Enzymes of Galactose Metabolism in Livers of Suckling and Adult Tammar Wallabies (*Macropus eugenii*) and Other Marsupials

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Abstract

The activities of galactokinase, hexose-1-phosphate uridylyl transferase and UDPglucose 4-epimerase in homogenates of livers of two adult and 20 suckling tammar wallabies aged from 6 to 50 weeks were investigated. The activities of all three enzymes were high until 24-30 weeks post partum, after which they declined to low levels.

The activities of the three liver enzymes were high in pouch young of six other species of marsupial. Comparison of the activities of the three liver enzymes in suckling tammar wallabies with those in suckling rats showed no difference between the two species in regard to galactokinase and uridylyl transferase, but the UDPglucose 4-epimerase activity in tammar wallabies was approximately double that found in rats. This may be related to the high galactose content of tammar wallaby milk compared with rat milk.

In suckling tammar wallabies, the liver had higher enzyme activities than other tissues studied.

It is concluded that, contrary to the suggestion of Stephens et al. (1975), pouch young marsupials are not deficient in their ability to metabolize galactose.

Introduction

In eutherian mammals, the major pathway for the metabolism of galactose leads to glucose-1-phosphate via reactions catalysed by galactokinase (ATP: D-galactose 1-phosphotransferase, EC 2.7.1.6), uridylyl transferase (UDPglucose: α-D-galactose-1-phosphate uridylyl transferase, EC 2.7.7.12) and UDPglucose 4-epimerase, EC 5.1.3.2 (Segal 1978).

There have, hitherto, been few investigations on the metabolic fate of galactose in marsupials. Stephens et al. (1974a, 1975) found that erythrocytes of adult red kangaroos (*Macropus rufus*) and various other species of marsupial had low galactokinase and uridylyl transferase activities relative to those of human erythrocytes, and suggested that young herbivorous marsupials are deficient in their ability to metabolize dietary galactose. Richardson et al. (1979) similarly found low uridylyl transferase activities in erythrocytes of adult western grey kangaroos (*M. fuliginosus*). The suggestion that young marsupials might be deficient with respect to galactose metabolism is, however, difficult to reconcile with numerous studies showing that the milk of marsupials is exceptionally rich in galactose (Gross and Bolliger 1958; Jenness et al. 1964; Bergman and Housley 1968; Messer and Mossop 1977; Messer and Green 1979).
The milk galactose of marsupials is present in the form of various oligosaccharides such as 3'-galactosyllactose (Messer et al. 1980) and tetra- to heptasaccharides which consist of β-(1 → 3)-linked D-galactose residues attached to lactose at their reducing ends (Collins et al. 1981). The small intestinal mucosa of tammar wallaby pouch young has high β-galactosidase activity and liberates free galactose from these oligosaccharides during incubation in vitro (Walcott and Messer 1980). Significant amounts of free galactose must therefore be absorbed by tammar wallabies during suckling and one would expect the pouch young to be capable of metabolizing it.

In rats and other eutherians, galactose is metabolized mainly by the liver, and the galactokinase and uridylyl transferase activities of erythrocytes may not therefore reflect an animal's ability to use galactose (Rogers et al. 1979). In addition, the ability to metabolize galactose is greater in immature animals than in adults of the same species (Haworth and Ford 1963; Segal et al. 1963; Beyreiss 1971) and conclusions based on data obtained with adult marsupials may not therefore be valid for suckling animals.

In this paper we report on the activities of the three major enzymes of galactose metabolism in the livers of tammar wallabies of various ages, and compare these with the activities in livers of rats. Data on the enzyme activities in other organs, and in livers of other species of marsupial, are also presented.

Materials and Methods

Materials

Livers and other tissues from tammar wallabies and from a native cat (Dasyurus viverrinus) were obtained from the Division of Wildlife Research, CSIRO, Canberra. Livers of other species of marsupial were provided by Dr D. W. Cooper, School of Biological Sciences, Macquarie University, N.S.W. All tissues were removed immediately after death of the animal, transported to this laboratory in dry ice, and stored at −60°C for up to 6 months.

The following radiochemicals were obtained from Amersham Australia Pty Ltd, Sydney: D-[U-14C]galactose, 60 mCi/mmol; D-[U-14C]galactose 1-phosphate, >200 mCi/mmol; uridine diphospho-D-[U-14C]galactose, >200 mCi/mmol.

Enzyme Assays

Galactokinase, uridylyl transferase and UDPglucose 4-epimerase activities were determined on freshly prepared tissue homogenates by the radiochemical methods previously applied to rat tissues by Segal and co-workers, with some minor modifications.

