Production of Aortic Aneurysms in Turkeys and Effect of Various Compounds in Potentiating Induced Aortic Ruptures.* (28474)

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Demonstration that β-aminopropionitrile (BAPN) was the agent responsible for the toxicity of *Lathyrus odoratus* seeds(1) was followed by reports that several groups of compounds produced similar skeletal deformities and aortic aneurysms in rats. Some of these compounds produced a similar syndrome in young turkeys(2,3,4). Among the compounds reported to cause osteolathyrism in the rat was β-mercaptoethylamine (MEA) (5), but this observation could not be confirmed by Ponseti *et al.*(6). Roy(7) found dietary levels of 0.35 to 0.40% MEA·HCl lethal to turkeys, while 0.20 to 0.25% depressed growth but produced no symptoms characteristic of BAPN toxicity. MEA is of special interest because it could be formed in the gut by bacterial decarboxylation of cystine.

Cyanoacetic acid has been isolated from urine of rats and rabbits as a final metabolic product of BAPN(8); a conversion assumed to occur under the influence of monoamine oxidase (MAO). Two known MAO inhibitors, 1-isonicotinyl-2-isopropyl hydrazine (IIH) and isonicotinic acid hydrazide potentiated the toxicity of BAPN in turkeys (9), as did the nitrofuran drug, Nitrofurazone(3).

Among various *Lathyrus* species, seeds of *L. latifolius* and *L. sylvestris Wagneri* are extremely toxic to the rat. Recently a neurotoxin was isolated from these species and identified as free L-α, γ-diaminobutyric acid (DABA)(10).

BAPN occurs in *L. odoratus* seeds as the N-γ-L-glutamyl derivative. Of a series of synthetic N-acyl derivatives of BAPN, only those in which the acyl group was derived from a natural amino acid were toxic to the rat, presumably because they were cleaved *in vivo* to yield free BAPN(11).

The object of the present investigation was to extend the above observations, most of which have been made with rats, to another experimental animal, namely the turkey poult. The turkey is of particular interest in this connection because in commercial production losses are frequently experienced from aortic rupture, one of the characteristic symptoms of BAPN poisoning. It was hoped that further investigation of the interrelationships between osteolathyrogens and possible potentiators such as MAO inhibitors, taurine, and even certain amino acids might throw some light on this disease.

Methods and materials. Methyl 3-aminopropyl sulfide (MAPS) was prepared in 45% yield by decarboxylation of DL-methionine according to the method of Tutiya(12). The product, obtained as the free base, was a colorless liquid, b.p. 169-171° (730 mm), picrate, m.p. 125.5-126.5° (literature value, 126-127° (13)). β-Mercaptoethylamine hydrochloride and α,γ-diaminobutyric acid dihydrochloride were obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio, and BAPN fumarate (2 BAPN·1 fumaric acid) from Abbott Laboratories, North Chicago, Ill. These chemicals were used as supplied by the manufacturers.

Animals, diets, and procedures. Day-old Broad White or Broad Breasted Bronze turkey poults, divided into groups of 10 birds each, were kept in electrically heated, wire floored batteries during a 5-week experimental period. Feed and water were supplied *ad lib.* The basal diet was the practical starting ration described by Roy and Bird(14), without the added methionine. The birds were weighed and severity of leg deformities assessed at weekly intervals and all birds dying during the experiments were autopsied. Many of the deaths were caused by massive inter-

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TABLE I. Effect of β-Mercaptoethylamine Hydrochloride (MEA) and Other Compounds on Weight and Aortic Rupture in Turkeys.

