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Defective Galactose Oxidation in a Patient with Glycogen Storage Disease and Fanconi Syndrome

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Summary

Carbohydrate metabolism was studied in a child with atypical glycogen storage disease and Fanconi syndrome. Massive glucosuria, partial resistance to glucagon and abnormal responses to carbohydrate loads, mainly in the form of major impairment of galactose utilization were found, as reported in previous cases. Increased blood lactate to pyruvate ratios, observed in a few cases of idiopathic Fanconi syndrome, were not present. [1-^{14}C]Galactose oxidation was normal in erythrocytes, but reduced in fresh minced liver tissue, despite normal activities of hepatic galactokinase, uridyltransferase, and UDP-glucose 4-epimerase in homogenates of frozen liver. These data suggest a defect in hepatic galactose metabolism not so far identified.

Abbreviations

AA, acetoacetate
GSD, glycogen storage disease
KRB, Krebs-Ringer bicarbonate buffer
B-OB, beta-hydroxybutyrate
PPO, 2,5-diphenyloxazole
L/P, lactate to pyruvate

The association of hepatic glycogenosis with Fanconi syndrome was first described in 1949 (10). Thirteen such cases have so far been reported (1, 2, 4, 5, 11, 12, 17, 20, 22, 23). In addition to the normal features of the Fanconi syndrome (phosphoglucoaminoaciduria, vitamin D resistant rickets and growth retardation) and unclassified glycogen storage disease, patients exhibit disturbed responses to carbohydrate loading, especially major impairment of galactose utilization.

The aim of this paper is to report the results of metabolic studies of a patient with hepatic glycogenosis and Fanconi syndrome, and to demonstrate the presence of abnormal galactose oxidation.

CASE REPORT

The propositus, a 5-year-old Algerian girl, was admitted for evaluation of a presumptive Fanconi syndrome with glycogen storage disease (GSD). She was born after a full term pregnancy of healthy consanguineous parents and had two healthy siblings. Her height was 81 cm (−6 S.D.) and her weight was 11 kg (−3 S.D.). She was extremely hypotonic and exhibited severe rickets. She had not yet begun to walk. Her abdomen was protuberant and a soft liver edge was easily palpable 5 cm below the costal margin.

Laboratory investigations revealed proximal tubular dysfunction (Table 1). Serum cholesterol was 6.40 mmole/liter and her triglycerides level 3.43 mmole/liter. Standard liver function tests were normal. Slit lamp examination of both eyes did not reveal cystine crystals. X-ray examination showed active rickets and osteoporosis.

The patient's diet was supplemented with 25-OH-cholecalciferol, phosphorus, calcium, and bicarbonate. With this treatment, the serum phosphate concentration increased, but remained between 0.8 and 1.0 mmole/liter, whereas the plasma carbon dioxide level returned to normal (18–22 mmole/liter). Rickets was only partially controlled.

METHODS

All studies of the patient and of the subjects who served as controls were undertaken after obtaining parental or personal consent.

In vivo studies. Metabolic and hormonal responses were determined under normal dietary conditions, and during a 24-h fast. Stimulation tests were performed with glucagon, glucose, galactose, and fructose.

Specific enzymatic techniques were used to measure the concentration of blood glucose, galactose, fructose, lactate, pyruvate, β-hydroxybutyrate, and acetoacetate. Serum phosphate was evaluated by colorimetric assay. Plasma free fatty acids were assayed by gas phase chromatography. Plasma insulin and cortisol were determined by radioimmunoassays.

In vitro studies. Blood samples and surgical liver specimens were taken after an overnight fast. Blood samples were collected in heparinized tubes and studied immediately. Control samples came from adult subjects with no hematological disorders.

Part of the liver tissue samples was divided into aliquots, which were immediately frozen in liquid nitrogen. The remainder was placed in ice cold normal saline and rapidly brought to the laboratory. Oxidation experiments with [1-^{14}C]galactose were performed on fresh liver tissue within 30 min of biopsy. Enzyme assays were carried out on frozen tissue, after 15 days at most of storage at −180°C (enzyme activities were shown to be stable under these conditions). Controls were liver specimens obtained during laparotomy for abdominal pathology without liver failure.

Glycogenolysis. The activity of phosphorylase kinase (EC 2.7.1.38) and of amylo-1,6-glucosidase (EC 3.2.1.33) was assayed in hemolysates (18).

