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Abnormal Galactosylation of Complex Carbohydrates in Cultured Fibroblasts from Patients with Galactose-1-Phosphate Uridyltransferase Deficiency

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ABSTRACT. An abnormality in galactosylation of complex carbohydrates may be important in the pathogenesis of the long-term complications of classic (galactose-1-phosphate uridyltransferase-deficient) galactosemia. The ability of nine galactosemic fibroblast preparations to be galactosylated with a purified galactosyltransferase was measured as an indicator of vacant sites where galactose would normally reside. The amount of galactose transferred to cell protein from galactosemic patients was significantly higher than that transferred to a group of seven controls ($p < 0.005$). Galactosyltransferase activity of the galactosemic cell preparation toward N-acetylglucosamine was also significantly higher than normal ($p < 0.01$), and there was a linear relationship between these two parameters in galactosemic but not normal cells. These findings suggest that there is defective galactosylation of galactosemic cell complex carbohydrates and that such cells increase their galactosyltransferase levels in an attempt to compensate for the defect. Defective galactosylation may be implicated as an etiologic factor in complications observed in galactosemic patients even when treated with galactose-restricted diets. (*Pediatr Res* 31: 508–511, 1992)

Abbreviations

UDPgalactose, uridine diphosphogalactose
UDPglucose, uridine diphosphoglucose
OPCA, olivopontocerebellar atrophy

Although dietary restriction of galactose has been the basis for treatment of classical galactosemia for over 50 years (1), the long-term efficacy of this approach has been seriously questioned. A survey of over 300 patients indicates that even well-treated patients whose galactose restriction was initiated at birth have developmental delay, speech abnormalities, and ovarian failure in females (2). Older galactose-restricted patients have developed an ataxia syndrome (3).

Two theories have been proposed, both involving the sugar nucleotide UDPgalactose, to explain the presence of the complications seen despite the absence of dietary galactose. Gitzelmann *et al.* (4) postulate a state of continuous self-toxicity due to a build-up of galactose-1-phosphate from the UDPgalactose derived from UDPglucose, a normal metabolite of glucose metabolism. Galactose-1-phosphate elevation in red cells of patients under rigid galactose-restricted diets has, indeed, been a constant finding. Alternatively, Ng *et al.* (5) have proposed that galactosemic patients may have impaired synthesis of complex carbohydrates and other macromolecules necessary for normal cell function. This defective synthesis would result from the observed low concentrations of UDPgalactose in red cells, cultured fibroblasts, and liver biopsies of galactosemic patients (5). Indeed, Haberland *et al.* (6), in 1971, reported an abnormal pattern of glycoproteins in the brain of a galactosemic patient. Dobbie *et al.* (7) recently found that the galactose to mannose ratio is abnormally low in a hydrolysate of the cultured fibroblasts of galactosemic patients, supporting the idea of defective galactosylation in these cells.

To test the latter hypothesis, we studied the galactosylation state of glycoproteins in cultured fibroblasts by determining the extent to which cellular glycoproteins would serve as galactose acceptors when extracts were incubated with a purified galactosyltransferase. The rationale for this approach was that an impairment of galactosylation would result in a greater number of glycoprotein oligosaccharides being terminated in N-acetylglucosamine. Normally, galactose is linked to N-acetylglucosamine in the biosynthesis of the carbohydrate moieties of glycoproteins by a galactosyltransferase (Fig. 1). We reasoned that if we incubated cell extracts with radiolabeled UDPgalactose and purified galactosyltransferase, radioactive galactose would be transferred to terminal N-acetylglucosamine sites (Fig. 1). McGuire *et al.* (8) have shown that an N-acetylglucosamine:UDPgalactose galactosyltransferase purified from human milk will transfer radiolabeled galactose from UDPgalactose into various glycoproteins from which galactose has been removed or transfer galactose to free N-acetylglucosamine. Extracts of cells from galactosemic patients accept significantly higher amounts of galactose than control fibroblasts, indicating that a greater number of vacant galactose acceptor sites exist. In addition, the cultured galactosemic cells themselves have galactosyltransferase activity toward N-acetylglucosamine as acceptor that is significantly higher than normal. This result could suggest a cellular response that attempts to compensate for factors responsible for defective galactosylation.

