Toxicity and Metabolism of 2-Cyanopropylamine in Rats, Chicks, and Turkeys.* (25490)

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Administration of beta aminopropionitrile (BAPN) to rats produces skeletal deformities and changes in walls of blood vessels resulting in internal hemorrhage and death(1-5). Similar toxic symptoms were produced in young turkeys(6) and in chicks(7) at lower levels of BAPN. Cyanoacetic acid was isolated from urine of rats fed BAPN(8). Our object was to study in turkeys, chicks, and rats the toxicity and metabolism of 2-cyanopropylamine (CPA), $NH_2CH_2CH(CH_3)CN$, a methyl analog of BAPN.

Methods and materials. 2-Cyanopropylamine was prepared by procedure of Dickey (9) in which concentrated aqueous ammonia was reacted with methacrylonitrile† in a rocking hydrogenation bomb for 2 hours at 135°C. The product was purified by distillation and obtained as colorless liquid, b.p. 75-76° (16 mm). Anal. Calcd. for $C_4H_8N_2$: N, 33.17. Found: N, 33.21. BAPN was used as neutral fumarate salt (2 BAPN · 1 fumaric acid).‡ 2-Cyanopropionic acid was synthesized by reacting beta-bromopropionic acid with sodium cyanide according to procedure of Hoffman and Barbier(10). *Animal diets and procedures.* The basal diet of Barnett *et al.*(6) was used in chick and turkey experiments, except that dried skimmilk replaced dried whey. Each group contained 10 birds, housed in heated batteries. Experimental diets and water were given *ad lib.* from first day after hatching. Birds were weighed at weekly intervals and severity of symptoms recorded by assigning a numerical score to leg deformities at each weighing. Gross autopsies§ were performed on animals which died during experiment. Day-old straight run, cross-bred (New Hampshire male X Single Comb White Leghorn female) chicks were used in Exp. 18. In Exp. 19 and 20 Beltsville Small White turkeys were used and the 2 experiments run for 5 weeks each. In the rat exp. 6 weanling male albino rats (Holtzman strain) were used. Rats had access to food for 8-9 hours each day and were kept in metabolism cages for collection of urine the rest of the time. Water was available at all times. Animals were fed a stock diet having the following composition in percent: ground yellow corn 28, ground wheat 28, powdered milk 11.2, soybean oil meal 9.3, linseed oil meal 9.3, alfalfa meal 7.5, butter 4.7, and NaCl 1.9. This diet has been used routinely as stock diet for rats in the Dept. of Biochemistry. Controlled feeding was continued for first 21 days of experiment after which food and water were given *ad lib.* for additional 14 days. At termination of experiment, 2 animals from each group were X-rayed.‖ All animals were then killed and gross autopsy observations made. Urine of rats from the 4 groups was collected under toluene for 21 days and stored in deep freeze cabinet until used. *Paper chromatography of rat urines.* Aliquots of approximately 20 μl of urine were spotted directly on Whatman No. 1 paper. The chromatograms were developed by an ascending technic with butanol-acetic acid-water (120:30:50)(11). After drying, amines and amino acids were detected by ninhydrin spray. Duplicate chromatograms were sprayed with 0.1% 2,6-dichlorophenolindophenol in alcohol(12) for detection of acidic components. *Isolation of cyanopropionic acid from rat urine.* Ninety ml of urine collected from rats on 0.48% CPA diet (group 3, Table III) were acidified with 2.5 ml of concentrated sulfuric acid and clarified by centrifuging. The clear acidified superna-

* Published with approval of Director of Wisconsin Agr. Exp. Station. Supported by Research Comm. of Graduate School from funds of Wisconsin Alumni Research Fn., Madison, and N.I.H., U.S.P.H.S.

† Generously supplied by Tennessee Eastman Co.

‡ Generously supplied by Abbott Labs.

§ We thank Dr. J. J. Lalich for autopsy results.

‖ We thank Dr. R. F. Douglas for X-ray pictures of the rats.