The glycogen content and the respective activities of glucose 6-phosphatase (EC 3.1.39) and amylo-1,6-glucosidase (EC 3.2.1.33), phosphorylase (EC 2.4.1.1), and phosphoglucomutase (EC 2.7.5.1) were determined in liver homogenates (14).

Galactose metabolism. The following enzyme assays were performed. Galactokinase (EC 2.7.1.6), uridyltransferase (EC 2.7.7.12), and UDP-glucose 4-epimerase (EC 5.1.3.2) activities were assayed in hemolysates and liver supernatants after centrifugation of the crude homogenates at 12,000 × *g* for 20 min. *(1)* Galactokinase assay: both blood and liver galactokinase activities were measured by a radioisotopic method, using purified [1-^{14}C] galactose and ATP as substrates. Incubation was carried out under the conditions used by Shin-Buehring *et al.* (25, 26) except for liver supernatants, which were only incubated for 15 min to preserve the linearity of the reaction. *(2)* Uridyltransferase assay:

Table 1. *Renal dysfunction: laboratory data*

	Blood (fasting values)	Urines
Creatinine	44 μmole/liter	—
Glucose	2.4 mmole/liter	167–363 mmole/24 h
Amino acids		generalized amino aciduria
Phosphate	0.39–0.55 mmole/liter	15–30 mmole/24 h
Calcium	2.13–2.35 mmole/liter	
Alkaline phosphatases	848 μmole/min (N < 470)	
Chloride	107 mmole/liter	
Carbon dioxide	15 mmole/liter	
Uric acid	60 μmole/liter	775 μmole/24 h

red cell transferase activity was determined by the UDP-glucose consumption method of Beutler and Baluda (6). Liver uridyltransferase activity was measured by the method of Segal *et al.* (24). *(3)* UDP-glucose 4-epimerase assay: to determine this activity in both red cells and liver, a spectrophotometric assay with a two-step procedure was used (13).

The *in vitro* production of radioactive carbon dioxide from [1-^{14}C]galactose by intact red blood cells and minced liver tissue was measured. Incubations were carried out in Warburg flasks, with Krebs-Ringer bicarbonate buffer (KRB), gassed with combined O_2 and CO_2 to obtain a pH of 7.4. A removable plastic cup containing 0.2 ml of 5.4 mole/liter KOH was positioned on top of the center well for CO_2 collection. At the end of incubation, 0.4 ml of 6 mole/liter H_2SO_4 was poured from the side-arm into the medium, and the flasks were mechanically shaken for 1 h at room temperature. One-tenth ml of [$^{14}CO_2$] trapped in KOH was then transferred into a counting vial, with 4 ml of methoxy ethanol and 9 ml of scintillation fluid (5.5 g/liter PPO in toluene) and counted in a liquid scintillation spectrometer.

A blank without the cell preparation was included in each series of experiments. The propositus and one control were tested simultaneously.

Intact erythrocytes: the incubation medium consisted of 1.6 ml of cell suspension (¼ dilution of packed red blood cells with KRB buffer, pH 7.4), 1.85 ml of KRB buffer containing 5.8 mmole/liter of glucose, and 0.55 ml of 3.0 mmole/liter [1-^{14}C]galactose (specific activity of 0.6 mCi/mmole). Flasks were incubated for 90 min at 37°C. Production of [$^{14}CO_2$] was adjusted to a cell count of 5×10^9. Results were the means of triplicate determinations.

Minced liver tissue: six to nine fragments of minced liver with total weight of approximately 100 mg were placed in each Warburg flask. The incubation medium consisted of 1 mmole/liter [1-^{14}C]galactose (specific activity, 0.33 mCi/mmole) in 3 ml of KRB buffer, pH 7.4. Flasks were incubated for 60 min at 37°C after the room air had been flushed for 8 min with O_2/CO_2 (95 v/5 v). Production of [$^{14}CO_2$] was adjusted to 100 mg of wet tissue. Results were the means of quadruplicate determinations.

Other determinations. The hemoglobin content of hemolysates was determined by a modified Drabkin procedure (27).

Proteins in liver homogenates and supernatants were assayed by Lowry's method (19).

