INHIBITION OF THE SYNTHESIS OF NUCLEAR RNA BY URETHAN IN REGENERATING LIVER[a]

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Summary Treatment of partially hepatectomized male rats with urethan 6 hr after operation resulted in 50-55% inhibition of the incorporation of orotic acid-5-^{3}H into nuclear ribosomal RNA and heterogeneous RNA 18 hr later. Neither partially hepatectomized female rats similarly treated with urethan nor operated male animals treated with an equitoxic dose of butyl carbamate presented evidence of an impairment of nuclear RNA synthesis.

Urethan (ethyl carbamate) can act as a multipotential carcinogen eliciting a variety of tumors in rodents (1). Attempts to promote the hepatocarcinogenic action of urethan have utilized animal model systems wherein the liver of the animals was in a highly proliferative state as well as possessing a lesser functional capacity for metabolism of the carcinogen, as exemplified by the enhanced formation of urethan-induced hepatomas in neonatal (2-5) and partially hepatectomized (6-8) animals. These studies have engendered recent examinations of the metabolic effects of urethan on the functional (9) and proliferative (9-11) capacities of regenerating liver. The present communication emphasizes the structural dependence of urethan and the sex dependence of the animal model for the

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ability of this carcinogen to act as an inhibitor of nuclear ribosomal RNA (rRNA) and heterogeneous RNA (HnRNA) in regenerating liver.

METHODS

Orotic acid-5-^{3}H (12 Ci/mmole) was purchased from New England Nuclear Corp. Urethan was obtained from Sigma Chemical Co. and butyl carbamate was acquired from Eastman Organic Chemicals. All other chemicals were of reagent grade and deionized water was used.

Sprague-Dawley rats weighing 125-175 g were purchased from Cherokee Laboratories, Atlanta, Ga. The animals were housed two per cage over corncob bedding with alternating periods of 12 hr darkness and 12 hr light.

Partial hepatectomies were performed under ether anesthesia between 2:00 and 3:00 p.m., according to the method of Higgins and Anderson (12). All animals were fasted for a total period of 18 hr and allowed access to water ad libitum.

Either urethan (1 g/kg) dissolved in 0.9% NaCl solution (200 mg/ml) or an equitoxic dose (13) of butyl carbamate (200 mg/kg) dissolved in propylene glycol (40 mg/ml) was injected 6 hr after partial hepatectomy; control animals received an equivalent volume of diluent.

For labeling nuclear RNA, orotic acid-5-^{3}H was diluted with 0.9% NaCl solution (120 μCi/ml) and injected via the portal vein at a dose of 600 μCi/kg.

The concentration and specific activity of UTP was determined according to the method of Hurlbert et al. (14).

Nuclei were prepared as described by Higashinakagawa et al. (15). Nuclear rRNA and HnRNA were extracted from isolated hepatic nuclei using the differential pH extraction procedure (method I) of Edmonds and Caramela (16). Electrophoresis of RNA fractions on 2.4% polyacrylamide gels was carried out according to the method of Loening (17) using an electrophoresis buffer (pH 7.4) containing: 0.5% sodium dodecyl sulfate, 40 mM Tris, 20 mM sodium acetate and 2 mM EDTA.

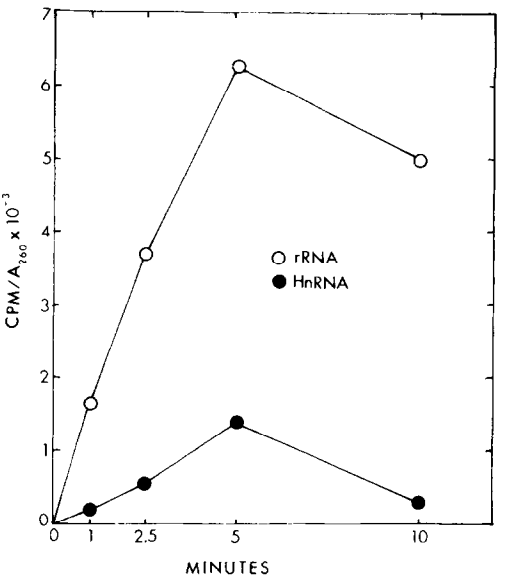

Fig. 1. Pulse labeling of nuclear rRNA and HnRNA. Animals were injected with 600 μCi/kg of orotic acid-5-^{3}H via the portal vein for the periods of time indicated. See Methods for experimental details. Each point represents the mean of individual determinations from 3 animals.

