Physiological changes in hairless mice maintained on an antioxidant supplemented diet[1]

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Summary. Several physiological parameters were measured in hairless mice maintained on a diet supplemented with antioxidants. Comparisons to animals on control diet revealed higher water-soluble antioxidant content of skin and increased liver weight. Only small differences in body weight occurred and no distinct histological changes were observed in skin or liver.

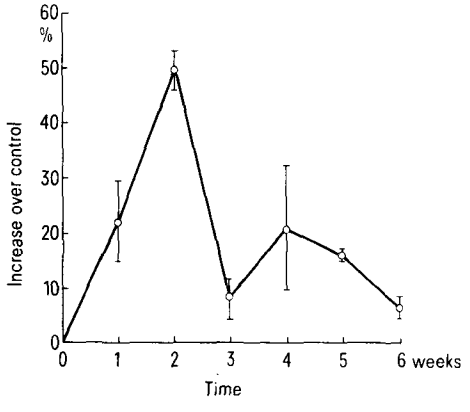
It was recently shown that a diet supplemented with a mixture of ascorbic acid, dl-α-tocopherol, butylated hydroxytoluene, and reduced glutathione was effective in suppressing the formation of ultraviolet light-induced squamous cell carcinomas in skin of hairless mice[2,3]. All of the constituents of this mixture have previously been demonstrated by others to elicit a number of physiological responses in host tissues[4–10].

During the course of our previous carcinogenesis studies, however, no toxic side effects or change in survival rates of the animals receiving antioxidants were noted. It is imperative, therefore, that host response(s) to antioxidant dietary supplements be closely documented should these antioxidants be considered for practical therapeutic use. Here we report on the effects of dietary antioxidants on skin antioxidant content, body weight and liver weight of the hairless mouse.

Effect of dietary antioxidants on liver weight

	Time (weeks)	Regular diet*	Special diet*	% difference in liver weight
Experiment 1	0	1.41	1.39	1
	2	1.40	2.11	51
	4	1.39	2.06	48
	6	1.46	2.42	66
Experiment 2	0	–	–	–
	2	1.32	1.86	41
	4	1.33	1.99	49
	6	1.09	1.77	62

* Values represent the mean weight of livers, in g, from 3 animals.



Levels of water soluble antioxidants in skin of mice receiving an antioxidant supplement diet (mean of 3 experiments expressed as % of control ± S. E. M.). Values were determined as μg equivalents of ascorbic acid per mg protein.

Female, hairless mice (hr/hr), 12 weeks of age, were divided into 2 groups. One group served as control and received a regular balanced laboratory diet (Wayne Labblox, Allied Mills, Chicago, Illinois). The other group received the regular diet supplemented with the same mixture of additives at concentrations used in previous studies[2,3]: 1.2% (w/w) ascorbic acid; 0.2% Dl-α-tocopherol powder (250 I.U. vit. E/g); 0.1% glutathione (reduced form) and 0.5% butylated hydroxytoluene.

3 mice from each group were randomly removed at 2 week intervals after initiation of feeding with the respective diets. The mice were weighed, decapitated, the body flushed thoroughly with deionized, distilled water before their dorsal skin was removed and skin biopsies were taken for future microscopic studies. Livers from each group of mice were pooled and were weighed. Uniform biopsies were removed for microscopic study. The dorsal skin was scraped clean of subcutaneous tissues, weighed and minced before it was homogenized in deionized water. The method of Glavind[11] was used for determination of water soluble antioxidants. 2 ml aliquots from comparable homogenates of the 2 treatments were removed and centrifuged for 45 min at 10,000 × g at 5 °C. One-tenth ml of the supernatant was mixed with 1.6 ml of deionized water. 3 ml of the stable free radical α,α-diphenyl-β-picrylhydrazyl (DPPH) in methanol (O.D. = 0.5) were added to the mixture. After 5 min, the reaction was stopped by addition of 4 ml of xylene, thoroughly mixed on a vortex, and centrifuged at 1000 × g for 5 min. The resulting xylene phase was removed and optical density (O.D.) determined at 517 nm. One drop of pyrogallol solution (1 mg/0.5 ml ethanol) was added, mixed and the O.D. was recorded again after 30 sec. The difference between the optical readings was determined.

