AN ABSTRACT OF THE THESIS OF

JOHN BARTLEY MIAULLIS (Name of student) for the M. S. (Degree)

in Biochemistry (Major) presented on March 27, 1968 (Date)

Title: IN VIVO AND IN VITRO METABOLISM OF 4-BENZO(b)THIENYL N-METHYLCARBAMATE AND 4-HYDROXY BENZOTHIOPHENE BY THE WHITE RAT

Abstract approved: [redacted]
Leon C. Terriere

In vitro studies of Mobam (4-benzo(b)thienyl N-methylcarbamate) metabolism have demonstrated that at least three ether soluble metabolites are formed in a microsomal system fortified with reduced triphosphopyridine nucleotide (NADPH). One of the metabolites was identical to 4HBT (4-hydroxy benzothiophene), the phenol of Mobam. The other two metabolites did not possess the carbonyl carbon of Mobam and therefore involved hydrolysis of the ester linkage.

Water soluble metabolites of Mobam and 4HBT were produced by NADPH-dependent microsomal reactions. One of the metabolites contained the carbonyl carbon of Mobam but not the ring moiety. The added cofactors UDPGA (uridine diphosphoglucuronic acid) and $ATP/SO_4^{=}/Mg^{++}$ resulted in the formation of specific metabolites of the ring moiety. One of these products is tentatively identified as the glucuronide of 4HBT. No reaction between the carbamic acid portion of the carbamate and UDPGA was observed.

In vivo metabolism of Mobam or 4HBT led to the rapid excretion or expiration of the metabolic products. Approximately 80% of the ring moiety and more than 40% of the carbonyl carbon were excreted in the urine within 72 hours after treatment of the rat. An additional one-fourth of the carbonyl carbon was expired as carbon dioxide indicating hydrolysis of the ester linkage and splitting of the C-N bond of the carbamic acid.

Besides expired carbon dioxide, four other in vivo metabolites were characterized by their paper chromatographic Rf values. One of these metabolites arose from the carbamic acid moiety and the other three contained the ring structure but not the carbonyl carbon. One of the ring metabolites is tentatively identified as the glucuronide of 4HBT and another may be the sulfate conjugate of 4HBT. Each of the major metabolites of Mobam was also excreted by rats treated with 4HBT, demonstrating the close relationship between the metabolic fate of Mobam and its phenol.

In Vivo and *In Vitro* Metabolism of 4-Benzo(b)thienyl-N-Methylcarbamate and 4-Hydroxy Benzothiophene by the White Rat

by

John Bartley Miaullis

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1968

APPROVED:

[redacted]

Professor of Biochemistry
in charge of major

[redacted]

Chairman of Department of Biochemistry-Biophysics

[redacted]

Dean of Graduate School

Date thesis is presented April 27, 1968

Typed by Marilyn Carl for John Bartley Miaullis

ACKNOWLEDGMENT

Words cannot express the author's appreciation to Dr. L. C. Terriere for his encouragement and advice during the course of this study.

Special thanks are extended to Dr. James W. Gillett and Mr. Timothy Chan for their assistance and useful suggestions.

The author also wishes to express his appreciation for the patience and understanding shown by his wife, Laura.

LIST OF FIGURES

TABLE OF CONTENTS

LIST OF TABLES

IN VIVO AND IN VITRO METABOLISM OF 4-BENZO(b)THIENYL-N-METHYLCARBAMATE AND 4-HYDROXY BENZOTHIOPHENE BY THE WHITE RAT

I. INTRODUCTION

The naturally occurring carbamate physostigmine was identified in 1925 as the active parasympathomimetic agent in extracts of plants (Casida, 1963). Since that time synthetic carbamates have been developed for medical uses, and as herbicides, fungicides and insecticides. The insecticidal carbamates first synthesized in the 1950's had the ROC(O)N R'R" structure where the R was a phenyl or substituted phenyl group and the R' and R" were hydrogen or methyl. Recently, the insecticidal activity of more than a hundred such carbamates has been reported (Metcalf and Fukuto, 1965; Lemin, Boyack, and MacDonald, 1965). Another class of carbamates in which the R is an oxime is typified by Temik (Figure 1) (Andrawes, Dorough, and Linquist, 1967).

Development of the carbamates as insecticides was stimulated by the undesirable consequences of the use of DDT and its analogs. Two such consequences were the resistance which arose in certain species and the residues of potentially toxic material caused by the slow biological and chemical degradation of these chlorinated hydrocarbons.

Carbaryl[1] (1-naphthyl-N-methylcarbamate), the most widely used and studied carbamate insecticide, typifies the carbamates in its rapid biological and chemical degradation to primarily nontoxic substances (Carpenter et al., 1961). Although insect resistance to carbaryl did develop and it is not effective against some species, carbaryl and other carbamates have successfully replaced the chlorinated hydrocarbons in many applications.

The insecticidal carbamates are reversible inhibitors of acetylcholinesterase, and presumably their biological activity is a result of this inhibition (Casida, 1963). Cleavage of the ester linkage yields products of reduced anticholinesterase activity, and therefore the effectiveness of the carbamates is dependent on their stability in the physiological environment. In addition to ester cleavage, other reactions could lead to inactive substances (Metcalf et al., 1966).

Although the inhibition of acetylcholinesterase by carbamates is reversible (Casida, 1963), metabolism of the carbamate could conceivably produce a more potent inhibitor. Several examples of insecticides which are converted to metabolites more toxic than the parent compound are parathion, Temik, and aldrin, the first two being anticholinesterase agents. Therefore, a characterization of the metabolites of a new carbamate is important from a toxicological

1. Generic name.

standpoint. A rather thorough report of the anticholinesterase activity of a number of carbamates and their in vitro metabolites has recently been published (Oonnithan and Casida, 1968).

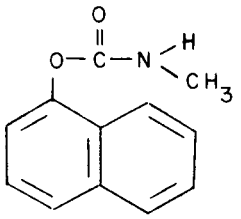
The metabolism of xenobiotics, a term used by Mason (1965) to describe compounds foreign to life, is commonly attributed to the endoplasmic reticulum of the cell. These membranes are isolated as microsomes, that fraction of the cell homogenate sedimenting between 9000g and 105,000g after removal of the nuclei, mitochondria and cell debris. The equivalence of the endoplasmic reticulum and the microsomes is based on electron microscopy. In the case of vertebrates, the microsomes are generally prepared from the liver or adrenal cortex. Microsomal enzymes acting on a wide variety of compounds have been designated as mixed function oxidases (Mason, 1965) and are characterized by their NADPH and oxygen requirement. The NADPH-dependent reactions demonstrated for pesticides include O- and N-dealkylation, alkyl and aryl hydroxylation, sulfur oxidation, epoxidation, phosphorothioate S to O conversion and the hydrolysis of phosphorathioates (Terriere, 1968; Nakatsugawa and Dahm, 1967).

Other enzymes present in the liver are responsible for conjugation reactions which form water soluble products (Brodie, Gillette, and La Du, 1958). These conjugations generally do not require NADPH, but often involve the transfer of a specific chemical group from a nucleotide to a hydroxyl group of the xenobiotic, forming for example, a sulfate or glucuronide conjugate of a phenol.

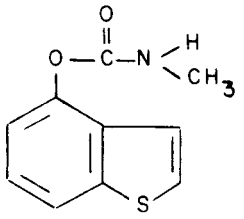
In Figure 1 are the structures of some carbamate insecticides and a diagram showing the three label position which have been used in the elucidation of the metabolic fate of several of the carbamates. Carbamates can undergo three types of reactions: hydrolysis, attacks of the ring portion of the molecule, and attacks involving the carbamic acid moiety.

In vitro oxidation of the N-methyl group was followed by measuring formaldehyde production which presumably results from an N-methylol structure (Hodgson and Casida, 1961; Hook and Smith, 1967). However, only certain of the carbamates tested in vitro form the respective N-methylol compound (Oonnithan, 1968). This same structure is probably the intermediate which leads to in vivo expiration of $^{14}CO_2$ from the N-methyl labeled carbamates, to be discussed in a subsequent paragraph.

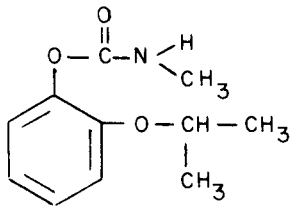
Five studies using microsomes fortified with NADPH have demonstrated the formation of organosoluble metabolites of carbaryl having the ester linkage intact (Dorough and Casida, 1964; Knaak et al., 1965; Leeling and Casida, 1966; Hassan, Zayed, and Abdel-Hamid, 1966; Oonnithan, 1968). The studies have indicated that microsomal preparations from the rat, guinea pig, mouse and rabbit metabolize carbaryl but with a slightly different distribution of products (Leeling, 1966).



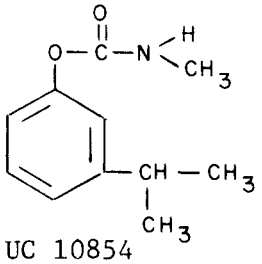
Carbaryl



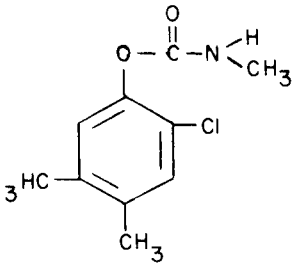
Mobam



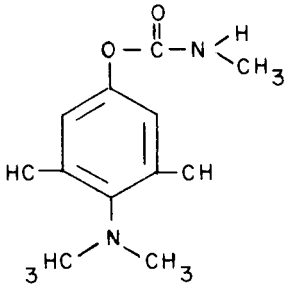
Baygon



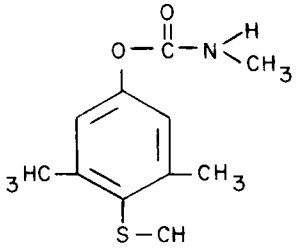
UC 10854



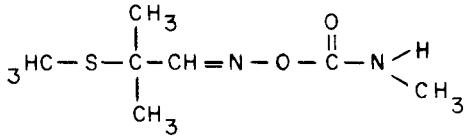
Banol



Zectran



Mesurol



Temik

Positions of the carbon commonly labeled for metabolism studies

Figure 1. Structure of representative insecticidal carbamates

In each case, a certain amount of 1-naphthol was produced indicating hydrolysis of the ester. Two of the studies suggested that hydrolysis was not a cofactor-dependent reaction, that is requiring no NADPH (Leeling, 1966; Oonnithan, 1968). Figure 2 illustrates the structure of microsomal metabolites of carbaryl which have been proposed or tentatively identified, but no structures have been suggested for other metabolites presently characterized by thin-layer chromatography. Microsomal metabolism of carbaryl also results in water soluble metabolites, but the chemical nature of the products has not been reported except for those systems which include specific conjugating agents such as UDPGA (uridine diphosphoglucuronic acid) (Knaak, 1965).

