THE STABILITY OF SOLUTIONS OF 5-HYDROXYTRYPTOPHAN

By J. W. Hadgraft, Shirley A. P. Price and G. B. West

From the Pharmaceutical Department, The Royal Free Hospital, Gray's Inn Road, W.C.1 and Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, W.C.1.

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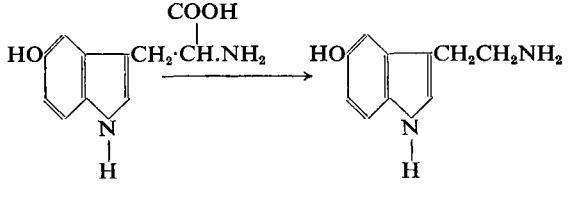
The stability of solutions of 5-HTP contained in ampoules has been studied using physical, chemical and biological methods. No loss of activity occurs when such solutions are sterilised by autoclaving provided the air in the ampoule is replaced by nitrogen. To prevent any formation of colour it is preferable to include 0·1 per cent sodium metabisulphite in the solution.

Recent investigations into the biochemistry of 5-hydroxytryptamine (5-HT) have provided evidence that this amine is formed in the body by decarboxylation of 5-hydroxytryptophan (5-HTP). Since various ataractic drugs mobilise 5-HT from the brain and other organs, the administration of 5-HT should conteract this loss, provided that it can penetrate to the sites where 5-HT normally resides and be stored there. However, it has been shown recently that 5-HT is incapable of readily penetrating the blood-brain barrier. This difficulty may be overcome by administering the amino acid precursor of the amine, as the brain possesses some 5-HTP decarboxylase activity, and injections of 5-HTP have raised the level of 5-HT in the brain and other organs. There is reason to suppose that 5-HT is related to the mechanism of nervous depression, and experimental work along these lines is in progress. Studies on the stability of solutions of this amino acid are therefore necessary.

In the present work, solutions have been maintained at a high temperature for short periods of time, since it is necessary to keep them for many months, or even years, to obtain considerable destruction at low temperatures.

Methods and results

This investigation into the stability of solutions of 5-HTP was made in two parts. In the first, the physical chemical properties of the amino acid were used; in the second, the amino acid was decarboxylated (I into II) and the resulting amine (5-HT) then tested biologically.



(I) 5-HTP (II) 5-HT

Spectrophotometric Estimation of 5-Hydroxytryptophan

The activity of the 5-HTP remaining in solution after heat treatment was determined by spectrophotometry. The material was diluted with 0·01N HCl to a strength of 25 μg./ml. and the ultra-violet absorption measured at 275·5 mμ, E (1 per cent, 1 cm.) = 264 and 295 mμ, E (1 per cent, 1 cm.) = 214[1]. Unheated solutions were similarly diluted with acid and served as controls (100 per cent).

To determine if any other indole compound was formed from 5-HTP by the heat treatment, two-dimensional chromatography was used. Solutions containing the equivalent of 5 μg. of the amino acid were applied to Whatman No. 1 paper and chromatograms were run in a glass tank by the ascending technique first with a solvent of *iso*propanol-ammonia-water (20:1:2) for 16 hours and secondly with *n*-butanol-acetic acid-water (12:3:5) for 6 hours[2]. The chromatograms were developed by dipping the paper in a tank containing an acetone solution of Ehrlich's reagent (dimethylaminobenzaldehyde). The R_F values for 5-HTP in the solvents are *iso*propanol-ammonia-water, 0·17 and *n*-butanol-acetic acid-water, 0·15.

The following solutions each containing 0·5 per cent (w/v) of DL-5-HTP were prepared and transferred to 2 ml. clear glass ampoules which were filled: (1) solution in distilled water; (2) solution in distilled water containing 0·1 per cent sodium metabisulphite; (3) solution in acetate buffer (pH 3·6); (4) solution in oxygen-free distilled water, filled in an atmosphere of nitrogen; (5) solution in oxygen-free acetate buffer (pH 3·6), filled in an atmosphere of nitrogen. All ampoules complied with the test of the British Pharmacopoeia for alkalinity of glass. The oxygen-free distilled water was prepared by boiling the water for 5 minutes in the presence of a stream of nitrogen. After dissolving the 5-HTP, nitrogen was passed through solutions (4) and (5) and also into each individual ampoule before sealing. A few ampoules in each group were kept unheated. The remainder were heated in an autoclave at 115° for 30 minutes. Some of these heated ampoules were then stored in the absence of light for 21 days at 45°. The colour and pH values of the solutions were noted and their ultra-violet absorption measured.

