Biochemical screening for inherited metabolic disorders in the mentally retarded

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Summary
A biochemical screening programme for the detection of inherited metabolic disease was carried out on urine and blood samples from inmates of the Alexandra Institute for the mentally retarded, Cape Town. Of the 1087 patients screened, positive results for phenylketonuria were obtained in 3, for cystinuria in 2 and for Hartnup disease in 1. The overall frequency of metabolic disorders was 0.6%. It is evident that genetic metabolic disease as detected by current screening procedures makes only a small contribution to the overall burden of mental retardation.

Materials and methods
The Alexandra Institute cares for almost 1000 mentally retarded White persons of all ages. All inmates were screened irrespective of clinical features. Early morning urine samples were collected in plastic containers (50 ml) with added preservative (2 drops of 10% thymol in isopropanol) and either analysed immediately or frozen (-20°C) until required. Blood samples were obtained by venepuncture or finger prick from those individuals who showed abnormal or raised levels of metabolites in the urine. Where not otherwise stated the methodology followed was that of Thomas and Howell and Shih. The battery of tests applied is given in Table I.

All urine samples were put through the screening tests and any apparent deviation from normal was noted. Those specimens yielding results indicating the possibility of underlying metabolic disease were subjected to further detailed and quantitative analyses. Where indicated, blood and repeat urine samples were examined to confirm or expand on the initial finding.

Results
Of the 1087 patients screened, 150 warranted repeat examination. For 144 of these the initial suggestive findings were found to be transient and of no metabolic significance. For the remaining 6, consistently abnormal results were obtained. These are discussed below.

Phenylketonuria
Marked ketonuria was found in 3 patients using 'Phenistix', ferric chloride and dinitrophenylhydrazine (DNPH) as reagents. Isolation of the DNPH derivatives and thin-layer chromatography revealed excessive excretion of phenylpyruvic acid in each case. Quantitative amino acid analyses showed an
Plasma tryptophan levels

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Phenylalanine* (µmol/l)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>33</td>
<td>1 250</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>48</td>
<td>1 453</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>38</td>
<td>1 516</td>
</tr>
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*Upper limit of normal = 64 µmol/l.

approximately 20-fold elevation in non-fasting plasma levels of phenylalanine (Table II).

Hartnup disease

Two-dimensional paper chromatography of urine from patient 1 (high-voltage paper electrophoresis in the first dimension and solvent chromatography in the second) revealed a marked hyperamino-aciduria (Fig. 1) involving specifically the neutral amino acids alanine, glycine, serine, glutamine, threonine, valine, tyrosine, leucine, isoleucine and phenylalanine, and, to a lesser extent, the dibasic amino acids lysine and ornithine. No proline or hydroxyproline was detected. Chromatography of the plasma amino acids showed a normal pattern.

Cystinuria

Urine samples from 2 sisters (35 and 41 years of age) gave positive results in the cyanide nitroprusside test for cystine. Solvent chromatography and high-voltage paper electrophoresis showed strikingly elevated levels (10 - 15 X normal) of cystine and the dibasic amino acids ornithine, lysine and arginine. Plasma amino acid patterns were normal.

Mucopolysaccharidoses

Urine samples from 6 patients were positive on screening for glycosaminoglycan (GAG) excretion. Isolation of the GAGs and separation by cellulose acetate electrophoresis revealed a normal pattern in each case, discounting the presence of any of the classic types of mucopolysaccharidoses. Further investigations were not carried out and it was assumed that the raised GAG excretion was secondary to increased connective tissue metabolism.
Miscellaneous

Patients with slight amino-aciduria of transient nature were not further investigated and were regarded as normal. Ten cases of proteinuria (> 0.3 g/l) were detected, all caused by urinary tract infection. Moderate glycosuria was discovered in 2 patients, both known diabetics. Tests positive for indicans were found to be due to bowel stasis in all cases except in the patient with Hartnup disease.

Discussion

The percentage of patients in this study with diagnosed inherited metabolic disease is 0.6%. This figure is low in comparison with the results of similar investigations carried out in Europe and America, where disorders have been detected in 1 - 6% of the populations screened. Comparative evaluation of these findings is difficult, however, as the range of tests applied in the various screening programmes has differed, as has the interpretation and listing of the results. In addition, some groups have been differently constituted, containing both institutionalized and referred cases. The latter group would presumably present with relatively higher positive frequency as they had undergone preliminary selection before referral.

Notwithstanding these differences there may be some justification for assuming a low frequency of phenylketonuria in white South Africans. Only 3 cases (3/1000) were diagnosed in this survey, a figure which is considerably lower than those (23/1000 and 48/1000) detected in similar studies in Northern Ireland and West Germany. Screening programmes for biochemical disorders in newborns in these countries have revealed the incidence of phenylketonuria to be high, i.e. 1/4505 and 1/6546 respectively. The figure from this study, from representative of institutions (White) in South Africa, is similar to results from surveys in Canada and Sweden (7/1000 and 5,7/1000), where screening of newborns has indicated a frequency of 1/17000 and 1/31621 respectively.

Although the incidence of phenylketonuria in Black South Africans is not known, it is likely to be lower than that of the white population group. Screening of newborns in the USA has revealed a very low occurrence of this condition among Negroes.

The finding of Hartnup disease in one patient is of interest in that to the best of our knowledge it is the first case to be reported in South Africa; for this reason detailed discussion is warranted. Before the advent of screening programmes for newborns, Hartnup disease was regarded as a rarity, but a revealed frequency of 1/20000 would indicate that many cases remain undiagnosed, presumably owing to the frequent asymptomatic presentation of the disorder. Our patient had none of the classic features — red pellagra-like skin rash or photosensitivity — but did present with the characteristic amino-aciduria resulting from a defect in the renal transport systems for the reabsorption of the neutral amino acids. Mental retardation is not normally associated with this condition, and it was therefore assumed that retardation in our patient resulted from a marked hydrocephalus and not the amino acid transport defect. Defective transport of neutral amino acids across the gut mucosa is well documented — hence the delayed and partial increase in plasma tryptophan levels in our patient following the oral tryptophan load. The reported but still undefined block in the metabolism of tryptophan in Hartnup disease was confirmed in patient 1 by a lack of kynurenine production following the tryptophan load which yielded increased plasma levels of this amino acid. In contrast, all 3 control patients showed continually increasing plasma levels of this important tryptophan metabolite during the 4-hour test period.

The incidence of cystinuria in this study is in accord with that of other surveys. Of interest is a report by Scrivener et al. indicating that the incidence of cystinuria in the mentally retarded is substantially greater than that in the general population.

In any critical appraisal of the low frequency of positive findings in these investigations it is important to recognize that the screening tests used will only detect disorders which present with a moderate to grossly abnormal metabolic picture, mainly of amino acid metabolism. Defects more subtle or transient in presentation are unlikely to be discovered. The true frequency of metabolic disease should therefore be higher than that found in this survey; this may call for a more detailed and broader biochemical approach to the mentally retarded patient. It is evident though that the inborn errors of metabolism, detectable by the screening tests used in this survey, make only a small contribution to the aetiology of mental retardation.

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REFERENCES