likely to be negligible. However, if desired, dilute HCl in which CO_2 is less soluble may be used as a substitute for the water in the measuring cylinder.

A few precautions should be pointed out here. The rubber stopper should be secured tightly enough to prevent a gas leak. The rubber tube connecting the vial and the measuring cylinder should be secured after the rubber stoppers are closed safely, so that there will be no air pressure changes in the system and the pressure inside and outside of the system will be equal before the chemical reaction takes place.

No attempt was made to distinguish between the calcite and dolomite. The technique gives only a calcium carbonate equivalent, or a total lime content.

The carbonate content of the 12 profiles as determined by this method is shown in Table III and also diagramatically in Figure 2. The duplicate analyses of 26 of 50 soil samples were identical and the remainder had 0.28% variation from their means. There were great variations in concentration and distribution of lime contents in the soils both vertically and horizontally. The distance between two adjacent profiles was usually less than 200 feet. The range of lime content of the soils tested was from 0 to 50%. The calcium carbonate equivalents of limestone from two profiles range from 70 to 100%. The wide variation in lime content within the soil catena studied is not unusual with the high heterogeneity of the soils in color, texture, structure, and horizon depth (Table III). However, most of the top soils were black when wet. It would be rather difficult for an individual, without a chemical test, to realize that there were such great differences in lime content among soil samples within such a small area of the farm. Agricultural experiments of a comparison nature, when carried out on soils with a similar characteristic, would expect considerable errors.

From the foregoing context it is concluded that the rapid method described herein for determining carbonates in calcareous soils and in liming materials appears to be satisfactory and useful.

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INSECTICIDE ACTIVITY IN MAN

Urinary Excretion of Paranitrophenol by Volunteers Following Dermal Exposure to Parathion at Different Ambient Temperatures

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The rate of absorption of parathion, as reflected by urinary excretion of paranitrophenol, was measured in human volunteers exposed to 2% parathion dust at different ambient temperatures. Rates of paranitrophenol excretion varied directly with temperature between 58° and 105° F. Maximum excretion occurred 5 to 6 hours following initiation of exposure. Neither depressions in cholinesterase activities nor clinical symptoms were observed following the exposures used.

THE ORGANIC phosphorus insecticide parathion is a useful material for controlling insects of economic and public health importance. It has a high toxicity to humans, however, and each year deaths are attributed to its use. In an effort to understand more fully the toxic hazards associated with parathion, studies have been made of factors that influence the mechanisms and rates of absorption. Two methods have been used to measure parathion absorption. They are determination of activities of cholinesterases, enzymes that are inhibited by parathion, and quantitation of a urinary metabolite, paranitrophenol (*3*).

Excretion of paranitrophenol and depression of cholinesterase activities reflect different physiologic functions. Changes in enzyme activity ordinarily cannot be determined by the Michel method following brief light exposures, because normal variations in the method are greater than the changes produced by the compound (*1*). Paranitrophenol assay is useful under these conditions however, since microgram quantities can be measured and the metabolite is absent from the urine of unexposed individuals. The quantity of paranitrophenol excreted indicates the magnitude of absorption and, less directly, the magnitude of exposure, but it does not necessarily measure the physiologic effects of the exposure. Changes in cholinesterase activities reflect physiologic effects resulting from parathion.

A direct quantitative correlation between changes in enzyme activities and paranitrophenol excretions has not been

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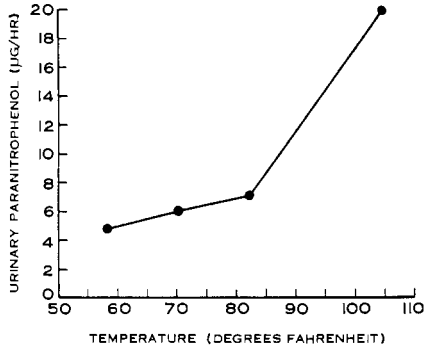

Figure 1. Average hourly rates at which p-nitrophenol was excreted in urine for 41 hours following dermal exposures sustained at different temperatures to 5 grams of 2% parathion dust by three volunteers

Table I. Paranitrophenol Excreted by Human Volunteers Following Dermal Exposures to 2% Parathion Dust at Different Ambient Temperatures

