

Plasma prednisolone levels and adrenocortical responsiveness after administration of prednisolone-21-phosphate as a retention enema

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SUMMARY Plasma prednisolone levels have been measured by radioimmunoassay after oral and rectal administration to healthy volunteers and to patients with idiopathic proctocolitis. The amount of prednisolone absorbed from a 20 mg retention enema given to patients with proctocolitis was about 44% of that absorbed from the same dose orally administered. Adrenocortical response to synthetic ACTH in patients receiving prolonged rectal therapy was either normal, or only slightly impaired, and this may be related to the pattern of steroid absorption rather than to the total amount absorbed.

With the development of various techniques for the assay of prednisolone in plasma, much has been learnt about the pharmacokinetics of this drug after oral administration in health and disease (Disanto and Desante, 1975; Meikle *et al.*, 1975; Schalm *et al.*, 1977). Although prednisolone-21-phosphate retention enemata are well established as effective treatment for idiopathic proctocolitis, particularly if the disease is localised to the sigmoid colon or rectum (Matts, 1961), very little is known about the pharmacokinetics of the drug after rectal administration. Whether the beneficial action of rectally administered prednisolone is due to local or systemic action, or a combination of the two, and, more importantly, whether prolonged therapy results in adrenocortical suppression have been the subjects of some dispute (Spencer *et al.*, 1960; Sanbar and West, 1961; Matts *et al.*, 1963; Wood *et al.*, 1964; Halvorsen *et al.*, 1969; Multicentre Trial, 1971; Powell-Tuck *et al.*, 1976). In the present study we have measured plasma prednisolone levels in normal subjects and in patients with distal proctocolitis, after oral and rectal administration, and have examined adrenocortical responsiveness after prolonged rectal therapy.

Methods

SUBJECTS STUDIED

Ten subjects received a standard 20 mg prednisolone

retention enema (Predsol: Glaxo) (Table 1). Eight had suffered either an acute relapse of their proctocolitis as judged by symptomatology, sigmoidoscopic appearance and rectal biopsy (Baron *et al.*, 1964), or were entering remission after exacerbation of their disease, after treatment with prednisolone retention enemata. One other subject (no. 9), had been diagnosed as suffering from proctocolitis in the past but at the time of study showed no evidence of the disease, and yet another (no. 10), was a healthy volunteer. All subjects remained supine for at least half an hour after administration of the enema, which was retained in every case for a minimum of four hours. At the end of the test period eight of the subjects received an intramuscular injection of 250 μ g synthetic ACTH (Synacthen: CIBA).

Nine healthy volunteers (six male, three female: age range 18-72 years) received oral prednisolone on a total of 12 occasions. The dose given was 0.3 mg/kg of bodyweight (range 17-22 mg: mean 20 mg), administered as crushed tablets made into a slurry with tap water and taken after an overnight fast. The tablets were given in this fashion to try to eliminate intersubject variations in plasma concentrations that may have been due to differences in disintegration times. Approximately two hours later the subjects were allowed coffee or tea, and subsequently throughout the day were allowed food and liquid *ad libitum*. Normal daily activities were permitted.

An indwelling cannula was inserted into a forearm

Received for publication 2 October 1978

Table 1 Details of patients who received prednisolone retention enema

Case no.	Age (yr) and sex	Extent of disease	Sigmoidoscopic appearances	Duration of previous local steroid therapy	
				(weeks)	(daily dose in mg)
1	23:F	Proctosigmoiditis	Inactive	3	20
2	75:M	Proctosigmoiditis	Moderately active	6	40
3	38:M	Proctosigmoiditis	Inactive	3	40
4	43:F	Proctosigmoiditis	Moderately active	2	40
5	30:F	Proctosigmoiditis	Active		Nil
6	66:F	Proctosigmoiditis	Active		Nil
7	29:F	Proctosigmoiditis	Active		Nil
8	48:F	Proctosigmoiditis	Active		Nil
9	73:F	—	Normal*		Nil
10	23:M	—	Normal†		Nil

*Colitis diagnosed in past. No recent evidence of disease.

†No history of any bowel disease.

vein at approximately 8.00 a.m., and 30 minutes later (after the subjects had become used to the cannula), a 5 ml blood sample was taken, and the prednisolone (administered orally or rectally) was given. Subsequent samples were taken at 20 minute intervals for four hours and then hourly for another three hours. The blood samples were taken into lithium heparin tubes and at the end of the test period these were centrifuged and the plasma stored at -20°C until assayed.