RESULTS

In vivo studies, basal studies (Fig. 1 and Fig. 2). Under normal dietary conditions, blood glucose, insulin and cortisol values varied within the normal range. However, the blood lactate concentration increased after 2 meals. Fractional sampling of urine showed permanent glucosuria, even when the blood glucose concentration fell to 2.4 mmole/liter.

During the 24 hour-fast, concentrations of blood glucose, lactate, β-hydroxybutyrate, free fatty acids and plasma insulin, remained within the normal range, as did the lactate to pyruvate and β-hydroxybutyrate to acetoacetate ratios (Table 2).

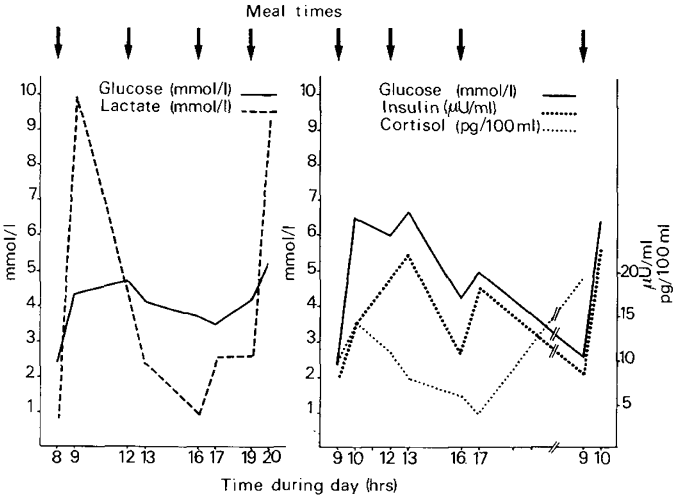
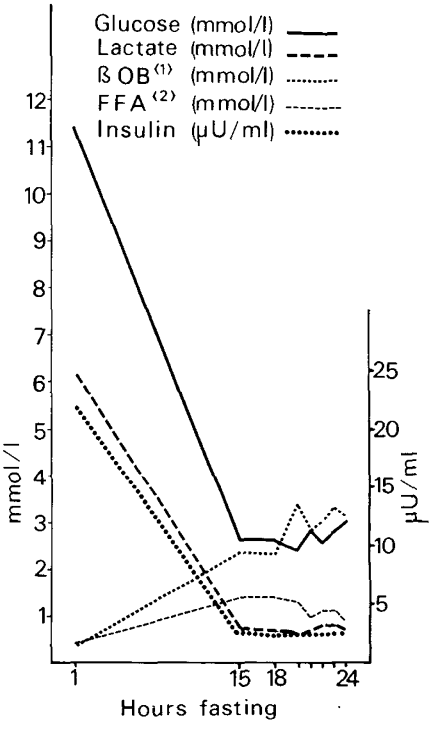


Fig. 1. Basal hormonal and metabolite levels.



(1) ß OB : ß hydroxybutyrate

Fig. 2. Fasting responses.

In vivo studies, stimulation tests. Administration of glucagon induced different responses (Fig. 3). *(1)* Two hours after a meal, a definite rise in blood glucose was observed, with a concomitant drop in blood lactate. *(2)* After an overnight fast, blood glucose only rose slightly and blood lactate remained unchanged. During the 2 h after glucagon injection, the glucosuria was 21.3 mmole *versus* 6.3 mmole during the 2 h before injection. There was no significant change in the lactate to pyruvate or β-hydroxybutyrate to acetoacetate ratios during this test (Table 2).

The results of the carbohydrate loading test are as follows. *(1)* Oral administration of glucose (Fig. 4) was followed by a large increase in the blood glucose concentration. A return to basal level was only observed 3 h after ingestion. Blood insulin-to-glucose ratios remained within the normal range as did the blood lactate-to-pyruvate and β-hydroxybutyrate to acetoacetate ratios (Table 2). *(2)* After I.V. administration of glucose (Fig. 4), a slow decline in the blood glucose concentration was noted, together

