Influence of Some Cations on the Intestinal Absorption of Maneb

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Orally administered maneb [polymeric manganese ethylenebis(dithiocarbamate)] is rapidly degraded in the intestine of rats, excretion being almost complete within 3 days. After administration of [^{14}C]maneb to female rats, about 50, 40, and 1% of ^{14}C activity was excreted in urine, feces, and exhaled air, respectively. Studies with [^{54}Mn]maneb showed that no absorption of manganese complexes occurred. Simultaneous administration of Fe(III), Zn(II), Hg(II), and Cu(II) salts significantly reduced the excreted ^{14}C activity in urine and in exhaled air. It was concluded that formation of stronger and less soluble complexes of the ethylenebis(dithiocarbamate) (EBDC) anion and its degradation products were responsible for this fact and that the presence of certain naturally occurring cations in food might reduce the amount of EBDC residues absorbed. This depression of the absorption rate has to be considered in estimating hazards due to low levels of maneb residues in food.

Ethylenebis(dithiocarbamates) (EBDC) have been used extensively as fungicides for more than 30 years. They are readily degraded in plants and in animals, yielding ethylenethiuram monosulfide (ETM), ethylenethiourea (ETU), ethylenethiuram disulfide (ETDS), and ethylenediamine (EDA) as major metabolites (Vonk, 1975; Lyman, 1971; Engst and Schnaak, 1970).

Toxicological investigations have suggested tumorigenic, (Graham et al., 1975; Ulland et al., 1972; Innes et al., 1969) and teratogenic (Khera, 1973; Ruddick et al., 1976; Teramoto et al., 1975; Smith, 1976; Larsson et al., 1976) as well as mutagenic properties (Antonovich et al., 1972; Teramoto et al., 1977) of ETU and maneb.

Most of the degradation products of EBDC as well as the EBDC anion itself possess potent chelation properties and the influence of some cations on the absorption rate of the EBDCs, i.e., maneb and its degradation products, must therefore be expected. It has been shown by Larsson et al. (1976) that $ZnCl_2$ acts in a protective way against teratogenic effects produced by maneb.

In this article, data are presented regarding the influence of Fe(III), Zn(II), Hg(II), and Cu(II) salts on the excretion rates of radiolabeled metabolites after a single oral dose of labeled maneb.

MATERIALS AND METHODS

Chemicals. Radiolabeled maneb was synthesized from $MnCl_2$ and Nabam [disodium ethylenebis(dithiocarbamate)] in aqueous solution. Nabam was prepared from ethylenediamine and carbon disulfide according to the methods of Klöpping and van der Kerk (1951) and Seidler et al. (1970).

[^{14}C]Nabam·6H_2O: 50 μCi [^{14}C]EDA·2HCl (sp act., 70.7 μCi/mg, Radiochemical Centre, Amersham, England) in 1 mL of water was mixed with 66 μL of EDA (1 mmol), followed by 300 μL of CS_2 (5 mmol) and 0.5 mL of 4 N NaOH. After 45 min at 40 °C, 5 mL of acetone was added and the top layer removed. One milliliter of methanol, 2 mL of absolute ethanol, and 5 mL of ethyl acetate were added to the bottom layer, and the solution was kept at 4 °C for crystallization. The colorless crystals were washed with ethanol, ethyl acetate, and petroleum ether. The yield was ≤65%.

[^{14}C]Maneb: 1 mL each of equimolar amounts of $MnCl_2$ and [^{14}C]Nabam·6H_2O (100 μmol) in water (pH 8.4) were mixed. After washing the precipitate with water, ethanol, ethyl acetate, and petroleum ether, a fine yellow powder with a specific activity of 131.5 nCi/mg from the first synthesis and 146.3 nCi/mg from the second synthesis was obtained. The yield was ≤95%.

[^{54}Mn]Maneb: 220 μmol of $MnCl_2$ in 0.5 mL of water was mixed with 10 μCi carrier free $^{54}MnCl_2$ (Radiochemical Centre, Amersham, England) in 20 μL of 0.1 N HCl and 20 μL of 0.1 N NaOH, followed by 220 μmol of inactive Nabam in 0.5 mL of water. The fine yellow powder was washed and dried as above. The yield was 95%, with a specific activity of 151.0 nCi/mg.