The microsomal metabolites of other carbamates have been investigated by Oonnithan (1968). In each case certain metabolites of the parent compound had the ester linkage intact. Reactions identified as being NADPH-dependent included ring hydroxylation, aryl-alkyl ether cleavage, sulfur oxidation to sulfinyl and sulfonyl derivatives, N-demethylation of tertiary and secondary amines attached to the ring, and hydroxylation of the N-methyl group of the carbamic acid moiety to form the N-hydroxymethyl carbamate. Although not all of the metabolites from the various carbamates were identified, each of the compounds tested underwent decomposition to several products in the presence of microsomes and NADPH.

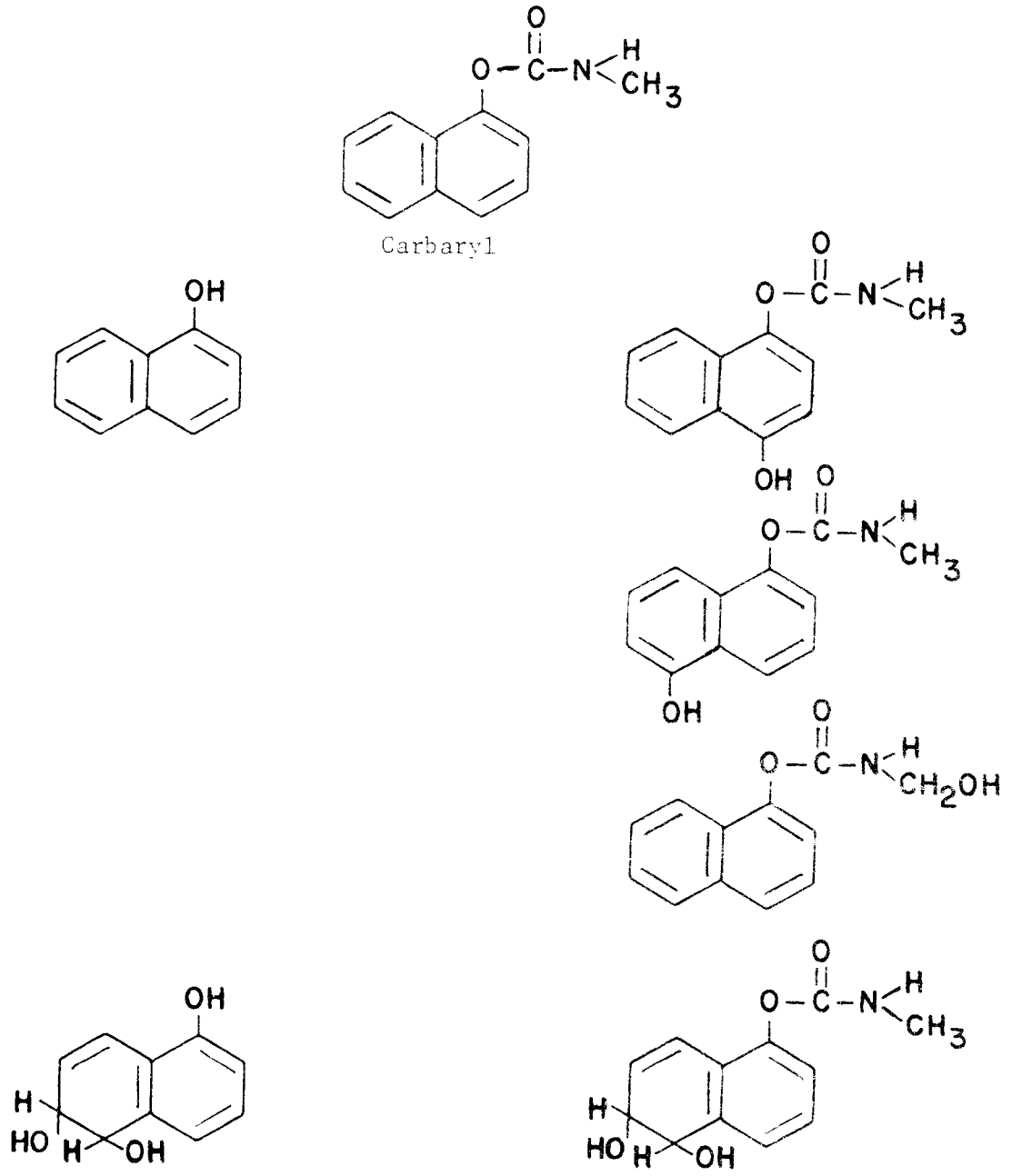


Figure 2. Proposed or tentatively identified metabolites of carbaryl.

Carbamate metabolism studied *in vivo* has involved the treatment of laboratory animals with the radiolabeled molecules and the collection of urine, feces, expired CO_2 and in some cases an examination of tissue residues. Identification of the urinary metabolites was made in some cases. *In vivo* metabolism of carbaryl in the rat was aided by the use of three labeled forms of the insecticide: naphthyl-^{14}C, carbonyl-^{14}C and N-methyl-^{14}C (Figure 1). From rats treated with the labeled molecules, recovery of ^{14}C after seven days was nearly complete with most of the radioactivity either expired as $^{14}CO_2$ or excreted in the urine (Knaak, 1965).

From the carbonyl and N-methyl labeled carbaryl, 32% and 11% respectively of the dose was expired as $^{14}CO_2$ (Knaak, 1965). Expiration from the carbonyl ^{14}C-carbaryl indicates hydrolysis and C-N bond cleavage, while radioactive carbon dioxide from the N-methyl moiety presumably involves hydroxylation of the methyl group which is further oxidized, releasing CO_2. *In vitro* results supporting this hypothesis have been presented (Hodgson, 1961; Hook, 1967). With both carbonyl and N-methyl labeled carbaryl, most of the radioactivity not expired as $^{14}CO_2$ was excreted in the urine (Knaak, 1965).

Rats treated with the naphthyl-^{14}C carbaryl excreted the major portion of the dose in the urine with less than 0.5% detected as expired products. Elimination of radioactivity in the feces amounted to approximately 10% of the dose with each of the labeled carbaryls (Knaak, 1965).

Similar results supporting the rapid metabolism and excretion of the carbamates were reported for Banol (Baron and Doherty, 1967) and for carbaryl and Baygon (Krishna and Casida, 1966) using the ring, carbonyl, and N-methyl labeled compounds. However, in the latter case, $^{14}CO_2$ was expired by rats treated with 1,3-^{14}C-isopropoxy labeled carbamate ("ring" labeled Baygon) indicating the breaking of a carbon-carbon bond (Table I).

Other in vivo studies report the metabolism of ring labeled carbaryl in a lactating cow (Dorough, 1967), ring labeled Zectran in the dog (Williams, Meikle, and Redemann, 1964b), ring and N-methyl labeled carbaryl in the dog (Knaak and Sullivan, 1967), and seven carbonyl-^{14}C carbamates in the rat (Krishna, 1966). These results confirm the excretion of the ring moiety in the urine and the expiration of radioactive carbon dioxide from the carbonyl and N-methyl labeled carbamates. Some evidence for fecal elimination of carbaryl metabolites in the cow and dog was also presented. Tissue residues from N-methyl labeled compounds have been reported (Krishna, 1966). Casida (1963) has suggested that carbamylation of an esterase at or near the active site could result in more tissue residues with the N-methyl and carbonyl labeled compounds than with ring labeled molecules.

Urinary metabolites of carbaryl were identified as the imido-glucuronide of carbaryl, the glucuronide of carbaryl, the glucuronide and sulfate conjugates of the carbamate through the four position of

the ring, and the glucuronide and sulfate conjugates of 1-naphthol (Knaak, 1965). Unidentified neutrals were also present but there was no evidence for the excretion of free carbaryl or 1-naphthol. Treatment of a guinea pig yielded similar results, but 1-naphthol glucuronide and sulfate conjugates were the only detected urinary metabolites from men exposed in an industrial situation (Knaak, 1965). In a parallel study by the same laboratory, none of the carbaryl metabolites from dog urine corresponded to those found with rats and guinea pigs, and none of the metabolites from the dog could be identified (Knaak, 1967). These results clearly show the differences in metabolism of the insecticides exhibited by various mammals.

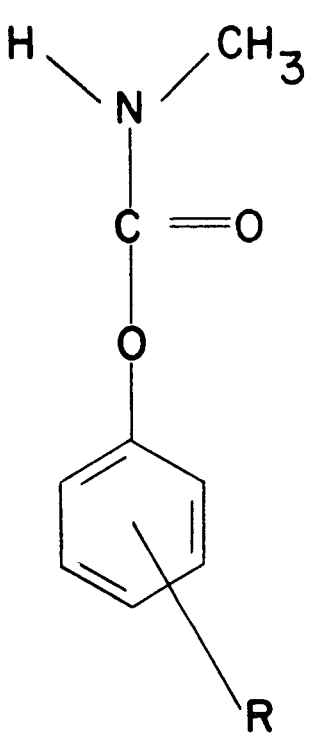
Hydrolysis of carbaryl accounted for 40% to 53% of the metabolism of carbaryl in the rat (Knaak, 1965), but for Zectran in dogs 90% of the metabolites had lost the carbamic acid moiety (Williams, 1964). The major urinary metabolite of Banol in the rat is the glucuronide of the 2-chloro-4,5-xylenol, but 34% of the urinary products had the ester linkage intact (Baron, 1967). Chemical characterization of the major metabolite containing the carbamic acid moiety suggested the N-glucuronide of Banol.

