TABLE I

RATE OF PHOSPHORYLATION OF THYMIDINE BY EXTRACTS OF FUDR-SENSITIVE AND FUDR-RESISTANT MAST CELLS

0.25 ml of supernatant (100,000 × *g* for 1 h) was added to 1.0 ml of a mixture compounded such that these final concentrations were obtained: $8 \cdot 10^{-5}$ *M* [^{3}H]thymidine (9 μC/μmole), $4.2 \cdot 10^{-3}$ *M* ATP, $6 \cdot 10^{-3}$ *M* 3-phosphoglycerate, $4.8 \cdot 10^{-3}$ *M* Mg^{++} and $1.25 \cdot 10^{-1}$ *M* tris(hydroxymethyl)-aminomethane, pH 8.0. Incubations were carried out at 36° and terminated by chilling and addition of 0.1 ml 70% $HClO_4$. $KClO_4$ was precipitated prior to the separation of phosphorylated compounds from thymidine using Dowex 1 × 10 formate[6]. Activity is expressed as moles product/min/mg protein.

Sensitive (P815Y)	*Resistant (P815Y/FUDR)*
$1 \cdot 10^{-10}$	$3 \cdot 10^{-12}$

phosphate, since a strain of P815Y selected for high resistance to FUDR[9], which is apparently deficient in thymidine kinase activity (Table I), is not inhibited by thymidine[5]. The results suggest that in sensitive cells a phosphorylated derivative of thymidine inhibits the conversion of cytidine 5′-phosphate to 2′-deoxycytidine 5′-phosphate, thereby limiting DNA synthesis and cell division.

This work was supported by grants from the American Cancer Society (T-17 and T-23) and from the National Institutes of Health (CRTY-5012 C2), U.S. Public Health Service.

Department of Pharmacology, Yale University School of Medicine, New Haven, Conn. (U.S.A.) N. RONALD MORRIS, GLENN A. FISCHER

[1] M. A. RICH, J. L. BOLAFFI, J. E. KNOLL, L. CHEONG, M. L. EIDINOFF, *Cancer Research*, 18 (1958) 730.
[2] M. T. HAKALA AND E. TAYLOR, *J. Biol. Chem.*, 234 (1959) 126.
[3] G. A. FISCHER, *Proc. N.Y. Acad. Sci.*, 76 (1958) 673.
[4] P. REICHARD, Z. N. CANELLAKIS AND E. S. CANELLAKIS, *Biochim. Biophys. Acta*, in the press
[5] N. R. MORRIS AND G. A. FISCHER, in preparation.
[6] F. J. BOLLUM AND V. R. POTTER, *Cancer Research*, 19 (1959) 561.

Received June 4th, 1960

Biochim. Biophys. Acta, 42 (1960) 183–184

Biological oxidation of N,N-dialkyl carbamates

N-Alkyl and N,N-dialkyl carbamates are widely used as insecticides [1,2] and medicinals for the relief of myasthenia gravis and other disorders[3]. The ultimate mode of action appears, in both cases, to be the competitive inhibition of cholinesterase. Cholinesterase[4] and plasma albumin[2,5] have been implicated in the biological degradation of N-alkyl carbamates. N,N-dimethyl carbamoyl fluoride is hydrolyzed by the plasma A-esterase and certain other esterases in the rabbit[6]. The N,N-dialkyl carba-

Abbreviations: DpNC, N,N-dimethyl *p*-nitrophenyl carbamate; TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide; SKF 525-A, β-diethylaminoethyl diphenylpropylacetate. The chemical designations for insecticides and synergists are given in papers cited[1,2,10,11].

mates derived from aromatic and heterocylic enols are not degraded by these mechanisms[2]. It appeared of interest to examine alternate pathways for metabolism of these compounds.

DpNC was used for preliminary studies because of the ease of analysis for degradation products. The *p*-nitrophenate ion was determined directly at 400 mμ and formaldehyde by the chromotropic acid method[7]. It was found that DpNC was attacked by an enzyme system in rat-liver microsomes requiring TPNH and oxygen. If the liver soluble fraction was present, TPN could be substituted for TPNH (Table I).

TABLE I

LOCALIZATION OF DpNC-DEGRADING ACTIVITY IN SUBCELLULAR FRACTIONS FROM RAT LIVER

Microsomes + soluble was the supernatant fraction ($S_{15\,000}$) after centrifugation of a 20% rat-liver homogenate in potassium phosphate buffer (pH 7.0, 0.05 *M*) at 15,000 × *g* for 30 min. Microsomes were the residue, and soluble the supernatant after centrifugation of $S_{15,000}$ at 105,000 × *g* for 1 h. Microsomes were resuspended in phosphate buffer. DpNC was added first to the flasks in ethanol solution and the ethanol evaporated. Flasks contained 1.0 mg DpNC, 3.7 mg co-factor, 1.5 ml enzyme preparation, 1.0 ml buffer and distilled water to a total volume of 5.0 ml. After 2-h incubation at 37° with shaking in air, the *p*-nitrophenol from subsequent degradation of the metabolite at pH 11.0 was measured.

