DEVELOPMENTAL EFFECTS ON LIVER D-GLUCURONOLACTONE DEHYDROGENASE LEVELS AND ON D-GLUCARIC ACID EXCRETION IN URINE; HORMONAL EFFECTS ON D-GLUCARIC ACID EXCRETION IN URINE

A. P. MOWAT*

Department of Child Health, University of Aberdeen and Rowett Research Institute, Bucksburn, Aberdeen

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SUMMARY

Total urinary d-glucaric acid excretion increased with age and weight gain in males and was found to be low in terms of unit body weight in the newborn. In premature infants, there was a direct relationship between the serum bilirubin level and urinary d-glucaric acid excretion. In women excretion was not found to vary with the menstrual cycle, but was increased during prolonged therapy with contraceptive agents.

INTRODUCTION

In rodents the activity of liver d-glucuronolactone dehydrogenase, the enzyme which catalyses d-glucaric acid formation, is virtually absent in the first 2 weeks of life. It increases gradually to a maximum at 6–8 weeks and then falls to the adult level; human foetal liver activity is less than half of that of the adult liver (Marsh & Carr, 1965). In view of the possible role of d-glucarolactone in the metabolism of materials which undergo conjugation with d-glucuronic acid, these findings are of interest, especially since the human newborn infant's limited ability to form glucuronides occasionally has disastrous consequences, as in hyperbilirubinaemia. Urinary d-glucaric acid excretion was determined in males ranging in age from 2 days to 18 yr., including premature infants. Liver d-glucuronolactone dehydrogenase activity in human foetuses of varying gestation, and in two infants, was also examined.

Recent studies have shown that the activity of d-glucuronolactone dehydrogenase in mouse liver is modified by ovariectomy and the administration of hormones (Mowat, 1968). Urinary excretion of d-glucaric acid in adult women in different states of hormonal balance was determined.

* Present address: Department of Medicine, Albert Einstein College of Medicine, Yeshiva University, Eastchester Road and Morris Park Avenue, Bronx, N.Y. 10461, U.S.A.
MATERIALS AND METHODS

Tissue sources. Livers of human foetuses, removed at surgical termination of pregnancy, were stored at -20° until assayed. Liver samples from newborn infants, obtained at autopsy, were removed within 6 hr. of death and were stored similarly. (In mice it was found that enzyme activity did not fall if the liver was left in situ for 6 hr., A. P. Mowat, unpublished observation.)

Urine specimens. Complete 24 hr. urine samples were obtained in all instances. No subject showed evidence of disease and received no drugs except as indicated. Infants designated premature were so in terms of gestational age. Diet and activity were unrestricted. Before acid treatment, urine from newborn infants was concentrated to 30% of its original volume by freeze-drying or by evaporation under reduced pressure at 40°.

Urinary D-glucaric acid estimation. The inhibition of the enzymic hydrolysis of phenolphthalein glucuronide by acid-treated urine gives a measure of total inhibitors present. The inhibition by materials other than D-glucaric acid was determined by measuring also the inhibition in the same enzyme assay by alkali-treated urine. From the standard curve used for acid-treated urine (Mowat, 1968), the concentration of adventitious inhibitor was derived. Urinary D-glucaric acid concentration was the difference between these two values (Marsh, 1963). It was necessary to concentrate the urine of newborn infants before acid or alkali treatment in order to produce inhibition of 30-70% in the assay. Results are expressed as μmoles/kg. body weight/24 hr.

Liver D-glucuronolactone dehydrogenase activity was determined as previously described (Mowat, 1968).

Serum bilirubin levels were determined by the method of Lathe & Ruthven (1958).

RESULTS

Liver D-glucuronolactone dehydrogenase activity

Table 1 shows that liver enzyme activity was not directly related to gestational age in the period from 14 to 23 weeks, and that the activity during this period was similar to the activity in the neonatal livers examined.

Table 1. D-Glucuronolactone dehydrogenase activity in human foetal and newborn liver
(Activity in μmoles/g. of moist tissue/hr.)

<table>
<thead>
<tr>
<th>Gestation in weeks</th>
<th>14</th>
<th>14</th>
<th>17</th>
<th>18</th>
<th>18</th>
<th>18</th>
<th>19</th>
<th>19</th>
<th>19</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
<td>2.79</td>
<td>2.9</td>
<td>2.15</td>
<td>4.34</td>
<td>3.8</td>
<td>2.63</td>
<td>2.88</td>
<td>3.02</td>
<td>5.9</td>
<td>5.55</td>
</tr>
<tr>
<td>Age (weeks)</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>4.13</td>
<td>2.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D-Glucaric acid excretion

Growth and development. The results in male infants and boys of various ages are summarized in Table 2. The mean urinary excretion of glucaric acid, expressed as μmoles/kg. body weight/24 hr., in the newborn, both premature and full-term, was significantly less than in boys. Excretion was significantly higher in the younger group of premature infants than in the other groups of newborn. As shown in Fig. 1,
Table 2. Changes in D-glucaric acid excretion, with development in males (means ± s.e.)

