BIOCHEMISTRY AND BIOPHYSICS

EFFECT OF MASSIVE DOSES OF ADRENALIN ON THE PROTEIN METABOLISM OF THE MYOCARDIUM OF RATS

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It is known that injection of large doses of adrenalin into animals causes destructive changes in the myocardium, for which reason it is frequently applied for the production of so-called "experimental myocarditis" [1, 3]. However, up till now such destructive changes in the myocardium have been studied by histological methods only. The present paper describes an attempt at elucidating the protein metabolism of the myocardium following administration of adrenalin. As an indicator of the trophism of the myocardium we took the rate of incorporation of S^{35}-labeled methionine into myocardial proteins. The view has gained general acceptance over the past few years that the rate of incorporation of radioactive isotope-labeled amino acids into the proteins of organs or tissues is a measure of the intensity of their metabolism and of the rate of replacement of these proteins.

EXPERIMENTAL METHOD

We produced experimental myocarditis in rats by single massive intramuscular injections of adrenalin, in doses of 0.5-0.8 ml of 1:1000 solution. According to the findings of O.P. Vishnevskaia [3] such doses cause pronounced morphological changes in the myocardium of all injected rats within the first few days after injection.

We examined the protein metabolism of the myocardium in experimental myocarditis two hours, and one and two days after injection of toxic doses of adrenalin, by measuring the specific radioactivity of the myocardial proteins. In some of the experiments, we supplemented these measurements by autoradiographic examination of fragments of myocardium. The simultaneous application of both these methods allowed us to draw conclusions regarding both the general rate of incorporation of S^{35}, and its distribution within the myocardium.

Intraperitoneal injections of S^{35}-labeled methionine were given to control rats and to rats which had been suffering from myocarditis for various lengths of time, at a dosage level of 8000 impulses per g body weight. The animals were killed by decapitation two hours later. The heart was removed, and perfused with physiological saline through a cannula inserted in the aorta, until the effluent was no longer bloodstained. The heart was then pulverized and triturated with quartz sand in a mortar. The proteins were precipitated with 10% trichloroacetic acid. The protein precipitate was washed with 5% trichloroacetic acid solution, until the washings no longer showed any radioactivity. The washed proteins were defatted by extraction with alcohol and alcohol-ether mixture. The precipitate was ground up with 2 ml of N potassium hydroxide. Portions (0.4 ml) of the resulting solution were transferred to a watch-glass for radioactivity counts, using an end-window counter, and a 0.4 ml portion was taken for determination of protein content, by the biuret method. Specific radioactivity was calculated from the number of impulses per minute per 10 mg of protein.

The specimens for autoradiography were fixed in Carnoy's fluid, and embedded in paraffin. Type R emulsion, manufactured by NIKFI, was placed on the sections, by the procedure described by Gracheva, Zhinkin, and Shcherban' (1956). The preparation of a supporting layer for the emulsion, and the development and fixation of

the films were performed according to NIKFI instructions. We prepared track autoradiographs with a duration of exposure of one, three, and five days, and contrast autoradiographs with exposures of 25 days.

The rate of incorporation of labeled methionine was taken as being proportional to the rate of darkening of the photographic emulsion, which we measured with the aid of an MF-4 microdensitometer, and expressed in arbitrary units.

We also followed the incorporation process by counting tracks formed in the emulsion by products of disintegration of S^{35}. The number of tracks was counted by means of a microscope with a grating fitted to the ocular. We counted the number of tracks in a square of the grating, of 100 μ^2 area.

EXPERIMENTAL RESULTS

At the dosage level of adrenalin used by us we were able to achieve definite dystrophic changes in the myocardium, as shown by both electrocardiographic data and by microscopic inspection. Marked changes appeared in the electrocardiograms during the first three to four hours after injection of adrenalin. The sinus rhythm was retarded, as a general rule. The P waves became flattened and dichrotic. The potential of the R waves fell,

