Carbamate Poisoning. Effects of Selected Carbamate Pesticides on Plasma Enzymes and Brain Esterases of Japanese Quail (*Coturnix coturnix japonica*)

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Lethal and sublethal doses (0.33 or 0.5 × LD_{50}, LD_{50}, and 2 or 3 × LD_{50}) of five commonly used carbamate pesticides (aldicarb, methiocarb, oxamyl, pirimicarb, and thiofanox) were administered to Japanese quail. Acetyl cholinesterase (AChE), cholinesterase (ChE), and α-naphthyl acetate esterase activities were measured in plasma at 2 and 24 h or at death. Brain AChE activities were measured in fresh tissue extracts and after 7 days post-mortem. Tissue-specific enzymes glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), and sorbitol dehydrogenase were also measured in the plasma at 2 and 24 h or at death. Lethal doses of carbamates gave >64% inhibition of plasma AChE and >75% inhibition of plasma ChE. Plasma esterase activities at 24 h after dosing generally returned to control levels or above. Lethal doses of carbamates produced ~40–60% inhibition of brain AChE although the maximal inhibition for pirimicarb treatment was 97%. A dose–response relationship for brain AChE was only found in lethally dosed quail given oxamyl and pirimicarb. No birds surviving the carbamate treatments at 24 h showed significant inhibition of brain AChE, and birds surviving doses of aldicarb, methiocarb, and pirimicarb showed elevated esterase levels. After 24 h, increases in plasma GOT levels were observed in survivors from all the carbamate treatments. Elevation in plasma GDH levels were observed only in survivors from the aldicarb treatments. The changes resulting from sublethal carbamate dosing are discussed and compared with the responses obtained on dosing organophosphate pesticides to Japanese quail.

The measurement of avian brain esterase levels following reactivation after electrophoresis has been used by Bunyan and Jennings (1976) to enable poisoning by carbamate pesticides to be differentiated from that by organophosphate pesticides. Other studies (Dieter, 1974, 1975; Dieter and Ludke, 1975; Ludke et al., 1975; Westlake et al., 1981) have measured esterase inhibition by organophosphate pesticides in avian plasma, although little concomitant characterization of carbamate poisoning has been made (Ludke et al., 1975; Dieter and Ludke, 1978). The levels of tissue-specific enzymes circulating in the plasma have also been studied to monitor sublethal effects of a number of pesticides in avian species (Dieter, 1974, 1975; Westlake et al., 1978, 1979, 1981).

This communication reports investigations into the changes in plasma and brain esterase levels and tissue-specific enzyme levels following the administrtion of various oral doses of several commonly used carbamate pesticides. This series of experiments was designed to complement those of the previous study with organophosphorus pesticides (Westlake et al., 1981). The biochemical effects observed in the two studies may provide a means of further differentiation between poisoning by these two types of pesticide.

EXPERIMENTAL SECTION

Animals. The details of Japanese quail and their treatment before and after dosing have been described previously (Westlake et al., 1981). The experimental treatment of the birds are summarized in Table I.

Pesticides. Five pesticides were chosen for this study, namely, aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime], methiocarb [3,5-dimethyl-4-(methylthio)phenyl methylcarbamate], oxamyl [*N*,*N*-dimethyl-α-[[(methylcarbamoyl)oxy]imino]-α-(methylthio)acetamide], pirimicarb [2-(dimethylamino)-5,6-dimethylpyrimidin-4-yl dimethylcarbamate], and thiofanox [3,3-dimethyl-1-(methylthio)-2-butanone *O*-[(methylamino)carbonyl]oxime].

Table I. A Summary of the Experimental Treatment of Japanese Quail with Carbamate Pesticides

carbamate	dosage level	dose, mg/kg	approx. LD_{50}	fate[a] survived to 24 h	fate[a] dead at 2 h
aldicarb	1	3.0	0.5	6 (3)	0
	2	6.0	1	4 (2)	2
	3	10.0	1.6	0	6[c] (3)
methiocarb	1	8.3	0.33	2 (1)	4 (1)
	2	25.0	1	3 (1)	3 (1)
	3	75.0	3	0	6[c] (3)
oxamyl	1	1.5	0.33	6 (3)	0
	2	4.6	1	1 (1)	5[c] (2)
	3	13.9	3	0	6[c] (3)
	1	1.5	0.33	6[b]	0
pirimicarb	1	24.6	0.5	6 (3)	0
	2	54.0	1	4 (2)	2
	3	108.0	2	0	6[c] (3)
thiofanox	1	0.4	0.33	6 (3)	0
	2	1.2	1	5 (2)	1
	3	3.6	3	0	6[c] (3)

[a] Figures in parentheses refer to birds in which organ removal and esterase analysis were not carried out until 7 days post-mortem. [b] Sacrificed at 2 h in the second dosing experiment. [c] Died within 30 min of dosing.

