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A POSSIBLE DEFECT IN THE INTER-CONVERSION BETWEEN CORTISONE AND CORTISOL IN PREPUBERTAL PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA RECEIVING CORTISONE ACETATE THERAPY

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Summary—Oral administration of cortisone acetate is widely used to treat prepubertal patients with congenital adrenal hyperplasia (CAH). However, efficient 'first pass' hepatic conversion of the biologically inactive cortisone (E) to cortisol (F) by the 11-reductase component of the 11β-hydroxysteroid dehydrogenase (11β-HSD) system is required for suppression of the hypothalamic–pituitary–adrenal (HPA) axis. 11-β-HSD activity can be assessed by measurement of urinary tetrahydroderivatives of E (tetrahydrocortisone, THE) and F (tetrahydrocortisol, THF), formed in separate hepatic compartments by reduction of the A ring. Inadequate HPA axis suppression is frequently encountered in peripubertal CAH patients receiving cortisone acetate therapy. In this paper, we describe THE and THF concentrations in 24 h urine samples collected every 3–6 months from 14 prepubertal patients with simple virilizing CAH. The patients had been receiving cortisone acetate and 9α-fluorohydrocortisone since diagnosis and were investigated for 2–4 years during which there was marked intra- and inter-individual variation in the level of suppression. Good and poor control of HPA axis suppression were defined on the basis of a profile of early morning serum 17-hydroxyprogesterone, androstenedione, plasma renin activity and 24 h urinary excretion of pregnanetriol, pregnanetriolone and 5β,17α-hydroxypregnanolone. Serum steroids were measured by RIA and urinary metabolites quantitated as methyloxime–trimethylsilylimidazole derivatives by gas chromatography and GC-mass spectrometry.

There were no significant differences in the THE/THF ratio between male ($n = 9$) and female ($n = 5$) patients during either good or poor therapeutic control. The data were therefore analyzed without consideration of patient sex. Urinary THE/THF (mean $\pm$ SD) was significantly higher in patients during periods of poor control (6.56 ± 2.51, $P < 0.001$) compared with periods of good control (3.73 ± 0.96) in the same patients. THE/THF levels were also significantly ($P < 0.001$) higher in CAH patients, irrespective of the level of control, than those for the normal subjects (1.79 ± 0.20). Furthermore, THE excretion was significantly higher during periods of poor control compared with good control at all doses of cortisone acetate administered (10–50 mg/day). There were no significant differences in THF excretion. THE levels also rose significantly ($P < 0.001$) in response to increasing total dose during periods of poor control. The increase in THF excretion was slight and significant only at doses >40 mg/day compared with doses <15 mg/day. A significant linear correlation could be drawn between THE excretion and total daily dose during periods of both poor ($r = 0.67$, $P < 0.05$) and good ($r = 0.68$, $P < 0.005$) control.

Excretion of 5α-THF, cortols and the cortolones, unlike that for normal subjects, was very low in cortisone acetate treated CAH patients. In contrast, the ratios of 5α/5β C19 steroid metabolites were no different from normal. The results from this study suggest that poor therapeutic control is unlikely to be due to: (i) failure of compliance with therapy; (ii) inefficient absorption from the intestine; or (iii) inefficient acetate group removal. The data are consistent with a hypothesis of rapid tetrahydroderivitization of E, present in excess of the capacity of 11β-HSD to form F; i.e. a preferential reduction of the A ring rather than the 11-keto group as a consequence of hepatic 'first pass' uptake of the exogenously administered E. The ability of prednisolone and dexamethasone, both of which are bioactive and not subject to significant A ring reduction, to suppress the HPA axis of patients poorly suppressed by cortisone acetate, lends support to this hypothesis.

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Abbreviations: THE, tetrahydrocortisone = 3α,17β,21-trihydroxy-5β-pregnane-11,20-dione; THF, tetrahydrocortisol = 3α,11β17β,21-tetrahydroxy-5β-pregnan-20-one; PT, pregnanetriol = 5β-pregnane-3α,17α,20α-triol; PTL, pregnanetiolone = 3α,17α,20α-trihydroxy 5β-pregnan-11-one; and 5B17HP, 5β,17α-hydroxypregnanolone = 3α,17α-dihydroxy-5β-pregnan-20-one.

INTRODUCTION

Cortisone acetate was introduced as a glucocorticoid replacement therapy for the treatment of congenital adrenal hyperplasia (CAH) over forty years ago [1] and despite the discovery of more potent synthetic agents, remains in widespread use. However, since cortisone (E) is biologically inactive and must undergo 11β-reduction to the bio-active corticosteroid, cortisol (F), its clinical use has been debated in terms of problems in absorption and bioconversion [2]. Studies comparing the effects of therapeutic equivalent oral doses of cortisone acetate and hydrocortisone on plasma F levels in the same subjects has yielded conflicting results [3–7].