Solutions prepared either in water or in acetate buffer in the presence of air were the only two to show a slight colouration on autoclaving and a slight loss of activity (see Table I). On autoclaving and storage at 45°,

TABLE I

THE EFFECT OF AUTOCLAVING OR AUTOCLAVING AND STORAGE AT 45° FOR 21 DAYS ON THE COLOUR, pH AND ULTRA-VIOLET ABSORPTION (MEASURED AT 275 mμ) OF SOLUTIONS OF 5-HTP. ABSORPTION VALUE IS RECORDED AS A PERCENTAGE OF THAT OF UNHEATED SOLUTIONS.

Solvent	Autoclaved			Autoclaved and stored	
	Colour	pH	275·5 mμ	Colour	275·5 mμ
Water	Light brown	6·8	97	Dark brown	99
Water + metabisulphite	Trace	4·5	101	Light brown	97
Acetate buffer	Light brown	3·6	98	Brown	99
Water (under nitrogen)	Colourless	7·2	101	Trace	100
Acetate buffer (under nitrogen)	Colourless	3·6	100	Trace	101

Similar values for activity were obtained when absorption was measured at 295 mμ.

these two solutions darkened in colour. The solution containing 0·1 per cent of metabisulphite developed a trace of colour on storage and also showed a slight loss of activity. The oxygen-free solutions were colourless after autoclaving but developed a trace of colour on storage. The pH of the solution did not appear to have any significant effect on the stability of 5–HTP. Even the darkest solution on chromatography showed only one spot characteristic of 5-HTP. It was concluded that solutions of 5-HTP may be sterilised by autoclaving without loss of activity if the containers (ampoules) are sealed under nitrogen or contain sodium metabisulphite.

Some ampoules containing sodium metabisulphite as well as being filled under nitrogen showed no sign of deterioration after storage at room temperature for six months, whereas a black precipitate formed in control ampoules filled in air and stored under similar conditions. From these experiments, it appears that sodium metabisulphite (0·1 per cent) only partially inhibits the oxidation of 5-HTP, but is of value to prevent the slight discolouration which occurs when solutions filled under nitrogen are stored for prolonged periods of time.

Biological determination of 5-Hydroxytryptophan

The activity of the 5-HTP remaining in solution after heat treatment was determined by decarboxylating the amino acid and testing the resulting 5-HT on the rat uterus. The 5-HTP decarboxylase was prepared by grinding fresh rat kidneys in a mortar with a little sand and M/15 phosphate buffer (pH 8·0, 1 g. tissue/2 ml. buffer), allowing the mixture to stand and then pipetting off aliquots of the homogenate into specimen tubes which served as the reaction vessels. The reaction mixture in these tubes consisted of rat kidney homogenate (0·2 — 1·6 ml.), the co-enzyme, pyridoxal phosphate (100 μg.), iproniazid, an inhibitor of mono-amine oxidase, (100 μg.), DL-5-HTP (0·05–0·4 ml., 10^{-3}), and M/15 phosphate buffer (pH 8·0) to 5 ml. The substrate was added last and after mixing the solutions incubation was allowed to proceed at 37° for varying periods of time. The mixture was then cooled and the pH adjusted to 5·0 to retard further decarboxylation.

The reaction is specific as no 5-HT activity was detected after incubation in solutions containing boiled enzyme and none was present before incubation. The yield of 5-HT was not increased by using more pyridoxal or iproniazid, and when iproniazid was omitted no 5-HT activity was detected after incubation, the 5-HT being deaminated immediately on formation by the amine oxidase in the enzyme homogenate.

Bioassays were made on the isolated atropinised uterus of the rat in oestrus, according to the method of Parratt and West[3]. Usually a 2×2 design was employed. On occasion, the specificity of the response was checked using the potent anti-5-HT drug, 2-bromlysergic acid diethylamide.