MATERIALS AND METHODS

Cultured cells. Human fibroblasts were cultured from skin biopsies from nine patients with congenital galactosemia, five males and four females, ages 2 mo to 15 y, and eight normal

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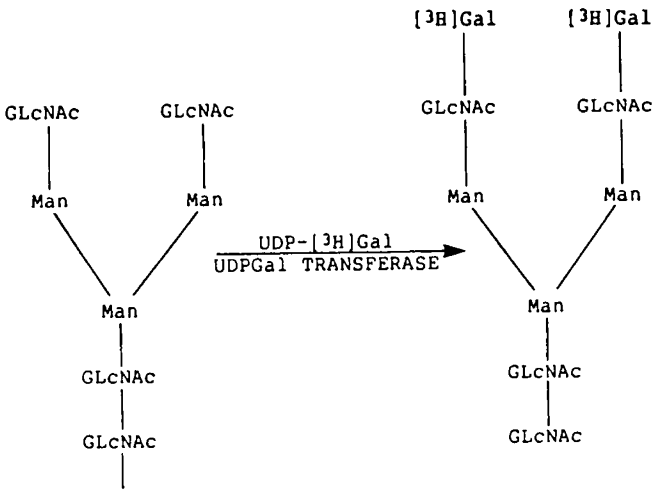

Fig. 1. Structure of an oligosaccharide showing sites of galactosylation and the reaction of galactosyltransferase. *GLcNAc*, N-acetylglucosamine; *Man*, mannose; *Gal*, galactose; *UDPgal*, uridine diphosphate galactose.

controls, four males and four females, age 4 mo to 34 y. Cells from four patients with diseases other than galactosemia were also examined: two twin females aged 29 with an unknown OPCA disorder and two children, one a 6-mo old with an unknown neurodegenerative disorder and the other a 6-mo old with an abnormality of ketone body metabolism. Except for two galactosemic cell lines that were in passages 11 and 12, all others were in passages 3 to 7. Cells were grown in T25 flasks to confluence in Eagle's minimal essential medium with 20% FCS and 2 mM glutamine. The cells were harvested by scraping after washing twice with HEPES buffer (5 mmol/L 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid pH 7.4) in physiologic saline. After suspension in 1 mL of PBS, 0.9%, the cells were centrifuged and the pellets resuspended in twice their volume of a buffer containing: 25 mmol/L cacodylate (pH 7.0), 1 mmol/L $MnCl_2$, and 1% Triton X-100. Pellets were kept frozen at −20°C until assayed.

Fibroblast complex carbohydrate UDPgalactose acceptor activity. The degree of galactosylation was determined by measuring the ability of the disrupted fibroblast preparation to serve as an acceptor for galactose in the presence of radiolabeled UDPgalactose and purified human milk galactosyltransferase. This purified enzyme transfers galactose in a β-1-4 linkage to glycoproteins (8). The standard assay consisted of 5 μL of the cell preparation containing 5–10 μg of protein, 10 μL of purified transferase containing 1 μg of protein (the enzyme was purified from human milk by gel filtration and affinity chromatography to a sp act of 70 pmol galactose transferred per μg/min), 10 μL of 50 mmol/L $MnCl_2$, and 10 μL of UDPgalactose (Sigma Chemical Co., St. Louis, MO) containing 0.2 μCi UDP-[^{3}H]galactose (Amersham, Arlington Heights, IL) and sufficient unlabeled compound to give a final concentration of 3.2 μmol/L in the total of 35 μL. The buffer consisted of MES [2-(N-morpholino)ethanesulfonic acid]/5′ AMP at pH 6.5, which was added to the 10 μL UDPgalactose (50 mmol/L MES and 15 mmol/L 5′ AMP). The mixture was incubated at 37°C for 15 min. For time and cell protein added, the reaction was in the linear range. The incubation was stopped by adding 10 μL of 0.25 mM EDTA in 1% sodium tetraborate. The radiolabeled macromolecular product was measured as the immobile entity after the reaction mixture was subjected to electrophoresis as described previously by McGuire *et al.* (8). Each measurement was performed in duplicate with less than 10% variation. The pmol of galactose transferred were expressed per μg cellular protein, which was measured by a modified fluorimetric assay (9).

Fibroblast N-acetylglucosamine: UDPgalactose galactosyltransferase activity. The galactosyltransferase of the cell itself in the absence of added purified transferase was assayed in a similar fashion except that each incubation contained 2 μCi and 32 μmol/L UDPgalactose. In this procedure, 10 μL of 50 mmol/L N-acetylglucosamine was added as acceptor. Acid phosphatase activity was measured according to the method of Walter and Schutt (10).