Fraction	*p-nitrophenol with cofactor indicated*		
	None	*TPN*	*TPNH*
Microsomes + soluble	15	94	120
Microsomes	0	16	120
Soluble	5	11	11
Reconstituted microsomes + soluble	7	120	120

This resembles the N-dealkylation systems which have been described for several drugs[8], but differs in that the methyl group is not liberated as free formaldehyde under the conditions of incubation. A material is formed, however, which yields formaldehyde under strong acid conditions and can be completely extracted into ether from aqueous solution. Free formaldehyde cannot be extracted in this manner. During enzymic oxidation no free *p*-nitrophenol is formed. The metabolite has a hydrolytic half-life of 37 min at pH 8.0 based on liberation of *p*-nitrophenol, which differentiates it from DpNC and N-methyl *p*-nitrophenyl carbamate which have half-lives of over one year and less than 1 min respectively under the same conditions.

The product from DpNC, as analyzed with or without ether extraction, yielded a 1:1 ratio of formaldehyde: *p*-nitrophenol on degradation. A similar product from N-methyl N-ethyl *p*-nitrophenyl carbamate also yielded a 1:1 ratio indicating that only one methyl group is attacked. This same metabolite was demonstrated in the liver of rats 4 h after oral administration of DpNC. By analogy with the mechanism proposed for N,N-dimethyl tryptophane metabolism[9], it appears that the metabolite might be N-methyl N-methylol *p*-nitrophenyl carbamate. The microsome preparation also degraded the higher N,N-dialkyl *p*-nitrophenyl carbamates, the activity decreasing with increasing chain length (N,N-dimethyl > N,N-diethyl > N,N-di-*n*-propyl > N,N-di-isopropyl = N,N-dibutyl). The metabolites formed were less stable to alkaline hydrolysis than that from the dimethyl compound, even though the original compounds are more stable[2].

The microsomal oxidation of DpNC and its N,N-diethyl analog were inhibited by $1 \cdot 10^{-4} M$ SKF 525-A, piperonyl butoxide and sesamex. Determination of the effective inhibitor levels was difficult owing to the insolubility of the substrates and the methylene dioxyphenyl compounds (piperonyl butoxide and sesamex). It is interesting to note that the two methylene dioxyphenyl compounds are effective synergists of the toxicity to house flies for certain N,N-dimethyl carbamates[10], and that toxicity studies with other insecticides and house flies have led to the hypothesis that these synergists may inhibit oxidative detoxification mechanisms[11]. Administration of DpNC to house flies yielded a metabolite which appeared to be the same as that formed in rats or by isolated liver microsomes.

45 N,N-dimethyl carbamates or closely related compounds were incubated with the liver soluble plus microsome fraction with or without TPN fortification to determine the structural specificity for oxidation. Many carbamates including the insecticides Dimetan, Dimetilan, Isolan, Pyramat and Pyrolan and the parasympathomimetic agents neostigmine and physostigmine were metabolized to formaldehyde-yielding derivatives. The formaldehyde yield was markedly increased by the addition of TPN. When the change in anticholinesterase activity during incubation was followed, it was found that Isolan and certain other compounds were rapidly destroyed. However, the loss of anticholinesterase activity did not always correspond with the ability to yield formaldehyde, indicating that certain of the intermediates may themselves be anticholinesterase agents.

This paper is approved for publication by the Director of the Wisconsin Agricultural Experiment Station. The study was supported in part by a Post-Doctoral Fellowship from the Graduate School to E. H. and by grants from the U.S. Public Health Service, National Institutes of Health (Contract RG-5304) and Regional Research Project N.C. 33 on Pesticide Residues. The skilled technical assistance of Miss ANDREA WOODWARD and Miss JUDITH ENGEL is gratefully acknowledged. The carbamates studied were kindly supplied by Dr. H. GYSIN of J. R. Geigy, Basle, Switzerland, Dr. J. A. AESCHLIMANN of Hoffmann-La Roche Inc., Nutley, New Jersey, U.S.A. and others.

Department of Entomology, University of Wisconsin, Madison, Wisconsin (U.S.A.)

ERNEST HODGSON
JOHN E. CASIDA

[1] R. L. METCALF, *Organic Insecticides; Their Chemistry and Mode of Action*, Interscience Publ. Inc., N.Y., 1955, p. 392.
[2] J. E. CASIDA, K-B. AUGUSTINSSON AND G. JONSSON, *J. Econ. Entomol.*, 53 (1960) 205.
[3] A. STEMPEL AND J. A. AESCHLIMANN, Synthetic analogs of physostigmine, in F. F. BLICKE AND R. H. COX, *Medicinal Chemistry*, Vol. III (1956), Chap. 4, pp. 238, John Wiley & Sons Inc., N.Y.
[4] A. GOLDSTEIN AND R. E. HAMLISCH, *Arch. Biochem. Biophys.*, 35 (1952) 12.
[5] J. E. CASIDA AND K-B. AUGUSTINSSON, *Biochim. Biophys. Acta*, 36 (1959) 411.
[6] K-B. AUGUSTINSSON and J. E. CASIDA, *Biochem. Pharmacol.*, 3 (1959) 60.
[7] W. R. FRISELL, L. A. MEECH AND C. G. MACKENZIE, *J. Biol. Chem.*, 207 (1954) 709.
[8] B. B. BRODIE, J. R. GILLETTE AND B. N. LA DU, *Ann. Rev. Biochem.*, 27 (1958) 427.
[9] M. S. FISH, C. C. SWEELEY, N. M. JOHNSON, E. P. LAWRENCE AND E. C. HORNING, *Biochim. Biophys. Acta*, 21 (1956) 196.
[10] M. E. ELDEFRAWI, R. MISKUS AND V. SUTCHER, *J. Econ. Entomol.*, 53 (1960) 231.
[11] Y-P. SUN AND E. R. JOHNSON, *J. Agr. Food Chem.*, 8 (1960) in the press.

Received May 27th, 1960