tant was extracted 6 times with 50 ml of ethyl acetate. Emulsions formed during extraction, were broken by centrifuging. The ethyl acetate extract was then washed 4 times in separatory funnel with small volumes of distilled water and the aqueous washings discarded. The clear ethyl acetate extract was evaporated at room temperature and the residue subjected to molecular distillation for 1 hour at 5 x 10^{-4} mm and 80-100°. The distillate was collected as an oily film on surface of condenser tube cooled with dry ice freezing mixture. Distilled material was washed from condenser with small volumes of ethyl acetate and the solution evaporated to about 2 ml. This concentrate was then examined by paper chromatography with butanol-acetic acid-water system(11) and with a solvent system composed of n-butanol saturated with 1.5 N ammonia(13). Presence of 2-cyanopropionic acid in the distillate was strongly indicated by appearance of paper chromatograms, and was confirmed by preparation of a crystalline cyclohexylamine salt. For this purpose the distillate was evaporated almost to dryness, the residue taken up in 2 to 3 ml of acetone and the acetone solution neutralized with cyclohexylamine. The mixture was cooled and the crystalline precipitate filtered off and recrystallized from acetone. Thirty mg of colorless needle crystals, m.p. 154-156.5 were obtained. Anal. Calcd. for $C_{10}H_{18}O_2N_2$: N, 14.13. Found: N, 14.36. For comparison, a sample of the same salt was prepared from synthetic DL-2-cyanopropionic acid and obtained as colorless needle crystals, m.p. 152-154.5. Anal. Calcd. for $C_{10}H_{18}O_2N_2$: C, 60.6; H, 9.15; N, 14.13. Found: C, 60.01; H, 8.68; N, 13.45. A mixed melting point of isolated and synthetic salts was 154-156°C. Infrared tracings on isolated and synthetic samples, taken in a potassium bromide pellet, were essentially indistinguishable.

TABLE I. Effects of BAPN Fumarate and 2-Cyanopropylamine (CPA) on Weight, Leg Deformity and Mortality of Chicks.

Additions to basal diet, %				
CPA	BAPN fumarate	Avg wt 4 wk, g	Leg score*	Mortality, %
.0	.0	282	5.	0
.0	.079	151	3.7	70
.036	"	85	1.	80
"	.0	288	5.	0
.072	.0	241	3.9	0
.108	.0	257	3.4	0

* Leg scores (avg of surviving birds): 1. Both legs severely bent; joints swollen. 2. One leg slightly, one severely bent; joints swollen. 3. Both legs slightly bent; joints swollen. 4. One leg normal; other slightly affected. 5. Normal.

Results. Animal experiments. Table I summarizes results of Exp. 18 on chicks. A level of 0.036% of CPA alone was not toxic but when mixed with 0.079% of BAPN fumarate, the mixture was appreciably more toxic than this level of BAPN alone. As percent of CPA was increased there was slight depression of growth rate, and severity of leg deformities increased. Table II shows results of Exp. 19 and 20. The symptoms produced by combination of CPA and BAPN were more severe in turkeys than in chicks, as all 10 poults died in the third week. As in Exp. 18, higher levels of CPA led to suppression of growth and increase in severity of leg and toe deformities. Exp. 20 (Table II) showed that 0.216% of CPA was at least as toxic as 0.079% of BAPN fumarate as judged by weight and by leg deformities. A level of 0.079% of BAPN fumarate is equivalent to 0.052% of CPA on a molar basis. It is clear that the "diluting effect" of added methyl group in CPA accounts for only a small part of the difference in toxicity between CPA and BAPN. Table III shows that growth and survival rate in rats were progressively reduced by increasing CPA levels. In all cases death resulted from aortic rupture. An additional toxic symptom observed in all animals on CPA was male priapism. Definite lathyritic deformities were not revealed by X-ray pictures of rats. Autopsy examination of surviving rats on 0.24% CPA revealed no significant difference from controls, although one rat died of aortic rupture during experiment. Of the 4 surviving animals on 0.48% CPA, one showed hemorrhage in area of aortic arch, normal aortae being present in the other three. Femurs, sterna, and vertebral columns were not obviously lathyritic.

Urinary metabolites. When the 4 unfractionated rat urines corresponding to groups shown in Table III were chromatographed on

TABLE II. Effects of BAPN Fumarate and 2-Cyanopropylamine (CPA) on Weight, Leg Deformity, Mortality and Massive Internal Hemorrhage in Beltsville Small White Turkeys.

Exp. No.	Additions to basal diet, % CPA	BAPN fumarate	Avg wt 5 wk, g	Leg score*	Mortality, %	Hemorrhage, % of original group
19	.0	.0	713	5.	0	0
	.0	.079	447	3.	90	90
	.036	"			100	30
	"	.0	651	4.7	0	0
	.072	.0	697	4.7	10	0
	.108	.0	587	3.9	10	0
20	.0	.0	510	5.	0	0
	.0	.079	433	3.	80	60
	.216	.0	316	1.	70	50

* Avg of surviving birds.

paper and the papers sprayed with ninhydrin, a purple zone with R_f approximately 0.56 was observed only in urines of Groups 2 and 3 and the zone was more pronounced in Group 3 than in Group 2. This zone was completely absent in both control and BAPN groups. Since known CPA gave an identical color with ninhydrin and showed the same R_f on paper chromatograms, it was concluded that unchanged CPA was present in above urines. Group 4 urine showed a green ninhydrin spot due to unchanged BAPN(8). Duplicate chromatograms of urines from Groups 2 and 3, when sprayed with diazotized sulfanilic acid (8), showed no orange spot characteristic of cyanoacetic acid.