Gifts of pesticides were obtained from the manufacturers. Aldicarb (analytical grade) was obtained from Union Carbide Corp., methiocarb (98.2% pure) from Bayer, Ltd., oxamyl (>99% pure) from E. I. du Pont de Nemours and Co., Inc., pirimicarb (97.6% pure) from Plant Protection, Ltd., and thiofanox (99% pure) from Diamond Shamrock Europe.

Treatment. One series of Japanese quail were dosed at one-third or half the LD_{50} dose, the LD_{50} dose, and one- and two-thirds, 2 or 3 times the LD_{50} dose in corn oil solution by gelatin capsule. Blood sampling and sacrifice were carried out as previously described (Westlake et al., 1981). A second series were dosed with oxamyl at one-third the LD_{50} value to obtain further 2-h brain esterase values.

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Table II. Control Esterase Levels in Japanese Quail[a]

	acetyl-cholinesterase[b]	cholines-terase[c]
plasma	151.6 (70)	913.0 (68)
brain (fresh)	1020.2 (20)	
brain (after 7 days post-mortem)	902.5 (15)	

[a] Mean esterase levels from the quail sample size in parentheses were taken as the respective controls (i.e., 100%). [b] Esterase activities are expressed as micromoles of NaOH used to maintain a constant pH per hour per gram of brain or per milliliter of plasma. [c] Esterase activity is expressed as International milliunits per milliliter of plasma.

These birds were sacrificed at 2 h and the brains removed immediately.

Preparation of Tissue Extracts and Enzyme Estimations. Acetyl cholinesterase (AChE), cholinesterase (ChE), α-naphthyl acetate esterase, glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), and sorbitol dehydrogenase (SDH) were measured as previously described (Westlake et al., 1981).

RESULTS AND DISCUSSION

A summary of the experimental treatments, times of bird deaths and survivors are given in Table I. The results of enzyme assays have been presented in three different forms to allow clearer interpretation. Esterase levels in plasma and brain are respectively presented in the form of histograms and modified scattergrams of percentage variation from the mean control values shown in Table II. Plasma AChE and ChE levels at 2 and 24 h post-dosing or at death when within 2 h of dosing are shown in Figure 1. The comparison of plasma enzyme data from birds dying prior to 2 h with that from the survivors at 2 h is necessary due to the limited number of birds on test. The comparison must be interpreted cautiously as the enzyme activities may vary with the time of sampling. Plasma esterase activities of the birds surviving to 2 h are likely to have been lower if the quail had been bled within 30 min of dosing along with the quail which died (Table I). Brain AChE levels at death, at sacrifice 24 h after dosing, or at 7 days post-mortem are shown in Figure 2. Tissue-specific enzyme levels are given in Table III. Plasma GOT was measured at 2 and 24 h post-dosing, but GDH and SDH levels were generally only measured at 24 h, as changes in these enzymes generally do not occur immediately after dosing.

The plasma and brain esterase levels resulting from the dosing of each pesticide have been considered statistically significant when the value was more than two standard deviations (2 × SD) from the control mean value (Bunyan et al., 1968a).

Aldicarb. The single oral LD_{50} reported for technical aldicarb in pheasant is 15.2 mg/kg (Bunyan and Jennings, 1976). The oral LD_{50} dose for aldicarb in Japanese quail was estimated to be 10 mg/kg, and groups of birds were dosed with 3, 6, and 10 mg/kg. Results show 6 mg/kg is a more realistic LD_{50} value for quail, since all the six birds given 10 mg/kg were found to die within 30 min. Plasma AChE (Figure 1) showed significant inhibition in the highest dose group at death and at the other dose levels after 2 h. By 24 h considerable recovery of AChE had occurred in all surviving quail, and the median-dosed group showed AChE levels above the control range. Plasma ChE (Figure 1) was also severely inhibited, especially in the two highest dose groups, but by 24 h had returned to control levels in survivors. Plasma GOT (Table III) was elevated after 2 h in all groups while by 24 h large increases were observed, especially in birds surviving the 6.0 mg/kg dose.

Table III. Plasma GOT, GDH, and SDH Activities in Japanese Quail after Dosing with Aldicarb, Methiocarb, Oxamyl, Pirimicarb, and Thiofanox at Various Levels

