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CHRONIC EFFECT OF ETHANOL ON THE METABOLISM OF MYOCARDIUM

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Abstract—Chronic administration of ethanol (2·5 g/kg for 10 weeks) to rats caused growth retardation, decrease of myocardial glycogen, and a decrease of myocardial homogenate respiration and of glycolytic activity. Heart mitochondrial respiration was slightly lowered but respiratory control decreased significantly. Heart weight was unchanged, as was blood glucose. These results are discussed with respect to the possibility of chronic damage to the heart muscle.

THE EFFECT of ethanol on the metabolic processes of the liver is well known. However, its effects on the heart muscle is not fully understood as the varying results of other authors show. During the last decade the term alcoholic myocardiopathy has been introduced by clinicians.[1–6] Although the "Münchener Bierherz" was described approximately 100 years ago,[7] the relation of ethanol to the damage of heart muscle remains unsolved. Histologic, electron-microscopic and metabolic alterations of the heart muscle due to ethanol have been discussed previously. However, the extent of their effect and their reversibility is largely dependent upon the period during which ethanol effects the myocardium.

In a previous communication[8] we reported acute metabolic alterations in the heart muscle after a single dose of ethanol. In the present experiments the chronic effects of ethanol on some biological and metabolic parameters of the myocardium were studied.

MATERIALS AND METHODS

Animals. The experiments were performed on male Wistar rats, weighing 220 ± 20 g. The animals were fed on standard Larsen diet and allowed water *ad lib.* The experimental group were injected intraperitoneally with 20% (v/v) ethanol in a daily dose of 2·5 g/kg body wt; administered each morning over a period of 10 weeks. The control animals were given an isocaloric dose of 20% glucose solution daily. After 10 weeks the animals were killed and samples of heart muscle were removed for biochemical analyses. Two or three samples were used for each assay.

In our chronic ethanol experimental model the following biological and metabolic parameters were analyzed: weight of animals and heart hypertrophy ratio, which was evaluated as follows:

$$\frac{\text{Weight of heart}}{\text{Weight of animals}} \times 10^3.$$

The dry weight of the myocardium was obtained after 24 hr at 105° and expressed as a percentage of the wet weight. Myocardial glycogen was measured using anthrone reagent,[9] after hydrolysis of the tissue in 30% KOH solution and precipitation of glycogen by ethanol.[10] Blood glucose was estimated by the orto toluidine method using standard tests of Lachema (Czechoslovakia). Respiration of heart homogenate was measured in a Warburg manometric apparatus. The medium comprised of the following phosphate buffer, pH 7·4 (100 μM), glucose (10 μM) and potassium chloride (160 μM) according to Schwartz and Lee.[11] Two ml of 20% myocardial homogenate were added to each vessel. Glycolytic activity of the myocardium was estimated according to the method of Reeves.[12] The medium contained: phosphate buffer pH 7·4 (20 μM), glucose (14 μM), NAD (1·8 μM), ATP (5 μM), histidin (50 μM), sodium chloride (34 μM), potassium chloride (110 μM) and magnesium chloride (6 μM). 0·5 ml of 10% cell-free homogenate of the heart muscle was added to each vessel. The glycolytic activity of the myocardium was calculated on the basis of lactate increase during 30 min incubation at 37° in nitrogen atmosphere in a Dubnoff shaker. Lactate was estimated according to the method of Barker and Summerson.[13] Heart muscle mitochondria were prepared according to Schneider and Hogeboom.[14] Hearts from ten rats were pooled and homogenized as one sample. The protein was estimated according to Lowry *et al.*[15] The respiration of the mitochondria was estimated manometrically in the Warburg apparatus using the medium of Schwartz and Lee:[11] phosphate buffer pH 7·4 (20 μM), EDTA (1 μM), sucrose (56 μM), ATP (2·5 μM), cytochrome *c* (0·017 μM), NAD (0·5 μM), bovine albumin, sodium succinate (13 μM), potassium chloride (50 μM), magnesium chloride (8 μM) and sodium fluoride (12 μM). The side arm of the vessel contained glucose (56 μM) and hexokinase (Sigma, type III) (20 units). Each manometric vessel contained 4–5 mg of mitochondrial protein. Respiratory control of mitochondria was calculated from the ratio between oxygen consumption with phosphate acceptor and without it. The phosphate acceptor system contained glucose and hexokinase.

The difference between the control and experimental group of animals was evaluated according to Student's *t*-test.

RESULTS

At the beginning of the experiment the average body weight of the control (221 $\pm$ 15·5 g) and experimental group of animals (225 $\pm$ 20·2 g) was not significantly different. During 10 weeks the weight of the control group increased by 11·0 per cent whereas that of the experimental group increased by 2·5 per cent only. The hypertrophy ratio of the heart muscle in the experimental animals (2·61 $\pm$ 0·21) did not differ from that of the control animals (2·54 $\pm$ 0·15). The dry weight ratio gave similar results (23·7 $\pm$ 1·20 and 23·6 $\pm$ 1·01).