All other chemicals were reagent grade (Fluka AG, Buchs, Switzerland).

Treatment of Rats. At 2.00 p.m. unfasted female rats (ZUR:SIV-Z, 200 g) were given by gastric intubation 4–10 mg/kg of [^{14}C]maneb or [^{54}Mn]maneb alone or together with an approximately tenfold molar excess of Fe(III), Zn(II), or Cu(II) salts as nitrates or chlorides suspended in 0.5% methylcellulose. Because of the low LD_{50} of Hg(II), the corresponding mercury salts were applied in equimolar amounts.

The rats were housed individually in polyethylene metabolism cages receiving commercial diet (Nafag AG, Gossau, Switzerland) and tap water ad libitum. Conditions were as follows: light from 6 a.m. to 6 p.m., temperature 23–25 °C, relative humidity 60 to 70%. Urine and feces were collected over a period of 72 h and excreta which could not be analyzed within 12 h were kept at −20 °C. Tissue and blood were analyzed after killing the animals by ether narcosis.

Respiration Study. The rats were housed in closed gas sealed glass metabolism cages (Jencons, Hertfortshire, England). Air was sucked through the cage with a flow of about 5–10 mL/s and $^{14}CO_2$ was trapped in a saturated solution of $Ba(OH)_2$. To prevent losses of ^{14}C, five flasks were seriated. CO_2 was liberated from 1 to 2 g of dry [^{14}C]$BaCO_3$ with H_2SO_4 according to Frohofer (1971) and was trapped in 4 mL of a 2:1 mixture of methanol and ethanolamine and counted in 10 mL of toluene/Triton X-100 (2:1) cocktail.

Analysis of ^{54}Mn Activity. All samples of feces, urine, blood, and tissue were counted in a Biogamma II counter (Beckman Instruments) without disintegration of the material.

Analysis of ^{14}C Activity. All samples were counted in a liquid scintillation counter (Berthold BF 5000/300, Wildbad, West Germany) after solubilization.

Urine. One milliliter of urine in a glass vial was decolorized with 200 μL of H_2O_2 (30%) and 200 μL of iso-

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Table I. Activities Excreted by Individual Animals within 72 h in Feces and Residual Activities in Liver 90 h after Application of 2×10^{-5} mol/kg of $^{54}MnCl_2$, 2×10^{-5} mol/kg of [^{54}Mn]Maneb Alone or Together with 2×10^{-4} mol/kg of $ZnCl_2$, $FeCl_3$, $CuCl_2$, or 2×10^{-5} mol/kg of $HgCl_2$

compound	molar ratio [M]/[Maneb]	recov. of ^{54}Mn in feces in %	residual activity in liver[a] $\times 10^{-3}$	residual activity in kidney[a] $\times 10^{-3}$
[^{54}Mn]maneb		94.3	2.84 ± 0.78	1.51 ± 1.15
[^{54}Mn]maneb		96.8	4.46 ± 1.04	<1.23
[^{54}Mn]maneb + $ZnCl_2$	10.1	94.5	1.71 ± 0.45	<1.23
[^{54}Mn]maneb + $ZnCl_2$	10.1	97.9	1.97 ± 0.61	1.35 ± 1.24
[^{54}Mn]maneb + $CuCl_2$	8.3	92.9	0.97 ± 0.51	<1.23
[^{54}Mn]maneb + $CuCl_2$	8.3	94.8	1.73 ± 0.60	1.23
[^{54}Mn]maneb + $FeCl_3$	30.9	103.4	1.14 ± 0.47	6.29 ± 4.92
[^{54}Mn]maneb + $FeCl_3$	30.9	94.5	1.55 ± 0.59	<1.23
[^{54}Mn]maneb + $HgCl_2$	1.0	98.0	1.67 ± 0.58	<1.23
[^{54}Mn]maneb + $HgCl_2$	1.0	99.7	1.64 ± 0.58	<1.23
$^{54}MnCl_2$[b]			0.79 ± 0.05	0.19 ± 0.03
$^{54}MnCl_2$[b]			0.47 ± 0.03	0.11 ± 0.01
$^{54}MnCl_2$[c]			0.34 ± 0.05	0.18 ± 0.11