Exp No.	Additions to basal diet	Avg wt 5 wk, g	Mortality, %*	Mortality due to hemorrhage, %*
1	None	902	10	0
	.10% MEA • HCl	991	0	0
	.20% MEA • HCl	801	30	10
	.04% IIH • PO_4	770	10	0
	.10% MEA • HCl + .04% IIH • PO_4	760	60	0
	.20% " + " "	602	40	30
2	None	946	0	0
	.02% BAPN • fumarate	915	10	10
	.20% MEA • HCl	760	0	0
	.04% IIH • PO_4	903	10	0
	.02% BAPN • fumarate + .04% IIH • PO_4		100	70
	.20% MEA • HCl + " "	765	60	40
7	None	827	0	0
	.02% BAPN • fumarate + .04% IIH • PO_4		100	60
	.185% MAPS + " "	796	20	0
	.37% " + " "	694	0	0
8	None	815	10	0
	.02% BAPN • fumarate	837	30	20
	" " " + .04% IIH • PO_4		90	70
	.20% MEA • HCl + " "	480	40	10
	.75% MAPS + " "	546	30	0
	.01% DABA • 2HCl	808	0	0
9	None	903	0	0
	.02% BAPN • fumarate	828	20	0
	" " " + .04% IIH • PO_4		100	90
	.20% MEA • HCl + " "		90	40
	.01% DABA • 2HCl + " "	696	0	0
	.02% " " + " "	819	10	0
Combined MEA data				
1, 2	.20% MEA • HCl	777	15	5
1, 2, 8, 9	" " + .04% IIH • PO_4	594	58	30

* % of original group.

nal hemorrhage due, in nearly every case, to aortic rupture (see below).

Results. The effect of feeding MEA to turkey poults is shown in Table I. A dietary level of 0.20% MEA • HCl, when fed alone, was of borderline toxicity as only 5% mortality by hemorrhage occurred (see Combined MEA Data, Table I). Including 0.04% IIH phosphate† in the ration markedly potentiated the toxicity of MEA, as evidenced by depressed growth and 30% incidence of aortic rupture. A level of 0.10% MEA • HCl produced no aortic ruptures even when fed together with IIH. These results with turkeys confirm the previous conclusion from rat data(5) that MEA is a lathyrogen.

Table I also shows that neither MAPS, which might arise by enzymatic decarboxylation of methionine, nor DABA produced the massive internal hemorrhages characteristic of BAPN toxicity in turkeys. Furthermore, DABA • 2HCl at 0.02% of the diet caused no noticeable neurological symptoms nor did the addition of IIH have any effect. It is difficult to compare these results with those of Ressler *et al.*(10) because of differences in the level and method of administering the compound, and because different species were studied.

The potentiating effect of certain additional compounds on the toxicity of BAPN in turkeys is shown in Table II. As found previously(8), IIH markedly increased mortality from massive internal hemorrhages. Furazolidone (3-(5-nitrofurfurylideneamino)-2-oxazolidinone),‡ a feed additive, and another

† IIH Phosphate (Marsilid Phosphate) was generously supplied by Hoffmann-LaRoche.

‡ Added to diet as an 11% Furazolidone mixture.

TABLE II. Effect of Several Drugs on BAPN Toxicity in Turkey Poults.

Exp No.	Additions to basal diet	Avg wt 5 wk, g	Mortality, %*	Mortality due to hemorrhage, %*
2	None	946	0	0
	.02% BAPN • fumarate	915	10	10
	.04% IIH • PO_4	903	10	0
	.03% Furazolidone	983	10	0
	.02% BAPN • fumarate + .04% IIH • PO_4		100	70
	" " " + .03% Furazolidone	848	50	50
13	None	1127	0	0
	.02% BAPN • fumarate	1030	20	10
	" " " + .03% Furazolidone	983	50	50
3	None	801	10	0
	.02% BAPN • fumarate	782	20	10
	" " " + .04% IIH • PO_4		100	80
	" " " + .01% Parnate • SO_4	648	30	30
	" " " + 1 ppm Reserpine	692	0	0
	" " " + 2 " "	654	0	0
	" " " + 4 " "	549	0	0
8	None	815	10	0
	.02% BAPN • fumarate	837	30	20
	" " " + .04% IIH • PO_4		90	70
	" " " + 8 ppm Reserpine	607	20	0
	.04% " "	643	80	60
	" " " + 4 ppm Reserpine	672	60	50

* *cf.* Table I.

