CHANGE IN THE SORPTIVE PROPERTIES OF A MYOCARDIUM AFFECTED BY ADRENALIN

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The fact that large doses of adrenalin have a toxic effect on the heart of warm-blooded animals is widely acknowledged in the literature. The morphological picture of this type of myocarditis has been described in detail by several researchers [1,4].

In earlier investigations [2,3], we found that a single intramuscular injection of a large dose of adrenalin (0.5-0.8 ml of a 1 :1000 solution) administered to rats causes destructive changes to develop in the myocardium. EKG changes indicating disturbance of myocardial trophicity were observed even during the first few hours after the injection: the voltage and form of the P, R, S and T waves changed, and the ST interval shifted away from the isoelectric line. Microscopic examination of fixed histological preparations obtained 3-12 hr after the adrenalin injection disclosed swelling and homogenization of individual muscle fibers, while preparations obtained after 18 hours or more showed disintegration of certain groups of muscle fibers and a sharply pronounced cellular reaction, both of which usually became more marked by the end of the second day. Later, connective tissue appeared, and the foci of muscle fiber disintegration were replaced by scar tissue. The destructive changes were concentrated primarily in the papillary muscle, the wall of the left ventricle and the interventricular septum.

By means of electrocardiography and microscopy of fixed histological preparations, therefore, we were able to trace the course of the pathologic process induced in the myocardium by the action of toxic doses of adrenalin. By these means, however, the myocardial injury can only be examined at the later stages of its development, as these methods can reveal nothing of the condition of the myocardium during the first hours following the action of harmful doses of adrenalin. In order to determine this aspect of adrenalin's effect, we resorted to the method of intravital staining developed by D. N. Nasonov. This method not only makes it possible to qualitatively demonstrate injury to cell structures resulting from the action of stimulants, but also permits quantitative analysis of such injury according to the change in sorption of a stain by the tissue [5,6].

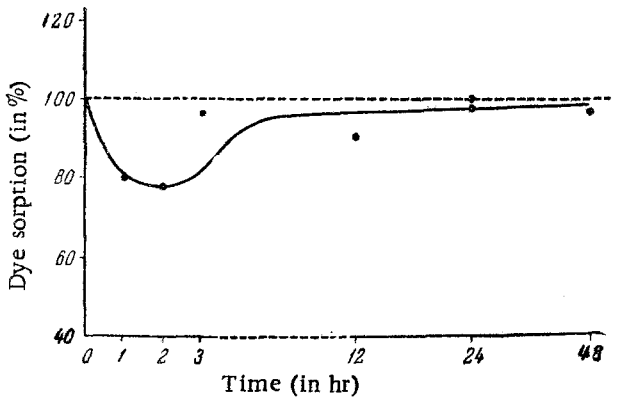
METHOD

The experiments were performed on rats weighing 180-250 g each. Adrenalin was injected intramuscularly in a dose of 0.5 ml of a 1: 1000 solution. The animals were sacrificed by decapitation after 1,2,3,12,24 and 48 hours. The heart was taken out of the thoracic cavity and washed out with a Ringer's solution administered through a cannula inserted in the aorta, after which 10 ml of a 0.05% solution of neutral red stain prepared in a Ringer's solution without soda was passed through this same cannula for five minutes. When the cavities of the heart and vessels had been washed out and filled with the stain solution, the heart was placed in a small glass containing the stain solution for a period of 20 min.

At the end of this period, it was taken out of the dye solution and washed in a Ringer's solution; then two transverse incisions were made through the whole thickness of the myocardium at the level of the papillary muscles, 1-2 mm apart. The resulting myocardial section was put into acidified ethyl alcohol in order to extract the stain. The alcohol extracts were measured photometrically with an electrophotocolorimeter. The amount of stain absorbed was determined in arbitrary units of extinction $\times 1000$ per 1 mg dry weight of the heart muscle, or expressed in percent of the control coloration, which was taken as 100%. The hearts of all the experimental and control animals were examined under the microscope. The Student-Fisher method of statistical processing was used to check the

TABLE 1. Change in Myocardial Sorption of Neutral Red Attending Adrenalin Myocarditis

