3. N. G. WATON, *Brit. J. Pharmacol.* **11**, 119 (1956).
4. B. SWEDIN, *Acta. med. scand.* **114**, 210 (1943).
5. R. KAPELLER-ADLER, *Biochem. J.* **44**, 70 (1949).
6. R. KAPELLER-ADLER and H. MCFARLANE, *Biochim. biophys. Acta* **67**, 542 (1963).
7. H. M. SINCLAIR, *Biochem. J.* **51**, 10P (1952).
8. A. N. DAVISON, *Biochem. J.* **64**, 546 (1956).
9. D. J. BOULLIN, *Brit. J. Nutr.* **15**, 577 (1961).
10. H. BLASCHKO, C. W. CARTER, J. R. P. O'BRIEN and G. H. S. STANLEY, *J. Physiol. (Lond.)* **107**, 18P (1948).
11. O. GREENGARD and M. GORDON, *Fed. Proc.* **22**, 232 (1963).

Biochemical Pharmacology, 1964, Vol. 13, pp. 805–806. Pergamon Press Ltd., Printed in Great Britain.

A sensitive assay for 5-hydroxytryptophan decarboxylase

(*Received* 6 *February* 1964; *accepted* 3 *March* 1964)

5-HYDROXYTROPTOPHAN decarboxylase (5-HTPD) activity is conventionally measured by manometric[1] or fluorometric[2, 3] techniques. The most sensitive of these methods[3] is capable of detecting the formation of 200 mμg of serotonin during the incubation period. This report describes a simple, sensitive, and specific radiometric assay for 5-HTPD activity which involves the measurement of ^{14}C-serotonin formed from DL-5-hydroxytryptophan-^{14}C. 5-HTPD activity can be routinely determined in tissues which enzymatically produce only 5 mμg of the radioactive amine during 1 hr incubation. Fifty or more assays can be performed in 3 hr. 5-HTPD activity has been measured in some tissues for which no quantitative estimates have been previously available.

METHODS

Tissues were homogenized in ice-cold 0·05 M phosphate buffer, pH 7·4. Enzyme preparation (0·1 to 0·6 ml), 0·1 ml of 0·5 M phosphate buffer (pH 7·4), 0·1 ml of a 1 mg/ml solution of iproniazid, and 0·1 ml of a 100 μg/ml pyridoxal phosphate solution and water to make a final volume of 0·9 ml were incubated in a 15-ml glass-stoppered centrifuge tube in air at 37°. After 3 min prior incubation, 11·4 mμmoles of DL-5-hydroxytryptophan-^{14}C (36,000 cpm, 2·64 mc/mm) was added, and the incubation was continued for 30 min. The reaction was stopped by the addition of 0·5 ml of 0·5 M borate buffer, pH 10. The mixture was saturated with sodium chloride, and the ^{14}C-serotonin formed was extracted by shaking into 6 ml of a 3:2 mixture of 1-butanol and chloroform. After centrifugation, the aqueous layer was aspirated and the organic phase washed with 1 ml of 0·05 M borate buffer, pH 10. Two ml of the resultant organic phase was transferred to a counting vial and evaporated to dryness in a stream of not air. The radioactivity was measured in a liquid scintillation spectrometer after the addition of 3 ml of ethanol and 10 ml of phosphor. Alternatively, the radioactivity in 1 ml of the organic phase was counted directly after the addition of 3 ml of ethanol and 10 ml of phosphor. In this case a correction was made for about 20% quenching. Under these conditions, recovery of ^{14}C-serotonin added to tissues was 90% (±4%), while less than 0·5% of added DL-5-hydroxytryptophan-^{14}C was extracted. All values were corrected for heated enzyme blank.

RESULTS AND DISCUSSION

The reaction was linear with time for at least 2 hr and proportional to tissue concentration in liver over a 5-fold concentration range (Table 1) and in all other tissues examined. At high tissue concentrations the enzymatic activity was not linear.

The product of the enzymatic activity was identified as ^{14}C-serotonin by co-chromatography with the authentic compound in 1-propanol–1 N NH_4OH (75:25) and 1-propanol–1 N acetic acid (75:25).

Specificity was determined by measurement of hepatic 5-HTPD in the presence of dihydroxyphenylalanine, a competitive inhibitor of 5-HTPD. Ninety per cent inhibition was obtained at 10^{-3} M and 63% inhibition at 10^{-4} M concentrations of the inhibitor.

5-HTPD activity was estimated in a variety of tissues (Table 2). Pineal gland tissue had the highest enzyme activity, and rat pineal exceeded bovine or quail pineal in activity. These results are in accordance with the extremely high tissue levels (20–100 μg/g) of serotonin reported for the rat pineal gland.[4,5] It is interesting that the rat duodenum is devoid of 5-HTPD activity, whereas the jejunum and ileum

TABLE 1. EFFECT OF TISSUE CONCENTRATION ON THE ENZYMATIC FORMATION OF ^{14}C-SEROTONIN

Liver aliquot (mg)	cpm	^{14}C-Serotonin (mμmoles formed/mg per hr)
0·2	261 ± 8	3·92
0·4	536 ± 9	4·02
0·6	798 ± 21	3·99
1·0	1,226 ± 5	3·68
2·0	1,800 ± 13	2·70
4·0	2,268 ± 40	1·70

Enzyme preparation, 50 mμmoles pyridoxal phosphate, 0·5 μmoles iproniazid, and 50 μmoles phosphate buffer (pH 7·4) were preincubated for 3 min at 37° in a total volume of 0·5 ml; 11·4 mμmoles of DL-5-hydroxytryptophan-^{14}C was added and incubation continued for 30 min. Results were corrected for blank values (150 cpm) obtained by incubating DL-5-hydroxytryptophan-^{14}C with heated enzyme. cpm are expressed as mean ± average deviation of duplicate samples.

TABLE 2. 5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN VARIOUS ORGANS

Organ	^{14}C-Serotonin (mμmoles formed/g per hr)
Rat liver	3,952
Rat duodenum	0
Rat jejunum	1,628
Rat ileum	1,675
Rat uterus	16
Rat eye	6
Rat salivary gland	2
Rat pineal gland	12,960
Bovine pineal gland	6,020
Quail pineal gland	5,340

have high levels. Gaddum and Giarman[6] examined 5-HTPD in different segments of guinea pig intestine and found that the duodenum has the highest level. The rat uterus, which does not normally have measurable levels of serotonin,[7] has an active 5-HTPD. This might be expected from the findings of Bogdanski *et al.*[7] that, after 5-hydroxytryptophan infusion, serotonin levels of 2–4 μg/g could be detected in the rat uterus.

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REFERENCES

1. P. HOLTZ and E. WESTERMANN, Naunyn-Schmiedeberg's *Arch. exp. Path. Pharmak.* **227**, 538 (1956).
2. A. BERTLER and E. ROSENGREN, *Acta physiol. scand.* **47**, 350 (1959).
3. R. KUNTZMAN, P. A. SHORE, D. BOGDANSKI and B. B. BRODIE, *J. Neurochem.* **6**, 226 (1961).
4. W. QUAY, *Analyt. Biochem.* **5**, 51 (1963).
5. A. PELLEGRINO DE IRALDI, L. M. ZIEHER and E. DEROBERTIS, *Life Sci.* **9**, 691 (1963).
6. J. H. GADDUM and N. J. GIARMAN, *Brit. J. Pharmacol.* **11**, 88 (1956).
7. D. F. BOGDANSKI, H. WEISSBACK and S. UDENFRIEND, *J. Pharmacol. exp. Ther.* **11**, 88 (1956).