Organophosphorus Poisoning

Chronic Feeding of Some Common Pesticides to Pheasants and Pigeons

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Pheasants and pigeons have been fed for varying periods with food dressed with 100 p.p.m. of demeton-methyl, diazinon, dimethoate, or phorate. Esterase levels have been measured both in liver and brain extracts and blood plasma. Electrophoresis coupled with histochemical staining has been carried out on liver, kidney, and brain extracts to demonstrate inhibition. Pesticide residue analysis of liver, fat, and breast muscle was undertaken. No deaths occurred as a result of feeding but esterase levels were altered. These together with the electrophoretic results provide a diagnostic pattern different from that found in fatally poisoned birds. The differing toxicities of the four pesticides and the relative susceptibility of the two species to pesticide intoxication are discussed.

Previous communications (Bunyan and Taylor, 1966; Bunyan *et al.*, 1968a,b) from this laboratory have reported mainly on the biochemical effects of acute dosage of seven widely used organophosphorus pesticides in pheasants (*Phasianus colchicus*). That work was undertaken to obtain a reliable method of diagnosing fatal poisoning in wild birds following the agricultural use of these compounds. With the exception of a limited number of pigeons (*Columba livia*) which were dosed with phorate [*O,O*-diethyl-*S*-(ethylthio) methyl phosphorodithioate], no comparisons were made among species during the course of the previously reported experiments. However, the control esterase levels necessary for a comparison between the pheasant and the pigeon were measured and reported (Bunyan *et al.*, 1968a). Acute oral administration of pesticide was employed throughout the initial work, since it allowed an exact dose rate to be calculated, and a fairly simple picture of the relationship between dose and effect to be built up. Such an approach, however, has the disadvantage that in practice birds may not be poisoned acutely, but may absorb pesticides on food over a long period.

The authors now report on the results of a study in which the four most acutely poisonous pesticides of the seven previously used were fed to pheasants and pigeons. The object was to induce a chronic condition in order to determine whether nonfatal exposure followed by death from other causes could be differentiated from fatal poisoning at autopsy. The pesticides involved were demeton-methyl (a mixture of 70% demeton-*O*-methyl [*O,O*-dimethyl *O*-2-(ethylthio) ethyl phosphorothioate] and 30% demeton-*S*-methyl[*O,O*-dimethyl *S*-2-(ethylthio) ethyl phosphorothioate], diazinon (*O,O*-diethyl *O*-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate), dimethoate (*O,O*-dimethyl *S*-methyl carbamoylmethyl phosphorodithioate), and phorate. Birds were fed for various periods and sacrificed, tissue extracts were examined electrophoretically, and esterase levels measured. Cholinesterase levels were measured only in brain, while triacetin esterase levels and 1-naphthyl acetate esterase levels were measured in the brain and liver, since these were the only esterases for which significant depressions could be demonstrated (Bunyan *et al.*, 1968a). Plasma cholinesterase, triacetin, and 1-naphthyl acetate esterase levels were measured in samples taken before the feeding of pesticide-dressed food commenced, and again immediately before death. Chemical examination for residues was extended beyond previous investigations (Bunyan and Taylor, 1966; Bunyan *et al.*, 1968b) to include specimens of liver, muscle, and omental fat from all poisoned birds. Histochemical staining and comparative examination of liver, kidney, and brain sections was also carried out on the freshly dissected tissue (to be reported elsewhere).

