CONTRASTIC EFFECTS OF ETHIONINE AND CARBONTETRACHLORIDE ON ASCORBIC ACID METABOLISM AND ON THE ACTIVITY OF MICROSOMAL DRUG-METABOLIZING ENZYMES IN RATS*

RYUICHI KATO

*Department of Pharmacology and Department of Toxicology, National Institute of Hygienic Sciences,
Setagaya-ku, Tokyo*

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It is well known that the administration of chloretone, phenobarbital or methylcholanthrene induces an increase in the urinary excretion of ascorbic acid and they also induce an increase in the activities of drug-metabolizing enzyme of liver microsomes (1-3). These results indicate that there may be some relationships in the increase in the activities of microsomal drug-metabolizing enzymes and the increase in the urinary excretion of ascorbic acid. The increased activities of microsomal drug-metabolizing enzymes are prevented by the joint administration of ethionine (4-6).

However, in a previous work, Kato *et al.* presented the evidence that the increase in the urinary excretion of ascorbic acid after the administration of chloretone or phenobarbital was not prevented by the joint administration of ethionine which completely prevented the increase in the activities of microsomal drug-metabolizing enzymes (7). These results were not in accord with the results presented by Tuoster *et al.* which the increased urinary excretion by chloretone or barbital is prevented by the joint administration of ethionine (8). One of the purpose of the present studies, therefore, is to reinvestigate the effect of ethionine on the increase in the urinary excretion of ascorbic acid induced by chloretone, phenobarbital or methylcholanthrene in comparison with the effect of carbontetrachloride.

On the other hand, in these studies Kato *et al.* observed an increase in the urinary excretion of ascorbic acid after the administration of ethionine, while the administration of carbontetrachloride markedly depress the urinary excretion of ascorbic acid. The both drugs are well known hepatotoxic agents, therefore the administration of ethionine must decrease the biosynthesis of ascorbic acid and, thus it should decrease the urinary excretion of ascorbic acid. The second purpose of the present studies, therefore, is to elucidate the mechanism by which ethionine produces the increased excretion of ascorbic acid into urine.

加藤 隆一

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The evidence was given that ethionine could not prevent the increase in the urinary excretion of ascorbic acid induced by chloretone, phenobarbital or methylcholanthrene with the doses which almost completely prevented the increase in the activities of microsomal drug-metabolizing enzymes.

On the other hand, the administration of carbontetrachloride markedly prevented the increase in the urinary excretion of ascorbic acid induced by the drugs with the doses which only partially prevents the increase in the activities of microsomal drug-metabolizing enzymes. Furthermore, ethionine decreased the liver content of ascorbic acid and thus increased the urinary excretion, while carbontetrachloride decreased the urinary excretion probably through a decrease in the ascorbic acid biosynthesis.

MATERIALS AND METHODS

Female rats of Long-Evans strain, weighing about 160–180 g were used. In some experiments, female rats of Wistar strain, weighing about 160–180 g were used. All rats were fed on an ascorbic acid free synthetic diet, the protein content was about 25%. Carbontetrachloride were diluted in corn oil and given intraperitoneally in volume of 2 ml/kg. Ethionine is dissolved in 0.9% NaCl solution and given intraperitoneally in volume of 10 ml/kg. Phenobarbital and chloretone were dissolved in distilled water and given intraperitoneally in volume of 2 ml/kg.

Determination of ascorbic acid: Ascorbic acid contents in tissue and serum were measured by the method of Roe (9), and amount of urinary ascorbic acid was determined by the method of dichlorophenolindophenol.

Enzyme assay: The rats were decapitated and 25% liver homogenate prepared with 1.15% isotonic KCl solution at 2°–4°C. The homogenates were centrifuged at 9,000×g for 15 minutes. The microsomal oxidations of drugs were carried out by incubation of 9,000×g supernatant fraction (3 ml) with 1.5 μ mole of pentobarbital, meprobamate or zoxazolamine for 60 minutes at 37°C under air, in the presence of cofactor mixture containing 0.1 ml of glucose-6-phosphate (20 μ mole), 0.1 ml of NADP (0.4 μ mole), 0.1 ml of MgCl_2 (50 μ mole), 0.1 ml of nicotinamide (50 μ mole) and 1.4 ml of 0.1 M sodium phosphate buffer pH 7.4. The final volume was 5.0 ml.

Chemical methods: The metabolisms of pentobarbital, meprobamate and zoxazolamine were determined by disappearance of the substrates according to the method of Brodie *et al.*, Hoffman and Ludwig and Conney *et al.*, respectively (10–12).

RESULTS

1. Effects of ethionine and carbontetrachloride on tissue content and urinary excretion of ascorbic acid

As shown in Table 1, the administration of ethionine markedly decreased the content of ascorbic acid in the liver, while it decreased only slightly the content in the kidney, thus the ratio between liver and kidney markedly was decreased. In contrast, the administration of carbontetrachloride slightly decreased the content of ascorbic acid in the liver, while it more clearly decreased the content in the kidney, thus the ratio between liver

TABLE 1. Effects of ethionine and carbontetrachloride on tissue content and urinary excretion of ascorbic acid.

