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Inhibitory Effect of Ascorbic Acid on the Acute Toxicity of Dimethylamine Plus Nitrite in the Rat (37761)

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In laboratory animals, the administration of sodium nitrite ($NaNO_2$) together with secondary amines or alkylureas can induce tumors, and this is attributed to the acid-catalyzed intragastric formation of *N*-nitroso compounds, *i.e.*, nitrosamines and nitrosamides [reviewed in (1)]. This process could conceivably occur in the human stomach and be involved in the etiology of some human cancers (2). In chemical experiments, ascorbate blocked the formation of *N*-nitroso compounds from nitrite plus amines and ureas, presumably because ascorbate reacted with nitrite and hence made it unavailable for the formation of *N*-nitroso compounds (1). Therefore, we suggested that ascorbate might be administered together with nitrosatable drugs, to lessen the possibility of *in vivo* formation of *N*-nitroso compounds. Subsequently, we found that the induction of lung adenomas in strain A mice by chronic treatment with piperazine in the food and $NaNO_2$ in the drinking water was inhibited when ascorbate was included in the food (3).

Intragastric administration of high doses of dimethylamine (DMA) plus $NaNO_2$ to mice produced acute liver necrosis, which was attributed to the intragastric formation of dimethylnitrosamine (DMN) (4). Similar administration of aminopyrine plus $NaNO_2$ to rats also produced acute liver necrosis and an elevation in serum glutamic-pyruvic transaminase (GPT) levels, attributed to *in vivo* formation of DMN (5). We report here the effect of sodium ascorbate on the liver necrosis and the elevation in serum GPT and glutamic-oxolacetic transaminase (GOT), produced by administration to rats of DMA·HCl plus $NaNO_2$ and of DMN.

Methods. Male Wistar rats weighing 250–400 g were used. They were routinely fed Wayne Lab Blox (Allied Mills, Chicago, IL) and water *ad libitum*, except that food and water were withdrawn for 1 hr after the intubations. Solutions for gastric intubation were freshly prepared in distilled water and the pH was not adjusted (DMN) or adjusted with HCl or NaOH to 7.0 (DMA·HCl, Na ascorbate and $NaNO_2$). Where two solutions were intubated, separate syringes were used. After 48 hr, the rats were killed with CO_2 gas. Rats that died early were discarded. Blood was collected from the heart, allowed to clot, and analyzed for serum GOT and GPT (6–8). The results are given as international units/liter at 30°. Two lobes of each liver were examined histologically using hematoxylin–eosin staining. The livers were graded for necrosis according to the following criteria: (—), absent; (+), slight centrilobular; (2+), moderate centrilobular; (3+), centrilobular and midzonal; (4+), centrilobular, midzonal and interlobular. For the ascorbate determinations (9), 5 *N* acetic acid was substituted for the standard acetic acid–metaphosphoric acid mixture.

Results and Discussion. Solutions containing sodium ascorbate (9 and 72 g/liter), and DMA·HCl (300 g/liter), similar to those used for intubation, were analyzed for ascorbate after incubation at pH 3, 5 and 7 for 3 hr at room temperature and 37°. The loss of ascorbate was $<9\%$, showing that the ascorbate remained almost unchanged in the intubation solutions until

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TABLE I. Effect of Varying Doses of Sodium Ascorbate on the Action of Dimethylamine·HCl Plus Sodium Nitrite.[a]

DMA·HCl (mg/kg)	$NaNO_2$ (mg/kg)	Na ascorbate (mg/kg)	No. of rats	GOT (IU/liter)	GPT (IU/liter)	Liver necrosis
1500	125	—	13	2060 ± 190	600 ± 210	All 4+
1500	125	720	5	51 ± 4	11 ± 1	All —
1500	125	360	5	49 ± 2	11 ± 1	All —
1500	125	180	4	59 ± 8	13 ± 2	All —
1500	125	90	8	56 ± 5	17 ± 3	All —
1500	125	45	7	490 ± 220	160 ± 80	2 of —, 3 of 1+, 1 of 3+, 1 of 4+
1500	125	22.5	4	3700 ± 650	400 ± 60	1 of 3+, 3 of 4+
—	—	—	7	47 ± 3	16 ± 3	All —

