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# TRANSPORT OF $Ca^{2+}$ IONS IN THE MITOCHONDRIA OF EHRLICH'S ASCITES TUMOR CELLS


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The pattern of $Ca^{2+}$ accumulation by tumor mitochondria (MC) was investigated under various experimental conditions. In the absence of penetrating anions tumor MC were shown to take up $Ca^{2+}$ in only one fifth the amount taken up by liver MC. In the presence of acetate this difference was greater still. Inorganic phosphate ($P_{in}$) abolished the observed defects of $Ca^{2+}$ transport and increased the $Ca^{2+}$ capacity of the tumor MC considerably. By contrast with liver MC, $P_{in}$ also had a stabilizing effect on membrane permeability of the tumor MC; this may be the cause of the increasee $Ca^{2+}$ capacity of these MC.



KEY WORDS: tumor mitochondria; $Ca^{2+}$ transport; membrane permeability.


A disturbance of $Ca^{2+}$ homeostasis in the cells is an essential stage in the development of pathological processes. It has been shown, in particular, that the mitochondria (MC) of tumor cells can take up and retain unusually large concentrations of $Ca^{2+}$ [4]. However, the causes of the increased Ca-accumulating capacity of the MC of tumor cells have not been studied.

The object of this investigation was to study $Ca^{2+}$ accumulation by tumor MC under different experimental conditions.

## EXPERIMENTAL METHOD

An Ehrlich's ascites tumor was induced in sexually mature male albino mice by intraperitoneal injection of $10^6$ cells of a diploid strain of Ehrlich's ascites carcinoma into each animal. The cells were harvested 10 days after inoculation and were separated from the ascites fluid by centrifugation. MC were isolated by a


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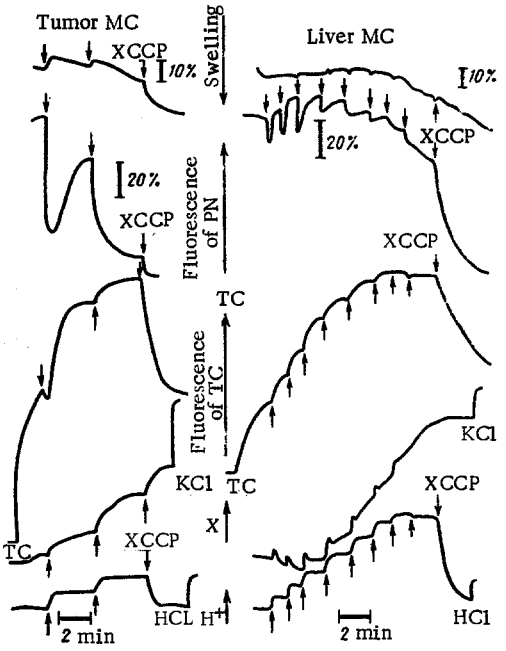


Fig. 1. Changes in light dispersion, fluorescence of PN and TC, and concentration of $K^+$ and $H^+$ in suspension of MC from tumor cells and liver MC during $Ca^{2+}$ accumulation. Incubation medium: 0.3 M sucrose, 3 mM Tris-HCl, 5 $\mu$M TC, 3 mg protein to 1 ml tumor MC, 6 mg protein to 1 ml liver MC. Arrows indicate successive additions of $Ca^{2+}$: 50, 50, 100, 100, 200, 300, and 400 $\mu$M. Here and in Figs. 2 and 3: XCCP 2.5 $\mu$M; KCl 100 $\mu$M; HCl 100 $\mu$M.

modified Nakamura's method [5]. The entirety and integrity of the isolated tumor MC were verified electronmicroscopically and polarographically. The respiratory control on succinate was 3.5-4 after Chance. MC were isolated from rat liver by the standard method [6].

Accumulation of $Ca^{2+}$ by the MC was monitored by measuring the fluorescence of tetracycline (TC), which forms a complex with membrane-bound $Ca^{2+}$ [1]. The state of the respiratory chain was judged from changes in fluorescence of pyridine nucleotides (PN). The intensity of dispersion of light was recorded at an angle of 90° and at $\lambda$ = 680 nm. The intensity of the optical signals was measured at three wavelengths simultaneously (NADH 450 nm, TC 550 nm, scattered light 680 nm) on a microspectrofluorometer with contact optical system [2]. Changes in the concentration of $K^+$ and $H^+$ in the extramitochondrial space during $Ca^{2+}$ accumulation were determined with the aid of ion-selective electrodes.

