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Chronic Administration of Hydroxylamine and Derivatives in Mice. (31969)

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The recent speculation on the possible carcinogenicity of the mutagen hydroxylamine (1) has prompted us to describe the results of several studies we have performed with hydroxylamine or its derivatives. Within recent years the mutagenic and teratogenic effects of hydroxylamine derivatives on micro-organisms and on *in vitro* systems have been demonstrated(2-4). However, relatively little has been reported of the chronic effects of such materials in animals. For this reason hydroxylamine, hydroxyurea, useful in treatment of some leukemias(5-8), and methylhydroxylamine (methoxyamine) reputedly an even more effective mutagen than hydroxylamine(9), were administered to mice.

Materials and methods. Compounds. Hydroxyurea was kindly provided by the Drug Development Branch, CCNSC, or through the courtesy of Dr. L. J. Lerner and Miss Barbara Stearns of the Squibb Institute for Medical Research. Hydroxylamine sulfate, O-methoxyamine hydrochloride and urethan (ethyl carbamate) were Eastman White Label chemicals.

Animals. Male random-bred Swiss-Webster or female C3H/HeN mice, 4 weeks old, from the NIH Animal Production Section were kept in plastic cages, usually 6-8 animals per cage. Purina Laboratory Chow was available *ad lib.* The mice were weighed weekly.

Toxicology. Tolerated dosage was determined on 6 mice at each level. The compounds were given in the drinking water at several concentrations for 2 weeks until signs of toxicity (death, loss of weight) appeared. From these results a suitable dose for chronic administration was selected.

Chronic administration of compounds. The compounds were given in the drinking water. With hydroxyurea enough solution was prepared for a 2-day supply to each cage to avoid the gradual hydrolysis of the material. Solutions of the other compounds were prepared

TABLE I. Effect of Hydroxylamine Derivatives in Male Swiss-Webster Mice.