The liver is the primary site for the interconversion of 11-dehydrocorticosteroids and 11β-hydroxycorticosteroids by the action of the 11β-hydroxysteroid dehydrogenase (11β-HSD) system [6–9], which comprises 2 kinetically distinct microsomal enzyme activities, 11-dehydrogenase and 11-reductase [10–12]. Other tissues, however, exhibit preferential capacity of either 11-reductase of 11-dehydrogenase activity. This has been well reviewed by Monder and Shackleton [10]. E and F are excreted principally as their respective tetrahydroderivatives, tetrahydrocortisone (THE), tetrahydrocortisol (THF) and allo-THF (5α-THF). Since there is negligible inter-conversion between these metabolites [13], they are thought to be formed in separate hepatic compartments by reduction of the A ring [14].

Several disorders of steroid metabolism have been clearly documented by gas chromatographic analysis (GC) of neutral and acidic steroid metabolites [15–19]. Peterson *et al.* [17] demonstrated increased 5β/5α urinary C19 steroid and THF/5α-THF steroid ratios in patients with male pseudohermaphroditism due to 5α-reductase deficiency. More recently, the diagnosis of type I apparent mineralocorticoid excess (AME) due to congenital 11β-HSD deficiency has been described in 4 children with low renin hypertension by GC measurement of urinary THE, THF and 5α-THF [19]. The diagnosis of AME by RIA of these metabolites has also been described [20].

In normal subjects THE is the principal excretion product [14, 18, 21] with levels almost twice those of THF. This contrasts with a THE/THF ratio of <1 for AME patients [20]. In a study of 7 normal subjects [pre-dexamethasone (DXM) suppressed] receiving high doses of hydrocortisone, it was shown that THF was the principal metabolite [16]. Where adrenal suppression could not be achieved in a 17-year-old hyperandrogenic girl receiving high doses of either cortisone acetate or hydrocortisone, THE was the exclusive excretion product of both glucocorticoids [18].

The aim of glucocorticoid therapy in the treatment of CAH is to maintain optimal suppression of the hypothalamic–pituitary–adrenal (HPA) axis so as to prevent excessive adrenal androgen secretion. Frequent monitoring of the level of suppression is therefore required and depends largely on the assessment of serum and urinary steroid profiles in relation to established criteria. Failure of cortisone acetate replacement therapy to maintain adequate suppression of the HPA axis is frequently encountered in prepubertal CAH patients. The present study was therefore undertaken to investigate the mechanism of cortisone acetate inefficacy in 14 prepubertal CAH patients by an analysis of urinary corticosteroid metabolites in relation to the level of HPA axis suppression over a range of therapeutic doses. Since untreated patients excrete negligible amounts of these metabolites quantitation of the latter will reflect the metabolic fate of exogenous E alone.

EXPERIMENTAL

Subjects

Fourteen prepubertal patients (9 males and 5 females) with simple virilizing CAH (SVCAH) due to 21-hydroxylase deficiency were randomly selected and studied for 2–4 years prior to the onset of puberty. The patients were aged between 4 and 14 years and had been receiving oral cortisone acetate replacement therapy and fluorohydrocortisone (0.10–0.15 mg/day) since diagnosis (which has been well described [22]) had been made in the first weeks of life. The total daily dose of cortisone acetate for each patient varied between 10 and 50 mg and was usually administered in 2 or 3 divided doses. The total daily dose was adjusted according to early morning serum levels of 17-hydroxyprogesterone (17OHP) and androstenedione (A), both sensitive markers of therapeutic control in girls and prepubertal boys [23], and urinary excretion of 17OHP metabolites relative to the criteria described in Table 1. Patients were evaluated every 3–6 months. Steroid metabolites

Table 1. Criteria for therapeutic control of CAH

Serum/urinary steroid	Poor control	Reference range
17OHP (nmol/l)	>30.0	<5.0
A (nmol/l)	>5.0	<3.0
PT (μmol/24 h)	>6.0	<3.0
PTL (μmol/24 h)	>1.0	<0.6
5B17HP (μmol/24 h)	>3.0	<1.0

For abbreviations see text.

were measured in 24 h urine samples, while serum steroids and plasma renin activity (PRA) were measured in blood samples drawn between 8 and 9 a.m. during the urine collection. All patients exhibited marked intra- and inter-individual variation in the level of HPA axis suppression. 24 h urine and early morning blood samples were also obtained from a group of normal subjects of similar ages, not receiving any medication, who were family members of hospital staff ($n = 13$).

