and Bönicke's sampling of sera from a population of patients with and without sarcoidosis may have been too small to represent adequately the two groups. (c) Different techniques were used to measure neutralization. It would appear that the standard neutralization technique used in the present investigation may be more sensitive than that of Mankiewicz or Bönicke because of its quantitative aspects and therefore identifies more sera with neutralizing activity. The method used by the latter was not quantitative. Eloquent descripitions of quantitative methods for neutralization have been described and that of Adams (6) for the *Escherichia coli*-T phage is classic. Bowman (7) showed that the same technique could be applied to mycobacterial phage–antiphage system.

The present experiments indicate that mycobacteriophage R1 would be the best mycobacteriophage for comparing antibodies titers to mycobacteriophages, particularly in the nonsarcoid sera group (see Fig. 3), since almost 100% of these sera had antibodies to this phage at K values in the 1.0–10 decade. These findings could be related to the fact that *M. butyricum*, which is naturally lysogenic with this phage (8), may be ubiquitously distributed in nature. However, this reason does not explain the failure of patients with sarcoidosis to produce R1 antibodies as extensively with K values in the 1.0–10 decade.

Summary. Of 70 sera from different patients with sarcoidosis serum neutralization activity could be demonstrated in 91–98% against mycobacteriophages D29, Leo, or R1. Of 81 sera from nonsarcoid patients, neutralizing activity was demonstrated in 83–85% against phages D29 and Leo, and in 100% against phages R1. In test using phage R1, the magnitude of the K values of sera of patients not having sarcoidosis was much larger than those of patients with sarcoidosis.

1. Mankiewicz, E., Arch. Environ. Health **5**, 122 (1962).

2. Mankiewicz, E., Can Med. Assoc. J. **88**, 593 (1963).

3. Bönicke, R., Arch. Klin. Exptl. Dermatol. **227**, 77 (1966).

4. Bowman, B. U., Proc. Soc. Exptl. Biol. Med. **128**, (1968).

5. Bradley, S. G. and Watson, D. W., J. Immunol. **90**, 782 (1963).

6. Adams, M. H., "Bacteriophages," Wiley (Interscience), New York (1959).

7. Bowman, B. U., Jr., J. Bacteriol. **76**, 52 (1958).

8. Bowman, B. U. and Redmond, W. B., Am. Rev. Respirat. Diseases **80**, 232 (1959).

Received June 19, 1968. P.S.E.B.M., 1968, Vol. 129.

Influence of Induction of Hepatic Microsomal Enzymes by Phenobarbital on Toxicity of Organic Phosphate Insecticides* (33402)

KENNETH P. DUBOIS AND FLORENCE K. KINOSHITA

Toxicity Laboratory, University of Chicago, Chicago, Illinois 60637

The possibility that the toxicity of pesticides and other environmental chemicals may be increased by prior exposure to drugs capable of inducing synthesis of hepatic microsomal drug-metabolizing enzymes is a problem of considerable concern. Since the presence in harvested crops of small quantities of the pesticides used in agriculture is frequently unavoidable, information is needed on the extent to which enzyme induction alters the toxicity of important pesticides. Most of the cholinergic organic phosphates that are commonly used as insecticides are phosphorothioates which must undergo oxidative desulfuration to become anticholinesterase agents. This activation reaction is catalyzed by a microsomal oxidase and drug-induced increases in its activity accelerate

* This study was supported by USPHS Grant No. ES-00109.

the conversion of phosphorothioates to toxic oxygen analogues (1). However, the detoxification of some phosphorothioates is also catalyzed by a microsomal enzyme system (2). Thus the effect of microsomal enzyme-inducing agents on the toxicity of various organic phosphates would be expected to depend upon the extent to which activation and detoxification processes are affected by enzyme induction. The effects of enzyme induction on the toxicity of cholinergic organic phosphates are not predictable for many of the members of this class. The present investigation was undertaken to ascertain the effect of a well-established (3) enzyme inducing agent, phenobarbital, on the toxicity of important cholinergic organic phosphates. It was anticipated that the results obtained with this broad-spectrum inducing agent would be applicable to other chemicals capable of stimulating the activity of microsomal enzymes. Thus, the results obtained using phenobarbital as the enzyme-inducing agent might eliminate the necessity of studying the effects of each potential enzyme inducer on the toxicity of organic phosphate insecticides, especially if enzyme induction by phenobarbital did not increase the toxicity, and consequently the hazard, of the insecticides.

