The fact that the 4-bromo-3-hydroxyl analog is most active may indicate certain steric requirements for binding of the inhibitor to the enzyme. The relatively poorer inhibition of the benzenesulfonyl group may reflect the inability of the bulkier group to enter the binding site of the enzyme.

The two aliphatic aminooxy compounds (VII and VIII) were less inhibitory than the aromatic aminooxy compounds. These aliphatic compounds cause elevation in the γ-aminobutyric acid content of the brain of animals.[10] Aminooxyacetic acid (VIII) inhibits the γ-aminobutyric acid-α-ketoglutaric acid transaminase activity *in vivo*, but does not affect the activity of the glutamic acid decarboxylase.[11] Both enzymes, found exclusively in the central nervous system, however, are inhibited *in vitro* by these compounds and, like histidine decarboxylase, are also PLP-dependent enzymes. The inhibition of the benzyloxyamines is, however, not simply due to a competition with PLP. NSD-1055 also inhibits dopamine-β-hydroxylase, which does not require PLP.[12] Preliminary kinetic studies indicate that the inhibition by NSD-1055 is competitive with substrate as well as with PLP. In addition, Dr. E. E. Snell (personal communication) has found that the molar I_{50} for NSD-1055 with the crystalline histidine decarboxylase from *Lactobacillus* 30*a* is about 10^{-3} M. This bacterial enzyme has been shown to be devoid of PLP by microbiological, enzymatic and spectrophotometric tests.[13] Further studies on the detailed mechanism of inhibition of the various aminooxy compounds and studies of theiri nhibitions *in vivo* are in progress.

Finally, the reaction vessel and procedure used for the assay of histidine decarboxylase, which was also used to assay nonspecific decarboxylase activity, is a useful, simple and rapid procedure for the measurement of the enzyme by the release of $^{14}CO_2$.

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Biochemical Pharmacology, Vol. 18, pp. 685–688. Pergamon Press. 1969. Printed in Great Britain

Oxidative degradation of diazinon by rat liver microsomes*

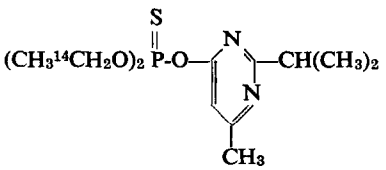
(*Received* 3 *July* 1968; *accepted* 13 *September* 1968)

* Journal Paper No. J-6004 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50010 (Projects No. 1351, 1435 and 1686). This investigation was supported by Public Health Service Research Grant ES-00205 from the Division of Environmental Health Sciences and by Research Grant 12-14-100-8033(51) from the Agricultural Research Service, U.S. Department of Agriculture; it is also a contribution from North Central Regional Project NC-85.

ORGANOPHOSPHORUS insecticides are degraded in animals by cleavage of phosphorus ester linkages. In the case of phosphorothioates, the common route of metabolism is the one leading to the production of dialkyl phosphorothioic acids.[1-4] It has long been assumed that these metabolites were produced by hydrolytic action of phosphatases. Attempts to demonstrate such phosphatases, however, have not been very successful, the only exception being a study concerning housefly enzymes that hydrolyze parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate) to diethyl phosphorothioic acid.[5]

Recent studies showed, however, that parathion is oxidatively cleaved by microsomal enzymes to give diethyl phosphorothioic acid and 4-nitrophenol.[6, 7] Analogs of parathion with 4-nitrophenyl ester structure also undergo similar degradation.[8] The purpose of the present work was to find if such oxidative enzymes also degrade diazinon [*O,O*-diethyl *O*-(2-*iso*propyl-4-methyl-6-pyrimidinyl) phosphorothioate].

Ethoxy-1 ^{14}C-labeled diazinon (1·3 mc/m-mole) was a gift from Geigy Agricultural Chemicals, Ardsley, N.Y.



It was purified by silica gel column chromatography with chloroform as the solvent. Purified diazinon gave single spots on thin-layer chromatograms (silica gel F-254 precoated plates from Brinkmann Instruments Inc., Westbury, N.Y.) when the following solvents were used: hexane:ethyl acetate (2:1); hexane:dioxane (2:1); hexane:*iso*propanol (10:1); chloroform. Positions of radioactivity were detected through a 2-mm slit in aluminum foil by using an end-window Geiger counter. R_f values for diazinon were 0·43, 0·53, 0·46 and 0·22 for the four solvent systems. Identity of the purified chemical was confirmed by infrared spectroscopy against the authentic nonradioactive sample of diazinon supplied by Geigy Agricultural Chemicals. The purified diazinon was dissolved in acetone containing 1% Triton X-100 as emulsifier, and stored at − 15°. Concentration of the radioactive diazinon was determined by gas chromatography as before,[8] except that column temperature was 175° and that $1{\cdot}34 \times 10^{-5}$M nonradioactive diazinon in toluene was used as the external standard. Potassium *O,O*-diethyl phosphorothioate was supplied by American Cyanamid Co., Princeton, N.J. $NADPH_2$ NADP, $NADH_2$ and NAD were purchased from P-L Biochemicals, Inc., Milwaukee, Wis.