TABLE III. Specificity of Toxicity of N-Substituted Derivatives of β-Aminopropionitrile and β-Mercaptoethylamine.

Exp No.	Additions to basal diet	Avg wt 5 wk, g	Mortality, %*	Mortality due to hemorrhage, %*
28†	None	738	20	0
	.079% BAPN • fumarate	401	80	70
	.123% Pantoyl-BAPN	768	10	0
	.246% " "	663	0	0
7	None	827	0	0
	.02% BAPN • fumarate	894	60	20
	" " " + .04% IIH • PO_4		100	60
	.026% Glycyl • BAPN + " "		90	50
	.029% Glutaryl • BAPN + " "	818	0	0
9	None	903	0	0
	.20% MEA • HCl + .04% IIH • PO_4		90	40
	.34% N-Octyl • MEA + " "	298	30	0

* *cf.* Table I.

† Exp 28 was conducted by Dr. D. N. Roy and Dr. S. H. Lipton.

drug, trans-2-phenylcyclopropylamine sulfate (Parnate sulfate),§ did likewise, but at the levels fed were less effective than IIH. Parnate sulfate has been reported to be a very potent *in vivo* MAO inhibitor as measured by the tryptamine potentiation test in rats(15).

Reserpine, a drug which has been reported to inhibit liver MAO *in vitro*(16), had no effect on the symptoms characteristic of BAPN toxicity when added at levels of 1, 2, 4, and 8 ppm to rations containing BAPN (Table II).

The data in Table III demonstrate that the turkey resembles the rat in ability to cleave N-acyl groups from BAPN(11). Thus N-glycyl-BAPN,‖ was as toxic as BAPN fu-

§ Generously supplied by Smith, Kline and French Laboratories.

‖ We thank Dr. S. H. Lipton and Mr. L. A. Ehrhart for N-acyl derivatives of BAPN.

