A new fluorimetric estmination of oxytetracycline in plasma: comparison with microbiological method

D. HALL, F. O'GRADY AND P. TURNER
Departments of Bacteriology and Clinical Pharmacology, St. Bartholomew's Hospital, London, E.C.1, U.K.

Accurate microbiological assay of antibiotics in plasma necessitates rigid control of the many variable parameters inherent to biological systems. As this is difficult to achieve in practice, a large number of replicated readings are required to reduce variations between individual results and consequently such assays are time-consuming. The tendency, therefore, is to reduce the number of replicates performed at the expense of accuracy with the result that most routine estimations only achieve an expected accuracy of $\pm 25\%$ of the true value. This communication describes a rapid fluorimetric method which achieves accuracy at least comparable with that of an accurate microbiological assay. The method adopts the same principle as that of Hayes & DuBuy (1964) in which a fluorescent anhydro-salt is formed by acid hydrolysis followed by measurement of alkaline solution using a spectrofluorimeter. It has, however, the advantages of greater reproducibility than that attributed to previous techniques whilst maintaining simplicity of operation.

Method: 1·0 ml plasma samples are shaken for 15 min with 0·1 ml 1·0N hydrochloric acid to release bound drug, immediately deproteinized by the addition of 0·3 ml trichloroacetic acid/hydrochloric acid (3:1) reagent and vortexed to produce through mixing. The precipitate is centrifuged at 3000 rev min^{-1} for 5 min and 0·9 ml of supernatant removed. 0·6 ml 1·0N HCl containing 1 mg ml^{-1} β-Thiopropionic acid is added and the tubes placed in a boiling water bath for 3 min. β-Thiopropionic acid acts as an antioxidant to prevent subsequent degradation of anhydro-oxytetracycline during assay (Scales & Assinder (1973). On removal, the tubes are rapidly cooled and maintained at 0°C in an ice/water bath; 0·6 ml 6·0N sodium hydroxide is added immediately before reading at 390 nm excitation and 510 nm emission. It is recommended that all samples are assayed in duplicate and the mean read against a standard plot prepared by the addition of oxytetracycline hydrochloride to drug-free plasma. If possible, a plasma blank correction should be made.

The results obtained by this method were compared with those derived from a carefully controlled large-plate microbiological assay using the same plasma samples. The test organism was *Staphylococcus aureus* (6571, Heatley Oxford) on Grove and Randall Medium 8 (pH 5·7). Results were obtained by averaging ten or more readings for each sample, A statistical analysis comparing the two methods showed the results to be highly correlated ($r = 0{\cdot}9805$, $n = 53$, $P<0{\cdot}001$). Furthermore, the fluorescent method offers a number of advantages. Assay time is reduced to 2–3 h compared with 16–18 h required for the microbiological method and sensitivity is increased, with readings possible from less than 0·1 $\mu g\ ml^{-1}$ with linear readings up to 7·0 $\mu g\ ml^{-1}$, thereby extending the measurable range. As it is a physicochemical technique, it offers greater reliability than the microbiological method and has the advantage of facilitating assay in the presence of other microbiologically active compounds without loss of accuracy.

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Modification of trypsin activity by anti-inflammatory drugs

A. J. BAILLIE AND W. ANDERSON
Department of Pharmaceutical Technology, University of Strathclyde, Glasgow, U.K.

Non-steroidal anti-inflammatory drugs (NSAI) can modify (activate and/or inhibit) trypsin (Anderson, Baillie & Gray, 1973) and trypsin or trypsin-like enzymes are believed to be involved in the inflammatory response. The role of the acidic function found in most NSAIs has been accentuated. The NSAIs ketoprofen (acidic), flazalone (non-acidic) and sodium flufenamate (acidic) are now compared as enzyme modifiers in a trypsin system. Solution of modifier (1 ml) was preincubated with azocoll (Calbiochem) substrate suspen-

sion (5 ml; 40 mg in 0·05M tris buffer pH 7·6) for 10 min at 37°. Trypsin (bovine crystalline 7·5−8 × 10^3 BAEE units mg^{-1}) solution (50 μl; 100 μg ml^{-1} in 10^{-3}N HCl) was added and the reaction (37°) stopped after 15 min by filtration. Absorbance (520 nm) of released dye was measured and the percentage change compared with the control calculated, giving the amount of inhibition or activation.

Concentration (mM in digest) of modifier required for 50% activation were found to be: flazalone, 2; ketoprofen, 4; flufenamate, >16. The effect of preincubating a solution of modifier and enzyme at 37° was also investigated by removing 1 ml aliquots of modifier-enzyme mixture into 5 ml substrate suspension (giving 1 mg modifier, 5 μg enzyme in 6 ml digest) and determining inhibition or activation as above.

Table 1. *Enzyme modification by NSAIs after preincubation with enzyme*

Preincubation time (min)	% change in enzyme activity		
	Flazalone	Ketoprofen	Flufenamate
0	+20	+18	−54
20	+24	+59	−75
40	+36	+156	−83

+, activation; —, inhibition

The time-dependent enzyme modification revealed by preincubating these NSAIs with trypsin (Table 1) suggests direct action on the enzyme leading to activation with flazalone and ketoprufen but inhibition with flufenamate. Activation by the latter compound when preincubated with substrate suggests substrate protection of the enzyme. Enzyme modification giving either activation or inhibition, as shown for the NSAIs here, is in accord with the existence of forms of trypsin more or less active than the native enzyme (Krieger, Kay & Stroud, 1974). The non-essential nature of the acid function shown here gives emphasis to the idea that NSAI-induced trypsin modification follows a hydrophobic modifier-enzyme interaction involving the hydrophobic pocket (Keil, 1971) near the active site of the enzyme.

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The effect of hydrocortisone on tryptophan metabolism in the rat

J. HILLIER, J. G. HILLIER AND P. H. REDFERN
Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

Curzon (1969) and Lapin & Oxenkrug (1969) have suggested that depressive illness may be caused by the induction of liver tryptophan pyrrolase by plasma corticosteroids leading to decreased synthesis and turnover of 5-hydroxytryptamine (5-HT) in the brain.

In this investigation the effects of hydrocortisone on brain tryptophan and serum 'free' and total tryptophan concentrations have been determined in the rat. In addition, the endogenous concentrations of 5-HT, the turnover of the amine in the brain and the activity of liver tryptophan pyrrolase have been determined after hydrocortisone injection.

Tryptophan was assayed by the method of Denckla & Dewey (1967); 5-HT by a modification of the method of Snyder, Axelrod & Zweig (1965); 5-hydroxyindole-3-acetic acid (5-HIAA) by the method of Giacalone & Valzelli (1966) and liver tryptophan pyrrolase activity by the method of Knox & Auerback (1955).

The activity of liver tryptophan pyrrolase was increased by 53% 3 h after the intraperitoneal injection of hydrocortisone (15 mg kg^{-1}). However, after the same period serum