Hydrolysis of Methylparathion in Soils

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The possible enzymatic hydrolysis of methylparathion insecticide in soil has been investigated. Methylparathion hydrolyzing activity was partially destroyed by heating and completely destroyed by autoclaving of the soil. The optimum conditions for methylparathion hydrolyzing activity in soil have been established, and found to have an optimum pH value at pH 7. Two saturation constants (K_m) were obtained from a Lineweaver–Burke plot which have the values 1.25×10^{-4} and 5.0×10^{-4} M. Clay minerals did not exhibit any measurable hydrolytic activity on the insecticide; however, preincubation of sterilized clays with the soil or the insecticide resulted in a reduced hydrolytic activity of the soil. Considerable evidence is presented to suggest that methylparathion was hydrolyzed enzymatically in soil.

Methylparathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate) is one of the organophosphorus insecticides widely used in agriculture. It has been tested for its possible use as a soil insecticide to replace the more persistent organochlorine insecticides. Its shorter life in soils was demonstrated to be partly because it is more readily attacked by soil microorganisms (Getzin and Rosefield, 1968; Griffiths and Walker, 1970). Lichtenstein and Schulz (1964) reported that microorganisms were associated with the breakdown of parathion and methylparathion in soil, and that the main pathway of breakdown was hydrolysis resulting in *p*-nitrophenol. Griffiths and Walker (1970) showed that both parathion and *p*-nitrophenol are decomposed by soil microorganisms. They isolated the microbes that attack *p*-nitrophenol and identified them as *Pseudomonas*; however, they did not isolate the organisms that degrade parathion.

Soil enzymes (e.g., phosphatases) may play an important role in the degradation and breakdown of parathion in the soil. They could be responsible or at least a major contributor to the first step in methylparathion degradation in soils in the mechanism suggested by Lichtenstein and Schulz (1964). Getzin and Rosefield (1968) found that sterilization of soil samples by autoclaving destroyed 90% of the degradation activity, while γ-radiation treatment (4 mrads, at 250000 rads/h) hardly reduced it. They were able to extract a fraction with 0.2 N NaOH that could actively degrade malathion.

This report gives results of an investigation on the possible enzymatic hydrolysis of methylparathion in soils. This was carried out by using methylparathion as a substrate and measuring the *p*-nitrophenol released after the hydrolysis of the insecticide. The effects of some factors such as soil pH and clay minerals on the hydrolytic activity were also investigated.

MATERIALS AND METHODS

Methylparathion, a pure crystalline white material, was obtained from Agricultural Division, Monsanto Co., St. Louis, Mo. *p*-Nitrophenol was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.

A surface sandy loam soil was collected from the University Farm at Abis, Alexandria, Egypt, air-dried, sieved through a 1-mm sieve, and stored in polyethylene bags. The soil contained 74% sand, 14% silt, and 12% clay. The organic matter and total N contents were 1.30 and 0.16%, respectively. The soil pH was 7.18 in 10:1 aqueous suspension. Pure clay minerals were obtained from Ward's Natural Science Establishment, sieved through a 400-nm sieve, and used in their natural state.

The hydrolysis assays were carried out in open 250-ml Erlenmeyer flasks at 25 ± 1°C on a rotary shaker at 70 rpm. Two grams of soil (based on dry weight) was buffered with 20 ml of 0.4 M sodium acetate solution to pH 7.0. Five micromoles of methylparathion (in 0.1 ml of ethanol) were added to the soil suspension and the mixture was shaken for 4 h. Incubation was stopped with 4 ml of 0.1 N NaOH. The suspensions were centrifuged at 3000 rpm for 15 min and *p*-nitrophenol was determined in the supernatant at 405 nm in a Bausch and Lomb spectrophotometer according to the method of Neal and DuBois (1965). Blanks were carried out simultaneously, utilizing substrate, buffer, and autoclaved soils for each determination. For heat sterilization, moist soil samples were autoclaved 1 h at 15 psi three times at weekly intervals. Clay minerals used in some experiments were sterilized. The results are expressed as nanomoles of *p*-nitrophenol formed per hour per gram of soil at 25°C (nmol of PNP/h per g of soil). All treatments included triplicate samples.