Urinary steroid measurements

Steroid conjugates were extracted from 10 ml aliquots of 24 h urine samples using Sep-Pak C18 columns (Waters Associates, Milford, MA, U.S.A.) as described previously [24]. This procedure is also thought to remove substances that may have inhibited enzymic hydrolysis. Each aliquot of urine was passed through a primed column at a flow rate of <1 ml/min. The columns were washed with 5 ml distilled water and the steroid conjugates eluted in 3 ml methanol. The methanol was evaporated under nitrogen and the steroid conjugates reconstituted in 3 ml acetate buffer (pH 5.0). Hydrolysis was by *E. coli.* β-Glucuronidase and β-glucuronidase-aryl sulfatase (Boehringer-Mannheim, Fed. Rep. Germany) at 55°C for 3 h [24]. A pooled urine sample served as a quality control for each assay and methodological losses were monitored by inclusion of 2 recovery samples containing known standard amounts of the principal C19 and C21 steroid metabolites measured. Liberated steroids were passed through Sep-Pak C18 columns and internal standards, *n*-tetracosane, *n*-dotriacontane, 5α-androstane-3α,17β-diol and cholesterol butyrate (Sigma Chemical Co., St Louis, MO, U.S.A. and Makor Chemicals Ltd., Sydney, Australia) were added to each methanol eluate in 500 μl cyclohexane, mixed thoroughly and each sample evaporated under nitrogen. Methyloxime and trimethylsilylimidazole (TMSI) derivatizations were both carried out for 16 h. Final purification was by Sephadex LH20 using pyridine–hexamethyldisilazane–cyclohexane (1:1:100) solvent system. Chromatographic separation was performed using a Varian 3400 GC through a 25 M × 0.22 mm (i.d.) bonded phase, fused silica BP-1 column (Waters Associates) with a temperature gradient programmed from 180–310°C at 3°C/min. The split ratio of the injection system was 40:1, inlet pressure was 30 KPa, carrier gas (helium) flow at 24 ml/min, make up gas (nitrogen) at 35 ml/min. A flame ionization detection device was used. Analysis of data was by Varian Chromatography Data System 402, which identified all components by retention time and integrated the data to quantitate steroid levels by peak area comparison with internal standards. The values were corrected by the appropriate response factors, calculated from standard samples. Percent recoveries varied between 97 and 102%. Further analysis and confirmation of steroid identity for each sample was carried out using gas chromatography-mass spectrometry (GCMS). Components were identified by characteristic principal ions using a Finnigan-MATT 212 mass spectrometer. The GC was similar to that described and the data analyzed using a Finnigan-MATT SS200 data system. Unconjugated F metabolites and the cortolic and cortolonic acids were not measured.

Serum steroid measurement

Serum levels of 17OHP were measured using a RIA kit method (Mallinkrodt, Sydney, Australia). A was measured by RIA using a previously described method [25]. Radiolabeled steroids were purchased from Amersham International Ltd., New England Nuclear-Dupont, Inc., Sydney and from the Department of Chemical Pathology, Prince Henry's Hospital, Melbourne, Australia. Unlabeled steroids were obtained from Research Plus (Bayonne, NJ, U.S.A.) and Sigma. PRA was measured by RIA kit (Radioassay Systems Laboratories, CA, U.S.A.). Intra and inter-assay variation for RIAs was <4 and <10%, respectively.

Statistics

All data underwent the Wilks–Shapiro and Anderson–Darling tests of normality and were then analyzed by Wilcoxon-Rank analysis and by Student's *t*-test. Equal variance was not assumed. Univariate linear regression analysis was also performed.

RESULTS

Urinary corticosteroid excretion is negligible in untreated CAH patients [15] and in our patients where treatment consists of either prednisolone (Pn) or DXM alone. Therefore measurement of these metabolites in all 14 patients receiving cortisone acetate replacement therapy alone was indicative of both compliance with medication and absorption of unesterified E into the circulation. Typical GC profiles of urinary metabolite excretion for patients in poor and good therapeutic control are shown in Fig. 1. These may be compared with that of a normal subject of a similar age (Fig. 2). It is clear that a 'poor control profile', i.e. elevated pregnanetriol, pregnanetriolone 5β,17α-hydroxypregnanolone (5β17-HP) and THE and very low THF excretion [Fig. 1(a)] differs markedly from that for a patient in good control [Fig. 1(b)]. Urinary levels of the cortols and cortolones were negligible in CAH patients (of both sexes) during periods of either poor or good control and were therefore not quantitated. The levels of 5α-THF were very low (frequently undetectable) compared with those for THF and were not included in the analaysis of the tetrahydroderivatives of F. This contrasts with a 5α-THF/THF ratio (mean ± SD) of 0.58 ± 0.23 for the normal subjects in our study and a ratio of between 2 and 3 recently described in AME patients [26]. The ratio of 5α/5β C19 steroid metabolites excreted by all our CAH patients (data not shown) did not differ from normal. Thus, orally administered cortisone acetate was excreted predominantly as either THE or THF. Measurements of urinary