Compound	Time (weeks)	Treatment	Number of mice	Organ weights (mean ± S.E.) Body, g	Liver, g	Liver/100 g body weight	Spleen, mg	Testes, mg	Red blood count (million)	White blood count ($\times 10^{-3}$)
Hydroxyurea	14	60 mM	6	28.8 ± 2.6	1.32 ± .14	4.58 ± .32	72.8 ± 9.6	42.1 ± 4.6		
		H_2O	3	38.3 ± 1.5	1.69 ± .10	4.49 ± .39	107 ± 13	241 ± 15		
	22	60 mM	6	34 ± 1	1.55 ± .12	4.60 ± .32	137 ± 43	40.2 ± 1.4	5.43	4.4
		H_2O	3	43.5 ± 2.9	1.97 ± .09	4.24 ± .37	136 ± 8	232 ± 7	8.01	3.7
	26	60 mM	4	32.4 ± .6	1.55 ± .08	4.76 ± .21	91.3 ± 21.0	43.3 ± 3.1	4.94	3.8
		H_2O	3	43.0 ± 1.8	1.84 ± .06	4.27 ± .07	171 ± 22	231 ± 17	9.22	5.97
Hydroxylamine sulfate	12	NH_2OH*	8	38.4 ± .8	2.17 ± .12	5.65 ± .34	1250 ± 146		6.6	8.9
		H_2O	4	37.3 ± 1.5	1.71 ± .12	4.59 ± .02	131 ± 16		11.1	3.5
$(H_2NOH)_2 \cdot H_2SO_4$	20	NH_2OH	8	39.4 ± 1.1	1.88 ± .10	4.78 ± .22	1060 ± 21		6.6	5.9
		H_2O	4	37.8 ± .8	1.34 ± .07	3.54 ± .11	100 ± 4		9.0	2.7
		$NH_2OH \rightarrow H_2O$ 12 wk 8 wk	8	42.9 ± 1.1	2.26 ± .26	5.24 ± .49	275 ± 26		10.4	2.9
	36	NH_2OH	8	40.8 ± .8	2.23 ± .21	5.42 ± .45	1141 ± 90		6.0	8.3
		H_2O	4	42.0 ± 1.0	1.82 ± .10	4.33 ± .22	158 ± 10		10.7	2.2
		$NH_2OH \rightarrow H_2O$ 12 wk 18 wk	8	42.5 ± 1.1	1.91 ± .18	4.46 ± .34	172 ± 10		11.6	3.6
	52	NH_2OH	5	41.0 ± 1.5	2.43 ± .18	5.72 ± .52	1270 ± 94	219	5.6	18.7
		H_2O	5	43.4 ± 1.6	1.76 ± .08	4.04 ± .15	125 ± 13	209	7.7	13.4
Methoxyamine hydrochloride	12	80 mM	8	29.1 ± 1.1	1.64 ± .07	5.68 ± .25	781 ± 100			14.1
		H_2O	4	42.3 ± 2.2	1.98 ± .13	4.68 ± .07	120 ± 6			7.5
$CH_3ONH_2 \cdot HCl$	20	80 mM	8	31.2 ± 1.3	1.71 ± .08	5.28 ± .17	707 ± 61			15.2
		H_2O	4	39.2 ± 2.3	1.77 ± .24	4.47 ± .37	151 ± 46			9.1
		$CH_3ONH_2 \rightarrow H_2O$ 12 wk 8 wk	10	38.7 ± 1.2	2.11 ± .12	5.40 ± .25	221 ± 17			9.4
	52	80 mM	11	34.2 ± 1.0	1.94 ± .06	5.74 ± .23	678 ± 58	214 ± 10	5.4	14.4
		H_2O	8	49.0 ± 1.3	2.16 ± .24	4.40 ± .44	145 ± 18	234 ± 11	10.2	9.0
		$CH_3ONH_2 \rightarrow H_2O$ 12 wk 40 wk	3	44.0 ± 2.0	2.16 ± .04	4.77 ± .40	472 ± 35	212 ± 17	7.5	8.5
Urethan	17	10 mM	4†	40.0 ± .5	1.49 ± .24	3.81 ± .25	67.8 ± 4.2			
		H_2O	4	49.8 ± 2.8	1.65 ± .13	3.31 ± .12	122 ± 5			
$C_3H_5O\text{-}C\text{-}NH_2$										

* It was found that there was no difference between the groups of mice given 10 or 20 mM/l of hydroxylamine. Therefore the data have been combined in the table.

† All these animals had numerous lung adenomas.

TABLE II. Effect of Hydroxylamine in Female C3H/HeN Mice.

Treatment	No. of mice	Mammary tumor: Time of appearance (wk)	Mammary tumor: Tumor wt (g)	Mammary tumor: % incidence	Death (wk)	Body wt at 52 wk (g)	Spleen wt (g)
H_2O	10	54 ± 2	8.4 ± 1.3	100	70 ± 4	29.2 ± 1.4	.202 ± .048
NH_2OH	10	—	—	0	84 ± 4	27.0 ± .5	1.05 ± .15

weekly and were kept cold until used.

Positive controls. As positive controls mice were administered urethan (10 mM) in the drinking water for 17 weeks. All these mice had numerous lung adenomas by that time, demonstrating the responsiveness of this strain.

Autopsy. The animals were killed with ether. The livers, spleens and select organs were weighed and fixed in 10% formalin. Blood for the various studies was obtained by heart puncture or from the tail. Hematocrit was determined using a Drummond micro-hematocrit apparatus.

Results and discussion. The results are presented in Tables I and II.

Hydroxyurea. With hydroxyurea the growth rate of the mice was decreased somewhat so that the experimental mice weighed about 10 g less than did the controls (Table I). Organ weights were not notably different except for the testicular atrophy, noted also by Murphy and Chaube(10). Although red cell counts were depressed, the white cell counts were not especially affected, comparable to previous reports(11,12).

Hydroxylamine. Hydroxylamine did not influence greatly the body or liver weights. However, there was remarkable splenomegaly, a decrease in red blood cells, and an increase in white blood cells, accompanied by much cellular debris. These effects were slowly reversible since animals on hydroxylamine for 12 weeks, and then on water for 8 or 18 weeks had virtually normal parameters.

