Effect of Low Dietary Levels of Parathion and Systox on Blood Cholinesterase of Dogs

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The subacute toxicity of two organic phosphate insecticides, parathion and Systox, has been investigated in dogs by measurement of plasma and erythrocyte cholinesterase changes. As little as 1 p.p.m. of parathion and 2 p.p.m. of Systox in the diet causes significant plasma cholinesterase inhibition. Erythrocyte enzyme inhibition occurs with 2 p.p.m. of parathion and 5 p.p.m. of Systox. When both insecticides are in the same diet, the effect on plasma cholinesterase is at least additive. A convenient method for determining these cholinesterase changes is described.

CHLORINATED AND PHOSPHATE INSECTICIDES are the two principal groups of organic compounds in the chemical control of insects, and both groups have found successful and extensive use. There are problems associated with the use of insecticides, however, which do not end with the destruction of the insect. As these compounds are toxic to warm blooded animals as well as insects, protection of the agricultural worker who handles and sprays the insecticide and the consumer who ingests the agricultural products must be assured. The hazards associated with handling these materials are usually of an acute nature and are more easily evaluated than those associated with the ingestion of contaminated food. Continued ingestion of chlorinated insecticides effects cumulative tissue changes (*4, 5, 9*), whereas experiments with organic phosphate insecticides have revealed an absence of such tissue changes (*1, 3, 7, 8*), but the presence of cumulative cholinesterase enzyme inhibition (*1, 6*). It is with this change hat the present study is concerned.

In 1951, the authors reported on the cumulative changes in rat brain, plasma, and erythrocyte cholinesterase following subacute administration of five organic phosphates (*6*). As little as 5 p.p.m. of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate) in the diet of rats for 8 weeks produced erythrocyte cholinesterase inhibition. In 1954 Barnes and Denz (*1*) reported similar changes with Systox [a mixture of the thiono and thiol isomers of *O,O*-diethyl-(2-ethylmercaptoethyl) thiophosphate] at dietary levels as low as 1 p.p.m. As knowledge of the sensitivity of several species is important in evaluating the hazard of a toxicant to the human consumer and the suggestion (*2, 11*) that the human may be much more sensitive to these compounds than the rat, experiments were conducted on the cumulative effects of parathion and Systox on the plasma and erythrocyte cholinesterase of dogs. A study was conducted also to ascertain whether additive cholinesterase changes occurred if they were administered simultaneously.

Methods

Eight male and eight female young adult dogs of mixed breeds were used for this investigation. All animals were individually housed in metabolism cages and were provided with food and water *ad libitum*. The source of food consisted entirely of a ground commercial laboratory chow, to which the toxicant was added in a 1% corn oil solution to yield the desired concentration. Stock diets of 100 p.p.m. of technical parathion and 100 p.p.m. of technical Systox (commercial mixture of thiono and thiol isomers) were prepared and diluted with control feed once a week to furnish dietary levels of 1, 2, and 5 p.p.m. Five control blood samples were obtained from each animal during a 3-week pretreatment period. Groups of one male and one female dog then received 1, 2, or 5 p.p.m. of either parathion or Systox; another group received, simultaneously, 1 p.p.m. of each compound, and still another group was maintained on control feed. All animals were maintained on the test diets for 24 weeks, at which time they were returned to control diet. Table I shows the daily milligram per kilogram dosage of toxicant ingested by these dogs during the 24-week test period. Blood samples were drawn at weekly intervals for the first month and biweekly intervals for the next 5 months. During the recovery period, blood samples were drawn at weekly intervals for the first month and at 2- or 3-week intervals thereafter until normal activity was restored. All blood samples were collected with heparin.

Table I. Daily Dosage of Parathion or Systox Ingested by Dogs Receiving Diets *ad Libitum*

Diet P.P.M. Insecticide	Average Daily Dose, Mg./Kg.
5 Parathion	0.117
2 Parathion	0.047
1 Parathion	0.021
5 Systox	0.149
2 Systox	0.047
1 Systox	0.025
1 Parathion and 1 Systox	0.026 each

Plasma and erythrocyte cholinesterase measurements were made on each blood sample using a modification of Michel's electrometric method (*10*) for human cholinesterase. The technique employed for dog plasma cholinesterase was identical to that previously reported for rat plasma cholinesterase (*6*). The low activity of dog erythrocytes necessitated additional modification and these were measured by incubating for 1 hour equal volumes of a 1 to 10 dilution of washed packed cells and a 0.002*M* sodium barbital, 0.0004*M* potassium monophosphate, 0.60*M* potassium chloride buffer with a starting concentration of 0.011*M* acetylcholine bromide. In all other respects the procedure was the same as that reported for rat eryth-

rocytes. The average change of pH obtained by this technique for 440 control determinations on 68 dogs to this date has been 1.14 for plasma and 0.92 for erythrocytes. The range for these same control dogs has been 2.14 to 0.54 for plasma and 1.48 to 0.40 for erythrocytes.