Microsomes were prepared from the livers of adult male rats (Dan Rolfsmeyer Co., Madison, Wis.). A 40% (40 g tissue/100 ml) homogenate in 0·25 M sucrose was differentially centrifuged in a Spinco model L2-65 as follows (relative centrifugal forces are the average at the center of the tube): 1600 *g* for 10 min, 10,000 *g* for 10 min and 269,000 *g* for 60 min. The pellet obtained from the last centrifugation was used as the enzyme source. Microsomes were stored at −15° and were resuspended in 0·25 M sucrose immediately before addition to the reaction system.

The standard reaction system contained 5 per cent microsomes, $2{\cdot}5 \times 10^{-5}$M ^{14}C-diazinon, 10^{-3}M $NADPH_2$, 0·15 M KCl, 8×10^{-3}M Na_2HPO_4, 2×10^{-3}M KH_2PO_4 and $6{\cdot}25 \times 10^{-2}$M sucrose. The total volume was usually 4 ml. After incubation for 60 min at 25°, the reaction mixture was shaken three times with 8 ml toluene to remove the substrate. The aqueous phase was filtered to remove coagulated protein. Metabolism was determined by counting the radioactivity of the remaining aqueous phase in a Packard model 3003 counter. Counting mixtures consisted of 0·5 ml of a sample and 20 ml of scintillation solution prepared by mixing 50 g PPO (2,5-diphenyloxazole), 2 g dimethyl POPOP [1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene], 65 ml ethanolamine, 5 l. of toluene, and methanol to make 10 l. Quenching was checked by internal standards.

Analyses for water-soluble metabolites was done by an ion-exchange chromatographic method of Plapp and Casida[9] by using a 1 cm (i.d.) × 21 cm column of Dowex 1-X8 anion-exchange resin. Eluting solutions were: A) elution gradient (100 ml) pH 2 to pH 1 HCl; B) elution gradient (80 ml) pH 1 HCl plus methanol (1:3) to 1 N HCl plus methanol (1:3); C) elution gradient (80 ml) 1 N HCl plus methanol (1:3) to 6 N HCl plus methanol (1:3).

Heated microsomes or microsomes without added cofactor produced no water-soluble metabolite from the ethoxy-1 ^{14}C-labeled diazinon. A very rapid metabolism occurred, however, when $NADPH_2$

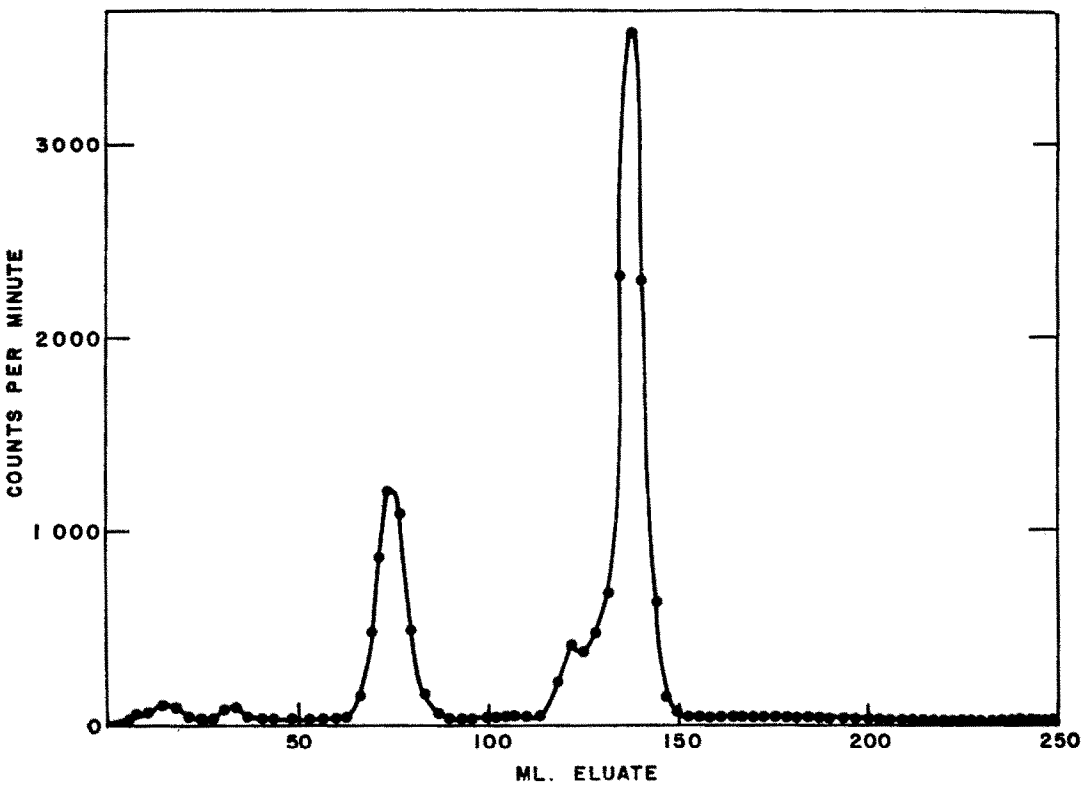


FIG. 1. Ion-exchange chromatogram of water-soluble metabolites of ethoxy-1 ^{14}C-labeled diazinon. Diazinon, $2{\cdot}5 \times 10^{-5}$M, was incubated with 5 per cent rat liver microsomes and 10^{-3}M $NADPH_2$ for 1 hr. After extraction with toluene three times, the aqueous phase was filtered to remove coagulated protein and 7 ml was chromatographed on a Dowex 1-X8 column.