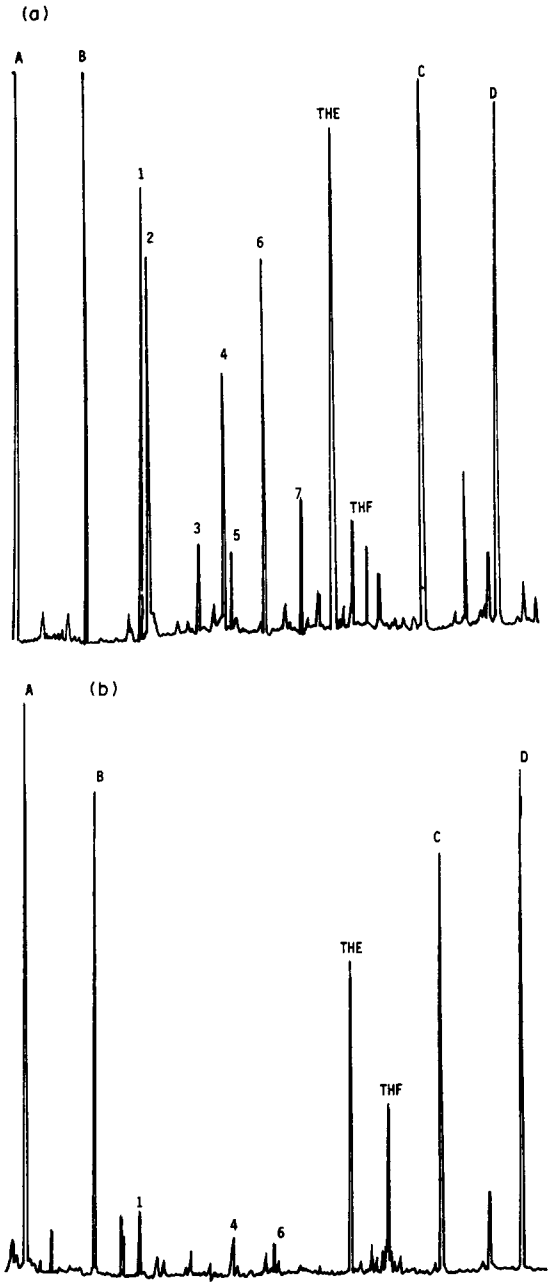

Fig. 1. Urinary steroid GC profile typical of peripubertal CAH patients in poor (a) and good (b) therapeutic control. Steroids were chromatographed as methyloxime-TMSI derivatives on a 25M × 0.22 mm (i.d.) bonded phase fused silica BP-1 capillary column. Internal standards: A = 5α-androstane-3α,17β-diol, B = *n*-tetracosane, C = *n*-diacontane, D = cholesterol butyrate. Steroids: 1 = androsterone, 2 = etiocholanolone, 3 = DHEA, 4 = 11-hydroxyandrosterone, 5 = 11-hydroxyetiocholanolone, 6 = PT and 7 = PTL.

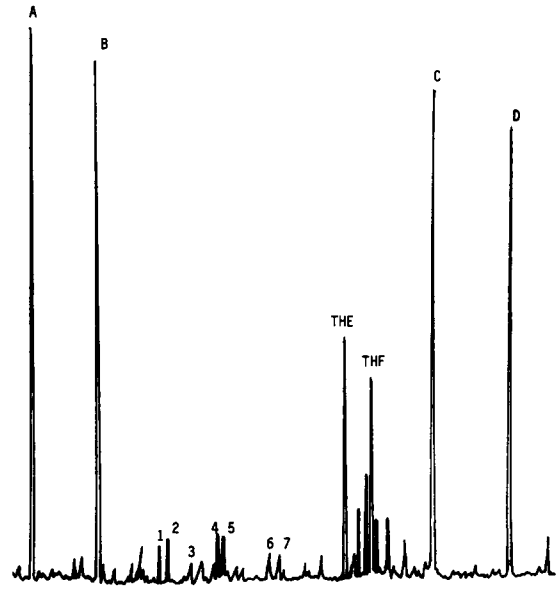

Fig. 2. Urinary steroid GC profile for a normal 11-year-old boy. Steroids were chromatographed as described in Fig. 1. Internal standards: A = 5α-androstane-3α,17β-diol, B = *n*-tetracosane, C = *n*-diacontane, D = cholesterol butyrate. Steroids: 1 = androsterone, 3 = etiocholanolone, 4 = 11-hydroxyandrosterone, 5 = 11-hydroxyetiocholanolone, 6 = PT and 7 = PTL.

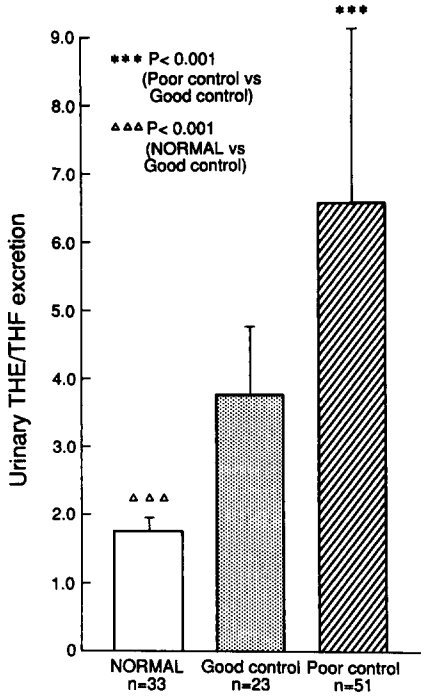

Fig. 3. Urinary THE/THF ratios (mean ± SD) for periods (>3 months) of both good and poor therapeutic control in all pre- and peri-pubertal CAH patients receiving cortisone acetate only compared with the ratio measured in normal children of a similar age not receiving any medication. For the CAH patients 'n' refers to the number of data points; approx. 5 to 6 values per patient.

