

Diagnosis of classical galactosaemia

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SUMMARY We report a child with classical galactosaemia whose diagnosis was missed until 12 weeks of age. The limitations of urine screening tests are discussed and the wider use of a qualitative enzyme assay for screening is recommended. Reference ranges for a quantitative enzyme assay using ^{14}C galactose-1-phosphate as substrate are presented.

The recognition and treatment of a case of classical galactosaemia in the newborn period usually effects a dramatic improvement in the patient's condition and is frequently life saving. Mass screening for this disorder is not practised widely because it is expensive considering the low incidence and does not always produce the diagnosis early enough (Shih *et al.*, 1971). It is therefore necessary to maintain a strong index of suspicion and test rapidly any infant with clinical signs that are suggestive of galactosaemia. This is particularly important if there is a previous family history of the condition.

The usual method of screening for galactosaemia has been a urine test for reducing substances, followed by chromatography in a positive case to confirm galactosuria. The danger of completely excluding the diagnosis because of a negative urine test is highlighted by recent cases which were not recognized for some time because galactosuria was not found (Harris, 1974; Johnson, 1975). We describe a similar example of delayed diagnosis in an infant with a history strongly suggestive of galactosaemia. By chance, this case arose in a family which carried both the gene for classical galactosaemia and the Duarte variant (Beutler, 1966). This gave us the opportunity to investigate the discrimination of the tests for red cell galactose-1-phosphate uridyl transferase (Gal-1-PUT, E.C.2.7.7.12). Recommendations for galactosaemia screening are made after these observations.

Materials and methods

Chemicals. Gal-1-P and UDPG were obtained from Sigma Chemical Co., ^{14}C -Gal-1-P from the Radio-

chemical Centre, Amersham and New England Nuclear.

Clinical samples. Blood was collected from galactosaemic patients ($\text{Gt}^{\text{a}}/\text{Gt}^{\text{a}}$), their parents ($\text{Gt}^{\text{+}}/\text{Gt}^{\text{a}}$), and other relations. As normal controls ($\text{Gt}^{\text{+}}/\text{Gt}^{\text{+}}$) blood was collected from 45 members of the laboratory staff and relations, where appropriate. We also investigated the family of our galactosaemic patient. This family carries the genes for both classical galactosaemia (Gt^{a}) and the Duarte variant (Gt^{D}).

Case history. A boy was delivered at term weighing 3.14 kg, after an uneventful pregnancy. He was the second child in the family; the first child had failed to thrive and died at the age of one month from a generalized fulminating septicaemia.

He was well until day 6 when he fed poorly, failed to gain weight and was found to have a *Klebsiella* septicaemia. He improved over the next week on antibiotics. During this time he became jaundiced with a maximum serum bilirubin of 100 $\mu\text{mol/l}$ (5.85 mg/100 ml) and his liver became palpable 2 cm below the right costal margin. He had recurrent thrush infections and a low serum IgA. At age 5 weeks he developed diarrhoea; stool cultures yielded *Alkalescens dispar* but other cultures were negative and he improved on antibiotics.

The infant was noted to have hepatomegaly (3 cm below the costal margin) at 6 weeks but was reported to be feeding well on Ostermilk. A urine specimen at that time was negative for reducing substances. At 8 weeks he was said to be feeding well and gaining weight but a Benedict's test for urine-reducing substances was again reported to be negative. However, a generalized aminoaciduria was found. He was well at 9 weeks but a further urine sample was obtained because of persistent hepatomegaly. This showed a trace of galactose and a similar amino acid pattern

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to the previous sample. A red cell assay for Gal-1-PUT showed no enzyme activity. He was started on a galactose-free diet and progressed well. He is now 2 years old and is making normal developmental progress and has no evidence of cataracts.

Assays. Infants were screened for galactosaemia using the enzyme screening method of Beutler and Baluda (1966) with either 10 μ l whole blood or a 6 mm punch from a blood spot on filter paper (Whatman grade 160), as collected in this region for phenylketonuria screening.

Gal-1-PUT was assayed quantitatively using the method of Ng *et al.* (1967) which measures the incorporation of radioactivity from 14 C-Gal-1-P into the nucleotide UDPGal. Each specimen was assayed in duplicate with a blank. The mean percentage difference between duplicates was 4.5 (range 0.9–9.0). Enzyme activity was expressed three ways: per ml packed cells, per g Hb, and per g protein. Hb was estimated by the cyanmethaemoglobin method; protein content by the method of Lowry *et al.* (1951).