was added. In 20 min, 85 per cent of the substrate was converted to water-soluble metabolites and, in 60 min, the metabolism was nearly complete. Ion-exchange chromatography of the metabolites gave two major peaks (Fig. 1). The smaller peak, representing 23 per cent of the metabolite radioactivity, was probably diethyl phosphoric acid judged by the position of elution. The larger peak contained 69 per cent of the radioactivity and chromatographed identically as diethyl phosphorothioic acid. A minor peak just preceding this larger peak is due to decomposition of diethyl phosphorothioic acid on the column.

The oxidative nature of this metabolism was demonstrated by its requirement for oxygen and a reduced pyridine nucleotide cofactor. Metabolites produced with various cofactors were (cofactor in parentheses): $2{\cdot}25 \times 10^{-5}$M ($NADPH_2$); $1{\cdot}29 \times 10^{-5}$M ($NADH_2$); $0{\cdot}29 \times 10^{-5}$M (NADP); $0{\cdot}67 \times 10^{-5}$M (NAD). Repeated evacuation and filling of Thunberg tubes containing the $NADPH_2$-incubation mixture with N_2 reduced the metabolism of diazinon by 76 per cent compared with a similarly treated system refilled with air.

Since little toluene-soluble material was left after 60 min, it is unlikely that diazoxon [*O*,*O*-diethyl *O*-(2-*iso*propyl-4-methyl-6-pyrimidinyl) phosphate] was present. Ion-exchange chromatography, however, indicated that diazoxon was produced and subsequently hydrolyzed to diethyl phosphoric acid. This acid was not produced from diethyl phosphorothioic acid because the latter acid is little metabolized by the microsomes (unpublished results with ^{35}S-labeled acid). It was probably produced by phosphatases rather than by oxidative enzymes, because degradation of diazoxon was extremely rapid without pyridine nucleotide cofactors. For the same reason, it could not be learned if diazoxon is a substrate for microsomal oxidases.

The results indicate that diazinon undergoes a dual oxidative metabolism similar to parathion, i.e. activation to diazoxon and degradation to diethyl phosphorothioic acid. Oxidative degradation by microsomes is not, therefore, limited to 4-nitrophenyl analogs of parathion, but probably occurs with many phosphorothioate insecticides. Furthermore, such oxidative degradation may be a common metabolic pathway to most P=S compounds. Our preliminary experiments have shown that ^{35}S-malathion (both S atoms labeled) (S-[1,2-*bis*(ethoxycarbonyl)ethyl] *O,O*-dimethyl phosphorodithioate) is also degraded oxidatively by rat liver microsomes (unpublished results); a requirement *in vitro* for oxygen and reduced pyridine nucleotide cofactors has been demonstrated, but the cleavage site (P—S bond or S—C bond) is yet to be determined. (The ^{35}S-malathion was a gift of the World Health Organization, Geneva, Switzerland.) Oxidative cleavage also occurs with at least one P=O compound, *n*-propyl paraoxon (*O,O*-di-*n*-propyl *O*-4-nitrophenyl phosphate).[8] These results emphasize the importance of microsomal oxidation in the degradation of organophosphorus esters. Many of the so-called phosphatase products or hydrolysis products may actually be oxidative metabolites. Existing concepts on organophosphate metabolism may have to be modified considerably.

Acknowledgement—The assistance of Carol S. Ostrem is gratefully acknowledged.

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Biochemical Pharmacology, Vol. 18, pp. 688–690. Pergamon Press. 1969. Printed in Great Britain

Effect of sugars and sugar derivatives on plasma free fatty acid in rats*

(*Received* 5 *August* 1968; *accepted* 11 *October* 1968)

THE INHIBITION of free fatty acid (FFA) release from adipose tissue can be achieved in two ways: by the use of nicotinic acid[1] or pyrazole derivatives[2, 3] which inhibit the lipolytic activity of adipose tissue, or by the administration of compounds such as simple sugars which compete with FFA as energy sources and promote reesterification of FFA in adipose tissue.[4] This study was carried out to test the effects of a number of sugar alcohols and of methylglucamine upon circulating FFA levels. Glucose and fructose were used as controls. In some experiments, partially hydrolyzed starch (Amidex) was also used.

Male Sprague–Dawley rats (150 g) were fasted, and the test compounds were administered orally or i.v. At intervals of 15–240 min, plasma FFA, blood glucose and liver triglycerides were determined

* Supported in part by grants HE-09971 and HE-03299 from the National Institutes of Health, USPHS.