RESULTS AND DISCUSSION

In the present investigation methylparathion hydrolyzing activity yielding *p*-nitrophenol in soil was studied. Preliminary experiments were carried out to establish incubation conditions of the soil with methylparathion. A study on the effect of the amount of the soil used on methylparathion hydrolyzing activity showed that the activity per gram of soil was constant and independent of the amount of soil used and 2.0 g of dry soil was suitable for measuring the hydrolytic activity. Another experiment was carried out to determine the effect of sodium acetate solution to soil ratio on the hydrolytic activity. The results indicated that the dilution ratio 10:1 gave the highest methylparathion hydrolytic activity. The effect of incubation time on the methylparathion hydrolytic activity was studied by varying the incubation time from 2 to 10 h. The results showed that the rate of hydrolysis of methylparathion decreased later in the incubation period, and so a 4-h period was chosen.

Figure 1 illustrates the effect of preheating of the dry soil at different temperatures, i.e., 75, 140, and 160°C for different time intervals, i.e., 1, 2, and 4 h. The data indicate that methylparathion hydrolytic activity decreased by increasing the temperature and the time of heating. Soil heated at 160°C still retained 1 to 4% of methylparathion hydrolyzing activity. These results are in agreement with the results of other workers that soil is an

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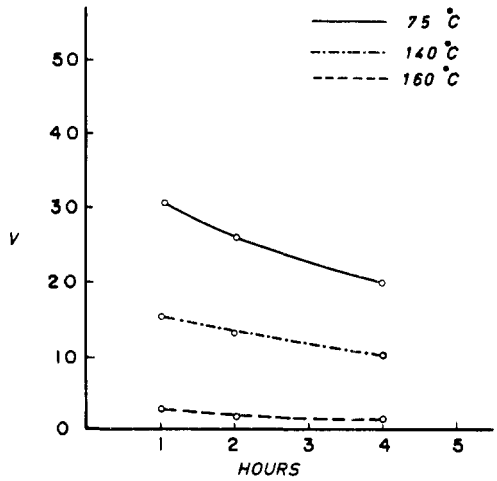

Figure 1. Effect of heat on methylparathion hydrolyzing activity in soils.

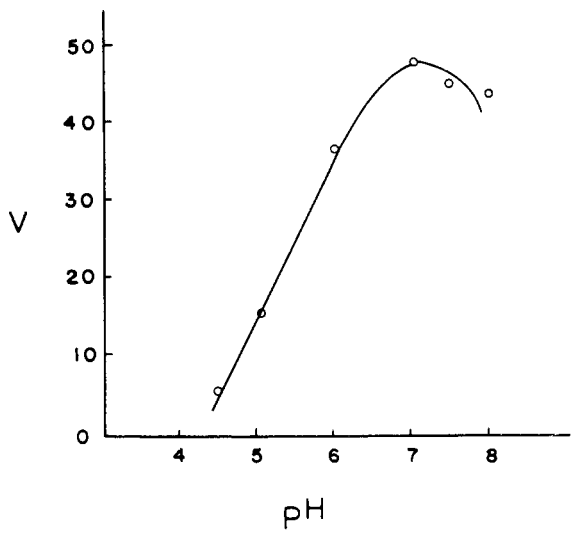

Figure 2. Methylparathion hydrolyzing activity as a function of pH.

excellent protection against heat sterilization for microorganisms and also for enzymatic activities (Skujins, 1967). However, methylparathion hydrolyzing activity was easily destroyed by autoclaving. Although these results are again in harmony with the effect of temperature on soil amylase (Hofman and Hofman, 1953), the possibility of nonenzymatic activity cannot be completely ruled out.