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EXPERIMENTAL

Animals. The origin of the pheasants and pigeons has been described (Bunyan and Taylor, 1966; Bunyan *et al.*, 1968a). Both pigeons and pheasants were kept in separate external communal aviaries until 2 weeks prior to feeding. The pigeons were fed a diet of grain and pulses, while pheasants were fed the same diet augmented with 20% protein chick crumbs. Pigeons were then weighed, individually caged, and kept at 18° C. with a constant daily photoperiod of 17 hours throughout the rest of their lives. They were all fed for 14 days on wheat, and then for a further 14, 28, or 42 days before sacrifice by cervical dislocation on wheat dressed with pesticide. Blood was collected from the brachial vein into heparinized tubes during the first 7 days of this regime and again just prior to sacrifice. Pheasants were weighed and caged in pairs (1 male; 1 female) in external runs approximately 6 × 10 feet in area with feeding from a covered hopper. The experiment was carried out during a mild winter and early spring, with a daily photoperiod of 8 to 14 hours. All these birds were fed for 14 days on 20% protein chick crumbs (Spillers, Ltd.), and then for a further 14, 28, or 42 days before sacrifice by cervical

Table I. Brain Esterase Levels in Pheasants and Pigeons Dosed with Pesticides

Pesticide	Feeding Time, Days	Enzyme Level[a] Pheasant Brain ChE	TE	1-NA	PB	Pigeon Brain ChE	TE	1-NA	PB
Demeton-Me									
	14	7.78[b]	5.13[b]	4.01	0.145	5.27[b]	5.95[b]	1.03[b]	0.093
		(−41)	(−48)	(−40)	(+3)	(−71)	(−54)	(−77)	(+12)
	14	11.45	6.75	5.62	0.115	5.90[b]	7.04[b]	1.07[b]	0.057
		(−13)	(−31)	(−18)	(−18)	(−67)	(−46)	(−76)	(−31)
	28	8.35[b]	5.41[b]	2.45[b]	0.118	7.45[b]	4.61[b]	1.17[b]	0.049
		(−37)	(−45)	(−64)	(−16)	(−59)	(−64)	(−74)	(−41)
	28	7.86[b]	6.63	4.05	0.180	5.91[b]	4.88[b]	1.11[b]	0.045[b]
		(−41)	(−32)	(−41)	(+29)	(−67)	(−62)	(−75)	(−46)
	42	8.07[b]	9.07	3.43	0.167	10.85[b]	7.50[b]	0.96[b]	0.010[b]
		(−39)	(−7)	(−50)	(+19)	(−40)	(−42)	(−79)	(−88)
	42	8.65[b]	10.35	2.68[b]	0.100	12.15[b]	6.77[b]	0.78[b]	0.019[b]
		(−35)	(+6)	(−61)	(−28)	(−33)	(−48)	(−83)	(−77)
Diazinon									
	14	12.45	8.55	4.70	0.219[c]	5.62[b]	3.62[b]	0.99[b]	0.093
		(−6)	(−13)	(−31)	(+56)	(−69)	(−72)	(−78)	(+12)
	14	14.10	7.82	3.68	0.333[c]	6.69[b]	4.00[b]	1.24[b]	0.126[c]
		(+5)	(−20)	(−46)	(+138)	(−63)	(−69)	(−72)	(+53)
	28	10.25	5.91[b]	2.42[b]	0.098	6.48[b]	6.20[b]	0.90[b]	0.048
		(−23)	(−40)	(−65)	(−30)	(−64)	(−52)	(−80)	(−42)
	28	10.90	6.77	2.83[b]	0.198	8.33[b]	5.82[b]	1.24[b]	0.062
		(−18)	(−31)	(−59)	(+41)	(−54)	(−55)	(−72)	(−25)
	42	8.78[b]	7.62	2.74	0.125	11.14[b]	4.28[b]	2.14[b]	0.045[b]
		(−33)	(−22)	(−60)	(−11)	(−38)	(−67)	(−52)	(−46)
	42	13.45	8.41	3.71	0.118	8.40	4.49[b]	2.79	0.056
		(+1)	(−14)	(−46)	(−16)	(−57)	(−65)	(−38)	(−32)
Dimethoate									
	14	8.84[b]	6.19[b]	3.53	0.162	9.94[b]	5.53[b]	1.98[b]	0.107
		(−33)	(−37)	(−48)	(+16)	(−45)	(−57)	(−56)	(+29)
	14	10.32	8.79	4.36	0.174	7.62[b]	5.02[b]	1.63[b]	0.092
		(−22)	(−10)	(−36)	(+24)	(−58)	(−61)	(−63)	(+11)
	28	10.70	6.12[b]	2.69[b]	0.216[c]	6.43[b]	3.80[b]	1.67[b]	0.149[c]
		(−19)	(−37)	(−61)	(+54)	(−64)	(−71)	(−63)	(+80)
	28	10.50	5.66[b]	3.53	0.178	7.34[b]	4.58[b]	2.52	0.677[c]
		(−21)	(−42)	(−48)	(+27)	(−59)	(−65)	(−44)	(+715)
	42	9.44[b]	6.73	3.95	0.174	8.35[b]	6.67[b]	2.01[b]	0.115
		(−29)	(−31)	(−42)	(+24)	(−54)	(−48)	(−55)	(+38)
	42	9.12[b]	6.45[b]	3.70	0.221[c]	5.44[b]	4.69[b]	1.31[b]	0.077
		(−31)	(−34)	(−46)	(+58)	(−70)	(−64)	(−71)	(−7)
Phorate									
	14	5.50[b]	2.34[b]	1.82[b]	0.046[b]	6.40[b]	3.73[b]	1.10[b]	0.044[b]
		(−58)	(−76)	(−73)	(−67)	(−65)	(−71)	(−75)	(−47)
	14	5.70[b]	4.15[b]	2.48[b]	0.073	6.44[b]	4.06[b]	0.98[b]	0.037
		(−57)	(−58)	(−64)	(−48)	(−64)	(−69)	(−78)	(−55)
	28	13.70	6.00[b]	3.43	0.114	7.88[b]	4.05[b]	1.84[b]	0.051
		(+3)	(−39)	(−50)	(−19)	(−57)	(−69)	(−59)	(−39)
	28	11.55	6.11[b]	4.19	0.063[b]	9.88[b]	4.52[b]	1.63[b]	0.050
		(−13)	(−38)	(−39)	(−55)	(−45)	(−65)	(−63)	(−40)
	42	10.95	6.30[b]	3.22	0.115	8.95[b]	4.97[b]	1.67[b]	0.102
		(−17)	(−36)	(−53)	(−18)	(−50)	(−62)	(−63)	(+23)
	42	14.70	7.35	4.08	0.188	8.53[b]	3.82[b]	1.58[b]	0.104
		(+11)	(−25)	(−40)	(+34)	(−53)	(−70)	(−45)	(+25)