RESULTS

UDPgalactose acceptor activity. Glycoproteins in fibroblasts from galactosemic patients incorporated more radiolabeled galactose from UDPgalactose in the presence of purified galactosyltransferase than did fibroblast glycoprotein from controls. The mean ± SD for the nine galactosemic patients was 0.146 ± 0.033 *versus* 0.098 ± 0.012 pmol/15 min/μg cell protein for the seven normal subjects ($p < 0.005$, t test). The array of individual values for the various cell lines is shown in Figure 2. Six of the nine galactosemic values are above the 95% confidence limit of the normal group. Of the four disease controls, the twins with OPCA had acceptor levels of 0.15 and 0.14 mmol/15 min/μg protein, clearly in the galactosemic range, whereas the other two disease controls had levels in the normal range 0.11 and 0.07.

Cell N-acetylglucosamine: UDPgalactose galactosyltransferase activity. The N-acetylglucosamine:UDPgalactose galactosyltransferase activity, with the solubilized cells serving as the enzyme source, was also higher in nine galactosemic cell lines compared with the eight normal cell lines, although there was considerable

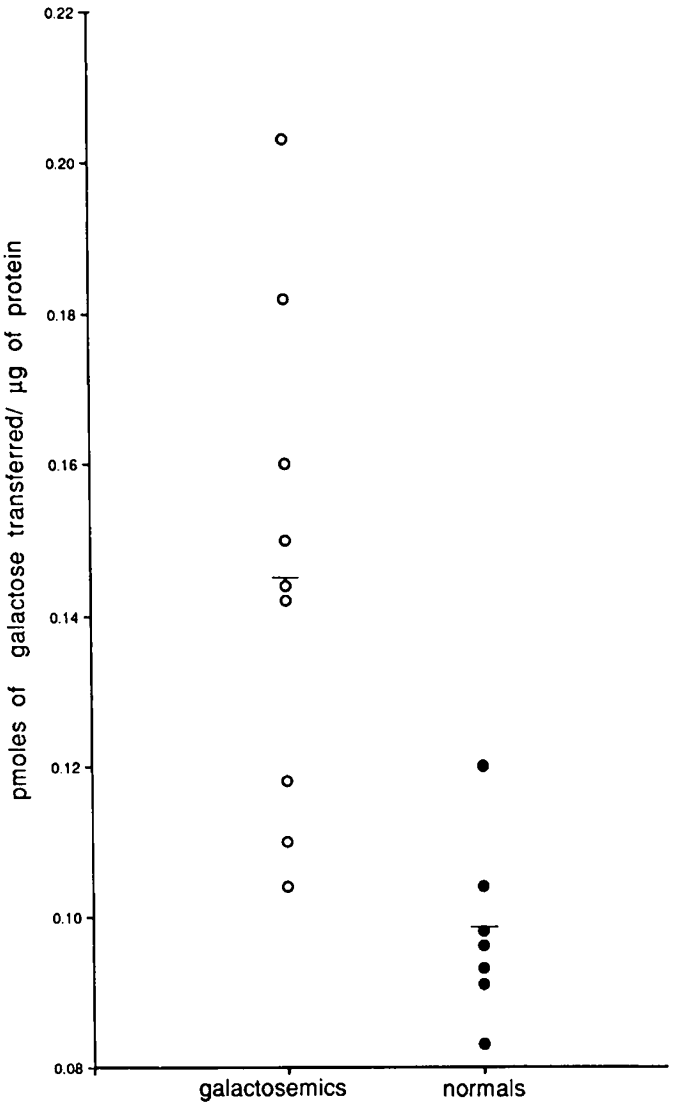

Fig. 2. Galactosylation acceptor activity of galactosemic and normal fibroblasts.

overlap in the data (23.5 ± 3.6 *versus* 16.3 ± 6.0 pmol/15 min/ μg protein; $p < 0.01$, Fig. 3). In two cell lines in which the activity was increased, the difference in activity was observed over a broad range of UDPgalactose concentrations between 10 and 160 μmol/L. To determine if the elevated galactosyltransferase level is specific or associated with a generalized increase in enzyme activity, acid phosphatase activity was measured in two galactosemic cell lines with high activity and two normal cell lines. The acid phosphatase levels did not differ between the galactosemic and control cell preparations.

Figure 4 shows the relationship between cell extract galactose acceptor levels and galactosyltransferase activity of the cells toward N-acetylglucosamine. There is no relationship between these parameters in normal cells. A linear relationship, however, exists between them in galactosemic cells as shown by linear regression analysis ($y = 9.75 + 94.5x$, $R^2 = 0.73$).