Galactokinase (Cuatrecasas and Segal 1965)

The homogenizing and incubation media contained N-acetylcysteine (Walker and Khan 1968) instead of mercaptoacetic acid. Serum albumin was omitted from the homogenizing medium. The enzyme assay incubation medium contained 0.40 mm [14C]galactose (1.0 μCi/ml), and its final pH was 7.8. Paper chromatography (to separate galactose 1-phosphate from galactose) was done by the ascending technique until the solvent front had reached the top of the DEAE-cellulose strip, which was 15 cm long (about 40 min). The radioactivity due to labelled galactose 1-phosphate was all found within 4 cm of the origin, and was well separated from that due to unreacted [14C]-galactose, which migrated close to the solvent front.

Hexose-1-phosphate uridylyl transferase (Bertoli and Segal 1966)

The incubation medium contained 0.34 mm [14C]galactose 1-phosphate (0.05 μCi/ml). After the incubation, unreacted galactose 1-phosphate was converted to galactose and inorganic phosphate.
by the addition of calf intestinal alkaline phosphatase (type VII, Sigma Chemical Co., St Louis, Mo.). Paper chromatography was done by the ascending technique until the solvent front had reached the top of the DEAE-cellulose strip (18 cm; about 1 h). The radioactivity due to labelled UDP-galactose was all found within 4 cm of the origin.

**UDPglucose 4-epimerase** (Cohn and Segal 1969)

The incubation medium contained 0·1 mm UDP-[14C]galactose (0·05 μCi/ml). After the incubation, 0·15 unit of UDPglucose dehydrogenase (type III, Sigma Chemical Co.) was added to convert UDPglucose to UDPglucuronic acid. Chromatographic separation of UDPglucuronic acid from UDPgalactose was done on commercial polyethyleneimine-cellulose plates (PEI-Cellulose F, Merck Art. 5725/0025). After chromatography, the radioactivity due to labelled UDPglucuronic acid was all found within 5 cm of the origin, whereas that due to labelled UDPgalactose had moved to within 10 cm of the solvent front.

Radioactivities were measured at 50% efficiency, in a liquid scintillation spectrometer in 10 ml of 0·5% (w/v) of 2,5-diphenyloxazole in toluene. The percentage conversion of labelled substrate to labelled product was calculated from the ratio of counts per minute of labelled product to the total counts per minute.

All enzyme assays were done in triplicate. Several control experiments were done to establish that in each assay the amount of product formed was proportional to the incubation time during the standard time of 6 min at 37°C, and that the conditions of pH and substrate concentrations used by Segal and co-workers for rat tissues were applicable to tissues of tammar wallaby.

Bertoli and Segal (1966) and Cohn and Segal (1969) reported that frozen rat tissues showed no loss of uridyl transferase and UDPglucose 4-epimerase activities, respectively, during storage at −45°C for periods of up to 1 month. According to Shin-Buehring et al. (1977), human galactokinase is less stable than uridyl transferase in frozen supernatants of tissue homogenates when stored at −20°C. We therefore compared the galactokinase activities of rat liver which had been stored at −60°C for 1 year, and of tammar wallaby liver similarly stored for 3 months, with those of fresh tissues; no significant differences between frozen and fresh tissues were found. It was therefore assumed that storage of tammar wallaby tissues at −60°C for up to 6 months had no effect on the activities of galactokinase, uridyl transferase, and UDPglucose 4-epimerase.

**Protein Estimation**

The protein contents of tissue homogenates, and of the supernatants from the 30 000 g centrifugations, were measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard. All protein estimations were done in duplicate. Except where otherwise stated, all specific activities are defined as micromoles of product formed per minute at 37°C per gram of tissue homogenate protein.

**Results**

Fig. 1 shows the specific galactokinase, uridyl transferase and UDPglucose 4-epimerase activities of homogenates of the livers of 20 suckling and two adult tammar wallabies. The results clearly demonstrate age-related changes. The activities of all three enzymes were highest between about 14 and 24 weeks post partum, and then gradually declined, reaching their low adult values by about 40 weeks. The decline in uridyl transferase activity appeared to begin somewhat later than that in the galactokinase and UDPglucose 4-epimerase activities, but the developmental changes in all three enzymes followed roughly similar patterns.

Table 1 shows the specific activities determined for the livers of suckling marsupials of five other species. Most of these activities were lower than those found in tammar wallabies aged 14–24 weeks, possibly because the animals used were mostly younger (cf. Fig. 1). However, all the activities were greater than those of adult tammar wallabies.
In Table 2, the activities of the three liver enzymes of galactose metabolism of tammar wallabies are compared with those of rats. The data for rats include results obtained in the present study, as well as mean values calculated from the results of Cuatrecasas and Segal (1965) for galactokinase, Bertoli and Segal (1966) for uridylyl transferase, and Cohn and Segal (1969) for UDPglucose 4-epimerase. For purposes of comparison, the uridylyl transferase and UDPglucose 4-epimerase activities are expressed per gram of protein of the 30000 g homogenate supernatant, since Bertoli and Segal (1966) and Cohn and Segal (1969) did not refer their values to the protein of the original tissue homogenate. The data for the suckling animals are the means of the values obtained during the period post partum in which the activities were

