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# PRELIMINARY STUDIES ON THE BIOSYNTHESIS OF 5-HYDROXYTRYPTAMINE

BY

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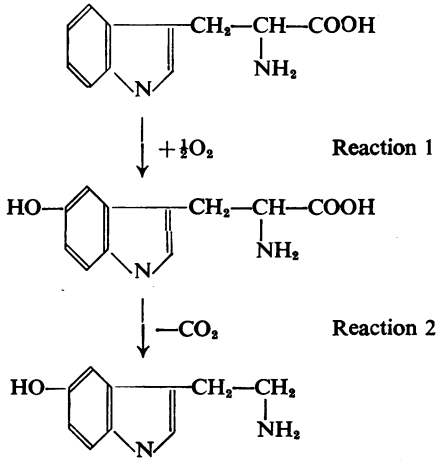
Recent evidence has suggested various theories concerning the origin and function of the 5-hydroxytryptamine (HT) in mammalian tissues (Erspamer, 1954). Its possible role in the central nervous system has been considered in some of the researches of Twarog and Page (1953); Amin, Crawford, and Gaddum (1954); Woolley and Shaw (1954); Shore, Silver, and Brodie (1955); and Paasonen and Vogt (1956).

Udenfriend, Clark, and Titus (1953a, 1953b) and Clark, Weissbach, and Udenfriend (1954) have demonstrated in mammalian tissues two enzymes which are concerned with the formation of HT; tryptophan oxidase, which catalyses the oxidation of tryptophan to 5-hydroxytryptophan (Reaction 1); and 5-hydroxytryptophan decarboxylase, which catalyses the decarboxylation of 5-hydroxytryptophan to HT (Reaction 2).




The work reported in this paper represents a preliminary study of the 5-hydroxytryptophan decarboxylase. A method capable of following the decarboxylation in small amounts of tissue has been developed, and the distribution of the enzyme in the tissues of various species has been traced.

## MATERIALS AND METHODS

*Preparation of Tissue.*—All tissues were freshly excised, carefully cleaned, and weighed in the wet state. Those tissues obtained from the abattoir were frozen with dry ice and carried to the laboratory in a thermos container. The desired amount of tissue was minced finely with scissors and then homogenized in an all-glass homogenizer with M/15 phosphate buffer at *p*H 8.0. The phosphate buffer was prepared after the method of Sørensen by mixing 94.5 ml. M/15-$Na_2HPO_4$ (9.47 g. anhydrous salt/l.) with 5.5 ml. M/15-$KH_2PO_4$ (9.08 g./l.).

Aliquots of the homogenates containing the desired quantity of tissue were measured into conical flasks (10 ml.) which served as the reaction vessels. The reaction mixture in these flasks was as follows:

| | | |
|---|---|---|
| 1. | Homogenate of tissue | 1 ml. |
| 2. | 10 μg. pyridoxal phosphate | 0.1 ml. |
| 3. | 300 μg. choline *p*-tolyl ether (inhibitor of monoamine oxidase) | 0.1 ml. |
| 4. | 5-hydroxytryptophan (55 μg. to 440 μg., i.e., final concentration $5\times10^{-5}$M to $4\times10^{-4}$M) | 0.025–0.2 ml. |
| 5. | M/15-phosphate buffer, *p*H 8.0 | 3 ml. |
| 6. | Isotonic saline to | 5 ml. |

The substrate, 5-hydroxytryptophan, was added last, and immediately following its addition the reaction vessels were loosely stoppered, to allow the free interchange of gases, and placed on a shaking device in an incubator maintained at 37° C. In preliminary studies the incubation was allowed to proceed for varying periods of time, but under our standard conditions all incubations were ended after one hour.

The 5-hydroxytryptophan was kindly supplied by the National Drug Company, Philadelphia, Pennsylvania.

