Effect of Acute Alpha-Naphthylisothiocyanate Administration on Hepatic Microsomal Drug Metabolism in the Mouse.* (30372)

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Acute administration of alpha-naphthylisothiocyanate (ANIT) has been found to produce hyperbilirubinemia and cholestasis in rats(1,2) and mice(3). Hepatocyte changes, as visualized by light microscopy, are minimal 24 hours after acute administration of ANIT. The tendency has been to attribute the hyperbilirubinemia to an inflammatory response of the bile ductules(1,2). Recent studies(3,4) have shown that the temporal aspects of ANIT-induced hyperbilirubinemia, and changes in sulfobromophthalein disappearance, are not consistent with those observed in experimental extrahepatic cholestasis (bile duct ligation). The results suggested that ANIT exerted an effect on the hepatocyte. Spycher and Rüttner(5) have shown that in rats ANIT causes alterations in the plasma membrane of the hepatocyte; these changes are seen in electron micrographs 24 hours after administration of ANIT.

The purpose of the present study was to see whether ANIT does cause changes in mouse hepatocyte function after acute administration. The ability of the hepatic microsomes to metabolize drugs was used as a measure of hepatocyte function. Preliminary comparative studies were carried out using 2 congeners of ANIT: phenylisothiocyanate (PIT) and beta-naphthylisothiocyanate (BNIT).

Methods. Male, Swiss mice (22-25 g) were used throughout. The aryl isothiocyanates were dissolved in corn oil and administered by gavage (0.01 ml/g) in the following dosages: ANIT, 20 and 80 mg/kg; PIT, 110 mg/kg; and BNIT, 80 mg/kg.

At either 2 or 4 hours later, the mice were killed by cervical dislocation. The livers were immediately excised and placed in vessels immersed in ice. Each liver was homogenized with 2 volumes of cold KCl (1.15%) in a Potter homogenizer having a plastic pestle. Homogenates were centrifuged at 9000 $\times$ *g* in a refrigerated high-speed angle centrifuge for 20 minutes to sediment nuclei, mitochondria and cell debris. The resultant supernatant fraction, composed of microsomes plus "soluble" fraction, was used for determination of enzyme activity.

The drug metabolic pathways studied were side chain oxidation of hexobarbital, ring hydroxylation of aniline and oxidation of the ring-sulfur of chlorpromazine. Hexobarbital metabolism was assessed by following substrate disappearance by the method of Cooper and Brodie(6). Aniline metabolism was followed by measuring the formation of the metabolite, p-aminophenol, by a method of Gillette as described by Hart and Fouts (7). Chlorpromazine metabolism was assessed by following substrate disappearance by the method of Salzman and Brodie(8). The conditions of incubation, cofactors used and their concentrations were as previously described by Dixon *et al*(9). The amounts of substrate used were as follows: hexobarbital, 3 micromoles; aniline, 13 micromoles; and chlorpromazine, 1 to 2 micromoles, in a total volume of 5 ml. One ml of liver 9000 $\times$ *g* supernatant fraction was incubated in a Dubnoff metabolic shaker-incubator for 1 hour at 37°C with oxygen as the gaseous phase.

The direct effect of ANIT and PIT on pooled mouse liver microsomes was also studied. ANIT and PIT were dissolved in 95% ethanol so that 0.2 ml of the solutions contained sufficent test agent to give final concentrations of 10^{-3} to 10^{-5} M in the incubation vessels. The pathways studied in these experiments were side chain oxidation of hexobarbital and oxidation of the ring-sulfur of chlorpromazine.

Results. Table I shows that when ANIT was administered orally to mice in a dosage of 80 mg/kg, the hepatic microsomal drug metabolizing activity for hexobarbital and for aniline was significantly depressed 2 hours

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TABLE I. Effects of Single Oral Dose (80 mg/kg) of ANIT on Hepatic Microsomal Drug Metabolizing Activity in the Mouse.

Time after ANIT†	Treatment	Amt of substrate metabolized* Hexobarbital	Ratio‡	Aniline	Ratio	Chlorpromazine	Ratio
2 hr	ANIT (6)	51 ± 12§	.27	34 ± 17§	.32	123 ± 16	1.08
	Oil (6)	187 ± 14		106 ± 27		114 ± 19	
1 day	ANIT (6)	49 ± 12§	.29	51 ± 16§	.40	129 ± 10	.95
	Oil (6)	166 ± 29		127 ± 18		136 ± 13	
5 days	ANIT (6)	125 ± 25§	.65	86 ± 25§	.65	128 ± 19	.98
	Oil (6)	193 ± 27		132 ± 13		131 ± 15	
9 "	ANIT (4)	149 ± 16§	.70	92 ± 17§	.68	131 ± 12	.90
	Oil (4)	214 ± 17		136 ± 15		146 ± 14	

* Values in Table represent metabolism by supernatant fraction expressed as mean micromoles drug metabolized ± standard deviation per g nitrogen in 1 hr. Numbers in parentheses represent No. of animals per group.