Laboratory methods

PREDNISOLONE ASSAY

Plasma prednisolone was measured using a radioimmunoassay technique derived from that originally described by Colburn and Buller (1973). The plasma was pre-extracted with hexane to remove interfering lipids, and the steroid was then extracted with dichloromethane. Appropriate aliquots of the extracts were pipetted into tubes and evaporated to dryness *in vacuo*. Antiserum and tritiated prednisolone in borate buffer were added to the residues and the mixture incubated overnight at 4°C . The unbound label was adsorbed onto dextran-coated charcoal, and after centrifugation the bound tritium in the supernatant was measured by liquid scintillation counting. A standard curve of the percentage of tritiated prednisolone bound over the range of 0-40 ng/ml was prepared for each assay, and the unknown concentration of prednisolone obtained by interpolation. The precision was determined by assaying all plasma samples in duplicate and the coefficient of variation for single samples within-batch did not exceed 10%. Quality control samples assayed in duplicate were used to monitor inter-batch precision. Most of the samples were assayed using an antiserum raised in sheep against dexamethasone, but which cross-reacted 100% with prednisolone (a gift from Racecourse Security

Services Research Laboratory Limited, Newmarket). The antiserum cross-reacted *in vitro* to a lesser extent with cortisol (19%) and prednisone (40%). To check the validity of the assay, the plasma prednisolone concentration in several samples was measured after separation by paper chromatography, and the results compared with those obtained in the direct assay. There was a highly significant correlation between the two sets of results ($r = 0.96$, $P < 0.001$), with no difference between them except at zero time—that is, before any prednisolone had been given—indicating that the cross-reacting steroids were not interfering with the assay. Cortisol was detected at zero time, because of its cross-reaction with the antiserum and this is why there was apparently prednisolone in the plasma at the start of the test. This ceased to be of significance, not only because of the increasing prednisolone concentration, but also because the plasma cortisol level decreased through the day.

The remaining samples were assayed using an antiserum raised specifically against prednisolone in rabbits (a gift of Dr M. Hayes, University of Surrey, Guildford), which had very little cross-reaction with cortisol (10%) or prednisone (5%). Comparability of the results using the two different antisera was confirmed by using the internal quality controls.

CORTISOL ASSAY

The cortisol concentration was measured in all plasma samples by an established fluorimetric technique (Townsend and James, 1968), which was unaffected by the presence of prednisolone.

CALCULATION OF PHARMACOKINETIC PARAMETERS

The peak plasma prednisolone concentration (C_{max}) and the time to reach peak concentration (T_{max}) were obtained from the plasma concentration-time

curves. The plasma half-life of prednisolone ($T_{\frac{1}{2}}$) was determined by plotting semilogarithmically the concentrations during the elimination phase of the drug against time, and measuring the slope of the line of best fit. The area under the plasma concentration-time curve (AUC) was calculated by a modification of the trapezoid method (Notari, 1971) using a specific programme on a model 9801A Hewlett Packard calculator. Assessment of statistical significance was made using Student's *t* test.

Results

The individual plasma concentration-time profiles for the two groups are shown in Figs 1 and 2. There was less inter-subject variation in plasma levels in the group who received prednisolone rectally than in those receiving the oral drug. There were no significant differences in the C_{max} , T_{max} or AUC

between patients with active, untreated proctocolitis and those whose disease had been treated with corticosteroids. However, the C_{max} in the two subjects with no evidence of bowel disease at the time of study was higher than in the rest of the group, but the numbers are too small for statistical comparison. The pharmacokinetic data are shown in Tables 2 and 3. After oral prednisolone, the C_{max} ($P < 0.001$) and the AUC ($P < 0.001$) were significantly greater than after rectal administration and the T_{max} was significantly shorter ($0.01 > P > 0.001$). However, there was no significant difference between the mean half-lives ($P > 0.5$).

The mean plasma concentration-time curves are shown in Fig. 3. Although oral prednisolone was given on a weight-related basis, the mean dose was similar to that administered by the rectal route. It has been demonstrated that it is not necessary to extrapolate the plasma concentration-time curve