[a] All levels measured in micromoles of substrate hydrolyzed per hour per milligram of protein.
[b] Significant inhibition compared to control mean.
[c] Significant elevation compared to control mean. Figures in parenthesis are per cent difference from control mean value.

dislocation on the same diet dressed with pesticide. Blood was collected as detailed.

Pesticides. The sources and purities of the pesticides have been listed (Bunyan *et al.*, 1968b). Food was dressed at an approximate nominal rate of 100 p.p.m. by the addition of the appropriate pesticide in acetone, followed by thorough mixing and then evaporation of the solvent in a stream of cold air. Wheat for the pigeons was first dressed with 0.5% technical white oil as a sticker. The concentrations were checked before and after each 6-week feeding period by continuous extraction of a sample with hexane and estimation of the pesticide in the extract by gas-liquid chromatography using an electron-capture detector. No correction was made for recovery. With dimethoate, the levels of 90 and 105 p.p.m. for pheasant and pigeon food, respectively, were near those planned, but losses of 43 and 31% over the 6-week period were high. With diazinon, the corresponding figures were 83 and 80 p.p.m. with losses of 17 and 15%; and with phorate 84 and 81 p.p.m. with losses of 0 and 17%. Demeton-methyl, which could not be checked by gas-liquid chromatography, was analyzed by an agar-agar diffusion method (Beynon and Stoydin, 1965) and showed about 50% loss of the nominal rate after 6 weeks with both diets.

