

## Effect of Different Lathyrogens upon Tissue Responses in Rats.\* (31446)

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A wide variety of tissue alterations has been described in experimental lathyrisms, most commonly in the skeleton and the arteries. All of the lathyrogenic agents are capable of producing osteolathyrisms which is characterized by extensive skeletal deformities. Alterations of the soft tissue, however, such as: degeneration of motor cells in the spinal cord(1), aortic rupture(2), herniation(3), colonic atony(4), and priapism(5) have not been observed consistently. For example, none of the known lathyrogens are comparable to b-b-aminodipropionitrile for production of neuro-lathyrisms(1,6,7). Although both mercaptoethylamine and semicarbazide can readily induce osteolathyrisms, they are considerably less effective in the function of arterial alterations than is b-aminopropionitrile (BAPN)(8,9). When estimates of biologic activity are limited to responses in the epiphyseal cartilage, semicarbazide and mercaptoethylamine will react in a different manner than b-aminopropionitrile(10,11). It has been suggested that aminoacetone nitrile resembles b-aminopropionitrile closely but that its action is more potent(12). Although it has also been documented that different concentrations of lathyrogens are necessary for production of equivalent tissue abnormalities(13), the relationship between manifestations of toxicity and the magnitude of tissue abnormalities has not been fully resolved. Comparisons between the severity of osteolathyrisms and the incidence of soft tissue alterations also require further clarification. Such an evaluation will reemphasize the fact that certain of these chemicals are better suited for some types of experimentation than others, and may suggest new avenues for investigation.

*Materials and methods.* Weanling male Sprague-Dawley rats were used. Finely granulated lathyrogens were added in different concentrations to a commercial diet.† The con-

centration of lathyrogens to be fed was based on the rat's weight gain and survival during a 49-day period. The diets were mixed by hand, kept at 4°C, and fed *ad libitum* for 49 days. Three rats were housed per cage during the assay. Autopsies were made on animals which died during the feeding period or were killed after 49 days. The rats were weighed every other week prior to the seventh. The presence and severity of bony malformation, herniation, aortic alterations and the condition of the colon and penis were recorded. A numerical approximation of the composite skeletal deformity in each group of rats was estimated as previously described(14). Malformations of the sternum, vertebral column, and femora were expressed numerically from 0 to 3 as follows: none = 0, minimal = 1, moderate = 2, and severe = 3 (Fig. 1). The mean for any group of rats was obtained by dividing the sum representing the deformities by the number of bones examined. As shown in Table I, a moderate composite deformity of the three sites examined is expressed numerically as 2.0.

*Results.* Concentrations of various lathyrogens fed, numbers of rats employed and significant gross alterations are recorded in Table I.

In 16 controls, weight gains of 4.8 g/day were observed in weanling male Sprague-Dawley rats fed a commercial diet for 49 days. Nothing of significance was encountered on gross inspection of the controls at autopsy. Five concentrations of di-BAPN-fumarate were fed in the diet. Rats fed 0.5 and 0.75 g/kg of diet did not develop any gross lesions. A slight depression in weight gain is evidence that the drug was producing some toxic effect. A concentration of 1.0 g of di-BAPN-fumarate (equivalent to 0.55 g of BAPN)/kg of diet was capable of producing aortic rupture. Ten of the 18 rats died of angorrhexis without a significant suppression in

\* This investigation was supported by grant AM-06444-03 from Division of Research Grants, USPHS.

† Lab-Blox, Allied Mills Inc., Chicago, Ill.

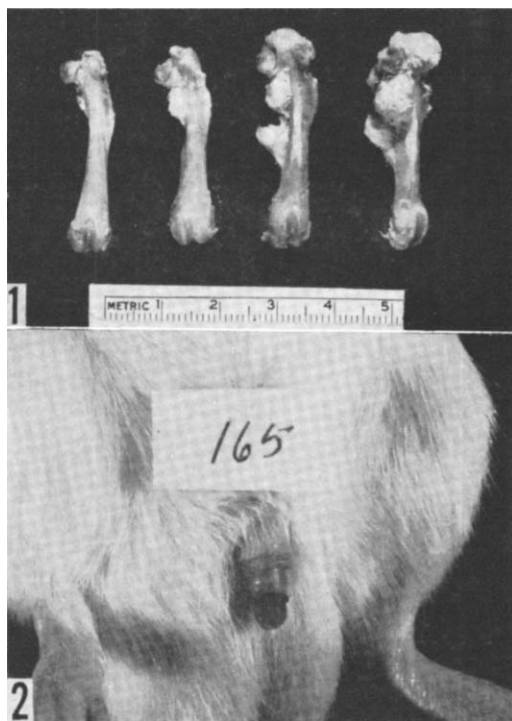


FIG. 1. Femurs are arranged in order of increasing severity of bony malformation from left to right. Normal femurs were assigned a numerical value of zero, whereas the most extensive deformities were equal to three. Similar numerical approximations of malformation were assigned to the sternums and vertebral columns in every rat. Numerical estimates of skeletal deformity shown in Table I are average values for all of the bones examined in one assay.

