Résumé. On a examiné le métabolism de la (C^{14})-DMN par des coupes de foie de truite, de poisson rouge et de trois espèces d'amphibiens, ainsi que l'excretion du cancérogène après injection par voie intrapéritonéale chez le triton et le poisson rouge. La relation entre le métabolisme et l'activité toxique et/ou cancérogène est discutée.

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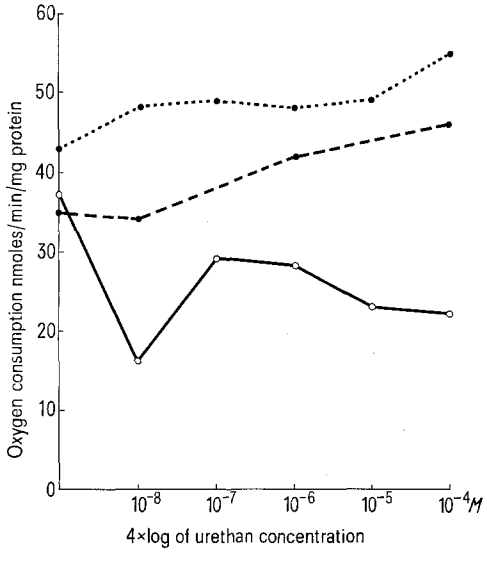
α-Tocopherol (Vitamin E): Reduction of Urethan-Induced Respiratory Depression in Mitochondria

Within the last few years, there have been several reports on the efficacy of vitamin E in retarding cancer growth. KOCUR et al.[1] reported that when vitamin E was injected i.p. to rats, the growth of transplanted tumors was inhibited and even showed complete regression in some cases. Harman found that the dimethylbenzanthracene-induced cancer was inhibited by dietary supplements of vitamin E[2].

It is also known that the chief biochemical characteristic of tumors is the increased dependence of the gycolytic pathway as opposed to the normal respiration pattern of normal cells. Recent work on the action of vitamin E has shown the usual antioxidant function of vitamin E is by no means the only function of the vitamin and recent emphasis has shifted towards the catalytic properties of tocopherols especially in regard to the role of the respiratory chain[3]. To our knowledge, the action of vitamin E on subcellular organelles in the presence of a carcinogen has not been investigated. Therefore we decided to observe the effect of vitamin E when the simple carcinogen, urethan, was added to a suspension of isolated mitochondria. As will be pointed out, the results led us to consider the in vivo action of the vitamin in the presence of urethan.



Oxygen consumption of isolated mitochondria as a function of varying urethan concentration with and without the addition of α-tocopherol. X····X, $1\times10^{-4}M$ α-tocopherol; X----X, $1\times10^{-2}M$ α-tocopherol; ·—·, zero α-tocopherol.

Methods and materials. 1. Isolated mitochondria. Male mice (CD-1, 20–30 g) were killed by cervical dislocation and the liver quickly removed. The mitochondria were isolated by the method of CARVALHO et al.[4] and finally suspended in ice-cold 0.44 *M* sucrose in 0.05 *M Tris*, pH 7.4. From this suspension, 0.5 ml aliquots were incubated with 100 µl of α-tocopherol (0.5 mg/ml of 95% ethanol) for 20 min. Then urethan in varying concentrations was added to the mitochondrial suspensions (containing usually 100 mg/ml protein) and allowed to incubate for 20 min at 20 °C. The oxygen consumption of the urethan-treated mitochondria from 6 mice was measured polargraphically in a chamber containing 4.5 ml respiration medium (sucrose 0.1 *M*, phosphate 0.003 *M*, KCl 0.02 *M*, *Tris* 0.005 pH 7.5) plus 60 µl of 0.5 *M* sodium succinate pH 7.0.

2. Urethan and vitamin E both injected intraperitoneally. Mice were given i.p. injections of vitamin E and urethan. The vitamin was injected every 24 h for 3 days at the dose of 1 international unit per g body wt. and was in the form of α-tocopherol. The urethan used was from a 10% solution and injected at the same time as the vitamin at the dose of 0.5 mg urethan per g body wt. The total urethan injection over 3 days was then 1.5 mg per g body wt. Each respiratory measurement was made on pooled mitochondria isolated from three mice approximately 24 h following the last administration of urethan and vitamin E.

A control group of mice was injected with urethan only, and killed at the end of 3 days. The mitochondria were isolated, and the oxygen consumption measured as above. The oxygen consumption of the 2 groups of mitochondria was compared on a per g mitochondrial protein basis.

3. Lipid Peroxidation (in vitro). In order to determine if lipid peroxidation was involved, we measured the amount of malondialdehyde production by the thiobarbituric acid test in the urethan-treated mitochondria incubated with and without tocopherol.