Before the histopathology was completed on this material fresh homogenates of some of the enlarged spleens were implanted into 8-week-old mice to determine whether there was any inherent leukemogenic effect. However, no lesions developed in the injected mice.

Almost 50% of the mice which had consumed hydroxylamine for 52 weeks presented sizable areas of bone formation in the spleen (Fig. 1). This was not seen in mice treated for shorter periods. No tumors were noted in the mice kept on hydroxylamine for as long as one year. Usually the condition and appearance of the experimental mice seemed better than that of the controls. Furthermore, in the female C3H mice which drank hydroxylamine solution for a year, there were no spontaneous mammary tumors, even in those surviving 2 years (Table II). The difference in body weights between the 2 groups was approximately 2 grams. This is not enough to allow an explanation for the results on the basis of caloric restriction (*cf.* 13).

Methoxyamine. Although methoxyamine had a more moderate effect than hydroxyl-

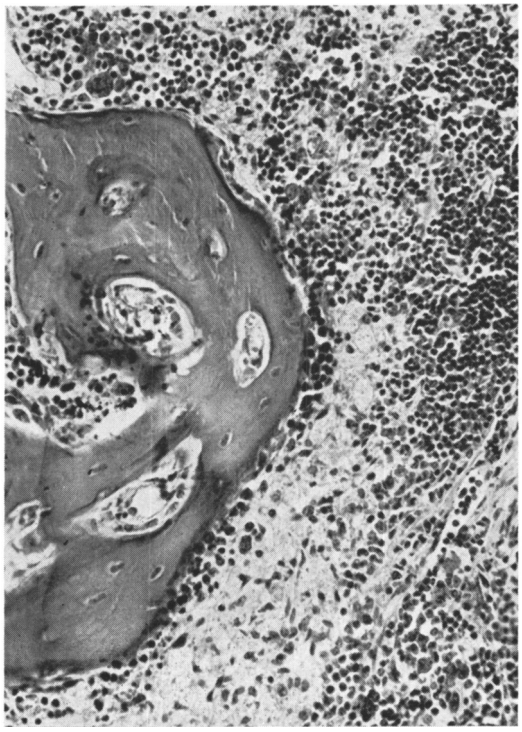

FIG. 1. Bone formation in the spleen. Hematoxylin and eosin. ×260.

amine in increasing the size of the spleen and the white cell count, this compound depressed the body weight somewhat more than hydroxylamine. Interestingly, the mice on methoxyamine also appeared to be in better condition than the controls, which sometimes showed dermatoses with alopecia, a condition not seen in treated animals. Mice which drank methoxyamine solution for one year had no tumors.

These experiments showed that the various hydroxylamine derivatives, administered in drinking water, were toxic. They led to anemia, probably through destruction of the red blood cells and increased the white cell count. The anemia may have been the cause of the splenomegaly. Long term administration of hydroxylamine caused bone formation in the spleen, a condition not observed with the derivatives.

However, none of the chemicals appeared to have any carcinogenic effect in the mice and rather inhibited some spontaneous tumors. These results are comparable to those of Harman(14) who reported that hydroxylamine at 1% in the diet increased the lifespan but decreased the spontaneous tumor incidence of C3H mice. With hydroxyurea the time was not sufficient to reveal a weak agent, but with the other 2 compounds the observation period was long enough to detect moderate carcinogenic potency, as demonstrated by the positive response to urethan.

Thus, even though at least 2 of the compounds administered to mice are very effective mutagenic agents in bacterial systems, no correlation between this effect and possible carcinogenicity in a mammalian system can be made at this time(15).

Summary. Long-term administration of hydroxylamine, methoxyamine, or hydroxyurea to Swiss-Webster mice did not cause tumor formation. Hydroxylamine inhibited spontaneous formation of mammary tumors in female C3H mice. The principal toxic effects of hydroxylamine were anemia, splenomegaly and bone formation in the spleen.

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