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5-Hydroxykynurenine Decarboxylase in Rat Intestine

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An enzyme preparation was obtained from rat small intestine catalyzing the decarboxylation of 5-hydroxykynurenine to yield 4,6-dihydroxyquinoline *via* 5-hydroxykynurenamine. The enzyme was highly specific and neither kynurenine, 3-hydroxykynurenine, tryptophan nor 5-hydroxytryptophan could replace 5-hydroxykynurenine. Some properties of the enzyme are presented.

Previous reports from this laboratory have shown that various tryptophan metabolites, such as 5-hydroxykynurenine, 6-hydroxykynurenic acid and 4,6-dihydroxyquinoline occur in the urine of various animals (*1—6*). Tryptophan may be metabolized *via* 5-hydroxykynurenine (*7*, *8*) and preparations of hen liver microsomes catalyze the conversion of kynurenine to 5-hydroxykynurenine (*9*). Further 6-hydroxykynurenic acid has been found to be formed from 5-hydroxykynurenine by kynurenine aminotransferase [L-kynurenine: 2-oxoglutarate aminotransferase, EC 2.6.1.7] (*8*). Moreover, evidence for the conversion of 5-hydroxykynurenine to 4,6-dihydroxyquinoline was obtained by Makino and Takahashi, using a mouse liver homogenate (*7*). The formation of 4,6-dihydroxyquinoline may involve two enzymes; decarboxylase and monoamine oxidase [monoamine: oxygen oxidoreductase, EC 1.4.3.4]. However, little or no information about these reactions is available.

Therefore, we investigated these enzymes, and a specific decarboxylase for 5-hydroxykynurenine was found in various tissues, especially rat small intestine. The present paper describes the subcellular distribution and some properties of this enzyme.

MATERIALS AND METHODS

Reagents—Kynurenic acid was synthesized according to the method of Rieger *et al.* (*10*) and recrystallized as described by Besthorn (*11*). Xanthurenic acid was prepared by the method of Furst and Olsen (*12*). 6-Hydroxykynurenic acid was synthesized according to the description of Takahashi (*13*). Kynurenamine was synthesized by the method of Butenandt and Renner (*14*). L-Kynurenine, DL-kynurenine, 3-hydroxy-L-kynurenine, 3-hydroxy-DL-kynurenine and 5-hydroxy-DL-kynurenine were the same preparations as those used in previous studies (*1—6*). 4,6-Dihydroxyquinoline was obtained as follows; 1 g of 6-hydroxykynurenic acid was suspended in 10 ml of diphenyl ether and refluxed at 260°C to 270°C for about 20 min. The resulting yellow solution was mixed with 70 ml of 10% ammonia water and washed with diethyl ether to remove diphenyl ether. The solution was adjusted to pH 7.0 with 6 N HCl and applied to a column (3 cm×5 cm) of Dowex 1 (acetate form, 12% cross linkage, 200—400 mesh), which was then eluted with 200 ml of deionized water and 300 ml of 3 N acetic acid. The eluate with the latter was dried by vacuum-distilation under nitrogen. The residue was dissolved in 30 ml of water, adjusted to pH 4.0 with 1 N NaOH and concentrated to dryness. The residue was recrystallized from acetone; yield 120 mg (15%), mp 293°C.

Anal.

Calcd. for $C_9H_7NO_2$: C, 67.08; H, 4.34; N, 8.69%.
Found: C, 67.14; H, 4.36; N, 8.65%.

4,8-Dihydroxyquinoline and 4-hydroxyquinoline were synthesized by the procedure described above from xanthurenic acid and kynurenic acid, respectively. 4,8-Dihydroxyquinoline was recrystallized from water; yield 100 mg (13%), mp 296°C.

Anal.

Calcd. for $C_9H_7NO_2$: C, 67.08; H, 4.34; N, 8.69%.
Found: C, 67.21; H, 4.29; N, 8.68%.