RESULTS

Pulse labeling of 18 hr regenerating liver via the portal vein with orotic acid-5-^{3}H revealed that optimum incorporation into nuclear rRNA and HnRNA occurred 5 min after injection (Fig. 1). Furthermore, the rate of decay in labeling was greater for nuclear HnRNA in comparison to rRNA.

Treatment of partially hepatectomized male rats with urethan 6 hr post-operation resulted in 50-55% inhibition of the incorporation of orotic acid-5-^{3}H into both nuclear rRNA and HnRNA with no apparent alteration in the percent binding of either fraction to Millipore filter membranes (Table 1). Of particular significance was the lack of inhibition as a result of either injection of an equitoxic dose of the non-carcinogenic analog, butyl carbamate, administered to male animals or an equivalent dose of urethan given to female rats. Analysis of the concentration and specific activity of UTP in the liver of control and urethan treated partially hepatectomized rats indicated that alterations in pool size were not evident as a result of this treatment regimen (data not shown).

Table 1

Effect of Urethan on Ribosomal and Heterogeneous Nuclear RNA in Partially Hepatectomized Rats

Animals were injected i.p. with (a) 1 g/kg urethan or an equivalent volume of 0.9% NaCl or (b) 200 mg/kg butyl carbamate or an equivalent volume of propylene glycol, 6 hr after partial hepatectomy and sacrificed 18 hr after operation. RNA was pulse labeled via the portal vein for 5 min with 600 μCi/kg of orotic acid-5-^{3}H. Ribosomal RNA and heterogeneous nuclear RNA were differentially extracted as described (16). RNA was adsorbed to Millipore filters by the method of Lee et al. (21). The values in parentheses represent percent of control for each set of experiments.

			rRNA		HnRNA	
Treatment	No. of Animals	Sex	cpm/A_{260} x 10^{-2}	% Bound to Millipore Filter	cpm/A_{260} x 10^{-2}	% Bound to Millipore Filter
0.9% NaCl	11	Male	496 ± 34 (100)	1.8 ± 0.3 (100)	137 ± 13 (100)	20.6 ± 2.7 (100)
Urethan	8	Male	250 ± 31 (51)[a]	1.5 ± 0.6 (83)	62 ± 13 (45)[a]	20.9 ± 4.0 (102)
Propylene Glycol	3	Male	651 ± 60 (100)	b	177 ± 18 (100)	b
Butyl Carbamate	3	Male	666 ± 70 (102)	b	157 ± 10 (89)	b
0.9% NaCl	5	Female	305 ± 27 (100)	1.8 ± 0.3 (100)	66 ± 5 (100)	22.5 ± 3.0 (100)
Urethan	8	Female	255 ± 31 (84)	1.5 ± 0.1 (83)	62 ± 4 (94)	24.1 ± 1.8 (107)

[a] Percent of control; $P < 0.001$.

[b] Not determined.

Electrophoretic profiles of nuclear rRNA revealed that urethan inhibition of orotic acid incorporation resulted in an impairment in the fabrication and processing of rRNA precursors to 28S and 18S RNA, as judged by the accumulation of radioactivity in 32S-35S RNA (Fig. 2A).