Results and Discussion

Figures 1 and 2 demonstrate the effect of 1, 2, and 5 p.p.m. of parathion on the plasma and erythrocyte cholinesterase activity, respectively. Each curve represents an average of the male and female dog and the cholinesterase level is expressed as a per cent of the mean of the five control determinations of each dog. The shaded areas indicate control feeding periods. All feeding levels of parathion (Figure 1) produced some plasma cholinesterase inhibition with rapidity. No significant additional accumulation occurred after 2 weeks of feeding. The inhibition at the 1 p.p.m. feeding level was consistently significant as measured by 2 standard deviations of the mean of pretreatment samples for one dog but only occasionally for the other. Only two of the 40 control samples drawn after establishment of the initial mean for these animals were outside these same confidence limits. Whereas the 1 p.p.m. feeding level caused minimal but significant inhibition of plasma cholinesterase, the 2 and 5 p.p.m. levels caused about 60 and 70% inhibition. The recovery of plasma activity on all levels was complete within 4 weeks after return to control diet and was frequently followed by a transient elevation above pretreatment activity. This rebound phenomenon has been observed and reported by this laboratory in the case of rats (*6*).

The effect of these same levels on erythrocyte activity was not as pronounced (Figure 2). The only level which produced significant depression was 5 p.p.m. The rate of depression, however, was much slower than for plasma and maximum inhibition was obtained only after 12 weeks. The recovery rate was also slower for erythrocyte activity, requiring 11 weeks to return to within 2 standard deviations of the mean of pretreatment levels.

Figures 3 and 4 show the plasma and erythrocyte activity of dogs fed 1, 2, and 5 p.p.m. of Systox. The rate of inhibition of plasma activity resulting from Systox was much slower than that resulting from parathion (Figure 3). Maximum inhibition occurred after about 12 weeks of feeding 5 p.p.m. and significant inhibition with 2 p.p.m. only after 16 weeks of feeding. The eventual degree of inhibition from 5 p.p.m. of Systox was of the same order as that obtained with 2 and 5 p.p.m. of parathion and that from 2 p.p.m. of Systox similar

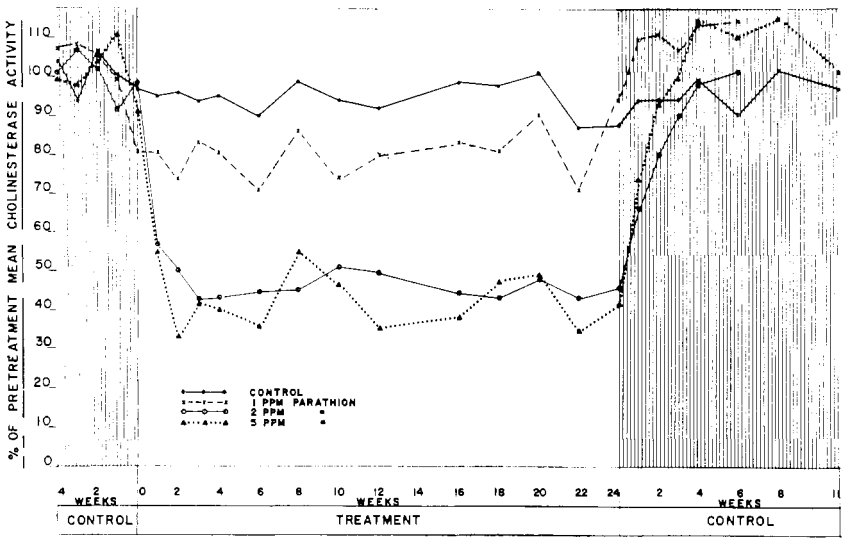

Figure 1. Plasma cholinesterase activity of dogs fed 1, 2, and 5 p.p.m. of parathion

