Nephrotoxic Action of Spermine. (19647)

S. M. Rosenthal, E. R. Fisher, and E. F. Stohlman.
(Introduced by Floyd S. Daft.)
From the National Institutes of Health, Bethesda, Md.

Spermine is an aliphatic amine [$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] that is widely distributed in mammalian tissues, with the highest concentration in the prostate gland (1,2). By the use of ion exchange resins we have developed a colorimetric method for the estimation of spermine, applicable to small amounts of material(3). As an illustration of the amounts present in the tissues of rats and mice, spleen contains from 300 to 600 μg per gram of wet tissue, and liver approximately half this quantity. Values within this range have been reported for man and large animals(1,4). Little is known of its physiological significance; it has been found to inhibit oxidation of carbohydrate in brain tissue *in vitro*(5); to be a growth factor for a strain of *Hemophilus parainfluenza*(6); to antagonize the bacteriostatic action of atabrine on *E. coli*(7); and to possess bacteriostatic action under certain conditions upon the tubercle bacillus(8). It has been generally considered, along with the related diamines putrescine and cadaverine, to be of low toxicity. The acute toxic effects of these amines have been attributed to a depressant action on the central nervous system(9).

It was observed that animals surviving the acute depressant action of spermine would often die after a lapse of several days. At autopsy marked kidney lesions were found, and further study has shown spermine to be a potent agent in the production of renal damage. Three commercial samples of spermine 4 HCl* bearing different lot numbers were used. One of them was recrystallized as the phosphate and another was put through one recrystallization as the hydrochloride and two as the phosphate. Satisfactory elementary analyses were obtained on these samples. A further sample of spermine phosphate was obtained through the courtesy of the National Institute for Medical Research of Great Britain. A sample of spermidine phosphate was obtained from Dr. Milton Silverman of this Institute, while putrescine and cadaverine (di HCl) were obtained from commercial sources. 0.5% solutions in 0.9% NaCl were employed. The phosphate was brought in solution by heating, and injections of this salt were made before the solution cooled sufficiently to crystallize out. All dosage values are expressed in terms of the base.

It is observed (Table I) that intraperitoneal injections into mice of 60 mg/kg or 2 daily injections of 30 mg/kg were uniformly fatal within 6 days to both male and female animals. One dose of 30 mg/kg killed 9 of 12 females. Eight of 10 mice receiving daily amounts of 15 mg/kg died after the 5th to 7th injection. Nine daily injections of 7.5 mg/kg were non-fatal.

Five rats received one injection of 33 mg/kg intraperitoneally. Animals sacrificed on the 3rd to 7th day showed moderate to advanced renal lesions.

Two small rabbits (1200 g) received single intraperitoneal injections of 33 mg/kg. No symptoms were observed until the 5th day when death in coma occurred. Advanced renal lesions were present. The non-protein nitrogen in the blood of these animals, up to the time of death, is shown in Table II. Similar determinations upon mice sacrificed at various times after one injection of 60 mg/kg are also shown.

Urines from the rats and rabbits contained considerable amounts of protein and many samples reduced Fehling's solution. Single intravenous injections of 5 to 15 mg/kg into rabbits produced proteinuria for several days.

This nephrotoxic action was not observed following putrescine or cadaverine (Table I). Mice were administered 200 mg/kg daily for 5 days and sacrificed on the 7th day. No renal lesions or elevation of blood N.P.N. were observed. Experiments with spermidine were

* From Hoffmann-La Roche, Nutley, N. J.

TABLE I. Toxicity of Spermine Salts for Mice. Females employed unless otherwise indicated.

Preparation	Daily dosage, mg/kilo*		Route	No. of mice	No. dead	Time of death
Spermine 4 HCl	30 mg × 1		I.V.	4	4	Immediate
Phosphate†	60	1		11§	11	1 in 2 hr, others 3-4 days
	30	1		10§	5	3-5 days
	15	1		10§	1	3 "
" ‡	60	1		6	6	1 in 2 hr, others 3-4 days
	30	1		6	4	3–6
	15	1		6	0	
HCl	60	2		10	10	2
	30	2		5	5	2–4
	15	7	I.P.	10	10	5–8
	7.5	9		5	0	
Phosphate	60	1		6	6	4–6 days
	25	5		10	10	4–6
Spermidine phosphate	60	1		3	0	
	30	1		3	0	
	17	1		3	0	
Putrescine 2 HCl	200	5		5	1	4
Cadaverine 2 HCl	200	5		5	0	

* Expressed as base.

† Recrystallized several times from the hydrochloride by Dr. E. L. Jackson of this Laboratory.

‡ Obtained from the National Institute for Medical Research, Great Britain.

§ Includes 4 male mice.

TABLE II. Blood Non-Protein Nitrogen Values Following Intraperitoneal Injections of Amines. Mice and rats were sacrificed at stated intervals to obtain blood, while rabbits were bled from ear vein.