[a] Solutions containing DMA·HCl (300 mg/ml) plus Na ascorbate (0–144 mg/ml) were intubated at a dose of 5 ml/kg body weight. Immediately afterwards, a solution of $NaNO_2$ (62.5 mg/ml, dose 2 ml/kg) was intubated. Transaminase results in all tables are given as mean ± standard error.

these were administered. The results also suggest that ascorbate was unlikely to react with DMA in the stomach. Ascorbate can react with amines by the "browning" reaction, but this usually requires more extreme conditions (10, 11).

Forty-eight hours after DMA·HCl (1500 mg/kg body wt) and $NaNO_2$ (125 mg/kg) were given to rats by stomach tube, the livers showed acute centrilobular necrosis [in agreement with Ref. (4)] and the serum GOT and GPT levels were raised to >35 times the normal values (Table I). When 720 mg ascorbate/kg was given at the same time, liver damage was completely blocked (Figs. 1 and 2) and serum GOT and GPT levels remained normal. This effect is attributed to the acid-catalyzed reaction of ascorbate with nitrite, as in our *in vitro* experiments (1). The blocking action of ascorbate remained completely effective down to a dose of 90 mg ascorbate/kg, with obvious necrosis and an elevation in transaminases occurring only at 45 mg/kg.

The individual results for GOT, GPT, and observed necrosis correlated well with each other. The GOT values were about 5 times the GPT values, but wide variations in this ratio were observed. Serum levels of creatine phosphokinase (12) were normal in 4 rats from the groups in Table I with elevated transaminases, indicating that the raised GOT was not due to muscle damage (13).

For comparison, the dose–response relationship was examined for DMN (Table II). The LD_{50} for DMN given *per os* is 40 mg/kg, death being due to liver necrosis (14). When rats given this dose were killed after 48 hr, the serum transaminases were >35 times the normal values. The effects remained considerable with 15 mg DMN/kg. With 10 mg/kg, GOT results were significantly above normal ($P < 0.01$) but GPT results were in the normal range. No effect was observed with 5 mg/kg. The effects were less marked and more variable when the rats were killed at 24 instead of 48 hr (untabulated results). Thus transaminase measurements and microscopic examination are good but rather insensitive indicators of DMN exposure.

A comparison of Tables I and II suggests that the treatment with 1500 mg DMA·HCl plus 125 mg $NaNO_2$/kg gave similar results to those produced by about 40 mg DMN/kg. Thus apparent DMN yield was estimated to be 30% from $NaNO_2$ and 3% from DMA. Furthermore, we conclude that in the DMA plus nitrite experiments of Table I, apparent DMN production was lowered by the ascorbate from 40 mg/kg to at most 10 mg/kg, *i.e.*, it was inhibited at least 75%.

