Organophosphorus Poisoning

Some Properties of Avian Esterases

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Measurements of the levels of various esterase activities in control pheasant and pigeon tissue extracts have confirmed the view that normal levels are consistent enough to allow abnormal levels to be detected when encountered. In general, the control levels were not significantly altered after leaving birds at ambient temperatures for up to 12 days post-mortem before dissection. Isoesterase patterns obtained by electrophoresis were also consistent under the same conditions. Death due to Thimet poisoning was indicated by complete inhibition of brain cholinesterase and certain specific changes in isoesterase patterns. These effects were still observed 12 days post-mortem. Attempts to reactivate inhibited isoesterases were only partially successful.

Following preliminary work on the oral dosing of pheasants by Thimet, [*O,O*-diethyl-*S*-(ethylthiomethyl) phosphorodithioate; British Standard common name phorate] changes in esterase levels and electrophoretic esterase electrophoregrams were promising as indicators of poisoning by organophosphate pesticides and possibly for identification of the particular pesticide involved (Bunyan and Taylor, 1966). The course of this work indicated that further investigation of the properties of avian esterases was necessary to determine the extent of variation which might be encountered in normal birds. Furthermore, the rate of decay of esterase activity in tissues from dead birds had to be determined if these levels were to be used as criteria of poisoning, since in most cases of deaths of birds in the field, there is a considerable time lapse before examination can take place. Further work on Thimet poisoning was also undertaken to test the stability of the esterase-phosphate complex after death, and the possibility of in vitro reactivation as a further aid to the diagnosis of organophosphate pesticide poisoning. At least two species of bird were required for comparative purposes; pheasants (*Phasianus colchicus*) and pigeons (*Columba livia*) were chosen as the experimental animals because they are easily caged and handled and are also common on agricultural land in England. Both species are herbivorous and may therefore be at risk from either spraying or seed-dressing operations.

As in the earlier work (Bunyan and Taylor, 1966), emphasis has been laid on spun aqueous tissue extracts, since they allow enzyme assay and electrophoresis to be carried out on the same preparation. Triton X-100 was used for brain tissue to ensure the extraction of organophosphate-sensitive bound esterases (Bernsohn *et al.*, 1964). Data on blood plasma have been included for completeness, although blood can rarely be obtained from field samples. Four esterase determinations have been made on the extracts. Cholinesterase activity was estimated since its inhibition is the classical effect produced by organophosphorus compounds. The activity towards three other ester substrates was also measured. These substrates are representative of the three major combinations of aliphatic and aromatic acids and alcohols found in esters. The values obtained for triacetin esterase suggest that this enzyme must be very similar to cholinesterase in its properties.

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EXPERIMENTAL

Animal. Pheasants and pigeons were purchased from animal suppliers and were of heterogeneous stock and unknown history. Birds were kept in a communal aviary until required. Control birds were taken at random, weighed, killed by cervical dislocation, and either dissected immediately, or hanged head down at 20° C. for the requisite time before dissection. Blood was collected into heparinized tubes from the brachial vein before sacrifice. Blood was similarly collected 7 days before dosing from birds where predose esterase levels were required. Birds, which were given Thimet (92.5% pure) in gelatin capsules, were weighed and individually caged immediately before dosing. Those which had not died within 18 hours were sacrificed and treated in the same manner as the control birds. For control electrophoresis patterns and esterase figures, batches of three male and three female birds were used.

Preparation of Tissue Extracts. Liver, kidney, and brain were removed from each bird and placed on ice immediately after dissection. Preparation of tissue extracts was undertaken within 4 hours of dissection in the manner previously described (Bunyan and Taylor, 1966). The protein concentrations of these preparations were measured by the method of Warburg and Christian (1941).

Electrophoresis. Starch gel electrophoresis was carried out according to Smithies (1955), using running conditions and staining mixtures as previously described (Bunyan and Taylor, 1966). To obtain satisfactory electrophoretic strips, electrophoresis of pigeon tissue extracts was performed with slightly larger aliquots (liver, 25 μl.; kidney, 50 μl.; brain, 50 μl.) than those of pheasant tissue (liver, 5 μl.; kidney, 25 μl.; brain, 50 μl.).

