CHANGES IN METABOLISM OF LIVER MITOCHONDRIA AFTER CARCINOGEN ADMINISTRATION

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A previous investigation demonstrated the effect of the carcinogen dimethylbenzanthracene (DMBA) on chemiluminescence of the mouse liver *in vivo* [1]. Chemiluminescence is an accurate indicator of the structural and energetic states of living systems, and also of certain metabolic changes [2, 3, 5]. Two maxima of emission from the liver were observed, one during the first few days after injection of DMBA, the other 2 weeks after injection, and they were separated by a considerable fall in theintensity of emission which lasted several days.

The facts discovered in earlier investigations, namely the rapid penetration of DMBA into liver cells [12], the early appearance in the liver of a quencher of radiation connected with structural changes in cell proteins [7, 9], and the later formation of an endogenous carcinogen (EC) in the liver, served as the basis for the following hypothesis.*

The initial peak of emission is due to penetration of DMBA into thecells and its accumulation therein. The weakly emitting middle phase corresponds to structural changes in the cell substrate activated by DMBA. The EC thus formed is responsible for the next rise in emission.

Meanwhile, according to observations made by several workers, mitochondrial activity is altered in the early stages of the process and mitochondria may actually be the primary target for carcinogens [11, 12, 14]. It has been suggested that substances formed at different stages of enzymic conversion of carcinogens, exposed to the action of the whole range of enzymes in the liver cells, exert a toxic action [12, 13].

It was therefore of definite interest to study respiration and phosphorylation of the liver mitochondria at approximately the same early periods after injection of DMBA as in the previous investigation [1], and this was the aim of the experiments described below.

EXPERIMENTAL METHOD

In total, 120 mice were used and were divided into three groups: 1) intact control; 2) mice receiving a subcutaneous injection of sunflower oil — the active control; 3) mice receiving an injection of 0.5-0.7 mg DMBA, dissolved in sunflower oil — the experimental group. The liver mitochondria were isolated by the usual method 1-4, 10-18, and 35 days after injection of DMBA. Mitochondria from control mice were studied at the same times. The rate of respiration was measured polarographically.

EXPERIMENTAL RESULTS

The endogenous respiration rate in the liver mitochondria of the mice of group 2 was 25% higher than in animals of group 1, and in the mice of group 3 it was 17% higher than in the active control.

The respiration rate of mitochondria with different substrates had the following values. When succinate was used it was 11% higher in the active than in the intact control, and in the animals of group 3 it was 13% higher than in the active control. When glutamate was used

*Both substances possess the property of fluorescence.

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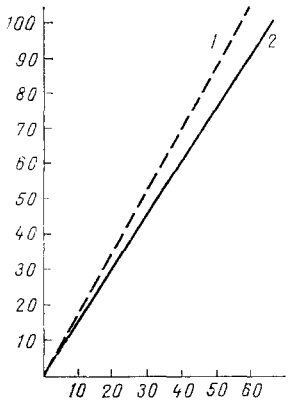

Fig. 1

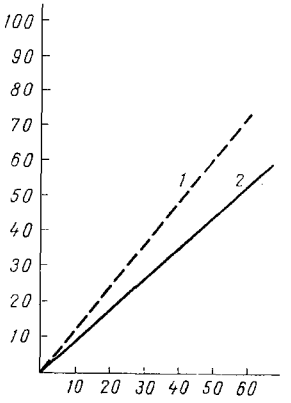

Fig. 2

Fig. 1. Rate of mitochondrial phosphorylation after addition of ADP. 1) Experimental group, 2) control group. Abscissa — time (in sec); ordinate — rate of phosphorylation (in nanoatoms O_2/mg protein/min).

Fig. 2. Mitochondrial respiration uncoupled by dinitrophenol. Legend as in Fig. 1.

the respiration rate of the mitochondria in the active control was 11% higher than in the intact control, and in the mice of group 3 it was 15% higher than in the active control. When a mixture of glutamate + malate was used, the respiration rate in the mice of group 2 was 14% higher than in the animals of group 1, and in the mice of the experimental group it was 10% higher than in the active control. However, these changes were not statistically significant.