4-Hydroxyquinoline was recrystallized from acetone; yield 100 mg (13%), mp 290°C.

Anal.

Calcd. for C_9H_7NO: C, 74.48; H, 4.83; N, 9.66%.
Found: C, 74.58; H, 4.76; N, 9.59%.

5-Hydroxykynurenamine (or 5-hydroxykynuramine or mousamine) was prepared by a modification of BUTENANDT and RENNER's method for kynurenamine synthesis (*14*) as follows: Twelve g of 2-nitro-5-methoxy-β-dimethylaminopropiophenone hydrochloride were dissolved in 120 ml of water and cooled to 0°C. Ninety ml of ethyl ether were added, followed by 20 ml of 2 N NaOH, cautiously with stirring. The organic phase was separated, washed with 10 ml of water and dried over anhydrous Na_2SO_4. Seven g of phthalimide were added and the ether was removed *in vacuo*. The residue was suspended in 30 ml of ethanol containing a small amount of sodium ethylate. The suspension was refluxed for two hours and then cooled to room temperature (20°C). Then 150 ml of water were added and the resulting product was extracted with three 300 ml portions of ethyl ether. The combined ether extract was washed successively with dilute HCl, 2 N NaOH and a large volume of water, and evaporated to dryness *in vacuo*. Two ml of ethanol were added to the residue and the mixture was cooled to −5°C. The crystalline precipitate was collected on a glass filter and dried; yield 650 mg (4.5%, 2-nitro-5-methoxy-β-phthalimidopropiophenone). The crystals (650 mg) were dissolved in 10 ml of acetic acid by warming the mixture and 2 g of stannous chloride dissolved in 2.5 ml of concentrated HCl were added. To the resulting yellow solution 30 ml of hot water were added. On heating the solution in a boiling water bath for 30 min it became turbid, and a crystalline deposit formed. The mixture was stored in a refrigerator to allow further crystallization, and then the crystals were collected by filtration; yield 450 mg (76%, 2-amino-5-methoxy-β-phthalimidopropiophenone). A solution of the crystals (450 mg) in 2 ml of acetic acid and 1.2 ml of concentrated HCl was heated at 150°C for one hour in a sealed tube. The resulting yellow solution was diluted with water, filtered and concentrated to dryness *in vacuo*. The residue was dissolved in dilute HCl and filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 3 ml of hot ethanol, treated with charcoal, concentrated to about 2 ml *in vacuo* and allowed to stand overnight at −5°C. The resulting crystalline precipitate was collected and dried; yield 210 mg (56%), mp 212°C (5-methoxykynurenamine dihydrochloride).

Anal. Calcd. for $C_{10}H_{16}N_2O_2Cl_2$:
C, 44.94; H, 5.99; N, 10.49%.
Found: C, 44.85; H, 6.03; N, 10.23%.

Two hundred mg of 5-methoxykynurenamine dihydrochloride were suspended in 57% hydriodic acid and refluxed in an oil bath for 16 hr under nitrogen. Then a large volume of water was added and the solution was applied to a column of Dowex 50W (H^+) (3×7 cm) which was eluted with 500 ml of 0.5 N HCl and 1,000 ml of 5 N HCl. The eluate with 5 N HCl was evaporated *in vacuo* under nitrogen. The residue was dissolved in 100 ml of 0.1 N HCl and subjected to a similar chromatographic procedure on one third the scale. The eluate with 5 N HCl was treated with charcoal, filtered and dried by distillation under a low pressure of nitrogen. The resulting yellow crystals were collected on a glass filter and dried; yield 65 mg (32%), mp 237°C (decomp.) (5-hydroxykynurenamine dihydrochloride).

Anal. Calcd. for $C_9H_{14}N_2O_2Cl_2 \cdot H_2O$:
C, 39.85; H, 5.90; N, 10.33%.
Found: C, 39.73; H, 6.01; N, 9.96%.