In a few instances, gel strips of extracts from birds poisoned with Thimet were treated with oximes in an attempt to reactivate inhibited isoesterases before histochemical staining. These strips were incubated for 1 hour with either $5 \times 10^{-4} M$ 2-pyridine aldoxime methiodide,

or $10^{-5}M$ hydroxylamine hydrochloride in 0.01M phosphate buffer, before washing and staining in the usual manner. In discussing the results, bands are numbered in order from the insertion slot.

Polyacrylamide gel electrophoresis was carried out in the apparatus described by Rogers (1965), using 70 × 7 mm. columns of 7.5% w./v. Cyanogum 41 (British Drug Houses Ltd.) in 0.375M Tris buffer pH 8.9. Aliquots of tissue extracts in quantities described under starch gel electrophoresis were added to 5-mm. layers of 0.25M Tris in 10% sucrose adjusted to pH 6.7 at the head of each column. Bridge buffers were those described by Davis (1964). Electrophoresis was carried out employing a constant current of 5 ma. per tube for 75 minutes. Gels were removed from the tubes by rimming with water. Electrophoregrams of each gel column were obtained using the same reagents as described for starch gels (Bunyan and Taylor, 1966), except that gels were preincubated in the mixtures without the substrate for 30 minutes. Electrophoregrams obtained by staining with Amidoblack 10B were destained electrophoretically in 7% acetic acid (Zingale *et al.*, 1963).

Enzyme Estimations. The electrometric methods (Bunyan and Taylor, 1966) for blood and brain cholinesterase were again used for a number of measurements, but as these suffer from the disadvantage that the results cannot be quoted in absolute terms, they are expressed as Δ pH × 100 per hour per 0.1 ml. of extract. The majority of the cholinesterase results were obtained using a Radiometer automatic titration apparatus comprising a titrator Type TTT 1c and a Titrigraph recorder Type SBR 2c coupled with a syringe buret Type SBU 1a, and a TTA 31 titration assembly. The reaction was carried out with stirring under nitrogen at 25° C. and was started by the addition of the undiluted tissue extract. The pH was then maintained at the predetermined optimum of 8 by the recorded addition of 0.03N sodium hydroxide. The estimations were made with optimum reagent concentrations. For the pseudocholinesterase of plasma, the reaction mixture had a final volume of 3.5 ml. and contained acetylcholine choride (British Drug Houses Ltd.) 0.02M, sodium chloride 0.145M, and potassium chloride 0.142M. For the true cholinesterase of brain tissue, the reaction mixture had a final volume of 3.5 ml. and contained acetylcholine chloride $4 \times 10^{-3}M$, sodium chloride 0.165M, and potassium chloride 0.162M. The reaction was allowed to run for a few minutes before addition of the enzyme to obtain the nonenzymic hydrolysis rate. Results are expressed as micromoles of acetylcholine hydrolyzed per hour per milligram of protein.

Triacetin esterase levels were measured under similar conditions with the automatic titration apparatus. Following the determination of the nonenzymic hydrolysis rate, the reaction was started by the addition of undiluted tissue extract, and the pH was maintained at the predetermined optimum of 7. The reaction mixture had a final volume of 3.5 ml. and contained triacetin (Sigma Chemical Co.) 0.2M and sodium chloride 0.145M. Results are expressed as micromoles of triacetin hydrolyzed per hour per milligram of protein.

α-Naphthyl acetate esterase was measured by an adaptation of the method of Gomori (1953). Tissue extracts were diluted with 0.05M potassium dihydrogen phosphate pH 7, and 1-ml. aliquots were incubated for 1 hour at 25° C. with 4 ml. of $1.86 \times 10^{-5}M$ α-naphthyl acetate in 0.05M KH_2PO_4, pH 7, freshly prepared by 100-fold dilution of an acetone stock solution of the substrate. The reaction was terminated by the addition of a 2.5% sodium lauryl sulfate solution (1 ml.) and a freshly prepared solution of 0.1% Fast red LTR salt (Gurr Ltd.) in 2.5% Triton ×100 (Lennig Chemicals Ltd. 1 ml.). After a further 30 minutes, the color was read at 530 mμ. Nonenzymic hydrolysis values were obtained by mixing the substrate and the tissue extract immediately before adding sodium lauryl sulfate. For this purpose pheasant liver extract was diluted 1 to 5000, kidney and brain 1 to 1000, and plasma 1 to 200 with phosphate buffer pH 7. Pigeon liver, kidney, and brain extracts were diluted 1 to 1000 and plasma 1 to 5000. Results are expressed as micromoles of α-naphthol liberated per hour per milligram of protein.

