Deoxyribose-5-Phosphate Aldolase Deficiency – a Harmless Inborn Error of Metabolism

Department of Paediatrics, University of Melbourne and Birth Defects Research Institute, Royal Children's Hospital Research Foundation, Melbourne, Australia 3052

Evidence is presented that a deficiency of 2-deoxyribose-5-phosphate aldolase was present in a previously described patient who excreted metabolites of 2-deoxyribose in his urine. Minor clinical abnormalities present did not appear related to this disorder.

In previous reports (Truscott et al., 1979a,b) we described the excretion of a number of metabolites of 2-deoxyribose in a patient who was thought to have cataracts and developmental delay. We now present evidence that the abnormal metabolites in the urine of this patient result from deficiency of the enzyme 2-deoxyribose-5-phosphate aldolase (EC 4.1.2.4). Follow-up assessment of the patient has revealed that he does not have true cataracts and that his mildly delayed development may be explained by his social circumstances. It is considered that deoxyribose-5-phosphate aldolase deficiency may be a harmless inborn error of metabolism.

CASE REPORT
The patient (J.S.) was the only child of unrelated Greek parents and has been under the care of the Royal Children's Hospital, Melbourne, since 3 months of age. Strabismus, associated with marked astigmatism, and bilateral ocular abnormalities, which were initially thought to be cataracts, associated with mild developmental delay, poor feeding and recurrent vomiting led to his investigation. Both parents had severe psychiatric disturbances and many of his problems have been due to emotional deprivation and abnormal rearing.

Slit lamp investigation at 9 months revealed that the suspected cataracts were really anterior lens capsular opacities. Urinary amino acids, organic acids and sugars were studied. Gas chromatography of the standard ether/ethyl acetate extracts of urine revealed a moderate amount of 2-deoxyerythropentono-1,4-lactone; use of different extraction systems showed that large amounts of a number of deoxyribose metabolites were present in the urine (Truscott et al., 1979a,b).

Since the initial presentations, the seriously disturbed family situation has persisted, making assessment of his mental development very difficult. Until 3 years of age brief periods at home with his schizoid mother alternated with periods in hospital or babies' homes. He has lived in an institution for the past 3 years and has made better developmental progress. However, his mother's regular visits cause emotional disturbance. Motor skills are normal for his age of 6 years, but speech and social skills are delayed. It is impossible to know whether any true mental retardation is present, but this is regarded as unlikely. Physical growth and health are normal.

MATERIALS
Eagle's basal medium and fetal calf serum were obtained from Flow Laboratories, polymyxin from Wellcome, Australia Ltd. and neomycin from Hoechst-Roussel Pharmaceuticals. Deoxyribose-5-phosphate was purchased from Sigma Chemical Co. and semicarbazide from B.D.H. Chemicals Ltd. Hoechst 33258 stain was obtained from Hoechst. Other chemicals were of analytical reagent grade.

METHODS
Fibroblast cultures were established from forearm skin biopsies and grown in Eagle's basal medium with 10% fetal calf serum and 1.5 μg/ml each of neomycin and polymyxin. The cultures were screened regularly for mycoplasma contamination using Hoechst 33258 stain as described by Chen (1977). The cells were harvested using trypsin/EDTA, washed in phosphate-buffered saline and resuspended in 50 mmol/l potassium phosphate, pH 7.4. They were disrupted by sonication on ice (Branson Sonic Power Co. Sonifier, 6 x 5 s pulses, output setting 20% of maximum) and the sonicates centrifuged at 10 000 g for 5 min. The supernatants were used for assay.

Haemolysates were prepared from 5–10 ml blood samples collected into heparinized tubes. The red cells were collected by centrifugation, washed twice with 0.154 mol/l sodium chloride and lysed with an equal volume of distilled water. Cell membranes were removed by centrifugation at 10 000 g for 5 min. Protein concentrations were measured by the method of Lowry et al. (1951) and haemoglobin by the optical density at 540 nm after conversion to cyanmethemoglobin.
Deoxyribose-5-phosphate aldolase assays
The formation of acetaldehyde from deoxyribose-5-phosphate was followed using a modification of the assay described by Burbridge et al. (1950). The reaction mixture contained 5 mmol/1 deoxyribose-5-phosphate, 5 mmol/1 sodium citrate, 40 mmol/1 potassium phosphate, pH 7.3 and haemolsates or cell extracts in a total volume of 0.5 ml. The reaction was carried out in a glass liquid scintillation vial in which was placed a small glass tube containing 0.2 ml 6.7 mmol/1 semicarbazide in 0.2 mol/1 potassium phosphate, pH 7.0. The vial was sealed with a gas tight rubber stopper. Acetaldehyde released during the incubation (60 min at 37°C) was trapped in the semicarbazide solution and its concentration determined from the absorbance at 224 nm after dilution to 1.0 ml with water. The amount of acetaldehyde formed during the reaction was determined from a standard curve over a range of 0–100 nmol. The standard curve was prepared from reaction mixtures without haemolsate to which were added various amounts of acetaldehyde. These were treated in the same manner as the assay samples to allow for recovery of acetaldehyde from the reaction mixture.