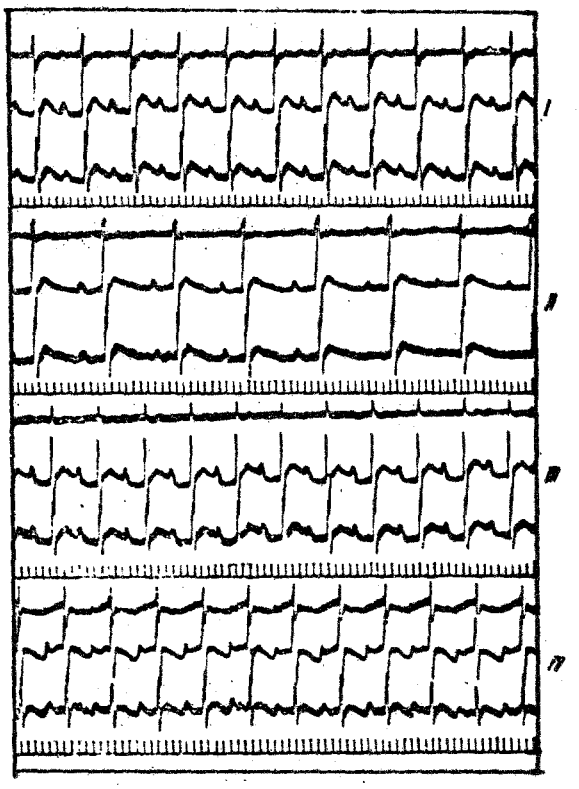

Fig. 1. Electrocardiograms of rats, taken with three standard leads: I) normal; II) after 20 minutes; III) after three hours; IV) two days after intramuscular injection of 0.5 ml of 0.1% adrenalin solution.

and of the S waves rose. The S–T interval was displaced above or below the isoelectric line. The T wave usually became accentuated during the first few hours after injection, but had become greatly diminished by the end of the first or second day, hardly rising above the isoelectric line, or even undergoing inversion (Figure 1).

The observed changes in the electrocardiograms were evidence of the presence of a serious myocardial lesion, and this was confirmed in a number of experiments by subsequent morphological examination. We could find no microscopically evident changes in the myocardium of rats killed within three hours of the injection. After one to two days, however, all the rats showed focal or diffuse fatty degeneration of the muscle fibers. Indi-

TABLE 1

Incorporation of S^{35}-Labeled Methionine Into the Heart Muscle of Rats (From the specific radioactivity of the myocardial proteins)

Date	Control		Experimental		Experimental/ /control (in %)*
	weight of animals (in g)	specific radioactivity	weight of animals (in g)	specific radioactivity	
	A. Two hours after injection of adrenalin				
5/16, 1956	310.0	80	300.0	127	159
5/16	330.0	152	300.0	130	85
7/5	240.0	84	230.0	121	144
7/5	250.0	125	250.0	121	97
9/23	280.0	71	280.0	91	128
9/23	260.0	69	270.0	96	139
6/5, 1957	280.0	177	270.0	217	123
6/5	256.0	197	260.0	188	95
Mean		119		136	
	B. One day after injection of adrenalin				
6/31,** 1956	180.0	86	220.0	118	137
6/31**	180.0	78	200.0	143	183
6/20	220.0	73	220.0	276	378
6/20	230.0	92	220.0	228	248
6/20	230.0	108	220.0	252	233
6/27	190.0	190	200.0	282	148
6/27	180.0	124	200.0	242	195
Mean		107		220	
	C. Two days after injection of adrenalin				
5/21, 1956	180.0	141	200.0	266	189
5/24	180.0	176	180.0	200	113
5/24	180.0	126	180.0	192	152
6/4	200.0	71	250.0	296	416
6/28	170.0	135	230.0	317	235
6/28	200.0	122	200.0	205	168
6/29	310.0	112	370.0	151	135
6/29	333.0	114	350.0	158	139
12/6	260.0	131	260.0	309	236
12/6	270.0	123	250.0	182	148
12/12	175.0	117	185.0	189	161
3/4, 1957	150.0	107	150.0	131	122
3/7	180.0	109	200.0	181	166
3/11	170.0	87	230.0	194	223
3/11	140.0	82	210.0	187	228
3/14	200.0	104	190.0	154	148
3/22	190.0	127	180.0	209	165
3/25	210.0	73	216.0	132	181
3/27	208.0	93	200.0	174	187
3/27	178.0	103	220.0	140	136
Mean		112		198	

*This is the ratio of the specific radioactivity of the myocardial proteins of the control animals to that of the experimental ones. The specific radioactivity of the control animal is taken as being 100%. In the subsequent tables (2 and 3) specific radioactivity, number of tracks per unit area, and optical density of the films for control animals are also taken as 100%.