## EXPERIMENTAL RESULTS

The ability of MC to regulate the $Ca^{2+}$ concentration in the cytoplasm depends on their Ca capacity.

In the absence of a penetrating anion the Ca capacity of the tumor MC was small. After the first addition of $Ca^{2+}$ (about 17 $\mu$M/mg protein) a gradual outflow of $K^+$ was observed (Fig. 1). The $Ca^{2+}$ freshly added to the incubation medium caused rapid oxidation of PN, and as fluorescence of TC showed, only part of it was accumulated by the tumor MC. These slowly swelled and became incapable of accumulating $Ca^{2+}$. Under these conditions liver MC accumulated much more $Ca^{2+}$ (about 20 $\mu$moles/mg protein for the tumor compared with about 100 $\mu$moles/mg protein for the liver). Accumulation of $Ca^{2+}$ by liver MC was accompanied by cyclic changes in fluorescence of PN with characteristic "rereduction" of PN [8] and a considerable increase in fluorescence of TC. The outflow of $K^+$ from the liver MC began only after the fourth addition of $Ca^{2+}$. A further increase in the $Ca^{2+}$ concentration led to the more rapid outflow of $K^+$, to slower oxidation of PN, and to swelling of MC. Just as in the tumor MC, the addition of an uncoupler to the liver MC led to alkalification of the extramitochondrial space and to the outflow of $Ca^{2+}$ from MC. It is interesting to note that the effect of "rereduction" of PN after $Ca^{2+}$ transport was absent in the tumor MC. Accumulation of $Ca^{2+}$ even led to a decrease in reduction of PN.

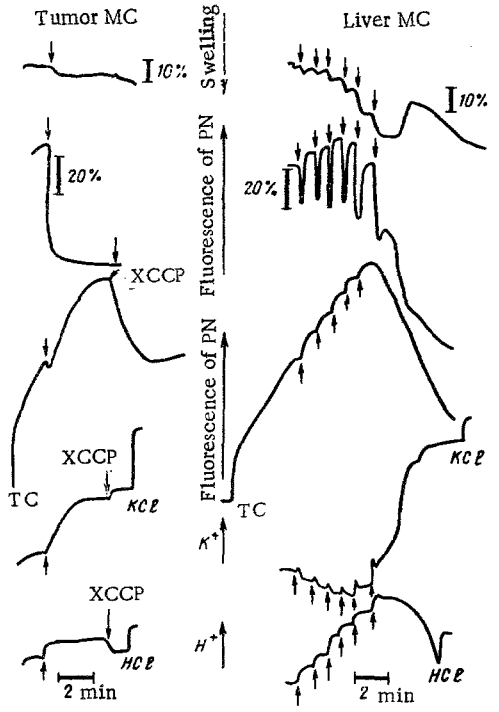


Fig. 2

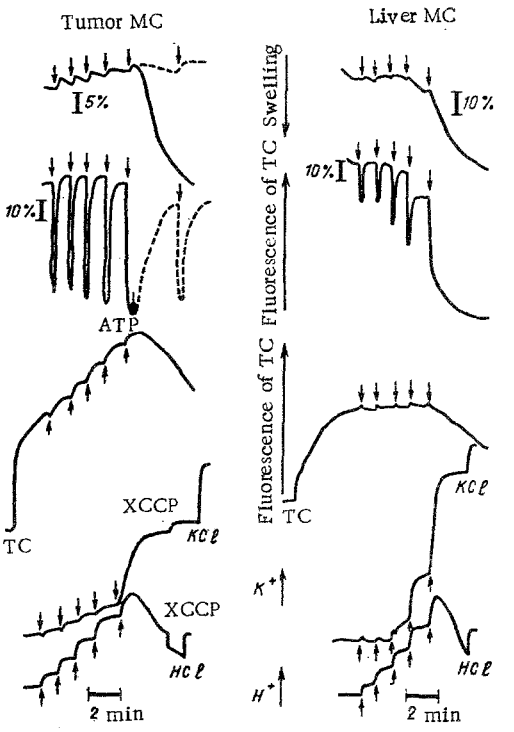


Fig. 3

Fig. 2. Changes in dispersion of light, fluorescence of PN and TC, at concentrations of $K^+$ and $H^+$ in suspension of MC from tumor cells and liver MC during accumulation of $Ca^{2+}$ in the presence of acetate. Incubation medium as in Fig. 1 with addition of 10 mM acetate; successive addition of $Ca^{2+}$: 50, 50, 100, 100, 200, and 200 $\mu$M.