Materials and Methods. Adult, female Holtzman rats and adult, male CF_1 mice were used. The toxicity of the following insecticides was measured in normal and phenobarbital-treated animals: *O,O*-diethyl 0-4-nitrophenyl) phosphorothioate (parathion), *O,O*-dimethyl *O*-(4-nitrophenyl) phosphorothioate (methyl parathion), *O*-ethyl *O*-(4-nitrophenyl) benzene thionophosphonate (EPN), a mixture of *O,O*-diethyl *O*-2-(ethylthio)ethyl phosphorothioate and *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothioate (Systox)®, *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate (Di-Syston)®, *O-O*-dimethyl *S*-[4-oxo-1,2,3-benzotriazin-3 (4*H*) ylmethyl] phosphorodithioate (Guthion)®, 2,3-*p*-dioxanedithol *S,S*-bis(*O,O*-diethyl phosphorodithioate) (Delnav)®,*O,O*-dimethyl 1-methyl-2-carbomethoxyvinyl phosphate (Phosdrin)®, *O,O,O',O'*-tetraethyl *S,S'*-methylenebis-phosphorodithioate (ethion), *O,O*-diethyl *S*-(*p*-chlorophenylthiomethyl) phosphorodithioate (Trithion)®, octamethyl pyrophosphoramide (OMPA), *O,O*-diethyl *O*-3-chloro-4-methyl-1-oxo-2*H*-1-benzopyran-7-yl phosphorothioate (Co-Ral)®, *O,O*-dimethyl *S*-[1,2-bis-(ethoxycarbonyl)ethyl] phosphorodithioate (malathion), *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate (ronnel), and tributyl phosphorotrithioite (Folex)®.

With the exceptions listed below the compounds were dissolved in ethanol (20%) and propylene glycol (80%) and the amount of toxicant in solution was adjusted so that an amount equivalent to 0.1% of the body weight was given intraperitoneally unless low toxicity necessitated administration of greater volumes. Co-Ral was dissolved in Velsicol AR-60. Folex was given as a suspension in ethanol and propylene glycol. Ronnel was melted and given in the undiluted form.

A 14-day observation period was used and death or apparent complete recovery occurred with all of the compounds within this period. The LD_{50} values were calculated from the mortality data by the method of Finney (4) programmed for the IBM 7094 computer by Oldfield *et al.* (5). Sodium phenobarbital was administered as an aqueous solution at a dose level of 50 mg/kg/day.

Results. Table I shows the toxicity of a number of cholinergic organic phosphate insecticides to normal female rats and to rats treated with phenobarbital (50 mg/kg) for 5 days. The toxicity of the organic phosphates was measured 24 hr after the last dose of phenobarbital. A previous study in this laboratory (6) demonstrated that maximal induction of the microsomal enzyme system that catalyzes the detoxification of EPN occurs after 5 days of treatment with phenobarbital. The same study demonstrated that phenobarbital treatment has no direct beneficial effect against EPN poisoning as a result of a pharmacological action by phenobarbital on the central nervous system.

Induction of microsomal enzymes by phenobarbital substantially reduced the toxicity of parathion, Systox, EPN, Di-Syston, Delnav, ethion, Trithion, and Co-Ral to rats (Table I). Smaller decreases in susceptibility to several other organic phosphates were observed. Only one of the compounds (OMPA)

TABLE I. Comparison of Acute Toxicity of Cholinergic Organic Phosphates to Normal and Phenobarbital-Treated Rats.

	Control		Phenobarbital-treated	
Organic phosphate	No. of rats	$LD_{50} \pm SE$ (mg/kg)	No. of rats	$LD_{50} \pm SE$ (mg/kg)
Parathion	41	2.5 ± 0.4	36	7.3 ± 1.3
Methyl parathion	44	7.0 ± 0.6	34	8.0 ± 0.5
EPN	44	7.3 ± 0.5	48	75.0 ± 5.8
Systox	60	1.4 ± 0.1	44	5.8 ± 0.6
Di-Syston	32	2.1 ± 0.1	40	17.0 ± 1.9
Guthion	37	8.7 ± 0.6	35	11.4 ± 1.1
Delnav	32	17.2 ± 0.9	48	118.7 ± 14.9
Phosdrin	35	1.2 ± 0.1	48	2.4 ± 0.4
Ethion	37	25.9 ± 1.8	72	302.6 ± 13.5
Trithion	34	10.1 ± 0.8	40	66.5 ± 5.7
OMPA	39	28.7 ± 1.3	34	14.5 ± 0.8
Co-Ral	45	7.5 ± 0.4	30	13.8 ± 1.1
Malathion	39	619.4 ± 25.0	40	949.9 ± 48.6
Ronnel	25	2822.6 ±137.4	58	3034.8 ±305.1
Folex	44	124.0 ± 8.8	32	171.9 ± 13.3

exhibited increased toxicity to phenobarbital-treated rats.

Table II summarizes the toxicity of several organic phosphates to normal male mice and to mice treated with phenobarbital for 5 days. The changes in suceptibility caused by phenobarbital were similar to those observed in rats but the amount of reduction in toxicity caused by phenobarbital in mice was usually somewhat less than in rats. Among the compounds that were studied the most pronounced reduction in toxicity following phenobarbital treatment was seen with methyl parathion, parathion, Di-Syston, EPN, Trithion, and Delnav. Smaller decreases in toxicity after phenobarbital treatment were observed with most of the other compounds. None of the organic phosphates exhibited increased toxicity to phenobarbital-treated mice.

