gest that this approach would produce data more reliable than red cell uptake of radioiron, especially during weak or short-lived effects. Hodgson *et al.*(12) have reached the same conclusion following use of this method to study bone marrow stimulation. These experiments are being extended to determine the limits of accuracy and sensitivity of this method.

Summary. We studied the depression of plasma radioiron turnover rates in rats following X-irradiation. The depression is prompt (maximal by 24-30 hours) and, at 24 hours post-irradiation, is directly related to dose between 50 r and 300 r. Recovery occurs by the 6th post irradiation day, indicating that subsequent anemia is more likely due to hemorrhagic diathesis than bone marrow dysfunction. Sensitivity of the radioiron turnover method permitted a study of bone marrow response at frequent short intervals after irradiation.

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1. Jacobson, Leon O. Hematologic Effects of Ionizing Radiations. *Radiation Biology,* Vcl. I, Part II, Ed. Alexander Hollaender, McGraw-Hill, 1954.

2. Hennessy, T. G., Huff, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 436.

3. Bothwell, T. H., Hurlado, A. V., Donahue, D. M., Finch, C. A., *Blood,* 1957, v12, 409.

4. Huff, R. L., Hennessy, T. G., Austin, R. L., Garcia, J. F., Roberts, B. M., Lawrence, J. H., *J. Clin. Invest.,* 1950, v29, 1941.

5. Wasserman, L. R., Rashkoff, J. A., Leavitt, D., Mayer, J., Port, S., *ibid.,* 1952, v31, 32.

6. Crosby, Wm. H., *Bull. N. Y. Acad. Sci.,* 1954, v30, 27.

7. Loffler, R. K., Rappoport, D. A., Collins, V. P., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 441.

8. Paterscn, J. C. S., *ibid.,* 1957, v96, 97.

9. Schade, A. L., Oyama, J., Reinhart, R. W., Miller, J. R., *ibid.,* 1954, v87, 443.

10. Kahn, J. B., Furth, J., *Blood,* 1952, v7, 404.

11. Chanutin, A., Ludwig, S., *Am. J. Physiol.,* 1951, v166, 380.

12. Hodgson, G., Eskuche, I., Yudilenich, D., Hernandez, P., Toha, J., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 826.

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Potentiation of Toxicity of Malathion by Triorthotolyl Phosphate.* (24668)

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The demonstration by Frawley and associates(1,2) that potentiation of toxicity results from simultaneous administration of ethyl p - nitrophenyl thionobenzenephosphonate (EPN) and S-(1,2-dicarbethoxyethyl) - O,O-dimethyl phosphorodithioate (malathion) to rats and dogs stimulated other investigations (3,4) to ascertain whether various pairs of insecticidal organic phosphates cause a similar effect. In view of widespread use of these insecticides on food crops, the testing of each organic phosphate in combination with each of the others currently used, is now an established procedure essential from standpoint of protection of public health. Potentiation of toxicity which occurs with EPN and malathion has recently been explained by Cook *et al.*(5) and Murphy and DuBois(6) on the basis of ability of EPN to inhibit detoxification of malathion. Detoxification of malathion consists of hydrolysis of 1,2-dicarbethoxyethyl side chains(5,7) and this reaction is catalyzed by non-specific esterase present in liver and some other tissues(6). Under these circumstances it seemed likely that any chemical agent capable of inhibiting non-specific tissue esterases could potentiate toxicity of malathion. Our attention was attracted to finding of Myers and Mendel(8) that triorthotolyl phosphate (TOTP) inhibits aliesterase activity of livers of rats. This compound has the chemical structure shown on page 484. The present investigation was conducted to ascertain whether this agent, used industrially

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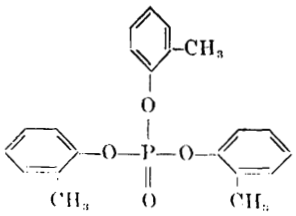

as plasticizer, potentiates toxicity of malathion through inhibition of its detoxification.

Methods and materials. Adult male Sprague-Dawley rats (250-350 g) were maintained in air-conditioned laboratories at 65-75° F and fed Rockland Rat Diet. TOTP was dissolved in sesame oil for intramuscular administration and given intraperitoneally in undiluted form at higher dosage levels or as propylene glycol solution when low doses were administered. For cutaneous application of TOTP the undiluted compound was spread evenly over a circumscribed area 4.5 cm diameter on shaved backs of rats. When doses of less than 250 mg/kg of malathion were given, the compound was dissolved in 10% ethanol and 90% propylene glycol and concentration of malathion was adjusted so that an amount equivalent to less than 0.3% body weight was injected. Doses exceeding 250 mg/kg of malathion were given in undiluted form. In toxicity studies, the animals were observed for 10 days after treatment and LD_{50} values were calculated from mortality data by logarithm-probability method. Cholinesterase measurements were performed manometrically by the method of DuBois and Mangun(9) and measurements of enzymatic detoxification of malathion were made, using the method of Murphy and DuBois(6).

