with that of authentic L-homoarginine monoflavianate, the decomposition of the base to lysine on alkaline hydrolysis, and the observed $[M]_D$ value of +42.4° would be consistent with the formulation of the compound as L-homoarginine.

In this connection it is interesting to point out that, while homoarginine is present in several species of *Lathyrus* (Bell, 1962a), *L. tingitanus* is distinguished in that it contains lathyrine (Bell and Foster, 1962). It is tempting to speculate that the biosynthesis of lathyrine in the above species is from homoarginine and that it proceeds via γ-hydroxy homoarginine. Should this be so, the presence of γ-hydroxy homoarginine and its lactone in *L. tingitanus* may be anticipated. The natural occurrence of the lower homolog, γ-hydroxyarginine, has recently been recognized (Makisuma, 1961). Since the present report was communicated, Bell (1962c) has published details on the isolation of L-homoarginine from *L. cicera*, and he has also suggested that L-homoarginine may be the natural precursor to lathyrine, which is formed by cyclization and dehydration, and that the difference between plants which contain lathyrine and those containing only homoarginine is the presence or absence of an enzyme system capable of bringing about this transformation.

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Preparation, Metabolism, and Toxicity of Certain Acyl Derivatives of β-Aminopropionitrile*

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Several N-acyl derivatives of β-aminopropionitrile were synthesized and tested in rats for their ability to produce osteolathyrism. Only those derivatives which were cleaved *in vivo*, as evidenced by the appearance of cyanoacetic acid and free β-aminopropionitrile in the urine, were effective as lathyrogens. The γ-glutamyl (whether L-, D-, or DL-), glycyl, L-leucyl, and DL-phenylalanyl derivatives were active, whereas the acetyl, succinyl, glutaryl, β-alanyl, DL-pantoyl, and 4-hydroxybutyryl derivatives were not. The results point to an absolute requirement for a free amino group for osteolathyrism production and are consistent with the hypothesis that β-aminopropionitrile acts by direct binding through its amino group to some structural feature of soluble collagen (tropocollagen) which must be free to allow the formation of highly polymerized, mature collagen fibers.

The connective tissue damage and metabolic disturbances produced by β-aminopropionitrile have been widely studied since 1955, when it was identified as the active toxic principle of *Lathyrus odoratus* (Strong, 1956; Schilling and Strong, 1955). In *L. odoratus* seeds the compound occurs combined with glutamic acid as the N-(γ-L-glutamyl) derivative, which elicits the same symptoms as β-aminopropionitrile when fed to rats. However, it was found that the N-acetyl derivative of β-aminopropionitrile is nontoxic (Bachhuber *et al.*, 1955). In the present study, a number of other N-acyl derivatives of β-aminopropionitrile have been synthesized and administered to rats, both by feeding and by intraperitoneal injection, in order to determine whether they would produce the characteristic symptoms of β-aminopropionitrile toxicity and whether they would give rise to urinary excretion of free β-aminopropionitrile and cyanoacetic acid (Lipton *et al.*, 1958) as does the N-(γ-L-glutamyl) compound.

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EXPERIMENTAL

Preparation of N-Acyl β-Aminopropionitrile Derivatives ($RCONHCH_2CH_2CN$).—Data on the compounds synthesized are summarized in Table I. Synthetic methods employed were derived from previously described procedures, with modifications as given in footnotes to the table. The L and DL forms of N-γ-glutamyl-β-aminopropionitrile were prepared as previously described (Schilling and Strong, 1955).

Method A (Anhydride).—The reaction of an acid anhydride with an equimolar amount of free β-aminopropionitrile (Buc, 1947) was run in dioxane solution at 5° as previously described (Schilling and Strong, 1955) unless otherwise noted. The products of these very exothermic reactions separated either by direct crystallization or after the addition of ether.

Method B (Lactone).—The reaction of equimolar amounts of a lactone and β-aminopropionitrile was carried out by heating the reactants together with or without a solvent.

