# FURTHER STUDIES ON THE BIOSYNTHESIS OF 5-HYDROXYTRYPTAMINE

By Shirley A. P. Price and G. B. West

*From the Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, London, W.C.1*

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The factors affecting the 5-hydroxytryptophan decarboxylase activity of the brain of animals have been studied. By altering the conditions of incubation and the amounts of the constituents of the incubation mixture, the activity as determined by the amount of 5-hydroxytryptamine formed has been increased about 10-fold. Intramuscular injections of cortisone and prednisolone did not reduce the enzyme activity although such treatment lowers the 5-hydroxytryptamine content of several tissues.

Knowledge of the distribution of 5-hydroxytryptamine (5-HT) in the tissues may give an indication of the part it is likely to play in the normal functioning of the body, but knowledge of its formation and destruction is equally important. Studies on the biosynthesis of 5-HT have been reported by various workers[1–3]. Whereas 5-HT is concentrated in the spleen and gastrointestinal tract of most mammals, the highest activity of the enzyme decarboxylating its precursor, 5-hydroxytryptophan (5-HTP), has been found in the kidney, liver and gut, with less in the brain[3]. When the activity of the decarboxylating enzyme in rat brain was measured using methods employed by the earlier workers very low values were obtained. The present experiments were therefore carried out to study the optimal conditions for the conversion of 5-HTP to 5-HT by the enzyme present in brain tissue.

## Methods

### *Preparation of the Tissues for Determining their Content of 5-HTP Decarboxylase*

Brains from freshly-killed animals were weighed, cut into small pieces, and ground in a mortar with a little sand and phosphate buffer (2 ml./g. tissue) of varying pH values. Such treatment extracts the enzyme but not the 5-HT. Aliquots of the homogenates containing the desired quantity of tissue were measured into specimen tubes containing varying quantities of the co-enzyme, pyridoxal-5-phosphate, and of an inhibitor of monoamine oxidase, iproniazid, to prevent the destruction of the 5-HT formed from 5-HTP. Phosphate buffer was then added to the desired volume and the substrate, DL-5-HTP, was added last. Immediately after its addition, the mixture (final volume 5 ml.) was shaken and incubation allowed to proceed at 37° for varying times. The reaction was then stopped by reducing the pH of the solution to 4·0 with N HCl, and the 5-HT content of the solution assayed. The amount of 5-HT formed per gram of tissue may be used as an indication of the 5-HTP decarboxylase activity of that tissue. Each value in Table I represents the mean of three

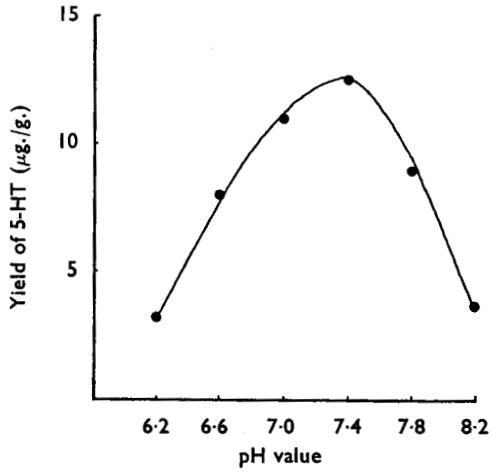


FIG. 1. Effect of pH on the 5-HTP decarboxylase activity of rat brain (as indicated by the yield of 5-HT).