To determine the optimal time of incubation, several experiments were performed using varying periods of time and substrate concentrations. Figure 1 shows the results of experiments with 100 and 800 mg. of kidney

homogenate and 50 μg. of 5-HTP, and similar types of graphs have been obtained with 400 μg. of substrate. The enzyme is a rapidly functioning one and detectable amounts of 5-HT were found within 1 minute after the addition of substrate to appropriate amounts of enzyme. It is clear that 800 mg. of kidney homogenate produced almost maximal conversion of 50 μg. of 5-HTP in 60 minutes, and in all subsequent work solutions were incubated at 37° for 60 minutes.

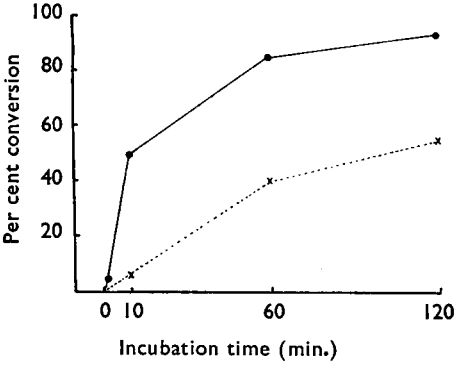


FIG. 1. The effect of varying times of incubation at 37° on the percentage of 5-HTP (50 μg.) converted into 5-HT by 100 m.g. x - - - x or 800 mg. ●—● of rat kidney.

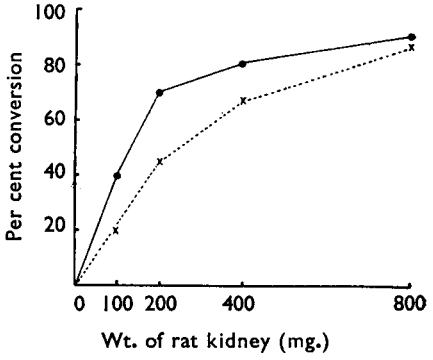


FIG. 2. The relationship between the concentration of 5-HTP decarboxylase activity of rat kidney and the percentage of 5-HTP (50 μg. ●—● or 400 μg. x - - - x) converted into 5-HT. Incubation time is 60 minutes.

The relation between the enzyme content and the concentration of substrate is shown in Figure 2. It appears that 800 mg. of rat kidney can efficiently decarboxylate 400 μg. of substrate in 60 minutes, and in the subsequent assay work the amount of tissue remained at 800 mg. but the substrate content was reduced to 50 μg.

The following solutions each containing 0·5 per cent (w/v) of DL-5-HTP were prepared and transferred to 1 ml. ampoules: (1) solution in distilled water; (2) solution in oxygen-free distilled water, filled in an atmosphere of nitrogen, as described earlier in this paper. The ampoules in the two sets were each divided into four groups—unheated and refrigerated, unheated and stored at 45° for 6 weeks, autoclaved and

TABLE II

THE EFFECT OF HEAT TREATMENT AND STORAGE AT 45° FOR SIX WEEKS ON THE COLOUR AND ACTIVITY, ESTIMATED AS 5-HT, OF SOLUTIONS OF 5-HTP CONTAINED IN AMPOULES SEALED UNDER AIR OR NITROGEN. STANDARD REFERENCE SOLUTION (100 PER CENT) IS UNHEATED FRESH SOLUTION SEALED UNDER NITROGEN

Treatment	Storage	Air		Nitrogen	
		Colour	Activity	Colour	Activity
Unheated ..	Fresh	Colourless	100	Colourless	100
Autoclaved ..	Fresh	Light brown	90	Trace	100
Unheated ..	45°	Dark brown*	52	Colourless	100
Autoclaved ..	45°	Dark brown*	22	Trace	90

* Small black precipitate also present in these ampoules.

refrigerated, and autoclaved and stored at 45° for 6 weeks. The results of the biological assays are shown in Table II. In each experiment, the standard reference solution was an unheated fresh solution sealed under nitrogen. When the ampoule was filled with air and heated (particularly when stored at 45° for 6 weeks), loss of activity was great and the solution darkened considerably. On the other hand, solutions under nitrogen showed no loss of activity and only a trace of colouration. The pH values of all these solutions were similar (7·0–7·2).

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