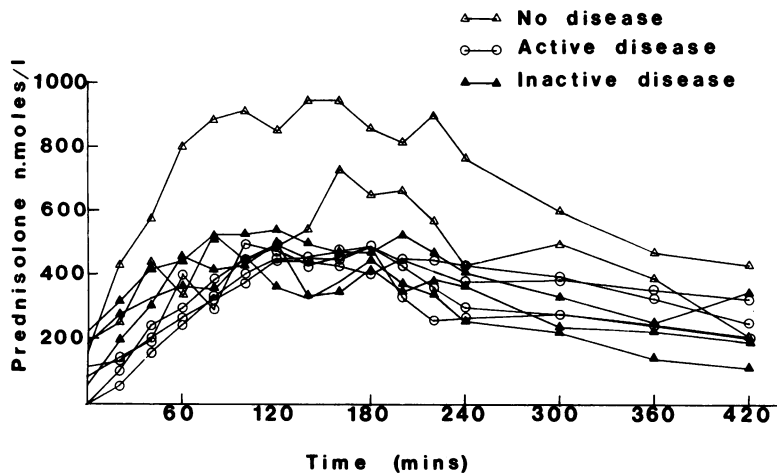


Fig. 1 Individual plasma concentration-time profiles after administration of a prednisolone retention enema. Conversion: SI to traditional units—plasma prednisolone: $1 \text{ nmol l}^{-1} = 0.36 \text{ ng ml}^{-1}$.

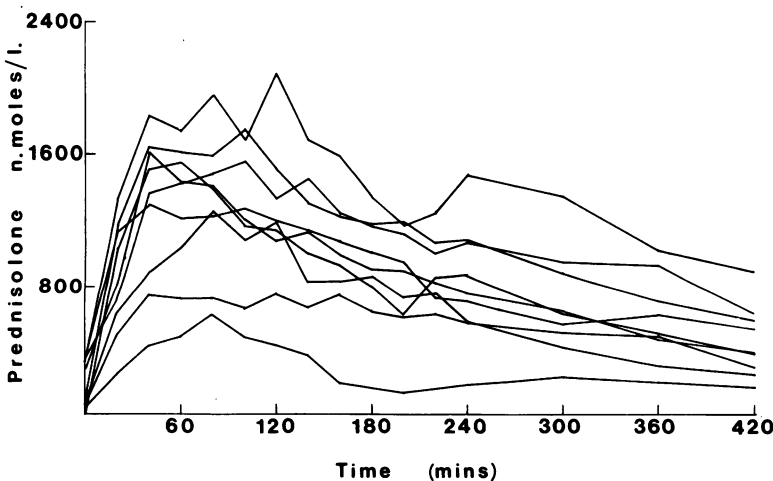


Fig. 2 Plasma concentration-time profiles after administration of oral prednisolone to normal subjects. Conversion: SI to traditional units—plasma prednisolone: $1 \text{ nmol l}^{-1} = 0.36 \text{ ng ml}^{-1}$.

Table 2 Pharmacokinetic data: details of 10 patients who received a prednisolone retention enema

Subject	C_{max} nmol l ⁻¹	T_{max} min	AUC μ mol l ⁻¹ min	$T_{\frac{1}{2}}$ min
1	455	100	118	136
2	497	120	130	220
3*	(902)	(240)	—	(160)
4	538	120	161	184
5	450	200	146	270
6	490	120	126	166
7†	483	180	125	—
8	497	100	139	490
9	946	140	286	232
10	731	160	190	204
Mean	565	138	158	229
SEM \pm	55	12	18	35

*Prednisolone detectable in plasma at start of test. Results excluded from calculation of mean values.

†Insufficient data to calculate $T_{\frac{1}{2}}$.

Conversion: SI to traditional units—plasma prednisolone:
1 nmol l⁻¹ = 0.36 ng ml⁻¹.

to infinity when investigating the relative bioavailability of two preparations of the same drug, provided that plasma levels have been measured for two half-lives (Lovering *et al.*, 1975) and thus the mean AUCs can be compared. The mean AUC of the group who received prednisolone rectally was 44% of that of the group who were given the oral drug.

The adrenocortical responses to Synacthen in the eight subjects who were tested are shown in Fig. 4. Synacthen was administered in the afternoon and the minimum response that would be considered normal at the 95% confidence limit, at that time of day in patients not receiving corticosteroid therapy is a stimulated cortisol concentration of 436 nmol/l

Table 3 Pharmacokinetic data: details of nine subjects who received oral prednisolone

Subject	C_{max} nmol l ⁻¹	T_{max} min	AUC μ mol l ⁻¹ min	$T_{\frac{1}{2}}$ min
11†	621	80	118	—
12	1601	40	358	184
13	2084	120	584	244
14*	758	120	241	253
15*	1742	100	463	179
16	1546	60	355	140
17	1249	80	276	130
18*	1546	120	455	255
19	1297	40	364	200
Mean	1383	84	357	198
SEM \pm	154	11	46	17

*Each tested on two occasions. Values are the means of the two results.

†Insufficient data to calculate $T_{\frac{1}{2}}$.