A blank was prepared in the same fashion from 1.7 ml deionized water. The differences in the change of O.D. between the experimental sample and the blank were used to determine the amount of antioxidant in microequivalents, using a standard curve constructed with pure ascorbic acid. The method of Lowry et al.[12] was used for determination of protein concentration.

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Water-soluble antioxidant content of skin of animals receiving the supplemental diet, compared to controls, was higher as seen in the figure. Control animal skin contained an average of 1.08 microequivalents of antioxidant per mg of protein. The maximum increase (50%) in skin antioxidant content of those animals receiving the supplemental diet occurred in the first 2 weeks of feeding, after which the level decreased and remained approximately 10% above controls. No distinct morphological differences were observed in the skin of those animals receiving the supplemental diet when skin biopsies were sectioned and examined under the light microscope.

The average body weight of animals on supplemental diet was slightly higher than that of animals on regular diet. However, the liver weight of animals on supplemental diet was significantly higher than controls (table). The liver weight of these animals reached a maximum in 2 weeks, at a level about 50% higher than the animals on regular diet, and maintained this higher level throughout the period of the experiment. Light microscopic examination of liver biopsies did not reveal any distinct histological differences.

It has been suggested that liver enlargement in rats chronically fed BHT was associated with increased activity of drug metabolizing microsomal enzymes[13]. The increases in liver weight observed in the current studies may also reflect an enhanced or altered ability to metabolize UVL-induced carcinogens of biogenic origin and thus explain the tumor-inhibiting properties of these antioxidants. On the other hand, elevated levels of skin antioxidants may suppress initiation and development of UVL-induced skin tumors by protecting against the direct deleterious effects of UVL. In support of the latter a recent study reported that the same antioxidant mixture protected against UVL-mediated erythema in hairless mouse skin[14]. Regardless, dietary antioxidants should provide a useful tool in elucidation of the mechanism(s) of UVL-carcinogenesis.

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Impaired proximal tubular transport functions in anaesthetized splanchnicotomized dogs

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Summary. The maximal tubular transport of inorganic phosphate, D-glucose and para-aminohippuric acid, respectively, was depressed on the denervated side in unilaterally splanchnicotomized, anaesthetized dogs. It is concluded that renal sympathetic activity might in general regulate proximal tubular transport functions.

The importance of sympathetic nervous activity in the regulation of salt and water balance by influencing the renal handling of sodium has been suggested in several studies. An increase of sympathetic activity achieved by low frequency stimulation of the renal nerves[2,3] or by haemorrhage[4], as well as by constriction of the thoracic inferior vena cava[5], were shown to decrease sodium excretion.

On the other hand, interruption of renal sympathetics by different procedures in different species[6–11] or administration of sympathetic blocking agents[2,10,12–14] were followed by an increase in urinary sodium excretion. The sodium retaining effect of sympathetic activition as well as the natriuresis following renal denervation are not related to any changes in glomerular filtration rate (GFR), renal blood flow (RBF), and/or in their intrarenal distribution. Micropuncture studies have yielded evidence that the primary site of action of both adrenergic activation and sympathectomy is the proximal tubule[3,5,8,10,11].

The urinary excretion of inorganic phosphate (Pi) results from filtration and active tubular reabsorption in mammals[15]. A predominant part of the reabsorptive process takes place in the proximal tubule[16], but distal tubular reabsorption has also been demonstrated[17]. The renal transport of D-glucose (G) by an active reabsorptive process can be localized mainly to the proximal tubule[18,19], but recent data raised the possibility of some distal reabsorption[20]. Para-aminohippuric acid (PAH) is excreted both in vivo[21] and in vitro[22] by active secretion at the proximal tubular level. However, following the verification of bidirectional transport in Necturus kidneys[23], such a process has been proved also in other species[24,25].

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