Treatment	Dose (mg/kg)	Tissue content ($\mu\text{g/g}$)		Liver/Kidney ratio	Urinary excretion ($\mu\text{g}/100\text{g/day}$)
		Liver	Kidney		
1) Controls	—	296 ± 3 (16)	150 ± 4 (16)	1.95	402 ± 43 (15)
2) Ethionine	50	203 ± 7 (8)	146 ± 6 (8)	1.39	989 ± 124 (12)
3) Ethionine	200	158 ± 5 (16)	134 ± 7 (16)	1.18	1158 ± 148 (12)
4) CCl_4	80	270 ± 10 (8)	123 ± 5 (8)	2.19	165 ± 29 (8)
5) CCl_4	300	253 ± 9 (8)	108 ± 7 (8)	2.34	133 ± 18 (8)
6) CCl_4	600	225 ± 9 (7)	95 ± 4 (7)	2.47	121 ± 21 (7)

Difference and significance :

	Liver Diff. (%)	Tissue content Signif.	Kidney Diff. (%)	Signif.	Liver/Kidney ratio Diff. (%)	Urinary excretion Diff. (%)	Signif.
1) — 2)	-31	$p < 0.01$	-3	N.S.	-29	+146	$p < 0.01$
1) — 3)	-46	$p < 0.01$	-11	N.S.	-39	+188	$p < 0.01$
1) — 4)	-8	N.S.	-18	$p < 0.01$	+12	-59	$p < 0.01$
1) — 5)	-14	$p < 0.01$	-28	$p < 0.01$	+20	-67	$p < 0.01$
1) — 6)	-23	$p < 0.01$	-37	$p < 0.01$	+27	-70	$p < 0.01$
3) — 6)	+42	$p < 0.01$	-29	$p < 0.01$	+109	-89	$p < 0.01$

Each value represents the averages $\pm$ standard error from 7-16 rats. The numerals in the brackets indicate numbers of animals used. Ethionine and CCl_4 were given intraperitoneally for 4 days and the rats were killed 24 hours after the last injection.

The urines were collected for 24 hours started immediately after the last injection.

and kidney was increased. For example, liver/kidney ratio of ascorbic acid was decreased by 29% and 39%, respectively, in the rats dosed 50 mg/kg and 200 mg/kg of ethionine, while the ratio was increased by 12%, 20% and 27%, respectively, in the rats dosed 80 mg/kg, 300 mg/kg and 600 mg/kg of carbontetrachloride.

Moreover, the administration of ethionine markedly increased the urinary excretion of ascorbic acid, while the administration of carbontetrachloride markedly decreased the urinary excretion of ascorbic acid. For example, the urinary excretion of ascorbic acid was increased by 146% or 188%, respectively by the administration of 50 mg/kg or 200 mg/kg of ethionine. In contrast, the urinary excretion of ascorbic acid was decreased by 59%, 67% or 70%, respectively by the administration of 80 mg/kg, 300 mg/kg or 600 mg/kg of carbontetrachloride.

These results suggested that ethionine may release ascorbic acid from liver and thus it increase the urinary excretion, while carbontetrachloride may inhibit the biosynthesis of ascorbic acid in liver and thus it decrease the urinary excretion.

2. Tissue content of ascorbic acid after the administration of ascorbic acid in rats pretreated with ethionine or carbontetrachloride

To proof the above-mentioned hypothesis ascorbic acid (200 mg/kg, i.p.) was given 4 hours before sacrifice to the rats pretreated with ethionine or carbontetrachloride.

TABLE 2. Tissue content of ascorbic acid after the administration of ascorbic acid in rats pretreated with ethionine or carbontetrachloride.

Treatment	Liver ($\mu\text{g/g}$)	Kidney ($\mu\text{g/g}$)	$\frac{\text{Liver}}{\text{Kidney}}$ ratio	Serum ($\mu\text{g/ml}$)
1) Controls	313 $\pm$ 8 (8)	143 $\pm$ 5 (8)	2.15	12 $\pm$ 2 (8)
2) Ethionine	172 $\pm$ 9 (8)	139 $\pm$ 8 (8)	1.24	14 $\pm$ 2 (8)
3) CCl_4	219 $\pm$ 9 (8)	89 $\pm$ 3 (8)	2.46	9 $\pm$ 1 (8)
4) Ascorbic acid	428 $\pm$ 7 (12)	197 $\pm$ 4 (12)	2.17	17 $\pm$ 3 (12)
5) Ethionine + Ascorbic acid	235 $\pm$ 11 (12)	193 $\pm$ 12 (12)	1.22	17 $\pm$ 2 (12)
6) CCl_4 + Ascorbic acid	352 $\pm$ 12 (11)	145 $\pm$ 9 (12)	2.43	15 $\pm$ 2 (11)

Difference and significance :

	Liver Diff. (%)	Signif.	Kidney Diff. (%)	Signif.	$\frac{\text{Liver}}{\text{Kidney}}$ ratio
1) — 2)	-55	$p < 0.01$	- 6	N.S.	-42
1) — 3)	-30	$p < 0.01$	-40	$p < 0.01$	+14
1) — 4)	+37	$p < 0.01$	+33	$p < 0.01$	+ 1
1) — 5)	-25	$p < 0.01$	+30	$p < 0.01$	-43
1) — 6)	+12	$p < 0.05$	+ 2	N.S.	+13
2) — 5)	+37	$p < 0.01$	+39	$p < 0.01$	- 2
3) — 6)	+61	$p < 0.01$	+63	$p < 0.01$	- 1

Each value represents the average $\pm$ standard error from 8-12 rats. The numerals in the brackets indicate numbers of animals used.

