Recovery of cholinesterase activity in mallard ducklings administered organophosphorus pesticides

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PLEASE SCROLL DOWN FOR ARTICLE
RECOVERY OF CHOLINESTERASE ACTIVITY IN MALLARD DUCKLINGS ADMINISTERED ORGANOPHOSPHORUS PESTICIDES

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Oral doses of the organophosphorus pesticides acephate, dicrotophos, fensulfothion, fonofos, malathion, and parathion were administered to mallard ducklings (Anas platyrhynchos), and brain and plasma cholinesterase (ChE) activities were determined for up to 17 d after dosing. In vivo recovery of brain ChE activity to within 2 standard deviations of the mean activity of undosed birds occurred within 8 d, after being depressed an average of 25-58% at 24 h after dosing. In vivo recovery of plasma ChE appeared as fast as or faster than that of brain, but the pattern of recovery was more erratic and therefore statistical comparison with brain ChE recovery was not attempted. In vitro tests indicated that the potential for dephosphorylation to contribute to in vivo recovery of inhibited brain ChE differed among chemical treatments.

Some ducklings died as a result of organophosphate dosing. In an experiment in which ducklings within each treatment group received the same dose (mg/kg), the brain ChE activity in birds that died was less than that in birds that survived. Brain ChE activities in ducklings that died were significantly different among pesticide treatments: fensulfothion > parathion > acephate > malathion (p < 0.05).

INTRODUCTION

Production of organophosphorus pesticides (OPs) in the United States increased from 60,099,175 to 95,161,411 kg during 1970-1975 (Fowler and Mahan, 1976). Of the 502 pesticides used in 1972, 92 were OPs (Derache, 1977). The number and quantities of OPs will probably increase for years to come. Accompanying the increased use of these chemicals is concern about their effects on wildlife.

Identification of OP exposure is an initial step in assessing the impact of these pesticides on wildlife. Inhibition of cholinesterase (ChE) activity is the primary mode of action of OPs and is easily detected by a variety
of analytical methods. Determinations of ChE activity have diagnostic value where exposure to OPs is suspected (Ludke et al., 1975).

Although it is generally agreed that most OPs bind almost irreversibly to the ChE molecule, some spontaneous reactivation of inhibited enzyme may occur in survivors of OP poisoning (Blaber and Creasey, 1960; O'Brien, 1967; Bunyan et al., 1968). Whether or not significant reactivation occurs, synthesis of ChE can lead to recovery of ChE activity. In mammals, the rate of recovery of acetylcholinesterase, the primary ChE in the central nervous system (as opposed to butyrylcholinesterase, which is found in the plasma), depends on the OP administered (Frawley et al., 1952; Davison, 1955; Kewitz and Nachmansohn, 1957) and seems to be largely a result of differences in spontaneous reactivation rates (Bunyan et al., 1968; Blaber and Creasey, 1960) rather than rates of de novo synthesis of ChE. In the present study we examined the recovery of brain and plasma ChE activity in mallard (Anas platyrhynchos) ducklings exposed to pesticides representing five classes of OPs.

METHODS

Experimental Procedures

Three experiments were conducted between October and December 1978 with mallard ducklings purchased from a commercial source (Whistling Wings, Hanover, Ill.). At 7-10 d of age, the birds were randomly assigned to 1-m$^3$ pens in accordance with the experimental designs (see below). Pens were equipped with brooder lights; food and water were provided ad libitum.

When ducklings were 2 wk old they were given a single oral dose by feeding needle of one of the six OPs listed in Table 1, or they were designated as controls. Except for acephate, OPs were mixed with corn oil so that administration of 5 $\mu$l of the mixture per gram of body weight would produce the intended dose (mg/kg). Acephate is not soluble in corn oil and was mixed with water by a similar protocol. Doses were adjusted to allow for differences in chemical purity. Controls received 5 $\mu$l corn oil per gram of body weight, except for the acephate control group in experiment 1, which received water.

