COMMUNICATIONS

Malathion Intoxication and Mitochondrial Damage

There was a decrease in the hexosamine content of intact liver mitochondria but not nuclei from malathion-fed rats. The decreased hexosamine content coupled with decreased $^{35}SO_4^{2-}$ uptake indicated loss of mucopolysaccharide from the mitochondria. Extraction procedures indicated that in addition to mucopolysaccharide loss there was also a loss in nitrogen from the mitochondrial membrane. A decrease in swelling confirmed the apparent loss in mitochondrial integrity following malathion feeding.

Much of the research on malathion has been concerned with its ability to inhibit hydrolyzing enzymes, particularly acetylcholine esterase (Albert, 1965; Bigley and Plapp, 1962). A previous report from our laboratory (Disney and Smith, 1970) demonstrated that the uptake of a test dose of $^{35}SO_4^{2-}$ by cartilage mucopolysaccharides was depressed in female rats fed malathion, presumably as a result of inhibition of the controlling enzymes of sulfation. However, it was also observed that malathion intoxication resulted in a mobilization and excretion of the tissue sulfur of rats (Disney and Smith, 1967), indicating tissue degradation rather than enzyme inhibition. Subsequently a difference in physical appearance and behavior of salt-extracted liver cell preparations from malathion-fed rats and their corn oil-fed controls has been observed. Since the membranes from nuclei and mitochondria are found in these salt-extracted liver cell preparations (Levin and Thomas, 1961), an investigation was conducted to determine if changes in gross composition and/or integrity of either nuclei or mitochondria from the livers of malathion-fed rats could be detected. It is the purpose of this paper to report the results of this investigation.

PROCEDURE

The malathion was fed by stomach tube with corn oil as carrier to inbred Long Evans–Wistar cross rats. The control rats received a sham feeding of corn oil. The rats were housed in group cages with rat chow and water available *ad libitum*. They were fed malathion and corn oil every 24 hr for 3 days, at which time the symptoms of organophosphorus intoxication become maximal (Stavinoha *et al.*, 1966). On the third day, at the time of the last malathion feeding, those rats whose tissues were to be used for ^{35}S analyses were injected subcutaneously with approximately 10 μCi of $Na_2{}^{35}SO_4$ in 0.5 ml of isotonic saline. All of the rats were killed 24 hr after the last malathion feeding and their livers were removed for the isolation of nuclei and mitochondria (Hogeboom, 1955). The hexosamine content of the nuclei and mitochondria was determined by the method of Boas (1953), and their ^{35}S radioactivity was determined by liquid scintillation counting after solubilization as described by Mahin and Lofberg (1966). The channels ratio method was used to correct for quenching. Nitrogen content of the nuclear and mitochondrial preparations was determined by the micro-Kjeldahl technique (Willets and Ogg, 1950), and the percent hexosamine and ^{35}S radioactivity was expressed per mg of N. Mitochondrial swelling was evaluated as described by Tedeschi and Harris (1955). Extracted mitochondria were prepared by the method of Smith *et al.* (1957), as modified by Levin and Thomas (1961).

RESULTS AND DISCUSSION

A previous report (Disney and Smith, 1970) demonstrated a decrease in the uptake of a test dose of $^{35}SO_4^{2-}$ by rib cartilage mucopolysaccharides from malathion-fed rats. Salt-extracted liver cell preparations similar to those in which the physical changes with malathion feeding were observed contain a mucopolysaccharide fraction which was shown to be altered by dietary modifications (Fulton and Smith, 1970). Therefore, it seemed reasonable to compare the hexosamine content of the nuclear and mitochondrial fraction of livers from malathion- and corn oil-fed rats. The data obtained from this comparison (Table I) show a statistically significant 25% decrease in the hexosamine content of the mitochondria from the livers of malathion-fed rats but no effect on their nuclei.

These data which show specificity of malathion intoxication for reducing mitochondrial but not nuclear hexosamine indicate a reduction in mitochondrial hexosamine as a result of malathion feeding. A comparison of the uptake of a test dose of $^{35}SO_4^{2-}$ by the mitochondrial fraction isolated from malathion- and corn oil-fed rats (Table II) shows a 42% decrease in the uptake of $^{35}SO_4^{2-}$ by the mitochondria from the livers of malathion-fed rats.

A decrease in hexosamine content and a decreased uptake of $^{35}SO_4^{2-}$ indicate that there is a loss of mucopolysaccharide material from the mitochondria of malathion-fed rats. In order to determine if the loss of mucopolysaccharide material from the mitochondria impaired their integrity, passive swelling of the mitochondria was determined. These data (Table II), based on a decrease in absorbance with swelling, show a significant decrease in the swelling of the mitochondria isolated from the livers of malathion-fed rats.