Preparation of Tissue Extracts, Electrophoresis, and Enzyme Estimations. These operations were carried out on all birds as previously described (Bunyan *et al.*, 1968a).

Table II. Plasma Esterase Inhibition in Pheasants and Pigeons Dosed with Pesticides

Pesticide	Feeding Time, Days	Pheasant, Sex	Pheasant Plasma Inhibition, %[a] ChE	Pheasant Plasma Inhibition, %[a] 1-NA	Pigeon, Sex	Pigeon Plasma Inhibition, %[a] ChE	Pigeon Plasma Inhibition, %[a] 1-NA
Demeton-Me							
	14	m	86	89	f	97	96
	14	f	66	83	f	94	94
	28	m	91	93	m	95	92
	28	f	87	50	f	98	97
	42	m	100	88	f	100	96
	42	f	94	84	m	99	95
Diazinon							
	14	m	100	75	m	91	93
	14	f	100	69	m	81	98
	28	m	94	97	m	92	94
	28	f	100	90	f	92	94
	42	m	95	62	f	83	95
	42	f	87	82	f	85	96
Dimethoate							
	14	m	84	55	m	93	43[b]
	14	f	70	57	m	100	23[b]
	28	m	86	60	m	98	4[b]
	28	f	82	57	m	94	40[b]
	42	m	79	74	f	100	81
	42	f	83	71	m	100	92
Phorate							
	14	m	100	72	m	93	94
	14	f	100	79	m	95	92
	28	m	80	65	f	89	81
	28	f	80	73	f	82	82
	42	m	96	79	f	63	96
	42	f	71	53	m	62	97

[a] All percentages based on plasma samples taken before and after feeding each bird. All levels before feeding were normal. All levels after feeding (except[b]) were significantly depressed compared with previous control mean values. [b] Percentage elevation.

Chemical Methods. Chemical analysis for the various pesticides was carried out on samples of liver, muscle, and fat from birds fed diazinon, dimethoate, and phorate. The procedure for liver and muscle samples was that described previously (Bunyan and Taylor, 1966; Bunyan *et al.*, 1968b). Fat samples were ground with sodium sulfate until friable, continuously extracted with hexane, and cleaned up either as described in the case of birds fed the dimethoate diet, or by partitioning with dimethylformamide (de Faubert Maunder *et al.*, 1964) where birds were fed the diazinon or phorate diets. In these instances, final identification and estimation were by thin-layer chromatography (Bunyan, 1964). Liver and muscle extracted with chloroform and fat extracted with hexane were also examined from birds fed a diet containing demeton-methyl. Final estimation was made by solvent transfer without cleanup to 2-propanol, and the use of an agar gel diffusion technique (Beynon and Stoydin, 1965) following bromine oxidation and readjustment to pH 7.

RESULTS AND DISCUSSION

The presentation of all the individual results obtained would be both difficult and space-consuming. Only those results are recorded which, in the case of esterases, differ significantly from normal (Bunyan *et al.*, 1968a), or in the case of the residue analyses have some particular interest. These together with the results from the electrophoresis are grouped under the heading of each pesticide employing previously used conventions (Bunyan *et al.*, 1968b). Significant results are defined as those which differ by more than two standard deviations from the control mean, and percentages are inhibitions or increased enzyme activity based on the control mean (Bunyan *et al.*, 1968a,b) unless otherwise stated.