Ducklings were asphyxiated with CO$_2$. Blood was collected from the cranium in heparinized capillary tubes. A sagittal half of each brain was placed in 10 times its weight of cold 0.05 M Tris buffer, pH 8.0. Samples were stored at 4°C for analysis within 2 d and at -18°C if 3 or more days were to elapse before analysis. Immediately before analysis, samples were homogenized with a tissue grinder. Samples collected in each sacrifice period were analyzed concurrently; control values were used to standardize the assay between sacrifice periods. ChE activity was determined colorimetrically (Ellman et al., 1961) with a Bausch and Lomb Spectronic 70.
TABLE 1. Nomenclature, Class, and Purity of Organophosphorus Pesticides Tested

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Common name</th>
<th>Chemical name</th>
<th>Chemical purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiophosphoric acid</td>
<td>Parathion</td>
<td>O,O-Diethyl O-p-nitrophenyl phosphorothioate</td>
<td>98.5</td>
</tr>
<tr>
<td>group</td>
<td>Fensulfothion</td>
<td>O,O-Diethyl O-[p-(methylsulfinyl)phenyl]phosphorothioate</td>
<td>91</td>
</tr>
<tr>
<td>Dithiophosphoric acid</td>
<td>Malathion</td>
<td>Diethyl mercaptosuccinate S-ester with O,O-dimethyl phosphorodithioate</td>
<td>95</td>
</tr>
<tr>
<td>acid group</td>
<td>Dicrotophos</td>
<td>3-Hydroxy-N,N-dimethyl-3-isocrotonamide dimethyl phosphate</td>
<td>85</td>
</tr>
<tr>
<td>Phosphoric acid group</td>
<td>Fonofos</td>
<td>O-Ethyl S-phenyl ethylphosphonodithioate</td>
<td>93</td>
</tr>
<tr>
<td>Phosphonic acid group</td>
<td>Acephate</td>
<td>O,S-Dimethyl acetylphosphoramidothioate</td>
<td>98</td>
</tr>
</tbody>
</table>

spectrophotometer coupled to a strip chart recorder. Acetylthiocholine iodide (Sigma Chemical Co., St. Louis, Mo.) was the substrate. All samples were analyzed in duplicate; the average value was used in calculations. The ChE activity of treated birds was expressed as a percentage of the average ChE activity of their control counterparts. Average ChE activity of controls (calculated from all control samples without correction for daily variation) was 8.9 ± 0.9 μmol acetylthiocholine hydrolyzed per minute per gram of wet brain tissue and 1282 ± 402 nmol hydrolyzed per unit per milliliter of plasma. All statistical tests were conducted with α = 0.05.

Experiment 1

It was first necessary to establish a dose level of each OP that would significantly depress brain ChE activity, preferably to about 40-50% of normal. Five or six ducklings were assigned per cage. Ducks within each cage were designated as controls or were dosed with different amounts of the same toxicant (Table 2), and brain and plasma ChE activities were determined. Additional dose levels were tested if a significant depression of brain ChE activity was not achieved with the first dose series. Each pesticide treatment was replicated in six cages. Ducklings were sacrificed 16 h after exposure because previous experiments with dicrotophos showed this time period to be representative of the maximum ChE inhibition produced.

Experiment 2

To determine the time required for recovery and the pattern of recovery of ChE activities, six pens of six ducklings were randomly assigned to each of six OP treatments or a control group (total, 42 pens). At 14 d of age, ducklings in each treatment group received a single oral dose of one of the following as determined from experiment 1: 350 mg/kg
W. J. FLEMING AND S. P. BRADBURY

TABLE 2. Brain and Plasma Cholinesterase Activities of Mallard Ducklings 16 h after Oral Exposure to Organophosphorus Pesticides, Experiment 1

