Br. J. clin. Pharmac. (1977), **4**, 597–603

PLASMA PREDNISOLONE LEVELS FROM ENTERIC AND NON-ENTERIC COATED TABLETS ESTIMATED BY AN ORIGINAL TECHNIQUE

P.J. MORRISON, I.D. BRADBROOK & H.J. ROGERS[1]

Departments of Forensic Medicine and [1]Clinical Pharmacology and Pharmacology, Guy's Hospital Medical School, London SE1 9RT

1 A quantitative thin layer chromatographic technique for the estimation of plasma prednisolone levels has been devised with a minimum level of estimation of 10 ng/ml.

2 A comparative study of the absorption of 5 and 10 mg prednisolone from enteric and non-enteric coated tablets (5 mg) was carried out in healthy subjects.

3 Mean plasma half-life and peak plasma concentrations obtained from the non-enteric coated preparation agree well with previous studies in normal subjects reported by other investigators using competitive protein binding or radioimmunoassay techniques. Intersubject variability in bioavailability was noted.

4 Enteric coating increased the lag time before prednisolone appeared in the blood but did not alter the bioavailability of prednisolone compared to the equivalent dose of the non-enteric coated tablet.

Introduction

The therapeutic efficacy of corticosteroids is well established and prednisolone is the most popular choice amongst the available glucocorticoids for oral administration. The potential hazards of these drugs are well-known and it has been suggested that peptic ulceration may be reduced by administration of an enteric coated preparation (West, 1959). Prednisolone has however been identified as a drug with a high risk of therapeutic inequivalence related to different bioavailability from various formulations (Academy of Pharmaceutical Science, 1973). The employment of alternate-day steroid therapy as a further means of reducing side-effects (Carter & James, 1972) makes reproducible bioavailability essential. Until recently detailed knowledge of the pharmacokinetics of prednisolone has been lacking and dosage regimes have of necessity been arbitrary. There is some evidence that monitoring plasma prenisolone levels may be helpful in patients developing side-effects (Kozower, Veatch & Kaplan, 1974). It is likely that more detailed knowledge about prednisolone pharmacokinetics will be of value in the design of appropriate therapies.

Two main types of assay have been employed for the estimation of prednisolone in plasma: radioimmunoassays (Colburn & Buller, 1973; Sullivan, Stoll, Sakmar, Blair & Wagner, 1974; Chakraborty, English, Marks, Dumasia & Chapman, 1976) and competitive protein binding methods (Turner, Carroll, Pinkus, Charles & Chattoraj, 1973; Leclercq & Copinschi, 1974; English, Chakraborty, Marks, Trigger & Thompson, 1975). Although both techniques are relatively specific for prednisolone and are capable of the precision required for the determination of therapeutic prednisolone plasma levels they are tedious and time consuming in practice. In this paper we present a new, rapid technique for the estimation of prednisolone in plasma and the results obtained in a study on the comparative bioavailability of the drug from standard and enteric-coated formulations.

Methods

Human volunteer study

Twelve healthy adult volunteers, having had the purpose of the trial explained, consented to take part in the study and were allocated to two groups. Group A consisted of four males (mean age 27 years, range 19–33 years; mean weight 65.8 kg, range 56.4–76.5 kg). Group B consisted of eight females (mean age 28 years, range 23–33 years; mean weight 54.0 kg, range 41.8–61.8 kg). No subject smoked more than ten cigarettes or their equivalent daily and no smoking was allowed on the day of the experiment. Subjects also abstained from alcohol for 48 h before and after each study. A balanced two-way crossover design was employed, the alternative randomly assigned treatments being uncoated 5 mg and enteric coated 5 mg prednisolone tablets (Deltacortril®, Pfizer). Subjects in group A received one tablet, subjects in group B two tablets (10 mg) 2 h after a light breakfast of toast and weak tea or coffee. The tablets were