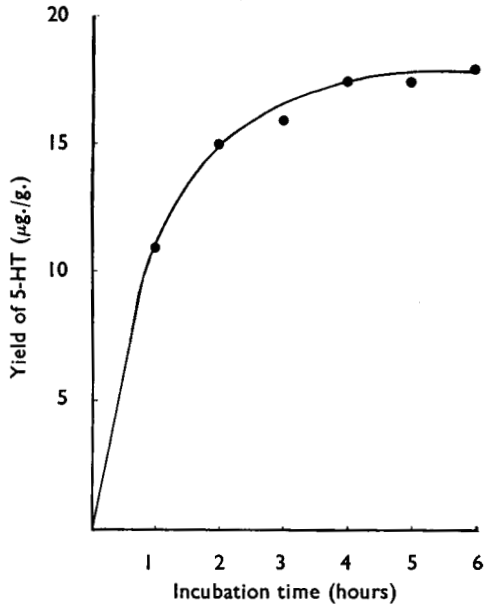


FIG. 2. Effect of incubation time on the 5-HTP decarboxylase activity of rat brain. Incubation at pH 7·4.

separate experiments. In all estimates, control tubes containing the boiled enzyme preparation were incubated and assayed; none showed 5-HT activity.

In a small series of further experiments, the brains from freshly killed rats which had received nine daily doses of cortisone or prednisolone (10 mg./kg. intramuscularly) were used to determine the effect of corticosteroid treatment on the 5-HTP decarboxylase activity in the brain.

Estimates were also made of the decarboxylase activity of mouse, guinea pig and rabbit brains.

*Bioassay Procedure*

Bioassays were made on the isolated uterus of the oestrus rat. An aerated 20 ml. bath of de Jalon's fluid containing atropine ($10^{-7}$) at

TABLE I

EFFECT OF VARYING THE CONDITIONS OF INCUBATION ON THE 5-HTP DECARBOXYLASE ACTIVITY OF RAT BRAIN (AS INDICATED BY THE YIELD OF 5-HT)

| pH | Time of incubation (hr.) | Contents of the incubation mixture | | | | Yield of 5-HT (μg./g.) | 5-HTP decarboxylated per cent |
|---|---|---|---|---|---|---|---|
| | | Pyridoxal (μg.) | Iproniazid (μg.) | Homogenate (mg.) | 5-HTP (μg.) | | |
| 8·0 | 1 | 100 | 100 | 800 | 400 | 3–7 | 3 |
| 7·4 | 1 | 100 | 100 | 800 | 200 | 9–13 | 8 |
| 7·4 | 5 | 100 | 100 | 800 | 200 | 16–20 | 16 |
| 7·4 | 5 | 200 | 100 | 800 | 200 | 25–30 | 24 |
| 7·4 | 5 | 200 | 800 | 800 | 200 | 60–75 | 58 |

TABLE II

THE 5-HTP DECARBOXYLASE ACTIVITY OF THE BRAINS OF DIFFERENT SPECIES (AS INDICATED BY THE YIELD OF 5-HT IN μG./G.). THE RESULTS OF PREVIOUS AUTHORS ARE COMPARED WITH THOSE FOUND IN THE PRESENT STUDY

| Species | Previous work[1,3] | Present work |
|---|---|---|
| Rat | 7 | 7 |
| Mouse | 9 | 155 |
| Guinea pig | 13 | 110 |
| Rabbit | 5 | 73 |

28° was used. On occasion, the extracts were also assayed on the rat colon suspended in a similar bath at 20°. Usually, both preparations were sensitive to 0·01–0·02 μg. of 5-HT (i.e., approximately $10^{-9}$ g.). The specificity of the reaction was checked by using the 5-HT antagonist, 2-bromolysergic acid diethylamide. The standard 5-HT was used as its creatinine sulphate, but values given in the text refer to the base.

## RESULTS

*The 5-HTP decarboxylase of rat brain.* With a phosphate buffer of pH 8·0 and an incubation time of 1 hour, the yield of 5-HT was only 3–7 μg./g. of brain tissue. This is shown in Table I where the amounts of pyridoxal, iproniazid, homogenate and 5-HTP in the incubation mixture are recorded. As only the laevo form of the amino-acid is converted into 5-HT by the brain enzyme, the rate of conversion was about 3 per cent.

