Biophys. 34, 1 (1951).
2. Harrison, A. P., Jr. and Raabe, V. E., J. Bacteriol. 93, 618 (1967).
3. Hollaender, A., J. Bacteriol. 46, 531 (1943).
4. Simon, M. I. and Van Vunakis, H., J. Mol. Biol. 4, 488 (1962).
5. Wacker, A., Turck, G., and Gerstenberger, A., Naturwissenschaften 50, 377 (1963).
6. Smith, K. C., Biochem. Biophys. Res. Commun. 8, 157 (1962).
7. Webb, R. B. and Kubitschek, H. E., Biochem. Biophys. Res. Commun. 13, 90 (1963).

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Influence of Cystine Supplements on the Incidence of Aortic Rupture in Rats Fed β-Aminopropionitrile* (32736)

JOSEPH J. LALICH AND WOO CHUN WHANG PAIK
Department of Pathology, University of Wisconsin Medical School, Madison, Wisconsin 53706

An appreciable difference in the incidence of aortic rupture has been encountered when *Lathyrus odoratus* seeds are fed to immature rats (1–4). It has been suggested that the concentration of protein in semisynthetic diets probably exerts some protective effect against the development of aortic rupture in *L. odoratus*-fed rats (2). In support of this hypothesis, supplements of casein, gelatin, and lactalbumin minimize both suppression of growth and severity of osteolathyrism (5, 6). Besides the concentration of protein in the diet, it appeared that the type of protein probably also exerts some influence on the production of tissue changes in lathyritic rats. Protein quality became a consideration when it was observed that at equivalent concentrations of BAPN, semisynthetic diets of crude casein were more protective against aortic rupture than commercial diets (7, 8). The type of protein also appeared influential in another study because only cottonseed meal and BAPN were able to produce fecal impaction and colonic atony (9). The beneficial effect of protein against BAPN intoxication is supported by other studies where supplements of *l*-cysteine or *l*-glutamine were found to be protective against growth suppression and osteolathyrism (10). Supplements of *dl*-methionine in a diet with insufficient casein, however, appeared to enhance the incidence of aortic rupture when rats were fed BAPN (7). The protective influence of amino acid supplements has been questioned in still other studies because they did not appear to minimize BAPN intoxication (6).

Additional studies on the relationship of amino acids or protein to BAPN intoxication are needed if this controversy is to be resolved. Such studies may also offer some explanation for the wide variation in the incidence of aortic rupture which has occurred in rats in experimental lathyrism.

The point of issue in this study was to establish whether the incidence of aortic rupture which is caused by the ingestion of BAPN could be reduced by supplementation of amino acid. At present, ample justification exists for controversy regarding the influence of protein or amino acid supplements on the development of osteolathyrism or angiolathyrism in immature rats. Comparable experimental conditions have not always been employed in experimental lathyrism when evaluating tissue alteration in rats. There have been variations in the duration of the assay period, concentration of lathyrogen, strain, age, and sex of rats, as well as the concentration of protein. Finally, the observations that suggested a beneficial effect from protein or amino acid supplementation have not satisfied statistical criteria because insufficient numbers of rats were employed.

Before attempting to study the relationship between aortic rupture and dietary manipulation in BAPN-fed rats, several arbitrary limitations seemed essential. We decided to

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feed the lowest concentration of BAPN capable of producing angiorrhexis in most of the rats (11). A period in excess of 5 weeks appeared necessary because appreciable numbers of rats continued to die from aortic rupture up to 49 days. Finally, weanling male Sprague-Dawley rats which are known to be susceptible to the development of aortic rupture were used in these assays (7–9).

Method. Rats weighing 39–46 gm were used in this experiment. Ground commercial diet[1] was fed to 24 control rats. Di-BAPN-fumarate (BAPN) 1.5 gm/kg of ground diet was fed to 52 rats. Fifty-two test rats were given 0.15% BAPN and 1.5% *l*-methionine in ground diet. A fourth group received 0.15% BAPN in ground diet supplemented with 1.5% *l*-cystine. Due to limitations in housing space, only one-third of the rats in each group were fed at one time. Intervals between individual assays were 28 days. The rats were fed *ad libitum* for the duration of the experiment or until they died. Initially, four rats were housed in one medium sized open bottom cage for 28 days after which 2 rats occupied one compartment. The animals were checked daily for signs of weakness. Weights were obtained at 2-week intervals during the first month and weekly thereafter. The thoracic and peritoneal organs were carefully examined for evidence of the cause of death. It was easy to recognize that aortic perforation had occurred by the presence of blood in the thoracic or peritoneal cavities. In addition to the aortic alterations, the final weight, the presence of hind limb paralysis, prolapsed penis or herniation were recorded when observed. Because both the gross and histologic alterations in bones and aortas of weanling rats fed BAPN have been described previously (1, 2, 5, 8), emphasis therefore will now be directed to the incidence and time interval during which aortic perforation occurred in the three test groups which received BAPN in their diet. Secondly, the probable implications of the protective effect of *l*-cystine supplementation in BAPN-fed rats will be considered.