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Dose (mg/kg)</th>
<th>Brain Mean</th>
<th>Brain Range</th>
<th>Plasma Mean</th>
<th>Plasma Range</th>
<th>Number surviving/number dosed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acephate</td>
<td>25</td>
<td>76</td>
<td>54-95</td>
<td>50</td>
<td>32-94</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>74</td>
<td>46-95</td>
<td>43</td>
<td>20-67</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>55</td>
<td>38-87</td>
<td>37</td>
<td>13-60</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>225</td>
<td>50</td>
<td>34-75</td>
<td>29</td>
<td>20-46</td>
<td>5/6</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>48</td>
<td>27-66</td>
<td>36</td>
<td>26-46</td>
<td>6/6</td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>0.3</td>
<td>93</td>
<td>88-111</td>
<td>57</td>
<td>41-69</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>87</td>
<td>75-95</td>
<td>58</td>
<td>50-67</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>62</td>
<td>49-67</td>
<td>44</td>
<td>38-51</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>39</td>
<td>25-52</td>
<td>38</td>
<td>19-51</td>
<td>6/6</td>
</tr>
<tr>
<td>Fensulfothion</td>
<td>0.063</td>
<td>102</td>
<td>84-136</td>
<td>99</td>
<td>79-126</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>87</td>
<td>72-99</td>
<td>85</td>
<td>12-123</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>94</td>
<td>81-114</td>
<td>101</td>
<td>54-151</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>94</td>
<td>81-108</td>
<td>97</td>
<td>64-157</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>40</td>
<td>30-45</td>
<td>52</td>
<td>40-64</td>
<td>3/3</td>
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<tr>
<td>Fonofos</td>
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<td>83-105</td>
<td>87</td>
<td>68-111</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>94</td>
<td>83-102</td>
<td>86</td>
<td>75-106</td>
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<tr>
<td></td>
<td>5.0</td>
<td>97</td>
<td>90-108</td>
<td>75</td>
<td>55-98</td>
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<tr>
<td></td>
<td>7.5</td>
<td>92</td>
<td>80-102</td>
<td>86</td>
<td>51-123</td>
<td>6/6</td>
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<tr>
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<td>17</td>
<td>42</td>
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<td>49</td>
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<tr>
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<td>62</td>
<td>—</td>
<td>37</td>
<td>—</td>
<td>1/3</td>
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<tr>
<td>Malathion</td>
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<td>94-123</td>
<td>97</td>
<td>72-167</td>
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<td>225</td>
<td>104</td>
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<td>36-86</td>
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<tr>
<td></td>
<td>700</td>
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<td></td>
<td>900</td>
<td>49</td>
<td>38-61</td>
<td>65</td>
<td>61-69</td>
<td>2/6</td>
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<tr>
<td>Parathion</td>
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<td>86-99</td>
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<td>66-103</td>
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<td>83-106</td>
<td>89</td>
<td>68-127</td>
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</tr>
<tr>
<td></td>
<td>0.6</td>
<td>96</td>
<td>92-102</td>
<td>91</td>
<td>72-137</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
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<td>86</td>
<td>70-106</td>
<td>69</td>
<td>44-81</td>
<td>6/6</td>
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<td>1.8</td>
<td>97</td>
<td>84-112</td>
<td>—</td>
<td>—</td>
<td>3/3</td>
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<tr>
<td></td>
<td>2.0</td>
<td>50</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
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<td>28</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
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<td>30-38</td>
<td>—</td>
<td>—</td>
<td>2/4</td>
</tr>
</tbody>
</table>

*Based on the survivors of the OP administration. ChE activity in treated ducklings is expressed as a percentage of the activity in controls. Control ChE activity averaged 8.9 µm of acetylthiocholine iodide hydrolyzed/min·g of brain and 1282 nm hydrolyzed/min·ml of plasma.
acephate, 2.4 mg/kg dicrotophos, 0.75 mg/kg fensulfothion, 14 mg/kg fonofos, 650 mg/kg malathion, or 1.75 mg/kg parathion. Controls received a single dose of corn oil or water. One duckling from each cage was sacrificed at 1, 2, 4, 7, 11, and 17 d after exposure and brain and plasma ChE activities were determined. Data on recovery of brain ChE activity were log transformed and tested for linearity (Neter and Wasserman, 1974). Brain ChE activity was determined for all ducklings that died within 20 h after dosing and was compared among OP treatment groups by a one-factor analysis of variance (Snedecor and Cochran, 1973) and Duncan’s multiple range test (Duncan, 1955) on arc sine transformed data.