Results. Isolated mitochondria. The oxygen consumption of urethan-treated mitochondria with and without addition of tocopherol to the suspension is shown in the Figure. The data show that there was a consistent alleviation of the urethan-induced depression in oxygen consumption in the mitochondria due to the presence of the tocopherol.

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The average respiration difference is more than several standard deviations. At zero urethan concentration, we observed no significant differences between the mitochondria with and without tocopherol, so the presence of the tocopherol did not stimulate mitochondrial respiration in our experiments.

The tests for malondialdehyde formation as an index of lipid peroxidation in these groups of mitochondria did not show a significant difference.

Injected mitochondria and tocopherol. The mitochondrial oxygen consumption of the mice which were given tocopherol was significantly greater than the urethan-induced controls as shown in the Table.

Discussion. Our results indicate that tocopherol protects mitochondria against the depressing effects of urethan on respiration both in vitro and in vivo. Although free radical scavenging is a frequently invoked mechanism for protective action of α-tocopherol, our failure to detect significant differences in lipid peroxidation suggests that another property of the tocopherol is operating here. In view of the recent emphasis on the catalytic effect of tocopherol in the respiratory chain, we are inclined to think in these directions.

The relationship of these findings to urethan carcinogenesis is interesting. There is evidence that urethan must first be metabolized before it can act as a carcinogen[5]. However, from studies on mitochondrial biogenesis, it is known that anaerobiosis leads to altered cellular morphology including enlarged nuclei, enlarged mitochondria with fewer cristae and other changes similar, to cancerous cells[6]. Therefore we have some evidence that in urethan carcinogenesis, the first steps may be a respiratory impairment.

Our results also suggest that experimentation on urethan carcinogenesis in the presence of tocopherol be undertaken. Large scale experiments, along the lines of the i.p. injections may yield an answer to the problem of whether the dominant mechanism in urethan carcinogenesis is a result of the respiratory impairment. Lysergic acid diethylamide counteracts the anesthetic effect of urethan but does not affect the carcinogenecity[7]. The relation of vitamin E to anesthetic action remains to be investigated. Therefore these experiments would be of practical and theoretical importance[8].

Résumé. La dépression induite par l'uréthane sur la respiration des mitochondries a été mesurée en présence de la vitamine E (α-tocophérol). Ces agents furent utilisés in vitro et aussi injectés intrapéritonellement. Nos résultats montrent que l'apport de vitamine enraye la diminution de la respiration causée par l'uréthane. Ils attestent le rôle que joue l'uréthane dans la carcinogénèse et la necessité de poursuivre ces expériences sur une plus grande échelle.

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Mitochondrial respiration (n*M* O_2/min/mg mito protein)

Injection	Run				Average S.D.
	1	2	3	4	
Urethan (0.5 mg/g body wt.)	19	21.8	26	19	21.5 ± 2.86
Urethan (0.5 mg/g body wt.) and α-tocopherol (1 IU/g body wt.)	31	31	31	34	31.8 ± 1.30

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Effect of Endotoxin and Dietary Phosphorus on the Proliferation of Liver Reticuloendothelial and Bone Marrow Cells[1]

Guinea-pigs fed diets containing high levels of phosphorus are more resistant to salmonellosis than controls[2]. We have recently observed that the RE clearance rate of ^{32}P-labeled, killed *Salmonella typhimurium* organisms is elevated in guinea-pigs 3 days after *Salmonella typhimurium* endotoxin administration[3]. The rate was significantly greater in guinea-pigs fed 1.0% than in those fed 0.4% of dietary phosphorus and clearance was effected primarily by the liver. KELLY et al.[4] also observed that endotoxin induced proliferation of liver reticuloendothelial (RE) cells 3 days after endotoxin challenge. This study was initiated to determine whether or not increased proliferation of liver RE cells accounts for the greater clearance rate observed in animals fed higher levels of phosphorus.

Materials and methods. 5-week-old female guinea-pigs were fed diets containing either 0.4% or 1.0% phosphorus[2] for 6 weeks then treated i.p. with *Salmonella typhimurium* endotoxin[3]. Three days after endotoxin treatment a pulse dose of ^{3}H-thymidine (specific activity, 20 Ci/mmole) was given i.p. (25 μCi/100 g body weight), and 24 h later the animals were anesthetized and heparinized. The livers were perfused in situ with a phosphate buffered (pH 7.4) glucose + KCl + NaCl solution[5]. The entire liver was homogenized in water, and the DNA extracted[6] and determined spectrophotometrically[7]. Radioactivity was determined[8] on the DNA extract. Femur bone

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