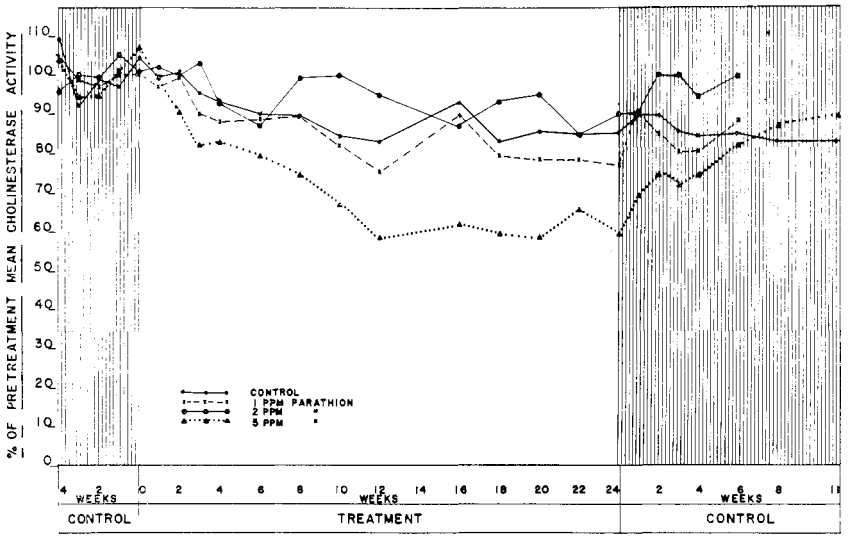

Figure 2. Erythrocyte cholinesterase activity of dogs fed 1, 2, and 5 p.p.m. of parathion

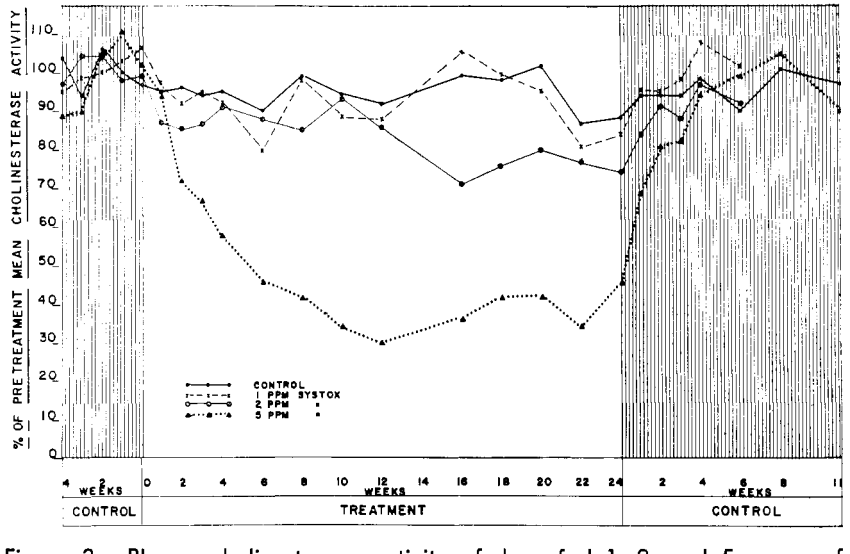

Figure 3. Plasma cholinesterase activity of dogs fed 1, 2, and 5 p.p.m. of Systox

to that obtained with 1 p.p.m. of parathion. No significant depression was found with 1 p.p.m. of Systox. The rate of recovery of plasma activity to within pretreatment limits after Systox administration was comparable to that following the feeding of parathion.

The effect of feeding these low levels of Systox on erythrocyte activity is shown in Figure 4. One and 2 p.p.m. caused no change, whereas 5 p.p.m. gave rise to some inhibition after 12 weeks. The significance of this inhibition, however, is obscured by the failure of these animals to regain their original activity, when placed on control diet, and the low activity of the control animals during the latter half of the experiment.

Figures 5 and 6 show the effect of a combination diet of 1 p.p.m. of parathion and 1 p.p.m. of Systox on plasma and erythrocyte activity. The effect of the combination is at least additive on plasma cholinesterase activity (Figure 5). Although 1 p.p.m. of Systox alone produced no significant effect, its pharmacological potential is revealed by its augmentation of the effect of 1 p.p.m. of parathion. The combination had no effect on erythrocyte activity (Figure 6).

The dog is many times more sensitive than the rat to plasma cholinesterase changes from parathion and Systox but erythrocyte changes in the dog appear to occur at about the same dosage for each compound as in the rat.

