α-HYDROXYTRYPTOPHAN, NOT AN INTERMEDIATE BETWEEN TRYPTOPHAN AND KYNURENINE

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Since the discovery of kynurenine by Kotake and Masayama (1), there have been extensive studies of the metabolism of tryptophan. Kynurenine is now regarded as an intermediate in the degradation of tryptophan by animal cells (1) and microorganisms (2). In addition there is evidence that kynurenine is a controlling factor in heredity (3).

However, very little is known about the process by which tryptophan is converted into kynurenine except for some limited enzymatic investigations (1) and indirect genetic experiments (3). Recently Mehler and Knox (4) have shown that N-formylkynurenine is enzymatically converted to kynurenine in a rat liver preparation.

Kotake, in his studies of tryptophan metabolism, postulated α-hydroxytryptophan to be a possible intermediate between tryptophan and kynurenine. Butenandt obtained natural α-hydroxytryptophan from H. Wieland and found it to be active, like kynurenine, in his experiments designed to control hereditary characters of Drosophila. At the same time, he admitted that, quantitatively, the former is less active than the latter and stated "Wir haben gefunden, dass l-α-Oxytryptophan qualitativ die gleichen physiologischen Wirkung entfaltet wie Kynurenin... In quantitativer Hinsicht ist es schwächer wirksam."

Recently, Suda, Hayaishi, and Oda (2) used the adaptive behavior of a Pseudomonas sp. to analyze the metabolic pathways of tryptophan, and their evidence suggests the following sequence of reactions: tryptophan → kynurenine → anthranilic acid → catechol → muconic acid.

Independently, Stanier and Tsuchida (5) working with a different strain of Pseudomonas sp. arrived at the following formulation: tryptophan → kynurenine → kynurenic acid.

Lately one of us (T. S.) (6) has succeeded in obtaining α-hydroxy-DL-tryptophan, and we have applied the method of "successive adaptation" to test Kotake's hypothesis that this substance is an intermediate between tryptophan and kynurenine.
**Behavior of Tryptophan-Adapted Cells and Non-Adapted Cells to α-Hydroxy-DL-tryptophan**—For the details of the principle and the methods of the so called "successive adaptation" the reader is referred to the original article (2).

The Iizaka strain of *Pseudomonas* sp. was used throughout the experiments. So called unadapted cells (i.e., cells not adapted to any compounds related to tryptophan) were grown in acid-hydrolyzed meat peptone medium (ordinary broth), the free tryptophan concentration of which is very low. To prepare the adapted cells, the specific substance, for instance tryptophan in this case, was added to this basal medium at a concentration of 0.2 per cent. The cells were harvested after 16 hours of incubation at 31–33°, washed twice with distilled water, and suspended in /15 phosphate buffer of pH 7.4.

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<th>Table I</th>
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<td><strong>Oxygen Consumption of Adapted Cells</strong></td>
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<tr>
<td>Cell suspension, ml.</td>
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<tr>
<td>Phosphate buffer, m/15, pH 7.4, ml.</td>
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<tr>
<td>Substrate, 0.2 ml.</td>
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<tr>
<td>Total oxygen consumption, c.mm.</td>
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<td>Atoms of oxygen per mole of substrate</td>
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Conditions, 30°, in air; 0.2 ml. of 10 per cent KOH in the central well.

All experiments were carried out in the Warburg respirometer, at a temperature of 30° in an atmosphere of air. All the substrates were neutralized.

As shown in Fig. 1, tryptophan-adapted cells show no marked enzymatic activity towards hydroxytryptophan, whereas they are active with tryptophan and kynurenine. When limited amounts of the substrates are oxidized, adapted cells consume approximately 7.5 moles (15 atoms) of oxygen per mole of tryptophan and 6.0 moles (12 atoms) of oxygen per mole of kynurenine (Table I).

**Presence of Inhibitor**—As stated in previous reports, the enzymatic activity is very sensitive to small amounts of metal ions such as Ag⁺ and Cu++. During the preparation of hydroxytryptophan, stannous chloride was used and the latter had to be excluded as a possible inhibitor.

Double arm vessels were used and tryptophan (0.2 ml. of a 0.005 M solution) and hydroxytryptophan (0.2 ml. of a 0.01 M solution) were tipped into the main compartment at the same time independently. The oxygen consumption curve plotted against the time is just the same as the
curve with tryptophan shown in Fig. 1 and one can conclude that no inhibitory substance was present in the hydroxytryptophan preparation.

*Adaptation to Hydroxytryptophan*—To the ordinary broth as specified above, sterilized hydroxytryptophan was added to a final concentration of 0.2 per cent. Hydroxytryptophan was sterilized by filtration. The activity of the cells was then tested with tryptophan, kynurenine, and hydroxytryptophan.

![Graph showing metabolism of tryptophan and kynurenine by Pseudomonas sp.](image)

**Fig. 1.** The oxidation of L-tryptophan (1 μM), L-kynurenine (1 μM), and α-hydroxy-L-tryptophan (2 μM) by *Pseudomonas* sp. adapted to L-tryptophan. The amount of bacterial cells was determined by turbidimetric measurement to give the same concentration.

**Fig. 2.** The oxidation of L-tryptophan (1 μM), L-kynurenine (1 μM), and α-hydroxy-L-tryptophan (2 μM) by *Pseudomonas* sp. which is grown on ordinary broth without adding excess tryptophan. The amount of bacterial cells was determined by turbidimetric measurement to give the same concentration.

Hydroxytryptophan was found not to be metabolized at all, and the oxygen consumption for tryptophan and kynurenine was quite similar to that of non-adapted cells, as shown in Fig. 2.

**DISCUSSION**

The data obtained show that tryptophan takes up 3 atoms of oxygen more per mole of substrate than kynurenine and therefore indicates the possibility of oxidative conversion of tryptophan to kynurenine. However, synthesized hydroxytryptophan, which was postulated as a possible...
intermediate by Kotake and by Butenandt, does not seem to be an inter-
mediate between tryptophan and kynurenine in the case of this micro-
organism.

The conversion of hydroxytryptophan to tryptophan or kynurenine is also doubtful in this case because hydroxytryptophan is not metabolized when the cells are grown in the presence of this substance.

SUMMARY

1. Hydroxytryptophan is not metabolized by tryptophan-adapted cells or by cells which were grown in the presence of hydroxytryptophan of Pseudomonas, whereas tryptophan and kynurenine are actively oxidized by the tryptophan-adapted cells.

2. It is most probable that α-hydroxytryptophan is not an intermediate between tryptophan and kynurenine in the case of Pseudomonas sp.

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BIBLIOGRAPHY

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