Metabolism of Maleic Hydrazide (MH) by Hepatic Microsomes from Phenobarbital Induced Rats

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Mammalian liver microsomes are capable of metabolizing a large number of xenobiotic substances, including pesticides (KAPPAS and ALVARES, 1975). The activity of these systems is enhanced by pre-treatment with inducers such as phenobarbital. Microsomes are responsible for degrading, and sometimes activating a large number of organic pesticides. The degradability of maleic hydrazide (MH) in these systems, however, is unknown. The objectives of the present experiment were to determine whether isolated rat liver microsomes could fragment MH to CO_2, whether hydrazine was produced as a byproduct, and the identity of other products formed by metabolism. This is part of a larger study on the environmental safety of MH.

METHODS AND MATERIALS

Two male white rats (Spague-Dawley, 450g) were pretreated for three consecutive days with phenobarbital (75 mg/kg,i. p.). On the fourth day the rats were killed by cerebral concussion and hepatic microsomes were prepared as indicated by conventional procedures (REMMER et al., 1967). Protein concentrations were determined by the method of LOWRY et al. (1951) and cytochrome P450 by the method of OMURA and SATO (1964).

Incubations with microsomes, an NADPH-generating system and maleic hydrazide were carried out in stoppered Warburg flasks. Each flask contained 4.0 mg of microsomal protein, 1.8 µ moles of NADP, 18 µ moles of glucose-6-phosphate (G-6-P), 0.4 Kornberg units of glucose-6-phosphate dehydrogenase (G-6-PD) and substrate in methyl cellosolve brought up to a total volume of 6.0 ml with 0.1 M phosphate buffer pH 7.4. The Warburg flasks allowed for trapping of $^{14}CO_2$ in 0.1 M KOH in the center well and hydrazine (H_2NNH_2) in p-dimethylaminobenzaldehyde (2g in 100 ml of 1N H_2SO_4)

Three sets of incubations employed the following solutions.

1. Flasks 1 thru 6 contained 64 nCi of 3,6-[^{14}C] MH (Sp. act.0.3m Ci/M mole) in 10 µl methanol.
2. Flasks 7 thru 12 contained 126 nCi of 4,5-[^{14}C] MH (Sp. act.2.82M mole) in 10 µl methanol.
3. Flasks 13 thru 18 contained 24.1 µg of unlabeled MH added in 10 µl methanol.
4. Flasks 19 and 20 contained 20 µg of unlabeled aldrin.

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For each set of incubations, there were four flasks of the complete incubation mixture and two flasks of heat inactivated controls minus the added cofactors. Incubations were for 1 hr at 37°C with mild shaking. Incubations were terminated by adding 1.0 ml of 20% TCA and restoppering the flasks. Aliquots of the incubation mixture and KOH trapping solution were counted by liquid scintillation counting with corrections by internal standardization. The hydrazine trapping solutions for replicate incubations were combined and read at 450 nm on a Bausch & Lomb Spectronic 20 colorimeter.

RESULTS AND DISCUSSION

The microsomal preparation was enzymatically active as indicated by the increased level of cytochrome P450 and the complete conversion of aldrin to dieldrin (flasks 19 & 20) in a positive control incubation. No $^{14}CO_2$ was detected from either 3,6- or 4,5-^{14}C-labeled MH as determined by the radioactivity counting data (Table 1).

TABLE 1

Table 1. ^{14}C-Radioactivity recovered after incubation of ^{14}C-MH with induced rat liver microsomes.

Labeling	& Sample number	KOH	% added ^{14}C Aqueous	Total
3,6-^{14}C	1 Complete[a]	.08	81.96	82.04
"	2 "	.06	82.61	82.67
"	3 "	.04	81.93	81.97
"	4 "	.03	81.95	81.98
"	5 Control[b]	.02	82.35	82.37
"	6 "	.01	79.86	79.87
4,5-^{14}C	7 Complete	.01	94.09	94.10
"	8 "	.01	96.66	96.67
"	9 "	.02	91.92	91.94
"	10 "	.00	94.54	94.54
"	11 Control	.01	91.89	91.90
"	12 "	.02	94.47	94.49

[a]Incubation Mixture
4.0 mg microsomal protein
1.8 μ moles NADP
18 μ moles G-6-P
0.4 Kornberg Units of G-G-P dehydrogenase
10 μl of Maleic Hydrazide solution all in 6.0 ml 0.1 M Phosphate Buffer pH 7.4

[b]Control
Same as above except microsomal fraction had been heated at 100° C for 10 min and no NADP, G-6-P or G-6-PD were added

The recovered radioactivity was consistently lower with 3,6-^{14}C labeled MH than with the 4,5-^{14}C labeled MH. This difference was found with both complete and control incubations, thus indicating it is not a difference due to metabolism. The amount of radioactivity trapped as $^{14}CO_2$ was insignificant in all incubations. Colorimetric examination of the dimethylaminobenzaldehyde (DMAB) trapping solution indicated there was no detectable amount of hydrazine liberated during the incubations.

The incubation mixtures were lipholized, extracted with acetone, and the extracts spotted on TLC plates (Brinkman silica gel G254 thin layer plates). The relative mobility of maleic hydrazide, maleimide, maleamic acid, maleic acid, fumaric acid, and succinic acid were compared with the radioactivity from the incubation mixture using benzene-dioxane-acetic acid (90:25:4); butanolacetic acid-water (120:30:50) and ethylacetate-isopropanol-water (18:2:1) as solvent systems. Only intact ^{14}C- labeled maleic hydrazide was detected on the TLC plates.

Hydrazine has been detected in samples of tobacco, tobacco smoke and maleic hydrazide using a trapping solution of pentafluorobenzaldehyde to form the decafluorobenzaldehyde azine (LIU et al., 1974). Pyrolysis of maleic hydrazide at 650°C yielded small amounts (0.06%) of hydrazine (HARKE et al., 1973). Hydrazine rapidly reacted with acetaldazine and acetonitrile in cigarette smoke. The reported fragmentation of maleic hydrazide to hydrazine, a known carcinogen inducing adenomas and adenocarcinomas in lungs of mice (USPHS, 1973), in these pyrolysis studies made it imperative to determine whether mammalian liver microsomes could affect the same reaction.

These microsomal results all suggest a lack of metabolism of MH by this *in vitro* system. This observation is supported by a previous *in vivo* study by MAYS et al. (1968). Administration of a single oral dose of [^{14}C] MH to rats was almost entirely eliminated by excretion in the urine, 65% in 12 hours, as the parent material or to a lesser extent a conjugate of the parent material. Our results based on the failure to detect CO_2, hydrazine or any suspected metabolites, strongly suggests that mammalian liver microsomal systems are incapable of degrading MH.

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