The rate of phosphorylation of ADP did not differ in the animals of the two control groups for all substrates used, but in the mice of group 3 (experimental) it was 15% higher than in the intact and active controls ($P < 0.05$; Fig. 1). Simultaneously with an increase in the rate of phosphorylation, the oxygen consumption during phosphorylation in animals of the experimental group was 8.5% less than in the two controls ($P < 0.05$).

The rate of mitochondrial respiration uncoupled by the action of 2,4-dinitrophenol was 34% higher in the animals of the experimental group than in mice of the control groups ($P < 0.01$), and the difference was most marked when succinate was used as the substrate (Fig. 2). The respiratory control (RC) did not differ significantly between the experimental and control groups when succinate and glutamate were used and its corresponding values were 3.2, 3.4, and 3.3, and 3.6, 3.8, and 3.4. When a mixture of glutamate and malate was used as the substrate, RC was 20% higher in the mice of the experimental group than in animals of the control groups ($P < 0.01$).

The results thus do not indicate any inhibition of metabolism in the liver mitochondria of mice after injection of carcinogens into the animals *in vivo* [10, 11, 12]. This conclusion evidently applies mainly to the later stages of exposure to the carcinogen. No sufficiently reliable activation of mitochondrial metabolism was found in the early stages after injection of DMBA, in harmony with the enhanced chemiluminescence of the liver *in situ* [1].

However, it would be premature to judge to what extent activation of the mitochondria and, in particular, the increased rate of phosphorylation observed in the experimental group, is specific for the very first phases of the process of malignant change. A further study of their energy metabolism by the use of the chemiluminescence method will probably bring the solution to this problem closer.

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INCREASED IMMUNOLOGIC REACTIVITY OF MTV-INFECTED C3H MICE AGAINST HEPATOMA 22a CELLS COMPARED WITH MTV-FREE (C3Hf) MICE

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KEY WORDS: MTV-infected (C3H/He) and MTV-free (C3Hf) mice; antitumor immunity; natural killers.

A virus of mammary gland carcinoma (MTV) is known to persist permanently in mice of certain strains and is the cause of the high frequency of mammary gland tumors in these animals [1, 15, 9, 13]. It has been shown that MTV-infected mice can easily be freed from the virus if the newborn mice are fed with milk from MTV-free mice of low-cancer lines [8]. As a rule, mammary gland tumor cells in MTV-infected animals grow more rapidly than in MTV-free animals. This is because of the stronger immune response of the MTV-free mice to antigens (MTV) present on such tumor cells [3, 10, 14, 16]. In the course of experiments with named sublines of the C3H strain of mice the present writers found that cells of some tumors of different tissue origin, including hepatoma 22a, if inoculated into mice of these sublines, behave in the directly opposite manner, i.e., they grow much less readily on MTV-infected than MTV-free animals.

The object of this investigation was to study the phenomenon of slower growth of hepatoma 22a cells in MTV-infected mice.

EXPERIMENTAL METHOD

Male mice aged 2-4 months belonging to MTV-infected (C3H/He) and MTV-free (C3Hf) sublines of the C3H strain were used. The C3Hf mice were obtained in the writers' laboratory by the standard method [8], fully described previously [2]. The following cell lines of the C3H phenotype, obtained by passage *in vitro* in the writers' laboratory, were used: 1) nonmalignat L cells (fibroblasts transplanted *in vitro*); 2) the malignant subline LS of these cells; 3) two cell lines of spontaneous mammary gland tumors — MMT1 and MT5; 4) cells of hepatoma 22a.

The animals were immunized by subcutaneous injection of $1 \cdot 10^6$ hepatoma cells into each limb. Lymph node cells were obtained 9 days, spleen cells and serum 14 days after immunization. The cytotoxic test with lymphocytes was carried out by the method described previously [4], by incubating effector and target cells for 48 h in an atmosphere containing 5% CO_2 at 37°C. The concentration of lymph node cells in the vessels was $2 \cdot 10^6$, and of splenocytes $1 \cdot 10^6$ cells/ml. In some variants of the experiments the target cells were irradiated to prevent them from dividing [4]. The cytotoxic test with freshly obtained rabbit complement (dilution 1:10-1:15) was carried out by the method in [6]. Cytotoxic activity of the lymphocytes and sera was determined by counting the number of living cells left on the slide after the reaction. In each variant of the experiment data at least 12 experimental and six con-

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