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Respiratory Enzyme Activities in Different Regions of the Mouse Heart.* (27977)

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The different work-loads of the chambers of the heart have led numerous investigators to study the oxygen consumption of the various parts. Three different results have been obtained in rat heart. First, oxygen consumption of the left ventricle was found by Goh and Dallam to be 25% greater than the atrium (1). Second, Olson *et al.* found that oxygen consumption of the rat ventricle was only 70% that of the atria(2), verifying the earlier results of Muus and co-workers(3). Third, Ullrick and Whitehorn found that oxygen consumption of atria and ventricles was the same (4).

To our knowledge the only study comparing the enzymatic activity of different myocardial tissues was by Cooper who found that succinic dehydrogenase activity of either ventricle was about twice that of the atria(5).

It was our belief that a more detailed study of the activities of electron transporting enzymes, such as the cytochrome *c* reductases and cytochrome oxidase, in different regions of the heart might aid in clarifying the conflicting results reported on oxygen consumption of the atria and ventricles. Information would be also obtained on the possible alternative pathways of electron transport in different myocardial tissues.

Materials and methods. Tissue samples were obtained from male mice (C57BL/6J strain, Jackson Memorial Laboratory) which had been reared on a commercial diet and were 59 to 80 days old at time of sacrifice. The mice were killed by separating manually their cervical vertebrae followed by decapitation. The hearts were removed quickly and dissected with the aid of a dissecting microscope in such a manner that each sample was composed of a maximal amount of myocardium, typical of a particular chamber wall, and a minimal amount of connective tissue. In this way, samples of paired atria, right ventricle, left ventricle, and inter-ventricular septum from the same heart were obtained.

For determination of enzymatic activity a sample was homogenized in 0.25 M sucrose using a loose-fitting Tenbroeck homogenizer. Samples were prepared and kept at 0-4°C until analyzed. Each sample was assayed for the different enzymatic activities within 2 hours after preparation. The cytochrome *c* reductases were determined spectrophotometrically at 23-25°C using the method of Lehman and Nason(6). To measure the different reductases, reduced nicotinamide-adenine dinucleotide (NADH), reduced nicotinamide-adenine dinucleotide phosphate (NADPH) or sodium succinate was added, and the rate of cytochrome *c* reduction was determined at 550 mμ. The validity of the reductase assays was examined by adding sodium amobarbital and antimycin A to homogenates of atria and of left ventricle. NADH-cytochrome *c* reductase activity was inhibited by both of these reagents, and succino-cytochrome *c* reductase by only antimycin A, as found in other heart preparations(7). Cytochrome oxidase activity was measured by the method of Cooperstein and Lazarow(8) with cyanide controls for each sample. A unit of both reductase and oxidase activity was defined as a change of 0.001 optical density unit at 550 mμ per

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TABLE I. Cytochrome Reductase and Oxidase Activities in Different Regions of Mouse Heart.

Section	No. of samples	Units per mg protein: Cytochrome c reductase NADH–	NADPH–	Succ.–	Cytochrome oxidase	% protein (fresh wt)
Atria	8	2.73 ± .16*	<.50	1.53 ± .11	7.45 ± .62	10.6 ± .67
Rt. ventricle	8	2.43 ± .13	<.50	2.61 ± .28	16.2 ± .78	13.9 ± .60
Lt. "	7	2.27 ± .16	<.50	2.52 ± .19	18.1 ± 1.3	16.8 ± .57
Septum	8	2.42 ± .12	<.50	2.34 ± .14	16.5 ± 1.5	14.8 ± .70

* Mean ± standard error of mean.
Animals were 59 to 80 days old, C57BL/6J male mice.

minute. The initial velocity of all activity measurements was proportional to protein concentration. Conditions of substrate saturation were used in the enzyme assays, and all results were corrected for the endogenous rate. Cytochrome *c* and the reduced pyridine dinucleotides, obtained from the Sigma Chemical Co., were of greater than 90% purity. In addition, a protein analysis was carried out on an aliquot of each homogenized sample(9).

To determine the chemical composition of different regions of the heart, another series of samples was frozen immediately at dry ice temperature. These frozen samples were weighed to determine the fresh weight which varied from 5-24 mg. These were then lyophilized and dried at 95° to constant weight, and the water content was calculated by difference. The dried samples were extracted in a Soxhlet apparatus with diethyl ether and again dried to constant weight. The lipid content was determined from the difference in weight before and after ether extraction. Protein concentration was determined in the dried, defatted samples by the procedure of Lowry *et al.*(9).