<table>
<thead>
<tr>
<th>Class of subject</th>
<th>Number studied</th>
<th>D-Glucaric acid excretion (µmoles/kg./24 hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prematurely born infants aged 2–4 days</td>
<td>8</td>
<td>0.63 ± 0.081</td>
</tr>
<tr>
<td>Prematurely born infants aged 5–7 days</td>
<td>5</td>
<td>0.28 ± 0.102</td>
</tr>
<tr>
<td>Full-term infants aged 2–4 days</td>
<td>11</td>
<td>0.44 ± 0.069</td>
</tr>
<tr>
<td>Full-term infants aged 5–7 days</td>
<td>5</td>
<td>0.38 ± 0.102</td>
</tr>
<tr>
<td>Boys aged 2–18 yr.</td>
<td>18</td>
<td>1.06 ± 0.09</td>
</tr>
</tbody>
</table>

Fig. 1. Ratio of D-glucaric acid excretion and serum bilirubin in individual premature infants aged 2–4 days (Ο) and 5–7 days (●).

in individual infants in this group it was possible to demonstrate a significant direct correlation between serum bilirubin levels and glucaric acid excretion \( r = 0.63; P < 0.05 \). In the older group of premature infants the relationship seemed similar, but the results were insufficient to examine the relationship in detail. In the full-term infants, serum bilirubin levels were not determined, the infants being anicteric.

Figures 2 and 3 show that, in boys, there was a relationship between D-glucaric acid excretion and either weight or age over the range of 1–18 yr. It was not possible to determine whether weight or age was the relevant factor. The mean excretion in µmoles/kg./24 hr. was significantly higher than that of infants or healthy adult females, but the variation between individual boys was significantly greater than that found in these other groups. The appearance of secondary sex characteristics did not influence excretion.

Healthy adult females. From urines collected at 7-day intervals, between five and seven values were obtained for the D-glucaric acid excretion from each of seven women, as shown in Table 3. The mean variation within individuals was expressed by s.d. = ± 0.24 µmoles/kg./24 hr., and there were significant differences between individuals \( P < 0.01 \). The mean excretion of the group as a whole was 0.58 ± 0.19 µmoles/kg./24 hr. No pattern of excretion was detectable over the menstrual cycle.

Effect of oral contraceptive agents. In healthy women who had received oral contraceptive agents containing synthetic oestrogens and progestogens, for social reasons, for periods of 2–5 yr., the mean urinary D-glucaric acid excretion was 1.04 ± 0.39 (s.e.)
\( \mu \)moles/kg. body weight/24 hr. These values were obtained from single urine collections in nine individuals who were actually taking the drugs when the collections were made. [Four patients were receiving Ovulen (mestranol 0.1 mg., ethyndiol acetate 2.5 mg., Searle and Co. Ltd., Bucks.), two Orthonovin (mestranol 0.1 mg., norethisterone 2.0 mg., Ortho Pharmaceuticals, Bucks.), two Gynovlar (ethinylestradiol 0.05 mg., norethisterone acetate 3.0 mg., Schering Chemicals Ltd.), and one Conovid E (mestranol 0.1 mg., norethynodrel 2.5 mg., Searle and Co. Ltd.). These particular preparations had been taken for at least 18 months, but not necessarily over the total period of therapy.] The excretion of the subjects taking oral contra-

![Graph](image1)

**Fig. 2.** Relationship of \( \text{D-glucaric acid excretion/24 hr.} \) and body weight in healthy males aged 2-18 yr.

**Fig. 3.** Relationship of \( \text{D-glucaric acid excretion/24 hr.} \) and age in healthy males.

**Table 3.** Urinary D-glucaric acid excretion in healthy women (means \pm S.D.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Number of specimens</th>
<th>D-Glucaric acid excretion (( \mu )moles/kg./24 hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.R.</td>
<td>5</td>
<td>0.58 \pm 0.19</td>
</tr>
<tr>
<td>M.S.</td>
<td>6</td>
<td>0.51 \pm 0.16</td>
</tr>
<tr>
<td>L.M.</td>
<td>5</td>
<td>0.31 \pm 0.09</td>
</tr>
<tr>
<td>A.M.</td>
<td>7</td>
<td>0.46 \pm 0.23</td>
</tr>
<tr>
<td>S.M.</td>
<td>7</td>
<td>0.57 \pm 0.35</td>
</tr>
<tr>
<td>M.D.</td>
<td>6</td>
<td>0.87 \pm 0.28</td>
</tr>
<tr>
<td>A.M.</td>
<td>5</td>
<td>0.79 \pm 0.19</td>
</tr>
</tbody>
</table>