Carbamates are also degraded by insects, plant and photochemical reactions (Crosby, Leitis, and Winterlin, 1965). Reports of insect metabolism of a number of carbamates nearly parallels that of the mammalian studies (Andrawes and Dorough, 1967; Gemrich, 1967; Metcalf, Osman, and Fukuto, 1967; Tsukamoto and Casida, 1967; Zayed, Hassan,

and Hussein, 1966). Limited work with plant metabolism shows the metabolites are analogous with mammalian and insect products, but a major portion of the metabolites either remain to be identified or are present as conjugates, presumably as glycosides (Abdel-Wahab and Casida, 1967; Abdel-Wahab, Kuhr, and Casida, 1966; Friedman and Lemin, 1967; Kuhr and Casida, 1967; Williams, Meilke, and Redemann, 1964a).

Figure 3 summarizes the reactions identified for carbamate metabolism and shows the portion of the molecule attacked. Though it is unlikely that any carbamate undergoes all the reactions listed, the work with carbaryl in particular shows that more than one mechanism may be involved in the metabolism of a carbamate. It should be pointed out that conjugation reactions which are not shown in Figure 3 play an important role in the elimination of the metabolites from the body, but in most cases, some oxidative or hydrolytic reaction must precede conjugation.

The need to examine the metabolic fate of each new carbamate is clearly demonstrated by the wide variety of reactions which the carbamates undergo and by the differences in metabolic fate of the individual carbamates. In the present study, the metabolism of Mobam (Figure 1), a recently developed insecticide, and 4-hydroxy benzothiophene, the phenol of Mobam, has been investigated using both in vivo and in vitro techniques. To aid in the characterization of the metabolites and the fate of each portion of the molecule, both



Oxidation
Dealkylation

C-N bond cleavage

Hydrolysis

Hydroxylation
Dihydro-diol formation

Hydroxylation
C-C bond cleavage
N-dealkylation
Aryl-alkyl ether cleavage
Sulfur oxidation to sulfinyl
and sulfonyl derivatives

Figure 3. Summary of identified or postulated reactions in carbamate metabolism for various positions on the molecule.

carbonyl and ring labeled Mobam and ring labeled 4HBT were employed in these studies.

II. MATERIALS

The ring labeled Mobam (4-(4,7-^{14}C)benzo(b)thienyl-N-methylcarbamate) and 4HBT (4-hydroxy(4,7-^{14}C)benzothiophene) with specific activities of 0.79 and 0.85 μc per μmole respectively were obtained from Mobil Chemical Company. Specific activity was determined as described in Methods for spectrophotometric determination of 4HBT.

Carbonyl labeled Mobam (4-benzo(b)thienyl-N-methyl (carbonyl-^{14}C) carbamate) was synthesized via the methyl isocyanate method of Krishna, Dorough, and Casida (1962) except that 1-^{14}C-acetic anhydride replaced 1-^{14}C-acetyl chloride. The final product has a specific activity of 0.32 μc per μmole. The synthesis was carried out by James Barbour of this laboratory. Both Mobams, purified as outlined in Methods, and 4HBT as received assayed 97+% by thin-layer chromatography.

Reference compounds were Mobam sulfone (4-benzo(b)thienyl sulfonyl-N-methylcarbamate), 4HBT sulfone produced by alkaline hydrolysis of Mobam sulfone, and a sample of 4,7-dihydroxy benzothiophene which was a gift from Professor Thomson (Blackhall and Thomson, 1954).

Biochemicals were purchased from Sigma Chemical Company or General Biochemicals. Tetrazotized o-dianisidine and p-nitrobenzenediazonium fluoborate were from Sigma and Distillation Products

respectively. Unless otherwise stated all other materials were of analytical or comparable quality.

III. METHODS

Analytical Methods

Scintillation Counting: Scintillation fluid was composed of toluene: methylcellosolve (2:1) with 5.5 grams 2,5-diphenyloxazole per liter. Standard counting procedures were observed, and efficiency was determined by internal standardization with ^{14}C-toluene.

Thin-layer Chromatography: Silica gel-G plates of 250 micron thickness were activated at 130°C for 45 minutes and desiccated until used. Samples to be chromatographed were concentrated under an air jet at room temperature before spotting. Migrations were carried out in glass tanks which were closed to provide a saturated atmosphere (Table I). To detect radiolabeled material, the plates were stored in contact with X-ray film (No-Screen Medical) for 5 to 15 days. Quantitative results were obtained by scraping the silica gel from the radioactive areas into scintillation fluid for counting. Preliminary tests showed that quantitative recovery was achieved with this method.

Paper Chromatography: Prior to spotting on Whatman No. 1 chromatography paper, samples were concentrated at 40°C with an air jet. Radioactive areas separated by 25 to 30 cm migrations were scanned with a Vanguard Autoscanner 880 to produce a chromatogram. From the recorded scan, the relative amount of each radioactive area was obtained by dividing each peak height by the sum of the peak heights for all radioactive areas. The relative amount multiplied by

the total mμmoles in the sample gave the approximate mμmole equivalents for each radioactive region of the strip. The peak height and analysis method results in a slight overstatement of the minor areas and a slight understatement of the major areas.

Detection of Unlabeled Products: To detect Mobam or 4HBT, chromatograms were sprayed lightly with a 5% NaOH solution, air dried, and immediately sprayed with a fresh 1% solution of p-nitrobenzenediazonium fluoborate in methanol (Krishna, Dorough, and Casida, 1962). The phenol, both free and newly hydrolyzed, appeared as a purple spot. Mobam sulfone and 4HBT sulfone fluoresced blue and yellow respectively without any treatment of the chromatogram or both fluoresced yellow after 5% NaOH solution treatment. The 4,7-dihydroxy benzothiophene sample yielded three dark purple fluorescent spots after thin-layer chromatography migration (Table I).

Column Chromatographic Purification of Mobam: A Florisil column (30 grams, 60-100 mesh dried at 130°C) was used to purify Mobam. The column was eluted with 50 ml hexane, 100 ml hexane:ether (9:1), 100 ml hexane:ether (1:1), and 100 ml ether to separate the radioactive materials. Identity and purity of the eluted products were determined by thin-layer chromatography.

Spectrophotometric Determination of 4HBT: Mobam was determined as 4HBT by the method of van Asperen (1962) as modified by Karinen et al. (1967). NaOH solution (1.0 ml of 0.5N) was added to the sample or its

residue upon evaporation of the solvent. After ten minutes acetic acid (0.5 ml of 1.0N) was added and the volume brought to 9.0 ml with phosphate buffer (0.2M, pH 7.0). One ml of a 0.28% tetrazotized o-dianisidine in 3.6% lauryl sulfate solution was added and the optical density measure at 600 nm after ten minutes.

In Vitro Experimentation

Preparation of Microsomes: A stock male albino rat (Wistar strain, Corvallis), weighing 250 to 500 grams and from six months to one year old, was anesthetized with ethyl ether and the liver immediately excised. All subsequent operations were at two to four degrees Centigrade. After being rinsed in cold 1.15% KCl solution, the liver was homogenized in nine volumes of KCl solution in a VirTis 45 Homogenizer run at full speed for 30 seconds. The homogenate was centrifuged for 30 minutes at 12,000 rpm (9,000g minimum) in a Servall refrigerated centrifuge. The decanted 9,000g supernatant (post mitochondrial supernatant) was centrifuged in a Beckman Model L ultracentrifuge for 45 minutes at 50,000 rpm (105,000g minimum) to sediment the microsomes. The material not sedimenting at 105,000g was designated as the 105,000g soluble fraction. With a glass tissue homogenizer fitted with a Teflon plunger, the microsomes were resuspended in Tris buffer (0.05M, pH 8.0 or 7.2) to a final concentration of one gram liver equivalents per ml of buffer. The preparation was stored at two to four degrees Centigrade until used

in incubations (less than six hours). Occasionally the ultra-centrifugation was omitted, and the post mitochondrial supernatant used as the tissue source.

NADPH Generating System: The generating system consisted of 2.0 mμmoles NADP, 20 μmoles glucose-6-phosphate, 6.0 units of glucose-6-phosphate dehydrogenase (one unit reduces one μmole of substrate per minute at 25°C, pH 7.4), and in some cases, 2.0 μmoles nicotinamide all brought to a final 5.0 ml volume with Tris buffer (0.05M, pH 8.0 or 7.2). Measurement of optical density at 340 ηm showed generation of NADPH was complete within 15 minutes at room temperature, therefore the generating system was prepared at least 15 minutes prior to addition of the tissue for incubation.

Incubations: Substrates to be tested for <u>in vitro</u> metabolism were incubated in a buffer medium with combinations of tissue, NADPH generating system, and additional cofactors. Incubations were carried out in 50 ml erlenmeyer flasks to which were added in order the substrate, water, generating system, other cofactors, and tissue for a final volume of 7.0 ml. When substrates were dissolved in ether, the solvent was evaporated prior to addition of the water. For substrates in methylcellosolve, concentrations were adjusted so that less than 0.2 ml of methylcellosolve were present in the final 7.0 ml incubation. This amount of solvent did not effect the system.