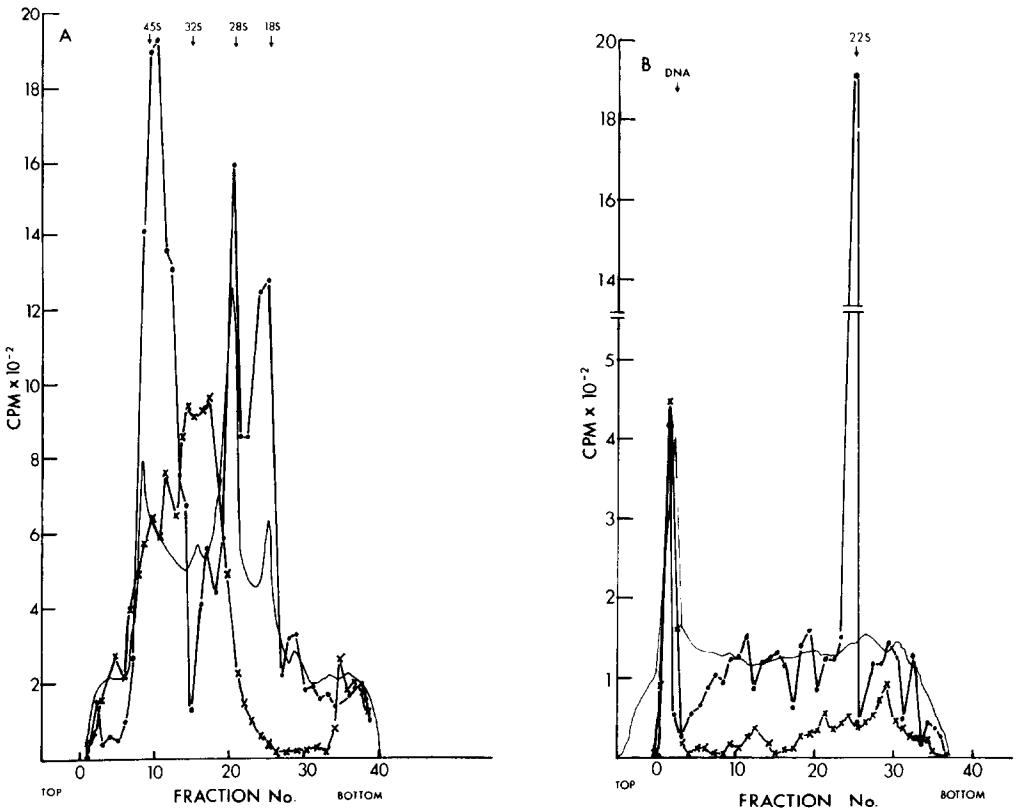

Fig. 2. The effect of urethan on nuclear rRNA and HnRNA of 18 hr regenerating liver. The experimental conditions were identical to those described in Table 1. See Methods for experimental details. A_{260} (———); 0.9% NaCl treated controls (•———•); urethan treated (x———x). A. Gel electrophoresis of rRNA. B. Gel electrophoresis of HnRNA.

Inhibition of nuclear HnRNA by urethan treatment indicated that suppression of the labeling of this fraction was associated predominantly with RNA of approximately 22S (Fig. 2B).

DISCUSSION

Studies designed to elucidate the interaction of urethan with macromolecules have shown that it is incorporated into RNA cytosine (18) as well as other subcellular constituents (19) of normal liver. However, the relationship of these effects to the replicative process in rapidly dividing hepatic tissue has not been delineated. A recent report suggesting that part of the inhibitory activity of urethan may pertain to interference with nuclear

function, indicated that ultrastructural abnormalities of the nucleolus of the liver cell could result from administering urethan to newborn mice (20). This is consistent with the present study showing that treatment of partially hepatectomized male rats with urethan can result in pronounced inhibition of the synthesis of nuclear rRNA and HnRNA. Interestingly, the degree of post-transcriptional polyadenylation of HnRNA as determined by Millipore filtration (21) was not impaired by urethan treatment, suggesting that inhibition of this species of RNA occurs at the level of the DNA template or RNA polymerase. Of equal significance was the evidence that at least two prerequisites were required for these inhibitory effects to occur: (1) the male sex of the experimental animals and (2) the ethyl ester moiety of urethan.

The sex specificity involved in the initiation of hepatomas by urethan has been noted by several investigators (7,8,22). In these studies, there appeared to be a lesser susceptibility of castrated male mice (22) or partially hepatectomized female animals (7,8), to urethan-mediated hepatocarcinogenesis. As shown in the present study, the insensitivity of partially hepatectomized female rats to urethan treatment suggests that the inhibitory potential of urethan on RNA synthesis is also dependent on androgen-mediated processes in rapidly proliferating liver.

The carcinogenicity of this carbamate ester is believed to depend on the structural integrity of the ethyl ester substituent (23-25). This has been substantiated biochemically by the absence of the effect of methyl carbamate on the ability of regenerating liver to synthesize rRNA (10). However, since the latter analog does not possess anesthetic activity, as does urethan, the present data showing the negligible activity of an equitoxic and equianesthetic dose of the non-carcinogenic derivative, butyl carbamate, further confers specificity to the anti-metabolic activity of urethan.

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