DISCUSSION

Our finding that there is a 50% increase in galactose acceptor activity in galactosemic cell extracts is consistent with the idea that galactosemic glycoconjugates exist in a hypogalactosylated state. This interpretation, however, requires clarification in view of the findings of Hart and his coworkers (11–13) that there are abundant O-linked terminal N-acetylglucosaminides that are not normally galactosylated. Nevertheless, these moieties act as acceptors of galactose in the presence of lactose synthetase and UDPgalactose in a procedure similar to ours. It is, therefore, possible that galactosemic cells may contain significantly more

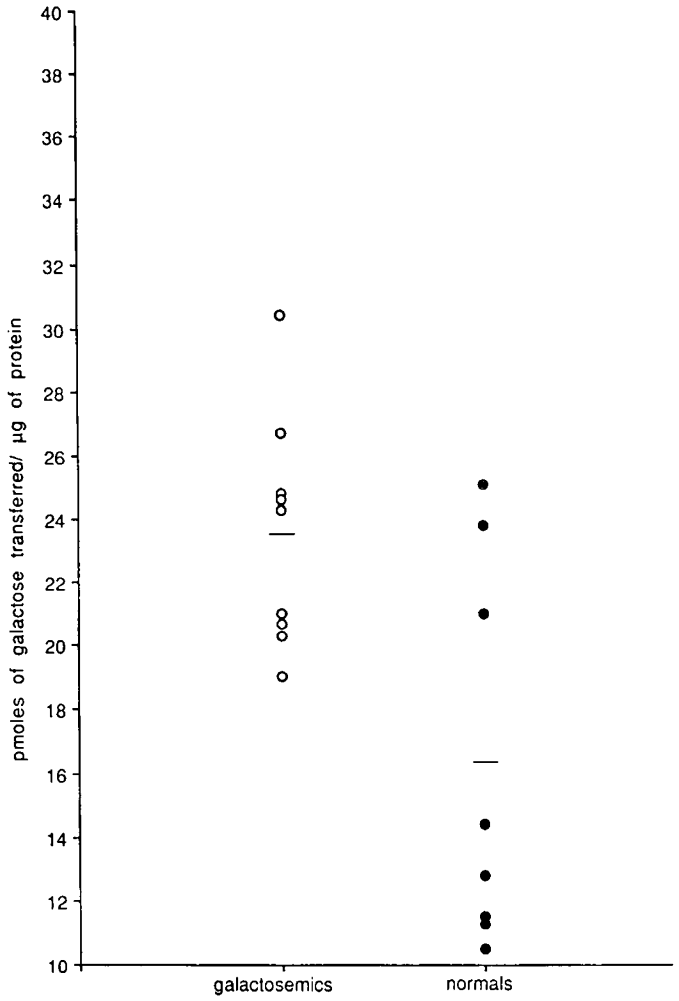

Fig. 3. Galactosyltransferase activity of galactosemic and normal fibroblasts.

of the O-linked N-acetylglucosaminide sites than normal, although there is no reason to believe this is the case. We favor the possibility that the O-linked N-acetylglucosaminides are similar in galactosemic and normal cells, but that the available galactosylation sites on galactosemic glycoconjugates are increased. Because Holt and Hart (2) showed that in many cell fractions over half of the available galactosylation sites are of the O-linked N-acetylglucosaminide variety, our estimate of the increase in glycoconjugate acceptor sites in galactosemic cells could actually be 2-fold or more higher. Further work is indeed needed to determine the actual structural alteration of the saccharides in galactosemic cells.

Our results give weight to the early findings of Haberland *et al.* (6) of deranged glycoproteins in a galactosemic brain and the recent report of Dobbie *et al.* (7) of an abnormal galactose to mannose ratio in galactosemic cell hydrolysates. Both observations support the postulate that galactose incorporation into glycoproteins is impaired in galactosemic patients. Such a defect indeed could be a contributing factor in the etiology of the long-term complications observed in this disorder. Haberland *et al.* (6) suggested that the basis for abnormal brain glycoproteins in galactosemia is an abnormal metabolism of nucleotide sugars. Ng *et al.* (5) found in red blood cells, liver biopsies, and cultured fibroblasts from galactosemic patients abnormally low UDPgalactose levels, which they felt could be responsible for defective galactosylation. Kaufman *et al.* theorized (14) that maintenance of a normal cellular UDPgalactose pool requires function of the missing galactose-1-phosphate uridyltransferase, which converts galactose-1-phosphate to UDPgalactose, and that the UDPgalactose pool cannot be maintained solely by epimerization of UDPglucose (15). This, however, cannot explain the abnormal UDPgalactose levels in cultured fibroblasts, because both normal and galactosemic cells are grown on glucose alone. Indeed, we have measured (16) by HPLC the sugar nucleotide concentration in two of our normal cell lines and two of the galactosemic lines with a high level of galactose acceptor activity. We found, as did Ng *et al.* (5) in four galactosemic lines, that the UDPgalactose concentration was low. We found, however, that UDPglucose was also only 25% of normal (unpublished results). The data support an abnormality in sugar nucleotide metabolism as an underlying cellular defect. This may be related to the hypothesis of Gitzelmann *et al.* (4) of self-intoxication by endogenous formation of galactose-1-phosphate because galactosemic fibroblasts accumulate galactose-1-phosphate even when grown on glucose alone (17). Galactose-1-phosphate is known to inhibit the activity of UDPglucose pyrophosphorylase, which could result in decreased synthesis of UDPglucose (18).