*Extraction of HT.*—This was accomplished by the method of Amin, Crawford, and Gaddum (1954), using

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95% acetone and defatting with petroleum ether. After evaporation to dryness *in vacuo* (at 35° C., external temperature), it was found that the residues could be kept for 48 hr. at −17° C. without loss of activity. Just prior to the assay these residues were dissolved in 0.9% w/v NaCl (1 ml.), and treated with a polyphenol oxidase preparation (Garven, 1956) in order to destroy any catechol amines (such as adrenaline or noradrenaline) in the extracts.

*Preparation of Polyphenol Oxidase.*—About 20 g. of commercially available mushrooms were put through a household mincer. The solid material was then ground further with silver sand for 15 min. and pressed through linen. Approximately 50 ml. of juice was obtained; this was left overnight in the refrigerator at about 4° C. The juice was then distributed into 0.5 ml. portions in small tubes and freeze-dried. The tubes were sealed and kept in the refrigerator until used. The content of each tube was dissolved in saline (5 ml.) and an appropriate aliquot was used according to potency. In general, 0.2 ml. of a 1:10 dilution was adequate for 0.8 ml. of tissue extract. This preparation destroyed all the catechol amines in extracts of sympathetic ganglia, but not of adrenal medullae.

*Bioassay Procedure.*—Bioassays were done on the isolated atropinized uterus of the rat in oestrus, according to the method of Amin, Crawford, and Gaddum (1954). Usually a 2 and 2 design was utilized. With certain extracts parallel assays were carried out by M. K. Paasonen, using the hearts of several species of mollusc (Gaddum and Paasonen, 1955). These results were not significantly different from those obtained with the rat's uterus.

These methods proved capable of dealing with quantities of tissue down to 5 mg., although whenever the amount of tissue was of no consideration in this work 250–333 mg. was used.

## RESULTS

*Action-Time Relationship.*—In order to discover the optimal time of incubation, several experiments were done for various periods of time with homogenates of guinea-pig's kidney, which was found to be the richest source of the decarboxylase, and with a relatively high substrate concentration, $8 \times 10^{-4}$M. The results of a typical experiment, plotted on a logarithmic scale, are shown in Fig. 1. It is clear that the selection of a time near the asymptote, such as 60 min., is likely to give satisfactory results; solutions were therefore incubated for 60 min. throughout this work.

These results demonstrate another point of some significance. The enzyme is a rapidly functioning one, in that detectable amounts (*ca.* 0.9 μg.) of HT were formed within 20 sec. after the addition of appropriate amounts (in this case 880 μg.) of substrate to homogenates of tissue rich in the enzyme.

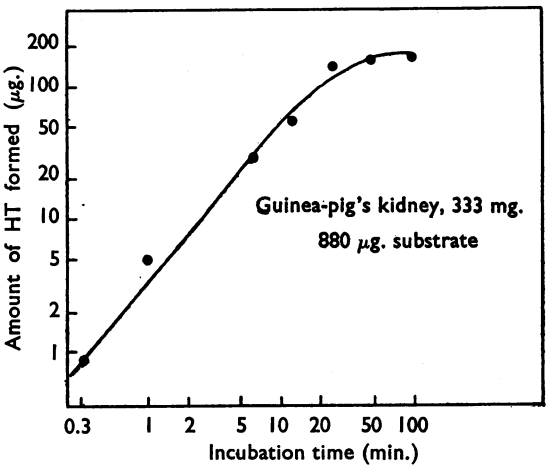


FIG. 1.—The amount of 5-hydroxytryptamine formed by guinea-pig's kidney after incubation for various times. Logarithmic scales. The relation is approximately linear for 45 min. and the reaction then ceased.