Method C (Acyl Chloride).—The reaction of an excess of free β-aminopropionitrile with phthalimidoacyl chlorides corresponding to the amino acids glycine, β-

TABLE I

PROPERTIES OF N-ACYL-β-AMINOPROPIONITRILE DERIVATIVES

$RCONHCH_2CH_2CN$

Name of Parent Acid	R—	Formula	M.p. (°C)	Analytical Data: Carbon (%) Calcd.	Carbon (%) Found	Hydrogen (%) Calcd.	Hydrogen (%) Found	Nitrogen (%) Calcd.	Nitrogen (%) Found	Yield (%)	Method of Preparation	Footnote Ref.
Acetic	CH_3—	$C_5H_8N_2O$	65–67.5	—	—	—	—	—	—	88	A	*a*
Succinic	$HOOCCH_2CH_2$—	$C_7H_{10}N_2O_3$	125–128	49.39	49.64	5.92	6.15	16.46	16.42	75	A	*b*
Glutaric	$HOOCCH_2CH_2CH_2$—	$C_8H_{12}N_2O_3$	77–78.5	52.17	51.96	6.57	6.66	15.22	15.17	69	A	*c*
4-Hydroxybutyric	$HOCH_2CH_2CH_2$—	$C_7H_{12}N_2O_2$	61–63	—	—	—	—	17.94	17.72	52	B	*d*
DL-Pantoic	$HOCH_2C(CH_3)_2CH(OH)$—	$C_9H_{16}N_2O_3$	77–79	—	—	—	—	13.99	13.92	57	B	*e*
L-2-Hydroxyglutaric	$NHCH_2CH_2CN$ | $COCH(OH)CH_2CH_2$—	$C_{11}H_{16}N_4O_3$	157–159	52.37	52.40	6.39	6.38	22.21	22.09	44	B	*f, g*
N-Phthaloylglycine	$C_6H_4(CO)_2NCH_2$—	$C_{13}H_{11}N_3O_3$	191–192.5	60.69	60.91	4.31	4.77	16.34	16.12	72	C	*h*
N-Phthaloyl-β-alanine	$C_6H_4(CO)_2NCH_2CH_2$—	$C_{14}H_{13}N_3O_3$	165–167	61.96	61.79	4.83	5.30	15.49	15.19	96	C	*i*
N-Phthaloyl-L-leucine	$C_6H_4(CO)_2NCH$— | $(CH_3)_2CHCH_2$	$C_{17}H_{19}N_3O_3$	111–112	65.18	65.44	6.10	6.11	13.41	13.41	63	C	*j*
N-Phthaloyl-DL-phenylalanine	$C_6H_4(CO)_2NCH$— | $C_6H_5CH_2$	$C_{20}H_{17}N_3O_3$	162–164	—	—	—	—	12.10	11.97	87	C	*k*
N-Phthaloyl-DL-glutamic	$C_6H_4(CO)_2NCHCH_2CH_2$— | $COOH$	$C_{16}H_{15}N_3O_6$	186–188	58.35	58.29	4.59	4.61	12.76	12.85	97	A	*l*
Glycine·HCl	$HCl{\cdot}H_2NCH_2$—	$C_5H_{10}N_2OCl$	148–150	36.71	36.21	6.16	6.33	25.68	25.16	62	D	*m*
β-Alanine·HCl	$HCl{\cdot}H_2NCH_2CH_2$—	$C_6H_{12}N_2OCl$	165–167.5	40.57	40.68	6.81	7.03	23.65	23.33	59	D	*n*
L-Leucine	$(CH_3)_2CHCH_2CH(NH_2)$—	$C_9H_{17}N_2O$	—	—	—	—	—	22.92	22.80	88	D	*o*
γ-D-Glutamic	$HOOCCH(NH_2)CH_2CH_2$—	$C_5H_{12}N_2O_3$	223–225	—	—	—	—	21.10	20.78	—	D	*p, q*
DL-Phenylalanine·HCl	$HCl{\cdot}H_2NCH$— | $C_6H_5CH_2$	$C_{12}H_{16}N_2OCl$	203–206	56.80	56.70	6.36	6.29	16.56	16.02	82	D	*r*