Results. The enzymatic activities found in the different myocardial regions are presented in Table I. No statistical differences in specific activity of NADH-cytochrome *c* reductase were observed in the atria, right ventricle, left ventricle, or inter-ventricular septum. NADPH-cytochrome *c* reductase activity was below our limit of detection and was less than 33% of the next lowest reductase activity. Furthermore, specific activities of succinocytochrome *c* reductase and cytochrome oxidase in the atria were only 57% and 46%, respectively, of those in the right ventricle, which was the same as the other ventricular samples. The lower values in the atria were statistically different ($P = <0.01$). Another difference between atria and ventricles was observed, *viz.*, the per cent protein of the atria was only 63-76% of that of the ventricles.

The different profiles of respiratory enzyme activity in the atria and ventricles suggested the possibility of other differences in chemical composition. Therefore, the water, protein, and nitrogen concentrations of various myocardial samples were determined in another series of animals (Table II). It was noted that the per cent water of the atrial tissue was greater than that of the other 3 regions while the per cent protein of the atria was less. These differences were statistically significant ($P = <0.02$). The sum of per cent water, per cent lipid, and per cent protein for all regions was the same. Like their enzymatic

TABLE II. Chemical Composition of Different Regions of Mouse Heart.

Section	No. of samples	% of fresh weight: % water	% fat	% protein	Total %*
Atria	5	77.2 ± .38†	1.35 ± .17	9.19 ± .58	87.79 ± .34
Rt. ventricle	5	74.3 ± .92	1.68 ± .07	11.9 ± .49	87.95 ± .42
Lt. "	5	74.6 ± .66	1.54 ± .05	13.0 ± .48	89.20 ± .36
Septum	5	74.1 ± .62	1.63 ± .18	12.3 ± .58	88.09 ± .38

* Sum of % water, fat and protein.
† Mean ± standard error of mean.
Animals were 82 days old, C57BL/6J male mice.

patterns the gross chemical composition of the right ventricle, left ventricle, and septum was the same.

Discussion. The conflicting results reported on oxygen consumption of various regions of the rat heart were the basis of the present investigation. Our experimental measurements of several enzymes of electron transport demonstrate differences in the enzymatic profile of the atria as compared to samples of ventricular myocardia. The present findings are consistent with those of Goh and Dallam (1) and of Cooper(5) and indicate that the atrial succino-cytochrome *c* reductase and cytochrome oxidase activities are about half those of the ventricles. NADH-cytochrome *c* reductase activity, however, was the same in both atria and ventricles. Another finding of these experiments is that the enzymatic profiles of right ventricle, left ventricle, and interventricular septum were the same. This observation is in contrast to that of Goh and Dallam who found the oxygen consumption of the left ventricle to be greater than that of the right ventricle(1). It is realized that a direct comparison between enzymatic activity and oxygen consumption may not be valid.

The gross chemical composition of atria *vs* ventricles was also different but cannot explain the differences in specific activities, for the per cent protein of the atria was lower than that of the ventricles.

Some of our preliminary data on cellular fractions of atria and ventricles indicate that the mitochondrial mass of these two tissues is approximately the same, but specific activities of succino-cytochrome *c* reductase and cytochrome oxidase of the atrial mitochondrial fraction are lower. This may account for the lower specific activities found in the atrial homogenates.

NADH-, NADPH-, and succino-cytochrome *c* reductases represent different and possibly alternative pathways of electron transport. The data in this study indicate that in the atria NADH-cytochrome *c* reductase activity is higher than that of the succinate enzyme, whereas in the ventricles both activities are the same. Furthermore, the over-all capacity of atrial tissues for electron transport is half that of the ventricular tissues, since cytochrome oxidase activity, which is an enzyme coupled to both reductases, is decreased in the atria. This lower capacity for electron transport may be a reflection of a lower oxygen requirement and work-load of atrial myocardium.

Summary. The enzymatic activities of NADH-, NADPH-, and succino-cytochrome *c* reductases and cytochrome oxidase were determined in homogenates of different regions of the mouse heart. It was found that the pattern of enzymatic activities in the atria was different from the right ventricle, left ventricle, and inter-ventricular septum. The atria contained only 57% of the succino-cytochrome *c* reductase and 46% of the cytochrome oxidase activity of the right ventricle, which was the same as the other ventricular regions. On the other hand, NADH-cytochrome *c* reductase activity was the same in both atria and ventricles. The atria also differed in gross chemical composition and contained more water and less lipid and protein than the ventricles.

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