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STATE OF CARDIAC PYRIDINE NUCLEOTIDES AND FLAVOPROTEINS DURING PRESERVATION IN 0.5% FORMALIN SOLUTION

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Papers have recently been published on the use of formaldehyde as a preservative for organs [4]. However, comparatively little can be found in the literature on the mechanism of its action on tissues.

It was accordingly decided to study whether formaldehyde can interact with the mitochondrial respiratory chain and, if so, to determine the site of such interaction.

EXPERIMENTAL METHOD

Rats' hearts preserved by intravenous injection of 0.5% formalin solution were studied. Intact hearts were used as the control.

The energy state of the mitochondria in heart tissue was assessed by luminescence analysis [1, 2]. The fluorescence spectra of the hearts were recorded at 10, 120, and 180 min by means of an expedition microspectrofluorometer with FMÉL-1 probe system [2]. The objective was focused on the muscle cells to record radiation predominantly from the myocardium [2]. The wavelength of the exciting light was 365 nm. Radiation recorded under these circumstances in the region of 460 and 530 nm was due mainly to fluorescence of mitochondrial NADH and flavoproteins (FP) [1, 5]. The energy state of the mitochondria was assessed by determining the ratio between the intensities of luminescence of oxidized FP and NADH [2]:

$$\xi = \frac{I_{530} - {}^1/_2 I_{460}}{I_{460}}.$$

EXPERIMENTAL RESULTS

In the control the coefficient ξ did not exceed 0.1, and it remained at the same level for 2 h (Fig. 1). A very small increase was observed only after 3 h, to 0.14. The value of ξ in the experiment 10 min after preservation was 0.17, and after 3 h it rose to 0.52 (Fig. 1).

Measurement of fluorescence of NADH (Fig. 2) showed that the decrease in the intensity of luminescence immediately after preservation of the heart was 34%, but after 3 h it was 57%. The increase in luminescence of FP of the preserved heart relative to NADH was most marked after 3 h (Fig. 1).

To assess the possibility of the direct effect of formaldehyde on fluorescence of mitocondrial NADH, luminescence of 1 mM solution of NADH was measured after addition of 0.5 mM formaldehyde to it; no change in the intensity of luminescence was observed.

The increase in ξ immediately after preservation of the heart indicates an increase in the degree of oxidation of the respiratory chain of the mitochondria.. In the ischemized organ this could be observed as a result of a block to the progress of reducing equivalents

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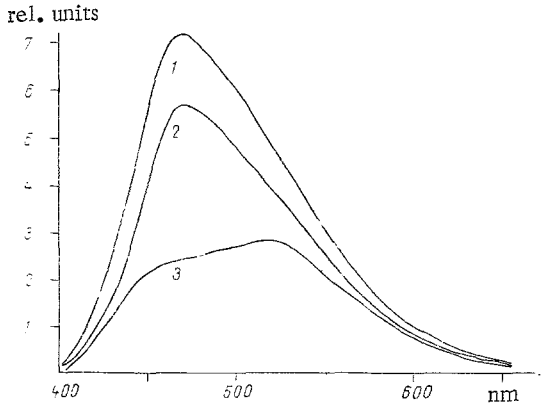

Fig. 1

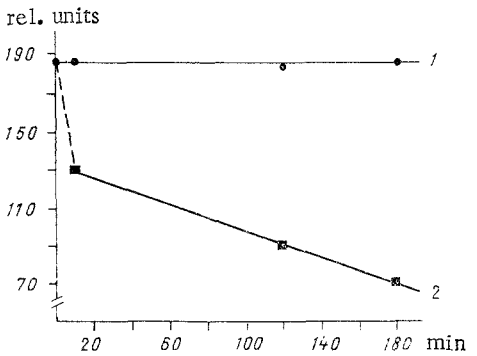

Fig. 2

Fig. 1. Fluorescence spectra during ischemia and preservation of the heart. 1, 2) Ischemia for 10 and 70 min, respectively (ξ = 0.1); 3) preservation for 3 h (ξ = 0.52). Abscissa, wavelength (in nm); ordinate, intensity of luminescence (in relative units).

Fig. 2. Changes in fluorescence of NADH during preservation. 1) Control; 2) preservation. Abscissa, time (in min); ordinate, intensity of luminescence (in relative units).

in the mitochondria from the Krebs cycle to the terminal region of the mitochondrial respiratory chain. In the case of a block of the respiratory chain after coenzyme NADH the value of the coefficient ξ would be changed only a little. If the respiratory chain was blocked by formaldehyde after FP, a marked decrease in ξ would be observed.

An almost twofold increase in ξ was found experimentally immediately after preservation. This increase indicates that the block to progress of the reducing equivalents lay before NADH. This also was confirmed by analysis of the kinetics of the fall in intensity of luminescence of NADH after preservation (Fig. 2).

Magomedova [3] showed that when the heart is preserved in a weak solution of formalin glycolysis is immediately suppressed. In muscles and, in particular, in the heart glycolysis is a source of substrates for the Krebs cycle rather than a mechanism of ATP production [2]. With these considerations in mind, the decrease in the supply of reducing equivalents to the mitochondrial respiratory chain during preservation of the heart may be associated with a decrease in the production of substrates by the glycolytic system in the region common to both aerobic and anerobic pathways of carbohydrate catabolism.

A further slow decrease in the intensity of NADH emission in the preserved heart for 3 h (Fig. 2), together with the relative increase in FP fluorescence, is evidence that formaldehyde also acts directly on the mitochondrial respiratory chain in the late stages of the process.

It can thus be concluded on the basis of these results that the action of formaldehyde on oxidation metabolism of myocardial cells is linked in the first stage, evidently, with inhibition of the glycolytic system. The subsequent effect of formaldehyde may be due to its penetration into the mitochondria, followed by inhibition of the Krebs cycle.

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