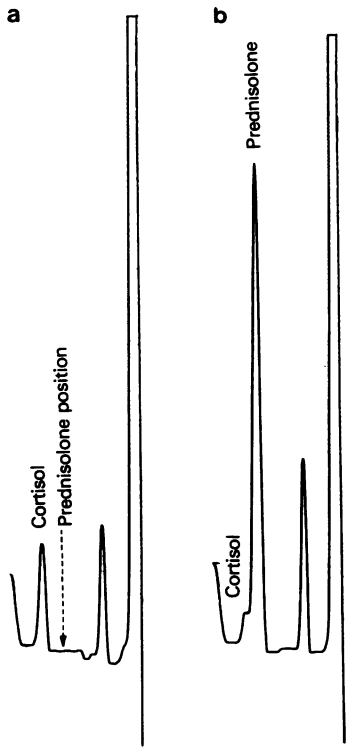

Figure 1 Chromatogram scan parallel to direction of solvent flow (see text). (a) scan of blank plasma showing position of endogenous cortisol peak, (b) scan of plasma taken 1 h after ingestion of 10 mg prednisolone and shows position of prednisolone peak which was equivalent to 220 ng/ml.

swallowed whole, not chewed or broken up, with 50 ml water. Venous blood samples (5 ml) were taken at 0, 0.5, 1, 2, 3, 4, 6 and 8 h after dosing. Plasma was separated immediately and stored at −20°C until assayed. Subjects took a light meal 3 h after dosing and were allowed to drink water at any time during the experiment. Following an interval of at least 7 days each subject was given the alternative treatment.

Prednisolone assay

Plasma (1 ml) was extracted by shaking on a Rolamix (Luckhams Ltd, Burgess Hill, Sussex) for 5 min with 3 ml ethyl acetate (AnalaR) in a 15 ml ground glass stoppered tube and then centrifuged for 5 min at 800 *g*. The supernatant organic phase (2 ml) was transferred to a conical glass centrifuge tube and evaporated to dryness under a stream of nitrogen in a 50°C water bath. The residue was taken up in 15 µl dry acetone (AnalaR) and applied by hand using a micro-syringe as a 5 mm band to a Merck

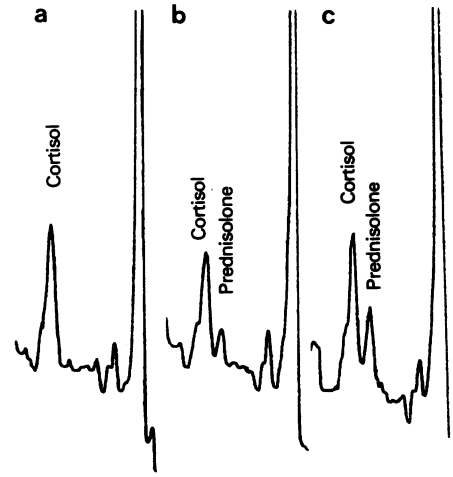

Figure 2 Chromatogram scans parallel to direction of solvent flow showing lower limit of detection. Scans from left to right are (a) blank plasma, (b) plasma with 10 ng/ml prednisolone added and (c) plasma with 20 ng/ml prednisolone added. Prednisolone and cortisone are well separated by this method.

10 cm x 10 cm High Performance Thin Layer Chromatography plate (Kieselgel 60, Type 5631 without fluorescent indicator, suppliers B.D.H. Ltd, Poole, Dorset). Samples (5 µl) were used from subjects in group A, but only 2.5 µl was applied to the plate in the case of samples from group B. The plasma taken before dosing (time 0) and plasma samples containing known amounts of prednisolone were also extracted and chromatographed on the same plate. The plates were developed for 15 min over 9 cm in a chloroform/ethanol (90:10) solvent system. After drying the plates at room temperature they were sprayed with a mixture of concentrated sulphuric acid (Aristar) and ethanol (3.25:1.75) which had been prepared on ice to prevent charring. The plates were then heated for 30–40 min in a 60°C oven.

The fluorescent intensity of the prednisolone bands was measured using a Vitatron TLD 100 flying spot densitometer (suppliers, M.S.E. Ltd, Crawley, Sussex), with a red filter (598 mm) inserted in the emission line.