† Time between administration of agent and time of sacrifice.

‡ Activity of ANIT-treated animals/activity of oil-treated animals.

§ ANIT-treated value is significantly different from oil control ($P < .05$).

after ANIT treatment, and the inhibition persisted for at least 9 days. On the other hand, ANIT treatment had no effect on the ability of the microsomes to metabolize chlorpromazine. The latter observation was more striking in view of the fact that the activities of the other two oxidative pathways were diminished to 30 to 40% of control, 2 to 24 hours after ANIT. A lower dose of ANIT (20 mg/kg) also inhibited the metabolism of hexobarbital and aniline, but not that of chlorpromazine, 2 hours after oral administration. The rates of metabolism (compared to controls) were 54, 67 and 102% for hexobarbital, aniline and chlorpromazine, respectively.

The two congeners of ANIT exhibited differing effects (Table II). Treatment with BNIT resulted in inhibition of both hexobarbital and aniline metabolism at 2 hours; by 24 hours, only hexobarbital metabolism was significantly depressed. BNIT had no effect on chlorpromazine metabolism. PIT had a significant effect on hexobarbital metabolism only at 2 hours; aniline and chlorpromazine metabolism were not affected.

ANIT and PIT added directly to liver microsomes decreased the hexobarbital metabolizing activity in concentrations as low as 10^{-5} M (Table III). On the other hand, the chlorpromazine pathway was affected only when the concentration was 10^{-3} M. The concentrations of ANIT and PIT required to

TABLE II. Effects of Single Oral Doses of ANIT Congeners on Hepatic Microsomal Drug Metabolizing Activity in the Mouse.

Time after treatment (hr)†	Treatment and dose	Amt of substrate metabolized* Hexobarbital	Ratio‡	Aniline	Ratio	Chlorpromazine	Ratio
2	BNIT, 80 mg/kg	134 ± 28§	.60	46 ± 8§	.42	121 ± 16	1.04
	Oil	224 ± 21		110 ± 23		118 ± 10	
24	BNIT, 80 mg/kg	171 ± 34§	.73	94 ± 24	.90	127 ± 17	.86
	Oil	234 ± 24		104 ± 14		147 ± 12	
2	PIT, 110 mg/kg	145 ± 19§	.67	79 ± 11	.78	117 ± 11	.98
	Oil	217 ± 28		102 ± 10		121 ± 16	
24	PIT, 110 mg/kg	279 ± 25	.98	90 ± 11	.83	118 ± 11	1.01
	Oil	285 ± 33		109 ± 12		117 ± 14	

* Values in Table represent metabolism by supernatant fraction expressed as mean micromoles drug metabolized ± standard deviation per g nitrogen in 1 hr. All groups consisted of 6 animals each.

† Time between administration of agent and time of sacrifice.

‡ Activity of aryl isothiocyanate-treated animals/activity of oil-treated animals.

§ Aryl isothiocyanate-treated value is significantly different from oil control ($P < .05$).

TABLE III. Effect of ANIT and PIT on Hepatic Microsomal Drug Metabolizing Activity when Added *in vitro*.

Agent	Molar concentration	Microsomal drug metabolizing activity* Hexobarbital	Chlorpromazine
ANIT	10^{-3}	0	34
	10^{-4}	0	100
	10^{-5}	35	100
PIT	10^{-3}	4	77
	10^{-4}	27	100
	10^{-5}	57	100

* Values in Table represent metabolism by supernatant fraction, in presence of ANIT or PIT, expressed as percent of metabolism measured in control (no treatment) supernatant fraction. Each value is mean of 3 determinations.

depress hexobarbital metabolizing activity to 50% of control were estimated to be about 8 $\times$ 10^{-6} M for ANIT and 2 $\times$ 10^{-5} M for PIT.

Discussion. The results in Table I show that acute ANIT administration has a rapid and relatively long duration of action on certain drug metabolizing pathways in mouse livers. The effects observed here on hexobarbital metabolism are quite consistent with the effect of ANIT on barbiturate sleeping time. Becker and Plaa(3,4) have shown that pentobarbital sleeping time in mice is significantly prolonged 2 hours after ANIT administration and that this response persists for over 12 days.