Results. Prior to potentiation tests the acute toxicity of TOTP and malathion to male rats was measured. LD_{50} values were 2500 mg/kg for TOTP and 1100 mg/kg for malathion when the compounds were administered in single doses intraperitoneally. Doses of malathion near the LD_{50} produced typical symptoms of poisoning by cholinergic organic phosphates which began in 15 to 30 minutes after injection and resulted in death following lethal doses in 1 to 3 hours. Symptoms produced by TOTP consisted of general debility, salivation and severe diarrhea but mortality was not observed for at least 18 hours. Differences in time of occurrence of symptoms and death aided in subsequent experiments in determining which compound was the potentiating agent.

Myers and Mendel(8) found that intramuscular injection of 0.1 ml of TOTP on alternate days for 5 days caused 97% inhibition of aliesterase activity of liver and serum of rats. Our initial experiments on the influence of TOTP on toxicity of malathion consisted of administration of 3 intramuscular doses of 440 mg/kg of TOTP on alternate days followed by one-half of the LD_{50} (550 mg/kg) of malathion given intraperitoneally 24 hours after last dose of TOTP. All 3 animals given this treatment died within one hour after malathion, whereas no mortality occurred in controls which received either drug alone. When 3 intramuscular doses of 110 mg/kg were administered, intraperitoneal administration of 550 mg/kg of malathion caused 100% mortality when given 24 hours after last dose of TOTP. When dose of TOTP was reduced to 10 mg/kg, death of all 5 rats given 550 mg/kg of malathion occurred. The particular solvent used for TOTP and malathion was not an important factor in producing potentiation since TOTP (110 mg/kg) given in undiluted form or as sesame oil or propylene glycol solutions caused 100% mortality of rats given 550 mg/kg of malathion 24 hours later. Then a more extensive study was made of the effect of TOTP on susceptibility of rats to acute toxic effects of malathion. Various routes, doses and dosage schedules were used for TOTP. The results are summarized in Table I. TOTP markedly increases susceptibility of rats to malathion. Potentiation occurred regardless of route of administration of TOTP which is explainable on the basis of subsequent experiments which demonstrated that TOTP inhibits detoxification of malathion. The greatest increase in susceptibility was noted when malathion was given 24 hours after intraperitoneal or cutaneous administration of TOTP. The greater toxicity of malathion to rats given TOTP 24 hours earlier as compared with simultaneous administration of the 2 agents indicated that relative time of administration of the 2 agents is

TABLE I. Effect of TOTP on Susceptibility of Rats to Malathion.

TOTP dosage, route of admin.	Time of admin. of malathion	No. of animals	Approx. LD_{50}* of malathion (mg/kg)
No TOTP		32	1100
10 mg/kg, intramuse. on alternate days for 5 days	24 hr after last inj. of TOTP	25	125
110 mg/kg, cutan., single application	24 hr after TOTP	34	12.5
110 mg/kg, intraper.	*Idem*	28	8.2
Idem	Simultaneously with TOTP	22	61
10 mg/kg, intraper.	*Idem*	25	175

* Malathion administered intraper. in all cases.

an important factor in determining degree of potentiation. Deaths usually occurred within 30 to 60 minutes after administration of malathion and TOTP, which corresponds with time of death of animals treated with large doses of malathion alone. No animals treated with TOTP exhibited any untoward effects until malathion was administered. These observations indicated that the only significant action of TOTP was to increase susceptibility of rats to malathion. Further evidence in support of this idea was obtained by experiments in which sequence of administration of malathion and TOTP was varied. When 110 mg/kg of TOTP were given intraperitoneally to 5 male rats 24 hours before injection of 550 mg/kg of malathion, symptoms of malathion poisoning developed rapidly and death of all animals occurred. In contrast, when malathion was administered 24 hours before TOTP, no symptoms or mortality were observed. Evidence that the potentiating action of TOTP is of long duration was furnished by experiments in which 550 mg/kg of malathion were given to groups each containing 5 rats at 1, 3, 7 and 14 days after single intraperitoneal dose of 110 mg/kg of TOTP. One hundred % mortality occurred within 1 hour after administration of malathion in all groups.