Changes in the passive swelling of mitochondria should reflect changes in the membrane. The salt-extracted preparations of Fulton and Smith (1970) contain the membranes of cells. Therefore, the mitochondrial fractions from malathion- and corn oil-fed rats were subjected to the salt extraction procedure to remove the matrix and the ^{35}S specific activity and hexosamine content of the residue determined. These data (Table III), which show an increase in the uptake of $^{35}SO_4^{2-}$ and in the percent of hexosamine of the mitochondrial residue isolated from the livers of malathion-fed rats, are the reverse of those found based on the nitrogen content of the intact mitochondria.

Table I. A Comparison of the Hexosamine Content of Liver Nuclei and Mitochondria from Malathion-Treated *vs.* Untreated Rats[a]

Malathion fed, mg/kg rat	Hexosamine concentration	
	Nuclei, mg/mg N	Mitochondria, mg/mg N
0	0.016 ± 0.001	0.034 ± 0.003
250	0.016 ± 0.001	0.026 ± 0.003
Difference	0	0.008
Significance	$p > 0.1$	$0.01 > p > 0.001$

[a] Each of four replications includes pooled livers of five male rats.

Table II. A Comparison of $^{35}SO_4^{2-}$ Uptake and Swelling of Liver Mitochondria from Malathion-Treated *vs.* Untreated Rats[a]

Malathion fed, mg/kg rat	^{35}S Sulfate uptake, cpm/mg N	Swelling, % decrease in absorbance
0	2462 ± 91	53 ± 1
250	1437 ± 233	49 ± 1
Difference	1025	4
Significance	$0.02 > p > 0.01$	$0.02 > p > 0.01$

[a] Data are averages ± *SE* of values obtained from five female rats.

The apparent reversal of the malathion effect on specific activity and hexosamine content which has been reported when whole mitochondria are compared with mitochondrial membranes may be explained by assuming a loss in membrane nitrogen. Based on the nitrogen content of the whole mitochondria, there is a 25% decrease in hexosamine content, but based on the nitrogen content of the membrane, there is a 10% increase in hexosamine content as a result of malathion intoxication. Therefore, in order to obtain these data, the mitochondria would have to lose 25% of the mucopolysaccharide and 35% of the nitrogen in their membranes. It can be argued that the apparent loss of membrane nitrogen and hexosamine is an artifact of the extraction procedure. However, the ^{35}S specific activity of the mucopolysaccharide in the malathion-fed and corn oil-fed mitochondrial membranes is 8.0×10^5 cpm and 8.5×10^5 cpm per mg of hexosamine, respectively. Since this difference is only 6%, preparation artifacts could not explain the differences observed. Thus,

Table III. A Comparison of $^{35}SO_4^{2-}$ Uptake and Hexosamine Content of Salt-Extracted Liver Mitochondria from Malathion-Treated *vs.* Untreated Rats[a]

Malathion fed, mg/kg rat	^{35}S Sulfate uptake, cpm/mg N	Hexosamine concentration, mg/mg N
0	2290	0.029
250	2729	0.032
Difference	439	0.003

[a] These data were obtained from the pooled livers of 15 female rats.

the apparent loss of membrane nitrogen, mucopolysaccharide, and a decrease in the swelling of mitochondria isolated from the livers of malathion-fed rats indicate that malathion intoxication damages mitochondria and impairs their function.

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A Pesticide Residue Data Information Retrieval System

The retrieval of laboratory, residue, and field data necessary for the formulation of safe and effective pest control programs is accomplished by a computer search of previously coded data collected from various locations. The retrieval program uses up to 16 search keys, including chemical, crop, and analytical method used. A printed report is prepared which contains 24 field and laboratory parameters, sample identification number, and residue found. The program is designed to run on a small computer using disk storage for all data.

The obvious culmination of any pesticide research program is the publication and interpretation of data and the intergration of all data available into practical recommendations for the safe and effective utilization of the chemical under study.

The data amassed by many investigators in countless controlled experiments, ranging over field and orchard, domestic and laboratory animals, and extending over a period of many years, present a difficult problem in information retrieval when recommendations for a particular pesticide are finally contemplated. Numerous retrieval systems were studied, including the notched edge cards (Elias and Warren, 1962). This method, however, becomes cumbersome as the data collection grows. The technique used by the pharmaceutical