The effect of sodium ascorbate (360

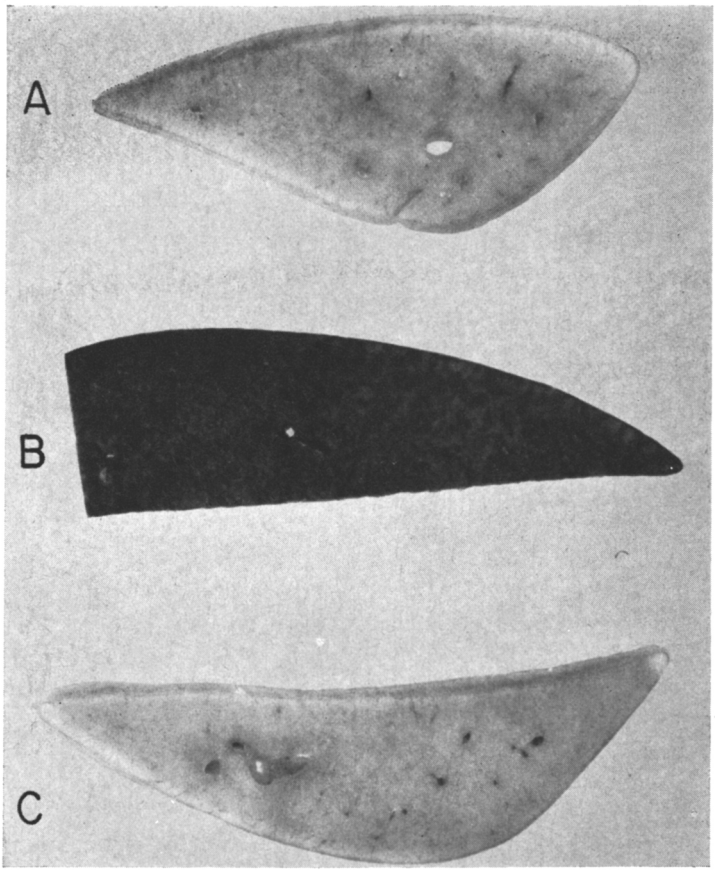

Fig. 1. Gross liver sections. (A) Liver of untreated rat. (B) Liver of rat treated with 1500 mg DMA·HCl and 125 mg $NaNO_2$/kg, showing diffuse necrotic and hemorrhagic changes. (C) Liver of rat treated with 1500 mg DMA·HCl, 125 mg $NaNO_2$ and 720 mg Na ascorbate/kg, showing normal appearance.

mg/kg) was examined on the action of 15–20 mg DMN/kg (Table III). Over this dose range of DMN, the response was most sensitive to small changes in dose but was also very variable (Table II). Table III shows that ascorbate had no significant effect on the action of DMN in 3 experiments with different feeding regimens. The controlled feeding experiments were designed to minimize the variation in response, which could be partly due to differences in the rate of gastric emptying. Thus the inhibitory effect of ascorbate on the DMA plus nitrite system was not due to inhibition of the action of DMN produced *in vivo*.

Ascorbate is required for certain *in vivo* hydroxylations, e.g., the conversion of proline to hydroxyproline in collagen (15), and DMN dealkylation probably involves a hydroxylation step, which may be the enzymic reaction leading to the active agent (14). Thus ascorbate might increase the effect of DMN by accelerating its *N*-dealkylation. However, this view is not supported by our present results.

During preparation of this paper, it was reported that ascorbate inhibited the elevation of serum GPT in rats by aminopyrine plus nitrite (16, 17). Contrary to our findings and those of Greenblatt (16), Kamm *et al.* (17) claimed that ascorbate may inhibit the toxic action of DMN. Also, ascorbate given to pregnant rats receiving ethylurea plus nitrite was reported to prevent hydrocephalus in the offspring (18).