Phenyl benzoate esterase was measured according to the method of Gomori (1949). For this purpose, both pheasant and pigeon liver extracts were diluted 1 to 1000, kidney and plasma 1 to 500, and brain 1 to 50 with phosphate buffer pH 6.3.

RESULTS

In general, more consistent results are obtained from control pheasants than from control pigeons. Starch gel electrophoregrams of pheasant and pigeon liver, kidney, brain, and plasma stained for α-naphthyl acetate esterase activity are illustrated in Figure 1. Figure 2 shows electrophoregrams of pheasant brain and plasma and pigeon kidney, brain and plasma stained for cholinesterase activity. The degree of variation has been described and discussed (Bunyan and Taylor, 1966). Electrophoregrams obtained using α-naphthyl acetate exhibit 11 bands for liver, 8 bands for kidney, and 4 bands for brain. Cholinesterase electrophoregrams exhibit 3 bands for brain (bands two and three true cholinesterase), 3 bands for kidney (all true cholinesterase), and 3 bands for plasma

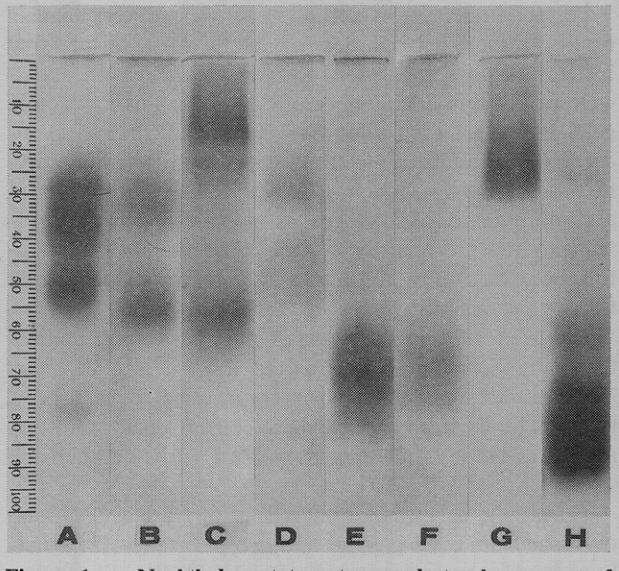

Figure 1. α-Naphthyl acetate esterase electrophoregrams of pheasant and pigeon organs and plasma

A. Pheasant liver *B.* Kidney *C.* Brain *D.* Plasma *E.* Pigeon liver *F.* Kidney *G.* Brain *H.* Plasma

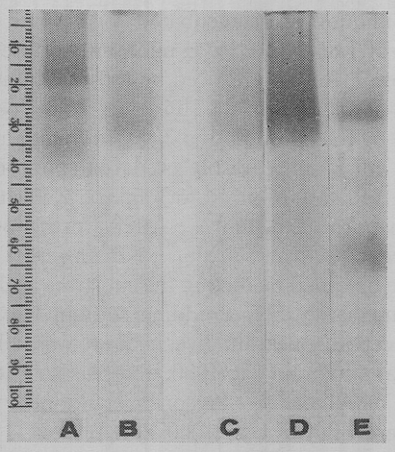

Figure 2. Cholinesterase electrophoregrams of pheasant and pigeon organs and plasma

