which synthesize special products. Although we have been unable as yet to link a specific cell product to the HSR's of neuroblastoma chromosomes, we are led to speculate from the observed association between the large chromosome regions and markedly elevated levels of dihydrofolate reductase in Chinese hamster cells that the regions are in some way functionally involved with excessive production of one or more proteins specific to the malignant neuronal cells. A first, essential question is whether the HSR's of the drug-resistant cells are transcriptionally active. The finding of increased levels of polysomal poly(A)-containing RNA in three HSR-containing sublines relative to control, drug-sensitive cells (*14*) is consistent with the large amounts of enzyme produced. The origin of the homogeneously staining chromosome region is also speculative. Possibly the region is the somatic mammalian cell chromosomal equivalent of the chromosome "puffs" of lower eukaryotes and/or is the cytological consequence of some process of chromosome amplification or magnification (*15*).

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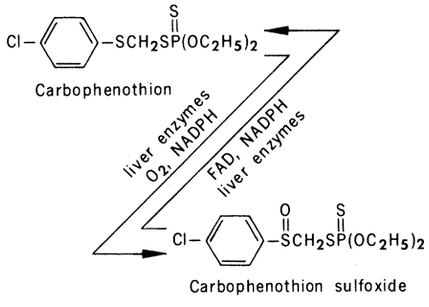
Sulfoxide Reduction in Relation to Organophosphorus Insecticide Detoxification

Abstract. *Carbophenothion sulfoxide, an oxidative metabolite of carbophenothion, is reduced to carbophenothion in the living rat and by an in vitro system containing rat liver enzyme, reduced nicotinamide adenine dinucleotide phosphate, and flavin adenine dinucleotide phosphate. Reduction of sulfoxides, formed metabolically from certain commercial organophosphorus insecticides, may be important in ameliorating the toxicity of these compounds.*

Many important insecticides contain both thionophosphorus and thioether groupings. These compounds undergo metabolic oxidation at both the thionophosphorus and thioether sites, converting them in vivo to potent cholinesterase inhibitors (*1*). This has been assumed to be an irreversible activation process, but we find that the sulfoxide or sulfinyl compound also undergoes reduction, re-forming the thioether or sulfide, under in vitro and in vivo conditions.

Our studies involved the insecticide and acaricide, carbophenothion or Trithion, and living rats and rat liver enzyme systems. These studies were performed with [phenyl-^{14}C]carbophenothion, [phenyl-^{14}C]carbophenothion sulfoxide, and [^{14}C]4-chlorothiophenol. Radiochemical purity of all administered compounds was 97 percent or greater, and [^{14}C]carbophenothion sulfoxide contained less than 0.05 percent carbophenothion.



For the in vitro studies we utilized a system reported by Mazel *et al.* (*2*) to reduce dimethylsulfoxide to dimethylsulfide, in which we replaced the dimethylsulfoxide with carbophenothion sulfoxide. A 20 percent rat liver homogenate was prepared in 0.05*M* tris(hydroxymethyl)aminomethane (tris)-HCl, *p*H 7.2, and centrifuged at 10,000*g*; 9 ml of the supernatant was treated in a Thunburg tube by repeated evacuation and purging with argon. Carbophenothion sulfoxide (1.0 μmole) was added in 0.2 ml of acetone and the evacuation and purge were repeated. Finally, 6.0 μmole of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 6.0 μmole of flavin adenine dinucleotide (FAD) were added in 1 ml of argon-flushed buffer. After no incubation or incubation for 2 hours at 37°C, the mixtures were extracted five times with 5-ml portions of ethyl acetate, and the combined extracts were analyzed by thin-layer chromatography on silica gel chromatoplates; plates were developed with each of three solvent systems: benzene; benzene + hexane (1 : 1); and 2,2,4-trimethylphentane + chloroform (3 : 2). The following products were found after incubation for 2 hours: unmetabolized carbophenothion sulfoxide, 78 percent; carbophenothion sulfone from oxidation, 1 percent; and carbophenothion from reduction, 12 percent. Two controls, one extracted without incubation and the other incubated for 2 hours but with supernatant boiled for 1 minute to destroy enzyme activity, gave essentially no metabolism: 97 percent carbophenothion sulfoxide; 0.2 percent carbophenothion sulfone, and 0.5 percent carbophenothion. Apparently the reduction is not a result of disproportionation of carbophenothion sulfoxide, as shown by the small amount of carbophenothion sulfone detected.