If CPA were metabolized similar to BAPN (8), one would expect to find 2-cyanopropionic acid in the urine. The isolation procedure, patterned after that used for cyanoacetic acid (8), gave a concentrate which when examined on paper chromatograms displayed an acid zone at an R_f of about 0.77 in the butanol: acetic acid:water system and at 0.19 in the butanol - 1.5 N ammonia system. Identical R_f values were observed for synthetic 2-cyanopropionic acid in the corresponding solvent systems. Presence of this metabolite in urine was then confirmed by isolation of the crystalline cyclohexylamine salt which proved to be identical with an authentic sample. Metabolism of amino and alkyl nitriles was discussed by Merkow *et al.*(14). Following the same reasoning, one would expect that oxidation of the amino group of CPA to an aldehyde group would be catalysed by monoamine oxidase, and that the aldehyde group then would be oxidized to - COOH. No attempt was made to determine optical configuration of urinary 2-cyanopropionic acid or CPA, but this might be of interest as the CPA fed was a racemic mixture and its optical forms might be metabolized to unequal degrees.

TABLE III. Effect of 2-Cyanopropylamine (CPA) and BAPN Fumarate on Weight, Mortality, and Massive Internal Hemorrhage in Rats.

Group No.	Diets	Avg wt 5 wk, g	Mortality, %	Hemorrhage, % of original group
1	Basal only	162	0	0
2	Basal + .24% CPA	145	17	17
3	+ .48% "	101	33	33
4	+ .36% BAPN fumarate	127	50	50

Summary. Lathyrogenic activity of 2-cyanopropylamine (CPA), a methyl analog of BAPN, was studied in chicks, turkey poults and weanling rats. Dissecting aneurisms of the aortae resulted in all 3 species. Although characteristic deformities of leg bones appeared in chicks and turkeys, lathyritic osseous changes were not produced in rats. A toxic symptom produced in all rats by CPA was male priapism. A level of 0.216% CPA in the diet of turkey poults was at least as toxic as 0.079% of BAPN-fumarate. CPA was excreted in urine of rats as unchanged CPA and as 2-cyanopropionic acid.

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Received September 22, 1959. P.S.E.B.M., 1960, v103.

Influence of Changes in Serum Phosphorus and Creatinine on Respective Concentration in Pancreatic Juice. (25491)

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Few studies have been devoted to the relation between chemical composition of plasma and of secretions of the gastrointestinal tract. This report concerns the influence of changes in plasma content of inorganic phosphorus and creatinine upon their respective concentrations in pancreatic juice. Both elements can be elevated artificially to a high level in blood without danger for the laboratory animal. Ball(1) has shown that calcium, phosphorus and magnesium in pancreatic secretion are much lower than in serum (¼ to 1/20th). Normally, inorganic phosphorus concentration in pancreatic juice equals 1/20 of its plasma value. When plasma phosphorus concentration is raised to 6 times greater than normal, there is a 12 fold increase in concentration of this ion in the pancreatic secretion, this being 1/10 of plasma value.

Technic. Experiments were performed on dogs (20 kg) with duodenal fistula. A modification of the Thomas(2) method was used, by which a fine glass cannula may be introduced to collect pure pancreatic juice. In fasted dogs, amount of juice so collected is minimal; it increases when secretin is injected intravenously. To establish a constant rate of secretion, secretin (Boots) was injected at constant rate with syringe for continuous injection. Rate of pancreatic juice secretion was adjusted betwen 1 and 1.5 cc/min. This was considered sufficient to avoid errors due to pancreatic dead-space. Extreme limits for each individual pancreatic juice collection period were 0.54 cc and 2.6 cc/min. The first 10 cc of pancreatic juice were discarded. Each experiment consisted in measuring output and concentration of inorganic phosphorus (Exp. 1-4) or creatinine (Exp. 2-4) in pancreatic juice at various levels of plasma inorganic phosphorus or creatinine. In Exp. 1 and 2, phosphorus excretion was measured at normal level of serum phosphorus and at various degrees of increased phosphorus levels, in Exp. 3 and 4 only at various levels of hyperphosphoremia. Increased serum phosphorus was induced by injection of buffered solution of inorganic phosphates at first in concentrated, then in diluted form. Rate of injection was such as to obtain levels as constant as possible. In Exp. 2-4, creatinine was added to the liquid of perfusion. Each collection period lasted 4 to 10 minutes. Blood samples were taken at brief and regular intervals throughout experiment. After each induced change in blood level of phosphorus and creatinine, the new pancreatic collection was begun only after at least 5 cc of