Fig. 3. Changes in dispersion of light, fluorescence of PN and TC, and concentration of $K^+$ and $H^+$ in suspension of MC from tumor cells and liver MC during accumulation of $Ca^{2+}$ in the presence of $P_{in}$. Incubation medium the same as in Fig. 1, with addition of 10 mM $P_{in}$. Additions of $Ca^{2+}$: 50, 50, 100, 100, and 200 $\mu$M; ATP 500 $\mu$M; ATP followed by 100 $\mu$M $Ca^{2+}$.

A low Ca capacity of the tumor MC can be assumed to be connected with absence of a penetrating anion. Acetate, which improves $Ca^{2+}$ transport in liver MC [3], can be used as the penetrating anion. However, as Fig. 2 shows, acetate modified the Ca-transporting system of the tumor MC so that even a small quantity of $Ca^{2+}$ caused rapid and total oxidation of PN and complete expulsion of $K^+$ from MC. The increase in TC fluorescence indicates that $Ca^{2+}$ was bound with the inner membrane of MC. Despite the fact that the MC did not swell and no spontaneous outflow of $Ca^{2+}$ took place, the tumor MC were unable to accumulate any more $Ca^{2+}$. In that case the liberation of $Ca^{2+}$ could be caused by the uncoupler.

Under physiological conditions the accumulation of $Ca^{2+}$ is accompanied by transport of $P_{in}$, in the presence of which $Ca^{2+}$ is transported faster and in greater amounts into MC [3]. However, high concentrations of $P_{in}$ (10-20 mM) impaired the ability of the liver MC to take up and retain $Ca^{2+}$ (Fig. 3). Under these conditions liver MC accumulated less $Ca^{2+}$ than in the absence of the penetrating anion or in the presence of acetate. All the accumulated $Ca^{2+}$ was evidently bound in MC with $P_{in}$ and the fluorescence of TC was unchanged.

By contrast with the liver MC, $P_{in}$ appreciably increased the Ca capacity of the tumor MC. In the presence of 10-20 mM $P_{in}$, tumor MC accumulated and retained large quantities of $Ca^{2+}$ (10 times more than in the absence of the penetrating anion and 3-4 times more than the liver MC under the same conditions: 200 $\mu$moles/mg protein for the tumor and 50 $\mu$moles/mg protein for the liver). The uptake of $Ca^{2+}$ by the tumor MC was accompanied by an appreciable increase in fluorescence of TC. This indicates that the insoluble Ca–$P_{in}$ complex is evidently not formed in these MC. Only complete oxidation of PN after the treatment of considerable quantities of $Ca^{2+}$ leads to the outflow of $K^+$ from the tumor MC, alkalification of the extramitochondrial space, swelling, and liberation of $Ca^{2+}$.

By contrast with liver MC, in every case the PN of the tumor MC, oxidized after accumulation of $Ca^{2+}$, could be reduced by the addition of ATP (Fig. 3, broken line). Under these circumstances the tumor MC again became capable of transporting $Ca^{2+}$.

If ATP was added at once to the incubation medium already containing $P_{in}$, the tumor MC were able to take up unusually high concentrations of $Ca^{2+}$: 300 times greater than in medium without PN and ATP and 5 times greater than liver MC under the same conditions (500 μmoles/mg protein for the tumor, 100 μmoles/mg protein for the liver). By contrast with the liver MC, the tumor MC did not swell and were not uncoupled, although PN could remain in the oxidized state for quite a long time. All $Ca^{2+}$ accumulated in the tumor MC was evidently firmly bound by the membrane and was only partially liberated by the action of the uncoupler, which produced hardly noticeable swelling under these conditions.

The $Ca^{2+}$-accumulating capacity of tumor MC is thus largely dependent on the conditions of $Ca^{2+}$ transport. In the presence of acetate, for instance, the tumor MC quickly lose their ability to accumulate even small amounts of $Ca^{2+}$, whereas $P_{in}$ considerably increases their $Ca^{2+}$ capacity. Under these circumstances $P_{in}$ had an unusual stabilizing effect on the membrane permeability of the tumor MC. Whereas in the liver MC $P_{in}$ induces uncoupling and outflow of $K^+$, in tumor MC this mechanism is evidently absent, and this could be one cause of the increase in the $Ca^{2+}$ capacity of the tumor MC.

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