Cellular Oxidation-Reduction State of Some NAD-linked Reactions in Diabetic Rat Liver, and the Effect of Coenzyme Q Administration

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The cytoplasmic reduction-oxidation state of some NAD-linked reactions has been investigated in the liver in vivo under normal and diabetic conditions with or without CoQ7 administration.

1. In the liver, the close relationships among lactate/pyruvate, malate/oxaloacetate and glycerol-1-phosphate/dihydroxyacetone phosphate ratios were found in all the metabolic conditions studied. In diabetic rats, these systems were found to be more reduced.

2. CoQ7 administration to diabetic rats for four days intramuscularly tended to restore these ratios to normal. This effect was followed by a significant drop in malate concentration.

Present findings would indicate that the effect of CoQ7 on the reduced state in diabetic rats seems to be mediated via malate removal.

CoQ, as a factor of the electron transfer system, intervenes between flavoprotein and cytochrome c1 in mitochondria (1, 2). The rat mitochondria usually contains CoQ9 and CoQ10 with the side chain longer than CoQ7 (3). However, some information is now available which may shed light on the role of CoQ7 on glucose oxidation and lipogenesis in diabetic rat in vitro or in vivo (4). Thus, it seems of interest to study the effect of CoQ7 on the cellular oxidation-reduction state in the tissue in vivo.

The NAD-linked reactions have been shown to react very fast to alterations of the metabolic conditions such as short ischemia and administration or deprivation of some hormones (5). Hohorst et al. (6) have concluded that the steady state of this system is very close to mass action equilibrium and therefore, it should be possible to estimate the NAD+/NADH ratio in the cellular compartment.

1 Following abbreviations are used: CoQ, coenzyme Q; NAD+ and NADH = oxidized and reduced nicotinamide-adenine dinucleotide, L/P = lactate/pyruvate, M/O = malate/oxaloacetate, G/D = glycerol-1-phosphate/dihydroxyacetone phosphate.

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of the liver cell.

In this report, we have investigated the relationships between the NAD-linked systems in the liver cells. The effect of CoQ7 on the reduced state in the diabetic rat liver is also studied.

**METHODS**

Male Wistar strain albino rats weighing about 200 g were fed *ad libitum*. Diabetes was produced by a single intravenous injection of 40 mg/kg of alloxan, and the animals were used 8 days after alloxan injection. The blood glucose in diabetic rats was over 300 mg/100 ml on the 6th day after alloxan injection.

The quick freezing method was employed; parts of the liver were cut off under pentobarbital anesthesia. Immediately after cutting off, the pieces were immersed in the liquid nitrogen. Frozen tissue powder was prepared by pounding the pieces in a mortar precooled with liquid nitrogen frequently added. After pulverization and perchloric acid extraction, lactate, pyruvate, glycerol-1-phosphate, dihydroxyacetone phosphate, malate and oxaloacetate were determined in the neutral extract by means of enzymatic method (7).

In the ischemic experiment, liver pieces after different duration of ischemia were obtained from normal rats. Comparison between normal and diabetic animals was made with the results of non-ischemic rat liver.

CoQ7 treatment in diabetic rats was started on the 3rd day after alloxan injection with a concentration of 40 mg of CoQ7/kg in surfactant intramuscularly for 4 days. Four hours after the last injection, liver pieces were taken as described above.

**RESULTS**

![Fig. 1 Oxidation-Reduction State of NAD-Linked Reactions in the Normal and Diabetic Rat Liver](image-url)

Data from 45 determinations on ischemia experiment of normal rat liver and 27 determinations on alloxan diabetic rat liver with or without CoQ7 treatment. Slope of M/O against L/P regression lines, 0.11 (0.08 < β < 0.13) in normal and 0.12 (0.10 < β < 0.15) in diabetic rat liver, slope of G/D against L/P regression lines; 1.65 (1.48 < β < 1.82) in normal and 1.79 (1.44 < β < 2.14) in diabetic rat liver.
In Fig. 1, M/O and G/D ratios after different duration of ischemia are plotted against the corresponding L/P ratio. The slopes of the normal rat liver with ischemia were +0.11 for M/O line, and +1.65 for G/D line. These relationships among L/P, M/O and G/D ratios could also be demonstrated in the alloxan diabetic rat liver of different degree in severity. No difference in regression lines between the ischemic normal and the alloxan diabetic rat liver was found.

