Toxicity of Beta-Aminopropionitrile for Turkey Poults.* (22857)

B. D. BARNETT,† H. R. BIRD, J. J. LALICH AND F. M. STRONG
Departments of Poultry Husbandry, Biochemistry and Pathology, University of Wisconsin, Madison.

Diets containing *Lathyrus odoratus* seeds or beta-aminopropionitrile (BAPN) cause extreme abnormalities of the skeleton, hernias, dissecting aneurysms of the aorta and reproductive failure in rats(1-4). A striking resemblance was noted between the degenerative changes of the aortas of rats described by Bachhuber and Lalich(4) and the changes observed by McSherry *et al.*(5) in aortas of commercially reared turkeys which died of dissecting aneurysms. This observation prompted an investigation of the possibility of a connection between BAPN, the active fragment of the toxin occurring in *Lathyrus odoratus,* and the field cases of dissecting aneurysms of turkeys.

Methods. Broad Breasted Bronze turkey poults were started in electrically heated batteries with raised wire floors and survivors were transferred to growing batteries with wire floors at approximately 4 weeks of age. Experimental diets were supplied *ad libitum* from 1 day of age. Inasmuch as the condition reported by McSherry *et al.*(5) occurred most frequently in rapidly growing birds consuming high protein rations, a high energy diet calculated to contain 28% protein was used (Table I). The birds were weighed at weekly intervals and the severity of symptoms was recorded at each weighing. In Exp. 77 the birds were scored for severity of leg deformities with a score from 0 for normal to 4 for severe deformities in both legs. Birds which died during the course of the experiment were examined grossly and microscopically for tissue changes. The BAPN used was prepared by addition of ammonia to acrylonitrile and was supplied by Abbott Laboratories.‡ For convenience in storage and incorporation into the experimental diet, the BAPN was converted to the hydrochloride (BAPN • HCl) which is a stable crystalline solid.

TABLE I. Basal Ration.

	g/kg
Ground yellow corn	305
Soybean oil meal	325
Fish meal	100
Oat groats	50
Alfalfa meal	50
Meat scraps	50
Choice white grease	50
Dried whey	50
Chick grit	10
Chick size oyster shell	5
Salt	5
Choline chloride	1
Vit. D_3 supplement (1500 ICU/g)	.9
DL-methionine	.2
B_{12} supplement (6 mg/lb)	.25
Penicillin supplement (4 g/lb)	.5
$MnSO_4 \cdot H_2O$	.33
Plus the following/kg:	
Vit. A palmitate	6,000 USP units
Riboflavin	6 mg
Niacin	40
Ca pantothenate	10
Alpha tocopherol acetate	20

Results. When BAPN • HCl was fed at the rate of 0.25% of the diet, a level on which rats can survive for several weeks, all poults were paralyzed by 1 week of age. Growth was markedly reduced (Table II) and death resulted in less than 2 weeks. Microscopic examination revealed degeneration of the anterior motor neurons (Table III). When

TABLE II. Effect of BAPN • HCl on Growth of Turkey Poults.

Exp.	% BAPN • HCl	Weekly wt in g 0	1	2	3	4	5
67	.0	52	121	267			
	.25	59	81				
69	.0	63	146	272			
	.125	60	117	130			
70	.0	61	116	226	357		
	.0625	66	110	203	307		
72	.0	59	123	274	460	766	1126
	.04	60	122	217	342	635	
	.0313	60	110	246	397	575	743

* Published with approval of Director of Wis. Agri. Exp. Station. Supported in part by grant from U.S.P.H.S.

† Present address: Poultry Department, Clemson Agricultural College, Clemson, S. C.

‡ Valuable information and materials furnished by Abbott Laboratories are gratefully acknowledged.

TABLE III. Symptoms of BAPN • HCl Toxicity for Turkey Poults.