THE and THF, where Pn formed part of the glucocorticoid therapy were not included in the analysis of data because the aim of the study was to examine the mechanism of cortisone acetate inefficacy during periods of poor therapeutic control.

There were no significant differences in the THE/THF ratios (mean ± SD) between male ($n = 9$) and female ($n = 5$) patients during periods of either good therapeutic control of adrenal suppression (3.52 ± 1.06 vs 4.29 ± 0.33) or poor therapeutic control (7.01 ± 2.41 vs 6.47 ± 1.44). We therefore analyzed the data without consideration of patient sex. Figure 3 shows urinary THE/THF (mean ± SD) for CAH patients during periods of poor control, periods of good control and for the normal subjects. The THE/THF ratio during poor control was significantly greater (6.56 ± 2.51, $P < 0.001$) than that during good control (3.73 + 0.96) in the same 14 patients. Furthermore, the THE/THF ratios for patients during good control and poor control were significantly higher ($P < 0.001$) than the ratio for the normal subjects (1.79 + 0.20).

Urinary THE and THF concentrations (mean ± SD) during periods of both poor and good therapeutic control are shown for each cortisone acetate dose range in Fig. 4. THE excretion was significantly higher during periods of poor control compared with good control at 10–15 mg/day (12.4 ± 4.0 μmol/24 h, $n = 10$ vs 6.9 ± 3.8 μ/24 h, $n = 6$, $P < 0.05$), 20–25 mg/day (19.2 ± 5.1 μmol/24 h, $n = 12$ vs

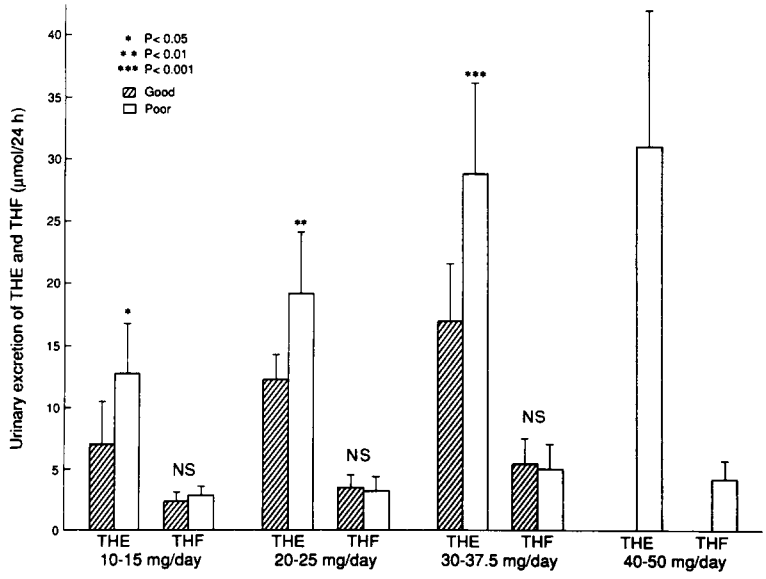

Fig. 4. Urinary excretion of THE and THF (mean ± SD) during periods of good vs poor therapeutic control in relation to the total daily oral dose of cortisone acetate only received by the CAH patients. The dose intervals were grouped according to dose increments of cortisone acetate normally prescribed (in 2 or 3 divided doses), e.g. there were no dose levels between 15 and 20 mg or between 25 and 30 mg. There were no patients in the good control category where the total daily dose was >40 mg. CAH patients not receiving cortisone acetate therapy excreted undetectable levels of THE and THF.

14.3 ± 2.0 μmol/24 h, $n = 10$, $P < 0.01$) and 30–37.5 mg/day (28.7 ± 7.4 μmol/24 h, $n = 24$ vs 17.0 ± 4.6 μmol/24 h, $n = 8$, $P < 0.001$). In contrast there were no significant differences in THF excretion at the same dose ranges (poor vs good): 10–15 mg/day (2.7 ± 0.7 μmol/24 h, $n = 10$ vs 2.3 ± 0.8 μmol/24 h, $n = 6$); 20–25 mg/day (3.2 ± 1.2 μmol/24 h, $n = 12$ vs 3.5 ± 1.0 μmol/24 h, $n = 10$); and 30–37.5 mg/day (4.9 ± 1.9 μmol/24 h, $n = 24$ vs 5.4 ± 2.0 μmol/24 h, $n = 8$). Furthermore, during periods of poor control, whereas the mean THE level was significantly ($P < 0.001$) higher at each increase in cortisone acetate dose range, there was no significant rise in THF levels (except at doses >40 mg/day compared with doses <15 mg/day). Urinary THE excretion correlated significantly with the total daily dose of cortisone acetate during periods of both poor ($r = 0.67$, $P < 0.05$) and good ($r = 0.68$, $P < 0.005$) therapeutic control.

