system from liver. Increasing the level of riboflavin to 200 ppm but not 20 ppm gives rise to maximal values of enzyme activity in cecal contents. Liver enzyme reached similar levels with 20 and 200 ppm supplementary dietary riboflavin.

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Comparative metabolism of selected *N*-methylcarbamates by human and rat liver fractions

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THE METABOLISM of *N*-methylcarbamates has been studied in plants,[1–3] vertebrate animals (*in vivo* and *in vitro*),[4–7] and insects.[8, 9] However, there is very little in the literature concerning their metabolism in the intact human or in isolated fractions of human liver. Matsumura and Ward[10] studied carbaryl metabolism by fractions from human livers obtained at autopsy. However, no attempt was made by these authors to identify the ether-extractable metabolites. Knaak *et al.*[6] investigated the metabolism *in vivo* of carbaryl by humans exposed to carbaryl dust by studying the urinary excretory products. They identified only the glucuronide and sulfate conjugates of 1-naphthol. More human studies are needed with many different types of compounds to increase our knowledge of the various metabolic pathways available (or not available) to the human and of possible differences between the human and other animals. The metabolism *in vitro* of the carbamates employed in this study by human liver fractions has not been investigated. The study, reported here, indicates there is a significant difference

in the metabolic pattern of these carbamates as produced by the human and rat liver. The human liver, in general, produces the same metabolites as does the rat liver, plus a number of metabolites not observed in the rat liver studies.

The qualitative comparative metabolism *in vitro* of the following carbonyl-^{14}C labeled *N*-methylcarbamates by human and rat liver fractions is to be reported: 1-Naphthyl *N*-methylcarbamate, Union Carbide, South Charleston, W. Va.; 4-dimethylamino-3,5-xylyl *N*-methylcarbamate, Dow Chemical Co., Midland, Mich.; 4-dimethylamino-3-cresyl *N*-methylcarbamate, Chemagro Corp., Kansas City, Mo.; and 4-methylthio-3,5-xylyl *N*-methylcarbamate, Chemagro Corp., Kansas City, Mo.

Methods

Sprague–Dawley strain rats (250–350 g) were sacrificed by cerebral concussion, the liver removed and homogenized in sufficient 0·25 M sucrose to make a 20% homogenate. The homogenate was centrifuged at 15,000 *g* for 30 min and reconstituted to the 20% concentration to obtain the enzyme preparation. Biopsy specimens of human liver were obtained at the time of surgery. The specimens were placed in cold 0·25 M sucrose, blended in a Waring blender for approximately 30 sec, and theu processed by the same procedure as used for the rat liver.

A benzene solution of the carbamate was deposited in a 25 ml Erlenmeyer flask as described by Dorough and Casida.[7] The usual incubation mixture (in duplicate) consisted of the liver homogenate equivalent to 200 mg of liver, 2 μmoles nicotinamide adenine dinucleotide phosphate (NADP) in 0·1 M phosphate buffer (pH 7·4), 20 μmoles glucose 6-phosphate, 0·1 ml propylene glycol (to redissolve the carbamates), 25 μmoles $MgCl_2$, and sufficient phosphate buffer to make 3·0 ml total volume. A control flask was prepared using boiled homogenate. The incubation mixture was incubated in air for 3 hr at 37° and extracted with 4 × 5 ml of ether. The extract was dried over anhydrous sodium sulfate and finally evaporated to dryness in a graduated centrifuge tube under a gentle stream of nitrogen. Absolute ethanol (0·1–0·2 ml) was added to dissolve the residue. The extract was spotted on 8 × 8 in. by 0·25 mm silica gel layers for two-dimensional chromatography.

Radioautography was carried out for 2–7 days using Singul-X/AP no screen film. The solvent system used for thin-layer chromatography in the work reported here was composed of chloroform–acetonitrile (4:1) in the first direction and anhydrous ether–hexane (4:1) in the second direction. Metabolites were tentatively identified by radioautography, co-chromatography, and elution and rechromatography with known compounds, either on the side of the two-dimensional thin-layer chromatographic plate and/or over-spotted and sprayed with one or more of the reagents for carbamates as suggested by Leeling and Casida.[11] Additional classical functional group reagents were used to assist in further characterizations of the suspected metabolites. The relative R_f's are averages of two or more plates from two or more experiments.

A gas chromatographic method was developed to analyze some of the ether extracts. A 4-ft glass U-tube column containing 3% OV-17 (w/w) on 100/120 mesh chromosorb AW-DMCS was used (Wheeler and Strother[12]). Some of the carbamates and their metabolites can be chromatographed with less than 8 per cent decomposition on the column. The preparation of various derivatives of the suspected metabolites for gas chromatography has provided additional information as to the possible chemical structure of the metabolites.

