Studies on Combined Effects of Organophosphates and Heavy Metals in Birds. I. Plasma and Brain Cholinesterase in Coturnix Quail Fed Methyl Mercury and Orally Dosed with Parathion

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Organophosphates are desirable for field application because they have much shorter half-lives than other pesticides. Since they do not accumulate in the organism, identification in field samples is usually by enzymatic rather than by standard chemical assays. Cholinesterase inhibition in the brain or blood is utilized to indicate the presence of organophosphates. We have reported that brain cholinesterase in Coturnix quail decreased after parathion ingestion (Ludke et al., 1974) and that decreases in plasma cholinesterase in quail fed malathion were linearly related to the concentration of pesticide in the feed (Dieter, 1974).

In that same study (Dieter, 1974), ingestion of graded concentrations of mercuric chloride also resulted in proportional decreases in plasma cholinesterase, raising the possibility that the enzyme response caused by a heavy metal might be confused with that caused by an organophosphate. Others have reported that exposure of rabbits or humans to mercury vapors inhibited serum cholinesterase activity (Wada et al., 1969; Kosmidor et al., 1966; Oikawa, 1955), and it was suggested that this enzyme could be of diagnostic value in early detection of mercury poisoning. Lead also has been shown to inhibit blood cholinesterase in rats and horses, and brain cholinesterase in sheep (Vallee and Ulmer, 1972).

In contrast to organophosphate pesticides, the properties of heavy metals, such as bioaccumulation, long half-lives, and severe neurotoxicity and nephrotoxicity, make this class of environmental contaminant particularly insidious. In addition, recent reviews indicated that toxic levels of lead and mercury residues were present in a wide variety of wildlife species (Peakall and Lovett, 1971; Femreite et al., 1970; 1971; Bagley and Locke, 1967; Vallee and Ulmer, 1972).

The direct effects of heavy metals, the possible interference of heavy metals in the cholinesterase bioassay for organophosphates, and the possible interaction of organophosphates and heavy metals on cholinesterase activity are all unknown quantities that deserve prompt investigation. We report here that the toxicity of orally administered parathion is altered in birds chronically exposed to morsodren and that the combination of morsodren and parathion becomes lethal at concentrations that are by themselves innocuous. In addition, the sublethal biochemical effects caused by the individual toxicants are greatly enhanced in combination.

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MATERIALS AND METHODS

Male Coturnix quail (Coturnix coturnix japonica) were kept 12 to a section in holding cages in air-conditioned rooms on 12h: 12h light:dark cycles. Birds 4 wk old were randomly selected to receive 4 ppm morsodren [1](dry weight as methyl mercury) or the carrier (1% propylene glycol) for 18 wk. Forty-eight hours after 2, 4, 6, 8, and 10 mg/kg oral dosages of parathion were administered, percentage of survival was measured in control and morsodren-fed birds and the LD_{50} in the two groups compared. Additional birds of these two groups were fasted 30 min and orally dosed with a sublethal concentration of parathion (1.0 mg/kg); 30 min later their plasma and brain cholinesterase activities were compared.

Blood was obtained by cardiac puncture, and the birds were sacrificed by cervical dislocation. Whole brains were immediately removed and homogenized in cold phosphate buffer (0.1 M, pH 8.0). Aliquots of plasma (20 µl) or whole homogenates of brains (200 µl of a 10-mg/ml homogenate) were used to determine cholinesterase activity with Boehringer-Mannheim kits (Ellman, 1961) on a Beckman Acta II recording spectrophotometer at 405 nm. Reactant concentrations were such that strictly linear kinetics could be maintained.

Livers and carcasses (skinned bird minus beak, feet, wings, and gastrointestinal tract) were analyzed for mercury by cold vapor atomic absorption at WARF, Inc., Madison, Wisconsin.

The LD_{50} data were analyzed by Probit analysis (Heath et al., 1972) and the enzyme data by Student's t test.

RESULTS

Morsodren did not cause mortality during the 18-wk feeding period. Weight gains or final body weights and brain weights did not differ in controls and morsodren-fed birds. However, the toxicity of parathion was greatly increased in morsodren-fed birds compared with that in birds maintained on clean feed (Table 1). The apparent LD_{50} of parathion in morsodren-fed birds was 4 mg/kg compared with 6 mg/kg in those on clean feed. The calculated values by probit analysis were 4.24 mg/kg in birds fed morsodren and 5.86 mg/kg in clean-fed birds; the 95% confidence intervals did not overlap the means. The increase in toxicity caused by morsodren, calculated by the ration LD_{50} parathion/LD_{50} morsodren + parathion, equalled 1.38.

Morsodren at 4 ppm resulted in a significant reduction in plasma cholinesterase activity that was 14% below values in control birds

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[1] methyl mercury dycandiamide

TABLE 1.

Percentage Survival of Coturnix Quail Fed Morsodren or Clean Food and Dosed with Parathion

Oral Dose (mg/kg)	Contaminant in Feed	
	None	Morsodren
2	100 (8/8)[a]	100 (8/8)
4	100 (8/8)	50 (4/8)
6	50 (4/8)	13 (1/8)
8	13 (1/8)	0 (0/8)
10	0 (0/8)	0 (0/8)

[a] Percentage survival (number birds surviving 48h per number birds dosed).

(Table 2). Even though plasma cholinesterase in birds fed clean diets was almost totally inhibited 30min after parathion dosage (90% reduction), further and significant inhibition occurred in morsodren-fed birds challenged with parathion.

