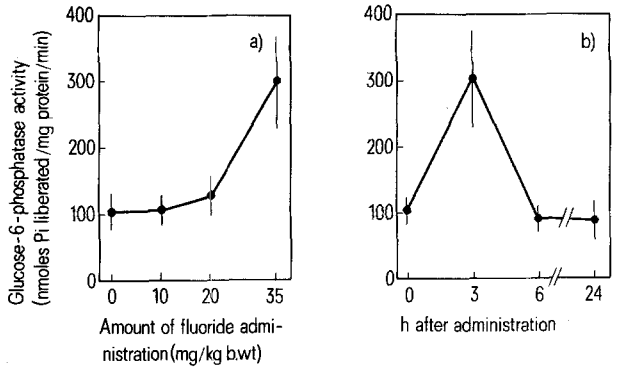
6-phosphatase activity with time after the injection of a single dose of fluoride were examined (figure, b). The enzyme activity reached a maximum 3 h after the administration of fluoride. On the other hand, parathyroid hormone (PTH) is known to stimulate the production of the renal glucose-6-phosphatase[8]. Plasma PTH levels are significantly elevated in classical endemic fluoresis[9] and endemic fluorosis with Genu Valgum[10]. Therefore, the elevation of the renal enzyme activity induced by a fluoride dose was examined with adrenalectomized or parathyroidectomized rats in this study. The enzyme activities were suppressed to 0.36- and 0.59-times those in intact rats by parathyroidectomy and adrenalectomy, respectively. Adrenalectomy almost completely suppressed the elevation of the enzyme activity induced by fluoride, but parathyroidectomy partially suppressed it. The ratio of elevation of the enzyme activity induced by fluoride was not altered by parathyroidectomy (table). In this experiment, it was postulated that the elevation of the renal glucose-6-phosphatase activity in fluoride-intoxicated rats is probably due to stimulation of the adrenal function rather than to stimulation of the parathyroid function by fluoride dosage.



a) Effect of fluoride on glucose-6-phosphatase activities in rat kidney. The rats were killed 3 h after a single i.p. administration of NaF.
b) The changes in glucose-6-phosphatase activities in kidney after a single i.p. administration of fluoride. Dosage of NaF was 35 mg/kg b.wt. Each value represents the mean of 6 rats. SE indicated by vertical lines.

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Effects of lipid soluble antioxidants on cytotoxicity induced by photochemical products of cholesterol[1]

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Summary. Lipid soluble antioxidants, butylated hydroxytoluene and vitamin E, were shown to suppress cytotoxicity induced by cholesterol-derived photoproducts in Chinese hamster embryo cells. These cholesterol-derived photoproducts were rather toxic.

Photochemical conversion of cholesterol to certain carcinogenic substances was suggested as a possible mechanism for the tumorigenic effect of UV light on skin[3]. Exposure of human skin to UV light resulted in the formation of myriads of polar cholesterol photoproducts[4]. A mixture of water soluble and lipid soluble dietary antioxidants have been shown to suppress the formation of UV light-induced squamous cell carcinomas in hairless mice[5]. This same mixture of antioxidants was also effective in inhibiting the production of premalignant lesions and the development of tumors from pre-malignant lesions induced by 3-methylcholanthrene in the skin of hairless mice[6]. Hairless mice maintained on such antioxidant supplemented diet showed a rapid increase of liver weight, however, only small differences in body weight occurred and no distinct histological changes were observed in skin or liver under a light microscope[7].

In Chinese hamster embryo cells, antioxidants such as ascorbic acid, DL-α-tocopherol, butylated hydroxytoluene and reduced glutathione were shown to reverse UV light-induced cytotoxicity[8]. Recently, the toxicity of some cholesterol derived photoproducts on Chinese hamster embryo cells has been studied. It was demonstrated that the toxicity of cholestan-3β,5α,6β-triol and cholesterol derived polar photoproduct was greater than that of dimethylbenz-(α)anthracene - a known chemical carcinogen[9]. It was the purpose of the present study to determine whether lipid soluble antioxidants alone could be effective in reducing the detrimental effect of cholesterol derived lipid soluble toxic compounds on Chinese hamster embryo cells.

Materials and methods. Chinese hamster embryo cells were used in this study. The maintenance of cell culture has been described before[8]. Cells between the 4th and 7th passages were used. Falcon 60 mm plastic dishes were seeded with 1×10^3 cells in 2 ml of conditioned medium (1:1, centrifuged used medium/fresh medium). 2 h after plating, butylated hydroxytoluene (BHT) and DL-α-tocopherol (vitamin E) were added, so that each Petri dish contained 0.02 µM/ml BHT and 0.002 µM/ml vitamin E. 24 h after plating the cells, groups of 8 Petri dishes were treated with the respective sterol compounds which were dissolved in dimethylsulfoxide (DMSO) such that the final concentration of DMSO in the culture medium was less than 0.5%. At this concentration, DMSO has no influence on the plating efficiency of Chinese hamster embryo cells. 8 days after plating, all dishes were fixed with glacial acetic acid:metha-

nol solution (1:3, v/v), air dried, stained with Giemsa solution. Colonies of more than 50 cells were scored. Plating efficiency of control culture was 12±3%. Relative plating efficiency is expressed as number of colonies per Petri dish in treatment group divided by number of colonies from Petri dishes in control group.