In instrument settings were: slit size number 2 (4 mm circle); scan mode lin II; damping = 1; level = f; span = 10; scan speed 1 cm/min. Tracings were recorded on a flat bed chart recorder (Vitatron, M.S.E. Ltd) at a chart speed of 1 cm/min.

Results

Chromatography

For the lower levels of prednisolone (<150 ng/ml) encountered in the plasma samples from subjects in

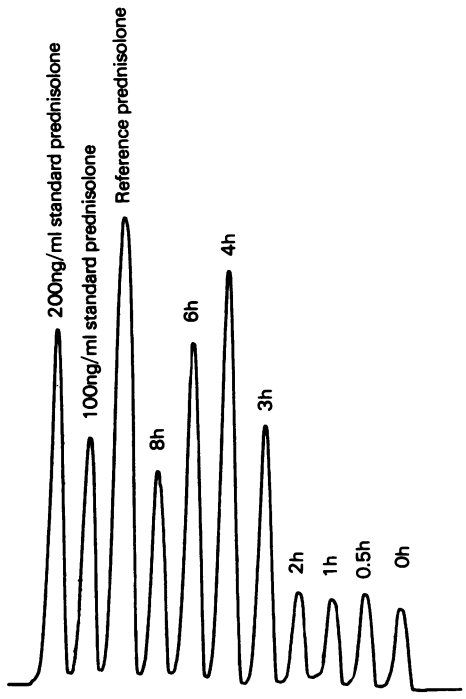

Figure 3 Chromatogram scan parallel to direction of solvent front (see text). Plasma samples taken from subject at times indicated after ingestion of two 5 mg enteric-coated prednisolone tablets.

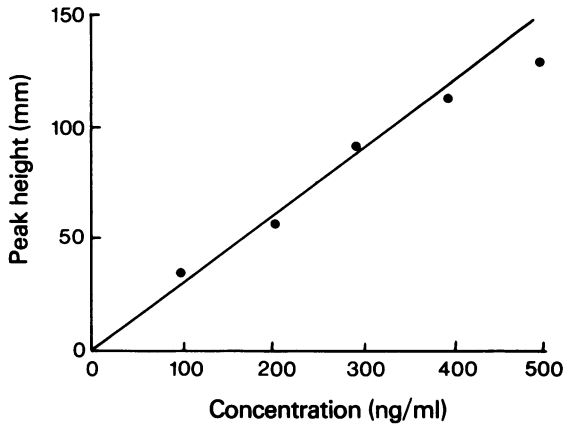

Figure 4 Calibration curve for prednisolone added to plasma and developed on a single plate with samples containing unknown amounts of prednisolone.

Group A the plates were scanned parallel to the direction of solvent flow, starting just before the point of application of the sample. This gave chromatograms not unlike those obtained in gas-liquid chromatography with the sample application point producing a large initial peak similar to the solvent peak (Figures 1 and 2). This method of scanning proved to be the most sensitive and accurate technique: blank plasma gives no interfering peak at the prednisolone position (Figures 1 and 2). For higher plasma prednisolone levels a more rapid technique was found possible by scanning the plates parallel to the solvent front i.e. across the plate. The position of the prednisolone peaks is localized by including a reference sample of prednisolone in each run, the scanner being aligned at the position of the reference sample. This method is extremely quick and proved to give equal linearity. Blank plasma does however give a small peak due to the sulphuric acid charring the track left by the sample (Figure 3).

In all assays a four-point calibration curve was constructed by simultaneously extracting and developing samples of fresh human plasma containing varying amounts of authentic prednisolone on the same plate as the unknown samples.

Sensitivity and precision of the assay

The technique described gave linear results up to concentrations of 400 ng/ml but above this fluorescent quenching may occur and the calibration curve flattens (Figure 4).

A 20 ml blood sample was taken from one subject at 2 h after dosing with 10 mg prednisolone (uncoated preparation) and eight replicate analyses performed on the sample. This gave a mean of 192 ng/ml (s.e. mean 2.3 ng/ml), the coefficient of variation being 3.4%. The lowest concentration of prednisolone that can be measured with confidence (duplicates differing by less than 10%) by the technique is 10 ng/ml.

