observed in the jejunal mucosa was different; an initial high level of DNA-associated ^3H decreased by 80% between 2 and 4 h then showed a slow exponential decline. RNA associated-^3H decreased from high levels at 2 h to a nadir at 16 h before rising to a small peak at 24 h (table). The high uptake of the drug in the jejunal mucosa may reflect direct absorption of the drug into the mucosal cells. However, why such absorption should occur is not clear since peritoneal absorption would be expected to involve the passage of the drug through the hepato-portal system into the liver and from there into the systemic circulation. Thus no early saturation of mucosal cells would be expected. This high drug uptake and atypical DNA-^3H association pattern may be of clinical significance in view of the high sensitivity of the mucosa towards cytotoxic agents.

Although cyclophosphamide differs from chlorambucil in requiring enzymatic activation, both the gross tissue distribution and the alkylation patterns appear to be similar[7], except in the jejunal mucosa. The 2 drugs differ only in the time of maximal nucleic acid alkylation, 24 h for chlorambucil and 48 h for cyclophosphamide. Hill et al.[8,9] have suggested that as a result of intracellular binding the cytotoxic effect of chlorambucil may be spread over a much longer period than would be assumed from the rapid hydrolysis and short chemical half-life of the drug[10]. The active alkylating moiety of cyclophosphamide, phosphoramide mustard, also has a very short half-life in vivo[11]; thus the above suggestions may be consistent with our previous postulate of a reversible drug-macromolecule complex from which there is slow release of an alkylating, and presumably cytotoxic, moiety. Such an intracellular chlorambucil-macromolecule complex could account in part for the observation that the maximum clinical effects of this drug are seen several months after administration[3]. More detailed studies are needed to establish the existence of such a complex and to determine if the drug moiety is chlorambucil or some metabolite of the parent compound.

The association of ^3H from chlorambucil with RNA and DNA of rat tissues at various times after administration of chlorambucil-^3H (100 μCi) at a dose of 12 mg/kg b. wt

Tissue		Time after chlorambucil-^3H administration (h)					
		2	4	16	24	48	72
Jejunum	DNA	71.0	13.5	8.6	7.0	4.4	4.0
	RNA	105.0	29.0	9.5	13.9	7.6	5.3
Spleen	DNA	3.0	2.7	3.8	7.3	6.9	4.3
	RNA	19.7	8.3	15.3	16.7	5.7	4.4
Liver	DNA	115.0	38.0	32.3	37.8	17.2	18.1
	RNA	82.9	32.1	36.7	30.4	10.5	7.6
Kidney	DNA	32.8	12.8	26.3	31.2	27.9	19.2
	RNA	105.0	49.1	152.0	86.3	53.4	40.7

Values are expressed as dpm × 10^{-3}/mg nucleic acid. All values are the mean of at least 4 animals.

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Effect of vitamin E on post irradiation death in mice[1]

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Summary. The 30-day survival after exposure to 800 Rad ^{60}Co gamma radiation has been compared for female mice maintained on vitamin E deficient, vitamin E supplemented or regular lab rations before and/or after irradiation. Pre- or post-irradiation dietary supplementation had no effect on survival; however, injection of α-tocopherol immediately after irradiation significantly reduced radiation lethality.

Vitamin E has antioxidant properties[3] and has been implicated in the activity of catalase[4], glutathione peroxidase[5,6] and possibly superoxide dismutase[6]. There are also reports stressing its role in immune responses[7,8] and altering cAMP levels[9]. Tocopherols are distributed slowly to all tissues[10,11] and associated primarily with membrane and chromatin[12] and as such are strategically located for a significant role in radioprotection or subsequent repair. The radioprotective action of vitamin E has been reported[13-17]; however, some authors have not been able to demonstrate reduced radiation damage with pre-irradiation injection or dietary supplementation[18,19]. Clearly, differences in effect may be associated with time and mode of administration of the vitamin relative to irradiation. Changes in serum tocopherol levels and tissue distribution of tocopherol following irradiation[19,10] have been reported. None of the studies reported to date have considered the effect of administration of tocopherol after irradiation.