Hours after Start of Exposure	Paranitrophenol Excreted, μg. per Hr.							
	58° F.[a]		70° F.[a]		82° F.[a]		105° F.[a]	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1–2	4.1–5.4	4.8	3.7	3.7	5.3–13.5	8.2	15.8–135.8	59.7
3–4	6.1–8.9	7.5	22.3	22.3	5.3–17.0	10.4	15.8–135.8	69.8
5–6	4.0–15.4	9.7	25.0	25.0	18.8–24.2	21.9	48.2–155.3	88.6
7–8	2.4–4.0	3.2	15.1	15.1	14.5–18.3	16.4	17.8–106.9	66.1
9–10	2.0–6.9	5.4	13.7	13.7	12.8–19.6	16.9	13.2–69.2	35.9
11–15	6.3–7.2	6.7	12.0	12.0	7.0–15.0	11.4	12.2–38.3	20.3
16–20	4.5–8.7	6.6	10.2	10.2	3.7–8.9	5.7	4.8–27.4	13.2
21–30	1.6–2.1	1.8	5.2	5.2	2.3–2.6	2.4	4.1–11.7	7.5
31–40	1.7	1.7	1.9	1.9	2.3–6.2	3.4	2.5–6.9	4.8
41+	...	...	1.2	1.2	2.1–4.0	3.0	1.5–4.3	3.2

[a] Two exposures were sustained at 58° F., one at 70° F., three at 82° F., and nine at 105° F.

demonstrated in humans under controlled conditions. Most of the available information on this relationship was obtained from studies of persons suffering occupational exposure under conditions that precluded precise measurement of the duration of exposure, quantities of toxicant per unit area of body surface, and temperature (*1*). This report presents data on sequential excretions of paranitrophenol and activities of cholinesterases in blood obtained from human volunteers exposed percutaneously to parathion dust under controlled conditions.

Procedure

Each of four volunteers sustained dermal exposures at different temperatures after 2% parathion dust was applied to the right hand and forearm. Volunteers were exposed at weekly or less frequent intervals at 58°, 70°, 82°, and 105° F. The order of exposure was selected at random. Two individuals sustained exposures at 105°, 82°, and 58° F.; one at 105° and 70° F.; and one at 105° F.

For each exposure, 5 grams of 2% parathion dust was placed in a polyethylene bag wide enough to accommodate the hand and long enough to cover the hand and forearm to the elbow. The formulation used was a commercial preparation of 2% parathion on pyrophyllite, the actual chemical content of which was not determined. The hand and forearm were secured in the bag with masking tape, and the toxicant was distributed as evenly as possible over the exposure area. The hand and forearm, still in the plastic bag, were then placed in a constant temperature chamber, and the chamber port was secured with rubber damming and masking tape. Exposures continued for 2 hours. To ensure only dermal exposure, the subjects breathed from a source of pure compressed air while being placed in the exposure situation and during decontamination. Following the 2-hour period, the hand and arm were decontaminated by scrubbing the exposed area with soap and water and a hand brush for 5 minutes. This was followed by two washings in ethyl alcohol contained in a polyethylene bag.

Urine was collected as individual voids for 40 or more hours following exposures. Voids were kept separate, and the time and date recorded on each sample bottle so that hourly excretion rates could be determined for each void. Intervals varied from 30 minutes to $8^1/_2$ hours. Each sample was assayed for paranitrophenol by the method of Elliott *et al.* (*3*). Average excretion rates were calculated at 2-hour intervals for the first 10 hours following exposure. This was done by dividing the total paranitrophenol excreted during each period by the time interval corresponding to the void. Rates were determined at 5-hour intervals for the next 10 hours and at 10-hour intervals for the remaining period.

Determinations for cholinesterase activity were made on blood samples taken prior to the first exposure, following decontamination, and thereafter for 4 days. Since no significant change in enzyme activities was noted, fewer blood samples were taken in connection with later exposures. Red blood cell and plasma cholinesterase activities were determined by the micro-Michel procedure (*4*).

Results

Data on paranitrophenol excreted by human volunteers who sustained discrete dermal exposure to 2% parathion dust at different ambient temperatures are presented in Table I. Maximum paranitrophenol excretion rates occurred 5 to 6 hours following initiation of exposures within temperature range 58° to 105° F. As the exposure temperatures were increased, a progressive increase in the rate of paranitrophenol excretion was observed, although other exposure conditions remained constant.

The average total excretion of paranitrophenol at different temperatures was: 58° F., 196 μg. during 40 hours; 70° F., 246 μg. during 41 hours; 82° F., 287 μg. during 41 hours; and 105° F., 804 μg. during 41 hours. These values permitted calculation of hourly rates used to plot the line in Figure 1, which shows a direct relationship between exposure temperature and the mean values for hourly rates of excretion of paranitrophenol on all exposures sustained at each temperature. The same relationship is indicated by mean values at the 5- to 6-hour time interval of Table I.

Red blood cell and plasma cholinesterase values were measured on 12 samples taken before exposures and on 11 samples following exposures. Mean value for RBC cholinesterase before exposure was 0.49 ΔpH per hr., standard deviation 0.247; after exposure, mean RBC value was 0.51 ΔpH per hr., standard deviation 0.264. Mean pre-exposure value of plasma cholinesterase was 0.74 ΔpH per hr., standard deviation 0.56; and mean postexposure value was 0.73 ΔpH per hr., standard deviation 0.55. These data indicate no significant changes in enzyme activities following these exposures. Under the conditions of the study, no positive relationship was demonstrated between cholinesterase activity and paranitrophenol excretion. A possible explanation for this observation may be that the largest excretion recorded over a 41-hour period was 1.2 mg. of paranitrophenol, corresponding to 2.5 mg. of parathion. Edson (*2*) reported that volunteers tolerated daily oral doses of 3 mg. of parathion without cholinesterase depression or clinical effect.