Since it was not possible to induce adrenal insufficiency in the normal subjects and subsequently administer oral cortisone acetate replacement therapy, we were unable to determine 'normal' urinary THE and THF excretion derived exclusively from A ring reduction of exogenously administered E only.

The serum and urinary steroid profiles from 3 of the patients (Fig. 5) clearly demonstrate the relationship that exists between the biochemical parameters defining the level of HPA axis suppression (17OHP, A, PT), therapeutic cortisone acetate dose and urinary THE and THF excretion in the individual patient. In periods of poor HPA axis suppression, urinary THE excretion was increased several fold, whereas THF excretion was relatively constant. Increasing the daily cortisone acetate dose without a concomitant improvement in control was associated with further increases in THE excretion and little change in the levels of THF.

The introduction of either Pn or DXM (potent bio-active synthetic glucocorticoids) resulted in prolonged periods of good HPA axis suppression in all patients, who were otherwise poorly suppressed with relatively higher therapeutic equivalent oral doses of cortisone acetate. Urinary THE and THF excretion declined concomitantly with the reduction of the cortisone acetate component in the therapeutic regimen. A return to poor HPA axis suppression followed withdrawal of either Pn of DXM in favour of the re-introduction of high dose cortisone acetate treatment. This is illustrated by patient AR (Fig. 5). Good control resulting from the replacement of the 20 mg evening cortisone acetate dose with 5 mg Pn at the age of 11.5 years was lost when the dose regimen reverted back to two daily cortisone acetate doses (10 and 20 mg) at the age of 12.3 years. The subsequent period of poor control was associated with significantly increased urinary THE excretion to levels which were similar to those observed prior to the introduction of Pn. Increasing the total daily cortisone acetate dose to 35 mg resulted in further increases in THE excretion with relatively little change in urinary THF and no apparent improvement in the level of adrenal suppression. However, improved therapeutic control occurred temporarily in patient CC in response to a 33% increase in daily cortisone acetate (12.5 mg). The patient reverted back to a state of very poor HPA axis control within a few weeks and exhibited a simultaneous increase in urinary THE excretion. The restoration of good HPA axis suppression in patient AR was clearly only affected by the re-introduction of a Pn component into the treatment regimen at 13.3 years of age. The effects of shorter intermittent periods of Pn therapy on suppression and urinary THE and THF excretion were similar in a younger CAH patient, SC.

DISCUSSION

Previous studies have shown that while E to F conversion in normal subjects is significantly greatly than that of F to E, the principal excretion product in normal subjects is THE and not THF [13, 18, 21] This is consistent with the fact that the metabolic clearance rate (MCR) of E is more than twice the MCR of F [13]. The results from this study and others confirm the mild predominance of THE excretion [15, 16]. It has been suggested that the markedly elevated levels of THE and THE/THF ratios observed in hyperthyroid patients [27] is largely due to diminished E to F conversion rather than increased formation of E from F [13]. However, the fact that the transfer constant favours E to F in normal subjects [13] may explain why THF (associated with negligible 5α-THF and THE levels) was the principal urinary corticosteroid metabolite in a study of normal DXM suppressed subjects treated with high doses of hydrocortisone [16]. Phillipou and Higgins [18] have reported significantly increased levels of urinary THE (and cortolones)