RESULTS
Blood samples from the patient, his mother and a group of ten normal young adult females were obtained. The father was unavailable for study. The results of 2-deoxyribose-5-phosphate aldolase assays of these samples are summarized in Table 1. No activity could be detected in any of a number of blood samples from the patient. Haemolsates from the mother had activity intermediate between the patient and controls.

A cultured fibroblast line was established from the patient and deoxyribose-5-phosphate aldolase activity in cell extracts compared to four control cell lines. These results are also presented in Table 1. It is not known if the very low level of activity measured in the patient’s cells is significantly different from zero in this assay.

DISCUSSION
The patient described in this report has a deficiency of 2-deoxyribose-5-phosphate aldolase as defined by unde-

Table 1 2-deoxyribose-5-phosphate aldolase activity in patient, mother and controls in haemolsates and in cultured fibroblasts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Patient</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Haemolsate</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>28.5 ± 3.1 (10)†</td>
<td>not detectable</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td></td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Fibroblast extracts</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>68.8 ± 3.5 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td></td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

*Activity expressed as nmol acetaldehyde formed/mg haemoglobin per h mean ± sd
†Activity expressed as nmol acetaldehyde formed/mg protein per h mean ± sd
‡Numbers in brackets are number of subjects or cell lines

tectable activity in red blood cells and minimal activity in cultured fibroblasts. This defect was first suspected because of the urinary excretion of 2-deoxyerythropentonic acid, 2-deoxyerythropentono-1,5-lactone, 2-deoxyerythropentono-1,4-lactone and 2-deoxyribitol-metabolites of 2-deoxyribose.

The 2-deoxyribose-5-phosphate generated during catabolism of DNA is metabolized by deoxyribose-5-phosphate aldolase to acetaldehyde and glyceraldehyde-3-phosphate. The enzyme was first described by Racker (1952), in mammalian liver and thymus and in several bacterial species. The enzyme has been purified from rat liver by Roscoe and Nelson (1964) and Groth (1967) and from human erythrocytes by Jedziñak and Lionetti (1970). The enzyme is subject to activation by a number of polycarboxylic acids, particularly citrate (Jiang and Groth, 1962), and appears to have an essential sulphydryl group (Roscoe and Nelson, 1964; Groth, 1967). The physiological role of the enzyme is at present poorly understood, although it is generally thought that the reaction in vivo is in the direction of deoxyribose-5-phosphate catabolism. The activity of the enzyme is increased in malignant tissues compared to normals (Boxer and Shonk, 1958) and the activity varies during the cell cycle in rat hepatoma cells with a peak in activity during the G2 phase (Lincoln and Hoffee, 1979).

While this patient is the only one so far described with 2-deoxyribose-5-phosphate aldolase deficiency, it is possible that this defect may be relatively common. In screening 58 normal subjects by the erythrocyte assay described, four were found with levels of activity comparable to that of the patient’s mother, suggesting that the heterozygote frequency may be quite high. Persons homozygous for this mutation could escape detection because the compounds excreted by the patient are poorly extracted from urine by the systems generally used in screening by gas chromatography–mass spectrometry and because there seems to be no specific clinical disorder.

We suggest that deoxyribose-5-phosphate aldolase deficiency is a benign condition.

Mr G. Keith assessed the ocular lesion. Dr Chappel was in receipt of the Elizabeth Sweet Fellowship of the University of Melbourne; Dr Truscott held a grant from the National Health and Medical Research Council of Australia.

MS received 10.3.83
Accepted for publication 6.5.83

References
Groth, D. P. Deoxyribonuclease and 5-phosphatase aldolase. Purification and properties of the rat liver enzyme. J. Biol. Chem. 242 (1967) 135–159