[a] See British patent 642,409, Sept. 6, 1950, for preparative procedure. The reaction was run in acetone at 0°. Yield calculated on β-aminopropionitrile used. All other yields based on amounts of acid chloride or acid anhydride used. All synthetic products were purified until homogeneous as judged by paper chromatography carried out as described in the text. [b] Recrystallized from acetone. [c] Recrystallized from ethyl acetate. [d] The reaction was run at 108° for 90 minutes. The product was extracted into and recrystallized from ether. [e] The reaction was run in ether by the procedure of Shive and Snell (1945), who reported m.p. 66–68°. [f] Equimolar amounts of β-aminopropionitrile and L-4-carboxy-4-butyrolactone were mixed, held 30 minutes at 145°, and cooled to room temperature, and a second mole of β-aminopropionitrile was added with stirring. The solid product which slowly formed was recrystallized from methanol. Observed $[\alpha]^{26}D = -21.5°$ (*c* 1, water). Reaction of 2 moles β-aminopropionitrile with 1 of the lactone at 145° or 90° gave only polymeric material. A crystalline salt (m.p. 108–110°) of β-aminopropionitrile and L-4-carboxy-4-butyrolactone was obtained when equimolar amounts were dissolved in dioxane at room temperature. Calcd. for $C_8H_{12}N_2O_4$: N, 13.99. Found: N, 13.70. [g] The L-4-carboxy-4-butyrolactone (Heilbron, 1953) was prepared by deamination of L-glutamic acid with excess sodium nitrite in aqueous acetic acid. The product (m.p. 72–73°) was extracted from the reaction mixture at *p*H 1 with ether and recrystallized from ether. [h] The product was isolated by the addition of 1 equivalent of cold pyridine to the reaction mixture followed by 8 volumes of ice-cold water. Recrystallization was from ethanol. [i] The crude product obtained from the reaction mixture by precipitation with ether was thoroughly washed with water to remove salt impurities prior to recrystallization from ethanol. [j] The molar ratio of β-aminopropionitrile to N-phthaloyl-L-leucine was 4:3. The addition of 2 volumes of ether to the reaction mixture in dioxane resulted in a precipitation of salts, which were removed by filtration. The desired product was obtained by the further addition of 3 volumes of petroleum ether (Skellysolve B). Recrystallization was from ethanol. [k] The molar ratio of β-aminopropionitrile to N-phthaloyl-phenylalanyl chloride was 2:1. [l] The DL isomer crystallized directly from the dioxane solution without addition of ether. All attempts to obtain crystalline D isomer or L isomer failed. [m] Halogen analysis. Calcd.: 21.67; Found: 21.69. [n] Halogen analysis. Calcd.: 19.96; Found: 20.23. [o] The HCl salt of N-L-leucyl-β-aminopropionitrile could not be crystallized. It was converted into the free base by dissolving the salt in water and passing the solution through a column of Dowex-1, OH^- form resin. After the solution was lyophilized and the sirup was dried *in vacuo*, the free base was obtained as an amorphous glass. [p] Cleavage was as described by Schilling and Strong (1955). For D isomer observed $[\alpha]^{24}D = -9.65$ (*c* 2, water). Observed $[\alpha]^{24}D = +9.05$ (*c* 2, water) for the L isomer. [q] The infrared spectrum of the D isomer observed in KBr pellet was practically identical to that of the L isomer. However, a spectrum of the DL mixture, similarly obtained, differed considerably. A peak of 6.55 μ present in the spectra of both the D and L isomers was absent in the tracing from the racemate. [r] In order to free the product of trace impurities visible on paper chromatograms, several recrystallizations from ethanol were necessary.

alanine, L-leucine, and DL-phenylalanine was run as in Method A in dioxane solution at 5°. Products were isolated by the addition of appropriate solvents to the reaction mixture. Recrystallization was from ethanol. The required phthalimidoacyl chlorides were prepared as described by Sheehan *et al.* (1952).