A. Pheasant brain *B.* Plasma *C.* Pigeon kidney *D.* Brain *E.* Plasma

(band one true cholinesterase). There is slightly more variation of intensity of bands in α-naphthyl acetate electrophoregrams from pigeon tissue, and in a few instances, extra bands have appeared. As with pheasant tissue extracts, cholinesterase electrophoregrams are very consistent (Figures 1 and 2). With electrophoregrams obtained using α-naphthyl acetate, pigeon liver exhibits 7 bands, kidney 7 bands, and brain 2 bands. In sharp contrast to the pheasant, pigeon plasma exhibits a pronounced reaction with α-naphthyl acetate, giving rise to 5 bands, with a particularly strong band in the albumin region. Cholinesterase electrophoregrams exhibit 2 bands for brain, and 5 bands for plasma, but kidney tissue extract does not exhibit a reaction under these conditions. No work has been done on inhibition by organophosphates in these electrophoregrams to allow an assignment of the cholinesterase type to be made, although it appears probable that in brain the faster moving band is a true cholinesterase.

Polyacrylamide gel electrophoresis yielded very similar results, although resolution of bands was considerably better, and in some cases large diffuse bands on starch were resolved into a number of bands on polyacrylamide gel. However, difficulties have been encountered in recording the results of electrophoresis in this medium, and because of the decrease in distance traveled, both natural and inhibitor-induced variations of pattern are more difficult to interpret than on starch. The method has therefore been used more for confirmation of results on starch than as a primary method.

The results of the various esterase assays on control birds are shown in Table I, in the form of a mean $\pm s$. These results were obtained by carrying out all the assays on tissue extracts from groups of six birds. There did not appear to be any difference in esterase levels between the sexes.

The results of a limited number of esterase assays done on tissue from eight pigeons and two pheasants which had been dead for some time are shown in Table II. The earlier work on decay of esterase activity in pigeons was restricted to cholinesterase and phenyl benzoate esterase of brain tissue, and only the latter can be quoted in absolute terms

Table I. Control Esterase Levels in Pheasants and Pigeons[a]

Esterase	Pheasant				Pigeon			
	Liver	Kidney	Brain	Plasma	Liver	Kidney	Brain	Plasma
Cholinesterase	...	...	13.24 ± 1.68	70.7 ± 11.3	...	...	18.02 ± 2.91	112 ± 16.4
Triacetin esterase	3.71 ± 1.10	1.22 ± 0.53	9.78 ± 1.62	23.0 ± 14.4	2.21 ± 1.15	1.74 ± 0.94	12.93 ± 2.15	...
α-Naphthyl acetate esterase	4.50 ± 1.23	1.08 ± 0.71	6.85 ± 1.83	35.3 ± 7.3	2.83 ± 1.34	0.88 ± 0.55	4.46 ± 1.05	473 ± 69
Phenyl benzoate esterase	1.60 ± 0.87	0.52 ± 0.31	0.14 ± 0.04	6.1 ± 4.1	1.85 ± 0.86	0.62 ± 0.60	0.08 ± 0.02	76.1 ± 30.1

[a] Cholinesterase and esterase activities are expressed as micromoles of substrate hydrolyzed per hour per milligram of protein, except for plasma, when the activity is expressed as micromoles of substrate hydrolyzed per hour per ml. Assay procedures are given in the text. The results are given as means ± standard deviation (s).

Table II. *Control Esterase Levels in Pheasants and Pigeons at Various Times Post-Mortem*[a]

Bird	Post-Mortem Lag Period, Days	Brain Cholinesterase	Triacetin Esterase			α-Naphthyl Acetate Esterase			Phenyl Benzoate Esterase			
			Liver	Kidney	Brain	Liver	Kidney	Brain	Liver	Kidney	Brain	
Pigeon *A*	0	178									0.09	
Pigeon *B*	1	162									0.06	
Pigeon *C*	2	158									0.12	
Pigeon *D*	3	170									0.19	Mean ± *s* =
Pigeon *E*	5	144	Mean ± *s* =								0.07	0.11 ± 0.04
Pigeon *F*	7	116	154 ± 18								0.12	
Pigeon *G*	9	156									0.08	
Pigeon *H*	12	146									0.13	
Pheasant, male	12	3.41	9.08	4.15	...	8.83	7.17	2.53	5.12	3.98	0.151	
Pheasant, female	12	10.60	7.76	2.51	5.45	9.93	4.07	4.05	3.95	2.96	0.10	