On oral administration to rats of carbophenothion or carbophenothion sulfoxide at 3 mg per kilogram of body weight, or 4-chlorothiophenol (4-ClTP) at 8 mg/kg, 71 to 80 percent of the radioactive [^{14}C]phenyl label appears in the urine obtained between 0 and 96 hours in each case. Figure 1 shows the identified metabolites, all of which are previously known compounds (*3*). The striking feature of the results is the quantitatively and qualitatively identical pattern of products formed from carbophenothion and carbophenothion sulfoxide. This suggests that one or both of these compounds may convert into the other, resulting ultimately in the same metabolites. Although somewhat similar results are found with 4-ClTP, they differ in several important respects. Both 4-chlorobenzenesulfinic acid (4-ClBSI) and 4-chlorobenzenesulfonic acid (4-ClBSO) are major metabolites of carbophenothion and carbophenothion sulfoxide, but are only minor metabolites of 4-ClTP. Thus, 4-ClBSI arises directly from cleavage of carbophenothion sulfoxide (reaction a in Fig. 1) or indirectly by a different type of cleavage (reaction b) and then oxidation of the 4-chlorobenzenesulfenic acid (4-ClBSE). 4-Chlorothiophenol is equally important as an intermediate in the metabolism of both carbophenothion and carbophenothion sulfoxide, since all metabolites formed through this intermediate, including 4-

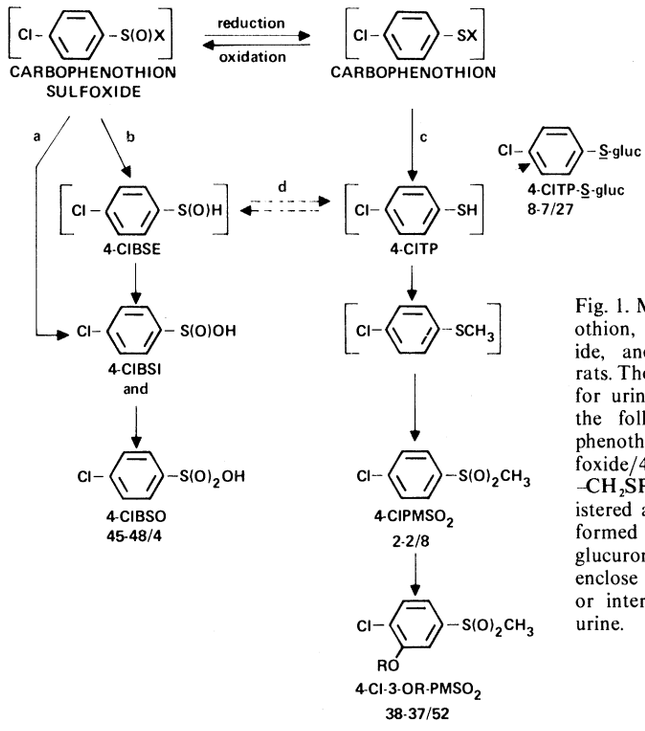


Fig. 1. Metabolism of carbophenothion, carbophenothion sulfoxide, and 4-chlorothiophenol in rats. The numbers are percentages for urinary metabolites given in the following sequence: carbophenothion–carbophenothion sulfoxide/4-chlorothiophenol. X = $-CH_2SP(S)(OC_2H_5)_2$ as administered and $-CH_2SP(O)(OC_2H_5)_2$ formed metabolically; R = H, glucuronide, or sulfate. Brackets enclose administered compound or intermediate not detected in urine.