Method D (Hydrazinolysis).—Cleavage of phthaloyl groups from the phthalimidoacyl-β-aminopropionitrile derivatives was carried out either by refluxing with one equivalent of hydrazine in alcohol solution (Sheehan *et al.*, 1952) or by use of aqueous hydrazine at 25° (Schilling and Strong, 1955).

Injection of Test Compounds.—Male albino rats of the Holtzman strain weighing approximately 200 g were used. The compounds to be tested were dissolved in water, and the solutions were adjusted to *p*H 7 by addition of sodium bicarbonate or acetic acid as needed and diluted to a concentration of 0.30 mmoles per ml. A single 1-ml dose was then administered to each of two rats by intraperitoneal injections.

The two animals of each group were then placed in individual stainless steel metabolism cages for urine collection. Food was withheld but water was supplied during the collection period of 16–18 hours. The urine, collected under toluene, was kept frozen until paper chromatography or other analyses were performed. Each injection experiment was repeated at least once. The results of the injection experiments are collected in Table II.

Feeding of Test Compounds.—Weanling male rats (six per group) of the Holtzman strain were fed the previously described rat stock diet (Roy *et al.*, 1960). Test compounds were incorporated into the stock diet at the levels indicated in Table III. Food and water were provided *ad libitum* during the 5-week experimental period. After 3 weeks of the diet, urine samples were obtained from the various groups by transferring the rats to metabolism cages for an overnight period of 16–18 hours. Three separate overnight urine samples were taken from each group for chromatographic study and other analyses. Weight gains, mortality, skeletal deformities, and urinary excretion of β-aminopropionitrile and cyanoacetic acid were noted, and are presented in Table III.

TABLE II

EXCRETION OF FREE β-AMINOPROPIONITRILE (BAPN) AND CYANOACETIC ACID (CAA) IN URINE AFTER THE INJECTION OF N-ACYL-β-AMINOPROPIONITRILE DERIVATIVES INTO RATS

Compounds Giving Rise to Urinary BAPN and CAA[a]	Compounds Not Giving Rise to Urinary BAPN and CAA
BAPN	N-Acetyl-BAPN
N-γ-L-Glutamyl-BAPN	N-DL-Pantoyl-BAPN
N-γ-DL-Glutamyl-BAPN	N-Succinyl-BAPN
N-γ-D-Glutamyl-BAPN[b]	N-Glutaryl-BAPN
N-Glycyl-BAPN	N-4-Hydroxybutyryl-BAPN
N-L-Leucyl-BAPN	N,N'-*bis*-(β-cyanoethyl)-L-2-hydroxyglutaramide
N-DL-Phenylalanyl-BAPN	N-β-Alanyl-BAPN

[a] Urinary excretion of free BAPN and CAA was considered positive if zones corresponding to these compounds were detectable on paper chromatograms. [b] Ambiguous results were obtained with N-γ-D-glutamyl-BAPN. In three injection experiments CAA was detected each time but free BAPN only once.

Analytical Methods.—Paper chromatography of the rat urine samples was run by an ascending technique. The solvent systems employed were: (1) butanol–acetic acid–water (120:30:50 by volume) (Smith, 1960) and (2) methylethylketone–propionic acid–water (75:25:30 by volume) (Clayton and Strong, 1954). The urines were spotted directly on Whatman No. 1 paper, with approximately 20 μl of urine at each position. Each series of samples was spotted in a repeating sequence several times on the same sheet of paper. After developing and drying, the chromatograms were cut so that the individual series of samples could be sprayed with different reagents. β-Aminopropionitrile and amino acids were detected by spraying with 0.1% ninhydrin in acetone, while cyanoacetic acid was detected with diazotized sulfanilic acid (Lipton *et al.*, 1958).