Typically, incubations consisted of substrate, 0.7 ml water, 5.0 ml NADPH generating system, 1.0 ml Tris buffer (0.05M, pH 8.0 or 7.2) containing 2.0 μmoles UDPGA, and 0.3 ml microsomal resuspension. The equivalent volume of water or buffer was added when a component was to be deleted. Immediately upon adding the tissue, the flask was capped and placed in a shaking water bath at 37°C. To halt the reaction after one hour, 10 ml of ether were added, and the flask was shaken vigorously several times. The flasks were then shaken mechanically for at least 30 minutes, after which the ether was separated from the water phase. The extraction procedure was repeated two more times. The final ether phase (three ether extracts combined) and the aqueous phase (incubations mixture after extractions) were adjusted to known volumes and ^{14}C determined by scintillation counting.

In Vivo Experimentation

Wistar strain, Corvallis, male rats (six months to one year old and weighing 250 to 500 grams) were lightly anaesthetized with ethyl ether and given either IP (intraperatoneal) injection or stomach tube treatment with a labeled compound. In either case the compound had been dissolved in dimethyl sulfoxide or methylcellosolve so that the total solvent volume per rat was less than 0.5 ml. The dose was determined by counting an aliquot of the treatment solution.

Urine and feces were collected for up to 72 hours after treatment, while the rats were housed in wire metabolism cages. When CO_2 was being collected, a glass cage which permitted trapping of expired gases was used. To trap CO_2, laboratory air was drawn through the cage into 3N NaOH solutions which were changed at least every 12 hours (100 ml per trap). Double aliquots were used to determine ^{14}C by liquid scintillation. An alternate method of recording $^{14}CO_2$ was to force the air from the cage through a vibrating reed electrometer instrument. Estimates of $^{14}CO_2$ expired were made by comparison with electrometer responses from known quantities of $^{14}CO_2$ released from $Na_2{}^{14}CO_3$.

Urine samples were collected at specified time intervals and the samples adjusted to volume with water or ethanol. A 0.2 ml aliquot was counted by liquid scintillation. Urine samples were spotted directly for paper chromatography. Feces samples were ground with water in the VirTis 45 homogenizer and a 0.2 ml aliquot of the mixture counted by liquid scintillation.

TABLE I. Rf VALUES OF KNOWN COMPOUNDS IN THIN-LAYER AND PAPER CHROMATOGRAPHY SYSTEMS

COMPOUND	CHROMATOGRAPHY SYSTEM[1]			
	Paper		Thin-layer	
	I	II	III	IV
Mobam	0.90	-	0.45	0.46
4HBT	0.94	0.94	0.66	0.53
Mobam Sulfone	-	-	0.15	0.09
4HBT Sulfone	0.88	-	0.30	0.21
4,7 Dihydroxy Benzo-thiophene (major spot)	-	-	0.45	0.28
Methyl Urea[2]	0.58	0.82	-	-

1. I. The organic phase of n-butanol-ethanol-water (17:3:20)

   II. Isopropanol-water-ammonia (7:2:1)

   III. Ethyl ether-n-hexane (4:1)

   IV. Hexane-acetone (3:2)

2. Prepared by ammonolysis of Mobam (Friedman, 1967).

IV. RESULTS AND DISCUSSION

In Vitro Experiments

In Vitro metabolism of ring labeled Mobam (Mobam-R), carbonyl labeled Mobam (Mobam-C) and 4HBT is summarized in Tables I, II, and III and Figures 4 and 5. The mμmole equivalents (^{14}C equivalent to one mμmole of substrate) of aqueous soluble radioactive products after ether extraction of the incubation mixture were recorded as a measure of metabolic activity since more polar products would be expected from both oxidative and conjugative processes.

Results in Table II for various tissue, cofactor and substrate combinations indicate that the microsomes are required for reactions producing water soluble radioactive products. Addition of the NADPH generating system to the microsomes markedly increased metabolic activity suggesting that mixed function oxidases are involved in the metabolism of Mobam and 4HBT (Table II).

Water soluble ^{14}C was greated when UDPGA (uridine diphosphoglucuronic acid) was added to incubations of ring labeled substrates, demonstrating that the system was capable of producing metabolites by secondary processes (Table II). The addition of UDPGA to 4HBT incubations gave, for example, 137 mμmoles of water soluble products compared with 23.4 mμmoles from a comparable incubation without UDPGA. Similar but smaller differences were observed in incubations of ring labeled Mobam with and without UDPGA. Without the NADPH generating

TABLE II. EXTENT OF METABOLISM WITH VARIOUS INCUBATION COMPONENTS AND SUBSTRATES

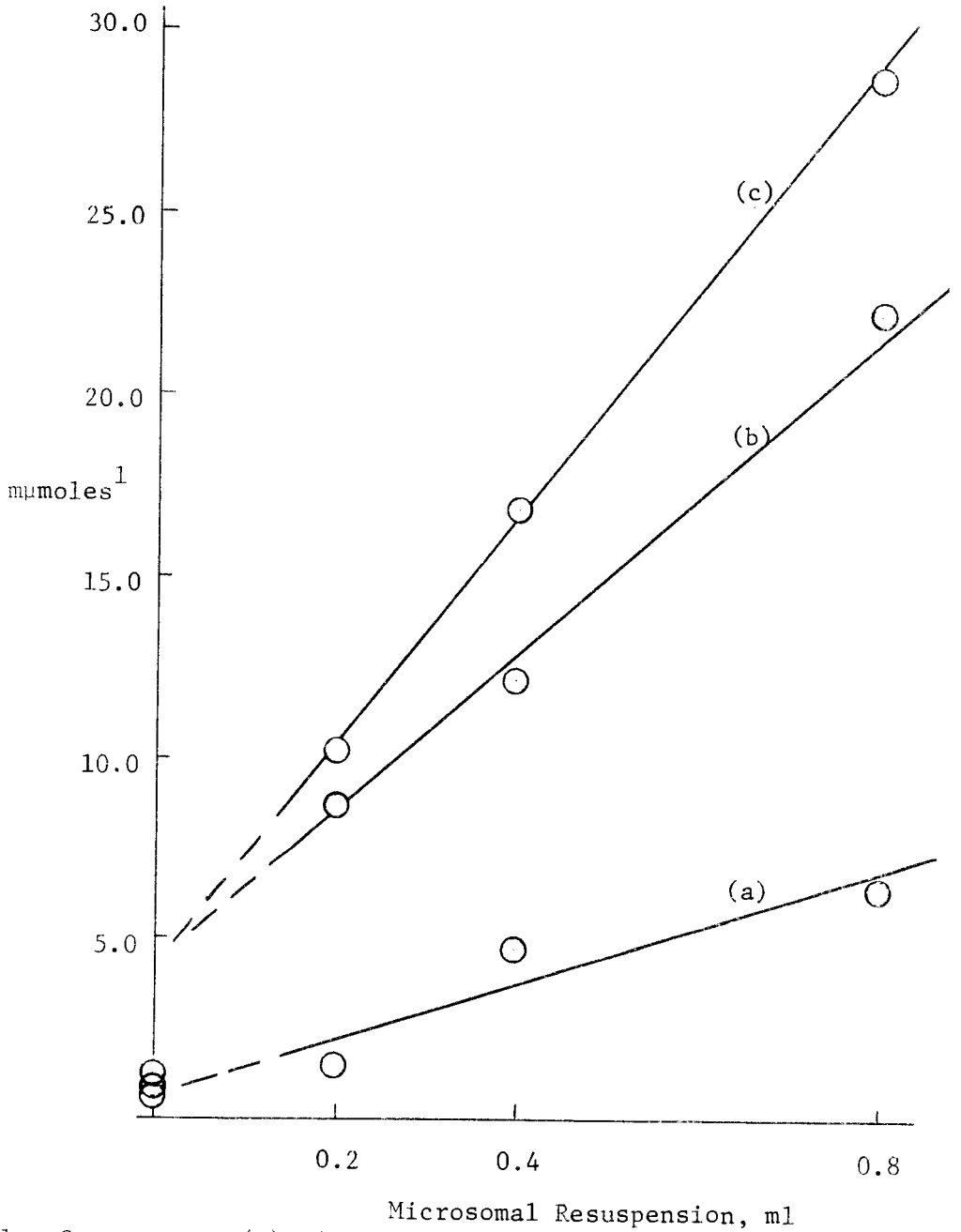
INCUBATION COMPONENTS			mμmole EQUIVALENTS OF WATER SOLUBLE PRODUCTS		
Microsomes 0.3 ml	NADPH 2.0 μmoles	UDPGA 2.0 μmoles	Mobam-R 220 mμmoles	Mobam-C[1] 220 mμmoles	4HBT 180 mμmoles
0	0	0	0.7	3.6	0.5
0	+	0	0.5	3.5	0.5
+	0	0	2.1	4.3	2.9
+	+	0	15.9	30.0	23.4
+	+	+	21.0	30.7	137.0
+	0	+	8.2	4.0	156.0
0	0	+	0.6	3.1	0.6

1. Average recovery ranged from 95% to 105% for different experiments with Mobam-C.

system, markedly lower water soluble activity was produced from Mobam-R while the mμmole equivalents from 4HBT incubations increased slightly (Table II). An explanation of these results is that 4HBT was conjugated with glucuronic acid by a transferase not requiring NADPH, but with Mobam there is a cofactor-dependent step prior to the conjugation reaction with UDPGA.

There was no glucuronic acid effect with Mobam-C showing that a glucuronide of Mobam was not formed in vitro (Table II). Evidence from in vivo studies has supported the identification of the N-glucuronide conjugates of Banol (Baron, 1967) and carbaryl (Knaak, 1965).

The relationship between the amount of tissue and the metabolic activity with incubations of Mobam-R is expressed in Figures 4 and 5. This activity is represented by the sum (curve c) of 4HBT in the ether phase (curve a) (as determined by thin-layer chromatography) and water soluble radioactive products (curve b). That these are linear with increasing microsomes (Figure 4) confirms the enzymatic nature of Mobam metabolism established in Table II. The "b" and "c" intercepts may reflect the endogenous activity of the microsomes not seen in the control with no microsomes (Figure 4).