Fig. 1. Specific activities of (a) galactokinase, (b) hexose-1-phosphate uridylyl transferase and (c) UDPglucose 4-epimerase of livers of tammar wallabies of various ages.
Table 1. Activities of galactose-metabolizing enzymes in livers of various species of marsupials

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (weeks)</th>
<th>Galactokinase</th>
<th>Specific activity</th>
<th>UDPglucose 4-epimerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern grey kangaroo</td>
<td>6</td>
<td>35</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>(M. giganteus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red kangaroo</td>
<td>2</td>
<td>16</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>(M. rufus)</td>
<td>3</td>
<td>19</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Wallaroo</td>
<td>3</td>
<td>9</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>(M. robustus)</td>
<td>13</td>
<td>19</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>Swamp wallaby</td>
<td>3</td>
<td>12</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>(Wallabia bicolor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native cat</td>
<td>7</td>
<td>43</td>
<td>42</td>
<td>16</td>
</tr>
<tr>
<td>(Dasyurus viverrinus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Comparison of activities of galactose-metabolizing enzymes of livers of rats and tammar wallabies

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Galactokinase (A)</th>
<th>Activity Uridyl transferase (B)</th>
<th>UDPglucose 4-epimerase (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat(^c)</td>
<td>Adult</td>
<td>12·3</td>
<td>21·0</td>
<td>2·0</td>
</tr>
<tr>
<td>Rat(^d)</td>
<td>Adult</td>
<td>12</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Tammar wallaby(^c)</td>
<td>Adult</td>
<td>6·0</td>
<td>13·0</td>
<td>2·0</td>
</tr>
<tr>
<td>Rat(^c)</td>
<td>0–16 days</td>
<td>31·3 ± 1·0</td>
<td>70·0 ± 6·0</td>
<td>19·0 ± 1·0</td>
</tr>
<tr>
<td>Rat(^d)</td>
<td>0–16 days</td>
<td>35·8 ± 1·4</td>
<td>78·0 ± 8·0</td>
<td>21·0 ± 1·0</td>
</tr>
<tr>
<td>Tammar wallaby(^c)</td>
<td>14–24 weeks</td>
<td>35·2 ± 1·5</td>
<td>61·0 ± 3·0</td>
<td>50·0 ± 1·0</td>
</tr>
</tbody>
</table>

\(^A\) \mu moles per minute per gram of homogenate protein.

\(^B\) \mu moles per minute per gram of 30 000 g supernatant protein.

\(^C\) Results of this study. The values for adult rats and tammar wallabies are each the mean of results from two animals. The values for the rats 0–16 days old and the tammar wallabies 14–24 weeks old are the means (± s.e.m.) of results from four and eight animals, respectively.

\(^D\) Values from data of Cuatrecasas and Segal (1965, fig. 5), Bertoli and Segal (1966, fig. 7) and Cohn and Segal (1969, fig. 3) for galactokinase, uridyl transferase and UDPglucose 4-epimerase, respectively. The values for the rats 0–16 days old are each the means (± s.e.m.) of five points read off the figures of these authors.

Table 3. Activities of galactose-metabolizing enzymes in various tissues of pouch young tammar wallabies

All tissues were from two pouch young aged 17 weeks

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Galactokinase</th>
<th>Specific activity Uridyl transferase</th>
<th>UDPglucose 4-epimerase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>34, 39</td>
<td>32, 29</td>
<td>29, 28</td>
</tr>
<tr>
<td>Small intestine</td>
<td>6·3, 6·1</td>
<td>7·5, 10</td>
<td>3·9, 3·3</td>
</tr>
<tr>
<td>Kidney</td>
<td>3·5, 4·0</td>
<td>2·6, 2·0</td>
<td>5·2, 4·7</td>
</tr>
<tr>
<td>Heart</td>
<td>0·9, 1·0</td>
<td>2·1, 2·1</td>
<td>0·9, 1·1</td>
</tr>
<tr>
<td>Lung</td>
<td>1·1, 0·8</td>
<td>1·1, 1·5</td>
<td>1·8, 2·2</td>
</tr>
<tr>
<td>Brain</td>
<td>2·0, 1·9</td>
<td>0·5, 0·6</td>
<td>2·6, 3·4</td>
</tr>
</tbody>
</table>
maximal, i.e. 14–24 weeks in tammar wallabies and 0–16 days in rats. It is seen that in adult tammar wallabies all three enzyme activities were lower than in adult rats. In suckling animals the galactokinase and uridyl transferase activities of tammar wallabies did not significantly differ from those of rats (\(P > 0.05\)), but the UDP-glucose 4-epimerase activities were very significantly greater (\(P < 0.001\)) in tammar wallabies than in rats.