Conversion: SI to traditional units—plasma prednisolone:
1 nmol l⁻¹ = 0.36 ng ml⁻¹.

with a rise above the basal level of at least 190 nmol/l (McGill *et al.*, 1967). The basal cortisol concentration in six of our patients was below the minimum expected value for that time of day (140 nmol/l) and equivocally lower in a seventh subject, suggesting suppression of endogenous ACTH caused by prednisolone that had been absorbed from the enema. However, six of the eight patients tested responded normally to synthetic ACTH. The remaining two subjects failed to achieve the minimum stimulated cortisol concentration of 436 nmol/l, but in both cases the increment after ACTH was greater than 190 nmol/l. The response to Synacthen in these two patients must therefore be considered to be impaired, and indicative of some degree of adrenal suppression.

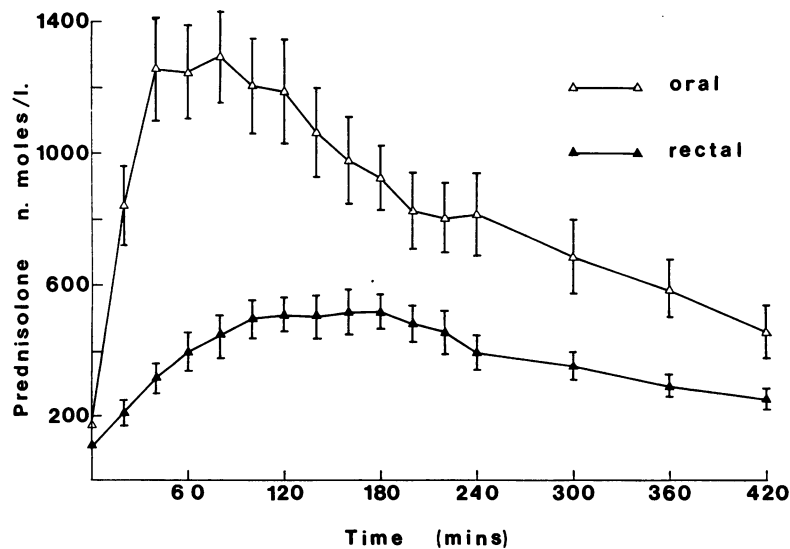


Fig. 3 Mean plasma concentration-time profiles after administration of prednisolone retention enema (\blacktriangle — \blacktriangle) and oral prednisolone (\triangle — \triangle) \pm SEM. Conversion: SI to traditional units—plasma prednisolone: 1 nmol l⁻¹ = 0.36 ng ml⁻¹.

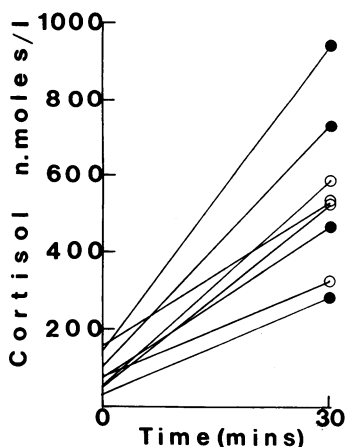


Fig. 4 Response of plasma cortisol to ACTH after administration of a prednisolone-21-phosphate retention enema. ○—○ No previous steroid therapy. ●—● Receiving local steroid therapy at time of study.

Discussion

We have used a direct radioimmunoassay to measure plasma prednisolone concentrations. The assay is simple and precise, and, although the antisera that were used were not entirely specific, there were insufficient amounts of cross-reacting steroids present in the plasma samples to affect the results. The plasma concentrations that were measured, and the large inter-subject variation after oral administration of prednisolone were similar to those reported previously where a radioimmunoassay technique was used (Colburn and Buller, 1973; Meikle *et al.*, 1975; Tembo *et al.*, 1977).

Although prednisolone retention enemata are widely used in the treatment of proctocolitis, it is not clear whether the steroid is exerting its beneficial effect by a local or systemic action. Clinical observations and experience with hydrocortisone enemata have suggested that the former explanation was likely (Truelove, 1960; Swarbrick *et al.*, 1974). Until the recent development of various techniques for the assay of prednisolone in plasma, only indirect methods were available for assessing the degree of prednisolone absorption after rectal administration. Wood *et al.* (1964), measured urinary free prednisolone after giving two 20 mg retention enemata 12 hours apart and found that the total amount of prednisolone absorbed was less than that after oral administration of 7 mg of the drug. This, they concluded, would be insufficient to explain the beneficial action of retention enemata or to cause adrenocortical suppression. These conclusions were supported by Sanbar and West (1961)

