THE INTERMEDIARY METABOLISM OF TRYPTOPHAN
BY CAT AND RAT TISSUE PREPARATIONS*

BY F. T. DE CASTRO,† R. R. BROWN, AND J. M. PRICE‡
(From the Cancer Research Hospital, Medical School, University of Wisconsin,
Madison, Wisconsin)

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Reports from several laboratories have shown that cats display a peculiar tryptophan metabolism as compared with other mammals. In 1941, Kotake and Nakayama (1) reported that L-kynurenine could be quantitatively converted to anthranilic acid when incubated with cat liver preparations. Gordon, Kaufman, and Jackson (2), on the other hand, found no kynurenic acid in urine of cats even when tryptophan was added to the high protein diet on which the animals were kept. Lepkovsky and Nielsen (3) and Miller and Baumann (4) showed that pyridoxine-deficient rats excreted large amounts of xanthurenic acid; however, Carvalho da Silva, Fried, and de Angelis (5) were unable to find xanthurenic acid in urine of pyridoxine-deficient cats even when supplemental doses of tryptophan were fed in the diet. Furthermore, they showed that L-tryptophan was not effective in relieving the avitaminosis symptoms of niacin-deficient cats in contrast to observations in other species (6). Previous studies in this laboratory (7) have shown that cats fed a high protein diet supplemented with single doses of L-tryptophan did not excrete any appreciable amount of most of the known metabolites of this amino acid. Only 0.07 per cent of the administered dose of tryptophan could be accounted for in the urine as the metabolites measured. Apparently, therefore, the cat has an active kynureninase, but is unable to use tryptophan in place of niacin and does not excrete significant quantities of the usual urinary metabolites of tryptophan.

The present work deals with measurements in vitro of the activities of some of the enzymes involved in tryptophan metabolism in an effort to explain the absence of tryptophan metabolites in cat urine. The enzymatic studies of Mason and Berg (8, 9) and of Knox and Mehler (10) served as valuable guides in the development of these studies. The enzymes compared in cats and rats were tryptophan peroxidase, kynureninase, kynure-

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† Fellow of the National Research Council of Brazil.
‡ Scholar in Cancer Research of the American Cancer Society.
nine transaminase, and the system which forms 3-hydroxykynurenine from kynurenine (11) for which the name kynurenine hydroxylase is proposed. It was found that homogenates of cat liver were considerably less active in tryptophan peroxidase and kynurenine transaminase than were those of rat liver. Kynureninase activities were similar in both species although mitochondria of cat liver were more active than those of rat liver in the hydroxylase. The urinary excretion of tryptophan metabolites by cats is discussed in light of these findings in vitro.

EXPERIMENTAL

Animals—Adult male rats of the Holtzman strain, maintained on a dog chow diet, were used throughout the experiments. Adult cats were purchased from local dealers and were fed a diet of commercial canned dog food.

Materials—The tryptophan metabolites used were those described previously (7, 11). Triphosphopyridine nucleotide (TPN), glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from the Sigma Chemical Company. α-Ketoglutaric acid was obtained from the Nutritional Biochemicals Corporation, and pyridoxal phosphate was obtained from the California Foundation for Biochemical Research.

Tissue Preparations—Only redistilled water from an all-glass still was used to make solutions. The animals were killed by exsanguination after being stunned by a blow on the head. The tissues were removed quickly and chilled in ice-cold 0.25 M sucrose solution and subsequent manipulations were carried on in the cold. Tissue homogenates were prepared in 0.25 M sucrose by use of an all-glass Potter-Elvehjem homogenizer. Tissue fractions were prepared according to the method of Schneider (12) with slight modifications. Nuclei were washed once with sucrose solution and the wash liquid was added to the supernatant fluid. Mitochondria were washed three times, the first time with a small amount of sucrose solution, usually one-fifth of the original volume, and the washing liquid was added to the supernatant fluid. The last two washings of mitochondria were carried out with large amounts of sucrose solution, and the wash liquid was discarded. The microsomes were not washed. All the fractions were made up to a final volume corresponding to a 20 per cent (w/v) suspension of fresh tissue. An International refrigerated centrifuge with a multispeed attachment was employed in the fractionation.