		dosage level[a]			
plasma enzyme		control	1	2	3
aldicarb					
GOT:	2 h	63.77 ± 12.38 (6)	72.30 ± 9.02 (6)	131.91 ± 51.93[c] (6)	84.68 ± 14.27[c] (6)
	24 h	74.27 ± 21.02 (4)	92.36 ± 39.61 (6)	214.78 ± 139.02[b] (4)	
GDH:	24 h	2.96 ± 2.21 (5)	13.39 ± 11.91[b] (6)	28.58 ± 10.99[d] (4)	
SDH:	24 h	1.10 ± 1.19 (5)	0.48 ± 0.34 (6)	1.62 ± 0.63 (4)	
methiocarb					
GOT:	2 h	75.33 ± 2.60 (6)	137.78 ± 33.99[c] (6)	71.40 ± 67.54 (6)	80.58 ± 31.49 (6)
	24 h	118.46 ± 49.14 (6)	194.16 ± 34.72[b] (2)	471.54 ± 568.44[b] (3)	
GDH:	2 h	1.73 ± 1.42 (4)	2.62 ± 1.82 (6)	3.08 ± 1.03 (5)	5.60 ± 5.28 (6)
	24 h	1.96 ± 1.80 (5)	5.94 ± 5.22[b] (2)	2.73 ± 3.85 (2)	
SDH:	2 h	1.15 ± 0.77 (6)	1.80 ± 1.29 (6)	1.12 ± 0.98 (6)	0.88 ± 0.52 (5)
	24 h	1.74 ± 0.72 (6)	NM	1.84 ± 1.07 (3)	
oxamyl					
GOT:	2 h	100.38 ± 28.45 (5)	96.31 ± 27.22 (5)	203.09 ± 147.59[b] (4)	110.39 ± 18.93 (5)
	24 h	136.11 ± 68.03 (4)	123.31 ± 33.65 (6)	422.70[c] (1)	
GDH:	24 h	5.82 ± 5.34 (4)	NM	6.10 (1)	
SDH:	24 h	2.40 ± 3.33 (5)	NM	1.92 (1)	
pirimicarb					
GOT:	2 h	68.71 ± 10.55 (6)	86.49 ± 15.38 (6)	95.27 ± 25.14[c] (6)	70.70 ± 23.90 (6)
	24 h	63.13 ± 8.28 (5)	121.35 ± 59.34[b] (6)	163.40 ± 50.73[d] (4)	
GDH:	2 h	1.14 ± 0.70 (6)	NM	4.19 ± 2.49[c] (5)	7.12 ± 5.70[c] (6)
	24 h	5.28 ± 2.46 (5)	1.02 ± 1.44 (6)	6.45 ± 2.15 (4)	
SDH:	2 h	4.80 ± 2.21 (6)	NM	5.25 ± 5.61 (6)	6.60 ± 1.46 (6)
	24 h	2.42 ± 1.43 (5)	1.62 ± 0.90 (6)	4.42 ± 1.81[b] (4)	
thiofanox					
GOT:	2 h	68.98 ± 21.92 (6)	56.74 ± 13.62 (6)	82.72 ± 53.74 (6)	59.17 ± 18.09 (6)
	24 h	81.40 ± 45.90 (6)	64.14 ± 26.08 (5)	135.76 ± 89.43 (5)	
GDH:	24 h	1.09 ± 0.72 (6)	0.62 ± 0.66 (5)	1.66 ± 1.29 (5)	
SDH:	24 h	0.42 ± 0.57 (6)	0.81 ± 0.58 (6)	1.63 ± 1.52[b] (5)	

[a] Enzyme activity is in International milliunits per milliliter of plasma; mean ± SD; NM, not measured; group size in parentheses. See Table I for dosage levels in milligrams of carbamate per kilogram of body weight. [b-d] Significant differences from the individual control values: [b] $p < 0.2$; [c] $p < 0.05$; [d] $p < 0.005$ (Student's t test).