Animals	Compound	Dosage, mg/kilo	Blood N.P.N., mg %: Days after onset of treatment 0	1	2	3	4	5–7
Mice	Spermine phosphate	60 × 1	45	70	64	350	290–367	
Rabbit	" "	33 1	40	62	83	161	322	
"	" "	33 1	40	53	108	170	294	
Rats		33 1	43–44					80–70–50*
Mice	Putrescine	200 5						43–59
"	Cadaverine	200 5						43–54
"	Spermidine	60 1						57†

* Values obtained on the 5th, 6th, and 7th days, respectively

† 6th day.

imited by lack of material, but no renal injury vas observed.

The early (1 day) morphologic changes following the injection of spermine consisted of ıyaline droplet degeneration and fatty change n the epithelial cells of the proximal convoluted tubules of the kidney. One and a ıalf days and in some instances even one day ollowing the injection there was a diffuse lesquamation of the epithelial cells of the ɔroximal convoluted tubules, apparently most narked in the distal portion (outer medullary zone). There were no reactive inflammatory changes associated with the tubular necrosis. The structures of the distal nephron appeared well preserved except for the presence of numerous casts within their lumina. These casts were for the most part of the hyaline type, being periodic acid-Schiff positive. On occasion amorphous as well as definitely granular basophilic material was noted in the casts (hematoxylin and eosin stained sections). This substance was also in instances periodic acid-Schiff positive as well as being positive

when stained by the Feulgen and by the Van Kossa methods. These latter technics indicate the protein, mineral (phosphate, probably calcium phosphate) and nuclear debris content of the casts. The lesions are similar in appearance from 3 to 10 days following the injection of spermine although at 10 days large lakes of mineral deposits (probably calcium phosphate) are noted within the lumina of the tubules of the distal nephron.

In all sections studied no glomerular, vascular or stromal changes were evident and lesions in the kidney as described above were similar in sacrificed animals to those observed in animals dying after the injection of spermine.

Other tissues failed to reveal significant morphologic change except for the presence of rare foci of necrosis in the liver of several animals surviving for 4 days after the spermine injection.

Animals injected with spermidine, putrescine and cadaverine failed to reveal any morphologic changes in the viscera.

Conclusions. Spermine administered parenterally is highly active in producing renal injury in mice, rats, and rabbits. This property was not observed for spermidine, putrescine, or cadaverine.

1. Dudley, H. W., and Rosenheim, O., *Biochem. J.*, 1925, v19, 1034.
2. Harrison, G. A., *Biochem. J.*, 1933, v27, 1152.
3. Rosenthal, S. M., to be published.
4. Hamalainen, R., *Acta Soc. Med. Duodecim.*, 1947 Series A, v23, 97.
5. Evans, E. A., Jr., Vennesland, B., and Schneider, J. J., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 467.
6. Herbst, E. J., and Snell, E. E., *J. Biol. Chem.*, 1948, v176, 989.
7. Silverman, M., and Evans, E. A., Jr., *J. Biol. Chem.*, 1944, v154, 521.
8. Hirsch, J. G., and Dubos, R. J., *J. Exp. Med.*, 1952, v95, 191.
9. Guggenheim, M., *Die biogenen Amine*, Basel S. Karger, 1951.

Received June 3, 1952. P.S.E.B.M., 1952, v80.

Studies on Nicotinamide Inhibitors. (19648)

L. COTÉ, J. J. OLESON, AND J. H. WILLIAMS.

From Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

Pyrazinoic acid was reported to be active in the cure of human pellagra(1). This compound, however, was later found to have no nicotinic acid activity for increasing the V-factor of human blood(2). Other investigators reported pyrazinoic acid and, in some cases, the corresponding amide to be inactive for the cure of canine blacktongue(3), growth of isolated pea roots(4), and for the growth of various microorganisms, for example, *Leuconostoc*(5), *Pasteurella*(6), *B. dysenteriae*(6), and *Proteus vulgaris*(7). These compounds have not been reported as nicotinamide antagonists for microorganisms.

Of a series of pyrazine and pyridine derivatives which were assayed for antinicotinamide activity with *Lactobacillus arabinosus*, strain 17-5, pyrazinoic acid, pyrazinamide and 2 sulfanilamido-5-nitropyridine are shown to be reversible inhibitors of nicotinamide. Experiments with pyrazinamide on rats and chicks are also reported.

Experimental methods. The details of the microbiological assays and the animal tests are given in a previous publication(8). The compounds tested for inhibitory activity with *L. arabinosus* were initially assayed at levels of 0.5, 1.0, 3.0, and 5.0 mg per ml alone and in the presence of 0.02 μg per ml of nicotinamide. This level of nicotinamide alone gives approximately half-maximum growth. The compounds showing inhibition were retested at 7 levels from 0.5 to 15 mg per ml against 7 levels of nicotinamide ranging from 0.005 to 0.2 μg per ml. An inhibition index was then calculated, which represents the ratio of inhibitor to vitamin producing a 50% inhibition of growth. The compounds were ad-