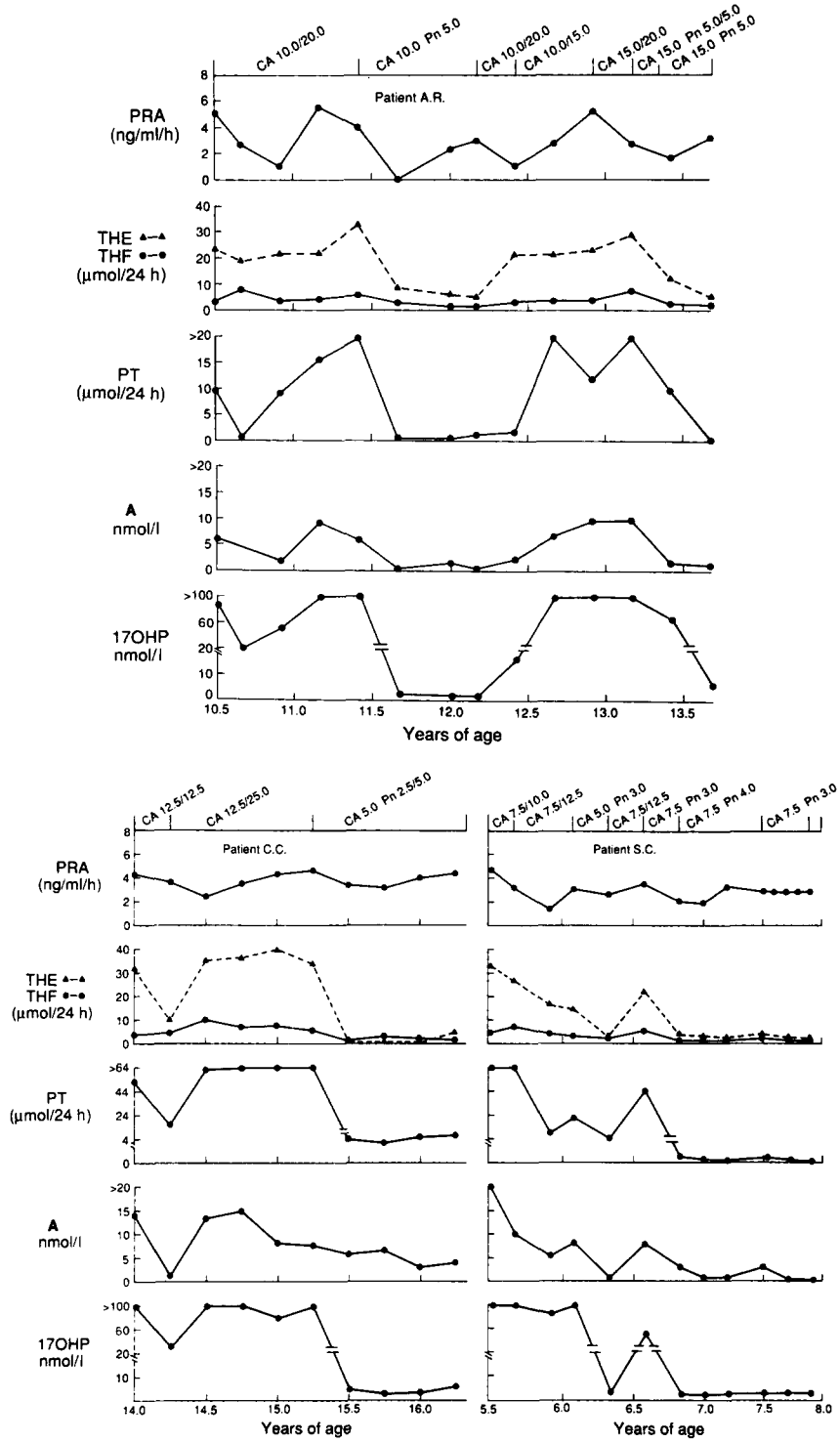

Fig. 5. Serum and urinary steroid profiles of 3 of the CAH patients studied illustrating the individual case relationship between the indices of adaquate or inadaquate HPA suppression, i.e. 17OHP, A, PT and PRA (PTL data not shown), oral dose of cortisone acetate (CA) and/or prednisolone (Pn) and the urinary of THE and THF. The data are plotted at approx. 3-month intervals since inpatient studies were performed at this frequency.

coupled with negligible levels of THF and 5α-THF in two hyperandrogenic pubertal girls. Treatment with either cortisone acetate or hydrocortisone (100 mg/day) failed to suppress the HPA axis in one patient and both glucocorticoids were excreted exclusively as THE.

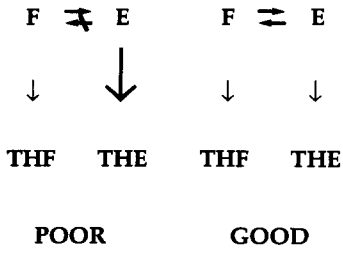

Fig. 6. Diagram to illustrate the effects of the putative net inhibition of E to F conversion in CAH patients in poor therapeutic control vs normal E to F inter-conversion in good control upon THE and THF excretion.

Suppression was achieved with a much lower therapeutic equivalent dose of DXM. This suggests that, in addition to a defect in E to F conversion, these patients may also demonstrate enhanced formation of E from exogenous F resulting in an E–F equilibrium very much in favour of E. A similar shift in equilibrium has also been described in hyperthyroid patients [26], but since 5α-THF excretion was increased in these patients relative to THF, the ratio of total 11-hydroxy to 11-keto corticosteroid metabolites differed little from normal. Elevated THE/THF and 5α-THF/THF ratios are also found in newborns [10]. The converse is seen in type I AME, where a deficiency of 11-dehydrogenase activity results in markedly increased excretion of the 11-hydroxy/11-keto corticosteroid metabolites [26]. A urinary THE/THF <1 is characteristic of this congenital defect [20, 29].