In a separate experiment, several clay minerals (kaolinite, bentonite, attapulgite, and allophane), gibbsite, brucite, hematite, and $CaCO_3$ were incubated after sterilization with methylparathion for more than 10 h to test for possible nonbiotic hydrolysis of methylparathion. The results indicated that there was no measurable hydrolysis produced by the tested materials. This supports further the conclusion that the hydrolysis of methylparathion was not due to the nonbiotic soil components but rather to a heat sensitive component, probably a hydrolytic enzyme system. This conclusion is in harmony with the finding of Getzin and Rosefield (1968) that organophosphorus insecticides were degraded by heat-labile substances in soil.

The pH–activity curve for methylparathion hydrolyzing activity is presented in Figure 2. Acetate buffer was used in the range pH 4.5–7.0 and Tris-HCl was used in the range pH 7.0–8.0. No difference was found between the two buffers. Appropriate blanks were carried out simultaneously, which included the substrate, appropriate buffer, and autoclaved soil. The methylparathion hydrolyzing

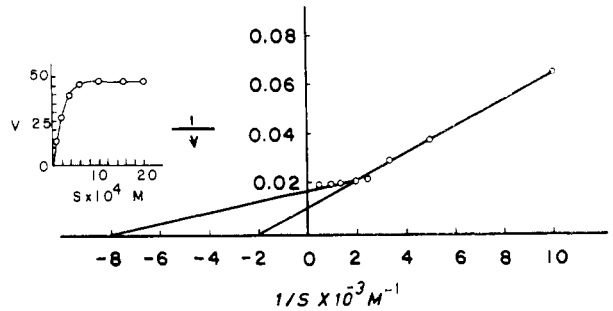

Figure 3. Lineweaver-Burke plot of $1/S$ vs. $1/V$ (S = molarity, V = nanomoles of PNP per hour per gram of soil; V_{max} = 55.6 nmol of PNP/hr per g soil) and effect of concentration of methylparathion (S) on the rate of its hydrolysis (V).

activity clearly demonstrated its maximum activity at pH 7.0. No attempt was made to pursue the reaction beyond pH 8 since methylparathion is unstable at higher pH values.

Although it is realized that Michaelis–Menten kinetics may not apply for these systems, the data suggest biological decay, that is essentially exponential. A reciprocal Lineweaver–Burke plot was made in order to obtain a saturation constant for this soil, a value that may be useful in characterizing the soil enzyme. Figure 3 shows two arbitrary saturation constants having values of 0.125 and 0.50 M obtained by extrapolating the two extremes, and the maximal velocities (V_{max}) were found to be 55.6 and 100 nmol of PNP/h per g of soil, respectively. The dependence of the velocity (V) of the reaction upon the substrate concentration is also shown in Figure 3, which illustrates that the maximal substrate concentration was 6.0×10^{-4} M. Since the system studied in this investigation was a soil suspension system, the maximal substrate concentration value of 6.0×10^{-4} M only pertains to this soil and soil–enzyme mixture. A true maximal substrate concentration for the system could only be obtained if it were possible to examine the kinetics of the enzyme in the absence of soil.

The presence of more than one form of methylparathion hydrolase may be inferred by the two saturation constants in the Lineweaver–Burke plot. The wide K_m range obtained may be explained by the existence of several groups of hydrolytic microorganisms in the soil in addition to the various plant sources. Aside from wide variability of hydrolase producing sources which are expected to give rise to different K_m values, the two extremes may prove to be constants pertaining to enzymes in a free state (1.25×10^{-4} M) and that in the highest state of adsorption on soil constituents (5.0×10^{-4} M). Thus, the latter value may only be an apparent constant related to the true value by an expression describing substrate distribution between the solid and solution phases.