*Effect of Substrate Concentration.*—Fig. 2 shows that the relationship of the enzyme to the concentration of substrate is more complex than its relationship to time. With homogenates of guinea-pig's kidney, enzyme saturation, and probably optimal substrate concentration, were reached at $8 \times 10^{-4}$ M (880 μg.). However, nervous tissue, such as the stellate ganglia of oxen, behaved in a qualitatively different manner. Optimal substrate con-

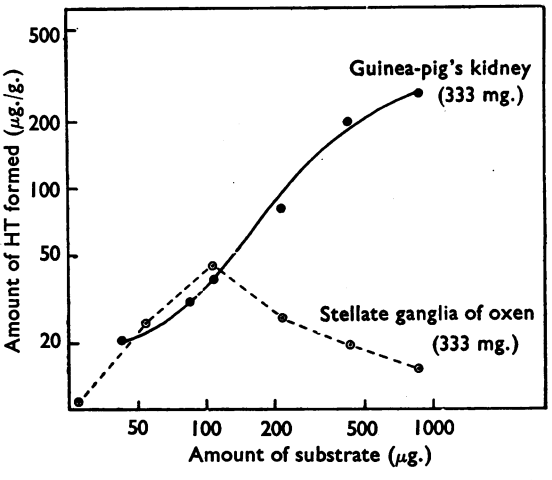


FIG. 2.—The effect of various concentrations of substrate on the amount of 5-hydroxytryptamine formed in one hour. Logarithmic scales. With guinea-pig's kidney 880 μg. of substrate gave a nearly maximal yield. With the stellate ganglia of oxen 110 μg. gave a maximum yield and higher concentrations appeared to inhibit the enzyme.

TABLE I

5-HYDROXYTRYPTAMINE (μG./G. TISSUE) NATURALLY PRESENT AND FORMED FROM ADDED SUBSTRATE ($4\times10^{-4}$M)

No. of observations in parentheses. G and E indicate data of Garven (1956) and Erspamer (1954) respectively

| Tissue | Formed | | | | Naturally Present | | | |
|---|---|---|---|---|---|---|---|---|
| | Guinea-pig | Rat | Rabbit | Man | Guinea-pig | Rat | Rabbit | Man |
| Kidney | 200 (9) | 120 (3) | 45 (3) | — | — | — | 0·03 G | — |
| Liver | 25 (3) | 20 | 15 | — | — | — | 0·4 G | — |
| Stomach | 33 (4) | — | — | — | 1·40 E | 1·40 E | 4·9 E | 3·35 E |
| Spleen | 3·3 | 1·02 | 4 65 | — | 1·06 E | 2 8 E | 19·6 E | 0 83 E |
| Serum | — | — | — | — | 0·21 E | 0·55 E | 3·53 E | 0·12 E |
| Plasma with platelets | 0 | — | 0 | 0 | — | — | — | — |
| Bone marrow | 0 | — | 0·65 | — | — | — | 0·33 G | — |

centration appeared at a lower level, $10^{-4}$M (110 μg.), and substrate concentrations greater than this led to a diminished production of HT.

*Tissue Distribution of 5-Hydroxytryptophan Decarboxylase.*—A number of tissues from different species of animals have been studied for their decarboxylase activity, an effort having been made to include those tissues which are possible sites of HT production.

Table I lists the data for the non-neural tissues studied, arranged in descending order of decarboxylase activity. For the purpose of comparison, estimates are also given, where possible, of the content of HT in these tissues. It is important to note that these values are not directly comparable with the values for decarboxylase activity, since the magnitude of the latter was determined by the amount of substrate put into the system. It will be observed that, while the HT found in spleen and serum (released from the platelets) was relatively high, the HT-forming capacity of these tissues was of low order or non-existent. Both values were low in bone marrow. A contrary lack of correlation was seen in the kidney and liver, where the ability to produce HT was of a high order, while the HT content was quite low.

The results of experiments on portions of the gastro-intestinal tract of the guinea-pig are summarized in Table II. These figures suggest that there is some correlation between the HT in these tissues and their HT-forming capacity. Both values are at a maximum in the duodenum and seem to decline in regions in either direction therefrom.