The rats were treated for 3 days with 200 mg/kg of ethionine or 600 mg/kg of carbontetrachloride and moreover on the fourth day half of non-treated and treated rat were given intraperitoneally 200 mg/kg of ascorbic acid and all rats were killed 4 hours later.

As shown in Table 2, the amount of increase in liver ascorbic acid after the administration of ascorbic acid was 115 $\mu\text{g/g}$, 63 $\mu\text{g/g}$ and 133 $\mu\text{g/g}$, respectively, in control rats, ethionine treated rats and carbontetrachloride treated rats. On the other hand, the amount of increase in kidney ascorbic acid after the administration of ascorbic acid was 39 $\mu\text{g/g}$, 54 $\mu\text{g/g}$ and 46 $\mu\text{g/g}$, respectively, in control rats, ethionine treated rats and carbontetrachloride treated rats.

These results may support the proposed hypothesis that the administration of ethionine may interfere a storage mechanism of ascorbic acid in liver, while carbontetrachloride may decrease the biosynthesis of ascorbic acid.

3. Effect of ethionine on the increase in the tissue content and urinary excretion of ascorbic acid induced by the treatment with chloretone, phenobarbital or methylcholanthrene

The administration of chloretone, phenobarbital and methylcholanthrene markedly increased the urinary excretion of ascorbic acid and it increased liver and kidney contents of ascorbic acid.

As shown in Table 3, the urinary excretion of ascorbic acid was increased by 970%, 300% or 573%, respectively, in the rats pretreated with chloretone, phenobarbital or methylcholanthrene.

TABLE 3. Effect of ethionine on the increase in the tissue content and urinary excretion of ascorbic acid induced by the treatment with chloretone, phenobarbital or methylcholanthrene.

Treatment	Tissue content ($\mu\text{g/g}$)		Liver Kidney ratio	Urinary excretion ($\mu\text{g}/100\text{g/day}$)
	Liver	Kidney		
1) Controls	280 $\pm$ 8 (12)	147 $\pm$ 5 (12)	1.92	424 $\pm$ 46 (12)
2) Ethionine (ET) 50	192 $\pm$ 9 (8)	145 $\pm$ 7 (8)	1.32	895 $\pm$ 98 (8)
3) Ethionine (ET) 200	138 $\pm$ 6 (8)	139 $\pm$ 4 (8)	0.99	1314 $\pm$ 121 (8)
4) Chloretone (CT)	354 $\pm$ 13 (12)	185 $\pm$ 8 (12)	1.91	4535 $\pm$ 345 (11)
5) ET 50 + CT	221 $\pm$ 12 (11)	196 $\pm$ 13 (12)	1.13	4941 $\pm$ 431 (12)
6) ET 200 + CT	165 $\pm$ 8 (12)	191 $\pm$ 10 (11)	0.86	4605 $\pm$ 455 (12)
7) Phenobarbital (PB)	330 $\pm$ 12 (12)	175 $\pm$ 9 (12)	1.89	1695 $\pm$ 210 (12)
8) ET 50 + PB	213 $\pm$ 9 (12)	170 $\pm$ 11 (12)	1.25	1705 $\pm$ 235 (11)
9) ET 200 + PB	148 $\pm$ 7 (11)	182 $\pm$ 10 (12)	0.81	1825 $\pm$ 310 (12)
10) Methylcholanthrene (MC)	337 $\pm$ 14 (12)	176 $\pm$ 12 (12)	1.92	2855 $\pm$ 295 (12)
11) ET 50 + MC	223 $\pm$ 7 (11)	167 $\pm$ 8 (11)	1.34	2690 $\pm$ 345 (11)
12) ET 200 + MC	150 $\pm$ 8 (12)	165 $\pm$ 10 (12)	0.91	2355 $\pm$ 260 (12)