Brain cholinesterase activity in morsodren-fed birds was not significantly different from that of controls. However, the enzyme activity was inhibited 26% in clean-fed, and 41% in morsodren-fed birds that had been dosed with parathion. These changes were significantly different from those in undosed birds and from one another ($P<0.01$).

Residues of mercury in the livers were 21.0 ± 1.3 ppm and in the carcasses were 8.4 ± 0.6 ppm (means $\pm$ standard errors, n=8). The livers and carcasses of four quail on clean feed contained less than 0.05 ppm mercury.

DISCUSSION

Organophosphate pesticides have been preferred over organochlorines because they rapidly degrade in the environment, detoxify in the organism, and are much less toxic to vertebrates than invertebrates (Boyd, 1972). Their mode of action, inhibition of cholinesterase, is reversible so that critical levels of the pesticide must be exceeded and maintained in the organism before mortality ensues. Other commmonly encountered environmental contaminants, the heavy metals, are also capable of

TABLE 2.

Plasma and Brain Cholinesterase Activity[a] in Coturnix Quail Fed Morsodren or Clean Food and Dosed with Parathion. Means ± S.E., 10 - 15 Birds/treatment

Contaminant in Feed	Not Dosed	Dosed with Parathion
	Plasma	
None	2360 ± 82	239 ± 33[c]
Morsodren	2034 ± 87[b]	138 ± 13[c,d]
	Brain	
None	11.50 ± 1.30	8.45 ± 0.53[c]
Morsodren	10.03 ± 0.29	6.79 ± 0.48[c,d]

[a] Activity expressed in milliunits (nmol substrate transformed/ min/ml plasma) or units (nmol substrate transformed/ min/mg brain).

[b] Significantly different from birds on clean feed, $p<0.05$.

[c] Significantly different from birds not dosed, $p<0.01$.

[d] Significantly different from dosed birds on clean feed, $p<0.01$.

cholinesterase inhibition (Vallee and Ulmer, 1972), but they do not degrade or detoxify and therefore, they accumulate in the organism. They are just as toxic to vertebrates as to invertebrates.

We initiated this study because of our concern that heavy metals might interfere with the bioassay for organophosphates, since both affect the same enzyme. Even though the statistically significant inhibition of plasma cholinesterase could be demonstrated in this and a previous study (Dieter, 1974) using low mercury levels, it is doubtful that this metal would interfere with the plasma bioassay for organophosphates. We found that cholinesterase in the plasma was already inhibited 90% by parathion alone (1.0 mg/kg oral dose). However, the effect of morsodren on brain cholinesterase was severe enough to seriously interfere with the diagnosis of organophosphate poisoning, as parathion inhibition of the enzyme in morsodren-fed birds was almost twice that in clean-fed ones.

More importantly, there was a marked increase in the toxicity of parathion in morsodren-fed birds. According to O'Brien's (1967) definition, morsodren in company with the cholinesterase inhibitor parathion exhibits synergism, since the value of the ratio LD_{50} parathion/LD_{50} parathion + morsodren was greater than unity. It was clear that both the toxicity and the physiological effects of parathion were potentiated by morsodren. Plasma and brain cholinesterase activities were inhibited to a significantly greater degree in morsodren-fed than in clean-fed birds dosed with parathion. A 41% inhibition of brain cholinesterase in morsodren-fed birds dosed with parathion very closely approximated that associated with mortality in a previous study (Ludke et al., 1974). It appears probable that the increase in parathion toxicity in the presence of morsodren is directly related to the increased inhibition of brain cholinesterase.

Toxicological interactions among pesticides are well known, involving potentiation between organophosphates or antagonism and potentiation between chlorinated hydrocarbons and organophosphates (Frawley, 1963). We believe the present study represents the first demonstration in vertebrates of a significant biochemical interaction between heavy metals and organophosphates. Phillips et al., (1973) injected parathion into immature rats, whose parents had been chronically exposed to lead, and measured their plasma and brain cholinesterase. Using these procedures they were unable to demonstrate biological interaction of the heavy metal-organophosphate combination.

In contrast, our data for Coturnix quail shows that mercury potentiates the toxicity and biochemical effects of parathion, an organophosphate. Similar lead-organophosphate and cadmium-organophosphate studies are being conducted since these heavy metals are also prevalent environmental contaminants. These also interfere with cholinesterase activity and produce detrimental biological effects like those of mercury.

SUMMARY

We found that mercury potentiated the toxicity and biochemical effects of parathion. Male Coturnix quail (Coturnix coturnix japonica) were fed a sublethal concentration of morsodren (4 ppm as methyl mercury) for 18 weeks. This resulted in an accumulation of 21.0 ppm of mercury in the liver and 8.4 ppm in the carcass. Birds fed clean feed and those fed morsodren-treated feed were orally dosed with 2, 4, 6, 8, and 10 mg/kg parathion, and their 48-h survival times compared. The computed LD_{50} was 5.86 mg/kg in birds not fed morsodren and 4.24 in those fed the heavy metal. When challenged with a sublethal, oral dose of parathion (1.0 mg/kg), morsodren-fed birds exhibited significantly greater inhibition of plasma and brain cholinesterase activity than controls dosed with parathion. Brain cholinesterase activity was inhibited

41% in morsodren-fed birds and 26% in clean-fed birds dosed with parathion, which suggested that the increase in parathion toxicity in the presence of morsodren was directly related to the inhibition of brain cholinesterase.

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