[a] All esterase levels are expressed as micromoles of substrate hydrolyzed per hour per milligram of protein except for pigeon brain cholinesterase which is expressed as Δ pH × 100 per hour per 0.1 ml. of extract.

similar to those of Table I. However, the enzyme levels are always similar to the control mean, and there is little fall in the levels during the 12-day period under investigation. The later work carried out on a cock and a hen pheasant, in which the whole range of tissue esterases were measured after 12 days, demonstrates that in absolute terms most levels rise. In the case of the cock bird brain, where sharp decreases of levels are recorded, the organ was so decayed as to render results extremely unreliable. Starch gel electrophoresis was also performed on these extracts, and there is little change from patterns obtained with fresh control tissue. Representative examples are shown in Figure 3.

The work reported earlier (Bunyan and Taylor, 1966) on pheasants was confirmed and extended with pigeons, although only blood and the particularly significant brain tissue were examined. In the confirmatory experiment, the birds were dosed with varying amounts of Thimet and blood and brain cholinesterase, and phenyl benzoate esterase levels were examined after 18 hours. The results are shown in Table III, with dosages and other relevant information. The plasma cholinesterase levels are even more erratic than was noted with pheasants, and brain tissue extracts undoubtedly give a clearer reflection of the condition of the birds. Some difficulty was encountered

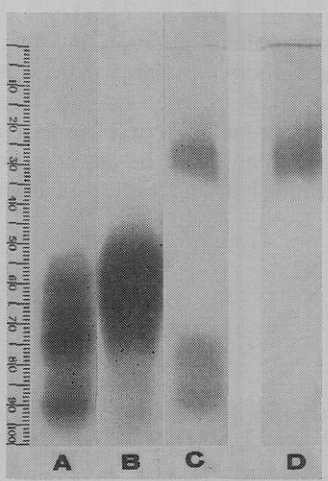

Figure 3. α-Naphthyl acetate esterase and cholinesterase electrophoregrams of pheasant tissue 12 days post-mortem

α-N.A. esterase of: *A*. Liver *B*. Kidney *C*. Brain *D*. Cholinesterase of brain

Table III. Effect of Treatment of Pigeons with Thimet on Activity of Esterases

Bird Wt., Grams	Thimet Dosage, Mg./Kg.	Condition after 18 Hours	Activities of Enzymes: Predose plasma ChE/[a] 0.1 ml.	Postdose plasma ChE/ 0.1 ml.	Brain ChE/0.1 ml.	Brain PBE/[b] mg. of protein
306	0.65	Alive	73	15	132	0.13
312	1.60	Alive	64	33	136	0.18
286	3.14	Alive	54	40	158	0.11
319	4.38	Alive	87	48	170	0.14
326	5.83	Dead	32	24	6	0.07
367	6.72	Alive	64	26	64	0.11
444	7.88	Dead	52	32	4	0.06
367	8.72	Dead	127	0	8	0.08

[a] Cholinesterase (ChE) levels are expressed as Δ pH × 100 per hour per 0.1 ml. of tissue.
[b] Phenyl benzoate esterase (PBE) is expressed as micromoles of substrate hydrolyzed per hour per milligram of protein.

with collection of blood samples from this batch of pigeons, and in a few instances slight hemolysis occurred which may account for some of the apparently low predose cholinesterase levels. Thimet appears to be even more toxic to pigeons than to pheasants. The starch gel cholinesterase electrophoregrams of all these brain extracts are normal unless death has occurred due to poisoning, when complete inhibition of band two and partial inhibition of band one occurs (Figure 4).

In addition to the earlier experiments, a further seven pigeons were given a lethal dose of Thimet, and brain cholinesterase and phenyl benzoate esterase levels were measured at various post-mortem time intervals (Table IV). Starch gel electrophoresis was also carried out on these brain extracts, and the gels were stained for cholinesterase activity. With the poisoned birds, sections of the gel were also incubated with 2-pyridinealdoxime methiodide, (2-PAM) or hydroxylamine hydrochloride before staining to attempt reactivation of any inhibited esterase bands (Figure 4).