Exp.	No. birds per group	% BAPN • HCl	Symptoms and lesions
67	11	.25	Paralyzed by 1 wk. Degeneration of anterior motor neurons.
69	6	.125	Paralyzed by 1 wk. 67% died of pericardial hemorrhage by 2 wk.
70	12	.0625	50% hock and toe deformities. 33% died of pericardial hemorrhage and 33% of aortic rupture by 3 wk.
72	12	.04	50% hock and toe deformities by 5 wk. 17% died of pericardial or pulmonary hemorrhage and 50% of aortic rupture.
72	13	.0313	92% hock and toe deformities in 5 wk. 15% died of pericardial or pulmonary hemorrhage and 15% of aortic rupture.

BAPN • HCl was reduced to 0.125% of the diet all birds were paralyzed by 1 week and 67% died of pericardial hemorrhage by 2 weeks. Growth was reduced at this level also. At 0.0625% growth was retarded. 33% of the birds died of pericardial hemorrhage and 33% of aortic rupture in 3 weeks and hock and toe deformities occurred in 50% of the birds. Of birds receiving 0.04% BAPN • HCl, 17% died of pericardial or pulmonary hemorrhage and 50% died of aortic rupture.

The aortae usually ruptured somewhere between the diaphragm and renal arteries. Massive retroperitoneal or peritoneal hemorrhages were most common. In some instances the hematoma would dissect along the aorta and extend into the thorax. A more detailed analysis of the histological changes characteristic of the arterial damage will be published elsewhere.

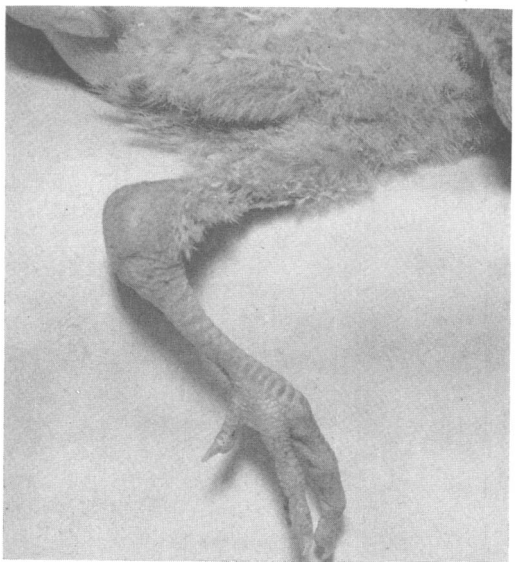

FIG. 1. Enlarged hock and bent tarso-metatarsus of bird receiving 0.0313% BAPN.

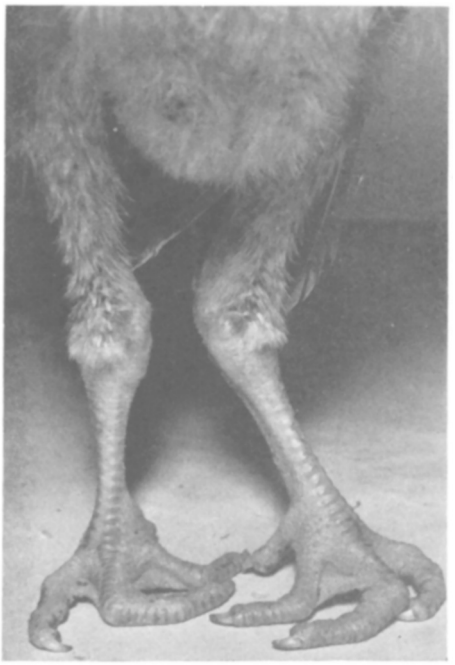

FIG. 2. Illustration of curled toes caused by 0.0313% BAPN.