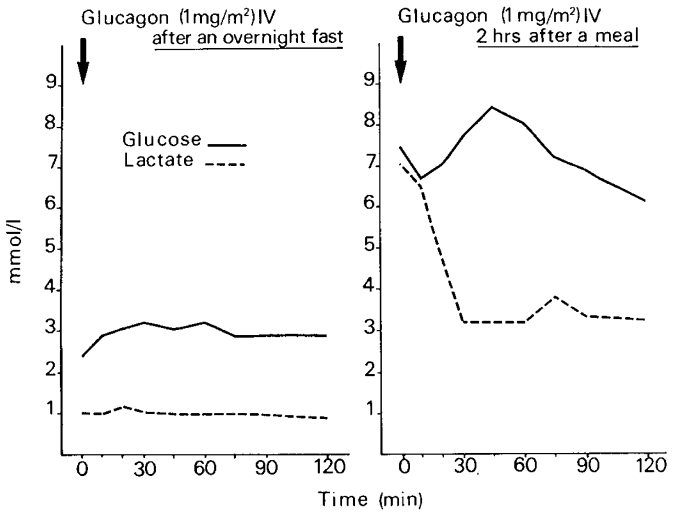


Fig. 3. Responses to glucagon administration.

with massive glucosuria (16 mmoles during the 1-h postinjection period). *(3)* After I.V. administration of fructose, blood glucose values rose less significantly than in control subjects (21), and changes in the blood lactate and serum phosphate values were found to be within the normal range (Fig. 5). *(4)* I.V. administration of galactose (1 g/kg) was followed by persistent galactosemia with no significant variations in blood glucose or lactate levels (Fig. 5). *(5)* After I.V. galactose administration of 0.35 g/kg, the half-life (t ½) of the injected product was 16.5 min (normal range at the patient's age: 6.5 ± 1.5 (16)), and about 50% of the dose administered was recovered as galactosuria.

There was no significant change in the lactate to pyruvate or β-hydroxybutyrate to acetoacetate ratios during the test (Table 2).

In vitro studies, glycogenolysis (Table 3). Increased liver glycogen content was observed, but no deficiency of glucose 6-phosphatase, amylo-1,6-glucosidase, phosphorylase or phosphoglucomutase activities were found in liver homogenates.

Normal activity was also found in hemolysates for phosphorylase kinase, and amylo-1,6-glucosidase.

In vitro studies, galactose metabolism (Table 4). Erythrocytes: normal activity was found in hemolysates for galactokinase, uridyltransferase and UDP-glucose 4-epimerase. [1-^{14}C]galactose oxidation by intact red blood cells was also shown to be within the normal range.

Liver tissue: activity was normal for the galactose metabolic pathway enzymes (galactokinase, uridyltransferase and UDP-glucose 4-epimerase) in the patient's liver supernatants, whereas significant impairment of [1-^{14}C]galactose oxidation was observed in minced liver tissue.

DISCUSSION

Our patient had an unclassified form of GSD associated with a Fanconi syndrome. As previously described in other patients with a similar condition, she displayed massive glucosuria, partial resistance to glucagon stimulation, and abnormal responses to carbohydrate loading, which was mainly a major impairment of galactose utilization.

Persistent galactosemia after galactose administration is very

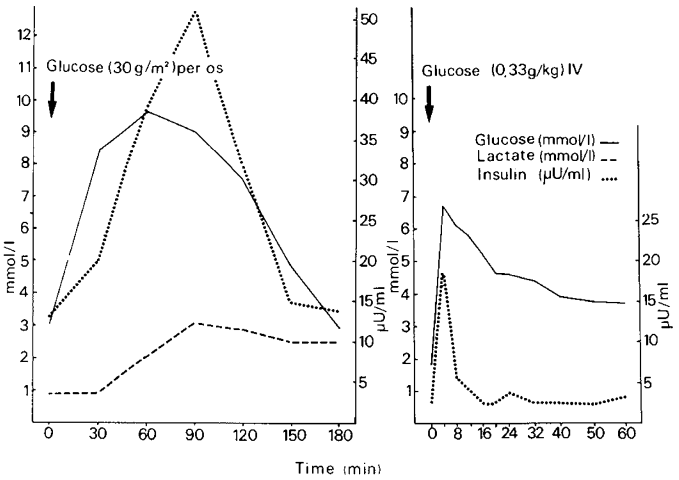


Fig. 4. Glucose loading tests.

