whom it was shown that significant elevation in gonadotrophin levels (LH and FSH) occurred, albeit significantly less in the diabetics.[9]

Nonspecific GH stimulation by hypophysiotropic-releasing hormones such as thyrotrophin-releasing hormone and LH-RH is a phenomenon which has been noted primarily in patients with acromegaly[5] and has also been shown to correlate with paradoxical GH suppression by dopaminergic drugs such as L-dopa and bromocriptine.[10] These observations in patients with acromegaly and the negative data obtained in our group of insulin-dependent diabetics evoke the speculation that dopamine receptor stimulants are unlikely to suppress the apparent GH hypersecretion of some diabetics. A recent report which notes the failure of bromocriptine to lower GH levels in a juvenile diabetic with proliferative retinopathy[11] supports this thesis.

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Isovaleric Acidaemia in Two South African Children

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SUMMARY

Two siblings who were repeatedly admitted to hospital with acute episodes of vomiting, dehydration and coma were found to be suffering from isovaleric acidaemia. This condition is a rare inherited abnormality of leucine metabolism, which is frequently fatal in the early weeks of life and leads to mental retardation in a high proportion of those who survive early attacks. However, both our patients were of normal intelligence. The clinical presentation, biochemical defect, diagnosis and suggested therapies are reviewed.

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Apart from obvious local and infective causes, severe vomiting in childhood may also be owing to metabolic disturbances. Included in this category are relatively common conditions, e.g. uraemia, as well as rare entities such as certain inborn errors of metabolism.

When vomiting is episodic and is associated with acidosis and an abnormal odour, a disorder of branched-chain amino-acid (leucine, isoleucine, valine) metabolism should be suspected. Several well-defined diseases in this group have been described and the enzyme deficiencies have been characterized.

In this article we describe 2 children who presented with these clinical manifestations and who were subsequently found to be suffering from isovaleric acidaemia — a defect in leucine metabolism. A search of the literature revealed 21 recorded cases from 13 families. To the best of our knowledge our 2 patients are the first South Africans in whom this condition has been reported.

CASE HISTORIES

The patients were a White girl and her younger brother, the only children of healthy, unrelated parents.

Case 1

This patient, born on 3 November 1969, was an apparently normal baby girl who weighed 3 kg. The preg-

nancy and delivery had been normal. During the neonatal period vomiting occurred occasionally after feeding. No untoward symptoms were noted at that time but during the first year of life the patient experienced at least two severe episodes of vomiting, apparently during attacks of tonsillitis. In retrospect the mother recalls that a peculiar odour was noticeable during these episodes. The child was admitted to hospital for the first time in February 1973 with vomiting, diarrhoea and drowsiness. Lumbar puncture revealed normal cerebrospinal fluid (CSF).

The patient was first admitted to Tygerberg Hospital on 6 July 1973 with a history of severe vomiting for 4 days and of having received treatment elsewhere for a sore throat. Examination revealed an acutely ill, stuporous and dehydrated child (temperature 37,8°C, pulse 120/min). There was a suspicion of an abnormal odour. Neck stiffness and nasal speech were noted but no localizing neurological signs were present. Her tonsils were enlarged and inflamed and tenderness in the lower abdomen was also found. After admission repeated vomiting occurred. No diarrhoea was present on admission, but it developed during her stay in hospital. As previously, lumbar puncture revealed normal CSF. Chemical and microscopical examination of urine showed a trace amount of protein. Haematological examination showed no abnormality. On admission, blood pH was 7,32, the bicarbonate concentration was 17,2 mmol/l (normal = 24 - 30 mmol/l), urea 6,9 mmol/l and sodium and potassium concentrations were within normal limits. X-ray films of the chest and abdomen and a barium meal and follow-through were normal. She was treated with intravenous fluids, including sodium bicarbonate solution, and with broad-spectrum antibiotics.

In September 1973 a tonsillectomy was performed elsewhere. The operation was followed by an acute attack resembling the previous ones. The child was subsequently readmitted to Tygerberg Hospital on two occasions — in January 1975 and again in June of the same year — after attacks similar to the previous ones. Her mother by now recognized a prodromal phase of 24 - 48 hours, involving malaise, abdominal discomfort and the presence of a peculiar unpleasant odour, reminiscent of sweaty feet. In addition to the previously mentioned findings, leucopenia was also noted during the latter two admissions. Treatment was as before.