Table III. Demeton-Methyl and Diazinon Residues in Pheasant and Pigeon Tissue[a]

Feeding Time, Days	Pheasant Tissue Residues, P.P.M. Fat	Pheasant Tissue Residues, P.P.M. Liver	Pigeon Tissue Residues, P.P.M. Fat	Pigeon Tissue Residues, P.P.M. Liver
		Demeton-Me		
14	260	180	3.8	25
14	436	138	<0.1	28
28	2.7	48	<0.1	22
28	<0.1	<0.1	<0.1	45
42	5	2	<0.1	13
42	0.3	2	0.7	5
		Diazinon		
14	0.1	1	15	1
14	1	<0.1	57	<0.1
28	<0.1	<0.1	0.4	10
28	0.5	<0.1	0.2	5
42	2	0.4	<0.1	<0.1
42	1.6	0.1	<0.1	<0.1

[a] Limit of detection, 0.1 p.p.m.

Demeton-Methyl. Electrophoretic results from the pheasants are similar to those found earlier in sublethally dosed birds (Bunyan *et al.*, 1968b), where in general bands were much weaker in intensity than controls, but none were completely and uniformly inhibited. The brain cholinesterase electrophoregrams are normal. With pigeons the effects are both uniform and marked on 1-naphthyl acetate esterase electrophoregrams. The three fastest moving bands (5, 6, and 7) in the liver, and the two slowest in the kidney (1 and 2) are consistently inhibited in all birds. There is a tendency to lose the fastest running bands (4) in both the kidney and brain in birds fed for 28 or 42 days. Brain cholinesterase electrophoregrams are normal.

Esterase levels in the pheasants give an over-all picture similar to that in sublethal acutely dosed birds. Liver esterases are not consistently affected, except in the hen bird fed for 42 days, where all three esterase levels are significantly raised. Brain esterase levels (Table I) are not consistently affected except for cholinesterase, where all birds but one have about 40% inhibition. The inhibition of triacetin esterase does not mirror that of cholinesterase as closely as previously noted (Bunyan *et al.*, 1968b). Plasma cholinesterase and 1-naphthyl acetate esterase (Table II) are, however, severely depressed (>80%) in all but two instances. Enzyme levels in the pigeon are more consistently affected, and the magnitude of change is greater. Most of the birds show significantly raised liver phenyl benzoate esterase levels and in those fed 42 days triacetin esterase levels are similarly raised. Brain esterases are almost all significantly depressed (Table I), although both the measured cholinesterase and triacetin esterase levels rise slowly as the feeding period lengthens. Considered alongside the increased liver esterase levels, this suggests some adaptation by the birds to the toxicant, although brain phenyl benzoate esterase levels fall with feeding time. Plasma esterase levels (Table II) are all depressed to an even greater extent (>90%) than those in the pheasants.

Residue levels measured in organs of birds fed demeton-methyl are somewhat unreliable, because of the analytical method used, but they appear to be high, although it is possible to discern a sharp drop as the feeding period continued, particularly in the pheasants (Table III).

Diazinon. Electrophoretic results from the organs of pheasants fed diazinon show little change from controls. Only the fastest band (11) in the liver 1-naphthyl acetate esterase electrophoregrams is inhibited in all pheasants. Kidney 1-naphthyl acetate esterase electrophoregrams show greater changes. Band 1 is consistently inhibited and bands 5 to 7 are inhibited in birds fed for 28 or 42 days. Brain electrophoregrams are normal in all pheasants. As with demeton-methyl, much greater effects are observed in the pigeons fed diazinon. In the 1-naphthyl acetate electrophoregrams, the three fastest bands (5 to 7) in the liver, the two fastest bands (4 and 5) in the kidney, and the fastest band (2) in the brain are inhibited in all birds. In a previous communication (Bunyan *et al.*, 1968a) the pigeon kidney 1-naphthyl acetate esterase electrophoregram was erroneously stated to have seven bands. It has five bands. Bands 2 to 4 in the liver and band 1 in the kidney are inhibited in birds fed for the shorter periods. Brain cholinesterase electrophoregrams are unaffected.

Esterase levels in both the pheasant and pigeon livers are not significantly altered by the diazinon feeding level employed. Esterase levels in the pheasant brain are generally unaffected, but those in the pigeon are nearly all significantly depressed, although there is some indication of a recovery of activity as the time of feeding increased (Table I). Plasma esterases are severely depressed in both species (Table II).