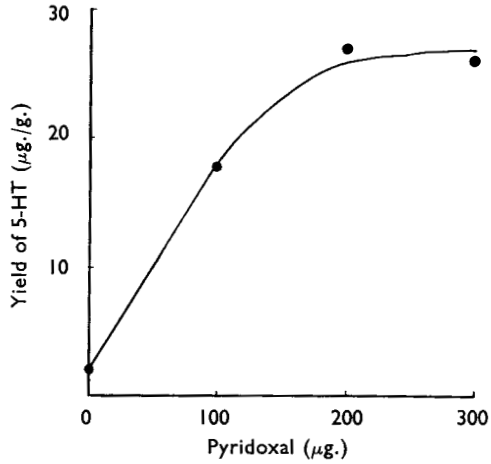


FIG. 3. Effect of pyridoxal on the 5-HTP decarboxylase activity of rat brain. Incubation at pH 7·4 for 5 hr.

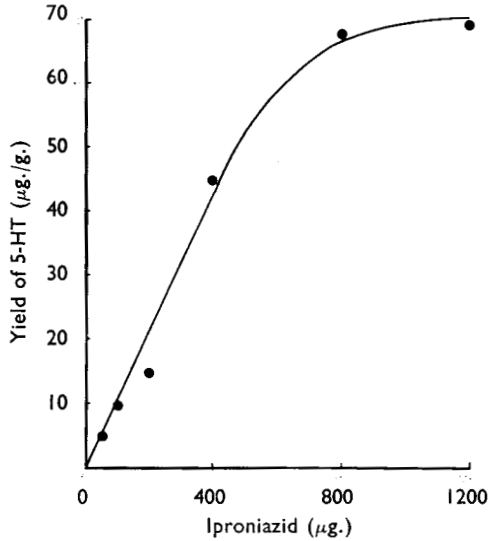


FIG. 4. Effect of iproniazid on the 5-HTP decarboxylase activity of rat brain. Incubation at pH 7·4 for 5 hr. with 200 μg. pyridoxal.

Efforts were therefore made to increase the conversion rate by altering the conditions of incubation.

*Effect of pH and incubation time on the enzyme activity.* Using a wide range of pH values and an incubation time longer than 1 hour, it was possible to increase the yield of 5-HT about four-fold. The optimal pH value was found to be 7·4 (see Fig. 1), incubation at this value for 1 hour yielding 9–13 μg. 5-HT per g. tissue. When incubation was extended to 5 hours the yield was 16–20 μg./g. corresponding to a conversion rate of about 16 per cent (see Fig. 2).

*Effect of pyridoxal and iproniazid on the enzyme activity.* By doubling the amount of pyridoxal in the incubation mixture, the yield of 5-HT was still further increased to 25–30 μg./g. (see Fig. 3). It was then possible to double this yield again by using 8 times as much iproniazid as in the original mixture (see Fig. 4). This large increase in the amount of iproniazid was necessary to prevent completely the action of the highly active monoamine oxidase known to be present in brain tissue.

*Effect of homogenate and substrate on the enzyme activity.* By using a wide range of amounts of both homogenate and substrate, it was not possible to increase the yield of 5-HT above 60–75 μg./g. The optimal value of homogenate was 800 μg. (Fig. 5) whilst the substrate value was 200 μg. (Fig. 6). The percentage of 5-HTP converted into 5-HT was also optimal when these amounts were used. Thus, in the present experiments, the yield of 5-HT (and therefore the 5-HTP decarboxylase activity of rat brain) has been increased 10-fold by altering the incubation conditions; the amount of 5-HTP converted has likewise been increased from 3 to 58 per cent.

*The 5-HTP decarboxylase of mouse, guinea pig and rabbit brains.* Using the optimal conditions for incubating rat brain, an estimate was then made of the decarboxylase activity of the brains of three other species. The results are shown in Table II. The activity has been increased about 10 times.