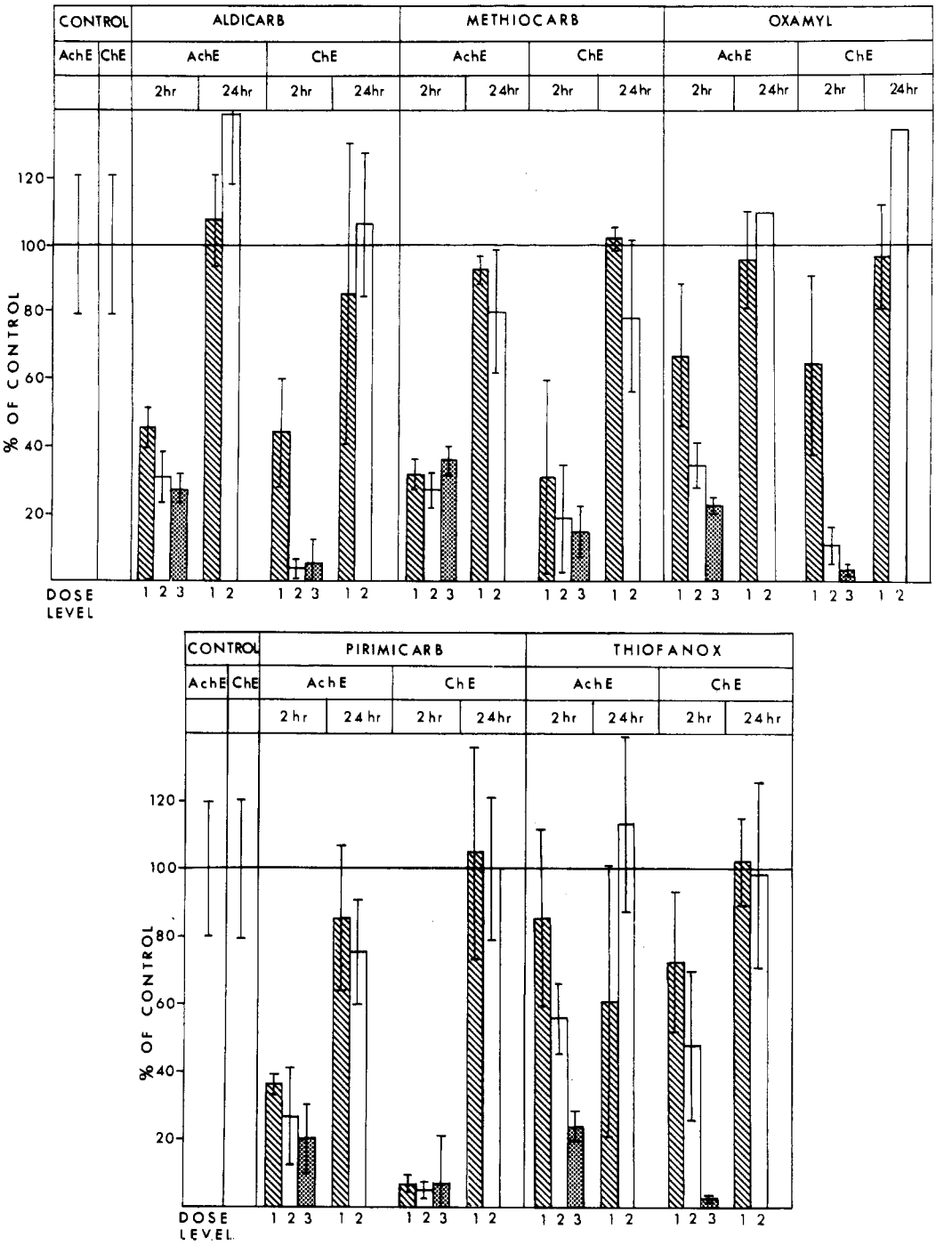

Figure 1. Plasma AChE and ChE activities in Japanese quail expressed as percentage of control mean 2 or 24 h after dosing or at death when within 2 h of dosing with aldicarb, methiocarb, oxamyl, pirimicarb, and thiofanox at various levels. Mean esterase activities from 70 control quail were taken as 100% (±SD). Dose levels (1, 2, or 3) are shown in Table I as milligrams of carbamate per kilogram of body weight.

Plasma GDH also exhibited dose-related increases after 24 h but no apparent elevation was found in plasma SDH (Table III).

Brain esterases (Figure 2) in birds dying in the median-dosed group were within the normal range. The birds given the highest dose showed 60% inhibition of AChE on death. The esterase level of one bird of this dose group recovered after hanging for 7 days; the difference in response from the other birds may be from an excess of free aldicarb preventing reactivation in these. By 24 h those birds which survived the 6 mg/kg dose had esterase activity above the control mean but were within the 2 × SD range. The birds in the lowest dose group showed no inhibition at 24 h with respect to AChE. In the median-dosed group significant depression of AChE levels was found after hanging in the birds which survived to 24 h compared with values for birds which survived to 24 h but were not hung.

Methiocarb. The single oral LD_{50} of methiocarb has been estimated as 50 mg/kg for the pigeon and 225 mg/kg for the pheasant (Bunyan and Jennings, 1976). Schafer et al. (1973) gave the LD_{50} dose to quelea as 4.2 mg/kg, to house sparrow as 18 mg/kg, and to redwing blackbird as 4.6 mg/kg. The oral LD_{50} for methiocarb to Japanese quail was estimated from this to be 25 mg/kg, and this figure proved to be satisfactory when used to calculate dosages. Plasma AChE and ChE (Figure 1) were both significantly inhibited for all groups after 2 h but after 24

