Urinary tetrahydrocortisone and tetrahydrocortisol glucosiduronates in normal newborns, children and adults

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Abstract. Utilizing a simple, highly specific radioimmunoassay (RIA), we measured the excretion of the glucosiduronate conjugates of tetrahydrocortisone (THE-gluc) and tetrahydrocortisol (THF-gluc) in adults (n = 16), children (n = 58) and newborns (n = 5), in order to establish a normal range of values for age and surface area. Both tetrahydroxometabolites showed a linear increase with age but became constant for all individuals except newborns when results were calculated per square meter. Newborns excreted disproportionately low levels of these metabolites for their size. In children of similar ages, when 24h urine collections (n = 13) were compared to spot AM specimens (AM-Sp) (n = 45) in which the daily volume was estimated by creatinine excretion, THE-gluc/m² levels were significantly higher in AM-Sp urines but THF-gluc/m² levels were similar. Levels of both metabolites were markedly elevated in two patients with hyperadrenal states and low in three patients with hyperadrenal states compared to normal values per m². These results indicate that the RIA for THE-gluc and THF-gluc can be a useful indirect test of cortisol secretion in children as well as in adults. Although 24h urine collections are more accurate, creatinine corrected AM-Sp urines may be clinically useful when values of these metabolites per m² are compared to appropriate controls.

The need for an accurate rapid method of evaluating glucocorticoid secretion, particularly in the paediatric age group has long been recognized. The Porter-Silber (Silber & Porter 1954) and tetrazolium-blue (Kingsley & Getchell 1961) reactions are non-specific methods that measure not only metabolites of glucocorticoids but also metabolites of other steroids as well as materials unrelated to steroids (Labhart 1974). Will et al. (1977) have developed a highly specific radioimmunoassay (RIA) for the glucosiduronates of tetrahydrocortisone and tetrahydrocortisol (THE-gluc and THF-gluc, respectively), the major urinary metabolites of cortisone and cortisol (Kornel & Saito 1975). While adult norms for these metabolites are well known (Will et al. 1977; Gross et al. 1977; Pal 1967; Vestergaard 1978b), little has been reported about their values in children and newborns (Ducharme et al. 1970; Hornig et al. 1971).

In the present study the RIA for THE-gluc and THF-gluc was utilized to establish a range of values for age and surface area in a population of normal children, and for comparison, in adults. In addition, AM-Sp specimens and 24h collections in children were compared.
Materials and Methods

Sixteen adults (18–60 years), 58 children (2 months to 18 years), and 5 full term newborns, all healthy, were studied. Five premature newborns from the intensive care nursery and five patients with abnormal adrenocortical function were also included in the study. Urine samples were obtained either from 24h collections or AM-Sp specimens obtained between 8 and 10 a.m. Urine creatinine was measured to evaluate the accuracy of collection of the 24h specimens, and to estimate the expected daily volume in the AM-Sp urines.

THE-gluc and THF-gluc were measured in unprocessed diluted (1:50 to 1:500) urine by RIA, according to the method of Will et al. (1977). Non-radioactive steroids were obtained from Makor Chemicals, Ltd., Jerusalem. [3H]1,2-THE (53.4 Ci/mmol) and [3H]1,2-THF (47.9 Ci/mmol) were purchased from New England Nuclear, Boston. THE and THF-20-oximes BSA antisera were a gift of Dr. P. Vécsei, Pharmakologisches Institut der Universität Heidelberg. Characteristics of these antisera have been reported elsewhere (Will et al. 1977), however, cross-reaction studies were also performed in our laboratory. Our studies confirmed 100% cross-reactivity between the free tetrahydrometabolites and their glucosi-
duronates as well as extremely low cross-reactivity with other interfering steroids. The intra- and interassay coefficients of variation for both assays were <6.5% and <9.0% (n = 45). The sensitivity of the assays was 50 pg/tube.

Statistical significance was evaluated by linear regression analysis (Figs. 1 and 2) and Student’s t-test (Table 1).

Results

Results are summarized in Figs. 1, 2 and in Table 1. In 58 normal children (13 = 24h; 45 = AM-Sp) absolute values of both metabolites showed a significant rise with age (THE-gluc: r = 0.596, P < 0.001; THF-gluc: r = 0.496, P < 0.001). No rise with age was seen in 16 normal adults (12 = 24h; 4 = AM-Sp) (Fig. 1). In children, when THE-gluc and THF-gluc were calculated per m² of body surface, the age effect on these metabolites disappeared (Fig. 2). Values of both metabolites were similar for males and females. The THE-gluc/THF-gluc ratio was 2.5 ± SEM 0.24 (n = 74).

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![Graph](image)

**Fig. 1.**

Absolute values of urinary THE-gluc and THF-gluc in mg/24 h in normal children and adults. Spot AM urines were corrected for 24h estimated volumes on basis of creatinine excretion. Babies less than 2 months of age are not included in Figs. 1 or 2.
Values of urinary THF-gluc and THF-gluc in mg/24h/m² in normal children and adults.

