods, fluorescence microscopy may be employed for the diagnosis of ischemia and early experimental myocardial infarction. It is thought that this method may have useful applications in pathology.

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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.**