**Experiment 3**

To determine whether reactivation (dephosphorylation) of the phosphorylated ChE plays a role in the *in vivo* recovery of brain ChE activity, six ducklings were dosed with corn oil and six with each of the chemicals listed in Table 1. Two ducklings from each treatment group were sacrificed at 4, 18, and 48 h after exposure. Brain homogenates from each bird were divided into three subsamples. One subsample was analyzed immediately for ChE activity. The second was incubated in a 27°C water bath for 1 h and then analyzed to determine the effect of incubation on ChE activity. The third subsample was incubated for 1 h with 2 μl 0.1 M 2-pyridine aldoxime methiodide (2-PAM) per 2 ml homogenate (Blaber and Creasey, 1960) before ChE determinations. 2-PAM catalyzes dephosphorylation (O’Brien, 1967). The portion of the inhibited ChE that could not be reactivated was considered irreversibly bound (Blaber and Creasey, 1960) and thus would not play a part in the *in vivo* recovery of brain ChE.

In contrast to many other OPs, Diazinon [O, O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] inhibited ChE can undergo considerable reactivation (Bunyan et al., 1968), indicating that its dephosphorylation occurs more readily than that of ChE inhibited by some other OPs. Thus diazinon was included in this study to serve as a positive control for the ChE reactivation technique. Diazinon was administered to six ducklings, which were treated in the fashion previously described.

Data were analyzed by repeated measures analysis of variance to determine whether there was a time-reactivation procedure (fresh versus incubated versus incubated with 2-PAM) interaction. For each treatment group, the percent recovery of ChE activity in samples incubated with 2-PAM was adjusted on the basis of the amount of inhibited enzyme in the samples \([(\text{ChE activity of 2-PAM treated homogenate minus ChE activity of fresh sample})/(100 — \text{ChE activity of fresh sample})]\). Differences in reactivation among the three sampling times were tested by analysis of variance on these adjusted data.
RESULTS

Experiment 1

ChE activities were inhibited by each of the OPs administered (Table 2). The sensitivity of brain ChE to increased doses of OPs varied among chemicals. For example, an increase in parathion from 1.8 to 2 mg/kg (11% increase) caused brain ChE activity to decrease to 50% of control levels. However, the dose of dichorofos had to be increased from 0.3 to 1.2 mg/kg (300%) to produce a similar brain ChE depression.

Dose levels used in experiments 2 and 3 were selected to produce ~50% depression of brain ChE activity. Where it was not possible to achieve this level of depression without mortality, a dose producing less depression was used. Dose levels selected for further experimentation were given in the preceding section on experiment 2.

Controls dosed with water were compared with those dosed with corn oil and there were no differences in brain ChE activity ($p > 0.05$). Thus all controls in experiments 2 and 3 were dosed with corn oil.