Animal group	Number of Animals	Time (in hr) after adrenalin injection	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	8	1	5.6± 0.49	0.968	80.0
Control	8	-	7.0± 0.46		
Experimental	8	2	5.5± 0.23	0.995	78.5
Control	8	-	7.0± 0.46		
Experimental	8	3	4.9± 0.80	0.721	96.0
Control	8	-	5.0± 0.28		
Experimental	8	12	4.0± 0.96	0.832	90.7
Control	9	-	5.4± 0.33		
Experimental	8	24	5.0± 0.25	0.500	100.0
Control	8	-	5.0± 0.28		
Experimental	8	48	5.3± 0.41	0.578	98.1
Control	9	-	5.4± 0.33		



Curve of myocardial sorption of neutral red during adrenalin myocarditis.

significance of the results obtained. When the difference probability α was at least 0.950, the results were assumed to be significant.

RESULTS

The experiments showed a considerable decrease in the sorption of the stain by the myocardial tissues during the first few hours after the adrenalin injection. Sorption was 80 and 78.5% of the control, for example, after 1 and 2 hr respectively. These results proved to be statistically significant. At the later stages (3-48 hr), sorption was somewhat less in the experimental animals than in the control, but this difference was not proven statistically significant (Table 1, see figure also).

Microscopy of the hearts of the control animals showed clearly striated muscle fibers containing single granules. The background was diffusely stained. Occasional (1-2 per preparation) nuclei could be seen. Vital staining of the hearts affected by adrenalin did not demonstrate any substantial changes in the muscle fibers. Predominantly lengthwise striation was only observed in a few cases. Besides this, a great quantity of connective tissue cells and formed white blood elements containing many granules of the dye was found in all the animals killed 1 and 2 days after the administration of adrenalin. They corresponded to the accumulations of cellular elements observed on the fixed histological preparations prepared at the same stages in the disease. However, neither a more intense background stain nor any muscle cell nuclei were observed in any case. This was an unexpected development, since the dynamics of the subject affection had been traced on histological preparations from the slight changes which occurred in the early stages to the disintegration of the muscle fibers and sharply pronounced cellular reaction evident at the later stages.

We were unable to find any information in the literature in regard to vital staining of the heart muscle of warm-blooded animals under normal conditions and after the infliction of injury. It could then be proposed that, in the first place, the myocardium of warm-blooded animals has its own specific characteristics and does not respond to injury by the manifestation of nuclear structures or increased sorption of dye and, in the second, that the failure of the sorption of neutral red to increase was due to change in the acid-alkali balance in the myocardial tissue attending the action of adrenalin.

TABLE 2. Effect of Hypotonic Medium on Myocardial Sorption of Neutral Red

Animal group	Number of Animals	Experimental conditions	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	7	Stain prepared in a four times diluted Ringer's solution	18.4± 3.23	0.998	279.0
Control	7	Stained in isotonic solution	6.6± 0.67		
Experimental	5	Stained in isotonic solution after removal of heart from hypotonic medium	7.2± 0.46		
Control	8	Stained in isotonic solution	5.5± 0.42	0.991	130.9

TABLE 3. Phenol Red Staining of Myocardium in Adrenalin Myocarditis

Animal group	Number of Animals	Time after adrenalin injection (in hours)	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	5	1	4.2± 0.37	0.578	102.0
Control	6	-	4.1± 0.18		
Experimental	4	2	4.6± 0.15	0.613	97.8
Control	4	-	4.7± 0.31		
Experimental	8	24	4.9± 0.22	0.721	104.2
Control	8	-	4.7± 0.22		
Experimental	5	48	4.1± 0.36	0.850	89.3
Control	5	-	4.6± 0.29		

TABLE 4. Effect of High Temperature on Myocardial Sorption of Phenol Red

Animal group	Number of Animals	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	3	24.1± 3.75	0.999	515
Control	8	4.7± 0.22		

To prove the first hypothesis, we performed experiments on rats' isolated hearts treated with a hypotonic medium. In the first series of experiments, the heart was first washed free of blood, then stained as described above except that the stain was prepared in a four times diluted Ringer's solution.

The experiments showed a sharp increase in sorption of the stain under these conditions, to 279% of the control, which was regarded as 100% (Table 2). Microscopic examinations of these hearts demonstrated a large number of nuclei in the muscle fibers.

