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A POSSIBLE SOURCE OF ERROR IN THE MEASUREMENT OF  
5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN RAT TISSUE

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A POSSIBLE SOURCE OF ERROR IN THE MEASUREMENT OF  
5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN RAT TISSUES

The biosynthesis of 5-hydroxytryptamine (5HT) occurs by the decarboxylation of its amino acid precursor, 5-hydroxytryptophan (5HTP). Many investigations on 5HTP decarboxylase have employed the rat or guinea pig kidney homogenate as a source of the enzyme. The homogenate usually is incubated with the 5 HTP, pyridoxal phosphate and iproniazid or some other monoamine oxidase (MAO) inhibitor in a medium buffered at pH 8.0-8.1. In some instances the incubation is carried out under a nitrogen atmosphere in order to prevent the MAO from inactivating the 5HT which is formed. After incubation aliquots of the mixture are measured for the quantity of 5HT formed.

In some of our recent work on the influence of pH on the metabolism of 5HT by rat tissue homogenates, it was observed that at pH 8.1 5HT was metabolized even in the presence of MAO inhibitors.<sup>1</sup> This process occurred only at the higher pH levels of from 8.1 to 9.5 and was found mainly with homogenates of heart and kidney. Subsequent work with this system indicated that the enzyme cytochrome oxidase might be responsible for attacking the hydroxy group of the indole nucleus. This was supported by the fact that the addition of cytochrome c markedly enhanced the metabolism of 5HT and other hydroxylated indole compounds. KCN also was found to be effective in preventing the oxidation of 5HT from taking place.

The present communication describes experiments demonstrating that when rat kidney homogenate is used as the source of 5HTP

decarboxylase, even in the presence of a MAO inhibitor, the 5HT measured after incubation is not a true representation of the actual amount of the amine synthesized. Rather, under these conditions, 5HT is simultaneously being degraded by the cytochrome oxidase present in the kidney homogenate. If, however, the incubation is done under anaerobic conditions, then the amount of 5HT is an accurate measurement of decarboxylase activity. This is shown in Fig. 1a. The amount of 5HT synthesized by rat kidney homogenate amounted to some 0.8 mole/90 min/flask in the presence of SKF-385 (tranylcypromine), a potent MAO inhibitor. Under anaerobic conditions ( $N_2$ ) the 5HT value rose to 1.3 mole/90 min/flask. The MAO inhibitor did not block the decarboxylation of 5HTP since under anaerobic conditions it has no influence on the biosynthesis of serotonin. KCN, in a concentration of  $10^{-4}$  also increased the final amounts of 5HT formed from 5HTP decarboxylation.

In the presence of pyridoxal-5-phosphate the biosynthesis of 5HT is markedly increased, especially under anaerobic conditions, averaging 3.3 mole/90 min/flask. KCN ( $10^{-4}$  M), however, hardly served to increase the yield of the amine as compared to preparations incubated in air (Fig. 1b). Higher concentrations of KCN decreased 5HT formation by inhibiting the activity of 5HTP decarboxylase.

The degradation of 5HT formed in the presence of MAO inhibitors, does not take place in all tissue preparations. Of those investigated, kidney homogenates of the rat and mouse are capable of exerting this effect at pH 8.0-8.1. The liver, intestinal mucosa and brain of the rat and guinea pig, as well as the kidney of the latter animal, do not exhibit this property. It appears to follow the distribution of the

4-hydroxyindole oxidizing enzyme (presumably cytochrome oxidase) which we reported in our studies on psilocybin and psilocin.<sup>3</sup> In those tissues which do not contain high levels of this enzyme, decarboxylase activity may be measured as well in the presence of adequate concentrations of inhibitors of MAO as when the reaction is carried out under anaerobic conditions.

From this study it is concluded that when heart or kidney homogenate of rat or mouse is used as a source of 5HTP decarboxylase, it is important that the reactions be carried out under anaerobic conditions. This is necessary to block effectively both MAO and cytochrome oxidase in these preparations. The latter enzyme especially requires the anaerobic state, for it can oxidize both the 5HT which is formed as well as the substrate, 5HTP.

#### References

1. A. Horita, Biochem. Pharmacol. 11, 147 (1962).
2. S. Udenfriend, H. Weissbach and B. B. Brodie, Meth. Biochem. Anal. 6, 95 (1958).
3. A Horita and L. J. Weber, Biochem. Pharmacol. 7, 47 (1961).