*The effect of cortisone and prednisolone on the 5-HTP decarboxylase of rat brain.* Treatment of rats with either corticosteroid failed to alter significantly the 5-HTP decarboxylase activity in the brain, the maximal change being an increase of 10 per cent in the activity. This result is surprising as treatment with glucocorticoids results in a considerable reduction in the 5-HT content of many tissues[3].

## Discussion

The 5-HTP decarboxylase of brain has been estimated as the amount of 5-HT formed by 1 g. of tissue. By altering the conditions of incubation, it has been possible to increase the yield about 10-fold in the brains of rat, mouse, guinea pig, and rabbit. The work also illustrates that the conditions for optimal enzyme activity vary widely from tissue to tissue. For example, the brains of these species show optimal activity at a pH value of 7·4, with much less activity at pH values of 8·0 and 6·2, whereas the kidney and liver produce maximal yields of 5-HT when the pH of the incubation mixture is kept at 8·0[1].

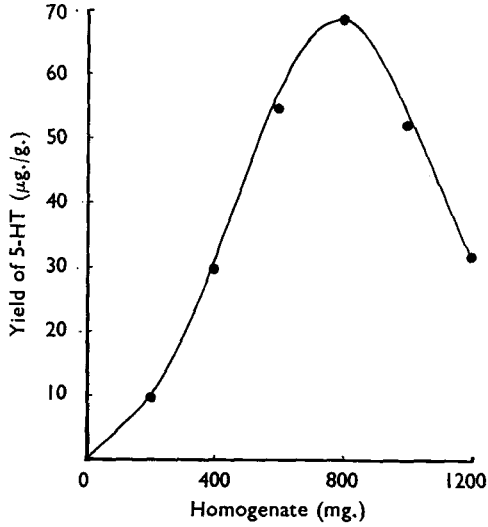


FIG. 5. Effect of varying the amount of homogenate on the 5-HTP decarboxylase activity of rat brain. Incubation at pH 7·4 for 5 hr. with 200 μg. pyridoxal and 800 μg. iproniazid.

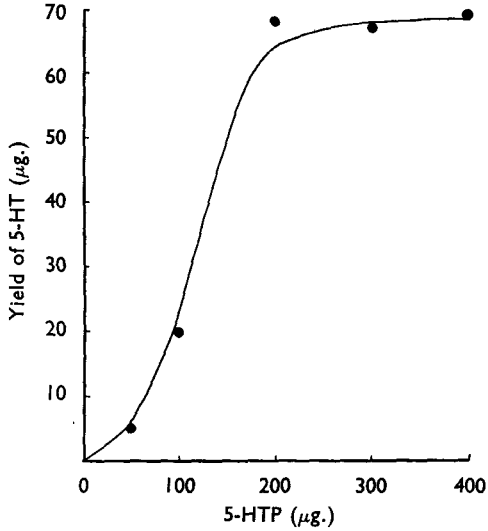


FIG. 6. Effect of varying the amount of 5-HTP on the 5-HTP decarboxylase activity of rat brain. Incubation at pH 7·4 for 5 hr. with 200 μg. pyridoxal, 800 μg. iproniazid and 800 mg. homogenate.

Further, the quantity of monoamine oxidase inhibitor needed in the kidney and liver experiments is some eight times less than that needed for brain incubation experiments. The values obtained for the 5-HTP decarboxylase activities of brain homogenates using the modified conditions of incubation and amounts of constituents show that the brain must be considered, together with the gut, kidney and liver, as one of the chief sources of this enzyme. It is known that 5-HT does not readily cross the blood-brain barrier although 5-HTP does, and the results of the present work indicate that the 5-HT-forming enzyme is particularly active in some animal species. This conclusion does not oppose the hypothesis that the 5-HT content of the brain may be an important factor concerned with the activity of nerve cells in the brain. Further work along this line is indicated, particularly as compounds which deplete the peripheral tissues of their 5-HT fail to modify the activity of the brain 5-HTP decarboxylase enzyme.

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