Homogenization of Intestine and Fractionation by Differential Centrifugation—Male Wistar albino rats, weighing 150 to 200 g were stunned, decapitated and exsanguinated in a cold room. All subsequent manipulations were carried out at about 4°C. The small intestine was removed immediately after decapitation, immersed in ice cold 0.25 M sucrose, cut open longitudinally and washed several times with 0.25 M sucrose. Then intestines were cut into small pieces, weighed and homogenized in a Waring blender for two minutes with 9 volumes of 0.25 M sucrose. The homogenate was centrifuged at 700×g for 10 min to yield a precipitate (F-I). The supernatant fraction was centrifuged at 5,000×g for 15 min yielding a precipitate (F-II). The successive centrifugations were carried out at 18,000×g for 10 min giving a precipitate (F-III) and at 100,000×g for 20 min to give a precipitate (F-IV) and supernatant (F-V). Each fraction was washed several times with 0.25 M sucrose at each step. The compact pellets were suspended in 0.25 M sucrose at a concentration equivalent to 1 g of intestine per ml. Protein was determined by the biuret method with crystalline bovine albumin as the standard (*15*).

Isolation of Reaction Products—For isolation of a sufficient amount of 4,6-dihydroxyquinoline for its identification, incubation was carried out as follows; 10 ml of reaction mixture containing 200 μmoles of potassium phosphate buffer at pH 7.5, 2 μmoles of 5-hydroxy-DL-kynurenine (or 5-hydroxykynurenamine), and homogenate corresponding to 200 mg wet weight of rat small intestine was incubated at 37°C for two

hours with vigorous shaking. The reaction was terminated by the addition of 1 ml of 50% trichloroacetic acid. The precipitate was separated by filtration and the filtrate was adjusted to pH 7.0 by dropwise addition of 2 N NaOH. An aliquot of this filtrate was applied to a column (1×5 cm) of Dowex-1 (acetate form, 12% cross linkage, 200—400 mesh), which was eluted successively with 30 ml of deionized water and 30 ml of 3 N acetic acid. The eluate with the latter was dried by distillation under a reduced pressure of nitrogen. The residue was dissolved in a small volume of methanol and subjected to thin layer chromatography using thin layers of silica gel G (Merck, Darmstadt, Germany) on glass plates as already described (*16*, *17*).

For identification of the 5-hydroxykynurenamine formed from 5-hydroxykynurenine, homogenate corresponding to 150 mg wet weight of rat small intestine was incubated at 37°C for two hours under anaerobic conditions in a reaction mixture containing 3 μmoles of 5-hydroxy-DL-kynurenine and 200 μmoles of potassium phosphate buffer (pH 7.5) in a final volume of 5.5 ml in a Thunberg type tube, flushing the tube thoroughly with nitrogen. After termination of the reaction by adding 1 ml of 50% trichloroacetic acid, the mixture was filtered. The filtrate was applied to a column of Dowex 50W (H^+, 12% cross linkage, 200—400 mesh) 1×5 cm) and eluted with 100 ml of deionized water, 10 ml of 2 N HCl and finally 50 ml of 5 N HCl. The eluate with 5 N HCl was dried by distillation under a reduced pressure of nitrogen at 50°C. The residue was dissolved in a small volume of distilled water and subjected to paper chromatography. Thin layer and paper chromatograms were examined under ultraviolet light and sprayed with various reagents (*18*, *19*). The reaction products were identified by comparison of their chromatographic behaviors with those of authentic compounds.