**Table 4.** Urinary D-glucaric acid excretion: effect of contraceptive agents

(\( \mu \)moles/kg. body weight/24 hr.; mean of observations in five subjects, contraceptives used: Ovulen (3); Synovlar (2).)

- Before therapy: 0.77
- After 1 week of treatment: 0.86
- After 3 weeks of treatment: 0.65
- 1 week after last dose: 0.55
Developmental effects on d-glucaric acid formation

ceptives was significantly higher than that of women receiving no drugs ($P < 0.01$).

As shown in Table 4, d-glucaric acid excretion in five healthy women who had not previously received contraceptive agents was not affected by these agents in the initial cycle of therapy.

**DISCUSSION**

The d-glucuronolactone dehydrogenase activity reported in human foetal and newborn liver is similar to that found in foetal liver by Marsh & Carr (1965) and significantly less than the activity they found in adult specimens. The low d-glucaric acid excretion in newborn infants and the gradual increase in excretion with age and weight gain in boys mirrors the increasing activity of liver glucuronolactone dehydrogenase with age in rats and mice (Marsh & Carr, 1965). The low level of excretion in the newborn is such that low tissue and enteric concentration of d-glucaro-(1-4)-lactone may be a contributory factor in the high faecal concentration of unconjugated bilirubin (Brodersen & Hermann, 1963), in addition to increased enteric β-glucuronidase activity (Karunairatnam, Kerr & Levvy, 1949) and the relative lack of bacterial degradation of free bilirubin in the bowel. Since Lester & Schmid (1963) have demonstrated that unbound bilirubin is rapidly absorbed through the intestinal mucosa and into the portal circulation, while bilirubin glucuronide is not absorbed, conditions in the newborn are particularly favourable to the establishment of a significant enterohepatic circulation of bilirubin. This could contribute to hyperbilirubinaemia although it may be mainly due to deficiency of UDPglucuronyl transferase or UDPglucuronic acid (Zuelzer & Brown, 1961). It seems likely that the physiologically important enterohepatic circulation of thyroid hormone, oestrogens, androgens and corticosteroids (Jayle & Pasqualini, 1966) would also be enhanced. Oral administration of glucarolactone or its increased formation after ingestion of glucuronolactone (Marsh, 1963) has been suggested as a possible means of controlling serum bilirubin levels in the newborn when kernicterus threatens (Anke, Fenichel & Barness, 1959). The administration of glucarolactone to a small series of mature infants was associated with higher serum bilirubin levels than in a control series who received glucose (L. A. Barness, personal communication, 1965). This is in keeping with the positive relationship reported here between the serum bilirubin levels and glucaric acid excretion in individual newborns of premature infants. Extension of these observations to other groups of jaundiced infants, as in rhesus iso-immunization, may clarify the significance of the relationship between serum bilirubin levels and d-glucaric acid excretion and the role, if any, of d-glucuronolactone dehydrogenase in the aetiology of jaundice in the newborn period.

d-Glucaric acid excretion did not appear to vary with the menstrual cycle in the group of healthy individuals studied. The main importance of the data reported from this group is that the differences in excretion between individuals reach statistical significance. This should be borne in mind if between-individual comparisons are made in assessing, for example, the effects of drugs on d-glucaric acid excretion. Although daily estimations together with basal temperature records and measurement of hormonal excretion may reveal changes during the menstrual cycle not evident on sampling at 7-day intervals, diet may be the over-riding factor and require control.
Elucidation of the role of endogenous hormones in D-glucaric acid excretion may be aided by the determination of its excretion in post-menopausal women. Since the over-all hormonal effect of oral contraceptives in some respects amount to medical castration (Klopper, 1965), the increased excretion of D-glucaric acid found after prolonged administration of these drugs to women might have been anticipated from the effect of ovariection on liver enzyme activity in mice. Oral contraceptives have already been shown to produce changes in carbohydrate metabolism in that 15–20% of women who have received them for more than 3 months show the features of steroid-induced diabetes (Wynn & Doar, 1966). Further investigation is required to find how long it is before their effect on D-glucaric acid excretion becomes apparent in women.

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REFERENCES