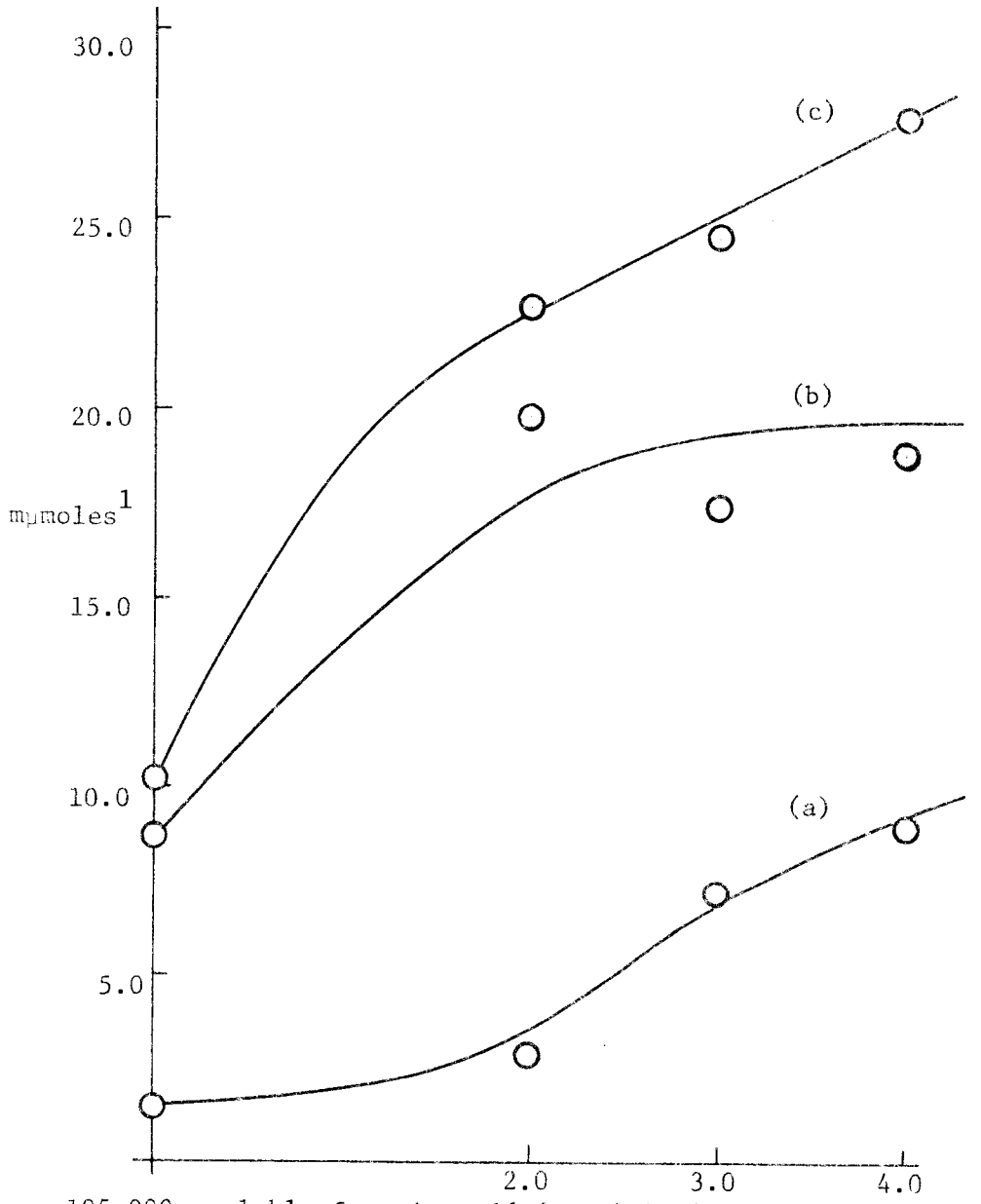


1. Curves are: (a), 4HBT in the ether phase, (b), aqueous soluble products, and (c), the sum of (a) and (b). Total mμmoles of substrate, 100.

Figure 4. Metabolic activity with various amounts of microsomal resuspension, NADPH, and ring labeled Mobam.

Incubation of the 105,000g soluble fraction (2.0 ml), with the NADPH generating system and Mobam-R gave 1.7, 1.4 and 3.1 mμmoles for "a", "b", and "c" respectively, demonstrating that this fraction has a low metabolic capability. However, initial combining of this soluble fraction (2.0 ml) with the microsomes (0.2 ml) resulted in a doubling of water soluble activity compared with similar incubations without the soluble fraction (Figure 5). Additional soluble fraction added to a constant level of microsomes promotes accumulation of 4HBT in the ether phase (curve a) but has no effect on water soluble radioactivity (curve b) (Figure 5). Under similar incubation conditions, the post mitochondrial supernatant fluid (2.0 ml) yielded 4.5, 18.9 and 23.4 mμmoles, values comparable to those of the equivalent reconstituted system of 2.0 ml soluble fraction plus 0.2 ml microsomal suspension (Figure 5).

The 105,000g soluble fraction apparently supplements the microsomal reactions that form water soluble products (curve b), but above a "normal" level (ten to one volume ratio allowing for dilutions during isolation procedures) a limiting factor presumably in the microsomes checks further increases in water soluble ^{14}C (Figure 5). However, the increases in 4HBT production (curve a) with 3.0 and 4.0 ml of soluble fraction added to the Mobam-R incubations suggest that an independent system hydrolyzing Mobam is not limited by the level of microsomes. This could be a soluble enzymes system requiring a specific microsomal cofactor or component



1. Curves are: (a) 4HBT in the ether phase, (b) aqueous soluble products, and (c) the sum of (a) and (b). Total of 100 mμmoles of substrate.

Figure 5. Metabolic activity with various amounts of the 105,000g soluble fraction added to microsomal resuspension, NADPH, and ring labeled Mobam.

for activity. Published results (Oonnithan, 1968) confirm the observation that the soluble fraction will increase the metabolic activity of the microsomal system in the metabolism of the carbamate insecticides.

The paper chromatographic separation of water soluble in vitro metabolites is summarized in Table III which gives representative values from several experiments. With the NADPH generating system, metabolites of the ring labeled substrates generally had Rf values of less than 0.20 and since these products were not well defined, they were summed as "other" in Table III.

Metabolism of Mobam-C yielded a single product of 0.57 Rf value upon incubation with microsomes and NADPH, but since no ring labeled metabolite had the same Rf value, the product must represent the carbamic acid moiety only (Table III). Although methyl urea has essentially the same Rf value (0.58) (Table I) as the Mobam-C metabolite, no direct evidence can be presented to show that they are or are not the same product. In the former case, however, a source of ammonia or an enzyme system capable of forming the product has not been established.

In an attempt to examine secondary (conjugative) microsomal reactions which the carbamate may undergo, two additional cofactor systems were employed. Both systems resulted in the formation of

TABLE III. PAPER CHROMATOGRAPHY OF WATER SOLUBLE IN VITRO METABOLITES

Substrate	Incubation Components: Microsomes	105,000g soluble fraction	NADPH	UDPGA	ATP/$SO_4^=$/Mg^{++}	Rf in Chromatography System I (Table I) and the mμmole Amounts Found			
mμmoles	O_2 ml	2.0 ml	2.0 μmoles	2.0 μmoles	20μmoles 10μmoles 5×10^{-4}M	0.27	0.57	0.65	other
Mobam-R 165	+	0	+	0	0	0	0	0	6.1
Mobam-R	+	0	+	+	0	17.2	0	0	5.1
Mobam-R	+	0	0	+	0	10.9	0	0	0
Mobam-C 220	+	0	+	0	0	0	30.0	0	0
Mobam-C	+	0	+	+	0	0	30.7	0	0
4HBT 112	+	0	0	+	0	109	0	0	0
4HBT 197	+	+	+	0	0	0	0	0	35.5
4HBT	+	+	+	+	0	162	0	0	0
4HBT	+	+	+	0	+	0	0	146	0

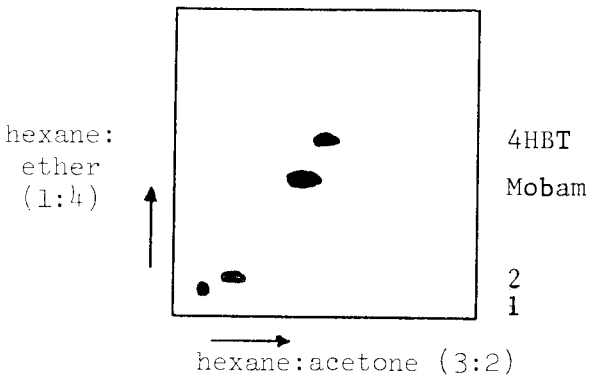
specific metabolites of Mobam or 4HBT. With the ring labeled substrates, incubations with UDPGA (uridine diphosphoglucuronic acid) contained a 0.27 Rf product, but there was no effect on Mobam-C metabolism (Table III). Results in Table II established the nearly quantitative nature of the reaction between UDPGA and 4HBT, and the appearance of the 0.27 Rf product in Mobam-R incubations coincided with markedly lower values for 4HBT in the ether phase (as determined by thin-layer chromatography). The results suggest the 0.27 Rf product is the glucuronide of 4HBT and that this product is formed in 4HBT incubations and in Mobam incubations after hydrolysis of the substrate. Knaak, Eldridge, and Sullivan (1967) have shown that the glucuronides of several phenols were produced in similar incubations containing microsomes and UDPGA. The UDP glucuronate glucuronyl transferase (2.4.1.17) may be responsible for the formation of these glucuronides in the microsomal systems.