Plasma level profiles

Plasma prednisolone levels in individual subjects at various time intervals for group A are shown in Figure 5. Those for group B subjects appear in Figures 6 and 7. In both groups prednisolone was present in the plasma 1 h after ingestion of the plain tablets and in most subjects was detected after 30 min. In all except two subjects prednisolone did not appear in the plasma for at least 2 h after the enteric coated preparation had been taken: in these two subjects the lag time for prednisolone appearance was identical with that for the standard tablets. The maximum plasma concentration (C_{max}) and the time at which it was attained (t_{max}) appear in Table 1. There was no significant difference between C_{max} for the two types

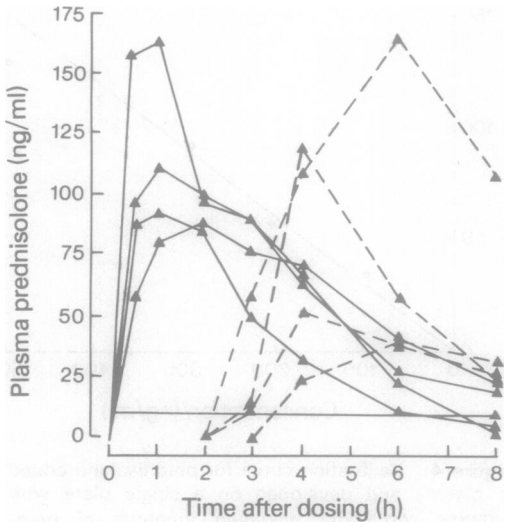

Figure 5 Plasma prednisolone levels in subjects of group A following 5 mg prednisolone (—— non-enteric coated preparation; ---- enteric-coated prearation; continuous line parallel to abscissa is lower limit of estimation of the method).

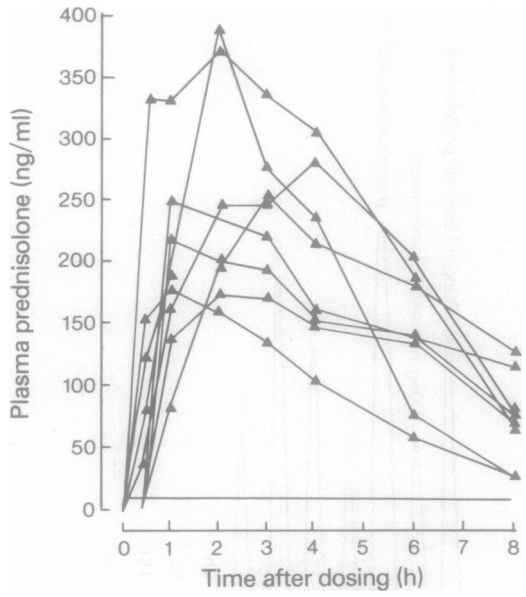

Figure 6 Plasma prednisolone levels in subjects of group B following 10 mg prednisolone as non-enteric coated preparation (continuous line parallel to abscissa is lower limit of estimation of the method).

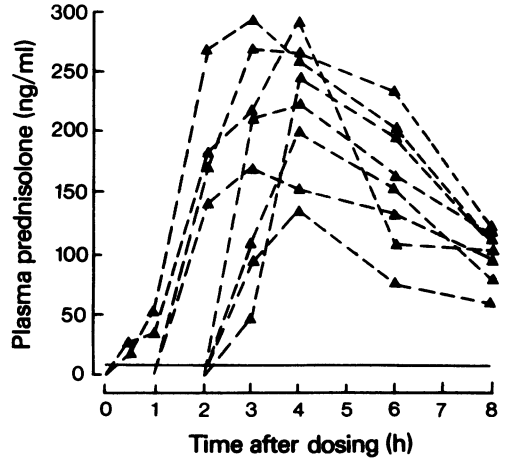

Figure 7 Plasma prednisolone levels in subjects of group B following 10 mg prednisolone as enteric coated preparation (continuous line parallel to abscissa is lower limit of estimation of the method).

of preparation (Student's paired t-test) although interindividual variations of C_{max} occur (Figures 5, 6 and 7). A parallelism between the curves obtained for each preparation in each subject was noted confirming the observations of Leclercq & Copinschi (1974).