Large doses of TOTP are known(10) to inhibit cholinesterase. It was, therefore, considered advisable to initiate our study of the mechanism of increased toxicity resulting from simultaneous administration of malathion and TOTP, by determining whether or not additive inhibitory effects by the 2 compounds occurred. Cholinesterase measurements on brain, submaxillary glands and serum of 3 rats showed that no inhibition of enzyme activity occurs at 24 hours after 3 intramuscular injections of 110 mg/kg of TOTP or after a single cutaneous application of 110 mg/kg of TOTP. In another experiment, groups each containing 3 male rats were given intraperitoneal injections of 110 mg/kg of TOTP, 150 mg/kg of malathion or both agents simultaneously. The animals were sacrificed 30 minutes after treatment and cholinesterase assays were performed on brain, submaxillary glands and serum. Average cholinesterase activity of tissues from 4 untreated rats which served as controls was 100.5 for brain, 24.9 for submaxillary glands and 7.4 for serum expressed in terms of microliters of CO_2/50 mg of tissue/10 minutes. The results of these measurements are summarized in Table II. Cholinesterase activity was not appreciably affected by doses of malathion or TOTP employed when the compounds were given separately. However, simultaneous administration of both compounds at the same dosage levels resulted in marked inhibition of cholinesterase activity of all tissues.

The existing evidence(5,6) that EPN potentiates toxicity of malathion by inhibiting its detoxification suggested that a similar mechanism was responsible for potentiation of toxicity observed with TOTP and malathion, particularly since TOTP is a strong inhibitor of non-specific liver esterases(8). This possibility was tested by measuring the effect of TOTP on enzymatic detoxification of malathion using the method of Murphy and

TABLE II. Effect of Malathion and TOTP Separately and in Combination on Cholinesterase Activity of Rat Tissues.

Compound	Dose (mg/kg)	% of control activity: Brain	Submaxillary gland	Serum
TOTP	110	100.5	91.2	81.1
Malathion	150	100.3	97.2	67.6
TOTP* Malathion	110 150	40.3	49.8	40.1

* TOTP and malathion given simultaneously.

DuBois(6). For this experiment 3 male rats were given 110 mg/kg of TOTP intraperitoneally. The animals were sacrificed 30 minutes later and liver and serum were assayed for ability to detoxify the oxygen analogue of malathion. Assays were conducted on tissues of 3 untreated controls for comparison. Liver and serum of TOTP-treated animals detoxified an average of 0.48 μg and 0.2 μg of malaoxon /mg of tissue /10 minutes respectively as compared with 3 μg for liver and 1.3 μg for serum obtained from control animals. The results indicate that TOTP increases susceptibility of rats to malathion by inhibiting the esterases responsible for its detoxification.

Discussion. The present investigation demonstrated that quantities of triorthotolyl phosphate(TOTP), which produce no apparent deleterious effects, markedly increase susceptibility of rats to malathion. Potentiation of toxicity by simultaneous administration of various pairs of organic phosphates including malathion plus EPN and malathion plus dipterex (O,O-dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate) has been observed by several investigators during past 2 years(1-4). However, in the past the search for agents capable of potentiating toxicity of malathion and other organic phosphates has been limited to testing pairs of compounds used as insecticides. The existing evidence indicates(5,7) that low mammalian toxicity of malathion is highly dependent upon rapid hydrolytic detoxification of the 1,2-dicarbethoxyethyl side chains and that EPN potentiates the toxicity of this compound by inhibiting its detoxification. It thus seemed reasonable to assume that any chemical agent capable of inhibiting hydrolytic detoxification of malathion would potentiate its toxicity. In this connection the known ability of TOTP to inhibit non-specific esterases(8) of liver stimulated our interest in conducting the present investigation. The data obtained during this study demonstrate that toxicity of malathion to rats is markedly enhanced by prior or simultaneous administration of TOTP and that potentiation of toxicity is due to inhibition by TOTP of enzymatic detoxification of malathion. TOTP is effective as a potentiator of toxicity of malathion when it is administered by any route through which a sufficient amount is absorbed to inhibit enzymatic hydrolysis of malathion. The degree of potentiation is greater when TOTP is given prior to malathion so that inhibition of the detoxifying enzyme is maximal before the latter compound is administered. Degree of potentiation obtained with TOTP and malathion was greater than that observed thus far with combinations of any 2 organic phosphorus-containing insecticides which have been studied.