Difference and significance :							
	Liver		Kidney		Liver Kidney ratio	Urinary excretion	
	Diff. (%)	Signif.	Diff. (%)	Signif.	Diff. (%)	Diff. (%)	Signif.
1) — 2)	-31	p<0.01	- 2	N.S.	-31	+ 111	p<0.01
1) — 3)	-59	p<0.01	- 6	N.S.	-48	+ 210	p<0.01
1) — 4)	+26	p<0.01	+26	p<0.01	- 1	+ 970	p<0.01
1) — 5)	-21	p<0.01	+33	p<0.01	-41	+1064	p<0.01
1) — 6)	-41	p<0.01	+29	p<0.01	-55	+ 986	p<0.01
1) — 7)	+18	p<0.01	+19	p<0.05	- 2	+ 300	p<0.01
1) — 8)	-24	p<0.01	+16	N.S.	-37	+ 302	p<0.01
1) — 9)	-47	p<0.01	-24	p<0.01	-58	+ 330	p<0.01
1) — 10)	+20	p<0.01	+20	p<0.05	0	+ 573	p<0.01
1) — 11)	-22	p<0.01	+14	N.S.	-30	+ 534	p<0.01
1) — 12)	-46	p<0.01	+12	N.S.	-53	+ 456	p<0.01
4) — 5)	-38	p<0.01	+ 6	N.S.	-41	+ 9	N.S.
4) — 6)	-46	p<0.01	+ 3	N.S.	-55	+ 2	N.S.
7) — 8)	-36	p<0.01	- 3	N.S.	-34	0	N.S.
7) — 9)	-55	p<0.01	- 4	N.S.	-57	+ 7	N.S.
10) — 11)	-34	p<0.01	- 5	N.S.	-30	- 6	N.S.
10) — 12)	-55	p<0.01	- 7	N.S.	-53	- 17	N.S.

Each value represents the average $\pm$ standard error from 8-12 rats.

The numerals in the brackets indicate numbers of animals used.

Chloretone (50 mg/kg, i.p.), phenobarbital (70 mg/kg, i.p.) or methylcholanthrene (25 mg/kg, i.p.) were given for 4 days and ethionine was given 30 minutes before the injection of the drugs.

The rats were killed 24 hours after last injection.

The urines were collected for 24 hours started immediately after the last injection.

However, ethionine decreased the liver ascorbic acid content in the rats treated with chloretone, phenobarbital or methylcholanthrene as well as in control rats. In contrast, ethionine did not significantly alter the kidney ascorbic acid in control rats and the drug-treated rats.

Moreover, the administration of ethionine did not prevent the increase in the urinary excretion of ascorbic acid induced by the chloretone, phenobarbital or methylcholanthrene.

4. *Effect of ethionine on the increase in the activities of drug-metabolizing enzymes of liver microsomes induced by the treatment with chloretone, phenobarbital or methylcholanthrene*

The administration of chloretone or phenobarbital increased the metabolism of pentobarbital by 94% and 220%, respectively, while they increased the metabolism of meprobamate by 113% and 230%, respectively. Moreover, the administration of methylcholanthrene increased the metabolism of zoxazolamine by 375%. As reported in previous papers (5, 6, 13), the joint injection of ethionine partially (50 mg/kg) or almost completely (200 mg/kg) prevented the induced increase in the metabolism of drugs (Table 4). However, as given in Table 3 these doses of ethionine could not block the stimulatory action of chloretone, phenobarbital or methylcholanthrene on urinary excretion of ascorbic acid.

These results suggested that the mechanism of stimulation of the activities of microsomal drug-metabolizing enzymes and of urinary excretion of ascorbic acid may be different.

5. *Effect of carbontetrachloride on the increase in the tissue content and urinary excretion of ascorbic acid induced by the treatment with chloretone, phenobarbital or methylcholanthrene*

As shown in Table 5, the administration of carbontetrachloride, in contrast to the effect of ethionine, partially blocked the increased urinary excretion of ascorbic acid induced by chloretone, phenobarbital or methylcholanthrene. For example, urinary excretion of ascorbic acid in chloretone treated rats decreased by 57% and 76%, respectively, by the joint treatment of 80 mg/kg and 300 mg/kg of carbontetrachloride.

Moreover, urinary excretion of ascorbic acid in methylcholanthrene treated rats decreased by 52% and 69% respectively by the joint treatment of 80 mg/kg and 300 mg/kg of carbontetrachloride. Since the decrease in kidney content of ascorbic acid in chloretone, phenobarbital or methylcholanthrene treated rats by the joint injection of carbontetrachloride was less markedly than that in normal rats, liver/kidney ratio of ascorbic acid was not significantly changed, while the ratio was increased in the rats treated only with carbontetrachloride.

These results, therefore, suggest that the administration of carbontetrachloride decrease the synthesis of ascorbic acid in liver and decrease kidney content through decrease urinary excretion of ascorbic acid.

6. *Effect of carbontetrachloride on the increase in the activities of drug-metabolizing enzymes of liver microsomes induced by the treatment with chloretone, phenobarbital or methylcholanthrene*

The administration of 80 mg/kg or 300 mg/kg of carbontetrachloride markedly decrease the metabolism of pentobarbital, meprobamate and zoxazolamine (Table 6). In contrast to ethionine which completely inhibited the induction of the drug-metabolizing enzyme, the administration of carbontetrachloride only partially inhibited. For example, the administration of 300 mg/kg of carbontetrachloride decreased the metabolism of pentobarbital, meprobamate and zoxazolamine by 67%, 67% and 58%, respectively, while, the

TABLE 4. Effect of ethionine on the increase in the activities of liver microsomal drug-metabolizing enzymes induced by the treatment with chloretone, phenobarbital or methylcholanthrene.