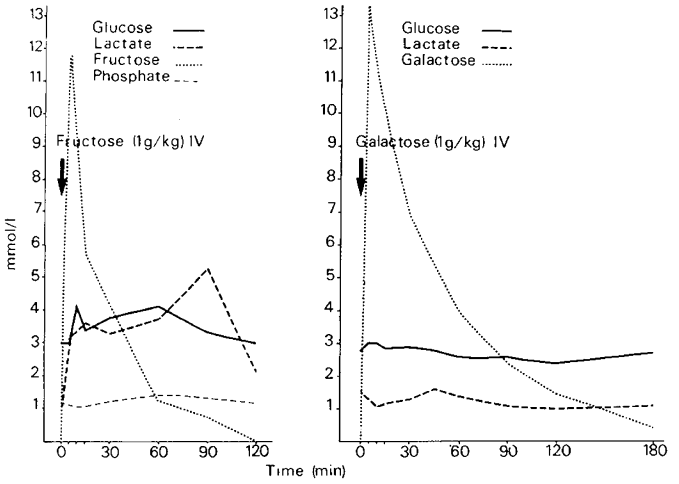


Fig. 5. Fructose and galactose loading tests.

Table 2. *Lactate to pyruvate (L/P) and β-hydroxybutyrate to acetoacetate (β-OB/AA) ratios in blood after four different types of stimulation*

Fasting			I.V. injection of glucagon after an overnight fast (1 mg/m²)			*Per os* glucose loading (30 g/m²)			I.V. injection of galactose (0.35 g/kg)		
Time (h)	L/P	β-OB/AA	Time (min)	L/P	β-OB/AA	Time (min)	L/P	β-OB/AA	Time (min)	L/P	β-OB/AA
0	12.8	2.0	0	9.7	2.5	0	9.8	2.3	0	9.1	3.3
1	19.4	4.1	10	9.5	2.5	30	10.8	1.9	5	11.8	3.4
15	10.9	1.9	20	10.6	2.2	60	10.9	1.8	10	13.3	3.6
18	11.5	2.0	30	9.7	2.0	90	10.9	2.3	15	11.9	3.4
20	12.4	2.0	45	9.0	2.2	120	10.6	3.0	20	11.7	3.5
21	10.0	1.6	60	10.1	2.4	150	9.9	3.0	25	10.6	3.2
22	12.0	1.9	75	8.7	2.3	180	12.0	2.5	30	11.0	3.2
23	11.3	2.0	90	9.5	2.2				40	11.2	3.0
24	9.9	2.0	120	9.3	2.5				50	11.0	2.8
									60	10.3	2.4

Table 3. *Exploration of glycogenolysis in patient's erythrocytes and liver tissue*

			Patient	Normal range
		Liver biopsy		(14)
Total proteins		% of wet liver	8.8	15–20
Glycogen content		% of wet liver	12.9	2.5–5.0
Glucose 6-phosphatase		μmole/min/g wet liver	5.7	2–10
Amylo 1.6-glucosidase		μmole/min/g wet liver	254	150–300
Phosphorylases		μmole/min/g wet liver	23	15–30
Phosphoglucomutase		μmole/min/g wet liver	73.5	52–86
		Erythrocytes		(18)
Amylo 1.6-glucosidase		U/g hemoglobin	2.7	1.1–3.7
Phosphorylase kinase	Activation of endogenous phosphorylase b	mU/g hemoglobin	2.6	0.7–4.0
	Activation of exogenous phosphorylase b	U/g hemoglobin	17.4	4.9–12.0

Table 4. *Exploration of galactose metabolism in patient's erythrocytes and liver tissue*

	Galactokinase	Uridyltransferase	UDP-Glucose 4-epimerase	[1-^{14}C]galactose oxidation
Erythrocytes	μmole/min/kg hemoglobin			cpm of [$^{14}CO_2$]/90 min/5 × 10^9 RBC
Patient (5½ y)	34.5	312	281	7479
Controls[1] (range)	19.0–42.3	267–533	146–385	2802–8973
	$N = 19$	$N = 57$	$N = 13$	$N = 13$
Liver biopsy	μmole/min/g soluble proteins			cpm of [$^{14}CO_2$]/60 min/100 mg wet liver
Patient (5½ y)	29.3	27.6	46.9	839
Controls[2]				
1 month			40.5	
2½ month	24.0		40.1	
3 month	15.8		49.0	
4 yr	14.3		34.0	
13 yr	30.6		43.6	
25 yr		27.1		
26 yr		31.0		
28 yr	18.6		49.0	
32 yr	19.3			
37 yr	20.2		36.7	
50 yr				5637
50 yr	16.7			3181
60 yr	25.2			
71 yr			44.2	4894

[1] Preliminary personal studies and data from the literature (25) showed that adult levels are reached at 5½ years, for all parameters assayed. This is why the patient's results were compared to adult controls.

