The impaired renal clearance of xylose in patients with pernicious anaemia ( Helmer and Fouts, 1937 ; Butterworth et al., 1959 ; Bezeman et al., 1959 ) has in part been attributed to the low haematocrit ( Bezeman et al., 1959 ). The 11 patients reported here who had a low urinary xylose excretion and a normal plasma xylose concentration all had widespread eczema. Patients such as these have an increased plasma volume ( Shuster and Wilkinson, 1963 ; Fox et al., 1965 ) as well as an increased venous pressure ( Shuster, 1963 ; Fox et al., 1965 ), and this could well explain the reduced renal excretion of xylose.

In practical terms, the standard δ-xylose absorption test may give false-positive results in patients with skin disease, although the incidence of such false-positive results is less than with the frul excretion test.

From the combined findings of the δ-xylose absorption test and small-intestinal biopsy, none of the 70 subjects studied was considered to have intestinal malabsorption: taking eczema by and large, therefore, intestinal malabsorption is unlikely to be an important precipitating cause. Eczema, however, comprises a very heterogeneous group of skin diseases, and it remains to be seen whether intestinal malabsorption is particularly associated with any one of these diseases.

Summary

The urinary xylose excretion test was carried out as a screening procedure for intestinal malabsorption on 50 consecutive patients admitted with all types of eczema and on 20 similar patients whose admission was not consecutive. Eleven of the 70 patients had a reduced excretion of xylose in the urine but with a normal increase in the plasma xylose concentration. This is thought to be due to impaired renal clearance of xylose. Small-intestinal biopsy was done in 10 of the 11 patients and was normal in each. Thus no evidence of gastro-intestinal abnormality was found in any of the 70 patients, and it is concluded that intestinal malabsorption is not a major precipitating factor in eczematous skin disease.

We are grateful to Miss Anne Stevington for her able technical assistance, to the staff of the in-patient branch of St. John's Hospital for Diseases of the Skin for their co-operation in this study, and to the Medical Research Council for a grant to S. S. and a grant to R. M. H. M.

Studies on Tyrosinosis: 2, Activity of the Transaminase, Parahydroxyphenyl-pyruvate Oxidase, and Homogentisic-acid Oxidase

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The original report on a case of tyrosinosis was given by Medes in 1932. She suggested that there was a complete block of the p-hydroxyphenyl-pyruvate oxidase with a slowing down of the steps before.

La Du (1960) re-evaluated the significance of the biochemical findings of Medes. To explain the different biochemical findings of Medes he suggested that the metabolic block was at the tyrosine transaminase step and not at the p-hydroxyphenyl-pyruvate oxidase step.

We have recently studied three cases with an excessive urinary excretion of tyrosine and p-hydroxyphenyl pyruvic, lactic, and acetic acids. In addition these patients had rachitis, renal tubular defects, cirrhosis of the liver, and elevated serum tyrosine. Two of them were sisters. These cases were similar to those reported by Sakai and Kitagawa (1957) and by Fritzell, Jagenburg, and Schnürer (1964).

To obtain more information about the pathogenesis of these cases we had already given one of them a diet restricted in phenylalanine and tyrosine (Halvorsen and Gjessing, 1964). The over-excretion of tyrosine and p-hydroxyphenyl metabolites disappeared and the level of tyrosine in the serum and the kidney function became normal. These results suggested that there was a block of the p-hydroxyphenyl-pyruvate oxidase.

In order to confirm this finding we examined the enzyme activity of the liver and the kidney from another of these patients.
Methods

Tyrosine-α-ketoglutarate transaminase was assayed manometrically by the method of Zannoni and La Du (1960). The incubation mixture was fortified with 30 µg of pyridoxal phosphate. For the assay of the transaminase enzyme in the tyrosinosis liver which lacked p-hydroxyphenyl-pyruvate oxidase the incubation mixture was fortified with 0.4 ml of step 3 preparaton of p-hydroxyphenyl-pyruvate oxidase prepared from a guinea-pig liver by the method of Taniguchi and Armstrong (1963). The added p-hydroxyphenyl-pyruvate oxidase had the activity of oxidizing 2.4 times the amount of p-hydroxyphenyl-pyruvate produced by the transaminase in the incubation mixture. This guinea-pig liver preparation was contaminated with a transaminase activity of 0.4 µmol/hr, and this was subtracted from the total value to ascertain the activity of the transaminase in the sample.

p-Hydroxyphenyl-pyruvate oxidase was assayed manometrically by the method of Hager, Gregerman, and Knox (1957).