Assuming that the absorption rate constants for each preparation are similar once the enteric coating has been removed calculation of the area under the plasma concentration, time curve (AUC) may be compared over equivalent times. This was done by adjusting the curve for the uncoated tablets by simple translation so that equivalent durations of plasma concentration, time data are considered for each formulation. Those areas were calculated by the trapezoid approximation and appear in Table 1. Alternatively the total area under the curve is approximated by

$$\int_0^{\infty} \text{Cpdt} \triangleq \int_0^{t} \text{Cpdt} + \frac{C_p^t}{k} = \text{AUC (Wagner, 1971)}$$

where C_p^t is the last measured plasma concentration at time t and k is the first order rate constant for elimination which was calculated from the half-life. These estimates appear in Table 1. AUCs estimated by either method were found to show no statistically significant difference by Student's paired t-test ($t=0.9$; $0.4<P>0.3$). Semilogarithmic plots were made of plasma concentration, time data and the slope of the line of closest fit to the logarithms of the plasma concentrations measured to obtain a value for the half-life. It was only possible to examine data from experiments with non-enteric coated tablets in this way. The mean half-life from the data of groups A and B was 3.2 h

(range 1.25–8.5 h). Excluding the single value of 8.5 h (from a subject in group B) gave a mean of 2.2 h (s.d. 1.1 h).

Discussion

Previously described assay methods for prednisolone have depended upon either competitive protein binding or radioimmunoassay. The competitive protein binding assay requires suppression of endogenous steroids able to displace tritiated cortisol bound to the plasma corticosteroid binding globulin used to assay prednisolone. This may be accomplished by administering dexamethasone to suppress the adrenal cortex (Leclercq & Copinschi, 1974; Sullivan *et al.*, 1974) or by studying patients already treated with steroids (Hulme, James & Rault, 1975). Alternatively the specificity of this type of assay can be improved by introducing a preliminary thin layer chromatographic step to separate prednisolone from other drugs and metabolites which might interfere with the subsequent protein binding assay (Wilson, Ssendagire, May & Paterson, 1975). This, however, also requires a tritiated prednisolone marker to be added to assess and compensate for extraction losses during the separation of prednisolone prior to elution. The lowest concentration of prednisolone which could be confidently estimated by this method was found to be 20 ng/ml. Contrary to the experience of these investigators we found ethyl acetate to be a useful extraction agent preferring it to dichloromethane since it can be isolated more easily after centrifugation and is also less lipophilic and therefore produces a cleaner extract for the purposes of this assay. A number of developing solvents were assessed for the thin layer chromatographic separation of prednisolone and we found chloroform/ethanol to be superior to dichloromethane/methanol/water in our system since it produced more uniform and denser bands of prednisolone.

Assays based on radioimmunoassay are also subject to cross-reaction by cortisol with the antibody. Colburn & Buller (1973) used a prednisolone conjugate to raise their primary antibody which showed approximately 10% cross-reactivity with endogenous cortisol and about 5% cross-reactivity with prednisone. A more recent assay (Chakraborty *et al.*, 1976) uses antibody raised against a dexamethasone conjugate. This has a cross-reactivity of 6.4% against cortisol and cannot be used in patients treated with dexamethasone although it has little affinity for prednisone. It is also slightly less sensitive than the Colburn & Buller (1973) assay having a useful range of 5–400 ng/ml. Although oral prednisolone will rapidly suppress endogenous cortisol to negligible levels (Shenfield, Paterson, Cøstello & Ijadvola, 1974) the interference with both types of assay will be considerable at lower prednisolone levels unless some form of separation step is used in the procedure. Furthermore, because of the curvilinear nature of the calibration curve obtained in both methods the precision of the assays at high prednisolone levels is relatively poor. Dilution of plasma samples by an empirically determined factor therefore becomes necessary so that prednisolone binding occurs in the most sensitive part of the binding curve.

The assay technique we have described is specific, subject to negligible interference from endogenous steroids, yields a linear calibration curve over the range 0 to approximately 350 ng/ml and can be rapidly carried out using simple apparatus. No special antisera or radioactive isotopes are involved. The estimation of plasma prednisolone levels therefore becomes feasible on a routine basis.