The bio-availability of orally administered cortisone acetate depends on (i) efficient absorption of unesterified E from the intestine and (ii) intact efficient 'first pass' hepatic conversion of E to F [3–7]. CAH patients not receiving treatment excreted negligible amounts of THE, THF, 5α-THF, cortols and the cortolones. These metabolites, shown in this study to be predominantly THE and THF, in patients on cortisone acetate therapy are derived from principally liver metabolism of the exogenous E. THE and THF are formed in separate hepatic compartments by reduction of the A ring of E and F, respectively [13]. The negligible inter-conversion between THE and THF [14] therefore enables these metabolites to serve as an accurate index of E–F inter-conversion. The results from this study demonstrate significantly higher urinary THE levels and THE/THF ratios during periods of poor HPA axis suppression compared with periods of good suppression in all 14 patients irrespective of the total daily cortisone acetate dose. Furthermore, there was a significant linear correlation between THE excretion and total daily dose. This suggests that the therapeutic inefficacy of oral cortisone acetate in these patients was unlikely to be due to: (a) failure of compliance with therapy; (b) inefficient absorption from the intestine; or (c) inefficient acetate group removal. Our data strongly suggest that poor therapeutic control of the HPA axis of CAH patients receiving cortisone acetate therapy is due to a net deficiency of E to F conversion, i.e. 11-reductase activity, in the liver. Since urinary THE/THF ratios were significantly higher in patients during periods of good HPA axis suppression compared with the ratios in normal subjects, well controlled patients may also exhibit an apparent mild impairment of net hepatic E to F conversion. These data are consistent with a hypothesis of rapid tetrahydroderivatization of E, present in excess to the capacity of 11β-HSD to form F, i.e. a preferential reduction of the A ring rather than the 11-keto group as a consequence of hepatic 'first pass' uptake of the exogenously derived E. Where the E dose was increased without concomitant improvement in HPA axis suppression, the enhanced excretion of THE compared with THF, also suggests that the excess E was increasingly subject to immediate A ring reduction to THE rather than 11-reduction to F. However, where periods of poor control were temporarily corrected by increasing the dose, the comparatively smaller increases in THE excretion point to increased hepatic 11-reductase activity as opposed to A ring reductase activity associated with elevated availability of E.

Urinary 5α-THF excretion was diminished compared with that for THF in all 14 patients irrespective of the level of adrenal suppression or the total daily dose of cortisone acetate. It is unclear whether this is due to diminished 5α-reductase activity or preferential 5β-reductase activity (or both). 5α-THF/THF ratios significantly below normal have also been demonstrated in patients with male pseudohermaphroditism due to 5α-reductase deficiency [17]. However, unlike the CAH and AME patients [26], those with 5α-reductase deficiency also exhibited a decrease in urinary 5α/5β C19 steroid metabolite excretion [17]. Interestingly a net decrease in 5β-reductase activity associated with 11-dehydrogenase deficiency has been described in AME patients, who present with 5α-THF/THF ratios between 2 and 3 [26]. No clear association is discernable

between 5α/5β reduction of C21 steroids and hepatic 11-reductase activity in the CAH patients because excretion of 5α-THF was very low and often undetectable during periods of both good and poor HPA axis suppression.

Restoration of HPA axis suppression was achieved in all 14 CAH patients with much lower therapeutic equivalent doses of either Pn or DXM. The mode of action of both Pn and DXM does not involve metabolism at C11 [30, 31] and neither are subject to significant A ring reduction [32]. This is therefore also consistent with the hypothesis of preferential reduction of the A ring rather than the 11-keto group of exogenous E as the mechanism for oral cortisone acetate inefficacy in HPA axis suppression.

It is unclear why this deficiency of 11-reductase activity becomes increasingly prominant during the pre- and peri-pubertal period. A recent review of the various factors and clinical conditions affecting 11β-HSD activity suggests that gonadal steroids may influence this activity in both rat liver and kidney [10]. Circulating T may favour E to F conversion, while estradiol inhibits it. However, we found no sex differences in both the THE/THF ratio or the ability to suppress the HPA axis with cortisone acetate at the onset of puberty. This is consistent with an earlier report describing no sex differences in rat liver 11β-HSD activity [9]. A number of other steroids are known to be inhibitors of 11β-HSD activity [10]. 11β-hydroxyprogesterone (11OHP) is a competitive substrate inhibitor of 11β-HSD and is formed by 11β-hydroxylation of progesterone. 21-desoxycortisol (21-DOC), formed by 11β-hydroxylation of 17OHP, has a structure almost identical to that of 11OHP and like 17OHP is present in very high concentrations in the serum of CAH patients in poor therapeutic control. It is tempting to speculate that 21-DOC (11-hydroxysteroid) and its metabolite, PTL (11-keto steroid) may together influence hepatic conversion of E to F in patients with CAH. Further *in vitro* studies are required to elucidate the causes of this hepatic 11β-HSD deficiency.

In summary, our data suggest that a relative deficiency of hepatic 11-reductase activity in combination with highly efficient A ring reduction of exogenous E (consequently present in large excess) thereby resulting in an E–F equilibrium very much in favor of E and significantly increased THE/THF excretion, may be responsible for the peripubertal demise of oral cortisone acetate effectiveness in the therapeutic control of CAH. GC profiling of urinary steroid excretion is a well established method for diagnosing the various enzymatic disorders underlying the clinical and biochemical heterogeneity of CAH [15]. The additional quantitation of THE and THF excretion, as markers of the hepatic E–F equilibrium, may serve as a valuable component in monitoring the complex management of CAH, particularly during the critical pre-pubertal period.

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