Table 1.
Urinary values of THE-gluc and THF-gluc in normal individuals (mean ± sd) and in hyper- and hypoadrenal states.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Urine</th>
<th>THE-gluc mg/24h/m²</th>
<th>THF-gluc mg/24h/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>18–60 years</td>
<td>24h</td>
<td>1.43 ± 0.44</td>
<td>1.35 ± 0.69</td>
</tr>
<tr>
<td>Children</td>
<td>2 months–18 years</td>
<td>24h</td>
<td>1.25 ± 0.91</td>
<td>0.84 ± 0.71</td>
</tr>
<tr>
<td>Children</td>
<td>2 months–18 years</td>
<td>AM-Sp</td>
<td>1.95 ± 0.97</td>
<td>0.98 ± 0.72</td>
</tr>
<tr>
<td>Newborns</td>
<td>2 days</td>
<td>AM-Sp</td>
<td>0.34 ± 0.19</td>
<td>0.25 ± 0.19</td>
</tr>
<tr>
<td>Prematures</td>
<td>6 weeks</td>
<td>AM-Sp</td>
<td>0.07 ± 0.02</td>
<td>undetectable</td>
</tr>
<tr>
<td>Primary adrenal insufficiency</td>
<td>4 years</td>
<td>24h</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Primary adrenal insufficiency</td>
<td>22 years</td>
<td>24h</td>
<td>&lt; 0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Panhypopituitarism</td>
<td>23 years</td>
<td>24h</td>
<td>0.06</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Primary hyperadrenocorticism</td>
<td>22 years</td>
<td>24h</td>
<td>8.25</td>
<td>18.50</td>
</tr>
<tr>
<td>Secondary hyperadrenocorticism</td>
<td>15 years</td>
<td>24h</td>
<td>13.80</td>
<td>16.36</td>
</tr>
</tbody>
</table>
Mean values of both tetrahydrometabolites, in mg per 24h per m², are listed in Table 1. When 24h collections from adults (n = 12) and children (n = 13) were compared, no significant differences were found. In children the mean value of THE-gluc in AM-Sp urines was significantly higher than in 24h collections (P < 0.01), but mean THF-gluc values were similar regardless of the method of urine collection. In a comparison of AM-Sp urines, newborns excreted less of both metabolites than children and levels in prematures were almost undetectable.

The three patients with adrenal insufficiency had lower levels of THE-gluc and THF-gluc than normal subjects. Both patients with hyperfunction of the adrenal cortex had abnormally high levels of both metabolites with a preponderance of THE-gluc (THE-gluc/THF-gluc ratios: 0.44 and 0.84) (Table 1).

Discussion

The use of the RIA for THE-gluc and THF-gluc in children pre-supposes the establishment of norms for age. Cortisol secretion is known to increase with surface area (Dohan et al. 1962) and muscle mass (Vestergaard 1978a). One would therefore except to be able to standardize the excretion of the metabolites of cortisol for individuals of different ages if results are calculated per m² or per g creatinine. Our findings confirmed this expectation since the excretion of both THE-gluc and THF-gluc was constant for all ages over two months when surface area was taken into account. This also appears to be true for creatinine excretion (data not shown)¹.

Our finding of higher mean values of THE-gluc/m² in AM-Sp urines as compared to 24h collections most probably reflects the early AM peak of cortisol secretion (Vestergaard 1978a). Beale & Tyrell (1974) studied the excretion of cortisol metabolites in two adults, at different times during a 24h period. They found a distinct circadian rhythmicity with highest values of all metabolites in the early morning hours. Although we did not succeed in demonstrating higher mean values of THF-gluc/m² in morning urines, the higher values of THE-gluc/m² found suggests that 24h collections more accurately reflect daily cortisol secretion. Despite the periodicity, we could establish norms for spot urines. This RIA performed on a single AM urine specimen may therefore prove to be a useful and rapid clinical tool, especially in the paediatric population, if results are interpreted based on the appropriate controls.

In contrast to our findings in adults, and children over age two months, premature and healthy newborns excreted amounts of THE-gluc and THF-gluc that were disproportionately low for their size. In addition to the fact that corticoids are generally found as minor components of steroid profiles in the neonate (Horning et al. 1971), there are several other reasons to explain the low values of THE-gluc and THF-gluc in newborns. First, newborns are known to have impaired hepatic glucuronidation ability, at least in the first few days of life (Ducharme et al. 1970). Second, they also have lower glomerular filtration rates than older children and adults, and subsequently have decreased clearance of the tetrahydrometabolites as glucuronides (Kornel 1960). Third, other investigators have found that newborns excrete THE and THF more as sulphates than as glucosiduronates (Ducharme et al. 1970; Aderjan et al. 1977). Preliminary studies performed in our laboratory in 9 newborns confirmed these observations, showing relatively higher values of both tetrahydrometabolites after solvolysis of the urine, when compared to the very low excretion as glucuronides (Aranoff & Rösl, unpublished data).

In summary, the RIA for THE-gluc and THF-gluc can be used as a specific clinical tool for evaluating glucocorticoid activity in children over age two months, as well as in adults, when results are calculated per m². The major limitation of its usefulness is its inapplicability to newborns and prematures.

Acknowledgments

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¹ A much wider range of values was observed when results were calculated per g creatinine than per m².
References


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