Materials and methods. Litters of Swiss albino mice (Canadian Breeding Farms and Laboratories) were raised with mothers maintained on normal lab chow (Purina) or tocopherol test-diet (U.S. Biochemicals) with or without 50 IU

Number of mice surviving exposure to 800 Rad 60-cobalt gamma radiation

Dietary/injection group Pre-irradiation diet	Post-irradiation injection	Post-irradiation diet	Time post irradiation (days) 0	8	16	23	30
A Deficient	None	Deficient	16	15	0	0	0
B Deficient	Carrier	Deficient	16	14	0	0	0
C Deficient	Vitamine E	Deficient	32	29	10	9	8
D Supplemented	None	Deficient	32	14	0	0	0
E Supplemented	Carrier	Supplemented	16	13	0	0	0
F Supplemented	None	Supplemented	16	13	0	0	0
G Normal	None	Normal	28	26	10	2	2
H Normal	Vitamine E	Normal	28	21	14	9	8

Groups C and H are significantly different from all other groups at the 5% level based on 2×2 χ^2 tests on days 23 and 30.

vitamin E (dl-α-tocopherol) added from birth until weaning and thereafter fed the same diet until females had attained a weight of 20–25 g.

Groups of 28–32 animals were exposed to 800 Rads ^{60}Co gamma radiation at a dose rate of 37 Rad/sec in an AECL Gammacell. Dose was determined by ferrous ammonium sulfate chemical dosimeter using absorbance at 230 nm.

Animals raised on vitamin E-deficient diet were treated as follows: 16 mice were irradiated and maintained on deficient diet for 30 days after irradiation (group A); 16 mice were injected i.p. with vitamin E carrier[20] containing 0.1 ml Emulphor EL620*, 0.1 ml ethyl alcohol, 0.1 ml propylene glycol, 0.1 mg disodium edetate, 9 mg NaCl, 0.3 mg sodium acetate, 2.5 mg acetic acid diluted to 1.0 ml with water, pH 4 immediately after irradiation and maintained on deficient diet for 30 days (group B); 32 mice were injected with 0.1 ml of the micellar-type aqueous dispersion of vitamin E (12.5 mg dl-α-tocopherol per ml of carrier) and subsequently maintained on vitamin E-supplemented diet (group C).

Mice raised on vitamin E-supplemented diets were treated as follows: 32 mice were irradiated and immediately placed on vitamin E-deficient rations (group D); 16 mice were injected with carrier immediately after irradiation and subsequently maintained on supplemented diets (group E); 16 mice were non-injected and maintained on supplemented diets (group F).

Mice raised on normal lab chow were divided into 2 groups of 28 animals and treated as follows: non-injected (group G) and injected with 1.25 IU vitamin E i.p. immediately after irradiation (group H).

Results. Survival data are summarized in the table. Injection of carrier into either vitamin E-supplemented or vitamin E-deficient animals had no significant effect on survival. There was significant mortality in all irradiated groups by the 8 day. This was particularly marked in vitamin E-supplemented mice on deficient diets after irradiation ($p<0.01$, χ^2 compared to other groups). It is interesting to note that after the 16th day, group C (deficient animals injected with vitamin E after irradiation and maintained on a vitamin E supplemented diet) had significantly greater survival than group A (deficient animals maintained on deficient diet after irradiation), group B (deficient animals injected with carrier immediately after irradiation and maintained on deficient diet), group D (vitamin E supplemented animals transferred to vitamin E deficient diet after irradiation), group E (vitamin E supplemented animals injected with carrier immediately after irradiation and transferred to deficient diet), and group F (vitamin E supplemented and maintained on supplemented diet after irradiation). Likewise, after the 23rd day, group H (normal diet but injected with vitamin E immediately after irradiation) had significantly ($p<0.01$) greater survival than group G (normal diet but non-injected).

Discussion. Radiation lethality does not seem to be greatly affected by the dietary intake of tocopherol either before or after irradiation. There is no significant difference between animals supplemented in vitamin E before irradiation and subsequently maintained on supplemented diets and those on deficient diet before and after irradiation. What seems to be of greater importance in reducing the lethality is the injection of tocopherol immediately after irradiation. This was noted in both deficient animals and those raised on regular lab rations. This suggests that tissue levels of tocopherol at the time of irradiation, which would be expected to be markedly different in the 3 groups at the time of irradiation (deficient diets, supplemented diet and normal diet), have had little effect on survival and would tend to discount a major radioprotective effect of tocopherol as an antioxidant. The reduced lethality associated with vitamin E injection after irradiation might be associated with enhanced immune response or enhanced recovery of bone marrow; however, further experimentation is required to evaluate these hypotheses.

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