J. Pharm. Pharmac., 1975, 27, 197

COMMUNICATIONS

An improved method for the determination of exogenous 5-hydroxytryptophan in plasma

The fluorometric method of Udenfriend, Weissbach & Brodie, (1958) for the determination of 5-hydroxytryptophan in plasma has low sensitivity. We have found that the fluorescence intensity resulting from the reaction of 5-hydroxytryptophan with *o*-phthalaldehyde devised by Maickel & Miller (1966) could be enhanced by the addition of L-cysteine, and the recovery of 5-hydroxytryptophan added to plasma could be increased by the addition of *o*-phenanthroline. The resulting procedure is highly sensitive and reproducible, and the recovery is satisfactory.

To assess the effect of a reducing agent on fluorescence intensity, 1 ml of 5-hydroxytryptophan solution (1 μg ml^{-1}) containing various concentrations of ascorbic acid, glutathione, cysteine and homocysteine was taken and 0·1 ml of 0·05% *o*-phthalaldehyde in absolute methanol and 2·0 ml of 10 N HCl were added. The solution was shaken and boiled for 30 min and then cooled to room temperature. The fluorescence intensity was measured at an activating wavelength of 360 nm and fluorescent wavelength 470 nm. Compounds having a thiol group doubled the fluorescence intensity therefore cysteine was used at 0·29 to 0·57 nm.

As 5-hydroxytryptamine and 5-hydroxyindole-3-acetic acid showed some 60% of the fluorescence intensity of DL-5-hydroxytryptophan, they must be removed for the assay of the latter in plasma. For that purpose the butanol and ethyl ether treatments of Udenfriend & others (1958) were used. The result was a reduction in interference to about 5%.

The recovery of 5-hydroxytryptophan added into plasma decreased as the concentration of ferrous ion was increased. To 0·5 ml of plasma obtained from adult rats were added 0·5 ml of various concentrations of 5-hydroxytryptophan and *o*-phenanthroline. The recovery of 5-hydroxytryptophan added into plasma (0·5 ml) in the presence of 0·5 ml of 80 mg% cysteine to which 0·6 N $HClO_4$ (2 ml) (containing 200 mg% disodium ethylenediamine tetra-acetate) was added was significantly higher when the concentration of added *o*-phenanthroline was more than 30 mg% (Table 1). There was no interference with the fluorescence of 5-hydroxytryptophan. A 40 mg% solution of *o*-phenanthroline was therefore used.

Table 1. *Effect of the addition of o-phenanthroline on the recovery of 5-hydroxytryptophan added into plasma.* To 0·5 ml of plasma containing 5·00 μg 5-hydroxytryptophan was added 0·5 ml of each concentration of *o*-phenanthroline. 5-Hydroxytryptophan was determined by the recommended procedure.

O-Phenanthroline (mg%)	Fluorescence intensity	5-Hydroxytryptophan added (μg)	5-Hydroxytryptophan detected (μg)	Recovery (%)
0	8·5	0		
0	40·0	5·00	2·03	41
15	94·0	5·00	4·39	88
30	100·5	5·00	4·69	94
60	98·0	5·00	4·57	91
100	95·0	5·00	4·43	87
100	7·0	0		

Table 2. *Recoveries of 5-hydroxytryptophan added to plasma or brain homogenate.* To 0·5 ml of plasma or brain homogenate was added 0·5 ml of 5-hydroxytryptophan. Fluorescence of 5-hydroxytryptophan was measured with the recommended procedure.

	Plasma		Brain	
Added	Detected	Recovery	Detected	Recovery
(μg)	(μg)	(%)	(μg)	(%)
1·25	1·05	84	1·15	92
2·50	1·95	78	2·10	84
5·00	4·00	80	4·10	82

Recommended procedure. One ml of plasma was pipetted into a conical centrifuge tube. One ml of 1 N HCl containing 0·4 mg of cysteine and 0·2 mg of *o*-phenanthroline and 2·0 ml of 0·6 N $HClO_4$ containing 4 mg of disodium ethylendiamine tetraacetate were added. The supernatant solution (3·0 ml) from centrifugation at 1500 *g* for 15 min was washed twice with about 5 ml of peroxide-free ethyl ether. The organic phase was removed and 0·5 ml of 20% Na_2CO_3 was added to the aqueous phase which was then extracted with about 10 ml of n-butanol. To 1 ml of the residual aqueous phase were added 0·1 ml of 0·05% *o*-phthalaldehyde in absolute methanol and 2·0 ml of 10 N HCl, and the solution was then boiled for 30 min and after cooling it was assayed for 5-hydroxytryptophan fluorimetrically at 360 nm and 470 nm.

The standard curve was linear from 0·01 to 1 μg in the final volume. Recoveries of authentic 5-hydroxytryptophan added to plasma or brain homogenate are shown in Table 2.

In vivo *animal experiment.* 5-Hydroxytryptophan (50–100 mg kg^{-1}) in physiological saline, or physiological saline alone (8 ml kg^{-1}, i.p.), were injected into normal rats 30 and 120 min before decapitation. Plasma 5-hydroxytryptophan was determined by the recommended procedure. Plasma from control rats contains no detectable amounts of 5-hydroxytryptophan but there are small amounts of material which react with *o*-phthalaldehyde HCl. The resulting fluorescence is maximum at fluorescent wavelength 400 nm, activation wavelength 360 nm and should be subtracted from the values obtained after 5-hydroxytryptophan administration. Plasma 5-hydroxytryptophan concentrations 30 and 120 min after 5-hydroxytryptophan (50 mg kg^{-1}, i.p.) were 58·5 ± 4·1 and 23·2 ± 6·8 μg ml^{-1}, respectively, which were expressed as means ± s.e.m. of four rats.

The authors wish to express their deep gratitude to Dr. Y. Kowa, Director of this Research Laboratory, for his encouragement in this study.

From the Safety Research Laboratory,
Tanabe Seiyaku Co., Ltd.,
Yodogawa-ku, Osaka, 532, Japan.

KIYOKAZU SHIMOMURA
TOMIKO FUKUSHIMA
TAMOTSU DANNO

October 2, 1974

REFERENCES

MAICKEL, R. P. & MILLER, F. P. (1966). *Analyt. Chem.*, **38**, 1937–1938.

UDENFRIEND, S., WEISSBACH, H. & BRODIE, B. B. (1958). *Methods of Biochemical Analysis*, Vol. 6, p. 95 New York: Interscience.