The glycogen content of the heart muscle in the experimental group of animals decreased (21·3 $\pm$ 5·13 in the control and 17·3 $\pm$ 5·94 in the experimental group). The blood glucose level remained unchanged. The respiration of the myocardial homogenate from experimental animals was significantly lower than that from the controls (1·99 $\pm$ 0·28 and 2·48 $\pm$ 0·52, respectively). The same trend was shown in the glycolytic activity of the cell-free homogenate of the heart muscle (1·82 $\pm$ 0·43 in experimental and 2·15 $\pm$ 0·62 in control group). The mitochondria showed a slight

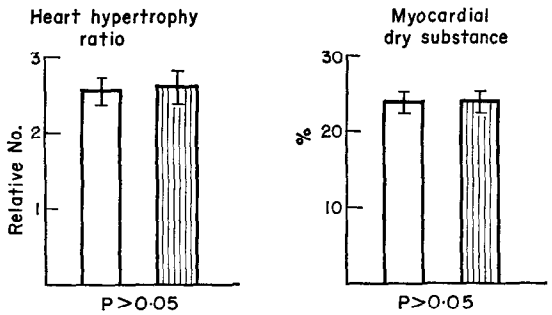

FIG. 1. Changes in biological parameters of the heart muscle. □, Control group; ▥, experimental group; I, ± S.D.

but insignificant decrease (8·7%) of respiration in the experimental animals. However, the respiratory control ratio of the mitochondria isolated from the heart muscle of the experimental group of animals (2·02 ± 0·51) was diminished by 28·2 per cent in comparison with the control animals (2·81 ± 0·99). This diminution is statistically significant.

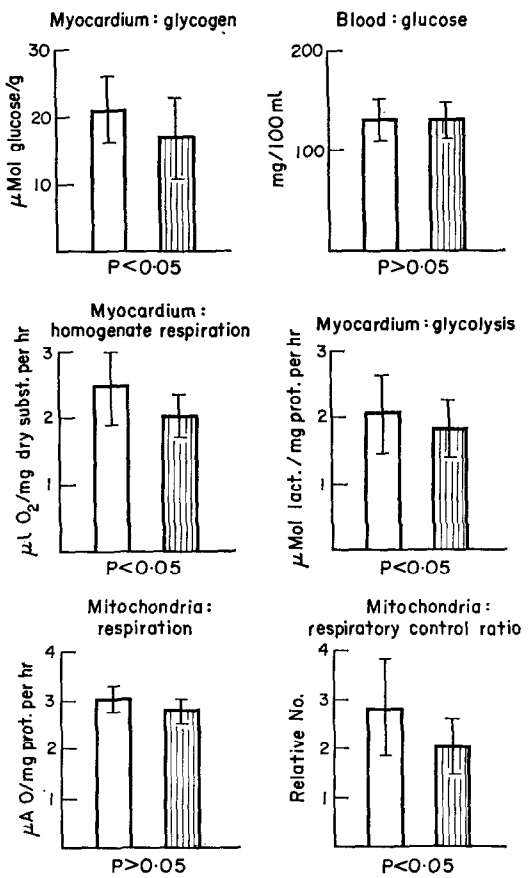

FIG. 2. Metabolic changes in the heart muscle. □, Control group; ▥, experimental group; I, ± S.D.

DISCUSSION

The influence of ethanol on the metabolism of the myocardium has drawn the attention of many cardiologists. However, the results of investigations in this field often vary, due mainly to different experimental conditions (acute or chronic administration of ethanol to the animal).

In our previous communication[8] we reported on the acute metabolic effect of ethanol. In the present experiments we studied the effect of chronic administration of ethanol for 10 weeks on the myocardium. Chronic administration of ethanol caused a retardation in the growth of animals. The heart weight decreased in parallel to the body weight of animals. The dry weight ratio of the myocardium was not altered.

However, chronic administration of ethanol provoked considerable alterations in myocardial glycolysis and oxidative phosphorylation.

Our experimental results coincide with the results of Wendt *et al.*[16] who studied the metabolism of heart muscle in chronic alcoholic men. They observed the liberation of some cytoplasmic enzymes (lactic dehydrogenase and aldolase) and intramitochondrial enzymes (malic dehydrogenase and isocitric dehydrogenase) from myocardial cells into the venous blood.

In spite of the relatively high dose of ethanol used in our chronic experiments we were unable to attain morphological changes in the heart muscle. Histological and electron-microscopical investigation of the myocardium did not reveal any alterations. Probably this is due to the relatively short period of ethanol influence on the myocardial cell in our model: over a period of 5 hr the level of ethanol in the blood, myocardium and liver decreased to zero.[17] It means that over the remaining 19 hr of the day the animals were not intoxicated by ethanol. This situation is contrary to that of the findings of Burch *et al.*[18] who described ultrastructural changes in the myocardium after ethanol.

On the basis of the results of our previous acute[8] and present chronic alcoholic experimental model, we conclude that it is necessary to distinguish the metabolic changes of the heart muscle. The alterations after a single dose of ethanol are transient[17] and are different from those caused by chronic alcoholic intoxication. They do not explain the metabolic and structural changes of the myocardium which develop in the case of chronic alcoholic intoxication.

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