**As in original. Probably 6/13 – Publisher's note.

vidual fibers were swollen, with fragmentation and necrotic changes in places. Extensive and numerous aggregations of infiltrated cells were visible throughout the thickness of the heart muscle (Figure 2).

Two hours after injection of adrenalin the rate of incorporation of S^{35} into the myocardial proteins differed only slightly from the normal value (Table 1,A), fluctuating in either direction from it.

After one to two days, however, the specific radioactivity of the myocardial proteins of the experimental rats was considerably greater than in the control series (Table 1, B and C). The increase in the amount of incorporation varied widely on different days and for different pairs of rats (experimental-control). In all cases, however, the specific radioactivity of the myocardial proteins of the adrenalin-injected rats exceeded that of the controls, on the average by a factor of 2 (the difference is statistically significant).

The autoradiographic data correspond closely with those for specific radioactivity. Simple inspection of the autoradiographs shows a striking difference between the densities of the films from injected and control rats (Figure 3). A precise quantitative expression of the rate of incorporation of S^{35}-methionine is given by the densitometric data, and by the track counts, giving a rate in the injected rats of nearly double that in the controls. Table 2 gives a comparison of the rates of incorporation of labeled methionine into myocardial proteins of a rat, as derived from specific radioactivity, densitometric, and track-counting data. If we take the rate of incorporation of S^{35} into myocardial proteins as 100% in control rats, then the rate for adrenalin-injected rats is 235% from specific radioactivity data, 223% from densitometric data, and 217% from track-count data.

TABLE 2

Incorporation of S^{35}-Labeled Methionine Into the Myocardium of Adrenalin-Injected and Control Rats, As Derived from Specific Radioactivity of Protein Measurements and from Densitometric Measurements of Radioautographs

Specific radioactivity			Number of tracks per 1000 μ^2			Optical density		
exptl	control	ratio: exptl/control (%)	exptl	control	ratio: exptl/control (%)	exptl	control	ratio: exptl/control (%)
317	135	235	15.2	7.0	217	41.4	18.6	223

As we have shown in an earlier paper [2], S^{35}-methionine is uniformly distributed throughout the thickness of the heart muscle of normal animals. In experimental myocarditis the rate of incorporation in the myocardium as a whole rises, as a result of increased incorporation both into muscle fibers and into connective tissue. The process is of particular intensity at sites of aggregation of infiltrated cells (Figure 3 and Table 3).

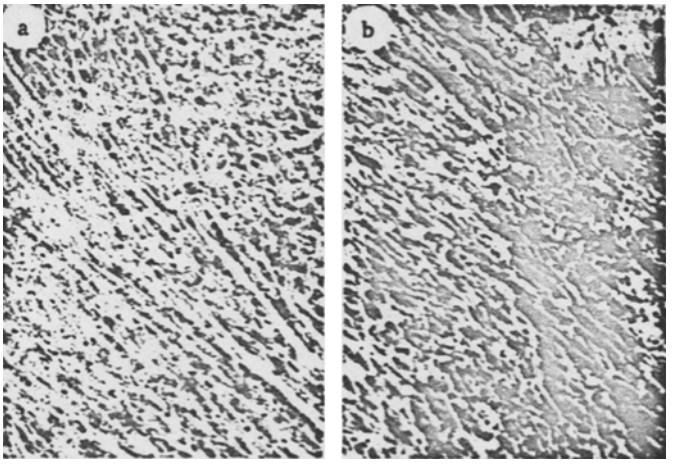

Fig. 2. Photomicrograph of rat myocardium: a) control; b) experimental. Objective 8x, ocular 10x. Stained with azure-eosin.

