

Prenatal Diagnosis of Galactosaemia

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Summary

We have monitored two pregnancies from families at risk for galactosaemia. The fetus was diagnosed as having galactosaemia in one and to be unaffected in the other. The accuracy of the predictions was confirmed postnatally. Assays for galactose 1-phosphate uridyl transferase involving the reduction of the coenzymes NAD or NADP are unsuitable for amniotic cells whereas estimation of ^{14}C -UDP-galactose produced from ^{14}C -galactose 1-phosphate detected the homozygous mutant fetus.

Introduction

While the acute clinical features of galactosaemia can be reversed rapidly by removal of galactose from the diet the long-term prognosis of mental development is less encouraging even when galactosaemia is diagnosed early and treated well. Thus, the intelligence of 18 out of 23 affected children in a British series was found to be lower than normal, the mean I.Q. being depressed by 34 points (Komrower and Lee, 1970). This trend has also been reported in two American series though measured intelligence was rather higher. In one of these (Donnell *et al.*, 1969) 29 out of 41 children (71%) had I.Q.s of 85 or higher and in the other (Nadler *et al.*, 1969) 26 out of 44 (59%) had I.Q.s of 90 or higher. In view of unequivocal evidence that poor dietary control leads to mental subnormality it is reasonable that parents who have proved unable to maintain strict galactose deprivation should elect for prenatal diagnosis and termination of pregnancy if the fetus is affected. Since transferase deficiency has been reported in cultured amniotic cells collected by amniocentesis performed immediately before caesarian section at 33 weeks (Nadler, 1968) it seemed probable that galactosaemia could be detected in utero at a stage when the pregnancy could be terminated if the fetus was affected.

After birth the diagnosis of galactosaemia is confirmed by showing a severe deficiency of galactose 1-phosphate uridyl transferase (EC 2, 7, 7, 12 "transferase") in haemolysates. The enzyme is assayed usually by monitoring either the consumption of uridine diphosphoglucose (UDPG) (Anderson *et al.*, 1957) or the production of glucose 1-phosphate (Beutler and Baluda, 1966; Nadler *et al.*, 1970). In the former assay residual UDPG is measured using the NAD-linked UDPG dehydrogenase reaction. The production of glucose 1-phosphate is measured after conversion of the glucose 1-phosphate to glucose 6-phosphate using the NADP-linked glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reactions. The specificity of these assays, however, depends on the absence of other NAD(P)-coupled dehydrogenases acting on components of the reaction mixture. We have confirmed that this condition is satisfied for erythrocytes but (as in the case of fibroblasts; Russell and DeMars, 1967) not for amniotic cells. Thus, when the substrate galactose 1-phosphate was excluded

from the reaction mixtures the rate of reduction of NAD(P) in amniotic cells was only about 25% less than in the complete systems.

Methods and Patients

AMNIOTIC CELLS

Clear amniotic fluid (12 ml) was collected by suprapubic amniocentesis after localization of the placenta by ultrasound. Amniotic cells were collected from the fluid by centrifugation, resuspended in 2 ml of HEPES buffered TC-199 medium supplemented with 20% human serum, other nutrients, and antibiotics. Growth was initially established in two Falcon petri dishes. Routine maintenance of the culture, including subculture, was carried out until enzyme assay.

ENZYME ASSAY

Transferase activity was measured by a modification of the method of Russell and DeMars (1967) which depends upon measurement of ^{14}C -UDP-galactose produced from ^{14}C -galactose 1-phosphate. Unreacted galactose 1-phosphate is then degraded by alkaline phosphatase treatment. The reaction product, ^{14}C -UDP-galactose, was recovered on DEAE-cellulose paper since this was simpler than charcoal powder (Nadler, 1968; Ng *et al.*, 1964) and because the latter method has been found to be unsatisfactory (Russell and DeMars, 1967; Fensom and Benson, 1974).

Amniotic cells were detached from the culture vessels using a solution of edetic acid (0.02%) in Dulbecco A phosphate-buffered saline (Oxoid), washed twice with saline (0.9%), and resuspended (about 5×10^6 cells/ml) in water containing triton X-100 (0.0025%). The cells were disrupted by sonication for one minute in an M.S.E. 150 W ultrasonic disintegrator. The reaction mixture contained the following components: 9.0 mM ^{14}C -galactose 1-phosphate (2.79 mCi/mmol), 250 μl ; 7.3 mM UDP-glucose, 125 μl ; 0.5 M glycine-NaOH buffer (pH 8.7), 250 μl ; and 12.5 mM cysteine HCl adjusted to pH 8.7 with 0.2 M NaOH just before use, 500 μl .

Then 50 μl of this mixture and 20 μl of the disrupted amniotic cell suspension were incubated at 37°C. The reaction was stopped after incubation at intervals for up to 30 minutes by placing the tubes in a bath of boiling water for two minutes.

To each tube was added 25 μl of a solution containing iodoacetamide (B.D.H. "biochemical grade") (25 mM) and sodium hydroxide (10 mM) followed by 20 μl of *Escherichia coli* alkaline phosphatase (Boehringer). The tubes were reincubated for two hours at 37°C to convert unreacted galactose 1-phosphate to galactose.