Treatment	Enzyme activity ($\mu\text{g/g/hr}$)		
	Pentobarbital	Meprobamate	Zoxazolamine
1) Controls	49 $\pm$ 4 (6)	53 $\pm$ 5 (6)	85 $\pm$ 6 (6)
2) Ethionine (ET) 50	48 $\pm$ 3 (6)	55 $\pm$ 4 (6)	89 $\pm$ 7 (6)
3) Ethionine (ET) 200	46 $\pm$ 5 (6)	52 $\pm$ 3 (6)	81 $\pm$ 5 (6)
4) Chloretone (CT)	95 $\pm$ 8 (6)	113 $\pm$ 12 (6)	—
5) ET 50 + CT	61 $\pm$ 7 (5)	67 $\pm$ 7 (6)	—
6) ET 200 + CT	44 $\pm$ 4 (6)	54 $\pm$ 5 (6)	—
7) Phenobarbital (PB)	157 $\pm$ 12 (6)	175 $\pm$ 11 (6)	—
8) ET 50 + PB	81 $\pm$ 9 (5)	86 $\pm$ 8 (5)	—
9) ET 200 + PB	56 $\pm$ 5 (6)	71 $\pm$ 10 (6)	—
10) Methylcholanthrene (MC)	—	—	403 $\pm$ 29 (6)
11) ET 50 + MC	—	—	179 $\pm$ 23 (6)
12) ET200 + MC	—	—	121 $\pm$ 16 (6)

Difference and significance :

	Pentobarbital		Meprobamate		Zoxazolamine	
	Diff. (%)	Signif.	Diff. (%)	Signif.	Diff. (%)	Signif.
1) — 2)	— 2	N.S.	+ 4	N.S.	+ 5	N.S.
1) — 3)	— 6	N.S.	— 2	N.S.	— 5	N.S.
1) — 4)	+ 94	p<0.01	+ 113	p<0.01	—	—
1) — 5)	+ 25	N.S.	+ 26	N.S.	—	—
1) — 6)	— 10	N.S.	+ 1	N.S.	—	—
1) — 7)	+ 220	p<0.01	+ 230	p<0.01	—	—
1) — 8)	+ 65	p<0.01	+ 62	p<0.01	—	—
1) — 9)	+ 14	N.S.	+ 34	N.S.	—	—
1) — 10)	—	—	—	—	+ 374	p<0.01
1) — 11)	—	—	—	—	+ 110	p<0.01
1) — 12)	—	—	—	—	+ 42	N.S.
4) — 5)	— 36	p<0.05	— 41	p<0.05	—	—
4) — 6)	— 54	p<0.01	— 52	p<0.01	—	—
7) — 8)	— 48	p<0.01	— 51	p<0.01	—	—
7) — 9)	— 64	p<0.01	— 60	p<0.01	—	—
10) — 11)	—	—	—	—	— 56	p<0.01
10) — 12)	—	—	—	—	— 70	p<0.01
2) — 5)	+ 27	N.S.	+ 22	N.S.	—	—
2) — 8)	+ 69	p<0.01	+ 56	p<0.01	—	—
2) — 11)	—	—	—	—	+ 101	p<0.01
3) — 6)	— 4	N.S.	+ 4	N.S.	—	—
3) — 9)	+ 27	N.S.	+ 37	N.S.	—	—
3) — 12)	—	—	—	—	+ 49	p<0.05

Each value represents the average $\pm$ standard error from 8–12 rats.

The numerals in the brackets indicate numbers of animals used.

Chloretone (50 mg/kg, i.p.), phenobarbital (70 mg/kg, i.p.) or methylcholanthrene (25 mg/kg, i.p.) were given for 4 days and ethionine was given 30 minutes before the injection of the drugs.

The rats were killed 24 hours after the last injection.

TABLE 5. Effect of carbontetrachloride on the increase in the tissue content and urinary excretion of ascorbic acid induced by the treatment with chloretone, phenobarbital or methylcholanthrene.

Treatment	Tissue content ($\mu\text{g/g}$)		Liver Kidney ratio	Urinary excretion ($\mu\text{g}/100\text{g/day}$)
	Liver	Kidney		
1) Controls	285 $\pm$ 10(12)	158 $\pm$ 4(12)	1.80	398 $\pm$ 35(12)
2) CCl ₄ 80	268 $\pm$ 12(8)	121 $\pm$ 3(8)	2.22	250 $\pm$ 48(8)
3) CCl ₄ 300	230 $\pm$ 17(8)	93 $\pm$ 4(8)	2.47	141 $\pm$ 41(8)
4) Chloretone (CT)	367 $\pm$ 14(12)	198 $\pm$ 7(12)	1.86	4452 $\pm$ 658(12)
5) CCl ₄ 80+CT	315 $\pm$ 15(12)	168 $\pm$ 5(12)	1.87	1913 $\pm$ 395(11)
6) CCl ₄ 300+CT	281 $\pm$ 9(11)	165 $\pm$ 6(12)	1.70	1055 $\pm$ 255(12)
7) Phenobarbital (PB)	325 $\pm$ 12(12)	185 $\pm$ 8(11)	1.76	2259 $\pm$ 425(11)
8) CCl ₄ 80+PB	306 $\pm$ 13(12)	165 $\pm$ 4(12)	1.85	1283 $\pm$ 206(12)
9) CCl ₄ 300+PB	275 $\pm$ 12(11)	156 $\pm$ 7(12)	1.76	975 $\pm$ 283(11)
10) Methylcholanthrene (MC)	332 $\pm$ 13(12)	207 $\pm$ 9(12)	1.60	3213 $\pm$ 493(12)
11) CCl ₄ 80+MC	294 $\pm$ 10(12)	178 $\pm$ 5(11)	1.65	1544 $\pm$ 216(11)
12) CCl ₄ 300+MC	271 $\pm$ 12(12)	159 $\pm$ 4(12)	1.70	1008 $\pm$ 148(12)