Summary. Gastric intubation of 1500 mg

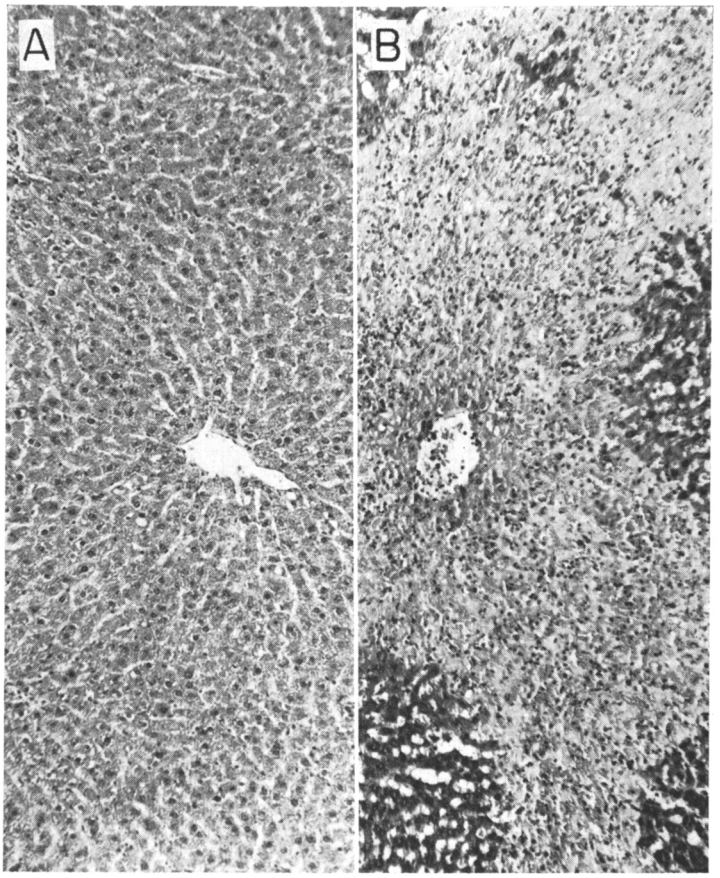

Fig. 2. Microscopic liver sections. (A) Liver of rat treated with 1500 mg DMA·HCl, 125 mg $NaNO_2$ and 720 mg Na ascorbate/kg, showing the integrity of the parenchyma around the centrilobular vein. (B) Liver of rat treated with 1500 mg DMA·HCl and 125 mg $NaNO_2$/kg, showing extensive necrosis around the centrilobular vein (hematoxylin–eosin staining, 100×).

dimethylamine·HCl (DMA·HCl) plus 125 mg $NaNO_2$/kg produced severe liver necrosis and an elevation of serum glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase levels. These effects, which are attributed to *in vivo* formation of dimethylnitrosamine (DMN), were completely prevented by simultaneous intubation of sodium ascorbate at doses of 90–720 mg/kg. A dose–response study on DMN indicated that apparent DMN yield in the DMA plus nitrite experiment was at least 40 mg/kg, and

TABLE II. Effect of Varying Doses of Dimethylnitrosamine (DMN).[a]

DMN (mg/kg)	No. of rats	GOT (IU/liter)	GPT (IU/liter)	Liver necrosis
40	4	2200 ± 480	630 ± 180	All 4+
30	5	1200 ± 280	430 ± 90	All 4+
20	5	660 ± 160	150 ± 40	4 of 3+, 1 of 4+
15	4	690 ± 270	210 ± 90	All 3+
10	16	92 ± 9	25 ± 6	8 of —, 7 of 1+, 1 of 2+
5	13	63 ± 3	17 ± 3	All —
—	16	58 ± 5	20 ± 3	All —

[a] A solution of DMN (1—8 mg/ml) was intubated at a dose of 5 ml/kg body weight.

TABLE III. Effect of Sodium Ascorbate on the Action of Dimethylnitrosamine (DMN).[a]

Expt no.	DMN (mg/kg)	Na ascorbate (mg/kg)	No. of rats	GOT (IU/liter)	GPT (IU/liter)	Liver necrosis
1	15	—	4	690 ± 270	210 ± 90	All 3+
1	15	360	4	910 ± 390	160 ± 50	2 of 2+, 2 of 4+
2	20	—	6	760 ± 130	180 ± 30	2 of 3+, 4 of 4+
2	20	360	6	480 ± 120	190 ± 30	3 of 3+, 3 of 4+
3	20	—	5	1330 ± 340	340 ± 110	4 of 3+, 1 of 4+
3	20	360	4	1140 ± 270	330 ± 70	1 of 3+, 3 of 4+

[a] A solution of Na ascorbate (72 mg/ml) was intubated at a dose of 360 mg (5 ml)/kg body weight. Where ascorbate was not given, water (5 ml/kg) was intubated. In Expt 1 the rats were given food and water *ad lib.* except for the first hr after treatment. In Expt 2 the rats were fasted but given water for 18 hr before treatment. In Expt 3 the rats were fasted (but given water) for 18 hr, presented with 4 g powdered food and drinking water for 1 hr, intubated and then deprived of food and water for 3 hr. After these treatments, food and water were given *ad lib.* till death. The subgroups of each experiment did not show significantly different transaminase results.

that the ascorbate reduced this yield to at most 10 mg/kg. Ascorbate (360 mg/kg) had no effect on the action of DMN itself and did not react *in vitro* with DMA, so that its effect was probably due to reaction with the nitrite. The results support our suggestion that ascorbate might be administered together with readily nitrosatable drugs, to inhibit possible *in vivo* formation of *N*-nitroso compounds.

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