ko[8], washed in buffer and dehydrated in ethanol. The dehydrated tissues were critical point dried using CO_2 as a transitional fluid. The dried corneas were mounted on aluminium stubs, coated with gold in a Hummer sputtering system and viewed in an ETEC autoscan scanning electron microscope.

The figure illustrates the results that were obtained. It can be seen, as early as 2 days after injection, that the thyroxine had a dramatic effect on the number of microvilli on the anterior corneal surface when compared to the normals or controls (normal and control groups were pooled because no differences could be detected). The precocious production of microvilli by the administration of exogenous thyroxine carries through the 4- and 6-day post-injection results. At the same time there is a slight decrease in the number of microvilli found on the anterior corneal surface of the thiouracil treated animals when compared to the control animals, indicating an inhibitory effect by this antithyroid agent.

The data from the thyroxine treated animals is in accordance with previous works[3-5] and the enhanced proliferation of microvilli is just as one would expect, if the density of microvilli is a true developmental parameter of the cornea epithelial cells. However, the data from the thiouracil treated animals did not give the clear results found when other parameters of corneal development were measured[3-5]. This could be the result of too low a dosage or the low solubility of the thiouracil.

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Tissue distribution and nucleic acid binding of chlorambucil-^{3}H in tumor-bearing rats

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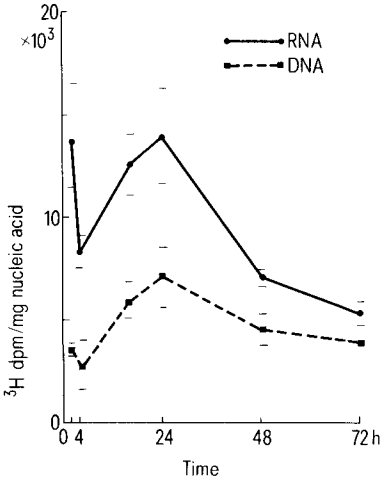
Summary. In tumour and normal rat tissues prolonged alkylation of DNA and RNA by chlorambucil-^{3}H occurs over periods of 24 h. It is suggested that this may indicate the slow release of an alkylating moiety from an intracellular drug-macromolecule complex.

The mechanism of action of chlorambucil is incompletely understood and its cytotoxic effects have been ascribed to both nucleic acid crosslinking[2-4] and covalent binding to nuclear protein[5]. The aim of the studies reported here was to compare the tissue distribution and nucleic acid alkylation patterns produced by chlorambucil-^{3}H with those previously reported for cyclophosphamide-^{32}P in the same animal system[6,7].

Materials and methods. The animals used were 5-6-month-old inbred female August rats bearing s.c. implants of the nonmetastasizing A-15 tumour, derived originally from a spontaneous anaplastic renal carcinoma. Animals were treated 10 days after transplantation when the tumour weighed approximately 1 g. Chlorambucil-^{3}H (4-4-di-(2-chloroethyl)-amino-3,5-^{3}H-phenyl butyric acid) (586 mCi/mmole) was supplied by Dr M. Jarman. The compound was dissolved in ethanol containing 1% HCl and diluted with propylene glycol and 0.5 cm^3 solution (100 µCi ^{3}H) was administered i.p. to ether anaesthetised rats at a total dose of 12 mg chlorambucil/kg b.wt. Tissue samples were prepared and analysed as described previously[7].

Results and discussion. In the tumour, liver, spleen, kidney and jejunal mucosa the concentration of ^{3}H from chlorambucil was greatest at 2 h post injection when the relative concentrations in these tissues were 1; 4.2, 0.9; 4.6 and 15.0 respectively. All these tissues showed a similar multicomponent clearance pattern over 72 h, with initial half-times of less than 2 h and final compartments, containing 25-50% of the 2-h ^{3}H-concentration, with half times about 60-70 h. There was no evidence of selective tumour uptake or retention of the drug. The time course of the association of ^{3}H from chlorambucil with DNA and RNA in the tumours is shown in the figure. This association, which survives the hot perchloric acid extraction in the Schmidt Thannhauser procedure, is considered to represent chemical binding of an alkylating ^{3}H-moiety derived from chlorambucil to the nucleic acid bases[8,9], since direct incorporation of ^{3}H released from drug metabolites into DNA is unlikely. A similar pattern of association occurred in liver, spleen and kidney, and in all the tissues studied the RNA-associated ^{3}H was greater than that of DNA. The pattern



The time course of nucleic acid alkylation in the A.15 rat tumour following administration of chlorambucil-^{3}H (100 µCi) by i.p. injection at a dose of 12 mg/kg b.wt. Each point represents the mean of at least 4 animals ±1 SD. Abscissa represents time after drug administration and the ordinate drug-nucleic acid association in dpm/mg DNA or RNA.

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