Assay of Enzymes

Incubations were carried out in stoppered 25 ml. Erlenmeyer flasks, shaken in a water bath at 37° with air as the gas phase. All the flasks

1 Friskies, the Carnation Company, Milwaukee, Wisconsin.
2 Red Heart, John Morrel and Company, Ottumwa, Iowa.
were made to a final volume of 3.0 ml. with water. After 90 minutes incubation, 1.0 ml. of 16 per cent trichloroacetic acid was added to each flask. The precipitate was removed by centrifugation, and the clear supernatant solution was kept frozen until analyzed. Flasks were run in duplicate and a zero time flask was included in each group of determinations. In all cases the activity of the enzyme was proportional to the amount of tissue added and increased linearly with time for at least 90 minutes. The enzyme activities were expressed as micromoles of metabolite formed per hour per gm. of fresh tissue.

Preliminary studies on the intracellular localization of the enzymes concerned showed that tryptophan peroxidase was located in cat liver supernatant fluid as found by Knox and Mehler (10) for rat liver. Kynureninase of rat and cat liver and kidney was located only in the supernatant fractions, whereas kynurenine transaminase was present in both the mitochondrial and supernatant fractions of these tissues. Therefore, whole homogenates were used to assay for the total transaminase. Kynurenine hydroxylase was located in the mitochondrial fraction of liver and kidney of both species.

Tryptophan Peroxidase Incubation Medium—L-Tryptophan, 5.0 μmoles; 0.05 M potassium phosphate buffer, pH 7.4; supernatant fluid from 20 per cent (w/v) liver homogenate, 2.0 ml.

Kynureninase Incubation Medium—L-Kynurenine, 5.0 μmoles; pyridoxal phosphate, 40 γ; 0.05 M potassium phosphate buffer, pH 7.4; supernatant fluid from 20 per cent (w/v) tissue homogenate, 2.0 ml.

Kynurenine Transaminase Incubation Medium—L-Kynurenine, 5.0 μmoles; pyridoxal phosphate, 40 γ; α-ketoglutarate, 30 μmoles; 0.05 M potassium phosphate buffer, pH 7.4; 10 per cent (w/v) whole homogenate, 2.0 ml.

Kynurenine Hydroxylase Incubation Medium—L-Kynurenine, 5.0 μmoles; triphosphopyridine nucleotide (TPN), 1.3 μmoles; 0.02 M nicotinamide; 0.004 M magnesium chloride; 0.001 M Versene ((ethylenedinitrilo)tetraacetic acid); 0.05 M potassium phosphate buffer, pH 7.4; 0.001 M cysteine; glucose-6-phosphate dehydrogenase, 0.4 Kornberg unit; glucose 6-phosphate, 10 μmoles; mitochondrial suspension, equivalent to 400 mg. of wet weight of tissue.

Quantitative Estimation of Metabolites—Kynurenine and kynurenic acid were measured spectrophotometrically by a modification of the procedure of Miller, Tsuchida, and Adelberg (13), and anthranilic acid by the method of Mason and Berg (9). Hydroxykynurenine was estimated colorimetrically by a modification of the nitrous acid method described previously (14). To duplicate colorimeter tubes, 0.3 ml. of the deproteinized sample, 1.0 ml. of 1.0 N HCl, and water, to a total volume of 3.0 ml., were added. Standard curves were run in a similar manner with hydroxykynurenine (10 to 40 γ) added to 0.3 ml. of the deproteinized sample. Blank tubes
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contained all materials, except that 0.2 ml. of water was used in place of 0.2 ml. of sodium nitrite solution. Optical density was measured at 390 mμ at which under these conditions the hydroxykynurenine-nitrous acid product showed maximal absorption.

In addition to the quantitative measurements, qualitative paper chromatograms were made in all cases to check the course of the enzyme reactions (11).

RESULTS AND DISCUSSION

The high kynurenine hydroxylase activity found in cat liver mitochondria together with the very low levels of kynurenine transaminase and the absence of kynureninase in these cytoplasmic particles presented a convenient system for study of the hydroxylase system. The use of a glucose-6-phosphate dehydrogenase system to maintain a supply of TPNH almost completely eliminated the formation of kynurenic acid, which was undoubtedly favored by the production of keto acids from the metabolism of citrate used in earlier studies on kynurenine hydroxylase (11).