[2] Only adult control subjects were tested, in cases when the values in the literature (24) were identical for children and adults.

unusual in cases where liver GSD is not associated with a Fanconi syndrome. On the other hand, Chesney *et al.* (7, 8) described a similar abnormality in three subjects with idiopathic Fanconi syndrome but no liver disease. Increased blood lactate to pyruvate ratios were also present in these three patients, suggesting an altered redox state. This last phenomenon might be involved in the defective galactose utilization, through a mechanism of UDP-glucose 4-epimerase inhibition due to an increased NADH to NAD ratio. For Chesney *et al.*, the abnormal redox state might also explain the disturbance in the galactose metabolism observed in cases of Fanconi syndrome associated with GSD. But our results seem to argue against this hypothesis because the redox state did not alter in our patient, either under fasting or fed conditions.

Galactose pathway enzyme activities and [1-^{14}C]galactose oxidation were normal in her red blood cells, but [1-^{14}C]galactose oxidation was markedly reduced in her liver. [1-^{14}C]Galactose oxidation by the liver involves the galactose metabolic pathway proper, [^{14}C]glucose 1-phosphate conversion to [^{14}C]glucose 6-phosphate and [$^{14}CO_2$] formation via the pentose phosphate shunt and the tricarboxylic cycle. As the patient's *in vivo* galactose-glucose interconversion was impaired, the metabolic defect seems to have occurred between the time when galactose enters the hepatocyte and when glucose is released from glucose 6-phosphate. Assays of phosphoglucomutase and glucose 6-phosphatase activities in a liver homogenate from the patient were normal. Consequently, the possibility of a metabolic disturbance of the hepatic galactose pathway itself was investigated. Normal activity

was found for the patient's liver galactokinase, uridyltransferase, and UDP-glucose 4-epimerase. Her defective hepatic galactose oxidation was therefore not connected with any *in vitro* deficiency of the key galactose metabolic pathway enzymes, so that two alternative possibilities were considered.

The first idea envisaged was impaired galactose transport across the liver cells. Little information is at present available on this transport mechanism in man, and one can only argue by analogy with what has been demonstrated in certain mammalian species like sheep and rat, thus, in the rat, galactose is transferred across the hepatocytes by mediated diffusion. But its carrier is not specific but is common to all the hexoses with graduated affinities: D-glucose, D-galactose, and D-fructose with K_M of 30 mmole/liter, 100 mmole/liter and > 100 mmole/liter, respectively (3, 9). The significance of this mediated diffusion mechanism is not known for physiologic circulating hexose concentrations, which are far below the K_M given above. Consequently, it is impossible to tell whether an abnormality in mediated hexose diffusion would have repercussions on hexose utilization. No abnormality of this type has yet been described. In our patient, we observed that poor *in vivo* galactose utilization was accompanied by slightly perturbed fructose utilization. The existence of a non-specific abnormality affecting hexose penetration into the liver was earlier mentioned by Odièvre (20) in a similar case. In this patient, reduced levels of intracellular [^{14}C]hexoses and [^{14}C]hexoses-phosphate were observed after a liver fragment from a needle biopsy was incubated in the presence of [^{14}C]glucose, [^{14}C]galactose, or [^{14}C]fructose.

The second possible explanation for defective galactose oxidation might be a dysregulation of the hepatic galactose pathway. But the absence in the propositus of clinical manifestations like those observed in subjects with deficient galactokinase, uridyltransferase or UDP-glucose 4-epimerase activities (15) argues against this hypothesis.

The abnormality observed in our patient is apparently the second localized abnormality to be described for galactose metabolism, the first being an UDP-glucose 4-epimerase defect restricted to red and white blood cells, with no clinical signs (13). Further investigations are required to confirm that defective hexose transport across the liver cell is indeed involved in this type of disorder, rather than a specific disturbance in galactose metabolism

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