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Effect of α-tocopherol on passive monovalent cation uptake by rat liver mitochondria

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THE BIOCHEMICAL function of α-tocopherol in cell metabolism is not known. As this compound was found in mitochondria, its participation in the mitochondrial electron transport system either as a cofactor or as a structural agent has been suggested.[1–3] Mollenar *et al.*[4] suggested that α-tocopherol plays a decisive role in membrane metabolism. Oliveira *et al.*[5] found that α-tocopherol is present in the inner mitochondrial membrane and totally absent in the outer membrane. It is generally accepted that the inner mitochondrial membrane takes part a key regulatory role in anion and cation transport. These data led us to examine the effect of α-tocopherol on the transport of monovalent cations in mitochondria. The permeability of the mitochondrial membrane to various solutes has been investigated by a number of different techniques. In this work we used the osmotic swelling method of Chappell and Crofts,[6] who took advantage of the osmotic response of inner membrane to test the permeability of the mitochondrion to cations. It is well known that isolated heart and liver mitochondria swell rapidly when suspended in isotonic NH_4–acetate or Na–acetate in the absence of a source of energy.[7,8] On the other hand isolated mitochondria suspended under this condition in the corresponding acetate salts of K^+ or Li^+ swell very slowly.[8] None of the cations Na, K, Li support swelling as the Cl^- salts either in the presence or in the absence of a source of energy at neutral pH.[9] In this short report the effect of α-tocopherol on swelling of rat liver mitochondria suspended in acetate Na, K, Li, Rb or in corresponding Cl^- salts in the absence of a source of energy has been studied.

It was found that addition of α-tocopherol to rat liver mitochondria suspended in isotonic K^+ acetate (Fig. 1) or Rb^+ acetate (Fig. 2) resulted in a decrease of absorbance. The effect of α-tocopherol was compared on these figures with gramicidin. When α-tocopherol was added to the mitochondria suspended in isotonic KCl or isotonic RbCl the decrease of absorbance was less pronounced (not shown here). As may be seen on the figures the effect of α-tocopherol on mitochondrial swelling in K^+ or Rb^+ acetate media is similar to gramicidin, however less pronounced. Brierley[8] reported that gramicidin was without effect on mitochondrial swelling in isotonic KCl, and caused swelling in K^+ acetate media in the absence of an energy source. Similar effect we observed with α-tocopherol. It seems that in our conditions α-tocopherol increases the permeability of rat liver mitochondrial membrane to K^+ and Rb^+ in the absence of source of energy. The effect of α-tocopherol on permeability of mitochondrial membrane in the absence of energy seems to be specific for K^+ and Rb^+; when mitochondria were suspended in Li^+ acetate, α-tocopherol was without effect, when suspended in Na^+ acetate even a slight inhibition was observed (not shown here).

A number of different chemical modifications of the mitochondrial membrane have been shown to result in an increase of the energy-dependent or non energy-dependent ion uptake. The modifications include addition of an ionophore or ion carrier such as gramicidin or valinomycin,[10] addition of Zn^{2+},[8] organic mercurials and other thiol-group reagents,[11] nonionic detergent[12] or addition of parathyroid hormone.[13] The presence of α-tocopherol may be added to the list as it activates the non energy-dependent K^+ and Rb^+ uptake. It seems that this effect is dependent on the chemical interaction of α-tocopherol with the molecular arrangement of mitochondrial membrane. It may be important for regulation of permeability of mitochondrial membrane. A more complete account of this study will be presented later.