Hydroxylation of kynurenine was not detectable if glucose 6-phosphate, its dehydrogenase, or TPN was omitted. Versene and cysteine were not necessary for hydroxylation, but together they caused about 50 per cent stimulation in activity, presumably due to their protection against heavy metal contamination and preservation of sulfhydryl groups. Versene or cysteine had no effect on the rate of TPNH formation by the dehydrogenase. Glutathione (0.001 M) stimulated the hydroxylation reaction 20 per cent and cyanide (0.005 M) 20 to 50 per cent, probably by preventing the loss of TPNH through the cytochrome system, although no evidence was obtained to suggest that production of TPNH was limiting. The addition of more glucose 6-phosphate or its dehydrogenase had no significant effect. Ferrous ion (0.001 M) inhibited 20 to 50 per cent but α,α'-dipyridyl (0.001 M), catalase (2 mg.), hydrogen peroxide (0.005 M), ascorbic acid (0.004 M), sodium diethylthiocarbamate (0.001 M), anthranilic acid (0.0033 M), o-aminobipyrurate acid (0.0033 M), and xanthurenic acid (0.001 M) had no effect on the amount of hydroxykynurenine formed by the rat liver mitochondria system.

The results of the comparative enzyme measurements are presented in Table I. The tryptophan peroxidase system was found to be about one-third as active in cats as in rats and the kynureninase activity was about the same in both species. Tryptophan peroxidase was not detectable in kidney of either species. Kynurenine transaminase was very active in the rat tissues but was relatively inactive in the cat tissues. Kynurenine hydroxylase, on the other hand, was almost twice as active in cats as in rats, both in liver and kidney. As much as 40 to 66 per cent of the added ky-
nurenine was hydroxylated by cat liver mitochondria under the conditions of incubation. On paper chromatograms, the only spots which reacted with diazotized sulfanilic acid were 3-hydroxy-L-kynurenine and traces of xanthurenic acid.

**Table I**

*Enzymatic Activities of Liver and Kidney Preparations from Cats and Rats*

The activities are expressed as micromoles of metabolite formed per hour per gm. of fresh tissue. The average values are given with the number of experiments indicated in parentheses. The range of values found is shown below the average. Compositions of the incubation media are given in the text.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tissue preparation</th>
<th>Tryptophan peroxidase</th>
<th>Kynureninase transaminase</th>
<th>Kynurenine hydroxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Liver homogenate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot; mitochondria</td>
<td>0.90 (5)</td>
<td>2.62 (4)</td>
<td>2.78 (7)</td>
</tr>
<tr>
<td></td>
<td>&quot; supernatant fluid</td>
<td>0.55–1.50</td>
<td>2.22–2.92</td>
<td>1.36–4.65</td>
</tr>
<tr>
<td></td>
<td>Kidney homogenate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot; mitochondria</td>
<td>12.0 (4)</td>
<td>11.1–12.8</td>
<td>1.28 (3)</td>
</tr>
<tr>
<td></td>
<td>&quot; supernatant fluid</td>
<td>0.50 (5)</td>
<td>0.40–0.60</td>
<td>0.83–1.66</td>
</tr>
<tr>
<td>Cat</td>
<td>Liver homogenate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot; mitochondria</td>
<td>0.37 (4)</td>
<td>2.16 (5)</td>
<td>4.75 (3)</td>
</tr>
<tr>
<td></td>
<td>&quot; supernatant fluid</td>
<td>0.23–0.45</td>
<td>0.98–2.98</td>
<td>3.53–5.50</td>
</tr>
<tr>
<td></td>
<td>Kidney homogenate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot; mitochondria</td>
<td>0.36 (3)</td>
<td>0.20–0.46</td>
<td>2.30 (3)</td>
</tr>
<tr>
<td></td>
<td>&quot; supernatant fluid</td>
<td>0.66 (5)</td>
<td>0.43–0.88</td>
<td>1.83–2.74</td>
</tr>
</tbody>
</table>

The results presented here confirm and partially explain some of the observations reported in the literature on the urinary excretion of tryptophan metabolites by cats and rats. According to the classical scheme of the metabolism of tryptophan along the kynurenine pathway (15), it is apparent that a low tryptophan peroxidase activity would supply a small amount of kynurenine for the subsequent metabolic pathways. The conditions of tryptophan peroxidase assay were probably not those for maxi-
mal possible activity since in mice, but not in rats, an added source of enzymatically generated hydrogen peroxide caused some stimulation of activity and less variability (16). Under the conditions of tryptophan peroxidase assay used in this study, the cat liver activity was significantly less than that of rat liver; however, it is felt that the cat liver tryptophan peroxidase activity was not sufficiently low to account entirely for the negligible amounts of kynurenine and its metabolites found in cat urine before or after dosage with L-tryptophan (7). Since in relation to the conversion of dietary tryptophan to niacin, the significant levels of enzymes would be those found in animals on normal diets, no enzyme studies were made with tissues from rats given the pharmacological doses of tryptophan used to stimulate tryptophan peroxidase adaptation (17). In the incubation mixtures of tryptophan peroxidase, kynureninase, and kynurenine transaminase, it was possible to account for essentially all the substrate added as the metabolites measured and as unchanged substrate. This is an indication that the substrate was not being used through pathways different from those under consideration. In the case of the kynurenine hydroxylase system it was possible to account for about 80 to 90 per cent of the added kynurenine, and it was not possible to identify any other metabolite by paper chromatography besides 3-hydroxykynurenine and sometimes traces of kynurenic acid and xanthurenic acid. Thus, at most, only small amounts of kynurenine could have been metabolized through unknown pathways, and only small amounts of the hydroxykynurenine formed could have decomposed non-enzymatically during the incubation.