Fifty percent of birds receiving 0.0313% BAPN • HCl survived to 5 weeks at which time they were transferred to the basal diet to see if recovery were possible. All surviving birds had hock deformities and crooked toes of varying degrees of severity at 5 weeks and only 1 of 13 birds failed to develop such deformities. This bird died prior to 2 weeks of

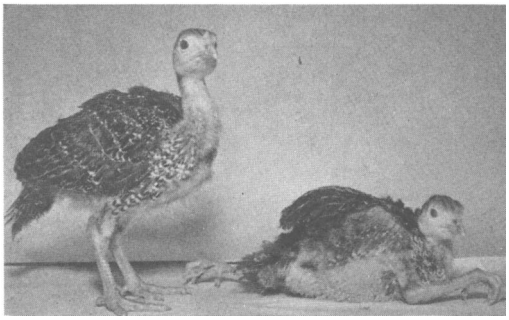

FIG. 3. Normal bird (left) compared with bird consuming 0.0313% BAPN (right).

age from pericardial hemorrhage. By 5 weeks 15% of the birds had died of pericardial or pulmonary hemorrhage and 15% died of aortic rupture. There was little if any recovery in the crippled birds when maintained from the 5th to 8th week on the basal diet. Figs. 1-3 illustrate the hock and toe deformities of birds receiving 0.0313% BAPN • HCl. The leg bones of some of the more severely deformed birds were removed for comparison to the leg bones of a normal bird. There was slight bending in some of the tarsometatarsi but very little if any of the other abnormalities observed in the skeletons of rats. It would appear that the extreme abnormality of the hock joint involves the ligaments and tendons rather than the bone.

The results of Exp. 77 are shown in Table IV. Growth was depressed by .03, .02 and .01% of BAPN • HCl although only slightly by the lowest level. Leg deformity scores of all groups receiving BAPN were much greater than controls although the scores of birds receiving 0.02% and 0.01% were somewhat less than the birds receiving 0.03%. When the 0.02% added DL methionine of the basal diet was omitted growth was slightly reduced but there appeared to be no effect on degree of deformity. The addition of 100 μg/kg of vit. B_{12} appeared to have no effect on the leg deformity; a high level of all B vitamins appeared to afford some protection against leg deformity but did not improve growth.

Discussion. Beta-aminopropionitrile in the diet of turkey poults at levels as low as 0.01% of the diet caused growth retardation and leg deformities. Also the occurrence of dissecting aneurysms of the aorta and other internal hemorrhages in poults less than 3 weeks of age with diets containing 0.0313% of BAPN • HCl indicates this species is much more susceptible to the toxin of *L. odoratus* than is the weanling rat. The work of Chang *et al.*(6) with *Xenopus larvae,* chick embryos and regenerating newt legs and the investigation of rat embryo resorption by Walker and Wirtschafter(3) indicate that age may be an important factor. The turkey poults in these experiments were one day old while the weanling rats in other experiments were approximately 21 days of age when normally started on experiment. Geiger *et al.*(1) were the first to recognize that age had an effect on degree of deformity of rats consuming *Lathyrus* seeds. Another factor which may contribute to the greater susceptibility of the turkey is its faster growth rate and greater feed con-

TABLE IV. Effect of BAPN • HCl on Growth and Leg Deformity—Exp. 77.

Alterations of basal diet	Weekly wt in g					
	0	1	2	3	4	5
None	53	111	213 (0.1)	362 (0.2)	597 (0.2)	930 (0.3)
+ .03% BAPN • HCl	54	97	187 (0.3)	327 (1.2)	563 (2.0)	836 (2.7)
+ .02% "	53	106	204 (0.9)	340 (1.1)	555 (1.6)	830 (1.9)
+ .01% "	54	104	206 (0.5)	354 (1.0)	584 (1.6)	881 (1.9)
+ .03% " + vit. B_{12}*	54	109	207 (0.5)	365 (1.3)	604 (2.3)	907 (2.2)
Omit DL-methionine	53	107	200 (0)	333 (0.2)	567 (0.2)	872 (0.1)
Omit DL-meth. + .03% BAPN • HCl	52	106	189 (0.5)	318 (0.9)	527 (2.2)	802 (2.3)
+ .03% BAPN • HCl + B vitamins†	52	107	203 (0.5)	346 (0.9)	554 (1.0)	844 (1.1)

* 100 μg/kg of diet.

