Further Studies on Lathyrism in the Rat.* (22480)

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It was reported(1) that β-aminopropionitrile and aminoacetonitrile fed to weanling male rats produced extensive skeletal lesions similar to those found in rats fed *Lathyrus odoratus* seeds(2). Bis(β-cyanoethyl)amine produced minimal skeletal lesions when fed for an extended period; the behavior of rats on this compound was suggestive of cerebellar lesions as reported by Bachhuber *et al.*(3). β-Aminopropionitrile and aminoacetonitrile, when injected subcutaneously, are at least as active in the production of skeletal lesions in rats as when given orally. Daily injections of .02 g and .01 g of β-aminopropionitrile and .003 g and .005 g of aminoacetonitrile hydrosulfate to weanling rats fed the standard laboratory pellet diet produced very severe skeletal lesions in 15 to 20 days. β-aminopropionitrile injected in daily doses of .005 g and .001 g produced only slight skeletal lesions in 27 days. Three rats fed diets‡ containing 0.1% methyleneaminoacetonitrile developed very severe skeletal lesions within a week. These animals died with severe inanition from 6-11 days after the onset of the diet. The roentgenograms demonstrated extremely abundant periosteal new bone formations in the femurs and humeri and extensive clefts in the widened epiphyseal plates of the long bones were observed in the histological sections. Another group of 3 weanling rats was fed a diet containing 0.05% of methyleneaminoacetonitrile for 64 days. These rats developed extensive degenerative arthritis, periosteal new bone formations in the femurs, and moderate widening and disarrangement of the epiphyseal plates. A diet containing 0.01% of the same compound was fed to another group of 3 weanling rats for 64 days. The roentgenological and histological examinations of the skeleton of these rats did not reveal any lesions. It is possible that after ingestion the methyleneaminoacetonitrile hydrolyzes into aminoacetonitrile and formaldehyde. However, this appears unlikely because the study of the severity of the skeletal lesions in relation with the daily intakes of these two nitriles indicates that the methyleneaminoacetonitrile is probably more active than the aminoacetonitrile. The report that β-mercaptoethylamine(4) produces the skeletal lesions of lathyrism could not be verified. Six weanling rats placed on a diet§ containing 0.3% of this compound for 35 to 64 days did not develop any skeletal lesions detectable in the roentgenograms or in the histological sections. In a previous paper(1) a number of nitriles were reported which did not produce lathyrism when fed to rats. Similarly, no lesions have been observed after feeding to weanling rats the following diets§ for periods of 5 to 10 weeks: 0.5% succinonitrile, 0.5% hydroacrylonitrile, 0.2% acrylamide, 0.1% acrylamide, 0.1% cyanamide, 1% α-cyanoacetonitrile, and 1% cyanoguanidine. It has been reported that diets high in vit. E(5), in casein(6), and in gelatin(7) give partial protection from the lesions of lathyrism. We have not been able to corroborate these reports. A group of 20 four-week-old rats fed a diet containing 50% *Lathyrus odoratus* seeds‖ were given alpha-tocopherol by mouth in doses of 120-300 mg per week for a period of 13 weeks. Another group of 20 rats of the same age and fed the same diet but without the supplementary feeding of alpha-toco-

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‡ The control diet consisted of casein, 18%; yeast, 10%; Crisco, 5%; Wesson Oil, 5%; sucrose, 29.6%; starch, 29.7%; salt mixture, 2.5%; haliver oil, .2%; choline, .04%.

§ Same diet as in footnote ‡.

‖ The 50% *Lathyrus odoratus* diet used in these experiments was as follows: casein, 10%; yeast, 10%; Crisco, 5%; Wesson Oil, 5%; sucrose, 8.6%; starch, 8.7%; salt mixture, 2.5%; haliver oil, 0.2%; *Lathyrus odoratus* pea, 50%; choline, 0.04%.

TABLE I. Antioxidants Incorporated into 50% *Lathyrus odoratus* Diet.

Compound	% in diet	Days on diet
2,6-Di-tert-butylphenol	1	35
Nordihydroguaiaretic acid	.5	63
N,N'-Diphenyl-p-phenylenediamine	.5	49
2,6-Di-tert-butyl-p-cresol	.5	49
Gum guaiac	.5	49
n-Propyl gallate	.5	49
Butylated hydroxyanisole	.5	49
4-tert-Butylpyrocatechol	.23	42
DL-β-3,4 dihydroxyphenylalanine	.28	42
Tyrosine ethyl ester	.29	42
Nordihydroguaiaretic acid + L-Methionine	.1 + .1	35
Nordihydroguaiaretic acid + Citric acid	.1 + .1	35
β-Conidendrol	1	23
α-Tocopherol	120-300 mg/wk	91

pherol served as controls. The incidence of dissecting aneurysm of the aorta and the severity of the skeletal lesions as judged from the roentgenograms and the histological examinations was similar in these two groups. In another experiment a group of 22 weanling male rats was fed a synthetic¶ vit. E deficient diet for a period of 4 to 7 months. All these rats developed severe testicular atrophy with degeneration of the germinal epithelium. The animals not sacrificed until the sixth or seventh month of this diet developed muscular dystrophy with paralysis and some died suddenly. Histological and roentgenographic studies of these animals revealed no lathyrism-like lesions.