TABLE II

5-HYDROXYTRYPTAMINE (μG./G. TISSUE) NATURALLY PRESENT IN GASTRO-INTESTINAL TISSUES AND FORMED FROM ADDED SUBSTRATE ($4\times10^{-4}$M)

No. of expts. in parentheses

| Guinea-pig's Tissue | Formed | Naturally Present (Erspamer) |
|---|---|---|
| Stomach, whole | 33·0 (4) | 1·4 |
| ,, fundus | 88·8 (4) | — |
| ,, body | 50·3 (4) | — |
| ,, pylorus | 112·5 (4) | — |
| Duodenum | 127·5 | 5·0 |
| Ileum | 88·4 | 3·4 |
| Caecum | 110·0 | — |
| Colon | 15·0 | 0·7 |

TABLE III

5-HYDROXYTRYPTAMINE (μG./G. TISSUE) NATURALLY PRESENT IN VARIOUS NEURAL TISSUES AND FORMED FROM ADDED SUBSTRATE ($10^{-4}$M)

A, data from Amin, Crawford, and Gaddum (1954); P, data from Gaddum and Paasonen (1955); M, parallel assay on heart of mollusc

| Tissue | Formed | | | Naturally Present | | |
|---|---|---|---|---|---|---|
| | Dog | Ox | Hog | Dog | Ox | Hog |
| Sympathetic ganglia* | 16·6 | 44·7 M | — | 0 P | 0 P | — |
| Peduncles | — | 6·7 | — | 0 A | — | — |
| Pons | — | 2·6 | — | — | — | — |
| Medulla (whole) | 4·7 | 2·6 M | — | 0·03 A | — | — |
| Caudate nucleus | — | 0·6 | 12·7 | 0·12 P | 0·2 P | 0·32P |
| Hypothalamus | — | 0·45 | 1·72 | 0·37 P | — | — |
| Floor 4th ventricle | — | — | 1·5 | 0·1 A | — | — |
| Cerebellar cortex | 0 | — | — | 0·01 P | — | — |
| Motor and premotor areas | 0 | — | — | 0·02 A | — | — |
| Area postrema | — | 0 M | 0 | 0·21 A | — | — |

* Combined stellate and superior cervical ganglia of dogs; stellate ganglia only of oxen.

Finally, sympathetic ganglia and several areas of the brain were examined for decarboxylase activity. The dissections were done with the aid of M. K. Paasonen. The results, together with values for the normal content of HT in the tissues concerned, are summarized in Table III. These tissues are arranged roughly in order of their decreasing ability to form HT. There is some suggestion of a correlation between the distribution of HT and of the enzyme. The values obtained for both were relatively high in the caudate nucleus and hypothalamus, while in the cerebellar and cerebral cortices the values observed were very low or zero. Truly anomalous situations exist, however, in the sympathetic ganglia and in the area postrema. The former tissue had very high decarboxylase activity, but a very low content of HT (Amin, Crawford, and Gaddum, 1954; and Gaddum and Paasonen, 1955). The area postrema, on

the other hand, presented the opposite picture—a relatively high HT-content (Amin, Crawford, and Gaddum, 1954), but no capacity to make HT.

## Discussion

Knowledge of the distribution of an active substance, such as acetylcholine, noradrenaline or HT, in extracts of tissues may give an indication of the part it is likely to play in the body. The discovery of high concentrations of acetylcholine in sympathetic ganglia (Chang and Gaddum, 1933) led to the discovery that it was released on stimulation of preganglionic fibres (Feldberg and Gaddum, 1934). On the other hand, such knowledge may be difficult to interpret. The presence of large amounts of acetylcholine in horse's spleen and human placentae has not as yet been satisfactorily explained.

The amount of an active substance in extracts may depend on a balance between formation and release or destruction, and a knowledge of the rates of these processes is likely to be more valuable than a direct knowledge of the sum of their effects. Active substances may be stored like histamine for long periods in situations where they can have little action because the turnover is small (Ciba Symposium, 1956).

Special difficulties arise with HT, since its presence in a tissue may be due to the accumulation of platelets, which are known to contain large amounts of the material.

The study of the distribution of enzymes concerned with formation is more satisfactory for various reasons: (1) it may give a more direct indication of turnover; (2) it is often applicable to smaller amounts of tissue (Feldberg and Vogt, 1948); (3) it is less likely to be affected by the mode of death of the animal.