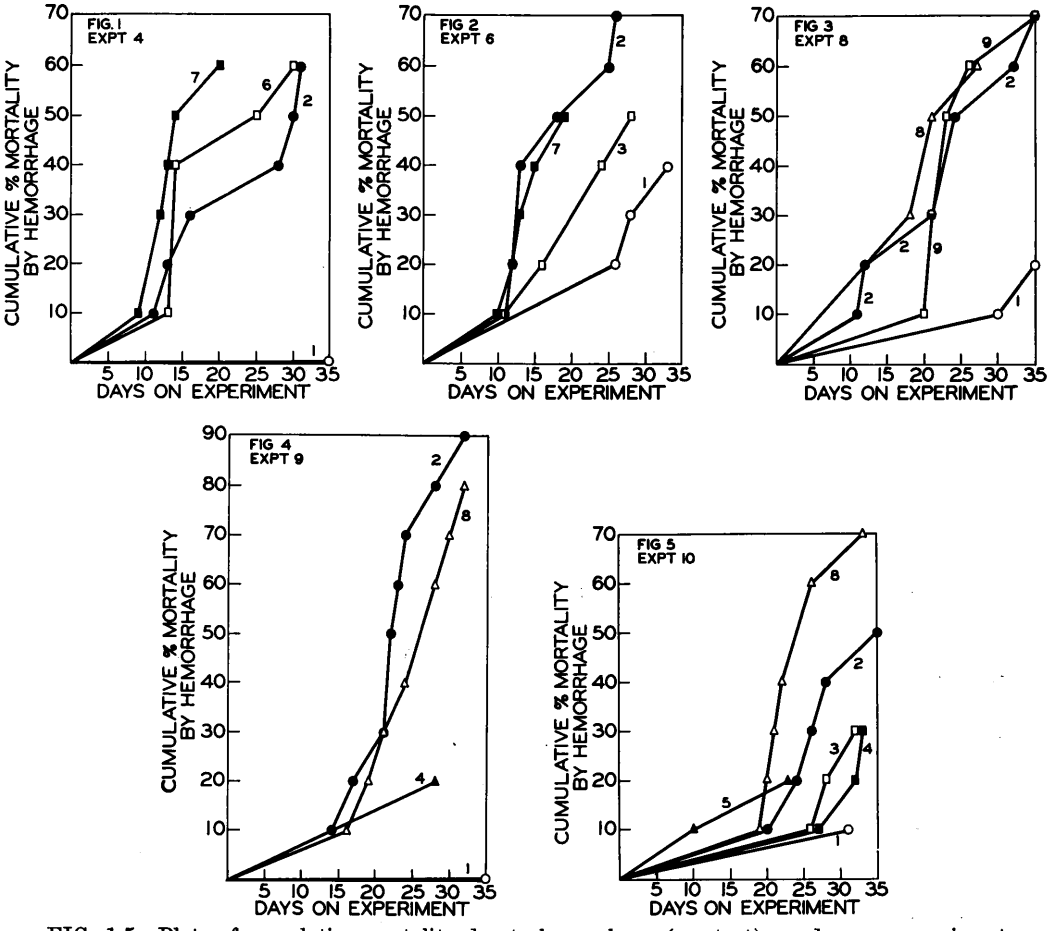

FIG. 1-5. Plots of cumulative mortality due to hemorrhage (see text) *vs.* days on experiment for 5 separate experiments. Results of each experiment are shown in separate figures. Since the same treatments were repeated in several experiments, each treatment is indicated in the several figures by the same number, as follows:

Curve 1—0.02% BAPN fumarate (Fig. 1, 2, 3, 4, 5)
" 2—0.02% BAPN fumarate + 0.04% IIH PO_4 (Fig. 1, 2, 3, 4, 5)
" 3—0.02% BAPN fumarate + 2% taurine (Fig. 2, 5)
" 4—0.02% BAPN fumarate + 3% L-lysine • HCl (Fig. 4, 5)
" 5—0.02% BAPN fumarate + 3% L-cystine (Fig. 5)
" 6—0.02% BAPN fumarate + 0.04% IIH PO_4 + 1% taurine (Fig. 1)
" 7—0.02% BAPN fumarate + 0.04% IIH PO_4 + 2% taurine (Fig. 1, 2)
" 8—0.02% BAPN fumarate + 0.04% IIH PO_4 + 3% L-lysine • HCl (Fig. 3, 4, 5)
" 9—0.02% BAPN fumarate + 0.04% IIH PO_4 + 3% L-cystine (Fig. 3)

Treatments 7 and 9 were included in Exp 10 and gave curves (not shown in Fig. 5) essentially identical to Curve 2.

marate whereas the N-pantoyl and N-glutaryl derivatives produced no aortic ruptures. A combination of IIH and the N-octyl derivative of MEA ($HSCH_2CH_2NH(CH_2)_7CH_3$)¶ likewise was nonlathyrogenic although it severely depressed growth. A molecularly equivalent level of MEA under the same conditions produced 40% mortality by hemorrhage (Table III).

Data from 5 experiments involving the feeding of taurine, cystine, and lysine to turkey poults together with BAPN or BAPN plus IIH are shown in Fig. 1-5. Adding these compounds to diets containing BAPN but no IIH (curves 3, 4, 5, Fig. 2, 4, 5) resulted in a slightly higher incidence of hemorrhages

¶ Generously supplied by Dr. D. P. Jacobus, Walter Reed Army Med. Center.

and occurrence of the hemorrhages at a much younger age. In 3 experiments no potentiating effect of taurine, cystine, and lysine was noted when both BAPN and IIH were present in the diets (compare curve 2 with curves 7, 8, or 9, Fig. 2, 3, 4). However, taurine in Expt. 4 (Fig. 1) and lysine in Exp. 10 (Fig. 5) did seem to be distinctly detrimental under these conditions. The additions of lysine, cystine, and taurine stimulated growth and this may account for their effects in potentiating BAPN toxicity. It has been reported (17) that fish meal, which promoted excellent growth, increased the incidence of hemorrhages in turkey poults.