To evaluate any possible curative or protective effects of other antioxidants on the development of lathyrism, the compounds listed in Table I were tested. Groups of 3 to 9 weanling rats were used for every compound. Roentgenograms of the rats under ether anesthesia were taken every 2 weeks and at the postmortem examination the femurs and upper tibiae were obtained for histological studies. Hematoxylin and eosin and Mallory stains were used. In no instance could a protective effect be demonstrated. Of interest, however, is the observation that the rats on these compounds often ate less than control rats. In these cases lesions in experimental animals were less severe than in controls. When the intake of control animals was limited to that of the experimental, no difference in severity of lesions could be demonstrated. To increase the intake of the *Lathyrus* pea diet plus the antioxidant, lower concentrations of nordihydroguaiaretic acid were used with the synergists citric acid and methionine. No protective effect could be demonstrated.

A high concentration of casein (27.3% of diet), when incorporated into 35% and 50% *Lathyrus odoratus* diet, showed no demonstrable protective effect on development of skeletal lesions. No rupture of the aorta occurred in the 7 weanling rats fed the high casein diet. However, a similar number of controls fed *Lathyrus* diet alone likewise did not show this lesion.

Other compounds tested for possible protective effects on lathyrism in the rat are listed in Table II. Rutin was tested because of its possible antifragility action; sodium thiosulfate, for its effectiveness in cyanide poisoning; γ-aminobutyronitrile, pantothenic acid, taurine, cystine, propylamine, ethylamine, propionaldehyde oxime, and acetaldehyde oxime as possible competitive inhibitors of aminonitriles; glucuronolactone, as a detoxifying agent for amines; sodium salicylate, because of its known clinical action in arthritis; glucosamine because of its importance in connective tissue structures. Groups of 3 to 9 rats were used for every compound

TABLE II. Compounds Incorporated into 50% *Lathyrus odoratus* Diet.

Compound	% in diet	Days on diet
Rutin	1	35
Sodium thiosulfate	.5	49
Casein	27.3	63
Cystine	2	63
γ-Aminobutyronitrile	4	42
d-Pantothenic acid	1	39
D-Glucosamine	1	39
Taurine	8.9	42
Glucuronolactone	10	41
Sodium salicylate	1	21
Propylamine*	1	24
Ethylamine*	2	24
Propionaldehyde oxime	2	12
Acetaldehyde oxime	2	10

* Fed as salt of HCl with $NaHCO_3$.

¶ The tocopherol deficient diet was obtained from the Nutritional Biochemical Corp., Cleveland, Ohio.

TABLE III. Excretion of Sulfur in Rats Fed Control (Stock Rockland Pellet) Diet, *Lathyrus* Pea Diets, and *Lathyrus* Pea plus 0.5% Nordihydroguaiaretic Acid (NGA) Diet.

Diet	No. of rats	No. of days	Total S, mg/day/rat, mean	Total S, SO_4-S, mean
		Series I		
Control	2	10	11.25	57.1
50% pea	4	20	5.95	65.5
		Series II		
Control	2	4	5.31	76
35% pea	2	4	4.17	85
50% "	2	4	3.37	69
.5% NGA	3	4	4.06	43.5
		Series III (intake controlled)*		
Control	2	2	3.36	61.4
50% pea	3	2	3.10	53.5
.5% NGA	2	1	3.71	4.3

* In Series III, intake of all 3 groups was limited to that of group ingesting the least food. Series I and II were permitted unlimited intake.

incorporated into a 50% *Lathyrus odoratus* diet. Roentgenograms of the animals under ether anesthesia were obtained every 2 weeks and at postmortem examination the femurs and upper tibiae were obtained for histological studies. No protective action could be demonstrated with any of these compounds.