Table 3 shows the specific activities of the three enzymes of galactose metabolism in various tissues of two tammar wallabies aged 17 weeks. All tissues examined had some activity, but the liver had the highest activities for all three enzymes. After the liver, the small intestine had the highest galactokinase and uridyl transferase activities, whereas the kidney had the highest UDPglucose 4-epimerase activity.

**Discussion**

The results show that up to about 30 weeks post partum the activities of the three major tammar wallaby liver enzymes of galactose metabolism were high relative to the activities found in adults. High activities were found also in the livers of pouch young of six other species of marsupials. We conclude that the livers of suckling marsupials, like those of eutherians, convert a major part of milk galactose to glucose. This is consistent with the finding of Janssens et al. (1977) that the livers of fed tammar wallaby pouch young contain significantly more glucose than those of fasted animals, and have significantly higher activities of glucose-6-phosphatase than those of adult animals.

The concentration of galactose in the milk of tammar wallabies increases gradually from about 6% (w/v) at 4 weeks post partum to 10% at 26 weeks, and then rapidly declines to 5% at 32 weeks and to 0.4% at 44 weeks (calculated from data of Messer and Green 1979). The activities of the liver enzymes of galactose metabolism similarly showed a marked decrease between about 24 and 40 weeks post partum (Fig. 1). Previous results (Walcott and Messer 1980) showed a similar time-course for the activity of tammar wallaby intestinal \(\beta\)-galactosidase. The period from 26 to 36 weeks post partum is one during which the young begin to leave the pouch and to consume herbage (Messer and Green 1979); therefore the temporal changes in all three parameters (milk galactose concentration, intestinal \(\beta\)-galactosidase activity and liver activities of galactose-metabolizing enzymes) are probably related to a change in diet from milk to herbage.

The developmental patterns shown by the galactose-metabolizing enzymes of tammar wallabies were roughly similar to those observed with rats (Segal 1978). However, the galactose content of rat milk is much lower than that of tammar wallaby milk, ranging from 0.7 to 1.9 g/100 ml (calculated from data of Luckey et al. 1954; Kuhn 1972), and it was therefore of interest to compare the activities of the galactose-metabolizing enzymes in suckling animals of the two species. There were no significant differences with respect to galactokinase and uridyl transferase, but the UDPglucose 4-epimerase activity was very significantly greater (by a factor of 2) in tammar wallabies than in rats (Table 2). This finding is consistent with UDPglucose 4-epimerase being the rate-limiting enzyme in galactose metabolism, in line with the suggestions of Segal (1978) and Berman et al. (1978). However, no definite conclusions can be drawn since we have no data enabling us to compare the rate at which a
given amount of galactose enters a given weight of liver during suckling in the two species.

In adult animals the activities of all three enzymes were lower in tammar wallabies than in rats (Table 2), consistent with the low values for erythrocyte galactokinase and uridylic transferase observed by Stephens et al. (1974a) and Richardson et al. (1979) in adult kangaroos, and with the fact that galactose is not a significant component of the diet of adult macropods.

Orphan kangaroos (Stephens et al. 1974b, 1975) and other marsupials (Slatter et al. 1980) hand-reared on cows milk sometimes develop cataracts. Since cataracts are symptomatic of congenital galactokinase or uridylic transferase deficiency in untreated human galactosaemia (Segal 1978), Stephens (1975) recommended that kangaroos be reared on lactose-free milk substitutes, such as those used for human galactosaemic infants. The present results do not, however, support the view that pouch young marsupials are deficient in galactose metabolism (Stephens et al. 1974a). Therefore the cataracts reported by these workers probably had some cause other than galactose toxicity.

Comparison of the present results with those of Walcott and Messer (1980) suggests that the decline in liver galactokinase activity may begin a few weeks earlier than that in intestinal $\beta$-galactosidase. This comparison may not be valid since the two enzymes were not assayed in the same animals, but it suggests the possibility that cataracts might be produced by lactose during a relatively short period late in lactation (roughly 30–34 weeks in tammar wallabies) in which the intestinal $\beta$-galactosidase activity could still be sufficient to digest lactose, but the liver galactokinase activity may be too low to metabolize galactose adequately. This period may be too short to permit development of cataracts (Mitchell and Dodge 1935); furthermore, any lactose consumed late in lactation may never reach the intestine, since at this stage the young are likely to have begun to develop the ruminant-type digestion typical of adult kangaroos (Tyndale-Biscoe 1973). It is, nevertheless, conceivable that cataracts might develop in young macropods if they are fed only cows milk at a time when they would normally have begun to consume significant amounts of herbage; this possibility should be amenable to experimental study and should not, perhaps, be dismissed out of hand.

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References


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