ESTIMATION AND URINARY EXCRETION OF TETRAHYDROAMINOACRIDINE

BY P. N. KAUL*

From the Department of Pharmacology, University of Melbourne

Received December 15, 1961

Two methods for the quantitative determination of tetrahydroaminoacridine in aqueous solutions and in urine are described. Four metabolites have been isolated from the rat urine. Two of these, constituting the major proportion of the total metabolites, have also been isolated from human urine and partially characterised.

1,2,3,4-TETRAHYDRO-5-AMINOACRIDINE (THA) was synthesised by Albert and Gledhill (1945) and later shown by Shaw and Bentley (1949) to be a morphine antagonist. It possesses anticholinesterase activity (Shaw and Bentley, 1953) and is a decurarising agent (Gershon and Shaw, 1958). Clinically, THA has been used with morphine in the treatment of intractable pain of terminal carcinoma (Stone, Moon and Shaw, 1961).

Although the pharmacology of THA has been studied at some length, largely in this laboratory, little is known about the metabolic fate of the drug. Studies on the metabolism of a compound like THA are particularly important, for the metabolic products and pathways may lead to an explanation of its pharmacological actions.

This paper reports methods for estimation of THA and some preliminary investigations on the metabolism of the orally and parenterally administered compound by means of urinary excretion studies in the rat and in man.

EXPERIMENTAL

Materials

THA (Monsanto), usually available as a faint yellow powder, was purified to a colourless crystalline product by extracting the base from an aqueous solution at pH 10 into light petroleum or benzene, drying the organic extract over anhydrous sodium sulphate and crystallising the base as hydrochloride. Chloroform and ethylene dichloride were of A.R. grade. Methyl orange reagent consisted of a mixture of equal parts of 0·5 per cent methyl orange solution in water and 0·5 M boric acid solution (pH 5), filtered and kept at 40° to prevent crystallisation.

Methods of Estimation

Method 1. Methyl orange under suitable conditions forms complexes with several organic bases. This property has been used to estimate cinchona alkaloids (Brodie and Udenfriend, 1945) and for example, morphine (Woods, Cochin, Fornefeld and Seevers, 1954). A modification of the original method of Brodie and Udenfriend was adapted for the quantitative determination of THA as follows.

* Present address, Hindustan Antibiotics Ltd., Pimpri, Near Poona, India.

To an impure aqueous solution or urine (10 ml.) containing 0·2 to 3·0 μg./ml. of THA, in a 50 ml. glass-stoppered centrifuge tube, was added saturated sodium carbonate solution (0·2 ml.) to produce a pH not lower than 10, and ethylene dichloride (26 ml.). The mixture was shaken for 7 min. on a mechanical shaker. The tube was then centrifuged at 2,000 r.p.m. for 5 min. A 25 ml. aliquot of the ethylene dichloride extract was transferred to another tube containing methyl orange reagent (0·5 ml.). The tube was shaken mechanically for 7 min. and centrifuged. A 24 ml. aliquot of the coloured organic layer was transferred to a third tube containing 0·5 N hydrochloric acid (4 ml.). The tube was shaken mechanically for 5 min., the acid layer was separated and its extinction at 508 mμ was read against 0·5 N hydrochloric acid on the spectrophotometer (Beckman DU).

Method 2. THA in aqueous solutions shows a characteristic absorption spectrum in the ultra-violet region, with peaks at 323 and 335 mμ. The ratio of the extinctions at these wavelengths characterises THA, whereas the extinction at 323 mμ may be used to measure its concentration in a solution. The procedure adopted for purification and assay was as follows.

To an impure aqueous solution or urine (10 ml.) in a 50 ml. glass-stoppered centrifuge tube were added concentrated ammonia solution (0·2 ml.) and chloroform (23 ml.). The tube was shaken for 7 min. and centrifuged. A 20 ml. aliquot of the organic layer was shaken for 5 min. with 0·5 N hydrochloric acid (5 ml.). After centrifugation, the extinction of the acid layer was measured at 323 mμ against 0·5 N hydrochloric acid saturated with chloroform.

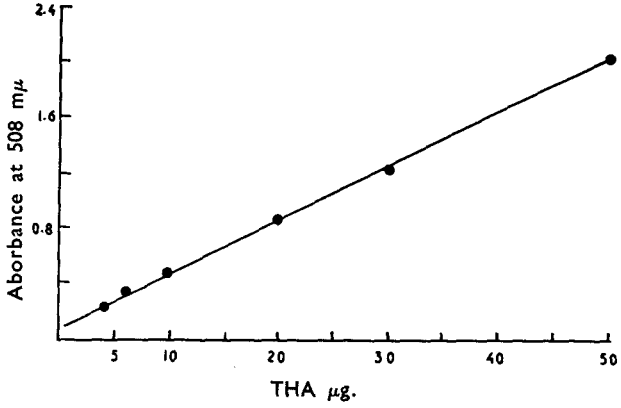

FIG. 1. THA-methyl orange complex.