The urinary excretion of sulfur in rats fed a *Lathyrus* pea diet and the same diet plus 0.5% of nordihydroguaiaretic acid was studied together with the sulfur excretion in control rats fed standard laboratory pellet diet (Rockland) (Table III). Total sulfur excretion by 3 series of control rats varied with their intake; 57 to 76% of the total sulfur was excreted as sulfate. The rats fed *Lathyrus* pea diets excreted 53 to 85% of the sulfur as sulfate. When nordihydroguaiaretic acid diet was fed to rats for one day (series III) only 4% of the excreted sulfur was in the oxidized form, compared to 53.5 and 61.4% in rats on pea and control diets restricted to the same intake of food as rats fed the antioxidant. However, rats fed nordihydroguaiaretic acid for 4 days showed considerable recovery of their ability to oxidize sulfur, excreting 43.5% of the total as sulfate. Partition of urinary sulfur by rats fed *Lathyrus* peas, in comparison with control rats, gave no evidence of altered sulfur metabolism in lathyrism.

The deposition of sulfur-35 in the epiphyseal plates, bones, and aorta of rats with Lathyrism was investigated by radioautography. Five 7-week-old male rats were used. Three rats received a 50% *Lathyrus odoratus* diet since 22 days of age. Two rats received the standard laboratory pellet diet and served as controls. Approximately 60 μc of carrier-free sulfur-35 in the form of sodium sulfate in 0.3 ml distilled water was injected intraperitoneally into each animal. They were sacrificed with chloroform 20 hours later. One rat on the pea diet was found dead with a hemothorax due to ruptured dissecting aneurysm of the aorta. The knees including lower femur and upper tibia, shoulders, hip joints, lower thoracic and upper lumbar vertebrae, and thoracic aorta were removed from each animal. Preservation was in neutral formalin and decalcification was done with versene. Sections were mounted on autograph emulsion(8) with exposures of about one week. The S^{35} is chiefly in the form of chondroitin sulfate(9).

Radioautographs demonstrated deposition of S^{35} in the epiphyseal plates in about equal amounts in experimental as in control animals. A high concentration of S^{35} was observed in experimental rats at the site of periosteal detachments overlying areas of new bone formations. This, of course, was not seen in control rats. S^{35} was deposited in small amounts in the thoracic aorta of both control and experimental animals. No greater concentration of S^{35} was observed in the aorta with the dissecting aneurysm. The uptake of S^{35} by chondroitin sulfate was the same in experimental as in control animals and, therefore, it may be assumed that the sulfur content of the chondroitin sulfate is not greatly altered in lathyrism. These findings appear to be in accordance with the work of Bauer *et al.*(10).

Summary. 1. Male rats injected subcutaneously with β-aminopropionitrile and aminoacetonitrile developed the same skeletal lesions of lathyrism as did those fed these compounds. 2. Methyleneaminoacetonitrile fed to rats produced very severe skeletal lesions and is probably more active than the

aminoacetonitrile. 3. Diets high in casein, gelatin, vit. E, and other antioxidants did not protect the rats fed *Lathyrus odoratus* seeds from developing skeletal lesions. 4. Radioautographs of the skeleton after injection of S^{35} and studies on sulfur excretion of rats fed *Lathyrus odoratus* peas and rats fed a control diet showed that the metabolism of sulfur is probably not altered in lathyrism.

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Some Clinical Conditions Affecting Urinary Excretion of Non-Dialyzable Hexosamine.* (22481)

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In a preceding communication(1), the normal levels of adult human urinary excretion of non-dialyzable conjugated hexosamine were reported to be 32.4 ± 7.2 mg/day for males and 25.9 ± 5.8 mg/day for females. The hexosamine appeared to be principally glucosamine. Other recent publications(*e.g.,* 2-5) related to this subject indicate or suggest that more than one conjugate contributes to the total hexosamine present in the non-dialyzable fraction of both normal and pathological urine, part being present as a result of glomerular filtration and part originating from the urinary epithelium.

This communication reports the results of investigations of "bound" hexosamine excretion in certain clinical conditions. These determinations were undertaken in an effort to distinguish some aberrations affecting bound hexosamine excretion, which in turn might further elucidate its origin and nature.

Methods. The procedure was the same as that previously described(1). Twenty-four-hour urine specimens were accurately collected and preserved with phenylmercuric nitrate. As a check on the entire procedure, samples of urine from healthy individuals were included at intervals.

Results. The results are expressed as mg glucosamine per 24 hours. Initially, urines from patients with some selected conditions were examined. These preliminary experiments indicated an increase in bound hexosamine in several of these (Table I). Urolithiasis, atherosclerosis, and pregnancy were further studied.

Urolithiasis. Neither the chemical type of urolite, a low-calcium diet, nor the presence of infection (in each of these cases minimized by concurrent antibiotic or chemotherapy) appeared to influence the results. An elevated bound hexosamine excretion was found in all cases (Table II). This was particularly striking in cases with large or actively growing stones.

Three male patients were included who had

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