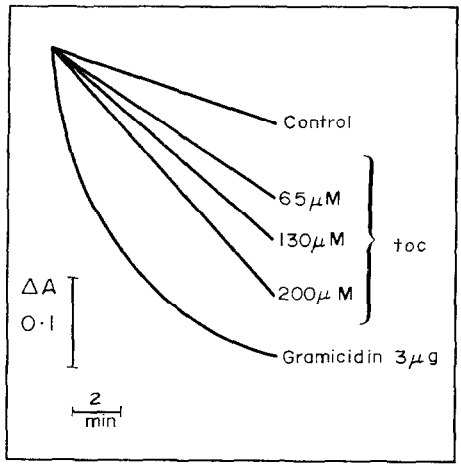

FIG. 1. Swelling of rat liver mitochondria suspended in K^+ acetate medium in the presence of α-tocopheral (toc). Mitochondria (3 mg of protein) were suspended in 3 ml of medium containing: 120 mM K^+-acetate, 4 mM Tris-acetate (pH 7·3), 4 μg rotenone, 2 μg oligomycin. Where indicated α-tocopherol and gramicidin were added. Swelling was monitored at 30° by absorbance at 546 nm. A sample (3 ml) of the medium was placed in a Unicam SP 800 spectrophotometer in a cuvette 1 cm light path. A time zero 20 μl of stock mitochondrial suspension in 250 mM sucrose + 10 mM Tris-HCl (pH 7·3) was added. α-Tocopherol was dissolved in ethanol and 10 μl added to the medium. In control 10 μl of ethanol was added. Rat liver mitochondria were prepared as described previously.[11] Protein was estimated by the biuret method.[14] Rotenone, oligomycin, gramicidin and α-tocopherol were obtained from Sigma Chemical Co.

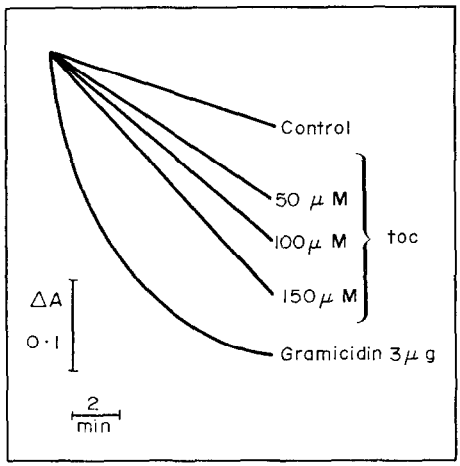

FIG. 2. Swelling of rat liver mitochondrial suspended in Rb^+ acetate in the presence of α-tocopherol. Experimental conditions as described in FIG. 1 except K^+ was replaced by Rb^+.

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Impairment of rat hepatic microsomal demethylating activity and structural protein after X-irradiation of the head

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RECENTLY, we observed a decrease of enzymic pethidine-demethylating system of isolated rat liver microsomes after *in vivo* whole body X-irradiation without concomitant decrease of cytochrome P-450 levels.[1] After whole body X-irradiation (1000 R), two components became undetectable on polyacrylamide gel electrophoreograms of a rat liver microsomal structural protein preparation.[2]

The present paper deals with the influence of X-irradiation (1000 R), when the body of rats was shielded and only the head was irradiated: electrophoretic patterns of rat liver microsomal protein together with the pethidine-demethylating activity, total microsomal protein and cytochrome P-450 levels were studied to elucidate whether the changes described earlier[1,2] were due to the direct effect of ionizing radiation or whether radiation exerted an indirect effect on liver endoplasmic reticulum.

Enzymic demethylating activity of isolated rat liver microsomes was assayed as formaldehyde (acetylacetone method[3]) released from pethidine on 60 min incubation in a NADPH-containing medium.[1] In a supplementary series of experiments the incubation lasted 2 min only. Cytochrome P-450 levels were also determined.[4] Proteins were estimated by the Folin reagent.[5] Polyacrylamide gel electrophoresis was carried out according to Takayama *et al.*[6] (7·5% acrylamide, 35% acetic acid, 5 M urea in the gels, 10% acetic acid as buffer, constant current of 5 mA per tube for 75 min at 4°) with aliquots of 0·25 mg of microsomal structural proteins. Staining of the gels was described previously.[2] Microsomal structural proteins were prepared by the method involving 6·5 per cent saturation with $(NH_4)_2SO_4$ of the material solubilized with deoxycholate.[7] The heads were exposed to a single irradiation with 1000 R,[1] the radiation received by the shielded rest of the body was less than 100 R.[8]

It can be seen in Fig. 1 that the pethidine-demethylating activity of isolated rat liver microsomes was significantly decreased on the second and third day after the irradiation of the head by 1000 R. Similar results were obtained in the supplementary experiments involving 2 min incubation periods. The concentrations of cytochrome P-450 and total microsomal protein (Table 1) were not changed at all intervals under study. Whole-body irradiation with 100 R showed no difference against non-irradiated controls.