The plasma profiles obtained show that on the non-enteric coated prednisolone tablets the drug is rapidly absorbed and is usually present in the blood within 30 minutes reaching a peak level within 1 to 2 h. These results are consistent with the findings of other

Table 1 Pharmacokinetic parameters characterising rate and extent of absorption of 5 mg (Group A) and 10 mg (Group B) prednisolone from standard and enteric coated tablets

	C_{max} (ng/ml)		t_{max} (min)		Truncated AUC by translation (ng h ml^{-1})		AUC by extrapolation (ng h ml^{-1})	
Group A	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Standard preparation	113	35	75	30	399	103	475	109
Enteric preparation	104	46	188	130	361	176	433	307
Group B								
Standard preparation	266	81	154	105	1196	302	1770	605
Enteric preparation	230	58	210	45	1038	332	1518	655

workers using higher doses of prednisolone (Colburn & Buller, 1973; English *et al.*, 1975; Hulme *et al.*, 1975; Wilson *et al.*, 1975) and comparable with the results of Sullivan *et al.* (1974) who administered 10 mg to their subjects. Expressed in terms of the mean maximum plasma prednisolone concentration attained per milligram dose of non-enteric coated preparation the subjects in group A attained 22.6 ng ml^{-1} mg^{-1}, subjects in group B, 27.0 ng ml^{-1} mg^{-1}. Sullivan *et al.* (1974) found a value of 20.9 ng/ml/mg for a 10 mg dose of prednisolone from tablets commercially available in the U.S.A.

The half-life of prednisolone observed in our subjects demonstrated greater variability than has been reported by others although the mean is similar to that reported by Wilson *et al.* (1975). No obvious difference was identified in the history and habits of subjects with long and short prednisolone half-lives.

An enteric coating is one that resists the action of stomach fluids and disintegrates in the intestines to release the drug from the dosage form (Wagner, 1971). Once in the small intestine the drug should be completely available. The enteric coated preparation studied here (Deltacortril R) appears to fulfil this function for the corrected AUCs (Table 1) for the standard and enteric-coated tablets are not significantly different. The rapid absorption of prednisolone suggests that comparative bio-availabilities based on truncated blood level curves as in this case will be valid (Lovering, McGilveray, McMillan & Tostowaryk, 1975). Two parameters of drug release retardation (Meier, Nuesch & Schmidt, 1974) are the retard quotients $R\Delta$ (half-value duration for enteric to non-enteric preparation) and R_c (ratio of C_{max} for enteric to non-enteric preparation). For the 10 mg dose $R\Delta = 0.9$ (s.e. mean 0.1); $R_c = 1.05$ (s.e. mean 0.1). The data for the 5 mg dose is more scattered but consistent with these values which suggest that the plasma concentrations produced by the two preparations are approximately equal in size. The same therapeutic effects would therefore be expected from both preparations. This may be contrasted with the results of the study by Hulme *et al.* (1975) on five renal transplant recipients who were given 30 mg prednisolone as either enteric or non-enteric coated tablets. In this case the enteric-coated tablets produced later peaks in only three patients and in all cases C_{max} was lower. This suggests either that the enteric coating was less effective in their preparation or that patients, who were also receiving azothioprine, absorb drugs from enteric-coated preparations less satisfactorily.

At present it is unclear which type of prednisolone preparation produces least suppression of the pituitary-adrenal axis. Some suggest that delayed release preparations produce less suppression than standard formulations (English *et al.*, 1975), others assert that long acting steroids are more suppressant (Carter & James, 1972) whilst other workers find that standard preparations of prednisolone produce no significant suppression (Turner *et al.*, 1973). Cortisol was also present as a green fluorescent band on the chromatography plate and a technique for estimating cortisol by this means has been devised (Morrison, unpublished observations). The peak for cortisol is visible in Figures 1 and 2. It was noted during the present work that the cortisol level rapidly fell as the plasma prednisolone concentration increased over the 8 h sampling period.

