size complementary DNA on the template of influenza virus hmwRNA either in the presence of oligo(dT) or if influenza virus 4S RNA is used as primer. The first result is in agreement with data on the absence of poly(A)-blocks in molecules of influenza virus virion RNA [6, 7], and the second result characterizes the template-primer properties of the complex of virion hmwRNA and 4S RNA in vitro. The results suggest that during interaction between influenza virus and cells its genome RNA cannot control synthesis of complementary DNA in the presence of RNA-dependent DNA polymerase of normal cells or of latent oncornaviruses.

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# MECHANISMS OF DISTURBANCE OF MITOCHONDRIAL ADENINE-NUCLEOTIDE TRANSPORT IN THE COURSE OF ACUTE LIVER ISCHEMIA

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KEY WORDS: liver ischemia; mitochondrial adenine-nucleotide transport; intramitochondrial adenine nucleotides; long-chain acyl-CoA; adenine-nucleotide translocase.

Disturbance of oxidative phosphorylation (OP) in acute ischemia of an organ is explained by structural injuries to the mitochondria. It has been shown that long-chain acyl-CoA, physiological inhibitors of mitochondrial adenine-nucleotide (AN) translocase (ANT) [6], accumulate in the ischemized myocardium [15]. Meanwhile, Shrago et al. [14] consider that the mechanism of disturbance of OP precedes structural damage to the mitochondria. However, AN transport in the mitochondria can be regulated not only by the acyl-CoA level; there is evidence that this process depends on the metabolic reserves of intramitochondrial AN ($AN_i$) [10]. It is therefore interesting to make a closer study of relations between disturbances of AN transport and OP in the course of acute liver ischemia.

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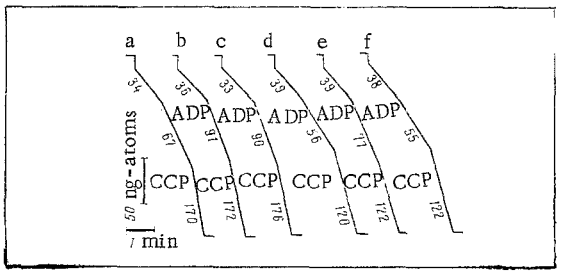


Fig. 1. Effect of carnitine and α-ketoglutarate on ADP-stimulated oxidation of succinate. Medium: KCl 125 mmoles, $KH_2PO_4$ 5 mmoles, $MgCl_2$ 5 mmoles, Tris-HCl 20 mmoles, pH 7.4, rotenone 2 μg/mg protein, succinate 10 mmoles after preincubation for 5 min with 1 mmole carnitine or 10 mmoles α-ketoglutarate, ADP 150 mmoles, chlorocarbonyl-cyanide phenylhydrazone (CCP) $3 \times 10^{-7}$ μmoles, protein 3.4 mg, volume of cuvette 1.5 ml. a, d) mitochondria from liver ischemized for 30 and 60 min, b, e) the same, d) the same mitochondria with carnitine; c, f) the same mitochondria with α-ketoglutarate.

## EXPERIMENTAL METHOD

Liver ischemia was induced in female Wistar rats by ligation of a. hepatica and v. portae for 30, 60, and 90 min without affecting the bile duct. Isolation and polarographic analysis of respiration of the mitochondria in different metabolic states were carried out as described previously [12]. Mitochondrial AN were determined on the Hitachi-556 differential spectrophotometer [8] and protein by the method of Cornall et al. [9].

## EXPERIMENTAL RESULTS

Mitochondria isolated from rat liver with an increased content of acyl-CoA, induced by starvation for 48 h or by injection of oil, have reduced rates of OP [4, 7]. The addition of carnitine increases the rate of OP to control values through the formation of acylcarnitines, which are inactive relative to ANT; this is a specific test for inhibition of ANT acyl-CoA [12].

Preincubation of mitochondria from the ischemized liver with carnitine (Fig. 1) increases the rate of oxidation of succinate in metabolic state 3 ($MS_3$) by 28% after 30 min and by 27% after 60 min of ischemia, but this value is still below the control levels. The fact that restoration of $MS_3$ by carnitine in mitochondria from the ischemized liver is incomplete indicates that inhibition of transport is due not only to the effect of acyl-CoA; another possible factor controlling AN transport could be a reduction in the metabolic reserves of AN, formed by the total of $ATP_i$ and $ADP_i$ [10].

The results of determination of $AN_i$ confirmed this hypothesis. The results in Table 1 show that after 30 min of ischemia the metabolic reserves of $AN_i$ were reduced by half. Preincubation of these mitochondria with α-ketoglutarate, which increases the metabolic reserves on account of phosphorylation of ANT formed in the course of substrate phosphorylation of GTP [13] (Table 1), stimulates the rate of respiration in $MS_3$ (Fig. 1). Consequently, the size of the metabolic reserves in this case limited the rate of OP. However, neither carnitine nor α-ketoglutarate led to complete restoration of the rate of OP. Another important fact is that, despite changes in the ratio between individual $AN_i$ after 30 min of ischemia, their total changed only very little. This demonstrates that the structural integrity of the inner mitochondrial membrane was preserved, for the intact membrane prevents nonspecific diffusion of AN [11]. Specific metabolism through ANT, on the other hand, maintains their total constant [10].