Analysis of Urine for Pantothenic Acid.—Urine samples collected during 16–18 hours from rats either fed or injected with N-pantoyl-β-aminopropionitrile were acidified and extracted with chloroform prior to microbiological assay[1] with *Lactobacillus arabinosus*. This treatment removed unchanged N-pantoyl-β-aminopropionitrile (confirmed by infrared spectrum, 4.45 μ C≡N band) from the urine and thus avoided possible inhibitory interference. Control rats received injections of pantothenic acid. Table IV presents the results of these assays. Intact pantoyl-β-aminopropionitrile in the urine could be hydrolyzed by mixing the urine with an equal volume of concentrated ammonium hydroxide and allowing the mixture to stand at room temperature overnight. Free β-aminopropionitrile was then detectable chromatographically.

Stability of Acyl-β-aminopropionitrile Compounds to Hydrolysis.—Stability of the N-acetyl, N-pantoyl, and N-γ-L-glutamyl derivatives was determined by allowing approximately 1% solutions of these compounds in 0.1 N hydrochloric acid, 0.1 N sodium hydroxide, or concentrated ammonium hydroxide to stand at room temperature. After a period of 16 hours the solutions were spotted on paper chromatograms. Splitting was determined by the appearance of free β-aminopropionitrile on the chromatograms. Zero time controls were run to be certain that the N-acyl-β-aminopropionitrile derivatives contained no free β-aminopropionitrile. Results of the stability studies are given in Table V.

DISCUSSION

N-DL-Pantoyl-β-aminopropionitrile.—The structural relationship of β-aminopropionitrile to β-alanine prompted a study of the toxicity of N-DL-pantoyl-β-aminopropionitrile. This structural analog of pantothenic acid proved to be nonlathyrogenic when fed to rats (Table III), and after its injection (Table II) urinary free β-aminopropionitrile and cyanoacetic acid could not be detected. Earlier, Bachhuber *et al.* (1955) had observed that N-acetyl and N-methyl derivatives of β-aminopropionitrile were nontoxic. Liberation of free β-aminopropionitrile from the derivative appeared to be necessary for toxicity, and was readily determinable by examination for urinary β-aminopropionitrile and cyanoacetic acid.

The toxicity of the naturally occurring γ-L-glutamyl derivative was considered during early phases of the work to reflect the ease with which this compound was hydrolyzed, perhaps nonenzymatically in the stomach. A reexamination of this derivative in regard to both its chemical stability and its toxicity and mode of excretion has been conducted, with a direct comparison of its behavior with that of the N-DL-pantoyl and N-acetyl derivatives. In Table V the relatively unstable chemical nature of N-DL-pantoyl-β-aminopropionitrile as

[1] The authors wish to thank Mr. Robert Prier and the Wisconsin Alumni Research Foundation for carrying out these microbiological assays.

TABLE III
EFFECTS OF FEEDING N-ACYL-β-AMINOPROPIONITRILE (BAPN) DERIVATIVES TO RATS

Expt.	Compound Fed	Level (mM/kg of diet)	Av. Wt. at 5 Wk. (g)	No. of Survivors at 5 Wk.[a]	Skeletal Deformities[b]	Urinary BAPN and CAA[c]
A	Stock diet	—	215	6	−	−
	BAPN[d]	20	110	1[e]	+	+
	DL-Pantoyl-BAPN	20	200	6	−	−
B	Stock diet	—	240	6	−	−
	BAPN	10	160	3	+	+
	BAPN	20	130	2[e]	+	+
	Acetyl-BAPN	20	260	6	−	−
	Succinyl-BAPN	20	250	6	−	−
	Glutaryl-BAPN	20	260	6	−	−
C	Stock diet	—	240	6	−	−
	BAPN	10	180	1[f]	+	+
	Glycyl-BAPN·HCl	20	150	2[g]	+	+
	4-Hydroxybutyryl-BAPN	20	240	6	−	−
	N,N′-*bis*-(β-cyanoethyl)-L-2-hydroxyglutaramide	10	240	6	−	−
	BAPN tetramer[h]	5	245	6	−	−
D	Stock diet	—	250	6	−	−
	BAPN	10	155	3	+	+
	Glycyl-BAPN·HCl	10	150	4	+	+
	L-Leucyl-BAPN	10	155	5	+	+
	DL-Phenylalanyl-BAPN·HCl	20	—	0[i]	+	+
E	Stock diet	—	220	6	−	−
	γ-D-Glutamyl-BAPN	10	210	5	+	+
	γ-DL-Glutamyl-BAPN	10	205	5	+	+
	γ-L-Glutamyl-BAPN	10	190	3	+	+
	BAPN	10	145	3	+	+