A few months later, after the diagnosis of isovaleric acidaemia had been confirmed, the patient again became febrile and complained of feeling unwell. Her mother detected the characteristic odour. Immediate institution of a high-liquid, low-protein diet and administration of antibiotics successfully aborted the development of acidosis and the full clinical attack. Between attacks she is free of symptoms apart from occasional abdominal pain. From an early age she has shown a spontaneous aversion for high-protein foods. Since the institution of a special low-protein dietary regimen, no further attacks have occurred.

The child's development, both physical and intellectual, has not been impaired. Her height and weight are normal for her age and her intelligence, at the age of 6 years, was rated as 127 on the New South African Individual Scale.

Case 2

This patient, the brother of patient 1, was born on 8 August 1972. As in his sister's case, the pregnancy, delivery and neonatal development were normal. He was admitted to hospital on 24 February 1973 during a first attack of vomiting associated with pyrexia and upper respiratory tract infection. In June 1973 he was treated at home for a similar but milder attack.

He was first admitted to Tygerberg Hospital on 26 September 1973 on account of fever, cough, vomiting and drowsiness. The same unpleasant odour of sweaty feet, as evidenced in the case of his sister, was observed. On examination he was found to be acutely ill and dehydrated. Tonsillitis was present and there were signs of bronchopneumonia. Neurological examination showed him to be stuporous and hypotonic. There was no evidence of neck stiffness or other signs of meningeal irritation. Lumbar puncture revealed normal CSF and a brain scan also yielded a normal result. Laboratory investigations showed the following: slight proteinuria, marked ketonuria, leucopenia (white cell count 4 400/μl, 27% atypical lymphocytes), thrombocytopenia (90 000/μl), metabolic acidosis (blood pH 7,34, plasma bicarbonate 11,3 mmol/l) and normal plasma urea and glucose concentrations. Treatment consisted of the administration of intravenous fluids, prochlorperazine and antibiotics. Recovery was slow, and on clearing of the sensorium, ataxia and jerky involuntary movements were evident for several days. The precipitating factor in this instance was probably glandular fever.

He was readmitted to Tygerberg Hospital in February 1974, October 1974 and June 1975 with acute attacks. The presentation on each occasion was as before, except that there was no disturbance of consciousness. After the third admission virtually all the patient's hair fell out.

Nasal speech was observed to accompany some of the attacks, as in the case of his sister. Like her, he has also experienced an episode of fever more recently, but no vomiting or abnormal odour developed, possibly as a result of early administration of antibiotics. He has also shown a spontaneous aversion towards high-protein foods from an early age. His physical and intellectual development has been normal. The McCarthy intelligence test, performed at the age of 3 years and 3 months, showed an IQ of 115.

Family History

A first cousin of the two patients described died at the age of 14 years in an institution for mentally retarded children. Initially this child was normal but at the age of 18 months salaam spasms and hyperactivity developed. These were accompanied by progressive mental retardation. At 2½ years of age an episode of intracranial haemorrhage of uncertain aetiology occurred. No attacks of the type experienced by our patients were ever recorded. CSF examination and tests of urine for evidence of aminoacidopathy, including isovaleric acidaemia, showed no abnormality.

Laboratory Investigations

Blood and urine samples were obtained from our patients at the time of an acute attack — in the case of the daughter immediately after admission on 15 January 1975 and in the case of her brother a few hours after admission on 20 October 1974. Urine collections (24-hour) were also made later during clinical remission.

Thin-layer chromatography of plasma and urine for amino acids showed no obvious abnormality. Keto-acids were demonstrated, according to the method of Dancis and Levitz,[1] in the urine of the girl during the acute phase but not in that of her brother. Screening tests for isovaleryl glycine with the thin-layer chromatographic method of Ando and Nyhan[2] were positive for urine obtained from both siblings during the acute attack and during remission.