Difficulties have been encountered in getting consistent results for the transferase assays using different batches of 14 C-Gal-1-P. These were attributed to impurities in some samples of labelled substrate. Similar conclusions were drawn by Ng *et al.* (1969b) and Russell (1969). Differences between batches of 14 C-Gal-1-P were apparent from high results in blank assays, carried out either by boiling the red cells before incubation or by omitting UDPG from the incubation mixture. It is important that all new batches of labelled substrate should be evaluated fully before use, including the investigation of blank values. Starch gel electrophoresis was done according to the method of Ng *et al.* (1969a) using a 13% starch gel.

Results

In the screening method for Gal-1-PUT, the presence of red cell enzyme results ultimately in the production of NADPH which fluoresces under UV light. Red cells of patients with classical galactosaemia produced no fluorescence, whereas cells from all other subjects investigated, including the Duarte variant/galactosaemic heterozygotes, gave obvious fluorescence within the 3-hour incubation period of the test. However, it was not within the scope of this test to identify the specific genotype of individuals. One factor which makes the screening test less precise is the variable amount of quenching of the fluorescence, due to different amounts of Hb in the blood samples.

A quantitative enzyme assay may be used to

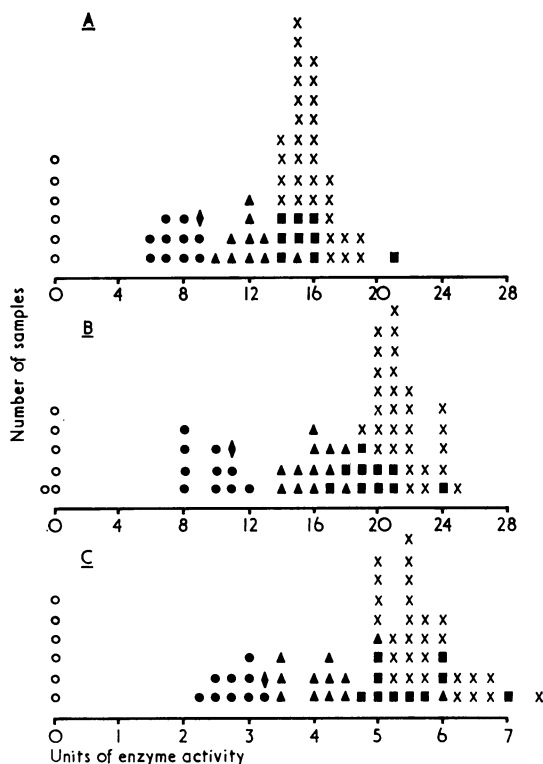


Fig. 1 Distribution of enzyme activities, expressed as μ mol of substrate converted per hour per g protein in A; as per g Hb in B; and as per ml packed cells in C. Genotypes are \bullet = galactosaemic homozygote; \circ = galactosaemic homozygote; \blacktriangle = Duarte heterozygote; \blacklozenge = Duarte homozygote; \blacksquare = wild-type homozygote; \times = presumed wild-type homozygote, but not confirmed by starch gel electrophoresis.

differentiate the genotypes of subjects with more certainty and the ranges of activity we found are shown in Fig. 1. Heterozygotes for the Duarte variant (Gt^+/Gt^D) were confirmed by starch gel electrophoresis. The individuals show an enzyme band which migrates faster than that of the wild-type enzyme.

The pedigrees of 3 families included in the enzyme studies and the assigned genotypes are shown in Fig. 2. Fig. 2a shows the family of our patient, in which both the gene for classical galactosaemia and the Duarte variant were found. Two individuals studied for reference range purposes had about half the normal enzyme activity. Starch gel electrophoresis and study of other members of the family suggested that one was genotype Gt^+/Gt^a and the other Gt^D/Gt^D (Figs. 2b and c respectively).