Assay of Decarboxylase Activity—The incubation was carried out at 37°C with shaking rather than at 30°C unless otherwise indicated. The reaction mixture consisted of 200 μmoles of potassium phosphate buffer (pH 7.5), an aliquot of intestinal fraction, rat liver mitochondria heated at 57°C for 5 min (protein, 10 mg; oxidase activity for 5-hydroxykynurenamine, 5.3×10^{-2} μmoles 4,6-dihydroxyquinoline formed/hr/mg of protein; this preparation does not decarboxylate 5-hydroxy-DL-kynurenine and is referred to have after as "RLM"), and 0.82 μmole of 5-hydroxy-DL-kynurenine in a total volume adjusted to 3.0 ml with distilled water. Incubation was also carried out without RLM. Reaction mixture to which 0.5 ml of 10% $HClO_4$ was added at zero time, was used as the control. After incubation for one hour 0.5 ml of 10% $HClO_4$ was added. The mixture was filtered and 1.0 ml of the filtrate was mixed with 5.0 ml of 0.2 M potassium phosphate buffer (pH 7.5) to adjust the pH to 7.5. The optical density at 333 mμ was measured against that of the control. For calculation of the amount of 4,6-dihydroxyquinoline formed the molar absorption coefficient of the product was taken as 6,500 (at 333 mμ), while for the products from kynurenine and 3-hydroxykynurenine extinctions of 10,000 (at 315 mμ) for 4-hydroxyquinoline and 8,500 (325 mμ) for 4,8-dihydroxyquinoline were used.

Assay of Monoamine Oxidase Activity—Oxidase activity for 5-hydroxykynurenamine (or kynurenamine) was assayed by measuring the amount of 4,6-dihydroxyquinoline (or 4-hydroxyquinoline) formed. The reaction conditions were the same as those for the assay of the decarboxylase activity described above, except that 5-hydroxykynurenamine and kynurenamine were used as substrates, and RLM was omitted. Tryptophan decarboxylase (L-tryptophan carboxy-lyase, EC 4.1.1.27] and hydroxytryptophan decarboxylase [5-hydroxy-L-tryptophan carboxy-lyase, EC 4.1.1.28] activities was assayed as described by CLARK *et al.* (*20*).

RESULTS

Formation of 4,6-Dihydroxyquinoline from 5-Hydroxykynurenine by the Homogenate of Rat Small Intestine.

5-Hydroxy-DL-kynurenine was incubated with the homogenate of rat small intestine and the reaction products were analyzed as described under "MATERIALS AND METHODS". An additional spot with blueish green fluorescence appeared on a thin layer chromatogram besides the yellowish green spot of 5-hydroxykynurenine. The compound formed had *Rf* values identical with those of 4,6-dihydroxyquinoline in the solvent systems used (Table I). Spots of the product and a synthetic sample of 4,6-dihydroxyquinoline gave identical color reactions with DSA and $FeCl_3$. The ultraviolet absorption spectrum of the product was identical with that of authentic 4,6-dihydroxyquinoline in 0.1 M phosphate buffer at pH 7.5. In the absence of substrate little 4,6-dihydroxyquinoline was detectable. Boiling the homogenate of intestine or stopping the reaction at zero time inhibited the reaction completely. In the above reaction, no 5-hydroxykynurenamine, 6-hydroxykynurenic acid or 5-hydroxyanthranilic acid were detected. But addition of α-ketoglutaric acid to the reaction mixture

TABLE I

Thin layer chromatography of the product and authentic material

	Product	4,6-Dihydroxyquinoline
Ethylacetate : isopropanol : 28% ammonia water (9 : 6 : 4 by vol.), *Rf*	0.56	0.56
Chloroform : methanol : acetic acid (15 : 6 : 4 by vol.), *Rf*	0.61	0.61
5% NaCl, *Rf*	0.51	0.51
Fluorescence under ultraviolet light at 3,650 Å	Blueish green	Blueish green
DSA[1]	Pinkish orange	Pinkish orange
$FeCl_3$	Brown	Brown
Absorption maximum at pH 7.5 (mμ)	333	333

[1] Diazotized sulfanilic acid.