Experiment 2

Dose levels selected from experiment 1 resulted in some mortality when given to ducklings in experiment 2. Mortality was confined to the 20-h period after dosing. Parathion, malathion, and acephate doses caused the deaths of 4 of 36, 7 of 36, and 11 of 36 ducklings, respectively. Dichorofos and fonofos treatment groups experienced no dose-related mortality in experiment 2. Brain ChE activity of the fensulfothion treatment group was unchanged at 1 and 2 d after dosing, possibly indicating that an improper dose was given. A new solution of fensulfothion was mixed and the remaining birds in the fensulfothion group were redosed 1 wk after the first exposure in an attempt to produce significant ChE depression so that recovery could be followed. The second dose was probably closer to the intended dose than the first, but resulted in 42% mortality within 1–20 h after dosing. Sacrifice schedules of treatment groups were altered as necessary to ensure that a minimum of three ducklings from each chemical treatment were available for sacrifice at each sacrifice period (number of ducks and time of sacrifice are included in Fig. 1; in one case a sample was accidentally lost, resulting in $n = 2$). The number of sacrifice dates remained unchanged for each treatment group except for the fensulfothion group. Birds redosed with fensulfothion were sacrificed at 1, 4, 10, and 15 d after the second dosing. Data from the first fensulfothion dosing were not used in the following results because of the problem with that dosing.

Within each treatment group, brain ChE activity in birds that died from OP poisoning was significantly less than in survivors dosed with similar amounts of the OP (Table 3). In addition, depression of brain ChE
activity at death was significantly different among chemical treatments; fensulfothion > parathion > acephate > malathion.

Patterns of ChE recovery after dosing are shown in Fig. 1. We attempted to linearize the recovery of brain ChE by fitting the data to the equation $Y = a + b \log X$, where $a$ is the minimum brain ChE activity observed, $b$ is the slope of the recovery, and $X$ is the number of days after dosing (Table 4). Although the model we used to linearize the recovery yielded low $r^2$ values for parathion, malathion, and fonofos, a test for linearity indicated that these values were due to individual variation within each sacrifice period rather than a poor fit of the mean daily ChE values. This can be seen in Fig. 1, where it is apparent that, in most cases, the mean ChE values for successive sacrifice periods are progressively higher.

The time required for brain ChE to recover to 80 and 100% of control levels was estimated from the regression equations (Table 4). The time to
recover to 80% was directly related to the initial ChE depression \( (r^2 = 0.93) \), whereas recovery rates (slopes) of brain ChE activity varied inversely with depression \( (r^2 = 0.97; \text{Fig. 2}) \).

At 24 h after dosing, plasma ChE activity was depressed more than brain ChE activity in the malathion \( (65 \pm 12 \text{ versus } 33 \pm 28\%; t = 3.03, \text{df} = 10) \), acephate \( (90 \pm 3 \text{ versus } 48 \pm 11\%; t = 8.26, \text{df} = 8) \), and dicrotophos \( (63.8 \pm 8 \text{ versus } 49 \pm 13\%; t = 2.45, \text{df} = 12) \) dosage groups, about equally in the fonofos \( (18 \pm 25 \text{ versus } 25 \pm 12\%; t = 0.18, \text{df} = 10) \) and parathion \( (37 \pm 7 \text{ versus } 26 \pm 30\%; t = 0.94, \text{df} = 10) \) groups, and less in the fensulfothion \( (11 \pm 16 \text{ versus } 50 \pm 18\%; t = 3.91, \text{df} = 8) \) group. Recovery of plasma activity appeared more erratic than recovery of brain activity and therefore no attempt was made to linearize the plasma ChE recovery data. To do so would have required subjective decisions about inclusion or exclusion of data collected after plasma ChE activity initially reached 100% of control activity. This problem would not have arisen if plasma ChE activity of OP-dosed ducks had not deviated so widely from control activity levels after returning to those levels. Even though it could not be tested statistically, plasma ChE activity appeared to increase to control levels more rapidly than did brain ChE activity in the acephate, dicrotophos, malathion, and parathion groups, but because of the fluctuations it cannot be said that the plasma values were normal after control levels were reached. Plasma ChE activity in the fensulfothion and fonofos groups appeared to recover at about the same rate as brain ChE activity.
TABLE 4. Recovery of Brain Cholinesterase Activity in Mallard Ducklings following Exposure to Organophosphorus Pesticides, Experiment 2°