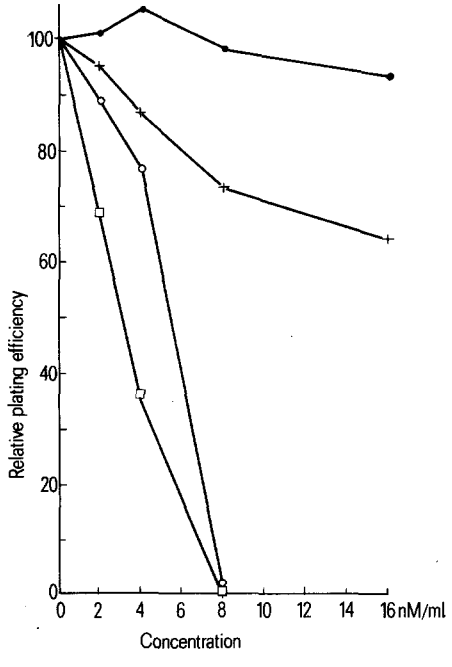
Cholesterol, cholesterol-5α,6α-epoxide, and cholestan-3β,5α,6β-triol were obtained from Steraloids, Inc. (Wilton, New Hampshire), while cholesterol derived polar photoproducts were isolated as described elsewhere[4]. Butylated hydroxytoluene and DL-α-tocopherol were purchased from Sigma Chemical Co. (St. Loius, Missouri).

Results and discussion. Treatment with photochemical products of cholesterol resulted in a rapid decrease in plating efficiency of Chinese hamster embryo cells as shown in the figure. The greatest decrease occurred with cholesterol derived polar photoproducts, followed by cholestan-3β,5α,6β-triol and he least with cholesterol-5α,6α-epoxide. A sterol concentration that would provide an approximately 50% inhibition of plating efficiency of hamster cells was chosen for studies on the effect of lipid soluble antioxidants on cytotoxicity caused by these compounds. The table demonstrates that a mixture of BHT and vitamin E was capable of allaying toxicity induced by cholesterol-derived photoproducts.

Effect of lipid soluble antioxidants on cytotoxicity induced by cholesterol-derived photoproducts on Chinese hamster embryo cell

	Relative plating efficiency	
	With BHT and vitamin E	Without BHT and vitamin E
Cholesterol-5α,6α-epoxide (12 nM/ml medium)	89± 6.6	43±4.5
Cholestan-3β,5α,6β-triol (5 nM/ml medium)	94± 6.0	60±5.7
Cholesterol-derived polar photoproduct (2.5 nM/ml medium)	86±10.3	40±8.4
Cholesterol (25 nM/ml medium)	102± 2.7	100±4.0

Concentrations of BHT and vitamin E used were 0.02 μM and 0.002 μM per ml medium respectively. The relative plating efficiency represents the mean±SEM. 8 replicates were used in each treatment group.



Cytotoxic effects of cholesterol-derived photoproducts on Chinese hamster embryo cells. The curves represent cholesterol (●——●), cholesterol-5α,6α-epoxide (+——+), cholestan-3β,5α,6β-triol (○——○) and cholesterol derived polar photoproduct (□——□).

It has been reported that vitamin E applied to mouse skin significantly reduced tumor formation induced by 7,12-dimethylbenz(α)anthracene and croton oil[10]. Rats fed tocopherol-rich wheat-germ oil had fewer mixed tumors after i.p. injection of 3-methylcholanthrene than rats fed a control diet[11]. Similarly, BHT supplemented diet has been shown to decrease the incidence of gastric cancer in mice[12], to inhibit UVlight-mediated tumor initiation and development[5] as well as to suppress 3-methylcholanthrene induced skin tumors in hairless mice[6]. However, the mechanism of such tumor-inhibitory action exhibited by these antioxidants is still unknown. In rats, it has been demonstrated that BHT inhibited the hepatic mono-oxygenase (mixed function oxidase) system[13]. This enzyme complex is known to play key roles in drug metabolism, carcinogenic activation and free radical pathogenesis. I.p. injection of BHT in mice produced increases in lung weight, total DNA, protein and pulmonary RNA[14]. Thymidine kinase activity and DNA polymerase activity were enhanced in the lungs of BHT-treated mice[15].

In Chinese hamster cells, it has been reported that neither excision repair nor postreplication repair of DNA treated with UVlight or the hepatic carcinogen, 2-acetyl-aminofluorene was affected by BHT[16]. However, both BHT and vitamin E have been shown to suppress carcinogen-induced 7,12-dimethylbenz(α)anthracene chromosomal breakage in human blood leukocytes[17]. Studies into the effect of cholesterol-derived photoproducts on chromosomes are in progress but it is clear that lipid soluble antioxidants are capable of alleviating some of the damaging effects of these sterols on hamster cells. The impact of such protection upon UV light-induced skin carcinogenesis is demonstrable by the recent report on the ability of cholesterol-5α,6α-epoxideone of the cholesterol-derived photoproducts reported in this study to induce transformation of hamster embryo cells[18].

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