[a] Residual activity as fraction of ^{54}Mn dose per gram wet tissue. [b] $^{54}MnCl_2$ together with 2×10^{-5} mol/kg of $MnCl_2$ as carrier. [c] $^{54}MnCl_2$ carrier free, corresponding to less than 2×10^{-10} mol/kg of $MnCl_2$.

propyl alcohol, to prevent excessive foaming, for 2 h at 50 °C. The sample was counted after adding 200 μL of ascorbic acid (15%), 1 mL of water, and 10 mL of a xylene cocktail [xylene/Triton X-100, 2:1 v/v, 5 g of PPO and 0.25 g of POPOP per liter of xylene (mixture of the isomers)].

Feces. The dry material was grinded and homogenized. An aliquot of <20 mg was placed in a glass vial and 100 μL of water was added. After 30 min 1 mL of Soluene-350 (Packard Instruments) was added and incubated for 2 h at 50 °C. After cooling to room temperature, 500 μL of isopropyl alcohol and 200 μL of H_2O_2 (30%) were added, and the screw cap was loosely attached, left to stand for 10 min, and then incubated at 50 °C for a further 2 h. Five milliliters of water was added and the sample counted in 10 mL of Dimilume (Packard Instruments).

An internal standard was used for quenching correction. The total relative standard deviation of the given data on percentage of ^{14}C activity is ±10%.

RESULTS AND DISCUSSION

Distribution of ^{14}C Activity in Excreta and Expired Air. Preliminary studies showed that after application of [^{14}C]maneb more than 99% of the total excreted activity in urine and feces is recovered in the first 72 h.

In four animals treated with maneb from synthesis 1, activity excreted in the urine ranged from 33–48%, compared to 48–57% in four animals treated with maneb from synthesis 2. Thus the amount excreted in urine seems to be dependent on the degree of polymerization of the maneb administered. Data of activity excreted in urine are consistent with results published by Seidler et al. (1970), whereas activity in the feces was greater, namely 40–63%. This difference is most probably due to the extraction method. When activity was extracted with dimethylformamide in a similar manner as these authors described, the yield was only 60% of the amount which was obtained with our method or after digestion of the feces with NaOH.

In the expired air of two animals we found less than 1% of the dose, 0.24% and 0.60%, respectively.

Distribution of ^{54}Mn Activity in Excreta, Tissue, and Blood. Following a single oral dose of [^{54}Mn]maneb alone or together with a tenfold molar excess of $FeCl_3$, $ZnCl_2$, $CuCl_2$, or an equimolar amount of $HgCl_2$, no significant amount of activity was found in urine, blood, or tissue with the exception of liver and kidney. As much as 95–100% of the dose administered appeared in the feces within 20 h. There was no difference in kinetic data between activities in feces following application of maneb alone or together with $ZnCl_2$ (Figure 1). The cations administered together with [^{54}Mn]maneb showed a weak effect by reducing the residual activity in liver tissue 90 h after application in the following order: maneb < Zn(II) < Hg(II) <> Cu(II) < Fe(III).

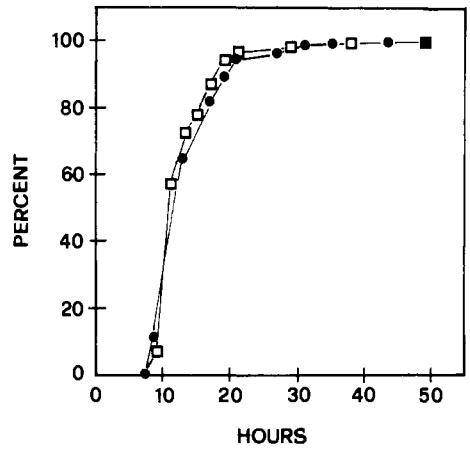

Figure 1. Kinetic data for the excretion of ^{54}Mn activity in feces after application of 2×10^{-5} mol/kg of [^{54}Mn]maneb, alone (□) or together with 2×10^{-4} mol/kg of $ZnCl_2$ (●).