The results with UDPGA and those in Table II suggest that the hydrolysis of Mobam may be an NADPH-dependent reaction. Otherwise it would be expected that Mobam-R plus UDPGA would yield the same result with and without NADPH, rather than the 21 mμmoles and 8.2 mμmoles observed (Table II). An alternate scheme is that a cofactor dependent reaction may produce a carbamate of modified structure which decomposes releasing 4HBT. In either case the result is at variance with those published for carbaryl (Krishna, 1966; Oonnithan, 1968), but agrees with the observed NADPH-dependent hydrolysis of the organophosphate insecticide parathion (Nakatsugawa, 1967).

A sulfate conjugation system requiring ATP/$SO_4^{=}$/Mg^{++} is known to exist in the liver homogenate, and when incubations of 4HBT with the post mitochondrial supernatant fluid were fortified with the three cofactors, a specific metabolite of 0.65 Rf was formed (Table III). Although it can only be surmised that the product is the 4HBT sulfate, a useful comparison with an in vivo metabolite will be made in a subsequent discussion.

The ether phase materials resulting from microsomal incubations were separated with thin-layer chromatography. Although most of the radioactivity was present as residual substrate, three ether soluble metabolites of Mobam-R were separated and appear to be produced by NADPH-dependent reactions (Table IV). One of the metabolites is identical to 4HBT. The other two products corresponding to areas "1" and "2" as shown in Table IV, were not detected in Mobam-C incubations and therefore must represent the ring moiety only. Rf values of areas "1" and "2" did not match those of any standard compounds available (Table I) and their nature can only be surmised. One possibility is that these products arise from alterations in the thiophene ring, for example a hydroxylation at the three position, but that they consist of the ring moiety only is apparent. Incubations of 4HBT also result in radioactivity in areas "1" and "2", but the difference in incubations with and without NADPH was not consistent.

TABLE IV. TWO DIMENSIONAL THIN-LAYER CHROMATOGRAPHY OF ETHER SOLUBLE *IN VITRO* METABOLITES



INCUBATION COMPONENTS			mμmole EQUIVALENTS AT			
Substrate[1]	Microsomes	NADPH	1	2	Mobam	4HBT
Mobam-R	0	0	2.7	4.0	210	3.4
Mobam-R	0	+	1.9	0.5	167	2.3
Mobam-R	+	0	1.9	5.3	209	2.4
Mobam-R	+	+	5.6	9.1	178	5.5
Mobam-C	0	0	0.1	0.3	218	-
Mobam-C	0	+	0.1	0.2	201	-
Mobam-C	+	0	0.1	0.4	204	-
Mobam-C	+	+	0.4	0.4	156	-

1. Substrate levels: 220 mμmoles for both Mobam-R and Mobam-C.

In Vivo Experiments

In ten experiments, rats treated with Mobam or 4HBT survived the 72 hour experiment period and showed no signs of toxicity from the 0.58 to 7.3 mg/kg doses. The major portion of the ^{14}C was excreted in the urine or expired as $^{14}CO_2$, while small amounts were generally present in the feces (Table V). In several experiments the feces were contaminated with urine, and therefore no conclusive evidence for direct fecal elimination of the metabolites can be presented. No attempt was made to trap expired metabolic products of the ring labeled compounds since published results show that no more than 0.2% of the dose could be trapped from rats administered ring labeled Banol (Baron, 1967) or carbaryl (Knaak, 1965; Krishna, 1966).

The average recovery of ^{14}C in two 4HBT experiments exceeded the average Mobam-R (ring labeled) value by 12.9% which suggests greater tissue residues may result from treatment with the carbamate (Table V). The presence of the free hydroxyl group in 4HBT would allow direct conjugation of the molecule, but with Mobam either hydrolysis or oxidation must occur first, and during these reactions, a portion of the Mobam may be converted to unexcretable residues. Preliminary tests showed that no tissue residues could be detected sufficient to account for the 10-15% expected, and therefore lower recovery with Mobam-R may also represent error in treatment and analysis.

TABLE V. RECOVERY OF RADIOACTIVITY FROM RATS TREATED WITH MOBAM OR 4HBT

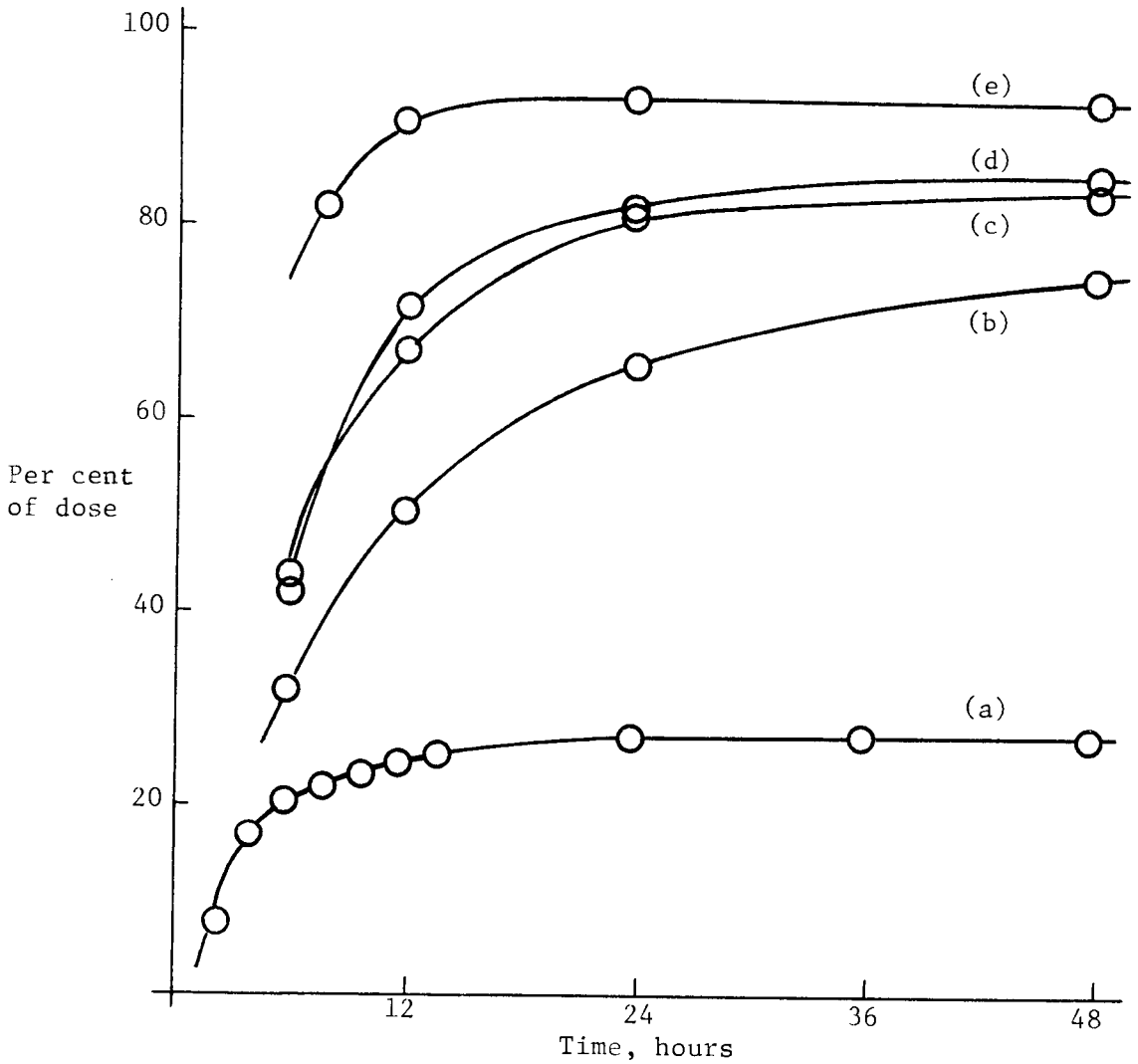
	Treatment	Dose µmoles	Per Cent of Dose Recovered in 72 Hours			
			Urine	Feces	$^{14}CO_2$	Total
1.	Mobam-R, IP[1]	0.96	73.1	11.8	-	84.9
2.	Mobam-R, IP	1.04	78.5	9.8	-	88.3
3.	Mobam-R, IP	1.04	77.9	0.7	-	78.6
4.	Mobam-R, IP	1.15	84.3		-	84.3
5.	Mobam-R, oral	1.0	73.5	9.7	-	83.2
6.	Mobam-C, IP	1.7	46.5	2.7	26.5[2]	75.8
7.	Mobam-C, IP	12.2	38.5	5.9	10	54.4
8.	Mobam-C, IP	12.2	41.5	15.0	10	66.5
9.	4HBT, IP	1.25	101.2		-	101.2
10.	4HBT, IP	1.0	92.4		-	92.4

1. Intraperatoneal route.

2. In two experiments $^{14}CO_2$ was estimated at 10% with a vibrating reed electrometer system; a third experiment in which $^{14}CO_2$ was trapped in NaOH solutions indicated 26.5% had been expired.

It was expected that rats treated with Mobam-C (carbonyl labeled) would expire considerable amounts of $^{14}CO_2$. Presumably carbamic acid formed by hydrolysis is decomposed at physiological pH releasing the carbonyl carbon as CO_2 (Krishna, 1966). In experiment six, 26% of the dose was recovered from NaOH solutions used to trap expired gases, and there was 76% overall recovery of the applied dose (Table V). In two other experiments an estimated 10% of the dose was monitored by a vibrating reed electrometer system, although urine and feces recovery was comparable to that of experiment six. The apparent failure of the vibrating reed system to register all $^{14}CO_2$ is probably responsible for the low overall recovery in the latter two experiments (Table V). Published results confirm the expiration of $^{14}CO_2$ from rats treated with carbonyl labeled carbamates and specifically 32% with carbaryl (Knaak, 1965), 52% with Banol (Baron, 1967) and from 25 to 77% in a study of ten carbamates (Krishna, 1966).

