Glucocorticoids also reduced weight gain. This may be due to their known catabolic action: they seem to induce an increase in free amino acids such as cysteine and methionine, precursors of taurine. So, it is possible that glucocorticoids increase the taurine content in the heart by increasing the levels of its precursors, although the metabolism of taurine in the heart is unknown. Another possibility is that transport of taurine into the heart is influenced by glucocorticoids.

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Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan

TOSHIO MATSUDA
SATORU YAMAGAMI
TAKUJI MIZUI
AKEMICHI BABA
HEITAROH IWATA

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In vitro degradation of malathion by mouse liver*

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In a series of recent communications [1–3], it was reported that malathion [*0,0*-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate] was hydrolyzed by two types of esterases from mouse liver. Malathion B-esterase was predominantly in the microsomal fraction and had an optimum pH between 6.4 and 6.6. Malathion A-esterase was localized in the 100,000 *g* supernatant fraction, was insensitive to inhibition by organophosphates, required reduced glutathione (GSH) or similar SH compounds, and had an optimum pH of 8.8. The A-esterase was believed to degrade malathion at the P-S linkage resulting in the formation of *0,0*-dimethyl phosphorothioate even though no diethyl thiomalate was isolated. This raised the question of whether malathion A-esterase was truly a hydrolase or possibly another enzyme system and whether the colorimetric method [4] utilized in these studies could detect the metabolite formed in the 100,000 *g* supernatant fraction.

Experimental

Chemicals. [^{14}C]-malathion [*0,0*-dimethyl *S*-(1,2-dicarbethoxy [1,2-^{14}C] ethyl) phosphorodithioate] was purchased from Amersham/Searle (Arlington Heights, IL), with a specific activity of 32.2 mCi/m-mole. Non-radioactive paraoxon, malathion, malaoxon, malathion mono- and di-acids and desmethyl malathion [*0*-potassium, *0*-methyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate] were kindly donated by American Cyanamid Co., Agricultural Division, Princeton, NJ. Reduced glutathione was purchased from ICN Pharmaceutical (Cleveland, OH).

Enzyme preparation. Male mice, (20–25 *g*), N. C. Board of Health Strain, were decapitated and the livers removed and homogenized as 10% homogenates in 0.05 M Tris–HCl buffer, pH 8.8, at 0–4°. Differential centrifugation was carried out as described previously [5]. The 100,000 *g* supernatant fraction was dialyzed against 0.05 M Tris–HCl buffer, pH 8.8, for 20 hr at 4° to remove possible endogenous cofactors.

Reaction. The reaction mixture consisted of 1.0 ml of the 100,000 *g* supernatant fraction and 2 μmoles GSH in 0.05 M Tris–HCl buffer, pH 8.8. Two μmoles malathion was added, and the reaction mixture was incubated at 37° for 30 min. In some experiments, 0.2 μmole paraoxon was added and the reaction mixture preincubated at 37° for 15 min prior to the addition of malathion. The total volume of the reaction mixture was 2.0 ml. After incubation, the reaction mixture was partitioned against an equal volume of chloroform and the radioactivity determined in each phase. The aqueous phase was adjusted to pH 1 and then re-extracted with chloroform. The chloroform phase was dried over $MgSO_4$, then concentrated for thin-layer chromatography (t.l.c.).

Chromatography. The radioactivity in the chloroform extracts was separated initially by t.l.c. using 5 × 20 cm silica gel N-HR/UV-254 (0.25 mm precoated) plates utilizing benzene–ether–acetic acid (80 : 20 : 10, v/v) as solvent system I.

The metabolites were detected by scanning the t.l.c. plates on a Packard 7201 radiochromatogram scanner. Known

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standards were visualized at 254 nm or with 2,6-dibromoquinone chlorimide [6].

After elution of unknown A from t.l.c. plates, it was separated further into two components on silica gel 60 F-254 (2.0 mm precoated) plates, using chloroform–petroleum ether (9:1, v/v) as solvent system II.

Table 1. Per cent water-soluble metabolites of malathion formed on incubation with 100,000 *g* mouse liver supernatant fraction

	Per cent metabolites	
	No paraoxon	1×10^{-4} M Paraoxon
No GSH	88.9 ± 0.7	0·00
1×10^{-3} M GSH	89.6 ± 0.4	18.5 ± 1.0

Results and Discussion

Table 1 shows the effects of paraoxon and glutathione on the degradation of malathion. In the presence of paraoxon and the absence of GSH, the formation of water-soluble metabolites of malathion was inhibited completely, whereas when GSH was added, 0.37 μmole of water-soluble metabolites was formed in 30 min. In the absence of paraoxon, the addition of glutathione had no effect on the formation of water-soluble metabolites. Dialysis of the 100,000 *g* supernatant fraction resulted in a slight decrease in activity in both the presence and the absence of glutathione as well as the absence of paraoxon.

Initially, three radioactive peaks were separated from the chloroform extracts of the pH I aqueous phase. Two of the three metabolites were identified by cochromatography as desmethyl malathion ($R_f \approx 0.11$) and malathion monoacid ($R_f \approx 0.69$). The third peak, unknown A, with an R_f of 0.89 was not identified (Table 2).

Table 2. Degradation of [^{14}C]-malathion to water-soluble metabolites (μmoles/ 30 min)

Reaction condition	Malathion monoacid	Desmethyl malathion	Unknown A
−Paraoxon, −GSH	1.78	0.0	0.0
−Paraoxon, +GSH	1.37	0.12	0.30
+Paraoxon, +GSH	0.06	0.09	0.22

Desmethyl malathion and unknown A were found only when GSH was added to the reaction mixture. This indicates a requirement for the cofactor. In the absence of any added GSH, the only metabolite isolated was malathion monoacid, the result of carboxylesterase activity. The formation of desmethyl malathion by the soluble fraction of the liver as well as the requirement for GSH would indicate that a glutathione transferase was probably involved in the formation of this metabolite. It is well documented that organophosphorus insecticides are dealkylated by glutathione *S*-transferases [7].

The incubation system was increased 1000-fold and rerun. Unknown A was isolated and then purified on preparative t.l.c. using solvent system II (R_f 0.42). Electron-impact-mass spectroscopy of the unknown resulted in an m/e value of 410.1085. This corresponded to the structure of tetraethyl dithiodisuccinate which has a calculated m/e value of 410.1068. Nuclear magnetic resonance and IR spectroscopy of the unknown resulted in spectra identical to that of synthetic tetraethyl dithiodisuccinate. A trace or unreacted malathion (R_f 0.73) also was isolated and identified as a component of unknown A.

It would appear that diethyl thiomalate was formed in the presence of glutathione and was oxidized subsequently during its isolation and purification to tetraethyl dithiodisuccinate. However, it is not clear whether diethyl thiomalate was formed directly as the result of A-esterase hydrolysis. When synthetic [^{14}C] desmethyl malathion was incubated in Tris–HCl buffer, pH 8.8, at 37° for 1 hr and subsequently extracted in chloroform at low pH, a large amount of [^{14}C] diethyl thiomalate was found. It would appear that the A-esterase which required reduced glutathione as a cofactor [4] is in reality a glutathione *S*-transferase which dealkylates malathion and the desmethyl malathion which is formed then hydrolyzes non-enzymatically to diethyl thiomalate. Therefore, the identification of diethyl thiomalate as a metabolite is not necessarily indicative of A-esterase hydrolysis.

Toxicology Program, Department of Entomology, North Carolina State University, Raleigh, NC 27650, U.S.A.

AMIN A. NOMEIR
WALTER C. DAUTERMAN

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