Since clay minerals were established to have no ability to hydrolyze methylparathion, their role in influencing methylparathion hydrolysis was investigated. Several clay minerals were used to cover a wide variation of clay surface properties, i.e., kaolinite, bentonite, illite, and vermiculite. The purpose of these experiments was to study the effect of adsorption of methylparathion by clay minerals on its subsequent hydrolysis by soil enzymes. Table I shows the effect of preincubation of sterilized clay minerals with methylparathion for 10 h before the addition of soil on methylparathion hydrolyzing activity. Controls were prepared by incubating the same amount of soil with the same quantity of methylparathion without the addition of clay. The effect of preincubation of sterilized clay

Table I. Effect of Preincubation of Methylparathion or Soil with Some Clay Minerals on Soil Methylparathion Hydrolyzing Activity

Clay mineral	Preincubation of methylparathion,[a] act., nmol of PNP/h per g of soil		Preincubation of soil,[b] act., nmol of PNP/h per g of soil	
	1 g of clay	2 g of clay	1 g of clay	2 g of clay
Control[c]	56	56	56	56
Kaolinite	54	46	38	36
Bentonite	49	34	34	32
Vermiculite	34	23	32	23
Illite	47	33	33	29
Attapulgite			44	30

[a] Sterilized clay minerals were preincubated with methylparathion for 10 h before addition of the soil. Methylparathion hydrolyzing activity was assayed as described under Materials and Methods. [b] Sterilized clay minerals were preincubated with soil for 10 h before addition of methylparathion. Methylparathion hydrolyzing activity was assayed as described under Materials and Methods. [c] Controls were carried out by incubation of methylparathion and soil without addition of clays.

minerals with soil for 10 h before the addition of methylparathion on its hydrolysis is shown in Table I. Controls of sterilized clays and methylparathion showed no activity. The effect of sterilized clay minerals in reducing or inhibiting methylparathion hydrolyzing activity is in harmony with the results of McLaren and Peterson (1967) and Galstyen et al. (1968). Aomine and Kobayashi (1964) found that allphenic clay inhibited protease, amylase, and cellulase. Clays were found to inhibit glucose oxidase and invertase (Zviaginstev and Velikanov, 1968). Galstyen et al. (1968) reported that the most active fractions of soil particles in fixing added enzymes were silts and clays. In this study kaolinite had the smallest effect which might be attributed to its smaller surface area. Methylparathion is known to have a dipole with cationic and anionic nature (Yaron and Saltzman, 1972). It may be suggested that both the clay and substrate compete for the same active sites of the enzyme. Since it is highly unlikely that the enzyme was transferred from soil to clay, the probable explanation is that adsorption of the substrate on the clay reduced the reaction rate. The nature of the substance in soil that hydrolyzes methylparathion to yield *p*-nitrophenol is under investigation. Its biotic nature, however, is suggested by the fact that neither sterilized pure clays nor autoclaved soil exhibited the capacity to hydrolyze methylparathion. Enzymes are a possible explanation for the biotic substances which could be derived from decaying vegetation or microorganisms.

The results of the present investigation suggest that methylparathion hydrolyzing activity might be attributed to soil enzymes; however, the possibility of nonenzymatic activity cannot be completely ruled out.

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Studies on the Adsorption and Interaction of 1,2-Dibromo-3-chloropropane. I. With Montmorillonites

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The adsorptive behavior of 1,2-dibromo-3-chloropropane on montmorillonite suspensions as affected by saturating cations yielded H class isotherms. Rapid initial adsorption pointed to chemisorption. The data agreed with the Langmuir equation. No desorption of the pesticide occurred on treatment of the complex with organic salts suggesting a strong nonionic electrostatic binding of the chemical to the clay surface. Electrical conductivity and pH observations were in accordance with the formation of an ion–dipole complex. X-ray diffraction showed a maximum interlamellar expansion of 6.42 Å, confirming an upright monomolecular ion–dipole bonded adsorption of the chemical with penetration and swelling of the substrate micropores by the solute.

While the adsorption and interaction of ionic polar pesticides (Nearpass, 1971; Weber, 1970) with clay minerals have been extensively studied in recent years, relatively little work has been done on the mechanism of adsorption of nonionic insecticides. A study of the adsorption and interaction of pesticides with clays and soils is of great importance, because processes such as effective pesticidal action, the persistence, the chemical and biodegradation, the leachability and translocation, and the toxicity of a pesticide depend to a great extent upon the nature of adsorption of the organic chemical by the silicates (Singhal and Singh, 1974; Bailey and White, 1970), although soil organic matter also has a profound influence on these factors. Montmorillonite is an important constituent of

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