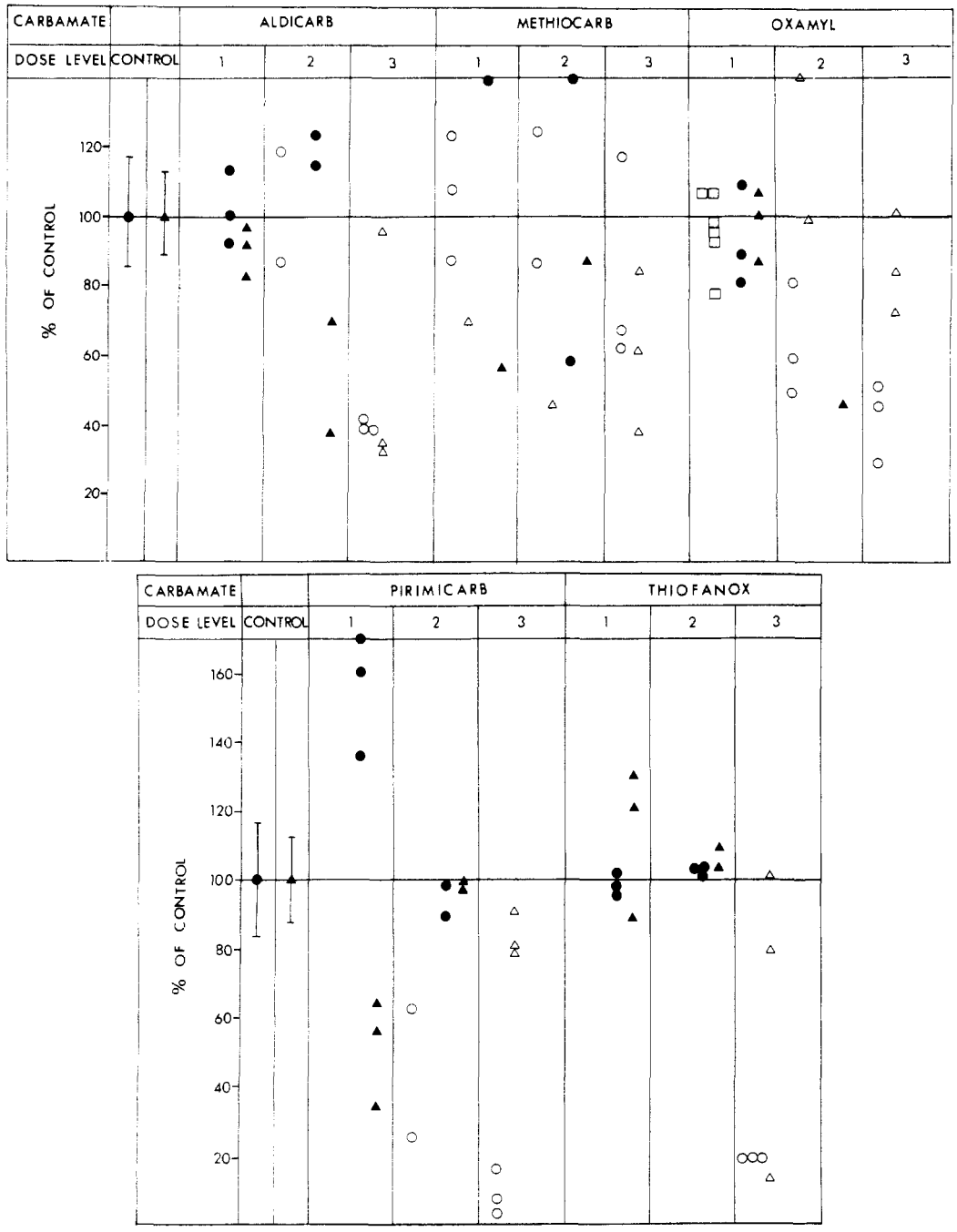

Figure 2. Brain AChE activities in Japanese quail expressed as percentage of control mean after dosing with aldicarb, methiocarb, oxamyl, pirimicarb, and thiofanox at various levels. Mean esterase activities from 20 control quail and 15 control quail after 7 days post-mortem were taken as 100% (±SD). Individual results are shown as follows: (○) level measured in birds dead at 2 h or within 2 h of dosing; (□) level measured in birds sacrificed at 2 h; (△) level measured 7 days post-mortem in birds dead at 2 h or within 2 h of dosing; (●) level measured in birds surviving to 24 h; (▲) level measured 7 days post-mortem in birds surviving to 24 h. Dose levels (1, 2, or 3) are shown in Table I as milligrams of carbamate per kilogram of body weight.

h both enzyme levels had recovered in survivors and returned to the control ranges. Methiocarb caused some increase in plasma GOT (Table III) in the lowest dose group after 2 h, but some increased individual values in the two other groups are hidden by the large standard deviations. Control GOT values had risen by 24 h, but the surviving test quail generally showed even larger elevations in level. Neither GDH or SDH were markedly changed at 2 h although some small dose-related increases could be seen in the mean GDH values at 2 h (Table III), and the survivors of the low-dosage level had increased GDH levels at 24 h.

At 2 h after dosing the lowest dose group had some elevated brain AChE levels (Figure 2), but all values were within the 2 × SD range of the control mean. One bird from the median-dosed group showed an elevated AChE level (124%) but again this was not significant. The mean AChE value for the highest dose group at 2 h was 82% of the control mean but the range was wide (62–117%) and none of the birds including those which died showed significant elevation or depression compared with the control range. At 24 h the AChE results were very variable in survivors and included elevated levels. After aging for 7 days there was some tendency for further AChE inhibition to occur in all birds, but generally levels were not low and often not significant.

Oxamyl. The acute oral LD_{50} for *Coturnix* quail has been estimated as 4.6 mg/kg (E. I. du Pont de Nemours

and Co., Ltd., 1978). Acute signs of poisoning were seen within 10 min in those birds given 4.6 and 13.9 mg/kg and death ensued within 20 min. Plasma AChE and ChE (Figure 1) were inhibited in excess of 50% in the two highest dose groups. After 24 h, surviving birds showed plasma AChE and ChE activities to be within or above the control range. The median-dose group showed some large increases in plasma GOT (Table III) by 2 h which remained very high in the surviving quail at 24 h. Plasma enzymes GDH and SDH were not altered within 24 h.