Figure 6 showing the cumulative recovery from all samples illustrates the rapid excretion of radio labeled products from Mobam-R, Mobam-C and 4HBT treated rats. The cumulative recovery of $^{14}CO_2$ in experiment six given in Figure 6 shows that by 24 hours expiration of $^{14}CO_2$ was complete. Similar rates of metabolism have been published for other carbamates (Knaak, 1965; Krishna, 1966), and amply confirm the rapid excretion and expiration of the metabolites.



(a). Experiment 6, Mobam-C; $^{14}CO_2$ only
(b). Experiment 6, Mobam-C; total urine, feces, and $^{14}CO_2$
(c). Experiment 5, Mobam-R; total urine and feces
(d). Experiment 4, Mobam-R; total urine and feces
(e). Experiment 10, 4HBT; total urine and feces

Figure 6. Cumulative recovery from rats treated with Mobam or 4HBT.

Ring and carbonyl labeled Mobam treatment resulted in a different urinary excretion rate (Figure 7). Krishna (1966) assumed that urinary radioactivity from rats treated with carbonyl labeled carbamates represented intact (unhydrolyzed) structures and that the extent of hydrolysis could be determined directly by the amount of $^{14}CO_2$ expired. This assumption is based on the presumed instability of N-methyl carbamic acid under physiological conditions.

However, the results shown in Figure 7 can not be interpreted in a similar manner, since in addition to the vastly different rate of excretion, recovery of the carbonyl label during several time periods consistently exceeded recovery from the ring labeled Mobam. If the carbonyl were attached to the ring moiety, that is unhydrolyzed, it would be expected that at least as much and probably more of the ring than carbonyl label metabolites would be excreted in every time period. No published results allow similar comparisons of the rate of recovery in the urine as shown in Figure 7 for Mobam.

Urine from five rats was examined for free Mobam or 4HBT and ether soluble metabolites by extracting the urine with ether, and in one case (experiment five) sufficient radioactivity was recovered for thin-layer chromatography (Table VI). Separation in the ether-hexane (4:1) system showed 80% of the solubles was 4HBT and the remaining activity migrated near the origin, but had Rf values less

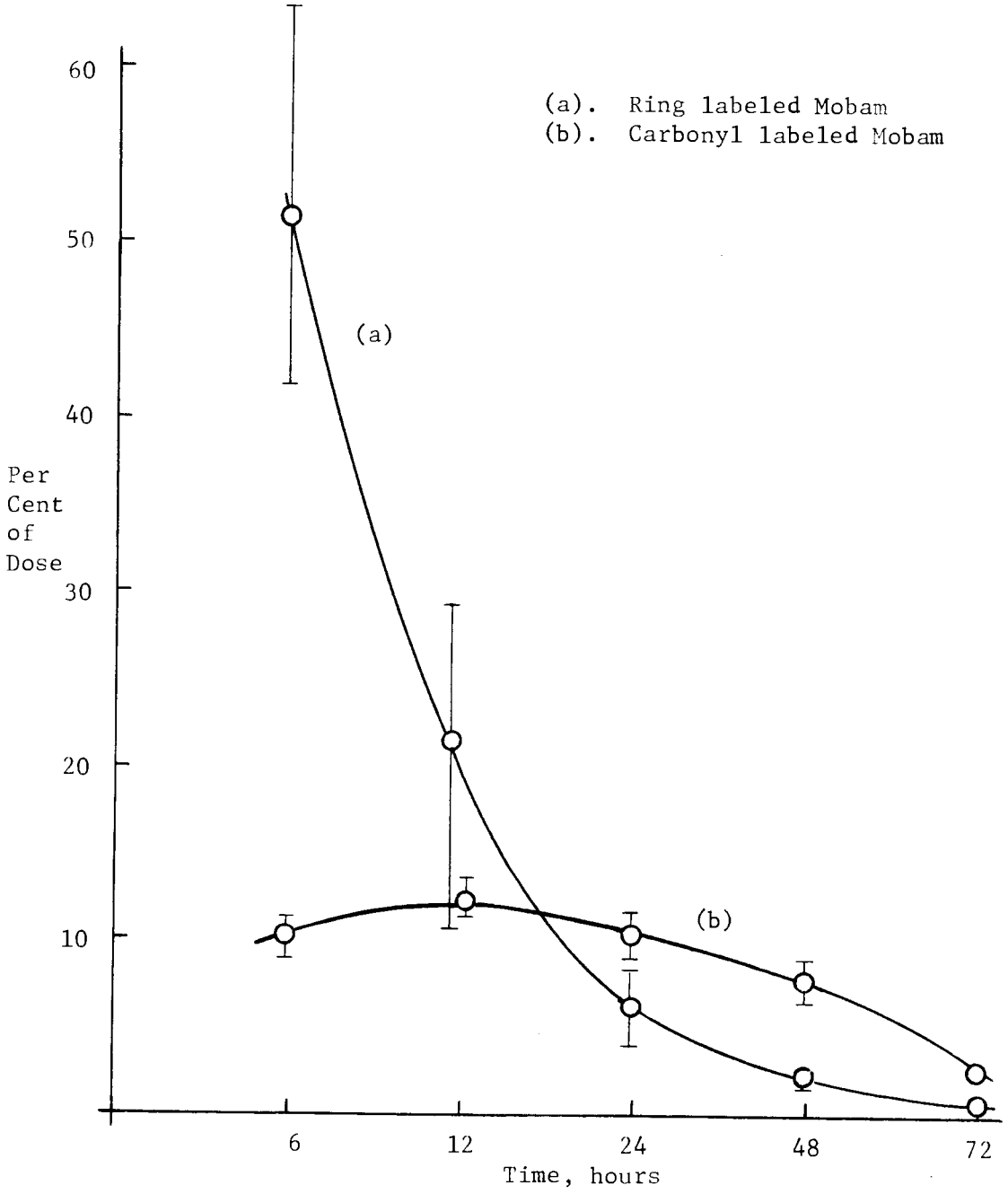


Figure 7. Urinary elimination rate from ring and carbonyl labeled Mobam treated rats.

TABLE VI. ETHER SOLUBLE MATERIAL FROM URINE OF RATS TREATED WITH MOBAM OR 4HBT

	Treatment	Time Urine Collected hours	Per Cent of Sample ^{14}C	Per Cent of Dose
3.	Mobam-R, IP	6	1.4	0.9
		12	-	-
4.	Mobam-R, IP	6	0.8	0.3
		12	0.5	0.1
5.	Mobam-R, oral	6	14.7	6.2
		12	11.7	2.6
7.	Mobam-C, IP	6	1.3	0.1
		12	0.6	0.07
8.	Mobam-C, IP	6	1.0	0.1
		12	0.6	0.07

than any reference compound available (Table I). There was no evidence of the excretion of Mobam.

Results of paper chromatographic separation of water soluble urinary metabolites are summaried in Table VII. From either ring labeled compound, the metabolites separated had comparable Rf values, and urine from rats treated with carbonyl labeled Mobam showed a single major metabolite having a 0.30 Rf value. Several minor metabolites were also observed from Mobam-R and Mobam-C, but no characterization of these products was attempted.

Comparison of the major Mobam-R and 4HBT metabolites not only shows the close relationship between the fate of the two compounds, but further suggests that the Mobam metabolites arose from 4HBT, and must involve hydrolysis of the ester linkage (Table VII). Comparison with *in vitro* metabolism of the ring labeled substrates permits tentative identification of the 0.30 Rf product as the glucuronide of 4HBT. Also, the 0.68 Rf metabolite *in vivo* is probably the same as the 4HBT metabolite from the ATP/$SO_4^{=}$/Mg^{++} fortified *in vitro* system. Cochromatography of *in vivo* and *in vitro* metabolites showed no separation of either of the suggested pairs.

Although the major metabolites of Mobam-C had the same Rf as a Mobam-R and 4HBT metabolite, they are probably not the same products. This can be shown by label position and amount excreted. In the

TABLE VII. PAPER CHROMATOGRAPHY OF AQUEOUS SOLUBLE URINARY METABOLITES

	Treatment	Time Urine Collected	Per Cent of Dose at Rf[1] 0.30	0.44	0.68	Other
1.	Mobam-R, IP	12 hours	6	37	21	8
2.	Mobam-R, IP	24 hours	5	46	16	7
6.	Mobam-C, IP	6 hours	45			1
7.	Mobam-C, IP	12 hours	38			1
9.	4HBT, IP	12 hours	23	23	55	-
10.	4HBT, IP	12 hours	30	30	33	-

case of 4HBT it is not possible that the 0.30 Rf metabolite could be the same as the Mobam-C metabolite since the labeled 4HBT only traces the ring moiety. The approximately 40% of the dose detected as the Mobam-C metabolite is nearly seven fold that of the 0.30 Rf ring labeled Mobam metabolite, thus excluding the possibility that all of the carbonyl label is attached to the ring. The identity of the carbonyl labeled metabolite is not known, but based on chromatography results with standard compounds (Table I), it is not N-methyl urea. The third major water soluble metabolite of Mobam-R and 4HBT (0.44 Rf) is also unidentified, but that it contains the ring moiety only is clear from Mobam-C results (Table VII).

Concluding Discussion

The metabolism of Mobam and 4HBT as discussed in the previous two sections could be summarized as follows:

1. In vitro metabolism by the liver microsomal system produced three ether soluble metabolites of Mobam none of which contained the carbonyl carbon. The three products appeared to be formed by NADPH-dependent reactions.

2. Cofactor dependent reactions also formed limited amounts of water soluble metablites from the carbamate and 4HBT. Only one of these metabolites was characterized by its paper chromatographic Rf value, and this metabolite contained the carbonyl carbon but not the ring moiety. The addition of UDPGA or ATP/$SO_4^{=}$/Mg^{++} as cofactors, resulted in the formation of specific metabolites of 4HBT. One of the metabolites is tentatively identified as the 4HBT glucuronide and the other may be the sulfate conjugate of 4HBT.