After ischemia for 60 min a further decrease not only in the metabolic reserves, but also in the total $AN_i$ was observed, on account of the outflow of AMP. As a result, α-ketoglutarate no longer affected $MS_3$ (Fig. 1). Comparison of the results of determination of $AN_i$ with those of polarographic investigations of the mitochondria showed close correlation in time between the decrease in the total $AN_i$ and the development of uncoupling of OP. The absence of uncoupling after 30 min of ischemia corresponded to little change in the total $AN_i$. After 60 min of ischemia, partial uncoupling of OP was accompanied by a decrease in total $AN_i$ by 67.5%.

TABLE 1. $AN_i$ in the Course of Acute Liver Ischemia (M ± m)

| Duration of ischemia, min | ATP | AN, nmoles/mg mitochondria protein | | Metabolic reserves | Total AN |
|---|---|---|---|---|---|
| | | ADP | AMP | | |
| Control (6) | 8,0±0,2 | 7,8±0,15 | 5,3±0,12 | 15,8±0,26 | 21,1±0,98 |
| 30 (6) | | | | | |
| a | 0,77±0,05 | 6,6±0,42 | 11,5±0,73 | 7,37±0,67 | 18,87±1,7 |
| b | 11,65±0,7 | 3,96±0,25 | 3,8±0,1 | 15,65±1,33 | 18,3±1,48 |
| c | 3,4±0,1 | 2,6±0,2 | 11,2±0,5 | 6,0±0,42 | 17,2±1,1 |
| 60 (5) | 0,17±0,01 | 2,88±0,08 | 3,8±0,1 | 3,05±0,13 | 6,85±0,27 |
| 90 (5) | 0,06±0,01 | 2,16±0,06 | 3,3±0,09 | 6,0±0,1 | 5,52±0,3 |

Legend. Number of rats used to determine AN given in parenthesis. In experiments with ischemia for 30 min AN were determined, as in all other cases, immediately after isolation of the mitochondria (a), and after preincubation with $\alpha$-ketoglutarate (b) or with succinate (c). Preincubation of mitochondria with these substrates repeated the conditions of the polarographic experiments.

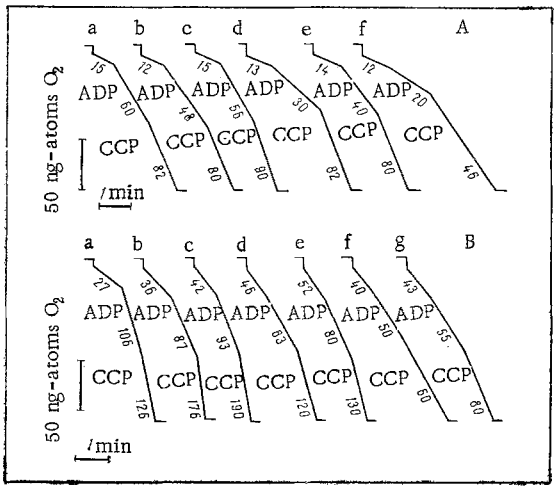


Fig. 2. Effect of dithiothreitol (DTT) on respiration of mitochondria. A: medium, volume of cuvette, ADP, and CCP as in Fig. 1; glutamate 10 mmole, malate 2 mmole, DTT 10 mmoles, protein 4.5 mg. a) intact mitochondria; b, d) mitochondria from liver ischemized for 30 and 60 min, respectively; c, e) the same mitochondria with DTT; f) mitochondria from liver ischemized for 90 min. B: medium, ADP, CCP, DTT, and volume of cuvette as in A; succinate 10 mmoles, rotenone 2 $\mu$g/mg protein, protein 2.7 mg. a) intact mitochondria; b, d, f) mitochondria isolated from liver ischemized for 30, 60, and 90 min, respectively; c, e, g) the same mitochondria with DTT.

The complete uncoupling of OP after 90 min of ischemia corresponded to an even greater loss of AN along nonspecific channels, as shown by the outflow of AMP (Table 1; Fig. 2).

Since the degree of coupling and the ability of the mitochondria to prevent nonspecific diffusion of AN are determined by integrity of the inner membrane of the mitochondria [15, 14], the trend of change of these parameters in the present experiments shows that structural disturbances of the inner membrane become well-marked only after 60 min of ischemia. In this period, the polarographic picture is identical with that arising during incubation of intact mitochondria in vitro with ischemic toxin (IT), which has the ability to interact with the protein components of mitochondrial membranes in the region of complex I [1] and to bind SH groups of membrane proteins [3]. Dithiothreitol, which restores functional activity of SH groups and abolishes certain effects of IT [2, 3], increases the rate of oxidation of glutamate with malate or succinate in $MS_4$ and

$MS_3$, and in the presence of an uncoupler, indicates a possible role of IT in the mitochondrial disturbance. It can be concluded from the effects of dithiothreitol that the effect of IT on ANT is nonspecific and is aimed at accessible SH groups of all sulfur-containing proteins of the mitochondrial membrane.

The structure of the liver mitochondria is thus resistant to unfavorable conditions of ischemia for a comparatively long time. Inhibition of AN transport as a mechanism of OP disturbance precedes structural injury to the mitochondria and is due to a decrease in the metabolic reserves of $AN_i$ and inhibition of ANT by acyl-CoA. It may perhaps intensify inhibition of AN transport by nonspecifically blocking SH groups of membrane proteins, but at comparatively late stages of ischemia, when structural injury to the mitochondria assume the greater importance. It can tentatively be suggested that inhibition of AN transport in the early stages of acute liver ischemia promotes preservation of cytoplasmic ATP, which might be hydrolyzed by mitochondrial ATPase if the mitochondria were de-energized.

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