Difference and significance :

	Liver Diff. (%)	Tissue content Signif.	Kidney Diff. (%)	Signif.	Liver Kidney Diff. (%)	Urinary excretion Diff. (%)	Signif.
1)---2)	- 6	N.S.	-24	p<0.01	+23	- 37	p<0.05
1)---3)	-19	p<0.05	-41	p<0.01	+42	- 65	p<0.01
1)---4)	+29	p<0.01	+25	p<0.01	+ 3	+1018	p<0.01
1)---5)	+11	N.S.	+ 6	N.S.	+ 4	+ 381	p<0.01
1)---6)	- 2	N.S.	+ 4	N.S.	- 6	+ 165	p<0.01
1)---7)	+14	p<0.05	+17	p<0.01	- 2	+ 468	p<0.01
1)---8)	+ 7	N.S.	+ 4	N.S.	+ 3	+ 222	p<0.01
1)---9)	- 4	N.S.	- 1	N.S.	- 2	+ 145	p<0.01
1)---10)	+17	p<0.05	+31	p<0.01	-11	+ 790	p<0.01
1)---11)	+ 3	N.S.	+13	N.S.	- 8	+ 329	p<0.01
1)---12)	- 5	N.S.	0	N.S.	- 6	+ 126	p<0.01
4)---5)	-14	p<0.05	-15	p<0.01	0	- 57	p<0.01
4)---6)	-23	p<0.01	-17	p<0.01	- 6	- 76	p<0.01
7)---8)	- 6	N.S.	-11	p<0.05	+ 5	- 43	p<0.05
7)---9)	-15	p<0.01	-16	p<0.05	0	- 57	p<0.01
10)---11)	-11	p<0.05	-14	p<0.01	+ 3	- 52	p<0.01
10)---12)	-18	p<0.01	-23	p<0.01	+ 7	- 69	p<0.01

Each value represents the average $\pm$ standard error from 8-12 rats.

The numerals in the brackets indicate numbers of animals used.

Chloretone (50 mg/kg, i.p.), phenobarbital (70 mg/kg, i.p.) or methylcholanthrene (25 mg/kg, i.p.) were given for 4 days and carbontetrachloride was given 30 minutes before the injection of the drugs.

The rats were killed 24 hours after the last injection.

The urines were collected for 24 hours started immediately after the last injection.

TABLE 6. Effect of carbontetrachloride on the increase in the activities of liver microsomal drug-metabolizing enzymes induced by the treatment with chloretone, phenobarbital or methylcholanthrene.

Treatment	Enzyme activity ($\mu\text{g/g/hr}$)		
	Pentobarbital	Meprobamate	Zoxazolamine
1) Controls	45 $\pm$ 5 (6)	52 $\pm$ 6 (6)	81 $\pm$ 5 (6)
2) CCl ₄ 80	29 $\pm$ 3 (6)	29 $\pm$ 5 (6)	56 $\pm$ 7 (6)
3) CCl ₄ 300	15 $\pm$ 3 (6)	17 $\pm$ 3 (6)	34 $\pm$ 6 (5)
4) Chloretone (CT)	91 $\pm$ 6 (6)	96 $\pm$ 7 (5)	—
5) CCl ₄ 80 + CT	60 $\pm$ 5 (6)	69 $\pm$ 4 (6)	—
6) CCl ₄ 300 + CT	42 $\pm$ 3 (5)	49 $\pm$ 5 (5)	—
7) Phenobarbital (PB)	144 $\pm$ 9 (6)	163 $\pm$ 11 (6)	—
8) CCl ₄ 80 + PB	93 $\pm$ 7 (6)	106 $\pm$ 8 (6)	—
9) CCl ₄ 300 + PB	66 $\pm$ 6 (6)	83 $\pm$ 3 (6)	—
10) Methylcholanthrene (MC)	—	—	451 $\pm$ 34 (6)
11) CCl ₄ 80 + MC	—	—	289 $\pm$ 32 (6)
12) CCl ₄ 300 + MC	—	—	193 $\pm$ 21 (6)

Difference and significance :