Residue levels in the pheasants were low (Table III). There was no relationship between the magnitude of the residues and the duration of feeding. A comparison of the levels in fat and liver suggests that diazinon may accumulate in the former to an extent not shown by the other three pesticides, nor demonstrated in the pigeon. Residues in the liver and fat of pigeons (Table III) were generally higher than those in the pheasants over the short feeding periods, but fell off rapidly with time. No residues were found in pigeon muscle extracts.

Dimethoate. Electrophoretic results obtained from pheasant tissue extracts are in general not so severely affected as those obtained previously from subacutely poisoned birds (Bunyan *et al.*, 1968b). In the 1-naphthyl acetate esterase electrophoregrams of the two pheasants fed for 14 days there is complete inhibition of liver band 1 and brain bands 1, 2, 3, and 6. Electrophoregrams return to normal after 42 days with the exception of bands 5 and 6 in the liver and band 4 in the kidney. Brain cholinesterase electrophoregrams are normal. 1-Naphthyl acetate esterase electrophoregrams from the pigeons show complete inhibition of bands 1, 5, 6, and 7 in the liver, while bands 2 and 5 in the kidney and band 2 in the brain are very weak when apparent. Of the remaining bands, there appears to be an increase of intensity in the liver, and a decrease in the kidney and brain as the feeding time lengthens. Brain cholinesterase electrophoregrams are considerably decreased in intensity, although neither band is completely inhibited.

Esterase levels are not generally altered in pheasant liver, although in one bird (female, 28 days) all three levels are significantly raised, and in two of the other birds fed for the longer periods phenyl benzoate esterase levels are also raised. Brain esterase levels (Table I) are generally only slightly depressed, although phenyl benzoate esterase levels are raised. Plasma cholinesterase (about 80%) and 1-naphthyl acetate esterase (about 55 to 75%) are not severely inhibited (Table II). Pigeons show a more marked but similar pattern of inhibition or elevation. Two birds exhibit raised liver 1-naphthyl acetate esterase levels, and two raised liver phenyl benzoate esterase levels. Brain cholinesterase, triacetin esterase, and 1-naphthyl acetate esterase levels (Table I) are with one exception significantly depressed. Two brain phenyl benzoate esterase levels are significantly raised. In all the pigeons plasma cholinesterase is severely depressed and plasma phenyl benzoate esterase is significantly elevated, but plasma 1-naphthyl acetate esterase is elevated only in pigeons fed for the shorter periods (Table II).

Residue Levels. Dimethoate was found only in the pair of pheasants fed for 42 days (0.5 and 0.4 p.p.m. in liver, and 3.8 and 4.5 p.p.m. in fat) and in the livers of the two pigeons fed for 14 days (0.4 and 1.2 p.p.m.).

Phorate. Electrophoretic results from pheasants fed phorate are similar to those described for demeton-methyl. In 1-naphthyl acetate esterase electrophoregrams there is a moderately severe over-all weakening of bands but little consistent inhibition. Brain cholinesterase electrophoregrams are weak but otherwise unchanged. The effects in pigeons are very similar, with over-all weakening and occasional inhibition. Brain cholinesterase electrophoregrams are weak and band 2 is inhibited in both birds fed for 42 days.

Esterase levels in pheasant liver show a greater change with phorate than with any other compound used. With one exception (female, 28 days) all the liver triacetin esterase and 1-naphthyl acetate esterase levels are significantly depressed in the pheasant liver, while phenyl benzoate esterase is unaffected. Brain esterase levels (Table I) are not seriously affected. Only two cholinesterase levels are significantly inhibited (about 58%), and few other esterase inhibitions exceed 60% of the control mean values. Most plasma esterase levels are severely depressed (Table II). In contrast to the pheasant, pigeon liver esterases are unaffected by phorate. All brain esterases with the exception of phenyl benzoate esterase in the four birds fed for the longer periods are significantly inhibited (Table I). Cholinesterase inhibition, however, does not exceed 65%, and appears to decrease as the length of time on the diet increases. Plasma esterases are more severely depressed than those of the pheasant (Table II).

