Malathion Intoxication and Sulfation of Mucopolysaccharides

The effect of malathion administration on brain acetylcholine esterase activity and the uptake of a test dose of $^{35}SO_4^{2-}$ by cartilage mucopolysaccharides were compared. The percentage decrease in the fixation of $^{35}SO_4^{2-}$ was larger than the percentage depression of brain acetylcholine esterase. The effect of malathion intoxication on both $^{35}SO_4^{2-}$ uptake and acetylcholine esterase activity was more pronounced in female rats than in male animals.

Malathion's effectiveness as an insecticide and pesticide is generally attributed to the ability of its oxygen analog, malaoxon, produced *in vivo* from malathion to inhibit acetylcholine esterase. In addition to acetylcholine esterase, organophosphates have been shown to block a number of other hydrolytic enzymes, including pseudocholine esterase, lipase, other esterases, trypsin, and chymotrypsin (Adrain, 1965; Bigley and Plapp, 1962). Matsumura and Brown (1963) found proof that malaoxon is a carboxyesterase inhibitor. Murphy (1967) found that malathion given repeatedly in nontoxic doses inhibited its own further hydrolytic detoxication by inhibiting the malathion hydrolyzing esterase. Frazier (1967) reported that inhibition of esterases other than acetylcholine esterase may be a significant factor in organophosphorus intoxication. If malaoxon is a general esterase inhibitor, as the above information suggests, it should have an inhibitory effect on the formation of ester sulfate *in vivo*. The investigation reported in this paper was designed to determine if organophosphate toxicity would affect sulfate uptake by cartilage mucopolysaccharides.

PROCEDURE

The malathion was administered by stomach tube, with corn oil as a carrier to male and female albino rats weighing approximately 250 g. Malathion is well absorbed by the oral route and disperses evenly in corn oil, a carrier which facilitates its absorption (Stolman and Stewart, 1960). There were three rats in each group for each replication: one receiving malathion in oil, one receiving a sham tube feeding of oil, and one receiving no treatment. The rats were fed lab chow and received food and distilled water *ad libitum*. They were housed individually in wire mesh cages. The concentrations of malathion in the corn oil solution were 100 and 200 mg per ml, the concentration used depending on the level of malathion to be administered. As a result of using these concentrations, all rats on the study received 2 to 3 ml of corn oil per kg per day, or a total of 6 to 9 ml per kg during the 3-day treatment period, regardless of the level of malathion given.

The rats were given malathion and oil every 24 hr for 3 days. Stavinoha *et al.* (1966) have shown that symptoms of organophosphorus poisoning become maximal at about the third day of administration. They observed that the acetylcholine content of the brain tissue rose to the highest level on the third day, indicating that acetylcholine esterase is most inhibited during this period. On the third day, the rats were injected subcutaneously with approximately 10 μc $Na_2{}^{35}SO_4$ in 0.5 ml isotonic saline. Bostrom (1952) has shown that maximal incorporation into chondroitin sulfuric acid occurred 24 hr following an intrapertioneal injection of $^{35}SO_4^{2-}$. Previous work in this laboratory (Fulton and Smith, 1970) has shown that maximal incorporation into cartilage mucopolysaccharides occurs 24 hr following a subcutaneous injection of $^{35}SO_4^{2-}$; therefore, the rats were killed by decapitation 24 hr after this injection. Immediately after decapitation, the brain and costal cartilage were excised from each rat. The costal cartilage was stored at $-20°$ C until assays for ^{35}S were made.

Table I. Effect of Corn Oil Administration on Acetylcholine Esterase Activity and ^{35}S-Sulfate Uptake by Cartilage Mucopolysaccharides

	Acetylcholine Esterase μl CO_2/mg N_2		Sulfate Fixation cpm/mM $SO_4^{2-} \times 10^{-4}$	
	$\bar{s}$ oil	$\bar{c}$ oil	$\bar{s}$ oil	$\bar{c}$ oil
Male	200 ± 16	187 ± 12	8.6 ± 2.8	7.3 ± 0.2
Female	190 ± 9	192 ± 9	5.0 ± 1.4	4.3 ± 0.8

The brain of each rat was immediately placed in cold Ringer's buffer solution and homogenized. The brain acetylcholine esterase activity was determined by the Warburg manometric technique (Augustinsson, 1957). A sulfomucopolysaccharide fraction was prepared from the costal cartilage according to the modified method of Bostrom (1952). The sulfate ester linkage was hydrolyzed (Dodgson and Rice, 1962). The sulfate was precipitated, collected, and counted (Katz and Golden, 1959). The precipitate was collected on a weighed glass-fiber filter paper disc and counted for 4000 counts with a Nuclear-Chicago, windowless, gas-flow chamber fitted with an automatic changer.