[a] Remaining from 6 rats started per group. Death was usually the result of aortic rupture. [b] Skeletal deformities characteristic of lathyrism are described by Strong (1956). [c] Detected on paper chromatograms as described in the text. CAA = cyanoacetic acid. [d] The BAPN was the neutral fumaric acid salt (2 BAPN·1 fumaric acid). This was kindly supplied by Abbott Laboratories. BAPN levels are stated as mM of BAPN rather than mM of salt. [e] At 3 and 4 weeks the numbers were 6 and 3, respectively. [f] At 3 and 4 weeks the numbers were 6 and 4, respectively. [g] At 3 and 4 weeks the numbers were 6 and 2, respectively. [h] Prepared by procedure of Tachikawa (1960). [i] At 4 weeks 5 rats survived. Growth was severely depressed.

compared with the other derivatives is illustrated. In Table II the stability of N-DL-pantoyl-β-aminopropionitrile and also of N-acetyl-β-aminopropionitrile after injection into rats is indicated by the absence of urinary β-aminopropionitrile and cyanoacetic acid, contrasting with the cleavage of N-γ-L-glutamyl-β-aminopropionitrile. The results of feeding these derivatives (Table III) confirmed the injection results, *i.e.*, toxic compounds were compounds which had undergone cleavage and *vice versa*.

The presence of intact DL-pantoyl-β-aminopropionitrile in the urine was demonstrated by infrared studies of the chloroform extract and by the appearance of free β-aminopropionitrile after treatment with ammonia.

TABLE IV
URINARY PANTOTHENIC ACID (PA) AFTER ADMINISTRATION OF N-DL-PANTOYL-β-AMINOPROPIONITRILE (BAPN) AND PANTOTHENIC ACID TO RATS

Route of Administration	Compound	Dose (mg/rat)	PA Excreted (mg/rat[a])
Injection	None	—	0.07
	PA[b]	66.3	64
	N-DL-Pantoyl-BAPN	60	0.07
Feeding, 1	None	—	0.08
	N-DL-Pantoyl-BAPN	[c]	0.05
Feeding, 2	None	—	0.20
	N-DL-Pantoyl-BAPN	[c]	0.07

[a] Average for 3 rats during 16–18 hours. [b] As calcium salt. [c] 20 mM per kg of diet fed *ad libitum*.

The stability of the amide group of N-DL-pantoyl-β-aminopropionitrile thus parallels that of pantothenic acid in its metabolism by the rat (Williams, 1943).

Metabolism and Toxicity of Other Acyl Derivatives of β-Aminopropionitrile.—The studies on N-DL-pantoyl-β-aminopropionitrile, which suggested that an acyl derivative of β-aminopropionitrile would be nontoxic unless enzymatically cleaved *in vivo*, were extended to the other derivatives shown in Table II. The results of the injection and feeding of these derivatives, summarized in Tables II and III, respectively, indicated that of the compounds tested only those in which the acyl groups corresponded to the amino acids, glutamic (whether L, D, or DL), glycine, L-leucine, and DL-phenylalanine were cleaved *in vivo*. In the injection experiments, besides β-aminopropionitrile and cyanoacetic acid, the free amino acids were observed in the urine. In the feeding experiments the animals showed the characteristic symptoms of β-aminopropionitrile toxicity, consisting of growth depression, skeletal deformities, and death due to aortic rupture. The other acyl-β-aminopropionitrile compounds apparently were not cleaved, since free β-aminopropionitrile and

TABLE V
LIBERATION OF FREE β-AMINOPROPIONITRILE FROM ACYL-β-AMINOPROPIONITRILE DERIVATIVES AT 27° FOR 16 HOURS[a]

Acyl Group	Conc. NH_4OH	0.1 N NaOH	0.1 N HCl
Acetyl	−	+	−
DL-Pantoyl	+	+	+
γ-L-Glutamyl	−	−	−

[a] Appearance of free BAPN is indicated by the + sign.

cyanoacetic acid were absent from the urine and symptoms of β-aminopropionitrile toxicity were not observed. The nontoxic compounds were those not containing an amino acyl group.