TABLE II

Paper chromatography of the product and authentic material

	Product	5-Hydroxykynurenamine
Methanol : benzene : butanol : water (2 : 1 : 1 : 1 by vol.), *Rf*	0.42	0.42
80% Isopropanol, *Rf*	0.60	0.60
5% NaCl, *Rf*	0.80	0.80
Fluorescence under ultraviolet light at 3,650 Å	Yellowish brown	Yellowish brown
Ekman's reagent	Blueish gray	Blueish gray
Ehrlich's reagent	Orange	Orange
DSA[1]	Orange	Orange
Absorption maximum (mμ) at pH 1.0	300	300
at pH 7.0	404	404

[1] Diazotized sulfanilic acid.

resulted in production of 6-hydroxykynurenic acid.

Formation of 4,6-Dihydroxyquinoline from 5-Hydroxykynurenamine by the Homogenate of Rat Small Intestine.

Using 5-hydroxykynurenamine as substrate with the above reaction system, the product was identified as 4,6-dihydroxyquinoline using a similar technique to that described in Table I.

Formation of 5-Hydroxykynurenamine from 5-Hydroxykynurenine by the Homogenate of Rat Small Intestine.

When 5-hydroxy-DL-kynurenine was incubated with the homogenate of rat small intestine under anaerobic conditions as described before, two spots corresponding to 5-hydroxykynurenine and 4,6-dihydroxyquinoline were obtained with an additional spot giving yellowish brown fluorescence. The *Rf* values in the different solvent systems and the color reactions of the latter compound were identical with those of synthetic 5-hydroxykynurenamine (Table II). No 6-hydroxykynurenic acid or 5-hydroxyanthranilic acid was detected. The formation of 4,6-dihydroxyquinoline even under anaerobic conditions may be because oxygen was not completely excluded from the reaction. In the absence of the enzyme or with boiled enzyme no 5-hydroxykynurenamine was detectable.

Distribution of Decarboxylase and Monoamine Oxidase Activity in Rat Small Intestine.

The subcellular distributions of decarboxylase activity for 5-hydroxykynurenine and

TABLE III

Subcellular distribution of decarboxylase activity for 5-hydroxykynurenine and oxidase activity for 5-hydroxykynurenamine and kynurenamine in rat small intestine

Cell fractionation of rat small intestine and assay conditions are described under "MATERIALS AND METHODS". The distribution is expressed as μmole product formed at 37°C in one hour by fractions corresponding to 800 mg wet weight of small intestine. RLM is rat liver mitochondria heated at 57°C for 5 min and 10 mg of this enzyme protein were used. F-I, precipitate on centrifugation at 700×*g*; F-II, precipitate at 700 to 5,000×*g*; F-III, precipitate at 5,000 to 18,000×*g*; F-IV, precipitate at 18,000 to 100,000×*g* and F-V, supernatant at 100,000×*g*.

Cell fraction	Substrate		
	5-Hydroxy-DL-kynurenine	5-Hydroxykynurenamine	Kynurenamine
	(μmole product/hr)		
Homogenate	0.30	1.26	2.34
Homogenate+RLM	0.32		
F-I	0.01	0.04	0.08
F-I+RLM	0.01		
F-II	0.19	0.34	1.50
F-II+RLM	0.19		
F-III	0.01	0.04	0.07
F-III+RLM	0.01		
F-IV	0	0	0
F-IV+RLM	0		
F-V	0	0	0
F-V+RLM	0		

oxidase activity for 5-hydroxykynurenamine and kynurenamine are shown in Table III. Formation of 4,6-dihydroxyquinoline from 5-hydroxy-DL-kynurenine was great using the precipitate formed to centrifugation at 700 to 5,000×*g* (F-II) as the enzyme preparation but was slight with other fractions (Table III). When RLM was added to oxidize the 5-hydroxykynurenamine formed rapidly, the amounts of 4,6-dihydroxyquinoline formed with the different fractions were similar. This shows that 5-hydroxykynurenamine formed by F-II was completely oxidized to 4,6-dihydroxyquinoline *via* 2-amino-5-hydroxy-benzoylacetaldehyde by the active monoamine oxidase of F-II. The oxidase activity for 5-hydroxykynurenamine and kynurenamine was also mainly localized in F-II.