FIG. 2. Penile prolapse usually appeared sometime after the fourth week of feeding. Following development of prolapse rats were thereafter incapable of drawing the penis back into its cutaneous covering.

growth or appreciable skeletal malformation. When 1.5 g of di-BAPN-fumarate was fed, survival time was further shortened to 31.3 days because 5 of 6 rats died of angiorrhesis. Despite evident arterial injury, weight gains were 4.0 g/day. When 2.0 g of di-BAPN-fumarate was fed, survival time was lessened only to 30.0 days whereas the weight gains dropped to 3.3 g/day. Skeletal deformities were more prominent than when 1.5 g of BA-

‡ B-aminopropionitrile fumarate was purchased from Abbott Laboratories, North Chicago, Ill. Aminoacetonitrile-bisulfate was obtained from Aldrich Chemical Co. Inc., Milwaukee, Wisc.

PN was fed. All of the rats in this group died of aortic rupture.

*Aminoacetonitrile-bisulfate*.<sup>‡</sup> Four concentrations of AAN were fed in the diet. Addition of 0.5 g of AAN reduced the weight gain to 4.0 g/day and produced prolapse of the penis in one rat (Fig. 2). At a concentration of 0.75 g of AAN/kg weight gains dropped to

TABLE I. Response of Rats to Ingestion of Different Lathrogens.

No. of rats	Dose of lathrogen fed, g/kg	Avg survival, days	Avg wt gain, g/day	Estimate of skeletal deformity	Gross alterations encountered				
					Aortic rupture	Distended small intestines	Hernia	Excess feces, in colon	Penile prolapse
16	.0	49.0	4.8						
6	.5 BAPN	49.0	4.4	none					
6	.75 "	49.0	4.3	"					
18	1.0 "	42.0	4.6	.3	10/18				
6	1.5 "	31.3	4.0	1.1	5/6	1/6			
6	2.0 "	30.0	3.3	1.7	6/6				
9	.5 AAN	49.0	4.0	none					1/9
6	.75 "	49.0	3.9	1.0					2/6
9	1.0 "	37.4	2.4	2.1	4/9	2/9	2/9	2/9	4/9
6	1.5 "	24.5	.9			3/9	5/9		1/6
14	.5 SC	47.0	3.5	.7	1/14				4/10
10	.75 "	48.0	2.7	1.7		1/10			
13	3.0 MEA	49.0	4.1	none					
10	4.0 "	49.0	2.0	1.4					1/10

an average of 3.9 g/day, skeletal malformations were minimal, and 2 of 6 rats developed penile prolapse. At 1.0 g/kg of diet, marked suppression of growth occurred because the rats only gained 2.4 g/day and survival time dropped to 37.4 days. Skeletal deformities were more extensive than in any previous assay and 4 of 9 rats died of aortic rupture. In 3 other rats, an empty gas distended and bile stained gastrointestinal tract was observed. Because nothing else of a morphologic nature was seen which could account for death, it was assumed that these 3 rats died either of starvation or of drug intoxication. Herniation at the costovertebral angle was observed in 2 and a dilated colon filled with soft pasty feces in 2 other rats. Four of 9 rats developed prolapse of the penis. When 1.5 g of AAN was fed, the survival time was reduced to 24.5 days. Weight gains dropped to 0.9 g/day. At autopsy, congestion of the lungs and liver, an empty and bile stained gastrointestinal tract and minimal sternal deformity were observed. Only 1 of 6 rats developed penile prolapse. It was again assumed that either starvation or drug intoxication was responsible for death in these 6 animals.

*Semicarbazide-HCl.*§ When fed to 14 rats at a concentration of 0.5 g/kg it reduced weight gains to 3.5 g/day. Inspection of the sternum, femur and vertebral column revealed the presence of minimal skeletal deformity, and only one of 14 rats died of aortic rupture. At a level of 0.75 g/kg of diet, weight gains were reduced further to 2.7 g/day. Skeletal deformities were considered to be of moderate severity. Neither aneurysmal dilatation nor aortic perforations were seen. Prolapse of the penis was seen in 4 of 10 rats after the 5th week. One of 10 rats died with an acute purulent orchitis and gastrointestinal distention.