Quantitative analysis of urine specimens was carried out by means of a two-stage gas chromatographic method.[3] Isovaleric acid was present in massive amounts in the volatile fatty acid fraction from the urine of both patients during the acute phase. Isovaleryl glycine was found in the initial ethereal extracts of these samples in amounts encountered only in isovaleric acidaemia. After continuous overnight liquid-liquid extractions of the urine specimens, isovaleryl glycine was methylated and determined by gas chromatography, with heptadecanoic acid as an internal standard. A mixture of synthetic isovaleryl glycine and heptadecanoic acid was used to establish relative response ratios.[3] The quantitative results are shown in Table I.

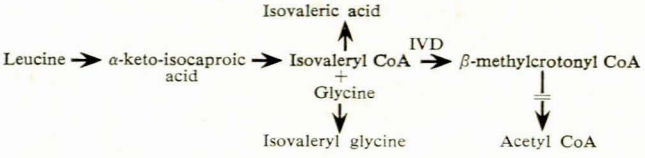
Analysis of the girl's urine during the acute phase was complicated by the presence of ketone bodies and long-chain dicarboxylic acids which are normally encountered in acute ketosis. However, this did not affect the measurement of isovaleric acid or its glycine conjugate. Isobutyric and propionic acids, which were also present but in smaller amounts, may have been due to faecal contamination. During clinical remission, only isovaleric acid and isovaleryl glycine were detectable. Urine from the boy during remission was analysed for isovaleryl glycine only.

Leucine loading tests were carried out on both parents and 2 normal volunteers as follows: urine was collected for 12 hours before and for 12-hourly periods up to 48 hours after oral administration of leucine (100 mg/kg body weight) and analysed for isovaleryl glycine. None of the samples from the parents or normal control subjects, either before or after leucine loading, was found to contain isovaleryl glycine.

DISCUSSION

The first defect of branched-chain amino acid metabolism to be described was maple syrup urine disease in 1954.[8] Laboratory diagnosis of this condition rests on examination of blood and urine for increased concentrations of the branched-chain amino acids and their corresponding α-keto acids. Oxidative decarboxylation of the latter compounds, which is defective in maple syrup urine disease, is irreversible. Consequently, in the more 'distal' enzymatic defects, the amino and keto acids do not accumulate. Diagnosis is dependent on demonstration of the relevant organic acid(s) and their metabolites in body fluids, which is technically more difficult. The discovery of the first of these more distal disorders, isovaleric acidaemia, was delayed until 1966[9] and the true incidence is probably still underestimated owing to the analytical problems. To date 21 cases have been recorded.[5,10,11,12] A detailed review[13] of the organic acidaemias has recently been published. Hence, only certain aspects will be discussed here.

Isovaleric acidaemia arises as a result of hepatic inability to convert isovaleryl co-enzyme A to β-methylcrotonyl co-enzyme A as shown below. The deficient enzyme is presumed to be isovaleryl CoA dehydrogenase (IVD).



During remission, plasma concentrations of isovaleric acid are typically 2 - 5 times the normal but during acute attacks they may be as much as 1 500 times the normal level.[14] From a diagnostic viewpoint, isovaleryl glycine is the most important metabolite appearing in the urine, during both acute attacks and remission. Since the urinary concentration is constantly elevated in these patients, it is possible to employ the relatively simple thin-layer chromatographic method of Ando and Nyhan[2] as a screening test for isovaleric acidaemia. If isovaleryl glycine is not available for use as a standard, β-hydroxybutyric acid should be included, since it can be confused with isovaleryl glycine with this procedure.[15]

Confirmation of the diagnosis requires quantitative gas chromatographic determination of isovaleric acid in urine and blood and of isovaleryl glycine in urine. There

TABLE I. URINARY METABOLITES IN PATIENTS WITH ISOVALERIC ACIDAEMIA

	Patient 1		Patient 2		
Metabolite	Attack	Remission	Attack	Remission	Normal values
Isovaleric acid (μg/mg creatinine)	68,4	9,9	26,6	—	0 - 0,2[4]
Isovaleric acid (mg/d)	—	2,2	—	—	0,3;[5] 0,4[6]
Isovaleryl glycine (μg/mg creatinine)	4 450	4 770	2 280	11 160	—
Isovaleryl glycine (mg/d)	—	1 088	—	3 156	0 - 2[7]
Molar ratio: $\frac{\text{isovaleryl glycine}}{\text{isovaleric acid}}$	38	287	51	—	—

is also increased urinary excretion of β-hydroxy isovaleric acid, particularly during acute attacks. However, the latter finding has been shown to be nonspecifically associated with keto-acidosis of any cause.[16] Beta-methylcrotonic acid concentration in body fluids is not increased in isovaleric acidaemia.

