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Evidence for a new oxidative pathway for tryptophan

The conversion of tryptophan to kynurenine is a well established biochemical reaction and with the recent work showing the conversion of kynurenine to 3-hydroxykynurenine[1,2] the overall sequence of metabolic steps from tryptophan to niacin appears to be substantially complete: tryptophan → kynurenine → 3-hydroxykynurenine → 3-hydroxyanthranilic acid → niacin.

The existence of these intermediates has been demonstrated repeatedly. Indeed, so much work has been reported in this area that it has almost obscured the possibility that tryptophan could also be metabolized by a second and hitherto unknown pathway. Although the sequence of reactions outlined above does not account for the ready oxidation of the benzene ring of tryptophan[3], it has been suggested that the enzymic opening of the 3-hydroxyanthranilic acid ring could lead to an oxidizable product instead of to niacin[4].

We wish to report evidence for a new pathway of tryptophan oxidation in rat liver which appears to be independent of kynurenine. The materials used for these experiments were L-[7a-^{14}C]tryptophan and L-[^{14}C]kynurenine singly labeled in the equivalent position of the benzene ring. The tryptophan was synthesized chemically, and resolved enzymically. The L-[^{14}C]kynurenine was prepared enzymically from the L-[7a-^{14}C]tryptophan. Both materials were pure as determined by paper chromatography and radioautography in two sets of solvents. The specific activity of each compound was 1 μC/μmole. Homogenates prepared from the livers of female Sprague-Dawley rats weighing approximately 200 g were used in these experiments. The livers were homogenized in four times their weight of 0.9 % KCl and then centrifuged at 2000 × *g* for 10 min to remove whole cells and debris. Incubations were carried out for 2–2.5 h at 37° in a Dubnoff shaking incubator. CO_2 was collected by means of alkali in a center well and precipitated and counted as $Ba^{14}CO_3$.

It can readily be seen that although tryptophan and kynurenine are quantitatively balanced in the medium, only the tryptophan is significantly oxidized under these conditions. Therefore, kynurenine cannot be an intermediate in the total oxidation observed in these experiments. The possibility exists, of course, that an activated form of kynurenine is produced from tryptophan; one which cannot readily be derived from free kynurenine itself.

Expt. 3 indicates that a reversal of oxidative rates for tryptophan and kynurenine occurred when 0.25 *M* sucrose was used for the medium instead of 0.9 % KCl. This result was also obtained in a second set of experiments. There is no question but that L-[^{14}C]kynurenine *can* be oxidized by broken cell preparations under appropriate conditions. However, in 0.9 % KCl, the evolved CO_2 never amounted to more than 0.05 % and was usually 0.02 % of the starting radioactivity.

TABLE I

OXIDATION OF L-[7a-^{14}C]TRYPTOPHAN AND L-[^{14}C]KYNURENINE TO $^{14}CO_2$ BY RAT LIVER HOMOGENATE

Each incubation flask contained 2 ml homogenate, 50 μmoles potassium phosphate buffer, pH 7.4, 3 μmoles ATP, 12 μmoles Mg^{++} and 3 μmoles each of potassium pyruvate and fumarate in a total vol. of 3 ml.

Expt.	*Substrate**	*Additions*	*% of activity recovered as* $Ba^{14}CO_3$
1a	[^{14}C]T + K	DPNH (3 μmoles)	0.94
1b	[^{14}C]K + T	DPNH (3 μmoles)	0.02
2a	[^{14}C]T + K	DPNH + Pyridoxine	0.27
2b	[^{14}C]K + T	DPNH + Pyridoxine	0.02
2c	[^{14}C]Ac	—	0.6
3a	[^{14}C]T + K	—	1.06
3b	[^{14}C]K + T	—	0.05
3c	[^{14}C]Ac	—	0.82
3d	[^{14}C]T + K	0.25 *M* Sucrose	0.01
3e	[^{14}C]K + T	Medium	0.44
3f	[^{14}C]Ac		1.8

* T = L-tryptophan; K = L-Kynurenine. One μmole of each substrate was used, representing 0.5 μC of activity in the case of the isotopic compounds. [^{14}C]Ac = Sodium [1-^{14}C]acetate (5 μmoles).

Mention should be made of the fact that in about 50 % of the livers used, no significant oxidation of either tryptophan or kynurenine to CO_2 took place. The use of fasted rats appears to have increased the percentage of "active" livers, but conditions have not yet been found which consistently produce a good rate of oxidation.

Additional work *in vitro* has shown that 400 mg of rat-liver slices in 3 ml of Krebs-Ringer phosphate solution can oxidize 2 μmoles L-[7a-^{14}C]tryptophan to $^{14}CO_2$ to the extent of 2–8 % in 2 h. In the case of liver slices from rats previously injected with tryptophan to boost the level of tryptophan peroxidase[5], CO_2 production was of the same magnitude. In the latter experiments, the amount of free kynurenine released into the medium was considerably increased. L-[^{14}C]kynurenine was converted to $^{14}CO_2$ to the extent of 1–3 % in the above experiments.

The authors recognize that the quantitative significance of the pathway whose existence is demonstrated here has not been established. It is hoped that further work will determine the importance of this pathway and the nature of the intermediates involved.

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