An aliquot from each tube was spotted on to a square (2.5 \times 2.5 cm) of Whatman DEAE-cellulose paper (type DE81) contained in a liquid scintillation phial. The papers were washed three times in the phials with 15 ml of distilled water at 55°C and dried overnight at 40°C, and the bound ^{14}C radioactivity was assayed in a liquid scintillation counter (Beckman model). Zero time blanks were 180-220 c.p.m. per sample. Protein was estimated by the method of Lowry *et al.* (1951).

Using this method we monitored two pregnancies in different families at risk for galactosaemia.

Case 1.—This 26-year-old woman had previously been delivered of a son with galactosaemia confirmed by blood transferase assays. In her subsequent pregnancy amniocentesis was performed at 13 weeks' gestation. Amniotic cells were cultured and assayed after

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three and five weeks. Transferase activity was 0.45% of the control mean of 38.9 units (μmol of UDP galactose formed/hr) per gramme of protein (range 33.7 U-54.1 U/g protein), the fetus being judged to be affected. The parents decided to continue with the pregnancy, and the mother was delivered at term when the diagnosis of galactosaemia was confirmed by blood transferase assay.

Case 2.—This 25-year-old woman had previously been delivered of a girl with galactosaemia, who at 2 years 9 months had a blood transferase activity of 3.9 units per gramme of haemoglobin. Both the girl's parents had activities in the heterozygote range (mother 21.2 U/g Hb; father 17.7 U/g Hb; normal adult mean 34.0 U/g Hb; range 29.5 U-38.5 U/g Hb). In the pregnancy we studied amniocentesis was performed at 15 weeks' gestation. After three weeks of culture amniotic cells were found to have normal transferase activity (43.3 U/g protein), the fetus being judged to be unaffected. We confirmed this at birth by showing normal cord blood transferase activity (41.6 U/g Hb).

Conclusion

These results suggest that assay of transferase activity on amniotic cells collected at amniocentesis and cultured for about three weeks allows accurate prenatal diagnosis of galactosaemia. Methods for assay which depend on reduction of the enzymes

NAD or NADP, however, are unsuitable for amniotic cell transferase assays.

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MEDICAL MEMORANDA

Gross Impairment of Hepatic Drug Metabolism in a Patient with Chronic Liver Disease

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The biotransformation of drugs occurs mainly in the liver. Patients with liver disease may therefore be unable to metabolize drugs normally and effects may be exaggerated and prolonged with an increased risk of toxicity. Selden and Sasahara, (1967). The results of drug metabolism studies in patients with liver disease have been conflicting, however, and some workers have reported a normal or even an increased rate of drug metabolism (Nelson, 1964; Held and Oldershausen, 1971). We found such gross impairment of lignocaine and antipyrine (phenazone) metabolism in a young woman with active chronic hepatitis that chronic administration of many drugs in conventional doses would undoubtedly have led to rapid cumulation with severe and probably fatal toxicity.

Case Report

A 28-year-old housewife was first admitted to hospital in 1968 with painless jaundice which resolved spontaneously after nine months. She remained well until 1970 when there was a recurrence of jaundice associated with dark urine, pale stools, and pruritus. Despite treatment with prednisolone in doses of up to 60 mg daily her condition did not improve, and in April 1973 she was readmitted to hospital because of anorexia, vomiting, upper abdominal pain, and increasing ankle oedema. One examination she was fully orientated and there was no evidence of hepatic precoma. She was deeply jaundiced, with multiple spider naevi, moderate hepatosplenomegaly, and ascites.

Laboratory investigations were as follows: prothrombin ratio 3.6:1, serum bilirubin 11.3 mg/100 ml, alkaline phosphatase 170 K. A. units, aspartate and alanine aminotransferases 129 IU/l. and 29 IU/l. respectively, total plasma proteins 8.7 g/100 ml (albumin 26.2% and gammaglobulin 57.4%), and plasma urea 21 mg/100 ml. Tests for antinuclear factor were positive, but antibodies to mitochondrial, smooth muscle, and hepatitis B antigen were not detected. There was no radiographical evidence of oesophageal varices. The patient was a Jehovah's Witness and a liver biopsy was considered an unjustifiable risk without permission to give a blood transfusion. A diagnosis of active chronic hepatitis was made. Drug therapy before admission had consisted of oral and intramuscular phytomenadione, prednisolone, Slow-K, and frusemide: spironolactone and folic acid were added after admission.

DRUG METABOLISM STUDIES

Twenty days after admission the patient ingested 400 mg lignocaine hydrochloride with 40 ml of water after an overnight fast. Venous blood was sampled at intervals for eight hours and urine was collected for three days. Five days later 18 mg/kg of antipyrine was given by mouth and the plasma half life was measured as described by O'Malley *et al.* (1971).

Control studies of lignocaine metabolism were carried out in seven healthy ambulant adult male volunteers aged 19-37 years. Plasma and urine samples were stored at -20°C . Lignocaine, its metabolites ethylglycylxylidide (EGX), glycylxylidide (GX), and 4-hydroxyxylidine, and antipyrine were estimated by gas-liquid chromatographic methods (Adjepon-Yamoah, 1973; Prescott *et al.*, 1973).

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