Although no significant changes were noted in cholinesterase activities and no clinical signs of poisoning were observed during or following exposures, local effects attributed to contact with

parathion were observed at the highest temperature. For several days following decontamination, more perspiration was noted on the exposed area than elsewhere on the body.

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INSECT METABOLISM OF INSECTICIDES

The Enzymatic in vitro Degradation of DDT by Susceptible and DDT-Resistant Body Lice

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Homogenates and acetone powders of both susceptible and DDT-resistant body lice catalyze the degradation of DDT in vitro. Reduced glutathione, cysteine, ascorbic acid, thioglycollic acid, and coenzyme A may be used as cofactors for activation of the enzyme system. Enzyme preparations when incubated with DDT under optimum conditions yield at least three metabolites. On the basis of their neutral or acidic character, ultraviolet and infrared absorption spectra, colorimetric analysis, and paper chromatography, the metabolites have been identified as 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene (DDE); 4,4-dichlorobenzophenone (DBP); and bis-(*p*-chlorophenyl)acetic acid (DDA). This is the first demonstration of DDA as a product of DDT metabolism in an insect.

DETOXICATION of DDT to innocuous derivatives has been implicated in the resistance of certain insect species to this insecticide, while in other species this correlation has not been conclusive. Perhaps the most challenging and fruitful investigations in elucidating the mechanism of DDT resistance have been those dealing with the metabolism of DDT by the housefly *Musca domestica* L. (*18*).

The demonstration of the enzymatic dehydrochlorination of DDT by DDT-resistant houseflies (*25*) and the subsequent isolation of the enzyme DDT-dehydrochlorinase (*24*) and its purification (*11*, *12*, *15*), have greatly strengthened the hypothesis that dehydrochlorination of DDT is an important factor in the over-all protective mechanism against the lethal action of this insecticide toward houseflies.

An enzyme system catalyzing the dehydrochlorination of DDT, DDD, and methoxychlor to their corresponding ethylene derivatives has been isolated from Mexican bean beetle larvae and pupae (*6*, *26*, *27*). Also, an oxidizing enzyme system capable of converting DDT to 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethanol has been demonstrated in the microsomal fractions of tissue homogenates of German and American roaches, the housefly, and the house mosquito (*1*). However, attempts to demonstrate the in vitro dehydrochlorination of DDT by DDT-resistant mosquitoes (*5*, *17*) and by the vinegar fly *Drosophila melanogaster* (*28*) have been unsuccessful.

In a previous communication, Perry and Buckner (*19*) reported on the in vivo metabolism of DDT by susceptible and DDT-resistant body lice *Pediculus humanus humanus* L. By incorporating C^{14}-DDT into citrated human blood and feeding it to adult lice through freshly dissected chickskin membranes, these authors showed that the DDT-resistant lice (Korean strain) but not the susceptible lice (Orlando strain) metabolized DDT to a nontoxic acidic conjugate. On the other hand, crude enzyme preparations of both strains of lice metabolized DDT in vitro at an approximately equal rate.

This report covers the methods used and the results obtained in isolating the enzyme system and in identifying degradation products resulting from the in vitro metabolism of DDT by susceptible and DDT-resistant body lice.

Materials and Methods

Enzyme Preparations. Homogenates and acetone powders were used for most of this work. Homogenates were prepared by macerating 3000 to 5000 adult lice of known weight in a mortar containing 3 ml. of a mixture of 0.1*M* NaCl and 0.1*M* $NaHCO_3$. The slurry was further homogenized in a blender at 14,000 r.p.m. for 2 minutes, after which it was strained through two gauze pads to remove gross particles and centrifuged at 14,000 × G in a refrigerated centrifuge. The precipitate contained very little activity and was discarded. The supernatant was adjusted with additional saline-bicarbonate to 10% concentration (1 gram of lice per 10 ml. of supernatant).

Acetone powders were prepared by a procedure similar to that described by Binkley (*3*) and Binkley *et al.* (*4*) for renal peptidases. Several thousand adult lice of known weight were triturated with acetone. After steeping for several hours at room temperature, the supernatant acetone was decanted, fresh acetone was added, and the mixture was boiled gently in a hot water bath at 70° C. for 20 minutes.

The supernatant acetone was removed, and the boiling with fresh acetone was repeated twice. Finally, the mixture was filtered while hot and the precipitate dried in vacuo. The resulting cake was pulverized and stored in an airtight container at −10° C. until used. For enzyme assay, 1 gram of powder was