Discussion. The results of this investigation demonstrated that pretreatment with phenobarbital did not increase the toxicity of any of the organic phosphates to mice and only increased the toxicity of one of the compounds (OMPA) to rats. The toxicity of several of the compounds that are widely used in agriculture was substantially decreased by pretreatment of the animals with phenobarbital. Previous work in this laboratory has shown that a microsomal oxidase system governs the detoxification of EPN (2, 6) and parathion (2). The present study indicates that several other organic phosphates are detoxified by enzymes whose activity is induced by phenobarbital.

The phosphorothioates and dithioates must undergo metabolic conversion catalyzed by a microsomal desulfurase in order to exhibit anticholinesterase activity and toxicity, and this system responds to enzyme inducing agents (1). The present experiments suggest that the toxicity of cholinergic organic phosphates more closely parallels changes in the microsomal detoxification enzymes than in the activation process. OMPA is an exception but its activation involves oxidation of an alkylamide linkage (7) rather than desulfuration.

This study has demonstrated a practical approach to investigation of the effects of enzyme induction on the toxicity of an important class of pesticidal chemicals. The use of a known inducing agent provides a method of predicting the possible effects of exposure to other agents capable of altering microsomal enzyme levels on the toxicity of pesticides. An obvious limitation to the procedure involves the fact that all inducing agents do not have the same potency as stimulators of all microsomal enzyme systems.

Summary. A comparison was made of the

TABLE II. Comparison of Acute Toxicity of Cholinergic Organic Phosphates to Normal and Phenobarbital-Treated Mice.

	Control		Phenobarbital-treated	
Organic phosphate	No. of mice	$LD_{50} \pm SE$ (mg/kg)	No. of mice	$LD_{50} \pm SE$ (mg/kg)
Parathion	57	8.1 ± 0.4	34	14.2 ± 1.1
Methyl parathion	52	9.3 ± 0.8	54	14.3 ± 1.7
EPN	70	23.9 ± 1.1	39	64.4 ± 3.8
Systox	35	3.9 ± 0.1	35	4.8 ± 0.2
Di-Syston	35	6.7 ± 0.6	50	16.3 ± 1.0
Guthion	37	4.0 ± 0.5	48	4.9 ± 0.4
Delnav	75	33.2 ± 2.2	44	87.0 ±10.0
Phosdrin	35	3.2 ± 0.3	30	3.2 ± 0.3
Ethion	36	34.5 ± 5.9	40	40.8 ± 5.5
Trithion	72	27.0 ± 0.8	58	44.0 ± 5.7
OMPA	78	9.6 ± 0.4	70	9.9 ± 0.5
Co-Ral	36	14.7 ± 1.4	36	14.9 ± 1.7
Malathion	48	193.0 ±25.9	42	234.0 ±18.1
Ronnel	38	118.0 ±16.2	40	137.4 ± 7.5
Folex	54	67.7 ± 5.0	60	61.8 ± 5.3

toxicity of 15 cholinergic organic phosphates to normal rats and mice and to animals pretreated with phenobarbital for 5 days. The purpose of the study was to determine whether the hepatic microsomal enzyme induction caused by phenobarbital changed the acute toxicity of the organic phosphates. The study demonstrated that phenobarbital treatment either decreased the toxicity or had no effect on the toxicity of any of the compounds in either species with one exception being an increased toxicity of OMPA to phenobarbital-treated rats.

1. Murphy, S. D. and DuBois, K. P., J. Pharmacol. Exptl. Therap. **124**, 194 (1958).

2. Neal, R. A. and DuBois, K. J., J. Pharmacol. Exptl. Therap. **148**, 185 (1965).

3. Conney, A. H. and Burns, J. J., Advan. Pharmacol. **1**, 31 (1962).

4. Finney, D. J., *in* "Probit Analysis," p. 264. Cambridge Univ. Press, London and New York (1952).

5. Oldfield, D. G., Doull, J., and Plzak, V., Radiation Res. **26**, 12 (1965).

6. DuBois, K. P. and Kinoshita, F., Proc. Soc. Exptl. Biol. Med. **121**, 59 (1966).

7. DuBois, K. P., Doull, J., and Coon, J. M., J. Pharmacol. Exptl. Therap. **99**, 376 (1950).

Received June 28, 1968. P.S.E.B.M., 1968, Vol. 129.

Protection of Mice against Radiation Injury by Phenylhydrazine* (33403)

L. H. SMITH AND T. W. MCKINLEY, JR.

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Phenylhydrazine administration evokes marked erythropoiesis in mammals. The chemical damages erythrocytes, thereby producing an erythropenia which in turn accelerates erythropoiesis. Use of this compound as a means of studying effects of ionizing radiation on hemopoietic tissue was pointed out by Jacobson *et al.* (1), who found less histologic evidence of injury to blood-forming tissue in irradiated rabbits pretreated with phenylhydrazine than in irradiated control animals. Hodgson and co-workers (2, 3) extended this observation and found that rats

* Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.