In both our patients there was clearly accumulation of isovaleric acid and isovaleryl glycine. The degree of ketosis and the high concentration of isovaleric acid in the first urine specimen of the girl are typical of the findings in the acute metabolic crisis of isovaleric acidaemia. The lower urinary concentration in the case of her brother was probably due to the fact that he had already received intensive therapy for several hours before his urine was collected. During remission the urinary levels of isovaleric acid in the case of the girl, and of isovaleryl glycine in the case of both children were found to be of the same order as published values.[8,11]

In the majority of cases, isovaleric acidaemia presents as an acute, neonatal illness. Of the 21 previously recorded cases 10 patients died within the first 3 weeks of life. After the neonatal period the typical clinical picture is that of acute attacks, beginning during the 1st year of life. Attacks are usually secondary to upper respiratory tract infection or other factors which increase leucine loading, and present with repeated vomiting and an unpleasant odour. The degree of metabolic keto-acidosis, dehydration and neurological symptoms is often disproportionately severe with respect to the vomiting. Signs of meningeal irritation, stupor, coma, convulsions or ataxia may cause confusion with primary neurological lesions. The disturbances of consciousness are probably due to isovaleric acid,[17] as is the characteristic odour which is decidedly unpleasant and is generally described as that of sweaty feet. Treatment is, therefore, directed at decreasing the endogenous and exogenous leucine load and reversing the metabolic acidosis. Rectal administration of glycine during acute attacks has also been claimed to attenuate and shorten the clinical course, presumably by promoting conversion of isovaleric acid to isovaleryl glycine.[11] Between attacks, patients with isovaleric acidaemia are free of symptoms and the odour is usually not noticeable.

Of the 9 patients hitherto described who did not die early in life and who did not receive treatment, at least 4 (and possibly 6) were mentally retarded. This makes the normal intelligence of our patients particularly noteworthy. Leucopenia and thrombocytopenia have been reported in a number of cases of isovaleric acidaemia and were also noted on more than one occasion in our patients. The pathogenesis of these manifestations is unknown.

Our patients' clinical presentation and course were thus typical of the less severe form of isovaleric acidaemia. Loss of hair, as occurred in the case of the boy, has been described previously in patients with this condition[11] but is possibly nonspecific. The reason for the transient nasal speech at the beginning of the attacks in our patients is obscure.

Isovaleric acidaemia, as far as is known, is inherited as an autosomal recessive condition. Detection of carriers in an affected family can possibly be done with the aid of radiochemical enzyme assays in fibroblast cultures,[18] but the facilities for performing these studies are not widely available. Guibaud *et al.*[5] reported a rise in urinary isovaleryl glycine excretion in the parents of their patient after oral administration of leucine 100 mg/kg body weight.

In contrast, we were unable to demonstrate any isovaleryl glycine in the urine of the parents of our 2 patients or in that of control subjects after leucine loading. This aspect clearly requires further investigation. Prenatal diagnosis with the use of cultured amniotic cells and early diagnosis in the neonate before development of symptoms are under investigation.[18]

In view of the fact that most of the neurological damage occurs in the first few months of life, it is questionable as to whether long-term preventive therapy should be implemented in patients in whom the diagnosis is made at a later stage. Furthermore, in some reported cases there have either been long intervals between attacks, or spontaneous improvement has occurred after a few years, and some untreated patients exhibit no intellectual impairment. We have nevertheless recommended a leucine-restricted diet (approximately 100 mg/kg body weight/day) for our patients in the hope of avoiding further acute episodes and of preventing possible mental deterioration. On account of the children's natural aversion for protein foods, the diet has not presented a problem of acceptability. In view of the clear prodromal phase of 24 - 48 hours preceding past acute attacks in at least 1 of our patients, we have also advised the institution of a high-fluid, protein-free regimen as well as antibiotic therapy at the first hint of symptoms or at the commencement of any upper respiratory tract infection. Similar precautions would naturally also be applicable in the case of any surgical procedures on the patients.

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