		Pentobarbital		Meprobamate		Zoxazolamine	
		Diff. (%)	Signif.	Diff. (%)	Signif.	Diff. (%)	Signif.
1) — 2)	— 36	p < 0.05	— 45	p < 0.05	— 31	—	—
1) — 3)	— 67	p < 0.01	— 67	p < 0.01	— 58	—	—
1) — 4)	+ 102	p < 0.01	+ 87	p < 0.01	—	—	—
1) — 5)	+ 33	p < 0.05	+ 33	p < 0.05	—	—	—
1) — 6)	— 7	N.S.	— 5	N.S.	—	—	—
1) — 7)	+ 220	p < 0.01	+ 213	p < 0.01	—	—	—
1) — 8)	+ 107	p < 0.01	+ 104	p < 0.01	—	—	—
1) — 9)	+ 47	p < 0.05	+ 59	p < 0.01	—	—	—
1) — 10)	—	—	—	—	+ 457	p < 0.01	—
1) — 11)	—	—	—	—	+ 257	p < 0.01	—
1) — 12)	—	—	—	—	+ 138	p < 0.01	—
4) — 5)	— 34	p < 0.01	— 28	p < 0.01	—	—	—
4) — 6)	— 54	p < 0.01	— 49	p < 0.01	—	—	—
7) — 8)	— 36	p < 0.01	— 35	p < 0.01	—	—	—
7) — 9)	— 54	p < 0.01	— 49	p < 0.01	—	—	—
10) — 11)	—	—	—	—	— 36	—	—
10) — 12)	—	—	—	—	— 57	—	—
2) — 5)	+ 106	p < 0.01	+ 138	p < 0.01	—	—	—
2) — 8)	+ 220	p < 0.01	+ 265	p < 0.01	—	—	—
2) — 11)	—	—	—	—	+ 416	p < 0.01	—
3) — 6)	— 180	p < 0.01	+ 188	p < 0.01	—	—	—
3) — 9)	+ 340	p < 0.01	+ 388	p < 0.01	—	—	—
3) — 12)	—	—	—	—	+ 468	p < 0.01	—

Each value represents the average $\pm$ standard error from 8–12 rats.

The numerals in the brackets indicate numbers of animals used.

Chloretone (50 mg/kg, i.p.), phenobarbital (70 mg/kg, i.p.) or methylcholanthrene (25 mg/kg, i.p.) were given 4 days and carbontetrachloride was given 30 minutes before the injection of the drugs.

The rats were killed 24 hours after the last injection.

joint administration of carbontetrachloride decreased phenobarbital induced increase in the metabolism of pentobarbital and meprobamate by 54 and 49%, respectively, and methylcholanthrene induced increase in the metabolism of zoxazolamine by 57%.

The effect of carbontetrachloride on the microsomal enzymes was similar to the effect on urinary excretion of ascorbic acid.

DISCUSSION

The administration of chloretone, phenobarbital or methylcholanthrene and other drugs an increase in the urinary excretion of ascorbic acid through an increased biosynthesis in liver (1). On the other hand, the administration of these drugs induces an increase in the activities of liver microsomal enzymes which metabolize foreign compounds (14, 15). The induction of the drug-metabolizing enzymes of liver microsomes was inhibited by joint injection of ethionine or puromycin (4-6, 16).

However, as demonstrated in the present paper, the administration of 200 mg/kg of ethionine completely prevent the stimulatory effect of chloretone, phenobarbital or methylcholanthrene on the activities of the drug-metabolizing enzymes, in contrast, the administration of same dose of ethionine did not prevent the stimulatory effect of chloretone, phenobarbital or methylcholanthrene on the urinary excretion of ascorbic acid.

Moreover, the administration of ethionine markedly decreased the ascorbic acid content in liver, but it markedly increased the urinary excretion of ascorbic acid and it did not decrease the activities of drug-metabolizing enzymes of liver microsomes. These results suggested that the administration of ethionine increase in the release of ascorbic acid from liver and thus increase the urinary excretion. This suggestion was supported by the evidence reported in Table 2 that the administration of ascorbic acid increased liver content less markedly in ethionine treated rats than in control rats. Since the administration of ethionine markedly depressed the content of ATP in the liver for more than 24 hours, thereby loss of ATP may resulted in a release of ascorbic acid from a storage pool (17, 18).

However, the lack of preventive effect of ethionine to the increase in the urinary excretion of acid by chloretone, phenobarbital or methylcholanthrene probably can not be attributed only to the increased urinary excretion by ethionine. The lack of preventive effect of ethionine therefore suggest that the mechanism of stimulation in the biosynthesis of ascorbic acid by the drugs may differ from that of the induction of the activities of drug-metabolizing enzymes and more the stimulation in the biosynthesis is not related to "de novo" synthesis of protein.

On the other hand, Touster reported that administration of chloretone or barbital increased the activities of uridinediphosphateglucose dehydrogenase (8), and a joint administration of ethionine completely prevented the increase in the excretion of ascorbic acid and in the activity of the enzyme.

These results are completely not in accord with the results of our preliminary and present investigations. Contrary to the present investigation, Touster employed male

rats and given ethionine in a diet. However, the effect of ethionine on the decrease in the liver ATP content and protein synthesis is markedly in female rats than in male (19). And single injection of ethionine markedly decreased the liver ATP content for more than 24 hours.