Some Properties of Decarboxylase in F-II.

Effect of Incubation Time—There was a linear relationship between the amount of product and the incubation time on incubation for one hour (Fig. 1). The quantity of 4,6-dihydroxyquinoline formed corresponded to 13% of the substrate after incubation for one hour and to 15% after incubation for two hours. Subsequently the amount of product did not change appreciably. The same result was obtained with reaction mixture containing RLM. The stoichiometry of the reaction was determined spectrophotometrically and the result is shown in Fig. 2. The amount of product increased with decrease in the amount of substrate, but the relation was not stoichiometric.

Effect of Enzyme Concentration—A linear relationship between decarboxylase activity and enzyme concentration was obtained with up to 10 mg of protein (Fig. 3), even on addition of RLM to the experimental cuvette.

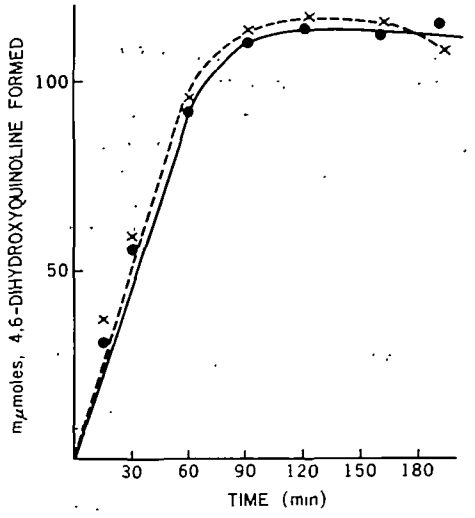

FIG. 1. Time courses of formation of 4,6-dihydroxyquinoline.

Assay conditions were as described in the text except for the incubation time. Ten mg of enzyme protein (F-II) were used.
—— Without RLM,
---- With RLM (protein, 10 mg).

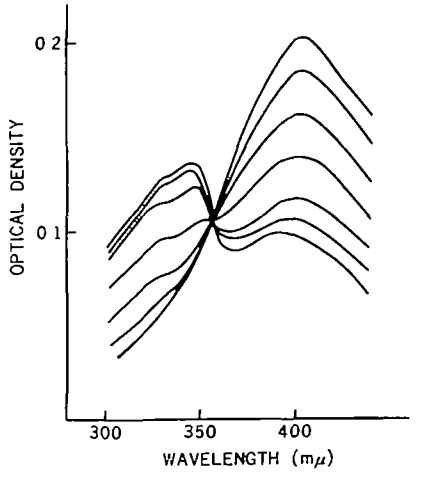

FIG. 2. Spectrophotometric assay of the stoichiometry of the reaction.

Assay conditions were as described in the text except for the incubation time. Spectra were examined after 0, 5, 10, 15, 30, 60, 90, and 180 min incubation. Ten mg of enzyme protein (F-II) were used. The molar absorption coefficient of 5-hydroxy-DL-kynurenine at 400 mμ is 4,500.

Effect of pH—The pH activity curve of the intestinal decarboxylase for 5-hydroxy-

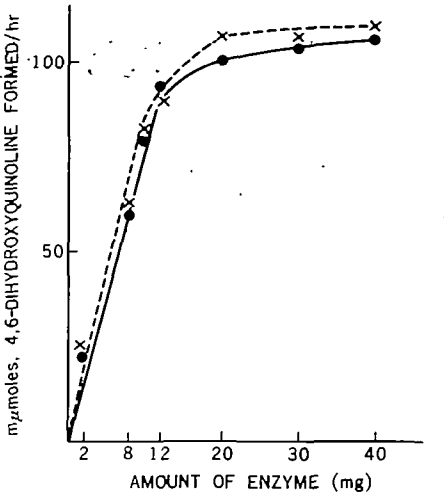

FIG. 3. Effect of enzyme concentration on reaction rates.