*Mercurptoethylamine-HCl.*|| At a concentration of 3.0 g/kg of diet, it did not suppress growth appreciably, produce any evidence of osteolathyrism in 14 rats, or affect the soft tissues. When the concentration in the diet

was increased to 4.0 g/kg, however, weight gain was reduced to 2.2 g/day. At this concentration, MEA produced minimal osteolathyrism and penile prolapse in 1 of 10 rats. In contrast to other lathyrogenic agents, MEA exerted a greater effect on the femur than the sternum or vertebral column. The femora were shorter, the condyles were larger and less periosteal fibrosis developed on the trochanteric ridges than is commonly seen in BAPN-fed rats.

*Discussion.* Estimates of biologic activity in intact lathyrific rats in the past have not been based on the same criteria so that conflicting reports in regard to the severity and type of tissue response may in part be due to this fact. Criteria used in evaluating the response in the present study have been: survival time, weight gain, concentration of lathyrogen fed, severity of skeletal malformation and the incidence of soft tissue alterations. Approximations of drug toxicity were obtained by a comparison of the consumption of a specified concentration of chemical with the animal's growth during a 7-week period. On the basis of these criteria, AAN and semicarbazide must be considered as the most toxic.

This study illustrates that the highly toxic concentrations of a lathyrogen are not necessarily the most preferable for production of a reproducible tissue alteration. Undoubtedly the most satisfactory concentration of a lathyrogen in any given experiment must remain arbitrary, because of the different objectives of various investigators. If one concedes that the lowest concentration of drug which is capable of producing morphologically discernible tissue alterations within a specified period of time is the most suitable, then comparisons of lathyrogenic activity become more meaningful. On the basis of this assumption, we considered the optimal drug concentration for a comparison of its lathyrogenic activity to be that amount capable of producing a maximal tissue response with the least suppression in weight gain. For this reason, the drugs were increased from levels which exerted only minimal suppression in weight gains to concentrations which reduced them by 50% or more. Using weight gains as guidelines, it is possible to exaggerate differences

§ Semicarbazide was obtained from Fisher Scientific Co., Chicago, Ill.

|| Mercurptoethylamine-HCl was purchased from Calbiochem, Los Angeles, Calif.

in the tissue alterations which can be produced by various lathyrogens. If one utilizes drug concentration in the diet as the sole criterion for comparison, mercaptoethylamine would be considered the least toxic. Yet, when the magnitude of skeletal malformation is evaluated with respect to growth suppression, mercaptoethylamine is less effective than aminoacetonitrile. It therefore appears to be a less suitable lathyrogen for production of osteolathyrism than semicarbazide or aminoacetonitrile. Choice of a lathyrogen dependent on the least weight suppression and the greatest incidence of aortic rupture would result in the selection of BAPN. On the basis of weight suppression with the least complications, semicarbazide appears to be most preferable for production of osteolathyrism. AAN is the most effective agent for the production of skeletal deformity and a variety of other soft tissue alterations including angiorrhesis. There is little evidence from this study to suggest that the soft tissue changes observed are related in any manner to the development of skeletal deformity. This, therefore, is in contrast to conclusions drawn when hind limb paralysis develops suddenly after dislocation of the vertebral bodies and spinal cord compression(13,15).

Since the lathyrogens are all capable of modifying the development of bone in growing rats, there has been a tendency to assume that these drugs may produce similar histologic or biochemical alterations. Experimental studies, however, do not support this hypothesis. Shintani and Taylor found that semicarbazide did not reduce the *in vitro* uptake of labeled sulfate by cartilage cells like BAPN or mercaptoethylamine(10). Ramamurti and Taylor discovered that BAPN produced widening of the maturing zone, whereas, mercaptoethylamine feeding results in hyperplasia of the proliferating zone of cells in the epiphyseal plates of rats(11). In this study the femoral deformities in MEA-treated rats did not resemble those seen in BAPN-fed rats. It has also been documented by others that neither mercaptoethylamine nor semicarbazide are as effective as BAPN for production of angiolathyrism(8,9). In agreement with previous observations, BAPN was found to be a more effective lathyrogen for produc-

tion of angiorrhesis in this study.

Under properly controlled conditions it has been shown that inclusion of BAPN in the diet predisposes to angiorrhesis while ingestion of semicarbazide primarily disturbs the skeletal development. Experimental models, therefore, exist now which can be employed to resolve how specific lathyrogens modify the metabolism of various proteins and by their combined action produce specific tissue alterations in growing rats.

*Summary.* All of the lathyrogens administered produced some degree of osteolathyrism and suppression of growth. Alterations in the skeleton and soft tissues appear to develop independently of each other following ingestion of the lathyrogens fed in this study. Aminoacetonitrile produces the greatest variety of soft tissue changes. Semicarbazide appears to be the most suitable for production of uncomplicated osteolathyrism. B-aminopropionitrile was found to be the most effective lathyrogen for production of angiorrhesis.

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