Results and discussion

4-Dimethylamino-3,5-xylyl N-methylcarbamate. The solvent system used produced an array of metabolites from the rat and human experiments which agree closely with the data from the rat, as reported by Oonnithan and Casida[5] (Table 1). However, results from the human liver studies indicate several metabolites not observed (NO) in the rat studies. Metabolite 1 is tentatively identified as 4-dimethyl-amino-3, 5-xylyl *N*-hydroxymethylcarbamate. The human liver apparently produces less of this metabolite than the rat, as evidenced from the radioautogram. The other NO metabolites are present in very small amounts and may not be observed if developing time for the radioautograms are less than about 5 days. Metabolite b is believed to be more than one compound because of its chromatographic behavior. It is consistently a larger spot on the radioautogram when obtained from human liver incubations. When the extracts from the rat studies are chromatographed, the radioactivity separates into two distinct smaller spots (b, s). From these studies there appears to possibly be 7 more metabolites produced from this carbamate by the human liver than by the rat liver.

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF EXTRACT FROM HUMAN AND RAT LIVER FRACTIONS INCUBATED WITH VARIOUS *N*-METHYLCARBAMATES*

	Carbamate-metabolites	Chloroform-acetonitrile Human	Chloroform-acetonitrile Rat	Ether-hexane Human	Ether-hexane Rat
	4-Dimethylamino-3,5-xylyl *N*-methylcarbamate				
a		0	0	0	0
s			8		0
b		13	8	0	10
c		24	NO	0	NO
d		27	NO	0	NO
e		24	NO	13	NO
f		33	NO	6	NO
g		33	NO	22	NO
h		45	45	0	0
i	4-Methylformamide-3,5-xylyl *N*-methylcarbamate	46	44	12	14
j	4-Amino-3,5-xylyl *N*-methylcarbamate	48	47	21	24
k	4-Methylamino-3,5-xylyl *N*-methylcarbamate	45	42	32	33
l	4-Dimethylamino-3,5-xylyl *N*-hydroxymethylcarbamate	43	44	41	44
m		65	NO	47	NO
n		72	NO	60	NO
	4-Dimethylamino-3,5-xylyl *N*-methylcarbamate	93	85	73	86
o		90		95	95
	1-Naphthyl *N*-methylcarbamate				
a		0	0	0	0
b		18	NO	4	NO
c		32	30	3	6
g	1-Naphthyl *N*-hydroxymethylcarbamate	48	43	34	33
d	4-Hydroxy-1-naphthyl *N*-methylcarbamate	60	56	37	39
e	5-Hydroxy-1-naphthyl *N*-methylcarbamate	70	65	47	48
f		78	NO	56	NO
	1-Naphthyl *N*-methylcarbamate	93	91	66	69

* $R_f = \frac{\text{Sample migration} \times 100}{\text{Sudan III migration}}$.

1-*Naphthyl* N-*methylcarbamate*. Metabolism of this carbamate by the human liver apparently produces 2 additional metabolites (b, f) not observed in the rat liver studies (Table 1). Radioactive area b appears to be more than 1 compound. Radioactive area c may also be more than 1 compound, since it usually has a trailing edge which overlaps with b. Both b and c may contain some 5,6-dihydro-5,6-dihydroxy-1-naphthyl *N*-methylcarbamate, but this has not been confirmed. Additional solvent systems did not provide any additional or helpful information. There apparently is slightly more of the compound tentatively identified as a 1-naphthyl *N*-hydroxymethyl-carbamate (g) produced by the rat liver than the human liver.

4-*Dimethylamino*-3-*cresyl* N-*methylcarbamate*. The human liver appears to produce 1 more metabolite (h) from this carbamate than does the rat liver (Table 2). Metabolite b from the human studies chromatographs very similar to b from 4-dimethylamino-3,5-xylyl-*N*-methylcarbamate and also appears to be more than one compound. The same area from the rat studies appears to be only one compound. This carbamate appears to be metabolized much less by the human liver than by the rat liver and also seems to be the more resistant of the carbamates studied to metabolism in either system. This is in agreement with the results of Oonnithan and Casida[5] in their studies with rat liver.