After administration of 2×10^{-5} mol/kg of $^{54}MnCl_2$ alone, similar results are obtained (Table I). This is in accordance with several other studies showing that Mn(II) is poorly absorbed from the intestine and that most of the absorbed manganese is trapped by the liver and excreted via the bile into the intestine (Sahagian et al., 1967; Thomson et al., 1971; Bertinchamps et al., 1966; Papavasiliou et al., 1966). It is apparent from Table I that residual ^{54}Mn activity in liver tissue is somewhat greater from [^{54}Mn]maneb than from equimolar or carrier free $^{54}MnCl_2$, suggesting that only a minute amount of manganese may be absorbed as chelate.

Ninety hours after intravenous injection of carrier free $^{54}MnCl_2$ in saline, the liver contains about 20% of the injected dose. The total amount of manganese ever absorbed from maneb can therefore be estimated as less than 2%.

Influence of Some Cations on Distribution of ^{14}C Activity. The excretion pattern of a single dose of [^{14}C]maneb together with a tenfold molar excess of some metal salts is presented in Figure 2. The results show a significant decrease of radioactivity excreted in urine in the order of maneb > Fe(III) > Zn(II) <> Hg(II) > Cu(II); but the lower [Hg]/[maneb] ratio of about 1.0 must be

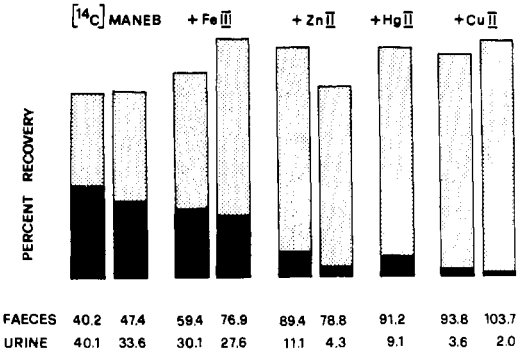

Figure 2. Percentage of activity excreted by individual animals within 72 h in urine and feces after a single oral dose of 2×10^{-5} mol/kg of [^{14}C]maneb (from synthesis 1) together with 2×10^{-4} mol/kg of $FeCl_3$, $ZnCl_2$, $CuCl_2$ or 2×10^{-5} mol/kg of $HgCl_2$.

Table II. Percentage of Activity Excreted in Urine of Individual Animals within 72 h after a Single Oral Dose of 2×10^{-5} mol/kg of [^{14}C]Maneb (from Synthesis 2) Together with 2×10^{-4} mol/kg of $Fe(NO_3)_3$, $Zn(NO_3)_2$, $Cu(NO_3)_2$, or 2×10^{-5} mol/kg of $Hg(NO_3)_2$

type of metal salt	molar ratio [M]/[maneb]	^{14}C activity in urine
none	0	57.3
none	0	56.1
none	0	53.3
none	0	48.5
$Fe(NO_3)_3$	13.2	78.7
$Fe(NO_3)_3$	19.2	24.0
$Fe(NO_3)_3$	11.5	32.7
$Fe(NO_3)_3$	9.9	16.0
$Zn(NO_3)_2$	10.2	28.9
$Zn(NO_3)_2$	16.1	27.1
$Zn(NO_3)_2$	10.3	15.8
$Zn(NO_3)_2$	11.9	8.2
$Hg(NO_3)_2$	1.0	18.7
$Hg(NO_3)_2$	1.2	13.9
$Hg(NO_3)_2$	1.6	7.1
$Hg(NO_3)_2$	1.5	15.2
$Cu(NO_3)_2$	12.5	7.8
$Cu(NO_3)_2$	5.9	6.7
$Cu(NO_3)_2$	11.2	3.9
$Cu(NO_3)_2$	16.6	2.4

taken into account. The amount of activity excreted in the feces increased accordingly. Total recovery ranged from 80–105%.