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose (mg/kg)</th>
<th>Minimum ChE activity</th>
<th>Regression statistics of ChE activity versus days after exposure(^b)</th>
<th>Projected days to recover to control activity level based on regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>a</td>
</tr>
<tr>
<td>Acephate</td>
<td>350</td>
<td>51.7</td>
<td>4.82</td>
<td>58.2</td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>2.4</td>
<td>42.8</td>
<td>5.4</td>
<td>32.0</td>
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<tr>
<td>Fensulfothion</td>
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<td>48.9</td>
<td>7.6</td>
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<tr>
<td>Fonofos</td>
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<td>67.1</td>
<td>4.9</td>
<td>76.5</td>
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<tr>
<td>Malathion</td>
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<td>67</td>
<td>11.6</td>
<td>63.2</td>
</tr>
<tr>
<td>Parathion</td>
<td>1.75</td>
<td>74.6</td>
<td>12.4</td>
<td>66.4</td>
</tr>
</tbody>
</table>

\(^a\) Activity expressed as a percentage of that of control birds. Control ChE activities averaged 8.9 μmol acetylthiocholine hydrolyzed per minute per gram of brain.

\(^b\) \(Y = a + b(\log X)\); see text.

\(^c\) The 80% of control level is about 2 SD below the control mean. Ludke et al. (1975) suggested that brain ChE activity below 80% of normal indicates exposure to anticholinesterases.

\(^d\) Although \(r^2\) values are low, tests for linearity were positive (\(p < 0.05\)), indicating that deviations of mean values from the predicted regression line were less than the variation of individual values around the daily means.
FIGURE 2. Relation between reduced brain ChE activity produced by OP pesticides in mallard ducklings and rate of recovery of brain ChE activity in vivo. Data were taken from Table 4, where the slope of the recovery rate \( b = (Y - a)/\log X \) and the intercept \( a \) is expressed as a percent of normal ChE activity.

Experiment 3

Diazinon-inhibited brain ChE was easily reactivated by 2-PAM treatment, as expected because of its potential for dephosphorylation (Fig. 3). It also appears from Fig. 3 that acephate-, fensulfothion-, fonofos-, and parathion-inhibited ChE were easily reactivated by 2-PAM. There was a significant interaction between time of sacrifice and reactivation procedures (fresh versus incubated versus incubated with 2-PAM) in the acephate, dicrotophos, diazinon, fensulfothion, and malathion groups but not in the fonofos or parathion groups. For the 2-PAM-treated samples, there was a significant difference in reactivation among times in the dicrotophos, fensulfothion, and malathion treatment groups but not in the acephate, diazinon, fonofos, or parathion groups.

DISCUSSION

Response of brain and plasma ChE activity to dose levels within each OP treatment group in experiment 1 was highly variable. This demonstrates a wide range of sensitivity or vulnerability to these pesticides even when administered under controlled conditions. Therefore it is not likely that uniform ChE depression will be found in birds from areas recently sprayed with OPs. Chronic exposure to OPs might result in more uniform effects on ChE activity, but because of the short half-lives of many of the OPs, chronic exposure is probably not common in the environment.

Contact with OPs does not always result in measurable ChE depression
in the brain or plasma. There appears to be a threshold level of exposure below which ChE activity is not significantly altered. Therefore failure to detect depression of ChE activity does not mean that birds have not been exposed to OPs.

We were unable to produce similar levels of brain ChE depression in different treatment groups in experiment 2, and therefore our data on recovery of brain ChE activity must be viewed as specific to each OP rather than comparative among OPs or their chemical classes. This is because, as suggested by Sutton and Salmon (1975), recovery rates of brain ChE activity were inversely correlated with the maximum degree of ChE depression, regardless of the OP administered. Therefore we could not separate the brain ChE recovery data for specific OPs from the effects due to different magnitudes of ChE inhibition among OP treatments. The pesticides used in this study were 85–98.5% pure. Thus, although we described the ChE responses as if only the chemical of interest were present, it is possible that other ingredients in the pesticide stock had toxic properties or were ChE inhibitors themselves. Such contaminants may also be present in commercial formulations to which wild birds are exposed.