The two highest dosage levels gave significant brain esterase inhibition (Figure 2) within 2 h. At 24 h brain AChE activity was not significantly inhibited in those birds which survived the lowest dose. Significant reactivation of brain AChE activity occurred in the two highest dose groups on hanging for 7 days post-mortem.

Pirimicarb. The acute oral LD_{50} for pirimicarb to Japanese quail has been estimated as 54 mg/kg (Westlake, 1980), and this was accepted as the LD_{50} in the study for the purposes of calculating dose rates (Table I). All the six birds given 108 mg/kg were found to die within 30 min. Plasma esterases (Figure 1) were severely inhibited at 2 h, but at 24 h both plasma AChE and ChE had recovered in survivors of all groups and returned to the control ranges. Plasma GOT (Table III) was marginally elevated after 2 h in all groups while by 24 h significant elevations were observed in the surviving birds. Plasma GDH exhibited small increases after 2 h in the two higher dosage groups compared to the control values. Plasma SDH was slightly increased only in the median-dose group at 24 h.

Brain esterases (Figure 2) in birds dying within 2 h were significantly inhibited, and the highest dosage group showed 85% inhibition. By 24 h those birds surviving the lowest dosage had AChE levels considerably elevated above the control mean, while those surviving the median dosage had returned to the control range. After the birds aged, significant reactivation of brain AChE activity was found in birds from the highest dosage group which died whereas significant depression of AChE was exhibited after aging in birds which survived the lowest dosage to 24 h.

Thiofanox. No data for single oral LD_{50} levels to avian species were available, and an estimate of 1.2 mg/kg for the Japanese quail was made based on values quoted for the rat and the rabbit. Groups of birds were dosed with 0.4, 1.2, and 3.6 mg/kg, and the highest dosage group all died within 30 min. Plasma AChE and ChE (Figure 1) showed a dose-related inhibition at 2 h with the highest dose being significantly inhibited for both enzymes. After 24 h, ChE levels had returned to normal in the birds which survived the two lower doses, but AChE showed further depression at the lowest dose and recovery to control levels in the median-dosed group. Thiofanox gave slight increases in plasma GOT, GDH, and SDH (Table III) in survivors of the LD_{50} dose group at 24 h.

Brain AChE (Figure 2) showed marked inhibition (~80%) in the highest dose after 2 h but considerable reactivation after aging. At 24 h the two lower dosed groups had AChE within the normal range, but reactivation and elevation of AChE above the control mean occurred in some individuals of the low-dose group after aging.

GENERAL DISCUSSION

The initial objective of feeding sublethal and lethal doses of a group of widely used carbamate pesticides to Japanese quail was generally achieved. Earlier communications from this laboratory have described some of the biochemical lesions of organophosphate poisoning in avian species (Bunyan and Taylor, 1966; Bunyan et al., 1968a,b, 1969, 1971; Jennings et al., 1975; Westlake et al., 1981). These effects have been used to diagnose poisoning in wild birds, and Bunyan and Jennings (1976) extended the observation to the diagnosis of carbamate poisoning in the pheasant and the pigeon. In this work the effects on brain ChE were particularly involved, but there is now a greater need to examine sublethal effects for field trials. The present work has reexamined brain AChE inhibition and reactivation following carbamate poisoning in Japanese quail and further characterized the lethal and sublethal effects by monitoring plasma AChE, ChE, and tissue-specific enzymes present in the plasma. The effects following the dosing of quail with a range of organophosphate pesticides have also been examined (Westlake et al., 1981), and these are contrasted with the effects found to result from carbamate treatment.

The administration of carbamates to birds produces a dramatic neuromuscular effect. Aldridge (1972) showed that carbamates react with esterases in a manner analogous to that of the normal substrate. The resultant excess of acetylcholine at neuromuscular junctions can act as a blocking agent, depolarizing the motor end plates. This effect was especially apparent with oxamyl, the onset of symptoms was extremely rapid, and this is probably due to its similarity to acetylcholine. A neuromuscular blocking effect could explain the rapid death of the carbamate-dosed birds and also the smaller degree of brain cholinesterase inhibition as compared with that produced by the organophosphates.