In a second experiment the nitrate salts were administered instead of the chlorides and Maneb from the second synthesis was used (Table II). The same decrease of ^{14}C activity in urine was observed. The reduction of activity in urine most probably reflects a reduction of the absorption rate from the intestinal tract. An alternative explanation, namely a shift from renal to biliary excretion, is unlikely for two reasons.

(i) The kinetic data shown in Figure 3 indicate that the velocity of excretion of activity in feces did not differ when maneb plus $CuCl_2$ was administered from that of maneb alone. In both cases excretion was almost complete within 10 h after dosing. If excretion would shift from renal to biliary, the excretion in feces, after administration of maneb plus $CuCl_2$, would be delayed.

(ii) Activity in exhaled air was decreased after dosing with maneb plus $CuCl_2$ concurrently with the activity in urine (Table III).

Formation of more stable and less soluble complexes with the EBDC anion and/or with its degradation products, being less able to penetrate the intestinal wall, are suggested as being responsible for these effects. This hypothesis is supported by the qualitative data for solubilities and stabilities of the corresponding metal diethyldithiocarbamates in vitro (Malatesta, 1941; Eckert, 1957). The smallness of the ^{54}Mn activity in urine and tissues (Table I) compared to the ^{14}C activity from labeled EBDC anion (Figure 2 and Table II) suggests that most of the administered maneb is degraded in the stomach and intestines and that practically all of the ^{14}C activity absorbed is in the form of EBDC anion or its metabolites not chelated to manganese ion. Type and quantity of these metabolites in the intestinal tract have to be determined in future work. ETU and its complexes will then attract major interest.

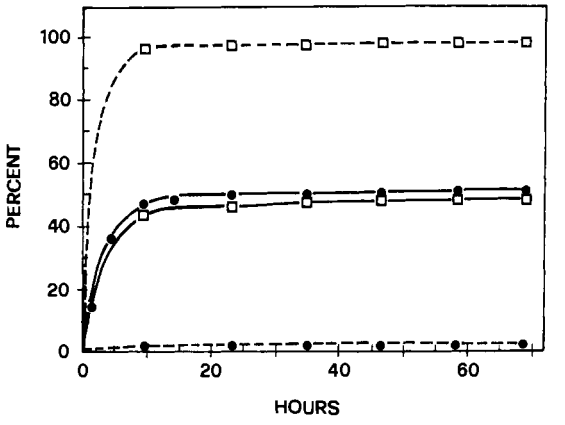

Figure 3. Kinetic data for the excretion of ^{14}C activity in urine (●) and in feces (□) after application of 2×10^{-5} mol/kg of [^{14}C]maneb, alone (—) or together with 2×10^{-4} mol/kg of $CuCl_2$ (- - -).

Table III. Percentages of Activity Excreted in Urine, Feces, and Exhaled Air of Individual Animals within 72 h after a Single Oral Dose of 2×10^{-5} mol/kg of [^{14}C]-Maneb Alone or Together with 2×10^{-4} mol/kg of $CuCl_2$

	[^{14}C]maneb		[^{14}C]maneb + $CuCl_2$	
% ^{14}C in urine	48.5	41.0	3.0	5.3
% ^{14}C in feces	63.2	53.7	116.0	113.2
% ^{14}C in exhaled air	0.24	0.60	0.11	0.10
total recov.	111.9	95.3	119.1	118.6

The presented data lead to the conclusion that the intestinal absorption of maneb will probably not be proportional to the dose given. It must be expected that the cations naturally occurring in food will decrease the absorption of maneb especially at lower dose levels.

The task of extrapolating toxicological results on maneb to man is difficult, particularly if experiments are performed with high dose levels and without simultaneous administration of a mixture of salts either alone or mixed with the diet.