Results. Response of rats to BAPN feeding in a ground commercial diet with and without amino acid supplements is tabulated in Table I.

Controls. The growth in the 24 rats was satisfactory. Nothing abnormal was seen on gross examination of the tissues. Weight gains were most rapid from the third to the fifth week.

BAPN. Fatal thoracic aortic rupture was most frequent from 21 to 35 days. However, an appreciable number also died during the following 14 days. Two of 43 rats which died of aortic perforation developed hemoperitoneum. Herniation occurred in one animal, seven developed prolapse of the penis and five manifested hind limb paralysis.

BAPN and methionine. The response was comparable to that seen in rats fed BAPN alone. Again, most of the rats died of angiorrhexis before day 35. Of 36 rats which died of aortic rupture, two had hemoperitoneum. The incidence of herniation, penile prolapse and hind limb paralysis was comparable to that seen in the BAPN-fed rats. While there is a suggestion of a decreased tendency for rats to die in the *l*-methionine supplemented group, the difference in the incidence of aortic rupture was not statistically significant, when compared with the BAPN-fed rats.

BAPN and cystine. The *l*-cystine supplementation definitely reduced the incidence of fatal aortic rupture in 52 BAPN-fed rats. In contrast to the previous two assays, more rats now died after than before day 35. Furthermore, only 17 of 52 test rats died of aortic rupture. This difference in the incidence of aortic rupture was found to be significant when compared with the two previous groups. Interestingly, the reduction in aortic rupture was attained despite the fact that the cystine supplemented rats gained more grams/day than the other two test groups. We believe this latter observation is important because the increased growth excludes the possibility that in the *l*-cystine supplemented rats, the decreased incidence of aortic rupture is due to a diminished consumption of the diet. On the other hand, more rats developed herniation and penile prolapse in the *l*-cystine supplemented group. It is not possible to resolve whether these soft tissue alterations are due to the longer survival of more test rats or the cystine supplementation.

[1] Lab-Blox, Allied Mills Inc., Chicago, Ill.

TABLE I. The Incidence of Aortic Rupture and Other Soft Tissue Complications Which Occurred at Various Times.

Animal group	No. of animals	Gross changes	Experimental days 14	28	35	42	49	Total observed
Control	24	Growth gm/day	4.8	6.3	5.8	4.3	3.3	
		Death						0
		Miscellaneous						0
BAPN[b]	52	Growth gm/day	3.8	4.5	2.1	0.3	0.4	
		Hemothorax		13	18	5	5	41[a]
		Hemoperitoneum				1	1	2[a]
		Miscellaneous		1		2		3[a]
		Herniation			1			1
		Penile prolapse				6	1	7
		Hind limb paralysis		4	1			5
BAPN[b] and methionine	52	Growth gm/day	3.3	4.6	2.7	1.7	0.3	
		Hemothorax		10	15	7	2	34[a]
		Hemoperitoneum			1	1		2[a]
		Miscellaneous		1	1	2		4[a]
		Herniation		2	1			3
		Penile prolapse				5	2	7
		Hind limb paralysis		1	1	2		4
BAPN and cystine	52	Growth gm/day	3.9	5.0	3.7	2.3	1.0	
		Hemothorax		3	5	5	4	17[a]
		Hemoperitoneum						0[a]
		Miscellaneous		1	1	3	2	7[a]
		Herniation		2	1	3		6
		Penile prolapse			1	7	4	12
		Hind limb paralysis			1	1		2

[a] Number of animals which died.

[b] The difference in mortality from aortic rupture was significant when compared with the cystine BAPN group; $p<0.005$.

The severity of osteolathyrism was considered to be moderate to severe in degree in all three test groups. The hind limb paralysis invariably developed suddenly which suggests that dislocation of the vertebral body and hemorrhage into the spinal cord had occurred (4, 12). Deaths which were observed in the miscellaneous group were found to be due to the presence of bronchopneumonia and starvation. Rats dying of other causes were not included in calculations of death from aortic rupture.