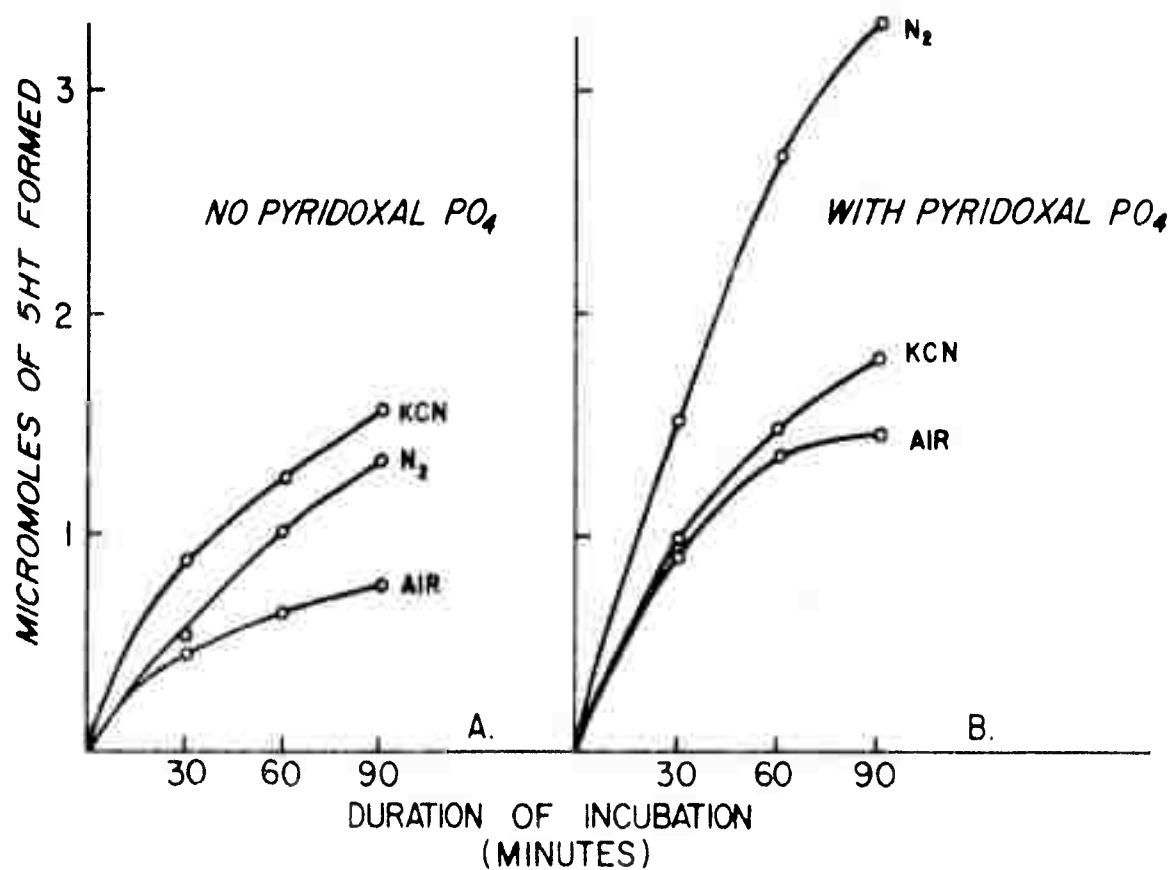


Fig. 1. Graph representing the rate of 5HT synthesis from 5HTP decarboxylation (1) under aerobic conditions, (2) in the presence of  $10^{-4}M$  KCN, and (3) under  $N_2$ . Each point represents the mean values of at least five experiments. Results of Fig. 1a were obtained in the absence of pyridoxal-5-phosphate; those in Fig. 1b were obtained in the presence of 100 mcg/ml pyridoxal-5-phosphate. All flasks contained 10  $\mu$ moles of 5HTP,  $10^{-4} M$  SKF-385, 1.0 ml of rat kidney homogenate (33 per cent), phosphate buffer, pH 8.1. Final volume 3.1 ml. 5HT determinations were made by the nitrosonaphthol method as described by Udenfriend *et al.*<sup>2</sup>

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