In the present study, of 174 birds dying with massive internal hemorrhages 153 were the result of rupture of the abdominal aorta. Massive hemorrhages into the pericardial sac were responsible for 17 deaths and 4 birds died of extensive hemorrhages into the leg musculature.

Discussion. Our results suggest that spontaneous aortic rupture among turkeys in commercial flocks could be caused by particular combinations of dietary ingredients and substances that might be produced from them in the gut or in the body proper. Thus there was a distinct tendency for ruptures to be more frequent and to occur at an earlier age when a low level of BAPN was fed together with cystine, lysine or taurine than when it was fed alone (compare curve 1 with curves 3, 4 and 5 in the various Figures). Inclusion of an MAO inhibitor (IIH · PO_4) partly obscured this effect although it was still evident in some experiments (curve 2 *vs* curves 7, 8 and 9). Although it has not been possible to find BAPN in commercial turkey feed, the effective lathyrogen could well be MEA derived from cystine in the gut. Since decarboxylation of cystine is presumably accomplished by bacterial action, variations in the intestinal flora among individual flocks might explain the erratic occurrence of spontaneous rupture in the field.

Summary. A number of compounds were tested for lathyrogenic activity, while others were investigated for their ability to potentiate BAPN induced aortic rupture in turkeys. β-Mercaptoethylamine hydrochloride when fed together with 1-isonicotinyl-2-isopropyl hydrazine phosphate was toxic, causing massive internal hemorrhages, and slower growth. Diaminobutyric acid and methyl 3-aminopropyl sulfide induced no aortic lesions. N-Glycyl-BAPN induced characteristic BAPN toxicity symptoms while 2 other acyl derivatives, N-pantoyl-BAPN and N-glutaryl-BAPN, did not. Furazolidone and Parnate sulfate, when fed together with BAPN, markedly enhanced the toxicity symptoms induced by BAPN. Additions of 3% L-lysine monohydrochloride, 3% L-cystine, or 2% taurine also potentiated BAPN toxicity in young turkeys.

1. Bachhuber, T. E., Lalich, J. J., Schilling, E. D., Strong, F. M., *Fed. Proc.*, 1955, v14, 175.

2. Barnett, B. D., Bird, H. R., Lalich, J. J., Strong, F. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 67.

3. Roy, D. N., Lipton, S. H., Bird, H. R., Strong, F. M., *Poultry Sci.*, 1961, v40, 55.

4. Lalich, J. J., Barnett, B. D., Bird, H. R., *A.M.A. Arch. Path.*, 1957, v64, 643.

5. Dasler, W., Milliser, R. V., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 759.

6. Ponseti, I. V., Wawzonek, S., Shepard, R. S., Evans, T. C., Sterns, G., *ibid.*, 1956, v92, 366.

7. Roy, D. N., Ph.D. Thesis, Univ. of Wisconsin, 1960.

8. Lipton, S. H., Lalich, J. J., Strong, F. M., *J. Am. Chem. Soc.*, 1958, v80, 2022.

9. Roy, D. N., Lipton, S. H., Strong, F. M., Bird, H. R., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 767.

10. Ressler, C., Redstone, P. A., Erenberg, R. H., *Science,* 1961, v134, 188.

11. Ehrhart, L. A., Lipton, S. H., Strong, F. M., *Biochemistry*, 1963, v2, 300.

12. Tutiya, Y., *J. Agr. Chem. Soc. Japan,* 1941, v17, 619.

13. Beilstein, F. K., *Handbuch der organischen Chemie,* 4 ed., Julius Springer Verlag, Berlin, 1929, v4, p434.

14. Roy, D. N., Bird, H. R., *Poultry Sci.*, 1959, v38, 192.

15. Tedeschi, D. H., Tedeschi, R. E., Fellows, E. J., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 680.

16. Bose, B. C., Vijayvargiya, R., *J. Pharm. and Pharmacol.*, 1960, v12, 99.

17. Waibel, P. E., Pomeroy, B. S., *J. Nutrition,* 1959, v67, 275.

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