† The following in mg/kg diet: Vit. B_{12} 0.16, biotin 1.6, menadione 4, pyridoxine 32, pteroylglutamic acid 32, riboflavin 48, Ca pantothenate 160, thiamin 80, niacin 400, para-amino benzoic acid 800.

Figures in parentheses represent avg leg deformity score of group based on a rating of 0-4 for severity.

sumption. The difference in symptoms and lesions between turkeys and rats, however, indicates that there is some species difference not associated with age or feed consumption.

The turkey poult is highly susceptible to hock disorders as indicated by investigations of Scott(7), Hunt *et al.*(8) and Slinger *et al.* (9). While these researches showed that supplemental niacin, vit. E and phosphorus can prevent enlarged hock disorder under certain conditions it is apparent from the last two reports that the problem is a complex one. Hock disorder of uncertain etiology appears in many commercial flocks but usually in only a few birds.

While the turkey poult consuming low levels of BAPN develops abnormalities similar to those which appear under field conditions, it is impossible to say whether or not BAPN is a causative agent in field cases. If BAPN is involved, its source under practical conditions is yet to be located.

Summary. (1) Beta aminopropionitrile hydrochloride was toxic to turkey poults at levels as low as 0.01% of diet. (2) Paralysis, degeneration of anterior motor neurons and growth depression were the chief abnormalities observed at 0.25% and 0.125% while pericardial and pulmonary hemorrhage, ruptured aortas, leg and toe deformities, and growth depression were the primary symptoms at lower levels. (3) Preliminary attempts to alter severity of symptoms by reducing the methionine level or by adding vit. B_{12} or a high level of all B vitamins were not very successful, but further investigation of the B vitamins appears to be justified.

1. Geiger, Beatrice J., Steenbock, Harry and Parsons, Helen T., *J. Nutr.*, 1933, v6, 427.

2. Bachhuber, T. E., Lalich, J. J., Angevine, D. M., Schilling, E. D., and Strong, F. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 294.

3. Walker, Donald G., and Wirtschafter, Zolton T., *J. Nutr.*, 1956, v58, 147.

4. Bachhuber, Thomas E., and Lalich, Joseph J., *A.M.A. Arch. Path.*, 1955, v59, 247.

5. McSherry, B. J., Ferguson, A. E., and Ballantyne, J., *J. Am. Vet. Med. Assn.*, 1954, v124, 279.

6. Chang, C. Y., Witschi, E., and Ponseti, I. V., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 45.

7. Scott, M. L., *Poultry Sci.*, 1953, v32, 670.

8. Hunt, J. R., Blaylock, L. C., and McGinnis, J., *ibid.*, 1954, v33, 1061.

9. Slinger, S. J., Pepper, W. F., and Arthur, D., *ibid.*, 1955, v34, 935.

Received October 11, 1956. P.S.E.B.M., 1957, v94.

Lipid Distribution and Metabolism in Two Areas of Aorta of Normal and Cholesterol-fed Rabbits. (22858)

ABRAHAM DURY

Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pa.

When cholesterol-fed rabbits are killed at successive periods gross, discrete atherosclerotic lesions appear first in the aortic arch and only much later is the rest of the aorta similarly affected. We have found a high incidence of atherosclerosis in the aortic arch only in rabbits on a high cholesterol diet for 60-70 days but rarely were lesions seen in aorta of rabbits only 40 days on the diet. Duff and McMillan(1) reviewed evidence indicating an importance of "local factors that operate in the vessel wall to determine the localization and to influence the subsequent development of individual atherosclerotic lesions." With due cognizance of the element of time as a factor in the development of atheromatosis, these observations raise the question of the nature of the factor(s) responsible for the localization and site of appearance of the earliest atherosclerotic lesions in the rabbits. The foregoing prompted us to study the distribution of lipids and phospholipid metabolism in 2 areas of the aorta, namely, the arch and the descending limb of the aorta of rabbits on the usual laboratory diet and rabbits kept for 40 and 70 days on a