Discussion. Weight gains in 24 control rats indicate that the commercial diet is nutritionally adequate. As has been observed on numerous occasions, any concentration of BAPN in the diet which produces any tissue alterations will also suppress growth in weanling rats. It is not certain whether growth retardation is caused by BAPN intoxication per se, or is due to the inactivation of some dietary essential in the diet. It has been suggested that protein supplements in BAPN-fed rats may produce a reversal of interference with amino acid metabolism (6). It has not been decided, however, whether all protein sources are equally protective when fed with BAPN. Comparisons made with diets containing cottonseed meal, crude casein or fishmeal with BAPN suggest that cottonseed meal is the least protective (9). There is a suggestion that some amino acids may be partially protective during the ingestion of lathyrogenic agents. Supplements of *l*-histidine and *l*-arginine in a commercial diet lessened the weight suppressing effect of

BAPN ingestion without altering the incidence of aortic rupture (9). Insufficient numbers of animals, however, were employed to be statistically significant. For this reason, it was considered essential to include sufficient numbers of rats in each BAPN assay so that valid statistical comparisons might be drawn.

We purposely selected a concentration of dietary BAPN which on the basis of past experience would produce angiorrhexis in most of the rats with the least effect on weight suppression (11). Observations in this study therefore do not imply that supplements of *l*-cystine will be equally protective against greater concentrations of BAPN in the diet. Within the limitations which have been imposed by this study, a statistically significant degree of protection against angiorrhexis has been demonstrated with a supplement of *l*-cystine when 1.5 gm of BAPN/kg is fed in a commercial diet to weanling rats. While it was possible to show that *l*-cystine is protective against angiorrhexis, it is questionable whether a maximum diminution in BAPN intoxication has as yet been attained by amino acid supplementation. It may be of interest to point out that diets with insufficient *l*-cystine have been observed to promote another type of experimental arterial injury. A deficiency of *l*-cystine has been shown to accentuate the development of atherosclerosis in cholesterol-fed Cebus monkeys (13) and rats (14). Mann has suggested that the arterial lipid accumulation was secondary to a disturbance of sterol metabolism which is mediated by a deficiency of *l*-cysteine precursosrs in the diet (15).

It is fairly obvious that the feeding of BAPN in some manner increases the requirement of *l*-cystine in the diet for rats. The mechanism responsible for an increased quantity of *l*-cystine in the diet is also of some nutritional importance. For example, is the requirement for more *l*-cystine caused by its direct reaction with BAPN in the intestinal tract or does the lathyrogen somehow interfere with the carrier mediated transport of *l*-cystine across the intestinal membrane? Other equally probable biochemical possibilities requiring experimental investigation are: Does a metabolic block in the conversion of *l*-methionine to *l*-cystine occur in the liver? Or is the resorption of *l*-cystine in the renal tubules reduced because of the presence of BAPN in the glomerular filtrate?

Summary. The *l*-cystine supplementation when fed with a proper concentration of BAPN in a commercial diet will prolong the survival of treated rats, reduce the incidence of death from aortic rupture and partially counteract the weight suppressing influence of this lathyrogen. Some of the BAPN intoxication therefore is mediated by the production of an increased need for more *l*-cystine. The mechanism by which the requirement for dietary *l*-cystine is increased by the ingestion of BAPN remains to be clarified.

1. Ponseti, I. V. and Baird, W. A., Am. J. Pathol. **28**, 1059 (1952).

2. Bachhuber, T. E. and Lalich, J. J., Arch. Pathol. **59**, 247 (1955).

3. Geiger, B. J., Steenbock, H., and Parsons, H. T., J. Nutrit. **6**, 427 (1933).

4. Lewis, H. B., Fajans, R. S., Esterer, M. B., Shen, C. W., and Oliphant, M., J. Nutrit. **36**, 537 (1948).

5. Dasler, W., Proc. Soc. Exptl. Biol. Med. **85**, 485 (1954).

6. Lee, J. G., Dupuy, H. P., and Rolfs, H. E., J. Nutrit. **58**, 433 (1956).

7. Lalich, J. J., Arch. Pathol. **61**, 520 (1956).

8. Merkow, L. P. and Lalich, J. J., Arch. Pathol. **70**, 752 (1960).

9. Lalich, J. J. and Turner, H., Exptl. Mol. Pathol. **6**, 1 (1967).

10. Dasler, W., Proc. Soc. Exptl. Biol. Med. **91**, 554 (1956).

11. Lalich, J. J., Proc. Soc. Exptl. Biol. Med. **123**, 214 (1966).

12. Hartmann, H. A. and Lalich, J. J., Federation Proc. **20**, 435A (1961).

13. Mann, G. V., Andrus, S. B., McNally, A., and Stare, F. J., J. Exptl. Med. **98**, 195 (1952).

14. Fillios, L. C. and Mann, G. V., Metab., Clin. Exptl. **3**, 16 (1954).

15. Mann, G. V., Circulation Res. **19**, 838 (1961).

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