Assay conditions were as described in the text except for the amount of enzyme (F-II) used.
—— Without RLM,
---- With RLM (protein, 10 mg).

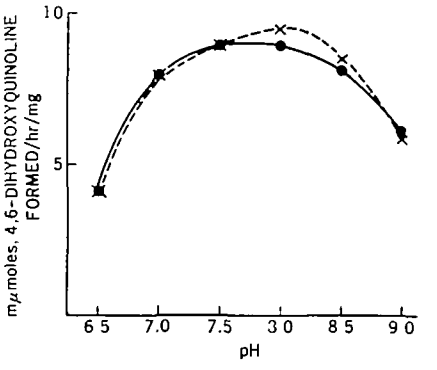

FIG. 4. Effect of pH on enzyme activities.

Assay conditions were as described in the text except for the potassium phosphate buffer used.
—— Without RLM,
---- With RLM (protein, 10 mg).

kynurenine is shown in Fig. 4. The enzyme showed maximal activity at pH 7.5 to 8.0 and the rate fell off symmetrically on the alkaline and acid sides. Addition of RLM did not affect the optimal pH.

Effect of Semicarbazide and Hydroxylamine—In the presence of 10^{-3} M hydroxylamine or 10^{-2} M semicarbazide formation of 4,6-dihy-

droxyquinoline from 5-hydroxy-DL-kynurenine (0.9×10^{-2} μmoles/hr/mg of protein) was almost completely inhibited (nearly 100%). The oxidase activity for 5-hydroxykynurenamine was not effected by the above inhibitors, so this decarboxylase seems to be vitamin B_6-dependent.

Substrate Specificity—L-Kynurenine, DL-kynurenine, 3-hydroxy-L-kynurenine, 3-hydroxy-DL-kynurenine, L-tryptophan and 5-hydroxy-DL-tryptophan could not be decarboxylated by F-II and only 5-hydroxy-DL-kynurenine acted as substrate for the enzyme. When kynurenine or 3-hydroxykynurenine was used as substrate, no formation of the corresponding quinoline compound was observed even in the presence of RLM.

Subcellular Distribution of Decarboxylase Activity for 5-Hydroxykynurenine in Rat Liver, Kidney and Heart.

Liver, kidney and heart were fractionated by the procedures used for intestine, and decarboxylase activity for 5-hydroxykynurenine was found to be mainly localized in the mitochondrial fractions of all tissues, obtained by centrifugation at 700 to $5{,}000\times g$. The decarboxylase activity was found to be high in fraction F-II of the small intestine but low in the mitochondrial fractions of the liver, kidney and heart. Addition of RLM to these fractions of kidney or heart did not affect the decarboxylase activity (Table IV).

TABLE IV

Decarboxylase activities for 5-hydroxykynurenine of F-II from rat small intestine and the mitochondrial fractions of rat liver, kidney and heart

Assay conditions were as described in the text except for enzyme preparations. Activity is expressed as the amount of 4,6-dihydroxyquinoline formed by 1 mg of enzyme protein at 37°C in one hour.

Enzyme preparation	4,6-Dihydroxyquinoline formed (mμmoles/hr/mg)
F-II[1]	9.0
Liver mitochondria	6.3
Kidney mitochondria	1.3
Kidney mitochondria+RLM[2]	1.6
Heart mitochondria	3.6
Heart mitochondria+RLM[2]	4.0

[1] Precipitate at 700 to $5{,}000\times g$ of homogenate of rat small intestine.

[2] Rat liver mitochondria heated at 57°C for 5 min.