The extent to which the stain was sorbed by the tissue in these experiments could be affected by the conditions

of hypotonia under which the heart muscle was stained. In the second series of experiments, therefore, the heart was stained in a stain solution prepared by the usual method, and myocardial injury was induced by placing the heart muscle for 20 min in a Ringer's solution diluted four times. Under these conditions, myocardial sorption of the stain was 130% (see Table 2), and microscopic examination disclosed well-defined nuclear forms.

In both these cases, therefore, the isolated heart responded to injury by a typical paranecrotic reaction: the appearance of nuclear structures and increased dye sorption. The symptoms of paranecrosis were considerably more pronounced in the first series of experiments than in the second. This was probably due to the fact that the paranecrotic symptoms (or "hypotonic numbing" of muscular structures) are reversible for a certain period, and the isotonic conditions under which the heart was stained considerably reduced these symptoms in the latter experiments.

A series of experiments in which the acid stain phenol red was used was performed in order to prove the second hypothesis-the effect of possible change in the pH of the medium on the fixation of neutral red. The pH is known to become more acid under conditions of inflammation. Therefore, if this holds true in adrenalin myocarditis, no increase in dye sorption would occur even in the presence of injury to the cellular structures if the dye used were the basic stain neutral red. Acid dyes, on the other hand, would produce a more intense stain.

The experimental procedure in this case was the same as that described above. Staining was done with a 0.03% solution of phenol red, prepared in a Ringer's solution. The staining time was 20 min. The experimental animals were examined 1,2,24 and 48 hr after the adrenalin injection (Table 3).

As Table 3 indicates, these experiments showed no increase in dye sorption either during the first two hours after the injury was inflicted or at the later stages (after 1-2 days), when the destructive changes in the myocardial tissue had become clearly apparent.

Dye sorption increased by more than five times when the isolated heart was injured by high temperatures (immersion for 5 min in a Ringer's solution at a temperature of 60° C). In this case, therefore, the isolated heart responded to injury by a typical paranecrotic reaction-increased dye sorption (Table 4).

The use of the vital staining method to study experimental adrenalin myocarditis disclosed considerable decrease in the sorption of the basic stain neutral red during the first few hours after the administration of adrenalin. This could be an early objective sign of the changes which occur in the protoplasm of the myocardial muscle cells during the initial stages of the myocardium's reaction to toxic doses of adrenalin. D. N. Nasonov and his co-workers [5] often observed the intravital stainability of the protoplasm to decrease under various influences. No exact explanation of this phenomenon has yet been offered. It could be a defense reaction of the tissue to irritation, increasing its resistance to various harmful agents [7].

In the later stages of the process of injury, however, we did not observe the increase in dye sorption which usually occurs in animals under certain influences. Perhaps, under conditions of an intact organism, reparative processes to heal the injury develop in response to the latter, and this effects dye sorption.

The lack of increase in the stainability of live myocardial cells could also be due to the fact that the injury induced by adrenalin did not encompass the entire muscle, as occurred, for example, in our experiments with the action of hypotonic solutions and high temperature on the heart, but affected only certain portions of it, and increased sorption by these small portions may not be reflected by the over-all result. However, the fact that microscopic examination of the sections never disclosed the appearance of nuclei, which is one of the first signs of injury to the cell protoplasm-despite the presence of cellular infiltration, and therefore, muscle fiber disintegration-would seem to negate the latter hypothesis.

Nor can the lack of increase in the stainability of the myocardial tissue under conditions of adrenalin myocarditis be ascribed to a shift of the pH of the medium toward the acid side, as is usually observed in inflammation, since the sorptive properties of the myocardium showed no change when acid dyes were used.

The experimental data obtained, therefore, permits the hypothesis that the failure of the stainability of the myocardial tissue to increase in adrenalin myocarditis under conditions of an intact organism is due to reparative processes which develop to heal the injury.

SUMMARY

A method of vital staining was used to investigate the sorptive property of rat cardiac muscle affected by adrenalin.

In 1-2 hours after injecting 0.5 ml of adrenalin solution (1: 1,000) intramuscularly the sorption of neutral red shows a marked reduction, whereas in 12-24 hours it does not differ materially from the control. The absence of increased vital staining of myocardial tissues, notwithstanding the presence of destructive changes in them (detectable by histological examination), may be explained by repair processes developing in the intact organism in response to injury.

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