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

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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

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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-