TABLE 3

Distribution of S^{35}-Methionine in the Heart Muscle of Rats Suffering from "Experimental Myocarditis" (From densitometric measurements of track autoradiographs)

Date	Time of exposure of films (days)	Number of tracks per 1000 μ^2			Ratio: experimental/control (%)	
		control	experiment		experiment	
		(muscle fiber)	muscle fiber	cell infiltrate	muscle fiber	cell infiltrate
6/28, 1956	1	7.0	11.5	19.0	164	271
6/28	3	17.0	25.5	42.0	150	247
10/22	1	2.4	5.0	12.2	208	508
10/22	3	7.9	13.3	24.2	168	306
10/22	1	4.1	6.8	13.6	166	332
10/22	3	11.4	13.0	26.7	114	234

The rate of incorporation of methionine varied within fairly wide limits. This is probably due to differences in the severity of the injury to the myocardium, since different animals reacted differently to the same dose of adrenalin. In some animals, the injuries had a focal distribution, while in others they were distributed uniformly throughout the heart muscle, and in some animals we observed extensive and numerous aggregations of infiltrated cells throughout the whole thickness of the muscle, while in others they were much smaller, and were located chiefly in the region of the papillary muscles. These observations explain, to a certain extent, the wide variations found for specific radioactivity of myocardial proteins (see Table 1). However, in all cases the rate of inclusion of S^{35} into the myocardial proteins was much higher in "experimental myocarditis" than in the controls, and this is evidence of the enhanced rate of resynthesis of the myocardial proteins. The heightened protein metabolism of the myocardium cannot be considered to be a direct reaction to adrenalin, since it does not appear earlier than a day after its injection, i.e., when any adrenalin in the tissues must have undergone de-

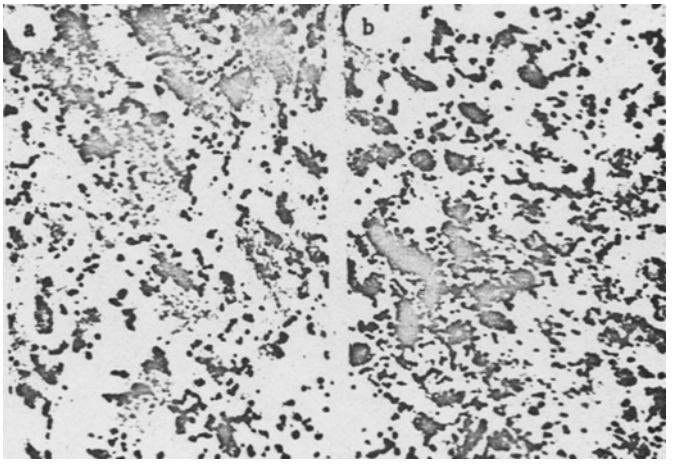

Fig. 3. Track autoradiograph* of rat myocardium two hours after introduction of S^{35}-methionine. Exposure time of the emulsion five days. Immersion objective 60, ocular 7x. Stain — hematoxylin-eosin. a) Control; b) experiment.

struction. The increased resynthesis of proteins must therefore be ascribed to the injurious action of adrenalin. It is difficult, at this stage, to explain the direct mechanism of this increase in protein synthesis. It is possible

*We wish to express our sincere gratitude to the Histology Laboratory, IEM, and to Prof. L.N. Zhinkin, for their help and advice in preparing the autoradiographs.

that in experimental myocarditis the specific activity of the myocardium, viz., its contractive function, is depressed, which in turn leads to intensification of metabolism, expressed as resynthesis of proteins. On the other hand, intensified resynthesis of proteins may be regarded as a consequence of regenerative processes proceeding in the myocardium in response to the destructive action of adrenalin.

SUMMARY

Myocarditis was experimentally induced in rats by a single intramuscular injection of a large dose of adrenalin (0.5 to 0.8 ml of 1:1000 solution).

Myocardial damage was supported by the data of electrocardiographic and morphological examinations. It was revealed that the rate of inclusion of S^{35}-methionine into the proteins of the cardiac muscle is greatly increased in experimentally-induced myocarditis. This takes place as a result of inclusion of S^{35}-methionine into the muscle fibers as well as into the connective tissue. Its inclusion is especially intensive in places of accumulation of the cellular elements.

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*In Russian.