Defective galactosylation of galactosemic fibroblasts occurs even though the activity of N-acetylglucosamine:UDPgalactose galactosyltransferase is elevated with a 73% correlation between high cell transferase activity and low galactose content of glycoproteins. This is in contrast to the finding of decreased galactosylation of IgG (19) in rheumatoid arthritis, where the explanation is a reduced B lymphocyte galactosyltransferase activity (20). The increase in galactosyltransferase activity may be a compensatory mechanism either to low levels of UDPgalactose or to low galactose incorporation into macromolecules. It is also possible that the increase as we measure it *in vitro* is a response to a functional depression of the enzyme activity *in vivo*, perhaps due to accumulation of galactose-1-phosphate or other metabolites. Indeed, low cellular concentrations of nucleotide sugars may be only one aspect of abnormal glycoconjugate metabolism in galactosemia.

Glycosylation of macromolecules has assumed some importance in clinical medicine. Abnormalities in the incorporation of galactose into glycoproteins and alteration of galactosyltransferase activity also appears in other diseases. In addition to hypogalactosylation of IgG (19), patients with rheumatoid arthritis have diminished B lymphocyte galactosyltransferase activity but monocyte activity is high (20). Elevated levels of a serum galac-

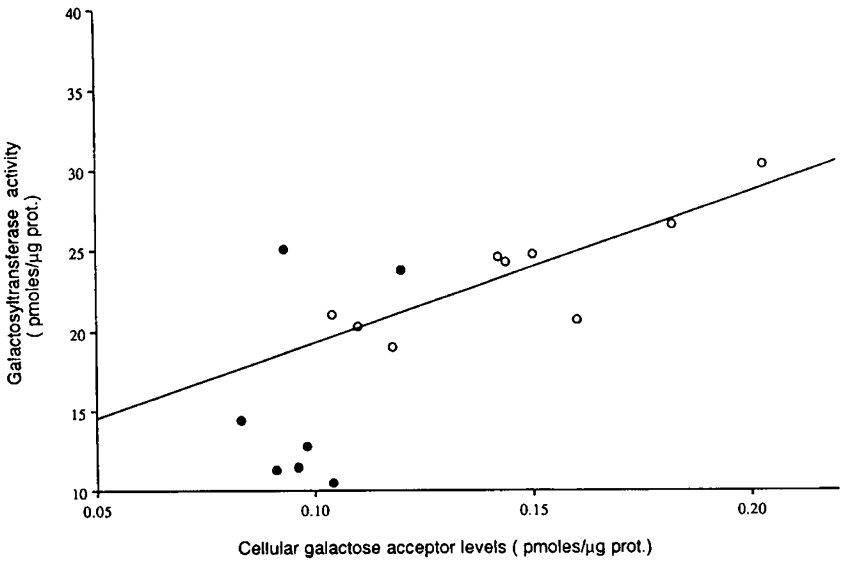

Fig. 4. Correlation of galactose acceptors and galactosyltransferase activity in fibroblasts from normal subjects (●) and galactosemic patients (○).

tosyltransferase occur in patients with some cancers (21), as well as in cultured cells and tissue from patients with various malignant disorders (22–24). Tissue glycosylation has been related to metastasis of breast cancer (25). Indeed, in our own study, cells of two siblings with an OPCA syndrome gave evidence of macromolecule hypogalactosylation. Curiously, one of the long-term complications of galactosemia may be cerebellar degeneration (3).

Our findings suggest that there is a defective galactosylation of macromolecules in galactosemic fibroblasts. As with other conditions mentioned above, whether this biochemical perturbation has a causal relationship to the enigmatic complications of galactosemia or is a biochemical abnormality to be added to others observed in the disorder (26) remains to be ascertained.

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