DISCUSSION

In the earlier communication on the effect of Thimet on pheasants, the authors postulated that, with a knowledge of the normal values of avian esterase levels and of the normal electrophoretic pattern of isoesterases, organophosphorus poisoning could be detected and the poison tentatively identified by the pattern of inhibition it produced. The measurements now reported on esterases in two avian species confirm the value of this approach, since in many instances control tissue esterase levels show remarkably small variation. If it is accepted that a measured esterase value more than two standard deviations from the control mean is significantly different, then a comparison with the values given in Tables I and II allows a number of conclusions to be drawn. In those cases where subtraction of two standard deviations from the control mean leads to a negative value, the levels are considered to have no statistical significance. This happens five times out of the 14 measurements on pheasant tissue, and four out of the 13 on pigeon tissue. Plasma triacetin esterase levels, which were measured for pheasant and found to have no significance, were not measured in pigeon since they were too low.

Analysis of the results from each tissue reveals that for brain tissue in both species, all four esterase levels are significant and extremely consistent. Many other measurements carried out in this laboratory on the brains of control birds have never revealed values outside the normal limits. Furthermore, Table II reveals that these values do not fall off significantly over 12 days after death. Thus inhibitions detected in brain esterase levels, even in old tissue from field samples, allow reliable conclusions to be drawn. By comparison, blood plasma, the tissue normally used in human and veterinary medicine, is far less reliable and shows a greater range. Indeed a small proportion of the control samples which have been collected in connection with other work, particularly with pigeons, have fallen outside the "normal" range (Table I). Furthermore, in connection with diagnosis of poisoning of birds in the field, blood can rarely be collected. Plasma is therefore only of interest in the diagnosis of sublethal or chronic poisoning, although even in this case, results may not be reliable as demonstrated by the cholinesterase values obtained after poisoning (Table III). Of the liver esterases, α-naphthyl acetate esterase levels are the only ones significant in both species and may well be useful in the diagnosis of poisoning. Triacetin esterase, which is significant in both pheasant liver and kidney, appears from other work with poisoned birds to be very similar to cholinesterase. Since levels of the latter obtained by the tissue extraction method used in liver and kidney are too low to measure, triacetin esterase could prove a useful substitute wherever the levels are shown to be meaningful. In general, the low esterase

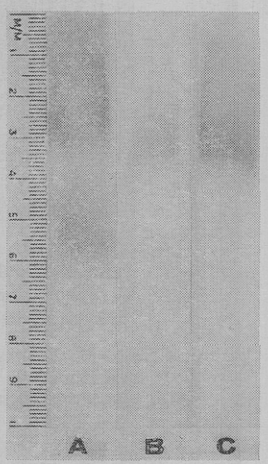

Figure 4. Reactivation of pigeon brain cholinesterases

A. Control *B*. Thimet poisoned 12 days post-mortem *C*. Thimet poisoned 12 days post-mortem, reactivated with 2-PAM ($5 \times 10^{-5} M$)

Table IV. Esterase Levels in Pigeons Measured at Various Times Post-Mortem Following Death Due to Thimet Poisoning[a]

Bird	Weight, Grams	Thimet Dosage, Mg./Kg.	Condition after 18 Hours	Post-Mortem Lag Period, Days	Brain ChE	Brain PBE
A	395	0	Alive	0	159	0.110
B	455	11.4	Alive	1	35	0.036
C	384	13.7	Dead	2	9	0.029
D	316	12.3	Dead	3	7	0.035
E	416	13.9	Dead	5	13	0.023
F	435	13.5	Dead	7	13	0.024
G	387	11.7	Dead	9	3	0.029
H	338	12.9	Dead	12	4	0.018

[a] Enzyme activities expressed as follows: cholinesterase (ChE), Δ pH $\times$ 100 per 0.1 ml. per hour; phenyl benzoate esterase (PBE), micromoles phenol liberated per milligram of protein per hour.

levels found in kidneys are of no use in diagnosis of poisoning, since they exhibit a wide range which does not allow a significance test to be applied.

The increased pheasant esterase levels in tissues extracted 12 days post-mortem are probably due to the rate of decrease in protein content outstripping the rate of decay of esterase activity. However, it does allow significant depressions of esterase activity measured in such tissue to be interpreted in the same manner as those in fresh control tissue.