It is clear from our results that the scope of the problem of potentiation of toxicity of organic phosphates extends beyond the possible interference by one insecticidal organic phosphate with metabolism of another compound having a similar chemical structure and use. A complete evaluation of the potential health hazards which might arise as a result of potentiation of toxicity of insecticidal organic phosphates would require consideration of drugs, industrial chemicals, food additives and other chemical agents which commonly gain entrance to the body. The problem is further complicated by the fact that a potentiating agent such as TOTP will inhibit detoxification of malathion at dosage levels which produce no detectable toxic effects. The use of toxicity tests for detection of potentiation by numerous possible combinations of chemical agents which can be ingested simultaneously is not possible from a practical standpoint. However, extension of our present knowledge of the mechanism by which certain compounds act as potentiating agents should greatly facilitate detection of combinations of insecticides and other agents which cause potentiation of toxicity.

Summary. Toxicity of malathion to male rats was markedly increased after treatment of animals with TOTP. At 24 hours after dermal application or intraperitoneal administration of 110 mg/kg of TOTP the LD_{50} of malathion was reduced from normal value of 1100 mg/kg to 12.5 and 8.2 mg/kg respectively. The increased susceptibility of rats to malathion persisted for as long as 14 days after treatment with TOTP. When TOTP was given 24 hours after malathion no potentiation was observed. TOTP inhibited enzymatic detoxification of malathion thus providing an

explanation for its potentiating action. These findings indicate that the problem of potentiation of toxicity of organic phosphate insecticides extends beyond those agents commonly used for insecticidal purposes.

1. Frawley, J. P., Hagan, E. C., Fitzhugh, O. G., Fuyat, H. N., Jones, W. I., *J. Pharm. Exp. Ther.*, 1957, v119, 147.

2. Frawley, J. P., Fuyat, H. N., Hagan, E. C., Blake, J. R., Fitzhugh, O. G., *ibid.*, 1957, v121, 96.

3. Rosenberg, P., Coon, J. M., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 836.

4. DuBois, K. P., *A.M.A. Arch. Indust. Health*, 1958, v18, 488.

5. Cook, J. W., Blake, J. R., Williams, M. W., *J. Assn. Off. Agric. Chem.*, 1957, v40, 664.

6. Murphy, S. D., DuBois, K. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 813.

7. March, R. B., Fukuto, T. R., Metcalf, R. L., Maxon, M. G., *J. Econ. Entomol.*, 1956, v49, 185.

8. Myers, D. K., Mendel, B., *Biochem. J.*, 1953, v53, 16.

9. DuBois, K. P., Mangun, G. H., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 137.

10. Coursey, M. M., Dunlap, M. K., Hine, C. H., *ibid.*, 1957, v96, 673.

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Temperature Tolerance and Kinetics of Thermal Inactivation in *E. coli communior* Phage of Various Concentrations.* (24669)

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Most studies of thermal inactivation of bacteriophages and of other viruses have indicated that the reaction is exponential over a wide temperature range and follows the first order equation $n/n_o = e^{-kt}$(1-4). This paper describes the thermal inactivation of an *E. coli communior* phage in peptone solution; under certain conditions, the kinetics of its destruction appear to deviate from the above relationship.

Materials and Methods. E. coli communior phage(5) suspended in a peptone solution (Difco Bacto-Peptone, 1%, NaCl, 0.5%, pH 7.6) was treated as follows. One ml aliquots of undiluted phage and of samples diluted 1/100 and 1/1000 in peptone solution were either dried *in vacuo* or stored as liquid preparations. For the inactivation studies, samples were heated in a water bath controlled to $\pm$ 0.5°C. The number of particles surviving 30 minutes at a given temperature was determined. In addition, estimates were made of survivors after varying times at different temperatures. The rate constant, K, was calculated from the data. The phage counts were made by the Adams technic(6).

Results. With the dried samples, a linear relationship was found between the logarithm of phage destroyed in 30 minutes and the temperature and this was independent of the initial phage concentration (Fig. 1a). On the other hand, the wet preparations behaved differently. The curves for the inactivation "rate" (logarithm of phage destroyed/30 min) plotted against temperature appeared to show a sigmoidal shape, (Fig. 1b, $1b_1$, $1b_2$). With undiluted phage (Fig. 1b) the change in inactivation rate was exponential between 50° and 75°C. Above this, there was a significant decrease in rate change/degree centigrade. In samples diluted 1/100 and 1/1000 (Fig. $1b_1$ and $1b_2$), the phage was inactivated exponentially between 55°-75°C and 58°-75° C respectively; in each case the rate change reached a plateau beyond 75°C.

The kinetics of heat inactivation in wet (undiluted) and dry conditions were studied by plotting logarithm n/n_o (the fraction of phage survivors), at any given temperature, against time, t, in seconds. In the dry state,

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