The degree of plasma AChE and ChE inhibition by the carbamates was not as large as that found with the organophosphate compounds, although there was greater variation in the extent of inhibition. Lethal doses of carbamates generally gave 75–97% inhibition of ChE and 64–77% inhibition of AChE within 30 min of dosing. The marked time response difference for lethal doses of carbamates (<30 min) and organophosphates (<2 h) to take effect probably reflects the more rapid depression of esterases observed with the carbamate pesticides. As with the organophosphates, larger depressions in plasma ChE were observed than with AChE at 2 h. In general, levels at 24 h had returned to those of the controls or above. Aldicarb, oxamyl, and thiofanox showed elevation of AChE above control levels at 24 h, and all of the carbamates showed some elevation of ChE. The method of Ellman et al. (1961) for assaying plasma ChE appears to be more sensitive than the titrimetric estimation of AChE for monitoring carbamate exposure as it is for organophosphate exposure (Westlake et al., 1981). Plasma α-naphthyl acetate esterase was found to be less inhibited (36–58%) than ChE in the quail given lethal doses of carbamates, and as found in the previous study, the enzyme was more variable in the range of response and does not appear to be a useful indicator of exposure.

GOT levels were generally increased in plasma at 2 h after dosing quail with carbamates, but there was no consistent dose–response relationship. The GOT levels in the high-dosage group at 30 min were generally lower than those in the median-dosage group (2 h). This may result from the latter birds having a longer time period for the effects of tissue damage to appear. After 24 h, increased plasma GOT levels were obtained in survivors from all five carbamates at some dose levels although these changes were not as large as those previously found in survivors from groups lethally dosed with the organophosphates carbophenothion and pirimiphos methyl. It is of interest that these changes in plasma GOT levels were found after 24 h when plasma esterase levels had recovered to control

levels or greater. Only aldicarb showed significant changes in plasma GDH at 24 h with the group given 3 mg/kg showing a 5-fold increase and the group given 6 mg/kg showing a 10-fold increase. No changes were found in plasma SDH with any of the carbamate treatments. Enzyme profiles in the plasma have been used as an indirect measure of exposure to both organochlorine and organophosphorus pesticides (Dieter, 1974, 1975; Westlake et al., 1978, 1979, 1981), but exposure to carbamate pesticides has not previously been similarly characterized except for plasma esterase inhibition by carbofuran (Ludke et al., 1975; Dieter and Ludke, 1978).

Bunyan and Jennings (1976) found 90% inhibition of brain AChE in pigeons lethally poisoned with aldicarb, methiocarb, pirimicarb, and propoxur while pheasants given the same compounds showed a lower, but still significant inhibition (>75%). Aminocarb and zectran gave 60 and 80% inhibition, respectively. Ludke et al. (1975) found 32% inhibition of cholinesterase in fresh brain tissue after giving Japanese quail 600 ppm of carbofuran for 4 h. The inhibition of brain AChE by lethal doses of carbamates in this study was ~40–60% although the maximal inhibition obtained with the pirimicarb treatment was 97%.

Pesticide inhibition of brain AChE was less severe in the carbamate-treated birds as compared with the 60–99% inhibition found in birds given organophosphates (Westlake et al., 1981). The differential response of brain AChE that is apparent in various avian species may be common to both carbamate and organophosphorus pesticides (Westlake et al., 1981). A dose–response relationship was found in brain AChE at 30 min with the oxamyl and pirimicarb treatments, whereas all the organophosphate treatments demonstrated a definite dose–response relationship (Westlake et al., 1981). Since the plasma esterases recover within 24 h, it is possible that brain AChE activities reached their greatest depression sooner after dosing with carbamates than with organophosphates. Birds which survived to 24 h showed no significant inhibition of AChE in any of the carbamate treatments, and birds dosed with aldicarb, methiocarb and pirimicarb showed elevated esterase levels. The organophosphate treatments still showed significant inhibition at 24 h in birds with the two highest levels with the exception of pirimiphos ethyl (Westlake et al., 1981). Reactivation of brain AChE occurred in quail that died within 2 h after receiving oxamyl, pirimicarb, and thiofanox and were then examined 7 days post-mortem, but it was further depressed following methiocarb treatment. Birds which survived to 24 h and were examined 7 days post-mortem showed depression of esterase levels with the aldicarb and methiocarb treatments and with the low-dosed pirimicarb group. Pirimicarb shows a trend similar to that reported by Bunyan and Jennings (1976) when some reactivation was found after 5 days post-mortem in pheasants although inhibition was still significant. Westlake et al. (1981) also found considerable variation in the reactivation of brain esterase following organophosphate treatments.

Bunyan and Jennings (1976) found elevation of brain esterases after electrophoresis in pheasants dosed with carbamates. It ws suggested that the starch gel acted as a molecular sieve and removed excess pesticide, enabling spontaneous reactivation to occur on an already induced enzyme system. In this study no electrophoresis was undertaken, but reactivation and elevation were still demonstrated in some instances. For an explanation the possibility of enzyme induction must be considered although changes in the availability of the enzyme for extraction may also be pertinent. Many xenobiotics are known to induce cellular microsomal enzymes while elevation of aliesterase and cholinesterase has also been demonstrated (Kay, 1966; Puyear and Paulson, 1972; Westlake et al., 1979). If induction of esterases by carbamates and certain organophosphates does occur soon after dosing, it may subsequently be masked by inhibition of the induced esterase activity. Removal of the pesticide by metabolism in vivo or by electrophoresis or dilution in vitro may then cause the increased levels of esterase to become apparent. The general lack of observance of reactivation and elevation of esterases following organophosphate poisoning may be due not only to the fact that the phosphorylated enzyme is more stable than the carbamoylated enzyme but also that they may themselves act as metabolic inhibitors, especially the thiophosphates. Uchiyama et al. (1973) showed that thiophosphate insecticides inhibit the microsomal oxidative metabolism of aminopyrine and aniline both in vivo and in vitro.