Our results suggest that the enteric-coated preparation studied here (Deltacortril®) behaves much like a standard release preparation with a longer lag-time. It is not appropriate to draw any conclusions from these findings with regard to therapeutic effects since the pharmacokinetic behaviour required for optimum clinical efficacy remains unknown.

We are grateful to Professor J.R. Trounce for his interest in this work and to Dr F. Bateman of Pfizer Ltd for his help during the study. Reprint requests should be addressed to H.J.R.

References

ACADEMY OF PHARMACEUTICAL SCIENCE (1973). An annotated list of drugs with a potential for therapeutic inequivalence based on current evidence of drug product bioavailability inequivalence. *J. Am. pharm. Ass.*, **13**, 279–280.

CARTER, M.E. & JAMES, V.H.T. (1972). Effect of alternate-day, single dose, corticosteroid therapy on pituitary-adrenal function. *Ann. rheum. Dis.*, **31**, 379–383.

CHAKRABORTY, J., ENGLISH, J., MARKS, V., DUMASIA, M.C. & CHAPMAN, D.J. (1976). A radioimmunoassay method for prednisolone: comparison with the competitive protein binding method. *Br. J. clin. Pharmac.*, **3**, 903–906.

COLBURN, W.A. & BULLER, R.H. (1973). Radioimmunoassay for prednisolone. *Steroids*, **21**, 833–846.

ENGLISH, J., CHAKRABORTY, J., MARKS, V., TRIGGER, D.J. & THOMPSON, A.G. (1975). Prednisolone levels in the plasma and urine: a study of two preparations in man. *Br. J. clin. Pharmac.*, **2**, 327–332.

HULME, B., JAMES, V.H.T. & RAULT, R. (1975). Absorption of enteric and non-enteric coated prednisolone tablets. *Br. J. clin. Pharmac.*, **2**, 317–320.

KOZOWER, M., VEATCH, L. & KAPLAN, M.M. (1974). Decreased clearance of prednisolone, a factor in the development of corticosteroid side effects. *J. clin. Endocrin. Metab.*, **38**, 407–412.

LECLERCQ, R. & COPINSCHI, G. (1974). Patterns of plasma levels of prednisolone after oral administration in man. *J. Pharmacokin. Biopharm.*, **2**, 175–187.

LOVERING, E.G., McGILVERAY, I.J. & McMILLAN, I. & TOSTOWARYK, W. (1975). Comparative bio-

availabilities from truncated blood level curves. *J. pharm. Sci.*, **64**, 1521–1524.

MEIER, J., NUESCH, E. & SCHMIDT, R. (1974). Pharmacokinetic criteria for the evaluation of retard formulations. *Eur. J. clin. Pharmac.*, **7**, 429–432.

SHENFIELD, G.M., PATERSON, J.W., COSTELLO, J.F. & IJADVOLA, O. (1974). The effect of prednisone treatment on the half-life of intravenous cortisol. *Br. J. clin. Pharmac.*, **1**, 237–240.

SULLIVAN, T.J., STOLL, R.G., SAKMAR, E., BLAIR, D.C. & WAGNER, J.G. (1974). *In vitro* and *in vivo* availability of some commercial prednisolone tablets. *J. Pharmacokin. Biopharm.*, **2**, 29–41.

TURNER, M.K., CARROLL, C.J., PINKUS, J.L., CHARLES, D. & CHATTORAJ, S.C. (1973). Simultaneous competitive protein binding assay for cortisol, cortisone and prednisolone in plasma and its clinical application. *Clin. Chem.*, **19**, 731–736.

WAGNER, J.G. (1971). *Biopharmaceutics and Relevant Pharmacokinetics*, Chapter 23. Hamilton, Illinois: Drug Intelligence Publication.

WEST, H.F. (1959). Prevention of peptic ulceration during corticosteroid therapy. *Br. med. J.*, **2**, 680.

WILSON, C.G., SSENDAGIRE, R., MAY, C.S. & PATERSON, J.W. (1975). Measurement of plasma prednisolone in man. *Br. J. clin. Pharmac.*, **2**, 321–325.

(Received December 12, 1976)