4-*Methylthio*-3,5-*xylyl* N-*methylcarbamate*. The major metabolite in both the human and rat liver is 4-methylsulfonyl-3,5-xylyl *N*-methylcarbamate (Table 2). There was generally more radioactivity in the aqueous phase and less of the parent carbamate left unmetabolized in the rat liver studies than in the human studies. The 4-methylsulfinyl-3,5-xylyl *N*-methylcarbamate, as found by Oonnithan and Casida,[5] has not been observed in these studies. A much longer exposure time to the X-ray film may be needed to observe this metabolite. Metabolites g, j and k appear to be produced only by the human liver since they could not be detected in studies with rat liver. Metabolite h and p obtained from the human liver studies are not believed to be the same as obtained from the rat liver studies since their

TABLE 2. THIN-LAYER CHROMATOGRAPHY OF EXTRACT FROM HUMAN AND RAT LIVER FRACTIONS INCUBATED WITH VARIOUS *N*-METHYLCARBAMATES*

	Carbamate-metabolites	Chloroform-acetonitrile		Ether-hexane	
		Human	Rat	Human	Rat
	4-Dimethylamino-3-cresyl *N*-methylcarbamate				
a		0	0	0	0
b		16	14	0	5
P		30	29	0	0
q		31	29	9	9
d		31	28	16	16
e	4-Dimethylamino-3-cresyl *N*-hydroxymethylcarbamate	32	30	29	26
c	4-Amino-3-cresyl *N*-methylcarbamate	41	40	14	15
f	4-Methylamino-3-cresyl *N*-methylcarbamate	67	63	39	36
h		77	NO	0	NO
	4-Dimethylamino-3-cresyl *N*-methylcarbamate	76	73	65	63
	4-Methylthio-3,5-xylyl *N*-methylcarbamate				
a		0	0	0	0
P		22	30 (?)	0	0
b	4-Methylsulfonyl-3,5-xylyl *N*-methylcarbamate	33	35	3	2
f		45	45	33	36
g		49	NO	46	NO
j		60	NO	72	NO
h		76	81 (?)	68	72
k		81	NO	98	NO
	4-Methylthio-3,5-xylyl *N*-methylcarbamate	89	88	80	80

* $R_f = \frac{\text{Sample migration} \times 100}{\text{Sudan III migration}}$.

chromatographic behavior is somewhat different as indicated by the question mark in Table 2. Suspected metabolite p appears to have a carbonyl group present as indicated by the results obtained from various functional group reagents. This could be the result of oxidation of one of the methyl groups on the ring or the methyl group attached to the sulfur atom.

The human liver appears to produce a somewhat different metabolic pattern of metabolites than does the rat from some of the carbamates studied. The metabolic pattern of 4-dimethylamino-3,5-xylyl *N*-methylcarbamate appears to be the most divergent from that of the rat. Metabolites tentatively identified and those not identified from incubating the carbamates with rat and human liver fractions appear to have the C-O-C(O)N-C group intact and would be expected to retain some anticholinesterase activity and possibly also some toxicity to man.

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Evidence for spironolactone as a possible inducer of liver microsomal enzymes in mice*

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SPIRONOLACTONE, currently employed as a diuretic, has recently been reported to have pharmacological effects in addition to aldosterone-blockade. Selye *et al.*[1] have shown that this agent offers protection against the sedative and anesthetic effects of such steroids as testosterone, progesterone, desoxycorticosterone and hydroxydione, as well as pentobarbital and ethanol. They emphasized that several days of pretreatment were essential for antagonism of this CNS depressant activity. Androgens, estrogens, glucocorticoids, anabolic and progestational steroids have been noted to influence the activity of drug-metabolizing liver microsomal enzymes.[2, 3]

To test the hypothesis that spironolactone was stimulating hepatic drug metabolism, we sought to examine the effects of its pretreatment on pentobarbital and testosterone-potentiated pentobarbita sleeping times and to measure liver microsomal enzyme activities.

Experimental

Animal pretreatment. Male Swiss mice, 18–25 g, were maintained *ad lib.* on a standard laboratory pellet diet and water. Animals were given a 100 mg/kg subcutaneous injection of spironolactone or its corn oil vehicle for 3 consecutive days. These injections, and all others described, were delivered in a volume of 0·1 ml/10 g body weight. Approximately 24 hr after the last injection, control and drug-treated animals were randomly divided into groups for the sleeping time studies and liver microsomal assays. Another group of animals, treated in an identical manner, were employed for the study of hexobarbital metabolism.

Sleeping time experiments. Details of the drug administration schedule are presented in Table 1. The duration of sleeping time was calculated from the time the animal lost its righting reflex until it was able to right itself three times in 30 sec.

Preparation of liver microsomes. Animals were sacrificed and livers excised immediately, rinsed and transferred to ice-cold 0·02 M Tris-HCl buffer, pH 7·4, containing 1·15% KCl. Livers were homo-

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