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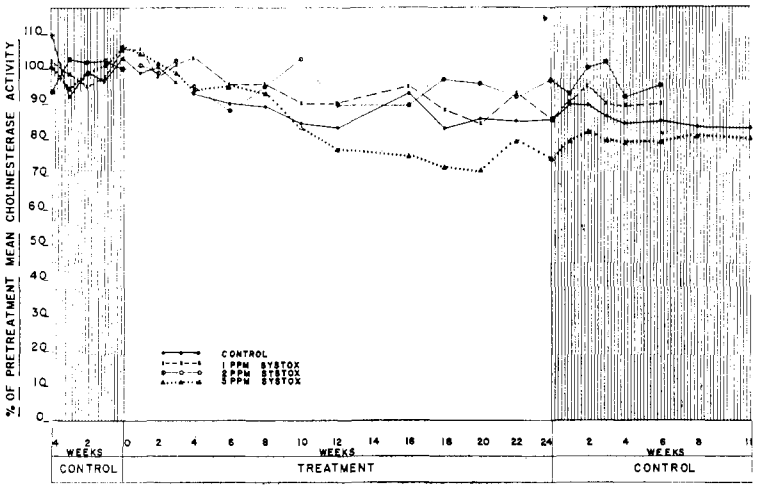

Figure 4. Erythrocyte cholinesterase activity of dogs fed 1, 2, and 5 p.p.m. of Systox

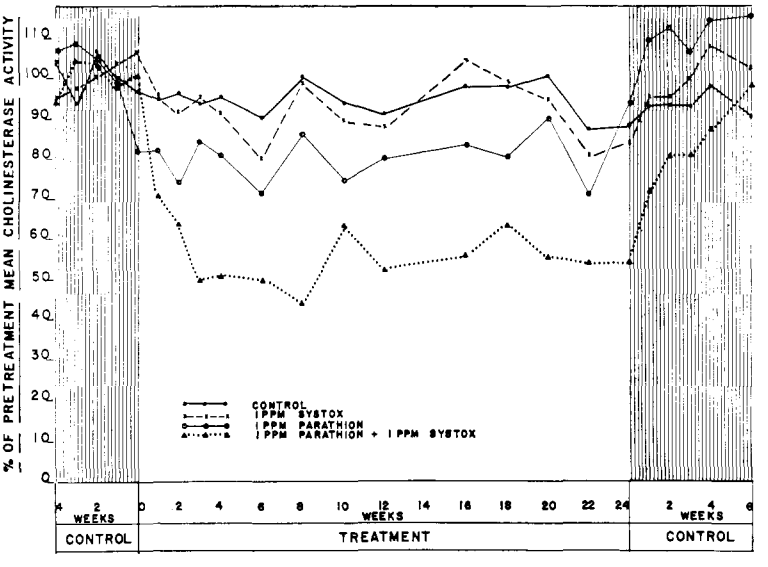

Figure 5. Plasma cholinesterase activity of dogs fed 1 p.p.m. of parathion or 1 p.p.m. of Systox or 1 p.p.m. of both parathion and Systox

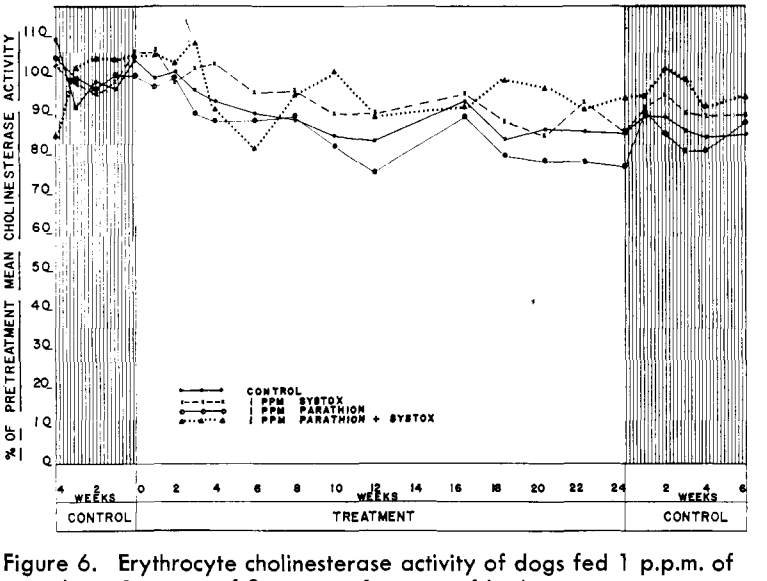

Figure 6. Erythrocyte cholinesterase activity of dogs fed 1 p.p.m. of parathion, 1 p.p.m. of Systox, or 1 p.p.m. of both