RESULTS AND DISCUSSION

To determine the optimum levels of malathion to use in this investigation, a survey was conducted by giving rats levels of malathion ranging from 100 to 1000 mg per kg of rat. Levels of malathion below 250 mg per kg were found to be of little value in this type of study, and illness or death occurred when levels higher than 500 mg per kg were given. Therefore, levels of malathion ranging from 250 to 500 mg per kg were used for more intensive investigation.

Baron *et al.* (1964) have shown that high levels of corn oil, 5 to 10 ml per kg, will inhibit certain esterases; therefore, the effects of the corn oil concentration used in this experiment, 2 to 3 ml per kg per day, or a total dose of 6 to 9 ml per kg on brain acetylcholine esterase activity and sulfate uptake by cartilage mucopolysaccharides were compared. The data obtained are shown in Table I. Although the differences are not statistically significant, there is a trend toward both decreased acetylcholine esterase activity and sulfate uptake by those rats given 2 to 3 ml of corn oil per kg of rat per day. Therefore, the data which are shown in Table II compare the effect of malathion intoxication on acetylcholine esterase and

Table II. Effect of Malathion Administration on Acetylcholine Esterase Activity and ^{35}S-Sulfate Uptake by Cartilage Mucopolysaccharides

Additions mg Malathion/ kg of Rat	Acetylcholine[a] $\mu l\ CO_2/mg\ N_2$	Sulfate Uptake[b] $cpm/mM\ SO_4^{2-} \times 10^{-4}$
	Male	
None	192 ± 10	7.7 ± 2.3
250	189 ± 37	17.9 ± 9.3
300	197 ± 9	3.0 ± 0.8
500	169 ± 8	6.4 ± 1.7
	Female	
None	173 ± 9	9.9 ± 0.9
250	135 ± 17	3.7 ± 1.1
300	162 ± 11	1.8 ± 0.0
500	144 ± 10	9.4 ± 4.0

[a] Data are averages ± the standard error of the mean of values obtained from assay of rat brain homogenates of at least five rats. [b] Data are averages ± the standard error of the mean of values obtained from assay of rib cartilage mucopolysaccharides of at least five rats.

sulfate uptake of those rats receiving an equal amount, 2 to 3 ml per kg of corn oil per day. These data are noteworthy, since assay of acetylcholine esterase is the classic method of detecting organophosphorus poisoning, and since Murphy (1967) has shown that inhibition of esterases other than acetylcholine esterase are more sensitive to poisoning by these compounds. When comparing the control with the values for rats receiving malathion, the depression in sulfate uptake is greater than brain acetylcholine esterase inhibition. The greater inhibition of acetylcholine esterase activity was 22%, the value obtained by comparing the control value with the value for 250 mg malathion per kg for the females. A 63% depression of sulfate uptake by females was obtained by comparing the control value with the value for 300 mg per kg, again in female rats.

If 500 mg per kg of malathion are given to a rat, the effect on sulfate uptake is less pronounced than when 250 to 300 mg per kg are given. These data are interpreted as an indication that the utilization of sulfate is different in mild and acute intoxication. In the latter case, it is possible that mobilization and rapid turnover of sulfate occurs, which is analogous to the mobilization of sulfur amino acids which occurs in wound healing (Fromm and Nordlie, 1959).

In agreement with data collected by other investigators (Casida, 1963; Murphy and Dubois, 1955; Taylor *et al.*, 1962) the data presented in Table II show that female rats are more sensitive to malathion intoxication, based on a depression of acetylcholine esterase activity, than are males. These data also indicate that the sulfation of cartilage mucopolysaccharides in female rats is more sensitive to malathion intoxication than in males. There is a significant inhibition ($0.05 > P > 0.02$) of sulfate uptake when comparing the values obtained from female rats receiving no malathion with those given 250 mg per kg while there is not a significant decrease in the corresponding values for male rats. The same is true for the inhibition of acetylcholine esterase activity when the values for the control and 250 mg per kg are compared in both males and females. There is a highly significant decrease ($0.01 > P > 0.001$) in the inhibition of sulfate uptake in females, when comparing the control value with the value for 300 mg per kg.

Previous data, Michels and Smith (1965), have shown the important role of dietary inorganic sulfur in sulfate utilization. The data reported in this paper have shown that sulfate utilization also is affected in malathion intoxication. Therefore, the uptake of sulfate by costal cartilage may be used as a model to investigate the interrelationship of dietary sulfur and malathion intoxication (Disney and Smith, 1969).

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