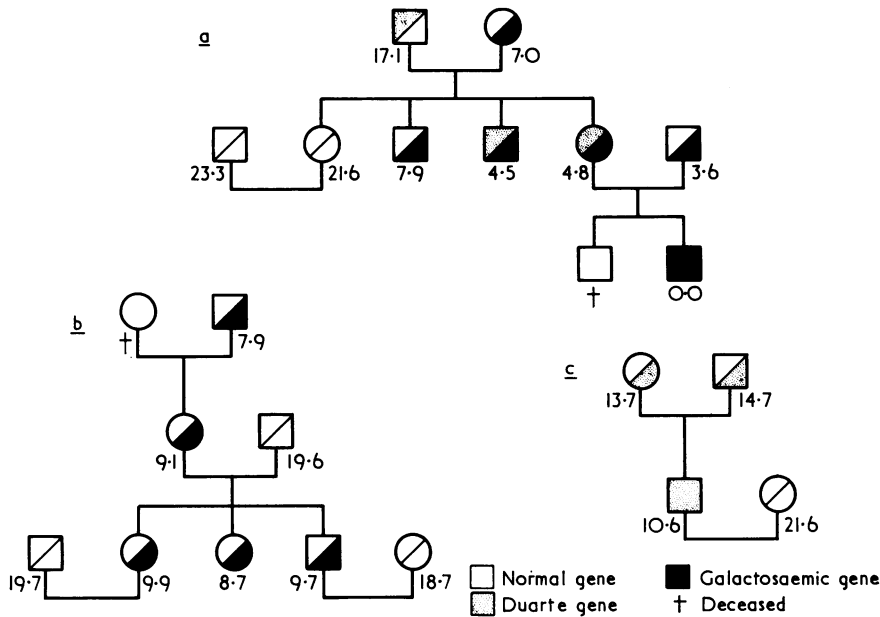


Fig. 2 Family trees investigated as described in the text. The numbers refer to the enzyme activities found in μmol of substrate converted per hour per g Hb.

Discussion

In the absence of mass screening in the newborn the paediatrician has the primary responsibility for detecting classical galactosaemia. If cases are not to be missed, a very high index of suspicion has to be maintained and the variability in the pattern of disease needs to be stressed continually. Some patients do not present with the features that are usually described. The practice of screening for galactosaemia those infants with severe or prolonged jaundice is well established. It is equally important to screen all infants who are severely ill, or fail to thrive, without obvious cause. The knowledge or suspicion of underlying infection is particularly indicative of a possible case of galactosaemia.

The most widely used method of screening for galactosaemia is the urine test for reducing substances, with chromatography in positive cases to confirm that the substance present is galactose. However, the inconsistency of galactose excretion in galactosaemia has been reported in many early cases (Hsia and Walker, 1961). The possibility of a diagnosis being delayed, or missed, if the urine test is relied on completely is emphasized by two recent reports (Harris, 1974; Johnson, 1975) in addition to this one.

The cause of the intermittent nature of the galactose excretion in classical galactosaemia is probably simply related to variations in galactose intake. On a steady intake of galactose there are no clear reasons

why the urine test for reducing substances should not be positive. However, blood levels of galactose fall rapidly and excretion stops when the source of this carbohydrate is withdrawn from the diet. A severely ill child may not be on normal milk feeds and even in the infant who is less sick, ward notes on feeding may grossly overestimate the amount of milk taken in.

The urine test may continue to be the basic test for galactosaemia, but it is obviously of no value in an infant who is not on regular milk feeds. In a sick infant a negative result for reducing substances should not be used alone to exclude galactosaemia if there is any clinical suggestion that this may be the diagnosis. In all cases of doubt an enzyme screening test should be performed. If the test is not readily available, and there may be some delay in obtaining a result, a lactose-free regimen should be started while awaiting the result of the biochemical investigation. The enzyme screening test is simple, rapid, and cheap and it seems logical that the aim should be to make it the primary method of screening.

The screening test should differentiate clearly cases of classical galactosaemia, with virtually no transferase activity, from all other genotypes. The lowest enzyme activity likely to be encountered in a clinically normal subject is in the patient heterozygous for both the Duarte and the galactosaemia genes. These patients show fluorescence in the screening test, but a false positive, i.e. no fluorescence, occurs in patients with glucose-1-phosphate dehydrogenase

deficiency, as the production of NADPH in the screening test relies on the presence of this enzyme in the red cells.

Although the diagnosis of galactosaemia does not demand it, patients and their families are frequently investigated further by a quantitative assay for Gal-1-PUT. This may be indicated for genetic counselling purposes and, if a prenatal diagnosis is considered, a precise knowledge of the familial genotypes is necessary. We found the best differentiation of genotypes was obtained by expressing enzyme activity per g Hb, but starch gel electrophoresis is necessary to confirm the presence of the Duarte variant.

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