While the over-all pattern of esterase levels in both species is similar, there are a few striking differences. Pigeon plasma exhibits higher levels of all measured esterases, while pheasant liver exhibits higher triacetin and α-naphthyl acetate esterase levels. These differences are mirrored by the sensitivity and complexity of electrophoregrams. Baker *et al.* (1966) suggested that the wide range of esterases present in pheasant tissue may have contributed to the success of this bird in regions of the U.S.A. where other avian species are adversely affected by pesticides.

Consideration of electrophoretic strip patterns from control birds has led the authors to believe that in both species the patterns are consistent, and that always with the pheasant and usually with the pigeon any variations under these conditions are only in relative band intensities or in over-all migration rate owing to slight changes in physical conditions. Thus, as with esterase levels, absolute changes in electrophoregrams may be acceptable as evidence of poisoning, and could prove exceptionally useful when dealing with old tissue from field samples, since such tissue extracts still yield good patterns (Figure 3). Unlike measured esterase levels, kidney electrophoregrams, which are relatively complex may also be able to contribute to this evidence. The authors hope that various pesticides will produce distinctive changes in these patterns and allow an even more positive identification. A similar approach to the problem has recently been proposed by Korolev (1965), although only involving cholinesterase levels in various tissues.

Extension of the earlier work on Thimet to the pigeon has confirmed most of the previous results. Brain tissue extract is of particular interest, because while cholinesterase levels are completely inhibited, if death occurs as a result of poisoning, they are relatively unaffected by sublethal doses. As with the pheasant, death is accompanied by the disappearance of the two faster running bands of the brain cholinesterase electrophoregram. Sublethal poisoning appears to have rather less effect on pigeon brain cholinesterase levels than on pheasant. Plasma cholinesterase levels are extremely erratic, but they do seem to be less affected than those of the pheasant, especially at higher dosages. This may be accounted for by the much higher nonspecific esterase levels of pigeon blood, which either provide alternative sites for the available inhibitor, or are able to hydrolyze it. Phenyl benzoate esterase levels do not appear to be depressed even in dead birds, but there is a significant rise in the levels measured in three of the four birds receiving the lowest doses of Thimet. This effect was noted in pheasants (Bunyan and Taylor, 1966).

Brain phenyl benzoate esterase levels are significantly depressed in the pigeons used for the aging tests (Table IV), when larger doses of Thimet were administered, and these levels fall very slightly over the 12-day experimental period. The results from this experiment again confirm that only death due to Thimet poisoning produces complete inhibition of brain cholinesterase. This is particularly well illustrated by the higher brain cholinesterase level of pigeon *B*, which survived a particularly large dose of Thimet for 18 hours before being sacrificed. They also demonstrate that there is no spontaneous recovery of activity in either of the two esterases measured. Later work has shown that spontaneous recovery of cholinesterase can occur following poisoning by certain organophosphate pesticides and that care must be taken when interpreting such results.

Unfortunately, the use of esterase levels and electrophoregrams as aids to the detection of organophosphorus poisoning constitutes a negative proof. Demonstration of reactivation of inhibited enzymes would be more positive. Thus a number of electrophoretic strips from heavily poisoned birds were preincubated with 2-PAM or hydroxylamine hydrochloride before staining for cholinesterase. These compounds are two of a number known to reactivate inhibited cholinesterase in certain instances. By comparison with the other half of the gel, some reactivation of band one of the cholinesterase electrophoregram was achieved by this method using 2-PAM, even on 12-day old tissues. Hydroxylamine hydrochloride had no effect. Bands two and three (probably true cholinesterase) were not reactivated, although in more recent work with other pesticides, a few completely successful reactivations have been achieved. No reactivation of α-naphthyl acetate electrophoregrams has been demonstrated.

An indication of poisoning by organophosphorus pesticides, by measurement of esterase levels continues to appear as a workable system provided that control esterase levels are known. As a result of the work reported here, a number of esterase levels are shown to be of no value for such a diagnosis. Further work (Bunyan *et al.*, 1968) has shown how this approach may be used when a number of pesticides are used in both lethal and sublethal single doses.

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