Suitability of Methods

Fig. 1 shows that the THA-methyl orange complex obeys Beer's law over a wide range of concentrations. The reagent blanks read 0·03 $\pm$ 0·01 O.D. units at the maximum wavelength whereas 0·1 μg./ml. THA read

0·12 units. The urine blanks prepared according to the method of Woods and others (1954), which involves trichloroacetic acid treatment, read 0·05 ± 0·01 while with the present method the blanks were 0·08 ± 0·01.

The urine blanks by method 2 read 0·017 ± 0·003 units at 323 mμ, whereas 5 μg./ml. THA read 0·304 units. This corresponds to a 62 per cent recovery based on direct ultra-violet measurements of pure aqueous solutions.

The metabolites of THA are extractable under the same conditions as the parent compound. They also show a similar absorption spectrum and may be estimated by measuring the extinction at 323 mμ. The total measurable substances in urine have been referred to as "THA".

Application and Results

Urinary Excretion

Rat. A 20 mg./kg. dose of THA was given subcutaneously to 6 rats weighing 192 ± 2 g. Estimations, by method 2, of "THA" in the urine of each rat were made on samples collected 5, 24, and 36 hr. after the injections. Table I, giving the percentages of the excreted "THA", shows that about 47 to 63 per cent of the injected dose is excreted over 36 hr.

TABLE I

Urinary Excretion of "THA" in Rats

	Per cent of the injected dose excreted during				
				Total	
Wt. of rat g.	0–5 hr.	5–24 hr.	24–36 hr.	Estimated	Absolute*
190	27·5	9·2	2·5	39·2	63·2
194	10·0	23·8	3·0	36·8	59·3
190	15·6	13·8	1·9	31·3	50·5
190	13·0	17·4	1·7	32·1	51·9
190	19·4	15·2	1·0	35·6	57·4
192	17·7	10·1	1·6	29·4	47·4

*Absolute value is the actual amount present in a sample assayed. Based on addition and recovery experiments, the estimated value is only 62 per cent of the actual amount present.

Man. Five normal human subjects were given 30 mg. of THA by intramuscular and oral routes, and the urinary excretion of "THA" was followed with time, method 2 being used. Table II gives the per cent of the given dose excreted during the time shown.

TABLE II

Excretion of "THA" in Human Urine

Subjects	Vol. voided	Total hr. of collection	Dose and route	Absolute* per cent dose as "THA"
1	1,570	24	30 mg. I.M.	16·8
2	730	24	,, Oral	7·9
3	1,203	23	,, ,,	6·8
4	1,651	24	,, ,,	5·3
5	470	12	,, ,,	4·7

* See Table I.

Isolation of the Metabolites

Rat urine samples collected after subcutaneous administration of 15 mg./kg. were pooled, adjusted to pH 10 and repeatedly extracted with chloroform. The organic extracts were combined and evaporated under reduced pressure on a rotary vacuum evaporator to a small volume suitable for paper chromatography. The concentrate was fractionated on 3 mm. paper by descending partition chromatography, with butanol: acetic acid: water (4 : 1 : 5) as the solvent system.

The chromatogram showed at least four spots which fluoresced under ultra-violet light. On spraying the paper with iodoplatinate reagent, all the four spots acquired a brown colour characteristic of alkaloidal or heterocyclic nitrogen function. The spots were tentatively labelled as metabolites 1 to 4 (M_1, M_2, M_3 and M_4) in order of their increasing R_F values. None of these four spots corresponded to the authentic THA spotted alongside the metabolite mixture obtained from the urine.

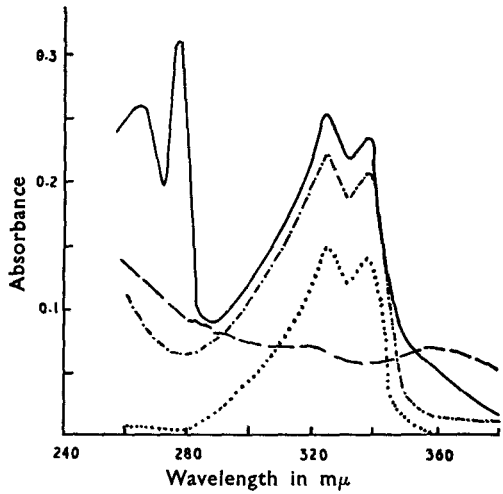

FIG. 2. Ultra-violet absorption spectra of THA and its metabolites. THA . . . ; M_1 — — ; M_3 —·—·— ; M_4 ———.