Residue Levels. No residues of phorate were found in any tissue examined. Lack of residues (< 0.1 p.p.m.) has been noted previously in pheasants dosed with phorate (Bunyan and Taylor, 1966).

GENERAL DISCUSSION

It is unlikely that wild birds would feed continuously on pesticide-contaminated food at the levels or the length of time described in these experiments. Since the effects observed from the chronic feeding of these pesticides are different both qualitatively and quantitatively from those observed earlier in acutely poisoned birds (Bunyan *et al.*, 1968b), it seems reasonable to assume that birds of unknown history, which are chronically poisoned by organophosphorus pesticides but had died from some other cause, are unlikely to be confused with those which had died from acute poisoning.

Previous communications revealed that brain esterase levels, especially cholinesterase, were consistent enough to allow abnormal values to be readily detected, and 90% inhibition of brain cholinesterase was suggested as the most reliable diagnosis of death due to organophosphate poisoning. This suggestion has received support from results obtained by Mehrotra *et al.* (1967) on the poisoning of sparrows (*Passer domesticus*) by malathion. In the current series of experiments no pheasant brain cholinesterase was inhibited more than 50%, and when diazinon and phorate were fed few significant inhibitions occurred. Pigeon brain cholinesterases appear more sensitive, and significant inhibitions were noted

in all fed birds, although few exceeded 65%. The previously postulated diagnostic rule that death due to organophosphorus poisoning is accompanied by >90% brain cholinesterase inhibition while nonlethal exposure leads to significant but lesser inhibition would seem to hold. Certain intoxications—e.g., diazinon—would not be revealed by this method.

Inhibition of triacetin esterase is similar to cholinesterase in the pigeon but appears to be greater in the pheasant. 1-Naphthyl acetate esterase exhibits the largest inhibitions and the biggest interspecies differences of the four brain esterases. Brain phenyl benzoate esterase is generally unaffected but is occasionally raised. This is particularly obvious when dimethoate is fed.

Liver esterases are not generally of use in diagnosing organophosphorus poisoning, since the levels are too erratic. However, both demeton-methyl and dimethoate cause elevations of phenyl benzoate esterase and occasionally triacetin and 1-naphthyl acetate esterase levels in pheasants and pigeons fed for longer periods.

The elevation of phenyl benzoate esterase levels seems characteristic of dimethoate. In addition to liver levels brain and plasma are significantly affected. Following acute dosage in pheasants, dimethoate inhibited liver esterases severely. The elevation reported in this communication suggests that after initial liver damage dimethoate is able to induce certain liver enzymes. Induction of liver phenyl benzoate esterase together with triacetin esterase but not cholinesterase was noted following administration of organochlorine pesticides in rats (Ball *et al.*, 1954; Crevier *et al.*, 1954). This is now recognized as part of the general increase in microsomal enzymes brought about by chlorinated hydrocarbons (Kay, 1966). Recent work, however, has suggested that many organophosphates inhibit microsomal enzymes (Nakatsugawa *et al.*, 1965; Rosenberg and Coon, 1958; Welch *et al.*, 1967). It would be of interest to ascertain if dimethoate induces other microsomal enzymes, since it appears to behave abnormally in raising phenyl benzoate esterase levels. This could then provide a valuable diagnostic feature of dimethoate poisoning.

Examination of esterase inhibition in plasma reinforces the authors' view that this tissue, even when obtainable from wild birds suspected of being poisoned, is seldom of diagnostic value, since it is both very variable and extremely sensitive to inhibition. Most of the birds used in these experiments exhibited very large plasma esterase inhibition without other apparent ill effects. In the case of diazinon, however, inhibition of blood esterases was generally the only observable effect following exposure to chronic poisoning.