The results recorded here are incomplete, but various conclusions may be drawn from them. The differences between the results for different nervous tissues recorded in Table III give strong support to the theory that HT plays a part in the activities of the central nervous system. Any lingering suspicion that the results of studies of the distribution of HT were due to accumulation of platelets can probably now be dismissed. From the available results it is difficult to judge whether the distributions of HT and the decarboxylase in nervous tissue are generally correlated or not, but it is evident that some tissues are exceptional. The area postrema contains particularly large amounts of HT, but no detectable enzyme. Amin, Crawford, and Gaddum (1954) have suggested that HT is probably not produced locally in this area but reaches it from the blood stream. The new results support this suggestion. HT was not found in extracts of sympathetic ganglia even when steps were taken to exclude complications attributable to the presence of adrenaline in the extracts tested (Amin, Crawford, and Gaddum, 1954; Gaddum and Paasonen, 1955; Garven, 1956), but this tissue contained particularly large amounts of the enzyme. This positive result suggests that HT may play a physiological role in this tissue. The nature of this role is obscure, but it may be connected with the sensitizing action of HT observed by Trendelenburg (1956).

The high enzyme activity of extracts of the alimentary canal (Table II) supports Erspamer's (1954) contention that this is an important site of HT production. The lack of enzyme in the platelets and bone marrow is in favour of the view that the HT in the platelets has been absorbed from the plasma (Humphrey and Toh, 1954). It is not in favour of the suggestion of Udenfriend and Weissbach (1954) that HT is synthesized at the site of platelet formation (presumably the bone marrow). The spleen contains large amounts of HT but little enzyme. This is presumably because platelets are broken down in this organ. The distribution of the enzyme through the alimentary canal appears to be similar to that of HT (Table II), maximal concentrations being found in the stomach and duodenum. The fact that comparatively low concentrations of HT were found in the kidneys and livers of rabbits, in spite of the presence of large amounts of the enzyme, may be due to the high activity of monoamine oxidase in these tissues (Blaschko, 1952). The function of the decarboxylase in these organs is particularly puzzling.

Fig. 2 shows that the enzyme in the stellate ganglia of oxen was inhibited by relatively high concentrations of the substrate, while the enzyme in the guinea-pig's kidney was not inhibited in this way. Other experiments, using various amounts of different tissues, seemed to show that this inhibition occurs consistently in nervous tissue and is weak or absent in other tissues. The reasons for this difference are obscure. In the case of cholinesterases a similar phenomenon has been shown to be due to the presence of different enzymes in different tissues (Alles and Hawes, 1940; Mendel and Rudney, 1943). The present observations might also be due to the existence of two enzymes, but there is no other evidence for this at present. Another possible explanation is that in nervous tissue a secondary reaction comes into play, converting the substrate into a derivative which competes with it for the enzyme.

Our method is open to refinement in two respects. The question of the optimal gas phase has not been studied systematically, and it is possible that an anaerobic system might prove more useful and less troublesome, since monoamine oxidase is inactive in the absence of oxygen. Secondly, the use of preparations of the subcellular component in which the decarboxylase resides might also eliminate the problem of enzymic oxidative deamination, because the enzymes responsible may be present in different parts of the cell. These modifications in method are now being studied.

## Summary

1. The enzyme 5-hydroxytryptophan decarboxylase, which forms 5-hydroxytryptamine (HT), was studied by incubating tissue homogenates with the substrate and estimating, by bioassay, the HT formed.

2. Kidney, liver and the gastro-intestinal tract showed high activity. Spleen, platelets, and bone marrow showed little or no activity. These results provide evidence against the theories that significant amounts of HT are normally formed in these last three tissues.

3. Sympathetic ganglia, and some parts of the central nervous system, showed activity; this observation supports the theory that HT plays a physiological role in some nervous tissues.

4. The enzyme in nervous tissues was inhibited by an excess of substrate, but in various other tissues such inhibition was not obtained.

5. There are many differences between the distribution of the enzyme and that of the HT which can be extracted from the tissues.

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