3. In vivo, both Mobam and 4HBT are rapidly metabolized to products which are excreted in the urine. More than 80% of the ring moiety is eliminated from the body in this manner. About 25% of the carbonyl carbon of Mobam was expired as carbon dioxide, while the remaining portion appeared in the urine during the first 72 hours after treatment. The expiration of carbon dioxide indicates that Mobam was hydrolyzed and the carbamic acid further decomposed with the breaking of the carbon nitrogen bond.

4. Five metabolites of Mobam have been characterized from the in vivo studies. The carbamic acid portion of the carbamate gave rise to carbon dioxide which was expired and a urinary metabolite not attached to the ring moiety. Three other metabolites were present in urine from rats treated with either 4HBT or Mobam, and they did not contain the carbonyl carbon. One of these metabolites is tentatively identified as the glucuronide of 4HBT and another may be the sulfate of 4HBT. No structure for the third metabolite has been suggested.

Although in vitro studies with other carbamates (Oonnithan, 1968) have demonstrated the formation of ether soluble metabolites containing the intact ester linkage, no similar metabolite or metabolites of Mobam have been observed in microsomal incubations fortified with NADPH. In vivo metabolism of Mobam and 4HBT has shown that the major metabolites of Mobam are also products of 4HBT metabolism. Published results (Knaak, 1965) indicate that carbaryl is only partially hydrolyzed in vivo, but the results Mobam suggest that Mobam is nearly all hydrolyzed in vivo.

These results suggest that Mobam is not readily metabolized by the microsomal mixed function oxidases of the microsomes, but rather the principal detoxication reaction is hydrolysis of the ester followed by conjugation of the phenol. A paper (Robbins, Bakke, and Feil, 1968) to be presented April 3, 1968 will confirm the results of the in vivo studies on Mobam reported here.

V. BIBLIOGRAPHY

Abdel-Wahab, A. M. and J. E. Casida. 1967. Photooxidation of two 4-dimethylaminoary methylcarbamate insecticides (Zectran and Maticil) on bean foliage and of alkylaminophenyl methylcarbamates on silica gel chromatoplates. Journal of Agricultural and Food Chemistry 15: 479-487.

Abdel-Wahab, A. M., R. J. Kuhr and J. E. Casida. 1966. Fate of ^{14}C-carbonyl-labeled aryl methylcarbamate insecticide chemicals in and on bean plants. Journal of Agricultural and Food Chemistry 14: 290-298.

Andrawes, N. R. and H. W. Dorough. 1967. Metabolic fate of carbaryl-naphthyl-^{14}C in Boll weevils and Bollworms. Journal of Economic Entomology 60: 453-456.

Andrawes, N. R., H. W. Dorough and D. A. Lindquist. 1967. Degradation and elimination of Temik in rats. Journal of Economic Entomology 60: 979-987.

Asperen, K. van. 1962. A study of housefly esterases by means of a sensitive colorimetric method. Journal of Insect Physiology : 401-416.

Baron, R. L. and J. D. Doherty. 1967. Metabolism and excretion of an insecticide (6-chloro-3,4-dimethylphenyl N-methylcarbamate) in the rat. Journal of Agricultural and Food Chemistry 15: 830-836.

Blackhall, A. and R. H. Thomson. 1954. Aromatic keto-enols. Part III. Some heterocyclic quinols. Journal of the Chemical Society, 1954, 3916-3919.

Brodie, A. and James R. Gillette and Bert N. La Du. 1958. Enzymatic metabolism of drugs and other foreign compounds. Annual Review of Biochemistry 27: 427-454.

Camp, H. B. and B. W. Arthur. 1967. Absorption and metabolism of carbaryl by several insect species. Journal of Economic Entomology 60: 803-807.

Carpenter, C. P. et al. 1961. Mammalian toxicity of 1-naphthylcarbamate (Sevin insecticide). Journal of Agricultural and Food Chemistry 9: 30-39.

Casida, J. E. 1963. Mode of action of carbamates. Annual Review of Entomology 8: 39-58.

Crosby, Donald G., Eriks Leitis and Ray L. Winterlin. 1965. Photodecomposition of carbamate insecticides. Journal of Agricultural and Food Chemistry 13: 204-207.

Dorough, H. W. 1967. Carbaryl-^{14}C metabolism in lactating cow. Journal of Agricultural and Food Chemistry 15: 261-266.

Dorough, H. W. and J. E. Casida. 1964. Nature of certain carbamate metabolites of the insecticide Sevin. Journal of Agricultural and Food Chemistry 12: 294-304.

Friedman, A. R. and A. J. Lemin. 1967. Metabolism of 2-chloro-4,5-dimethylphenyl N-methylcarbamate in bean plants. Journal of Agricultural and Food Chemistry 15: 642-647.

Gemrich, Edwin G. II. 1967. Enzymatic degradation of 2-chloro-4,5-dimethylphenyl N-methylcarbamate by fat bodies of Blaberus giganteus. Journal of Agricultural and Food Chemistry 15: 617-621.

Hassan, A., Smad Zayed and F. M. Abdel-Hamid. 1966. Metabolism of 1-naphthyl-N-methyl carbamate (Sevin) in rat. Biochemical Pharmacology 15: 2045-2056.

Hodgson, E. and J. E. Casida. 1961. Metabolism of N,N-dialkyl carbamates and related compounds by rat liver. Biochemical Pharmocology 2: 179-183.

Hoo G. E. R. and J. N. Smith. 1967. Oxidation of methyl groups by grass grubs and vertebrate liver enzymes. Biochemical Journal 102: 504-510.

Karinen, J. F. et al. 1967. Persistence of carbaryl in the marine estuarine environment. Chemical and biochemical stability in aquarium systems. Journal of Agricultural and Food Chemistry 15: 148-156.

Knaak, J. B., Jane M. Eldridge and L. J. Sullivan. 1967. Systematic approach to preparation and identification of glucuronic acid conjugates. Journal of Agricultural and Food Chemistry 15: 605-609.

Knaak, James B. and Lloyd J. Sullivan. 1968. Metabolism of carbaryl in the dog. Journal of Agricultural and Food Chemistry 15: 1125-1126.

Knaak, J. B. et al. 1965. The metabolism of carbaryl in the rat, guinea pig, and man. Journal of Agricultural and Food Chemistry 13: 537-543.

Krishna, Jataprolu G. and John E. Casida. 1966. Fate in rats of the radiocarbon from ten variously labeled methyl- and dimethyl carbamate-^{14}C insecticide chemicals and their hydrolysis products. Journal of Agricultural and Food Chemistry 14: 98-105.

Krishna, Jataprolu G., H. Wyman Dorough and John E. Casida. 1962. Synthesis of N-methylcarbamates via methyl isocyanate-^{14}C and chromatographic purification. Journal of Agricultural and Food Chemistry 10: 462-466.

Kuhr, Ronald J. and J. E. Casida. 1967. Persistent glycosides of metabolites of methylcarbamate insecticide chemicals formed by hydroxylation in the bean plants. Journal of Agricultural and Food Chemistry 15: 814-824.

Leeling, Norman C. and John E. Casida. 1966. Metabolites of carbaryl (1-naphthyl methylcarbamate) in mammals and enzymatic systems for their formation. Journal of Agricultural and Food Chemistry 14: 281-290.

Lemin, Alen J., Gerald A. Boyack and Robert M. MacDonald. 1965. Alkyl and halo-phenyl N-methylcarbamates. Journal of Agricultural and Food Chemistry 13: 214-215.

Mason, H. S. 1965. Oxidases. Annual Review of Biochemistry 34: 595-634.

Metcalf, Robert L. and T. R. Fukuto. 1965. Effects of chemical structure on intoxication and detoxication of phenyl N-methylcarbamates in insects. Journal of Agricultural and Food Chemistry 13: 220-231.

Metcalf, R. L., M. F. Osman and T. R. Fukuto. 1967. Metabolism of ^{14}C-labeled carbamate insecticides to $^{14}CO_2$ in the housefly. Journal of Economic Entomology 60: 445-450.

Metcalf, R. L. et al. 1966. Mode of action of carbamate synergists. Journal of Agricultural and Food Chemistry 14: 555-562.

Nakatsugawa, Tsutomu and Paul A. Dahm. 1967. Microsomal metabolism of parathion. Biochemical Pharmacology 16: 25-38.

Natoff, I. L. 1967. Influence of the route of administration on the toxicity of some cholinesterase inhibitors. Journal of Pharmacy and Pharmacology 19: 612-616.

Oonnithan, E. S. and J. E. Casida. 1968. Oxidation of methyl- and dimethylcarbamate insecticide chemicals by microsomal enzymes and anticholinesterase activity of the metabolites. Journal of Agricultural and Food Chemistry 16: 28-44.

Robbins, J. D., J. E. Bakke and V. J. Feid. 1968. Metabolism of 4-benzo-thienyl N-methylcarbamate (Mobam) in rats. Paper to be read before the 155th American Chemical Society meeting, San Francisco, California, April 3, 1968.

Terriere, L. C. 1968. Insecticide-cytoplasmic interactions in insects and vertebrates. Annual Review of Entomology 13: 75-98.

Tsukamoto, M. and J. E. Casida. 1967. Metabolism of methylcarbamate insecticides by $NADPH_2$-requiring enzyme system from housefly. Nature 213: 49-51.

Williams, Elinor, Richard W. Meikle and Carl T. Redemann. 1964a. Identification of metabolites of Zectran insecticide in broccoli. Journal of Agricultural and Food Chemistry 12: 453-457.

Williams, Elinor, Richard W. Meikle and Carl T. Redemann. 1964b. Identification of metabolites of Zectran in dog urine. Journal of Agricultural and Food Chemistry 12: 457-461.

Zayed, Smad, A. Hassan and T. M. Hussein. 1966. Metabolism of carbamate drugs. Degradation of 1-naphthyl-N-methyl carbamate (Sevin) in adult larva of the cotton leaf worm (Prodenia Litura F.). Biochemical Pharmacology 15: 2057-2064.