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Microbial Decomposition of Sodium Pentachlorophenolate

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During the decomposition of sodium pentachlorophenolate (**1a**) by *Alcaligenes eutrophus*, *Aeromonas hydrophila* var. *hydrophila* and var. *anaerogenes*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Flavobacterium aquatile*, *Pseudomonas fluorescens*, *Cytophaga johnsonae*, *Corynebacterium aquaticum*, *Brevibacterium testaceum*, and *Arthrobacter globiformis* the following were identified as metabolites: pentachlorophenol acetate (**2**), pentachloroanisole (**3**), 2,3,4,5-tetrachloroanisole (**4**), 2,3,4,6-tetrachloroanisole (**5**), 2,3,5,6-tetrachloroanisole (**6**), 2,3,4,5-tetrachlorophenol (**7**), 2,3,5,6-tetrachlorophenol (**9**), tetrachlororesorcinol (**11**), tetrachlorohydroquinone (**12**), and tetrachlorocatechol diacetate (**13**), the principal metabolite being pentachlorophenol acetate (**2**). Up to 6.2% of the sodium pentachlorophenolate (**1a**) used was recovered as pentachlorophenol acetate (**2**), while all other metabolites were found in amounts less than 1% of the starting compound.

Pentachlorophenol (**1b**) is used in large amounts as a fungicide and herbicide (Bevenue and Beckman, 1967). Numerous publications are concerned with its abiotic decomposition including photomineralization (Hiatt et al., 1960; Mitchell, 1961; Gäb et al., 1975) and microbial degradation (Suzuki and Nose, 1970; Chu, 1972; Chu and Kirsch, 1972; Kirsch and Etzel, 1973; Watanabe, 1977). Suzuki and Nose (1971) found pentachloroanisole (**3**) and tetrachlorohydroquinone dimethyl ether as decomposition products in the culture medium. Cserjesi (1967) found a decrease in pentachlorophenol (**1b**) under the influence of *Trichoderma* sp. In soil 2,3,4,5-tetrachlorophenol (**7**), 2,3,4,6-tetrachlorophenol (**8**), 2,3,5,6-tetrachlorophenol (**9**), 2,4,5-trichlorophenol, 2,3,5-trichlorophenol, 2,4-dichlorophenol, and 3-chlorophenol were identified as metabolites of pentachlorophenol (**1b**) (Ide et al., 1972).

The wide application range of pentachlorophenol (**1b**) and the possible ecological consequences make it important to obtain a more precise understanding of its transformation by specific bacterial strains. The present work describes the results of the experimental decomposition of sodium pentachlorophenolate (**1a**) by two different strains each of *Alcaligenes eutrophus* and *Aeromonas hydrophila*, by two species of *Azotobacter*, by *Flavobacterium aquatile*, *Pseudomonas fluorescens*, *Cytophaga johnsonae*, and three *Coryneform* strains.

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MATERIAL AND METHODS

Chemicals. Ninety-nine percent pure pentachlorophenol (**1b**) (Pestanal, Riedel-de Haën AG, Seelze-Hannover, West Germany), 2,3,4,5-tetrachlorophenol (**7**), and tetrachloro[*o*]benzoquinone (EGA-Chemie KG, Steinheim, West Germany), 2,3,4,6-tetrachlorophenol (**8**) (Fluka, Buchs, Switzerland), and 2,3,5,6-tetrachlorophenol (**9**) (Aldrich Chemical Co., Milwaukee, WI) were used. All other chemical substances were of the purest grade available (E. Merck, Darmstadt, West Germany). Pentachlorophenol acetate (**2**) was prepared from pentachlorophenol (**1b**) and acetic anhydride by the method of Chau and Coburn (1974). Pentachloroanisole (**3**) was obtained by methylation of pentachlorophenol (**1b**) with diazomethane. 2,3,4,5-Tetrachloroanisole (**4**), 2,3,4,6-tetrachloroanisole (**5**), and 2,3,5,6-tetrachloroanisole (**6**) were prepared by methylation of the corresponding tetrachlorophenols (**7**, **8**, and **9**). In addition the following substances were synthesized.

Tetrachlorocatechol (**10**): 10 g of tetrachloro[*o*]-benzoquinone was dissolved in 100 mL of ethanol and $NaBH_4$ was added until the color changed. The dark-colored precipitate was filtered off, the alcoholic solution was poured into water, and the crystalline precipitate was filtered off and recrystallized from benzene, mp 194–195 °C.

Tetrachlororesorcinol (**11**): 30 g of 3,5-dihydroxybenzoic acid was suspended in 150 g of glacial acetic acid saturated

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