Blaber and Creasey (1960) described two patterns of brain ChE recovery in rats. Rapid initial recovery followed by much slower recovery characterized phosphorylated ChE that was capable of spontaneous reactivation. Phosphorylated ChE with little potential for reactivation recovered

![Graphs showing recovery of ChE activity](https://example.com/graph.png)

**FIGURE 3.** *In vivo* reactivation of mallard brain ChE by incubation of brain homogenate with 2-PAM. Mallards were dosed with OP pesticides and sacrificed at 4, 18, and 24 h after exposure. (Solid line) Fresh brain ChE; (dotted line) ChE activity after incubation with 2-PAM. Vertical connecting lines show differences in ChE activity of brain before and after treatment with 2-PAM.
at a rate described as exponential (Blaber and Creasey, 1960). These two
types of patterns were not obvious in our work, but ChE activities were
not depressed sufficiently in some cases to make this distinction. Davison
(1955) reported a change in the slope of ChE recovery at about 55% of
normal in the brains of rats and suggested that this change was character-
istic of mammalian phosphorylated true ChE. However, the rapidity of the
initial recovery and the point of inflection of the recovery curve appeared
to depend on the anti-ChE compound administered. Brain ChE activity of
fish (Benke and Murphy, 1974) and ducks exposed to some OPs (W. J.
Fleming, unpublished data) also exhibits an apparent decrease in the slope
of ChE recovery at about 50–60% of normal ChE activity.

Whether the “biphasic” type of recovery pattern is really biphasic or
whether it simply represents logarithmic recovery can be debated. How-
ever, the equation \( Y = a + b \log X \) seems to adequately describe the
recovery pattern of brain ChE activity if sample means are used instead of
individual values. If individual values are used the fit of the data to the
regression is not as good because of the high degree of individual variation
within treatment groups for each sample period. Differences in an
individual's initial ChE response to the doses of each chemical might be
responsible for some of this variation. Figure 2 makes this point.
Therefore we conclude that the low \( r^2 \) values for individual regression
lines describing recovery of brain ChE activity are due not to a poor fit of
the means for each sample period, but to the high degree of variation in
individual ChE activities.

Brain ChE activity was inhibited 90% or more in pheasants (Phasianus
colchicus) that died from exposure to four of six OPs (Bunyan et al.,
1968). In malathion-poisoned house sparrows (Passer domesticus), brain
ChE was inhibited 36–100%, but was most frequently inhibited about 80% (Mehrotra et al., 1967). Ludke et al. (1975) stated that more than 50%
inhibition of brain ChE activity in dead birds was sufficient to diagnose
anti-ChE involvement in death. We found that the degree of brain ChE
inhibition associated with death was influenced by the anti-ChE com-
 pound involved, but inhibition in dead birds exceeded 50% in all but one
individual.

The applications of our findings to environmental studies can be
summarized in four main points. (1) There is a threshold dose of each OP
that must be exceeded before brain ChE activity is inhibited. Failure to
find inhibited ChE activity does not indicate that birds have not been
exposed to OPs. Once the threshold is exceeded, brain ChE activity is a
good indicator of recent OP exposure. (2) Plasma ChE does not necessarily
respond to the OP in the same way as brain ChE. Therefore the relation
between plasma ChE activity and brain ChE activity must be established
before one can be used to predict the other. This is important when
plasma samples are collected from animals that are not to be sacrificed for
brain ChE analysis. (3) Although recovery of brain ChE activity can be
described by \( Y = a + b(\log X) \), the predictive value of this equation is uncertain because of the high variance in individual responses to the OP administered. (4) The brain ChE depression in birds dying from OP exposure is at least partially dependent on the OP involved.

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