Although the mode of action of β-aminopropionitrile has not been defined, previous studies (Follis and Tousimis, 1958; Levene and Gross, 1959; Smiley *et al.*, 1962) have clearly associated increased levels of soluble collagen with lathyrism. Attempts to demonstrate an *in vitro* effect of β-aminopropionitrile on collagen fibril formation (Levene and Gross, 1959) were not successful. Whether the *in vivo* effects of β-aminopropionitrile involve a direct interference with conversion of soluble into native collagen or an indirect effect is the main question now under consideration. The emphasis which the present studies place upon the necessity for enzymatically liberating free β-aminopropionitrile[2] before these acyl derivatives can produce symptoms of osteolathyrism suggests further studies with radioactive β-aminopropionitrile.[3] An incorporation of the radioactivity into soluble collagen would support the view of a direct effect of β-aminopropionitrile, possibly by chemical binding through its amino group. Strong evidence that β-aminopropionitrile is directly bound to collagen *in vivo* has in fact been very recently obtained by Levene (1962) and by Stalder and Stegemann (1962) with nonradioactive material.

[2] Enzymes capable of cleaving the γ-glutamyl group from α-aminonitriles and from 4-hydroxymethyl-phenylhydrazine have recently been encountered in human blood serum (Szewczuk, 1959; Szewczuk and Orlowski, 1960) and in the edible mushroom, *Agaricus bisporus* (Levenberg, 1961), respectively.

[3] Earlier studies (Garbutt, 1958) with β-aminopropionitrile of relatively low specific activity indicated that only traces of the radioactivity were incorporated into rat tissues. Similar low incorporation of the radioactivity of C^{14}-labeled aminoacetonitrile was observed by Ponsetti *et al.* (1956).

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Metabolic Fate of Kynurenic Acid-C^{14} Intraperitoneally Administered to Animals*

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Kynurenic acid-2- or 3-C^{14} was intraperitoneally administered to the rat, mouse, hamster, rabbit, guinea pig, cat, and dog in a dose of 0.6 μmole per 100 g body weight. With all the animals tested, more than 90% of the dose given was excreted in the urine during the first 24 hours, with little or no radioactivity in expired CO_2 or carcass. Most of the radioactivity of the first-day urine was present as unchanged kynurenic acid (80–100%). The excretion of small amounts of quinaldic acid (1.3–4.8%) and quinaldylglycine (0.3–1.8%) was also detected.

In contrast to the recent accumulation of experimental evidence indicating a rapid metabolism of kynurenic acid to L-glutamic acid by tryptophan-adapted cells of *Pseudomonas fluorescens* (Hayaishi *et al.*, 1959, 1961; Behrman and Tanaka, 1959; Horibata *et al.*, 1961), no such active utilization of kynurenic acid by animals has been reported. Instead, earlier papers (Kotake and Ichihara, 1931; Correll *et al.*, 1938) showed that kynurenic acid subcutaneously administered to rabbits and dogs was nearly quantitatively recovered as such within 24 hours from the urine and bile. However, in view of the fact that the daily output of kynurenic acid in the urine of an untreated animal (Brown and Price, 1956) is far less than the dose those investigators applied, the possibility still existed that an appreciable metabolism of kynurenic acid could be detected if the latter were administered to the animal in a dose close to its physiological output. The present paper deals with the results of such experiments where a small dose of kynurenic acid-2- or 3-C^{14} was administered to several species of animals and the

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