The use of a more integrated approach to the diagnosis of poisoning by pesticides is likely to be of increasing importance in future toxicological and monitoring studies. At the present time, the measurement of brain esterase levels is widely used as an indication of both carbamate and organophosphate poisoning although subsequent demonstration of the presence of pesticide residues by chemical analysis is still essential for confirmatory purposes. Blood sampling and the measurement of tissue-specific enzymes and cholinesterase in the plasma of wild-trapped animals has so far had limited application for measuring environmental pollution (Dieter, 1975; Dieter et al., 1976; Westlake et al., 1980). Before these enzymes can be fully utilized for this purpose in conjunction with tissue esterase assay and chemical residue analysis, a more detailed understanding of their various responses to the administration of a wide range of pesticides and other xenobiotic compounds is required.

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Isolation and Structure of Red Pigments from *Aspergillus flavus* and Related Species, Grown on a Differential Medium

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A group of 25 strains of *Aspergillus* spp., most of them known for producing aflatoxins or other mycotoxins, was cultivated on *Aspergillus* differential medium, containing ferric ions. Two red pigments produced by the test-sensitive strains have been isolated and identified as ferriaspergillin (1) and ferrineoaspergillin (2). The formation of the yellow pigmentation on ADM is related to the production of aspergillic acid (3) or neoaspergillic acid (4).

Among the causal agents of toxicoses by foodstuffs, fungi of the genera *Aspergillus* and *Penicillium* play a major role. They are widespread and are common in stored products such as grains, field crops, and harvested forage, which are generally used for feeding domestic animals and man (Moreau, 1979).

Therefore, simple and rapid methods for the detection of mycotoxins in deteriorated substrates or for identification of the species responsible for the damages are of utmost importance.

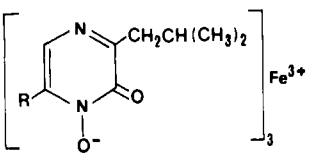
Bothast and Fennell (1974) proposed a diagnostic method for the identification of *Aspergillus flavus* and closely related species, which are often connected with the presence of aflatoxins. The method is based on the development of a characteristic yellow pigmentation, when the mold is cultivated on a particular medium (*Aspergillus* differential medium, ADM), containing iron citrate. Only a few species other than *A. flavus* give the same pigmentation.

In order to check whether there was any correlation between the yellow pigment and fungal metabolites, we isolated the pigments produced by test-sensitive strains. This paper reports their structural elucidation.

RESULTS AND DISCUSSION

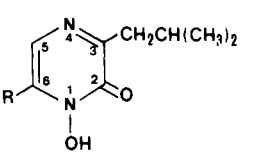
A group of 25 strains of *Aspergillus* spp., most of them the same ones that were examined by Bothast and Fennell (1974), were cultivated on Petri plates of ADM, and the crude EtOAc extracts were compared by TLC (hexane–AcOEt, 9:1) on silica gel plates. Two red pigments 1 and 2 were produced by most of the strains examined. Table I reports the results of this screening.

Scheme I



1 R = $CH_3CH_2CH(CH_3)-$

2 R = $(CH_3)_2CHCH_2-$



3 R = $CH_3CH_2CH(CH_3)-$

4 R = $(CH_3)_2CHCH_2-$

The strains *A. flavus* IPV-F 16L and *Aspergillus melleus* CBS 546.65 were used for large-scale cultivation, in order to obtain a sufficient quantity for structural elucidation of 1 and 2, respectively (see Scheme I for structures). The pigments were again isolated by chromatography of the hexane extract of the dried mycelia, grown on stationary liquid culture.

Although the pigment 1 appears as an oil, of low polarity, its color, its absence in cultures grown on iron-deprived media, and the disturbance of the NMR spectrum by paramagnetic atoms led us to suspect that it contained iron.

Alkali hydrolysis of the pigment 1 gave $Fe(OH)_3$ and a single, pure compound. Examination of the spectral (UV, mass, ^1H NMR, and ^{13}C NMR; see Experimental Section) data indicated that this compound is (+)-aspergillic acid (3), mp and optical rotation also being consistent with literature data (Wilson, 1971). Aspergillic acid is a known metabolite of various isolates and substrains of *A. flavus* and related *Aspergilli*. From the value of the molecular ion in the mass spectrum of 1, it can be inferred that the pigment is a compound which contains three molecules of aspergillic acid and one atom of iron, which can coordinate

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