DISCUSSION

In the present study with various rat tissues, a homogenate of small intestine was found to catalyze the conversion of 5-hydroxykynurenine to 5-hydroxykynurenamine and 4,6-dihydroxyquinoline. The decarboxylase activity for 5-hydroxykynurenine was localized in the fraction of the small intestine precipitated by centrifugation at 700 to $5{,}000\times g$ and the decarboxylase activities of liver, kidney and heart were mostly present in the mitochondrial fraction. No standard procedures have been established for fractionation of the small intestine but F-II probably represents mitochondria from the procedures used for fractionation. This enzyme was highly specific for 5-hydroxykynurenine. The enzyme activity of F-II can be determined without addition of monoamine oxidase, because under the present conditions 5-hydroxykynurenamine formed by the decarboxylase of F-II was completely oxidized to 4,6-dihydroxyquinoline *via* 2-amino-5-hydroxybenzoylacetaldehyde by the monoamine oxidase present in the fraction, as shown in Table I. The effects of the reaction time and enzyme concentration on the decarboxylase activity for 5-hydroxykynurenine were examined. Although little or no formation of either 6-hydroxykynurenic acid or 5-hydroxyanthranilic acid was observed in either experiment (pH 7.5), the disappearance of substrate was larger than the amount of 4,6-dihydroxyquinoline formed. However, in systems without enzyme or with boiled enzyme, no change of optical density at 333 mμ (the absorption maximum of 4,6-dihydroxyquinoline at pH 7.5) or at 400 mμ (the absorption maximum of 5-hydroxykynurenine at pH 7.5) was observed. The decrease in the amount of substrate, 5-hydroxykynurenine, was not proportional to increase in the amount of 4,6-dihydroxyquinoline formed possibly be-

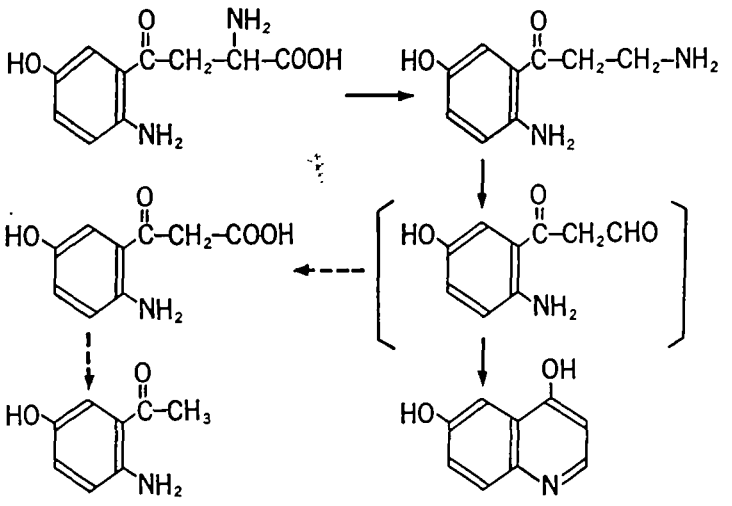

FIG. 5. Proposed route of biosynthesis of 2-amino-5-hydroxyacetophenone from 5-hydroxykynurenine.

cause of formation of some other compound. This compound is presumably 2-amino-5-hydroxyacetophenone, because 5-hydroxykynurenamine and the corresponding aldehyde may be precursors of acetophenone. This compound has been isolated in this labolatory from the urine of hens after injection of L-tryptophan (*21*). Acetophenone may be formed from 5-hydroxykynurenine through the pathway shown in Fig. 5. The optimal pH of 5-hydroxykynurenine decarboxylase was 7.5—8.0. In reaction mixture without enzyme, the absorption at 333 mμ did not change during incubation for one hour at pH 6.0 to 10 but that at 400 mμ decreased at pH 8 to 10. No formation of 4,6-dihydroxyquinoline, 6-hydroxykynurenic acid or 5-hydroxyanthranilic acid was detectable. Therefore, reactions should be carried out at below pH 8.0. Studies are now in progress on the mechanism and site of biosynthesis of the new compound.

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