further concluded that the tubules of the gastric glands in which the rate of flow of water is greatest are not an important site of absorption.

The gastrin pentapeptide was a generous gift of Imperial Chemical Industries.

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Inhibition of Rat Liver Microsomal N-Demethylase by α-Naphthylisothiocyanate: Studies with Puromycin Aminonucleoside.* (32584)

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Plaa *et al*(1) have shown that α-naphthylisothiocyanate (ANIT) inhibits the mouse liver enzyme(s) that catalyzes the side chain oxidation of hexobarbital and the ring hydroxylation of aniline, but does not inhibit the enzyme(s) responsible for the sulfoxidation of chlorpromazine. Inasmuch as ANIT was shown to be inhibitory over a longer time period than SKF 525A, it was of interest to study its effect on rat liver microsomal N-demethylase, *in vitro* and *in vivo*. The demethylation of puromycin aminonucleoside (PA) was used as the criterion of activity.

Materials and methods. α-Naphthylisothiocyanate, obtained from Eastman Organic Chemicals, New York, was recrystallized from hexane (decolorized with charcoal), m.p. 55.5-56.5° corr. Puromycin aminonucleoside was generously supplied by Lederle Laboratories Division, American Cyanamid Company. Other chemicals were purchased from commercial sources. Male albino rats were purchased from Simonsen Laboratories, White Bear Lake, Minn.

ANIT, in corn oil, was administered orally to rats at a dose of 80 mg/kg 2 hours before sacrifice or further treatment. The liver microsomes were isolated and analyzed for N-demethylase activity by measuring the formaldehyde formed according to Mazel *et al*(2). To study the effect of ANIT pretreatment on the metabolism of PA *in vivo*, PA (100 mg/kg, i.p.) was administered to rats 2 hours after ANIT, and urine was collected over the subsequent 72 hours. The urine was fractionated by ion-exchange chromatography and the aminonucleoside fraction separated by thin-layer chromatography into PA and its monodemethylated analog, MMPA,† as previously described(3). The ratio of PA/MMPA in the urine was used as the measure of microsomal N-demethylase activity *in vivo*.

Results and discussion. Pretreatment of rats with ANIT significantly inhibited the N-demethylation of PA (Table I). This was demonstrated by assay of the isolated micro-

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† 6-Methylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine.

TABLE I. Effect of ANIT Pretreatment of Rats on Liver Microsomal N-Demethylase Activity *in vitro* and on the N-Demethylation of PA *in vivo*.

	μmoles HCHO liberated/g liver/hr			Ratio PA/MMPA in urine 72 hr post PA	
Rat wt (g)	Control	ANIT pretreated	P	Control	ANIT pretreated
60– 70	4.89 ± 1.03 (6)*	3.52 ± 0.53 (6)	<0.05	2.45 ± 0.01 (4)	10.1 ± 1.8 (6)
110–120	3.41 ± 0.11 (3)	2.68 ± 0.30 (4)	<0.02		

* Mean ± standard deviation. No. of animals in parentheses.

somal enzymes from 60-70 and 110-120 g rats, as well as by examination of the urinary excretion products which showed a 4-fold increase in ratio of PA/MMPA in the ANIT treated rats relative to controls. In addition, pretreatment (1 hour) of rats with SKF 525A (20 mg/kg, 6 rats), which has been shown previously to inhibit rat liver N-demethylase (2), increased the ratio of PA/MMPA in the urine from 2.45 to 32.3 ±14.4. This greater inhibition by SKF 525A over ANIT under these *in vivo* conditions may be a reflection of the relative concentrations of these inhibitors in the liver after administration by the intraperitoneal (SKF 525A) and oral routes (ANIT), since *in vitro*, their inhibitory activities (hexobarbital metabolism) are comparable(1). Moreover, since PA is rapidly excreted by the rat—about 50% of that excreted in 72 hrs would be excreted within the 1st hour(3)—the longer action of ANIT would not be observed. Thus, the data on inhibition of the mouse liver microsomal enzymes(1) can be extended to include rat liver N-demethylase. It is likely that ANIT also inhibits the N-demethylase in mouse liver microsomes. The activity of the N-demethylase per unit weight of liver appears to decrease with the age of the rats in agreement with the results of Kato *et al*(5).

Pretreatment of rats with ANIT or SKF 525A did not reduce the nephrotoxicity of subsequently administered PA(4) indicating that the toxicity of PA is not *primarily* mediated by metabolic conversion to MMPA as suggested(6). However, since MMPA has been shown to be nephrotoxic(7), the nephrotoxicity exhibited by administration of PA to the rat must be due to the intrinsic nephrotoxicity of both PA and its metabolite, MMPA.

Summary. Pretreatment of rats with α-naphthylisothiocyanate (ANIT) significantly inhibited the N-demethylation of puromycin aminonucleoside (PA) as measured *in vitro* by assay of the isolated liver microsomes, and *in vivo* by determination of the ratio of PA to its N-demethylated metabolite excreted in the urine after PA administration. ANIT inhibition of the demethylation of PA did not reduce the latter's nephrotoxicity indicating that the monodemethylated analog of PA is not *the* nephrotoxic agent in the rat.

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