As shown in Table 1, concentrations of lactate, malate and glycerol-1-phosphate were significantly greater, whereas those of pyruvate and dihydroxyacetone phosphate were significantly less in the diabetic than in the normal liver. Oxaloacetate showed a slight but not significant decrease. As a result, the ratios of the NAD-linked reactions in the diabetic rat liver were increased by a factor of about two as compared to the normal controls.

CoQ7 treatment in diabetic rats for 4 days tended to restore the L/P, M/O and G/D ratios to normal, but in none of them was the effect statistically significant. Concentration of malate was significantly decreased by CoQ7 treatment.

**Table 1**

| Tissue Concentrations and Reduction State of Lactate/Pyruvate, Malate/Oxaloacetate and Glycerol-1-Phosphate/Dihydroxyacetone Phosphate in Rat Liver. |
|---------------------------------|-----------------|-----------------|-----------------|
| No. of experiments             | Normal          | Diabetes        | Diabetes with CoQ7 |
| Lactate (μmoles/g tissue)      | 1.278 ± 0.118    | 1.652 ± 0.170    | 1.680 ± 0.159   |
| Pyruvate (μmoles/g tissue)     | 0.111 ± 0.008    | 0.070 ± 0.007    | 0.088 ± 0.008   |
| Lactate/pyruvate               | 11.5 ± 0.6       | 25.8 ± 3.1       | 21.2 ± 2.8      |
| Malate (μmoles/g tissue)       | 0.329 ± 0.022    | 0.519 ± 0.056    | 0.375 ± 0.011   |
| Oxaloacetate (10^-5 μmoles/g tissue) | 4.03 ± 0.30    | 3.36 ± 0.34      | 3.21 ± 0.24     |
| Malate/oxaloacetate           | 87.5 ± 7.0       | 184.3 ± 31.3     | 125.9 ± 16.7    |
| Glycerol-1-phosphate (μmoles/g tissue) | 0.167 ± 0.018  | 0.326 ± 0.044    | 0.258 ± 0.054   |
| Dihydroxyacetone-P (μmoles/g tissue) | 0.030 ± 0.018  | 0.026 ± 0.001    | 0.025 ± 0.001   |
| Glycerol-1-phosphate/ dihydroxyacetone-P | 5.4 ± 0.3       | 13.2 ± 1.4       | 9.9 ± 1.6       |

*a All values represent the mean±standard error.

b *p*<0.05 for difference from normal rat liver.

c *p*<0.05 for difference from diabetic rat liver.

**DISCUSSION**

Hohorst et al. (5,6) studied the intracellular NAD-linked reactions in the liver cells during ischemia and have concluded that the steady state equilibria of these systems are very close to a mass action equilibrium. Theoretical values calculated from a mass action equilibrium are $k_{\text{malate}}/k_{\text{lactate}}=0.185$ and $k_{\text{glycerol-1-phosphate}}/k_{\text{lactate}} =1.68$. In the present experiments, the slope of L/P against G/D was practically
equal to the theoretical value, while that against M/O was not. It has to be considered that oxaloacetate was usually measured at the extreme range of sensitivity of the enzymatic method.

Diabetic rats showed a more reduced state in the NAD-linked systems in the liver cells than the normal. The ratios of the NAD-linked reactions in the diabetic rat liver were increased by a factor of about two as compared to the normal controls. Under conditions of chronic alloxan diabetes, the state of reduction was found to be increased by a factor of five in comparison to the non-diabetic control (5).

Much contradictory evidences have been reported on the oxidative phosphorylation of carbohydrate metabolism in diabetes. Hall et al. (8) demonstrated that the defect in the oxidative phosphorylation existed in mitochondria from the diabetic liver. The question whether the reduced state in the NAD-linked reactions found in the diabetic rat liver resulted from the decreased activity in the Krebs cycle or the respiratory system cannot be answered. The content of CoQ in the liver mitochondria decreased significantly in rats with diabetes and increased after administration of CoQ7, CoQ9 or CoQ10 (9). These results were paralleled to the change in succinic dehydrogenase activity in the liver. In the present experiment, concentration of malate was significantly decreased by CoQ7 treatment. Thus, the effect of CoQ7 on the reduced state in the diabetic rat liver seems to be mediated via malate removal either by partially relieving a block in the Krebs cycle or respiratory system, or by malate shuttle of Young et al. (10) to fatty acid synthesis.

Wieland et al. (11) discussed a possible influence of free fatty acid on the reduction grade of the NAD system in the liver cells. His findings are also of interest in connection with our results that CoQ7 accelerated fatty acid synthesis from glucose in the epididymal adipose tissue of rats in vitro (4). Experiments with liver mitochondria are currently planned.

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**References**