who gave their patients radiolabelled methyl prednisolone and found that the amount of radioactivity recoverable in the urine after rectal administration was 22% of that after oral administration, and thus the beneficial effect was likely to be due to a local action. By contrast, Spencer *et al.* (1960) found that up to 64% of a dose of radiolabelled methyl prednisolone administered rectally appeared in the urine, a result confirmed by Halvorsen *et al.* (1969) who recovered 72% of a dose of radiolabelled prednisone. Both sets of authors concluded that the beneficial action of these steroids administered rectally may have been partly due to a systemic action. Powell-Tuck *et al.* (1976), using a competitive protein binding assay, found similar plasma prednisolone levels in patients with ulcerative colitis after giving equal oral and rectal doses. They concluded from this that, as well as a local action, rectally administered prednisolone also has a systemic effect. However, the plasma levels that they measured after oral prednisolone were generally lower than ours and those previously reported, even though after rectal administration the prednisolone levels that they observed were very similar to the ones that we had obtained.

We have demonstrated that a dose of prednisolone is absorbed after rectal administration and that this is unaffected by disease activity or concurrent local steroid therapy. The amount absorbed, however, may well depend on the length of time that prednisolone is effectively in contact with the mucosa (although not necessarily on the distance travelled by the enema). Although all our subjects retained their enemata for at least four hours, the excessive mucus production that is a feature of active proctocolitis would have the effect of diluting the prednisolone and therefore reducing its concentration gradient across the bowel wall. As the absorption of prednisolone is a passive process, this dilution might result in less being absorbed and may possibly explain why the two subjects with no bowel disease achieved higher concentrations of plasma prednisolone, even though the pattern of absorption was similar to that seen in the other subjects. We have found a similar pattern of absorption and low plasma prednisolone levels after administration of a retention enema in a patient suffering from irradiation proctocolitis, another disease in which excessive mucus production is a prominent feature.

It has been reported previously that prolonged therapy with prednisolone retention enemata does not cause adrenocortical suppression (Matts *et al.*, 1963), although this has been disputed (Multicentre Trial, 1971). In only two of our subjects was the adrenocortical response to synthetic ACTH slightly impaired, and one of these people (subject 5) had

been using large quantities of betamethasone valerate cream for several years for the treatment of dermatitis. In all of the others the response was normal, indicating that the sensitivity of the adrenal cortex had not been impaired by therapy with rectally administered prednisolone. The adrenocortical response to synthetic ACTH has been reported in the past to be impaired by prolonged therapy with 7.5-10 mg daily of oral prednisolone (Wood *et al.*, 1965). In our study the amount of prednisolone that was absorbed from a 20 mg retention enema, as indicated by the mean AUC, was 44% of that absorbed from an oral dose. From this it could be postulated that 20 mg prednisolone administered rectally is equivalent to approximately 8 mg of oral prednisolone. Three of our subjects who were being treated with rectally administered steroids at the time of the study had been taking 40 mg prednisolone daily by enema for up to six weeks. This could be equivalent to 16 mg taken orally each day, a dose which would certainly be expected to cause a diminished response to Synacthen. There is, however, a major difference in the pattern of absorption *via* the two routes. After either route of administration prednisolone is detectable in the plasma within 20 minutes and the $T_{\frac{1}{2}}$ is similar. However, a clearly defined peak occurs at about 80 minutes after oral prednisolone, whereas after rectal administration the prednisolone concentration tends to plateau, reaching a maximum level at about 150 minutes. When 10 mg prednisolone are given orally the pattern of absorption is similar to that seen after a 20 mg oral dose (Lee, D. A. H., unpublished observations), although the C_{max} is lower. It is possible that the important factor in causing adrenocortical suppression in patients on prolonged steroid therapy may be exposure of the hypothalamic-pituitary axis to peak plasma prednisolone concentrations, rather than to the total amount absorbed. There is other evidence to support this hypothesis. It has been found that giving a sustained release preparation of prednisolone orally produces a pattern of absorption similar to that observed after administration of a retention enema and causes less adrenocortical suppression than that caused by an equivalent oral dose of the standard preparation (English *et al.*, 1975).

In conclusion, when prednisolone is given rectally to patients with proctocolitis, it appears likely that its effect is exerted mainly through a local action, but the plasma concentration achieved suggests that enough is absorbed to exert a beneficial systemic effect. However, therapy for a period of time that is usually sufficient to induce a remission of the disease, does not impair the adrenocortical response to synthetic ACTH.

This work and D.A.H.L. were supported by the Medical Research Council, Grant No. G.974/615/S. G.M.T. was supported by grant No. C/16/407/1 from the North West Thames Regional Health Authority. The authors gratefully acknowledge the assistance of Dr F. Short who performed the plasma cortisol estimations.

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