RESPIRATION AND RESPIRATORY CHAIN PHOSPHORYLATION IN HOMOGENATES AND MITOCHONDRIA OF RABBIT CARDIAC MUSCLE DURING EXPERIMENTALLY INDUCED MYOCARDITIS

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The process of oxidative phosphorylation has considerable significance for the normal activity of cardiac muscle, in that it leads to the accumulation of high-energy phosphate bonds in the form of adenosine triphosphate. Inhibition of tissue respiration disrupts the activity of the heart by slowing down the resynthesis of adenosine triphosphate [7].

The results of biochemical investigations, devoted to studying the processes of respiration and respiratory chain phosphorylation in cardiac muscle during various pathological states of the heart, are not unequivocal. For example, it was observed that the rate of oxidative phosphorylation in cardiac muscle does not decrease during diphtheria toxicosis [5] or thyrotoxicosis [6]. On the other hand, during myocarditis arising as a result of adrenaline injections, a decreased in oxidation-reduction reactions in the heart [4] and weakening of respiratory chain phosphorylation [3] were noted.

In the present investigation, the processes of respiration and respiratory chain phosphorylation in the tissue of rabbit cardiac muscle were studied during experimentally induced myocarditis.

EXPERIMENTAL METHODS

As experimental animals we chose male rabbits, weighing 2-2.5 kg. Myocarditis was produced in the animals by injecting a 1% solution of theophylline (20 mg per kg body weight), followed after two minutes by an injection of 0.2 ml of a 0.1% solution of adrenaline.

The processes of respiration and respiratory chain phosphorylation were studied in homogenates of cardiac muscle on the third or fourth day after injection of theophylline and adrenaline. Experimental techniques did not differ from those used in a previous investigation [2].

The added respiratory substrates were pyruvic, α-ketoglutaric, succinic, malic, citric, and β-hydroxybutyric acids. The terminal concentration of these acids in the incubation tube was 0.025 M.

To isolate the mitochondria of cardiac muscle (after perfusion with cool 0.15 M KCl solution), the tissue was cut up with scissors and homogenized in the cold in two volumes of 0.25 M sucrose solution with 0.001 M ethylenediaminetetraacetate, and the pH of the solution was brought to 7.4 with 2 N KOH. After homogenizing

* From histological evidence, the characteristic changes of myocarditis are already detectable on the third day after injection of these compounds.
for 30 seconds, seven more volumes of the same solution were added to the minced tissue, and after filtration through several layers of gauze, the homogenate was fractionated into cellular components by differential centrifugation in the cold.

Mitochondria, isolated at 7000 g, were subjected to a single rinse.

The isolated mitochondria were suspended in a 0.25 M sucrose solution at pH 7.4. A quantity of sucrose solution equal to the original weight of cardiac muscle was used. The mitochondrial suspension was rapidly poured out into Warburg vessels. Each vessel contained 1 ml of incubation mixture, consisting of 0.5 ml of mitochondrial suspension (corresponding to 0.9-1.2 mg of protein nitrogen), 0.3 ml of buffer solution, 0.1 ml of sucrose solution (0.25 M) with respiratory substrate, and 0.1 ml of hexokinase solution. The concentrations of the various components in the buffer solution (pH 7.4) were: $K_2HPO_4$, $3 \times 10^{-2}$ M; $MgSO_4$, $1.5 \times 10^{-2}$ M; adenosine triphosphate, $6 \times 10^{-3}$ M; glucose, $5 \times 10^{-2}$ M. The respiratory substrates were $\alpha$-ketoglutaric acid (along with malonic acid), succinic acid, and $\beta$-hydroxybutyric acid. The terminal concentration of these acids in the incubation tube was the same as in the experiments with homogenates. Hexokinase was obtained from yeast [9].

Experiments with mitochondria were conducted under an atmosphere of air, and oxygen absorption was measured in the Warburg apparatus. Incubation was carried out at 26° for 18 minutes. After incubation, inorganic phosphate [8] was determined in experiments with mitochondria, while inorganic phosphate and creatine phosphate [1] were determined in experiments with homogenates.

The rate of phosphorylation was computed from the disappearance of inorganic phosphate and formation of creatine phosphate. The quantity of protein in the incubation tubes was determined by the biuret reaction.

**EXPERIMENTAL RESULTS**

Figure 1 depicts results describing the rate of respiration and respiratory chain phosphorylation in homogenates of rabbit cardiac muscle under normal conditions and during experimentally induced myocarditis.

![Figure 1](image-url)  
*Fig. 1. Respiration and respiratory chain phosphorylation in homogenates of rabbit cardiac muscle under normal conditions (N) and during myocarditis (M). $\Delta O_2$, Oxygen absorption (in micromoles); $\Delta P$, binding of inorganic phosphate (in micromoles), cross-shaded columns. Obliquely shaded columns give formation of creatine phosphate (in micromoles of phosphorus); black columns give the P:O ratio.*