chlorothiophenyl-*S*-glucosiduronic acid (4-ClTP-*S*-gluc), appear in nearly equal amounts from either precursor. If the 4-ClTP arises from cleavage of carbophenothion (reaction c in Fig. 1), approximately half of the carbophenothion sulfoxide may undergo in vivo reduction to carbophenothion. Although there are possible alternative pathways which might account for the observed metabolites (reaction b, hydrolysis, and then reaction d, disproportionation; or direct disproportionation of carbophenothion sulfoxide to yield carbophenothion), the in vitro enzyme studies support the hypothesis that the enzyme-mediated reduction of the sulfoxide to carbophenothion is prerequisite to the liberation of 4-ClTP.

Detection of carbophenothion in extracts of rat liver 10, 20, and 30 minutes after intraperitoneal administration of carbophenothion sulfoxide (30 mg/kg) provides unequivocal evidence for this conversion in vivo. This evidence was obtained by homogenization of the liver in acetone, extraction of the resultant precipitate with methanol, concentration of the combined acetone-methanol extracts, and ethyl acetate extraction of the aqueous residue. Carbophenothion in the ethyl acetate extracts was confirmed by cochromatography in two different two-dimensional systems with the standard having (i) 2,2,4-trimethylpentane + *p*-dioxane (2 : 1) in the first direction and 2,2,4-trimethylpentane + benzene (3 : 2) in the second direction of development; and (ii) 2,2,4-trimethylpentane + chloroform (3 : 2) in the first direction and hexane + benzene (3 : 2) in the second direction of development. The maximum concentration of carbophenothion (2.2 percent of the total liver ^{14}C; 0.1 percent of the administered ^{14}C) was recovered in the liver extract of a rat killed 30 minutes after treatment. In this extract the ratio of carbophenothion sulfoxide to carbophenothion was 2.7.

Reduction of the sulfoxide moiety of other organophosphorus insecticides in nonmammalian systems has been reported for fensulfothion sulfoxide in the bean plant (*4*) and for disulfoton sulfoxide (*5*) and phorate sulfoxide (*6*) in the soil. Other studies with compounds that do not contain phosphorus have established that sulfoxide reduction occurs in vivo in several mammalian systems, including man (*7*).

Carbophenothion is one of several commercial organophosphorus insecticides with thioether substituents that undergo oxidation to the corresponding sulfoxide and sulfone derivatives. Since these sulfoxidized derivatives are more potent cholinesterase inhibitors than the parent sulfides, the reduction, described here for carbophenothion sulfoxide, may represent a general detoxification pathway in rats and possibly other animals, plants, and environmental systems.

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Cytoplasmic Microtubule Organizing Centers Isolated from *Polytomella agilis*

Abstract. *Basal body rootlets in* Polytomella *function as organizing centers for cytoplasmic microtubules in vivo. A method is described to isolate intact basal body–rootlet complexes. The integrity of the isolated complexes is confirmed by electron microscopy, and the rootlets are shown to be competent as initiation sites for the in vitro polymerization of brain microtubule protein.*

In many developing systems microtubules appear to assemble, at specific times and in characteristic patterns, at preformed intracellular sites. Basal bodies are examples of highly structured assembly sites for the axonemal microtubules of cilia or flagella. Both the pattern and directionality of microtubule assembly in the axoneme appear to result, at least in part, from distal polymerization onto an existing pattern of tubules in the basal body (*1*). In addition, the in vitro assembly of chick brain tubulin onto isolated basal bodies has been shown to occur principally by distal addition of subunits (*2*). The spatial and temporal regulation of cytoplasmic (and spindle) microtubule assembly has also been postulated to reside in intracellular "structures" which function as initial sites of microtubule polymerization (*3*). In