Some of the previously reported effects of ANIT on serum bilirubin and sulfobromophthalein retention(3,4) in the mouse suggested that this substance was affecting hepatocyte function, but the later development of cholestasis with the doses of ANIT employed complicated the interpretation. The 80 mg/kg dose of ANIT employed in the present study is about an ED_{99} for production of hyperbilirubinemia(4). This dose does not cause cholestasis in 2 hours; even after 24 hours only 20 to 40% of the mice usually show complete bile stasis (unpublished data). The 20 mg/kg dose of ANIT, shown here to affect both hexobarbital and aniline metabolism, does not cause hyperbilirubinemia or cholestasis. Therefore, it seems reasonable to conclude that the depression of these metabolic pathways represents a direct effect on hepatocyte function. The observation that addition of ANIT to liver microsomes *in vitro*, in a concentration of 10^{-5} M, also depresses hexobarbital metabolism supports this conclusion.

The effect of oral dosage with PIT and BNIT on drug metabolizing activity seems much less than that of ANIT. The degree of depression of hexobarbital metabolism was neither as great nor as persistent. BNIT depressed aniline metabolism only at 2 hours; PIT had no significant effect on this pathway. The addition of PIT *in vitro* seems to show this agent to be less effective than ANIT. PIT and ANIT have been found to have differing quantitative effects on pentobarbital sleeping time(4,10). Sleeping time is significantly prolonged in only about 50% of the mice 2 hours after administration of PIT; by 24 hours, sleeping time prolongation is seen in only about 10% of the mice(10). BNIT has been found to exert little or no effect on liver function after acute administration(10). Differences between these aryl isothiocyanates have also been seen regarding lethality(10) and their effect on iodide uptake by the thyroid(11); in both of these instances, ANIT is more active than either PIT or BNIT.

One of the more widely studied inhibitors of hepatic microsomal drug metabolizing enzymes is the diethylaminoethanol ester of diphenyl propylacetic acid (SKF 525-A)(12). The duration of the SKF 525-A-induced inhibition of hexobarbital metabolism is 12 to 24 hours(13,14,15). In contrast, ANIT prolongs pentobarbital sleeping times(3,4) and inhibits hexobarbital metabolism by hepatic microsomes for periods up to 9 days. Diethylaminoethanol esters of aryl alkanoic acids appear to be among the more potent of the known inhibitors of microsomal drug metabolizing enzymes(12). The concentration of SKF 525-A required for 50% inhibition of the *in vitro* metabolism of hexobarbital by mouse liver microsomes is about 5 $\times$ 10^{-6} M (16). The concentration of ANIT required for 50% inhibition of the metabolism of hexobarbital in mice appears to be less than 10^{-5} M. Therefore, ANIT appears to be one of the more active inhibitors of microsomal drug metabolizing enzymes and may inhibit a wide

variety of microsomal reactions. The long duration of the inhibitory action of ANIT requires further study, but it may be due to binding of the inhibitor to the endoplasmic reticulum membranes, a non-specific alteration of enzyme structure, or suppression of enzyme formation.

Although SKF 525-A inhibits a very wide variety of drug metabolizing reactions(12), the sulfoxidation of chlorpromazine is one of the enzymic pathways which seems unaffected (17). ANIT resembles SKF 525-A in its lack of inhibitory action on the sulfoxidation of chlorpromazine.

Summary. When alpha-naphthylisothiocyanate was administered orally to mice, the hepatic microsomal drug metabolizing activity for hexobarbital and aniline was significantly depressed 2 hours after treatment; the inhibition persisted for 9 days. Chlorpromazine metabolism was not affected. Phenylisothiocyanate and beta-naphthylisothiocyanate also inhibited some drug metabolizing pathways at 2 hours, but were neither as effective, nor as persistent, as alpha-naphthylisothiocyanate. Alpha-naphthylisothiocyanate and phenylisothiocyanate added directly to hepatic microsomes inhibited hexobarbital metabolism in concentrations as low as 10^{-5} M. Chlorpromazine metabolism was affected only when the concentrations were 10^{-3} M.

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Parathyroid Size and Uptake of Alpha-Aminoisobutyric Acid in Intact and Hypophysectomized Rats.* (30373)

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Parathyroid function appears to be regulated largely by changes in serum ionized calcium concentration(1), but the possibility that pituitary or other hormones may modulate this regulation requires further study(2). There are as yet no direct methods for measuring synthesis or secretion of parathyroid hormone *in vivo*. Previous studies(3-5) indicated that the parathyroid uptake of a non-metabolized amino acid, α-aminoisobutyric acid (AIB) could provide a useful index of parathyroid function both *in vivo* and *in vit-*

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