From a series of unsprayed papers, the corresponding spots were eluted with methanol and their ultra-violet absorption spectra recorded. Fig. 2 shows the spectra of M_3 and M_4 to be similar to that of THA. These two metabolites constitute most of the total metabolites isolated.

The spectrum of the M_4 metabolite shows pH-dependent reversible absorption. At basic pH, hypsochromic and hypochromic shifts occur, indicating the presence of an ionizable nitrogen.

Two metabolites were isolated from the urine of general-surgical subjects receiving intramuscular THA plus morphine. Their partition ratios, R_F values and the electronic absorption patterns suggest that these two substances corresponded to M_3 and M_4 isolated from the rat urine.

Fig. 3 shows an infra-red spectrum of the metabolite M_4 (from rat) compared with that of THA. A strong C = O band at about 5·8 μ is conspicuous.

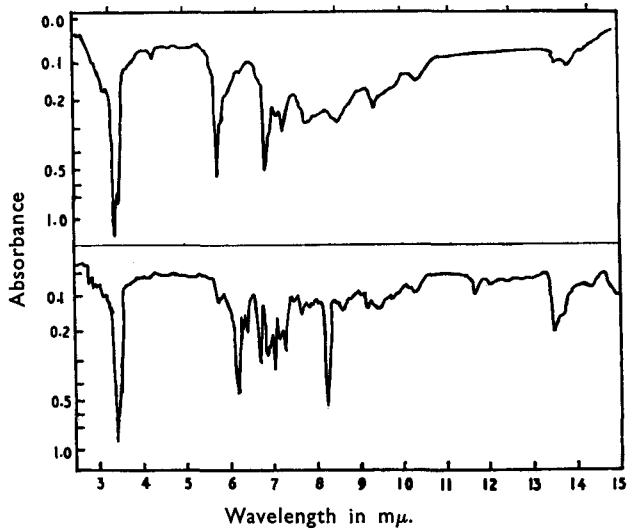

FIG. 3. Infra-red absorption spectra of THA (lower) and M_4 metabolite (upper).

DISCUSSION

The THA-methyl orange complex obeys Beer's law over a wide range of concentrations and therefore this method appears to be sensitive and suitable for solutions from which THA can be isolated before analysis. Woods and others (1954) reported that the morphine complex with methyl orange did not obey Beer's law and suggested the inclusion of a standard morphine sample with each analysis. However, in the present studies morphine was run as a check and it also obeyed Beer's law. Deviation from this law appears to be due to traces of methyl orange carried over while transferring the aliquot of the organic layer, containing the complex, into the acid. A complete separation of the two phases by centrifugation before transfer and careful transfer produces a linear concentration-absorption plot. As little as 0·5 μg./ml. can be determined with 5 per cent accuracy.

Removal of the protein matter from urine with trichloroacetic acid according to the method of Woods and others (1954) appears to be necessary to obtain lower blank values in the methyl orange method.

From the results in Table II it appears that a lower percentage of the administered dose of THA is excreted by the subjects receiving the drug orally. This may indicate that the absorption of oral THA is less complete than the intramuscularly administered drug. Application of the methods to the estimation of THA in blood was unsuccessful.

The ultra-violet absorption spectra of M_3 and M_4 show a close resemblance to that of THA. This may mean that the ring system of the THA

molecule remains almost unchanged. However, a strong absorption band at 5·75 μ in the infra-red spectrum of M_4 reveals the presence of a cyclic carbonyl group in this metabolite. One possible route of bio-origination of this C = O function would be through an oxidative deamination of THA which in biological systems would involve pyridoxal as the co-factor.

Acknowledgement. I am indebted to Professor F. H. Shaw for providing me the funds and facilities for this work and for his interest in it. I am grateful to Miss Anna Winiarski for technical assistance.

REFERENCES

Albert, A. and Gledhill, W. (1945). *J. Soc. chem. Ind.*, **64**, 169–172.
Brodie, B. B. and Udenfriend, S. (1945). *J. biol. Chem.*, **158**, 705–714.
Gershon, S. and Shaw, F. H. (1958). *J. Pharm. Pharmacol.*, **10**, 638–641.
Shaw, F. H. and Bentley, G. (1949). *Med. J. Aust.*, **2**, 868–874.
Shaw, F. H. and Bentley, G. (1953). *Aust. J. exp. Biol. med. Sci.*, **31**, 573–576.
Stone, V., Moon, W. and Shaw, F. H. (1961). *Brit. med. J.*, **1**, 471–473.
Woods, L. A., Cochin, J., Fornefeld, E. G. and Seevers, M. H. (1954). *J. Pharmacol.*, **111**, 64–73.