In further experiments, we failed to demonstrate the prevention of the increased urinary excretion in female rat by feeding on ethionine containing diet. On the other hand, the administration of carbontetrachloride decreased the ascorbic acid content in the liver and kidney and the urinary excretion of ascorbic acid. Moreover, it decreased the activities of drug-metabolizing enzymes of liver microsomes. However, the administration of ascorbic acid increased the liver ascorbic acid content more markedly in the carbontetrachloride treated rats than in control rats.

These results suggested that the administration of carbontetrachloride decreased biosynthesis of ascorbic acid in liver and then decreased the ascorbic acid content in the liver and kidney and the urinary excretion of ascorbic acid. Moreover, carbontetrachloride partially prevent the stimulatory action of the chloretone, phenobarbital or methylcholanthrene on the urinary excretion of ascorbic acid as well as on the activities of microsomal enzymes.

Thus, the action of ethionine may be mainly due to the decrease of liver content of ATP and consequently decrease the capacity of storage of ascorbic acid and block the induction of the drug-metabolizing enzymes of liver microsomes, while the action of carbontetrachloride is mainly due to nonspecific damage of liver cell and consequently decrease the biosynthesis of ascorbic acid and the activities of the microsomal drug-metabolizing enzymes.

SUMMARY

Administration of ethionine to rats markedly decreased the ascorbic acid content in liver and increased the urinary excretion of ascorbic acid. In contrast, administration of carbontetrachloride decreased the ascorbic acid content in liver and kidney and the urinary excretion. Thus, the ratio of ascorbic acid content between liver and kidney decreased in the rats treated with ethionine, contrary it increased in the rats treated with carbontetrachloride.

On the other hand, the joint administration of ethionine prevented the stimulation of urinary excretion of ascorbic acid as well as the induction of drug-metabolizing enzymes of liver microsomes by the administration of chloretone, phenobarbital or methylcholanthrene.

In contrast, the joint administration of carbontetrachloride only partially prevented the stimulation of urinary excretion of ascorbic acid and the induction of drug-metabolizing enzymes.

It was postulated that ethionine may interfere the mechanism of storage of ascorbic acid in liver and then increased the release from storage site and consequently increased the urinary excretion of ascorbic acid, while carbontetrachloride resulted a specific damage

of the liver and decreased biosynthesis of ascorbic acid and consequently increased the urinary excretion of ascorbic acid.

REFERENCES

- 1) BURNS, J.J., CONNEY, A.H., DAYTON, P.G., EVANCE, C., MARTIN, G.R. AND TALLER, D. : *J. Pharmac. exp. Ther.* **129**, 132 (1960)
- 2) CONNEY, A.H., DAVISON, C., GASTEL, B. AND BURNS, J.J. : *Ibid.* **130**, 1 (1960)
- 3) KATO, R. AND CHIESARA, E. : *Atti Acad. Med. Lambarda* **15**, 443 (1960)
- 4) CONNEY, A.H., MILLER, J.A. : *J. biol. Chem.* **228**, 753 (1957)
- 5) KATO, R. : *Med. Exp.* **3**, 95 (1960)
- 6) CONNEY, A.H., MICHELSON, I.A. AND BURNS, J.J. : *J. Pharmac. exp. Ther.* **132**, 202 (1961)
- 7) KATO, R., VASSANELLI, P. AND FRONTINO, G. : *Experientia* **18**, 9 (1962)
- 8) TOUSTER, O. AND HOLLMAN, S. : *Ann. N.Y. Acad. Sci.* **92**, 318 (1961)
- 9) ROE, J.H. : *Methods of Biochemical Analysis*, Vol. **1**, Edited by GLICK, D., p. 115, Interscience, New York (1954)
- 10) BRODIE, B.B., BURNS, J.J., MARK, L.C., LIEF, P.A., BERNSTEIN, E. AND PAPPER, E.M. : *J. Pharmac. exp. Ther.* **109**, 266 (1953)
- 11) HOFFMAN, A.J. AND LUDWIG, B.J. : *Am. Pharm. Ass.* **68**, 740 (1959)
- 12) CONNEY, A.H., TROUSOF, N. AND BURNS, J.J. : *J. Pharmac. exp. Ther.* **128**, 333 (1960)
- 13) KATO, R., CHIESARA, E. AND VASSANELLI, P. : *Biochem. Pharmac.* **11**, 211 (1962)
- 14) REMMER, H. : *Metabolic Factors Controlling Duration of Drug Action*, Edited by BRODIE, B.B., p. 235, Pergamon Press, London (1962)
- 15) CONNEY, A.H. AND BURNS, J.J. : *Adv. in Pharmac.* Vol. **1**, London (1962)
- 16) CONNEY, A.H. AND GILMAN, A.G. : *J. biol. Chem.* **233**, 3682 (1963)
- 17) SHULL, K.H. : *Ibid.* **237**, PC 1734 (1962)
- 18) VILLA-TREVINO, S., SHULL, K.H. AND FARBER, E. : *Ibid.* **238**, 1757 (1963)
- 19) NTORI, Y. : *Ibid.* **238**, 2075 (1963)