The low levels of kynurenine transaminase present in cat tissues is probably the chief reason why no appreciable amounts of kynurenic acid or xanthurenic acid have been found in the urine of these animals. On the other hand, the active kynureninase present in both liver and kidney of cats would be expected to convert part of the kynurenine available from tryptophan to anthranilic acid, which may eventually be excreted as a conjugate. Kotake and Nakayama (1) also found high levels of kynureninase in cat liver. However, contrary to the claim of these workers, the enzyme did not show higher activity in cats than in rats in the present studies. A previous report (7) indicated that o-aminohippuric acid was the only tryptophan metabolite definitely present in cat urine, and this was present only in very low concentrations. The high activity of kynurenine hydroxylase in cat tissues suggests that perhaps a major portion of the kynurenine available from tryptophan may be metabolized through this pathway. Experiments performed in this laboratory\(^3\) have shown that, when 3-hydroxy-DL-kynurenine was incubated with extracts of cat liver acetone powder in the presence of Versene, o-phenanthroline, or \(\alpha,\alpha'\)-di-

pyridyl (18, 19), 3-hydroxyanthranilic acid accumulated, as was expected from the kynureninase activity present. Moreover, when hydroxyanthranilic acid was incubated with cat liver tissue, it disappeared rapidly as shown by paper chromatography, and no other metabolite was identified under ultraviolet light or with diazotized sulfanilic acid.

It seems, therefore, that in the metabolic sequence from tryptophan to 3-hydroxykynurenine in unadapted cats, the tryptophan peroxidase system may be, in part, a limiting step. The low kynurenine transaminase activity is probably not able to compete effectively with the other more active enzymes for the small amounts of kynurenine produced. The high activity of kynurenine hydroxylase in cats, as compared to the other enzymes metabolizing kynurenine, suggests that the pathway to 3-hydroxykynurenine is an active one in this species. The observation that cat tissues can metabolize hydroxyanthranilic acid points to the availability of the metabolic process in this direction. The explanation of the low kynurenine transaminase in cats is not clear. Glutamic-oxalacetic transaminase in cat tissues was reported to be within the range of activities found in rats (20). Recently it was found (21) that kynurenic acid was converted to quinaldic acid by rats and humans. The role of this new pathway in the general picture of tryptophan metabolism is still to be studied. The results presented here indicate that cats have appreciable activities of all of the enzymes needed for the conversion of tryptophan to 3-hydroxyanthranilic acid; hence, the explanation of the inability of cats to make niacin from dietary tryptophan is more likely to be found in the enzyme systems in metabolic sequences after 3-hydroxyanthranilic acid.

SUMMARY

A number of enzymes involved in tryptophan metabolism were studied comparatively in liver and kidney of cats and rats in an effort to explain the absence of tryptophan metabolites in cat urine. The activities and distribution were measured for tryptophan peroxidase (liver supernatant fraction), kynureninase (liver and kidney supernatant fraction), kynurenine transaminase (mitochondria and supernatant fractions of liver and kidney), and kynurenine hydroxylase (mitochondria of liver and kidney).

The ratio of activities in cat and rat tissues was for tryptophan peroxidase 0.4, for kynureninase 0.8, for liver kynurenine transaminase 0.1, for kidney kynurenine transaminase 0.03, and for kynurenine hydroxylase 1.7.

In view of the observed enzyme activities, it appears that tryptophan peroxidase is probably the initial slow step in the cat which results in limited production of kynurenine. The absence of detectable kynurenic and xanthurenic acids in cat urine was probably a result of the very low activity
of kynurenine transaminase which competes unsuccessfully with the very active kynureninase and hydroxylase systems for the limited amount of kynurenine.

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