Electrophoregrams, particularly cholinesterase from brain tissue extracts, are not generally affected by demeton-methyl, diazinon, or dimethoate, in pigeons or pheasants, which agrees well with previous findings in subacutely poisoned pheasants (Bunyan *et al.*, 1968b). The greatest effect appears in phorate-fed birds, where the only complete inhibition of a cholinesterase isoenzyme is noted in pigeons fed for 42 days. Changes in liver and kidney electrophoregrams are erratic and difficult to interpret, but generally they are less than those noted previously in acutely poisoned pheasants, and could not be confused with birds which had died of poisoning. There is a tendency for patterns to return to normal as the feeding period increases. Detailed results may be of value in confirming suspected exposure to a particular organophosphate.

Residues when found were small and generally decreased as the feeding period lengthened, suggesting a rapid metabolic rate which might further increase as exposure continued. In these experiments the tendency of diazinon to be stored in fat is contrary to the findings of Crofts and Noakes (1965) on the feeding of diazinon to domestic fowl. In the latter, the limit of detection was 3 p.p.m. and feeding was continued for 30 weeks, when the type of adaptation noted in this communication may have occurred. These results support the authors' previous conclusions from acutely poisoned pheasants that, in the absence of more sensitive methods of analysis, the detection of residues in the tissues of wild birds feeding on far smaller quantities of organophosphate pesticides than those employed in this study is of limited value as an aid to diagnosis of exposure.

Although none of the birds showed any toxic symptoms while on the pesticide-dressed diets, a simple comparison of weight losses during the regime suggested that the pheasants were less affected by feeding than the pigeons in all cases. The change of diet from mixed grain to wheat, necessary to feed the pesticides, may account for some weight loss in pigeons.

Using as criterion of toxicity the total number of significant esterase inhibitions caused by each of the four pesticides employed in this study (irrespective of species), phorate appears to be the most toxic, with demeton-methyl rather more toxic than either of the other two. Similar conclusions may also be drawn by examining either the average values for the inhibition (per cent) of each brain esterase produced by each pesticide, or the average inhibition of all brain esterases produced by each pesticide. When these results are considered separately for pheasants and pigeons, however, it becomes apparent that phorate produces the largest effects in the pheasant, while demeton-methyl produces the largest effects in the pigeon. Phorate appears only as effective as diazinon in the pigeon, although this may be due to a greater loss of phorate from pigeon food than from pheasant food.

The comparative approach may also be utilized to expose differences between the two species, which are more striking than those noted between pesticides. With the exception of phenyl benzoate esterase following dimethoate and phorate poisoning, every pigeon brain esterase is inhibited 20 to 30% more than the corresponding pheasant brain esterase. Although liver esterase levels do not show a similar difference, this may be due to the greater inherent variation in these values. This is confirmed by examination of the differences exhibited by the electrophoregrams, where in the cases of demeton-methyl, diazinon, and dimethoate, feeding effects are far greater in pigeon than in pheasant patterns. Phorate presents a curious reversal of the general trend, since although it inhibits pigeon brain esterases more markedly than those of pheasant, it significantly inhibits many pheasant liver esterases, while not affecting those of the pigeon. Although all plasma esterase levels are severely depressed, those in the pigeons are generally lower than those in the pheasants.

Baker *et al.* (1966) have suggested that the wide range of esterases found in the pheasant contribute to its resistance to pesticidal poisoning. The extent of this resistance may be gaged by comparing the complexity of pheasant and pigeon esterase systems previously demonstrated (Bunyan *et al.*, 1968a) and the much greater effect obtained in pigeons than in pheasants after chronic ingestion of pesticides described in this communication. Further work in this laboratory, to be reported later, has revealed even more striking differences between pigeons and other avian species following ingestion of certain organophosphates, suggesting that differences in the sensitivity of cholinesterase to inhibition or metabolic differences may also be involved.

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