Phenyl-pyruvate oxidase was assayed by the method of Taniguchi and Armstrong (1963). Because of the presence of diazo-positive compounds in crude tissue homogenate, the product, α-hydroxyphenylacetic acid, was determined by visual comparison of the spots with reference spots after separating the products by paper chromatography and visualizing them by diazotized sulphanic acid spray.

Homogentisic-acid oxidase was assayed manometrically by the method of Zannoni and La Du (1960).

Material

The liver and kidney material was obtained from a boy who died at the age of 4½ months. Samples of the liver and kidneys were removed from the body within nine hours after death. This material was compared with that of an adult male who had died suddenly from an accident. Samples of kidney and liver were taken 24 hours after death. All material has since been kept frozen in a deep freeze at ~20°C.

Results

The transaminase and homogentisic-acid oxidase were present in the tyrosinosis liver, but p-hydroxyphenyl-pyruvate oxidase was not detected. Nor was phenylpyruvate oxidase found by the method used, which could detect 0.004 µmol/hr./g. tissue of activity (see Table).

<table>
<thead>
<tr>
<th>Enzyme Activities in Normal and Tyrosinosis</th>
<th>Normal (µmol/hr./g)</th>
<th>Tyrosinosis (fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transaminase (liver) . . . .</td>
<td>10-6</td>
<td>9-7</td>
</tr>
<tr>
<td>p-hydroxyphenyl-pyruvate oxidase (liver) .</td>
<td>26-7</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Phenylpyruvate oxidase (liver) . . . .</td>
<td>9-1</td>
<td>&quot;</td>
</tr>
<tr>
<td>Homogentisic-acid oxidase (liver) . . .</td>
<td>0-099</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>1-7</td>
<td>1-3</td>
</tr>
</tbody>
</table>

Discussion

In her classic report Medes suggested that the "tyrosyluria" of her patient was due to the lack of p-hydroxyphenyl-pyruvate oxidase. This proposal was confirmed in the present study by the finding that p-hydroxyphenyl-pyruvate oxidase could not be detected in vivo in the liver and kidney. It was also reported by Medes that her tyrosinosis case could oxidize exogenous homogentisic acid effectively, and homogentisic-acid oxidase does exist in the tyrosinosis liver tested in our laboratory.

Although the activities of transaminase and homogentisic-acid oxidase were lower than those of normal liver used as a control, it is not certain whether this was due to the low content of the enzymes, or to difference in age, or to post-mortem changes during the storage and transportation of the sample.

This absence of p-hydroxyphenyl-pyruvate oxidase in the liver and kidney from one of these cases with tyrosinosis confirms our previous results with phenylalanine- and tyrosine-restricted diet. These results are also in agreement with those of Sakai, Katagawa, and Yoshioka (1959) and of Gentz, Jagenburg, and Zetterström (1965). In liver biopsies from cases similar to ours they found that p-hydroxyphenyl-pyruvate oxidase activity was completely lacking. The activity of tyrosine-α-ketoglutarate transaminase, however, was the same as in control livers.

As Medes termed the disease of her patient tyrosinosis, assuming that there was a block of p-hydroxyphenyl-pyruvate oxidase, we have used her term, whereas Gentz et al. prefer genuine tyrosyluria.

Another point of interest is that phenylpyruvate oxidizing activity could not be detected in a tyrosinosis liver. Taniguchi, Kappe, and Armstrong (1964) suggested the possible identity of phenylpyruvate oxidizing activity with that of p-hydroxyphenyl-pyruvate oxidase, and this absence of both activities in tyrosinosis liver may lend further support to this idea. On the other hand, this tyrosinosis case excreted in the urine an approximately normal amount of o-hydroxyphenylacetic acid, evaluated by two-dimensional paper chromatography. The excretion of 0-hydroxyphenylacetic acid during phenylalanine- and tyrosine-restricted diet also seems to be constant and normal. This o-hydroxyphenylacetic acid may therefore be formed through an o-tyrosine pathway.

Summary

Examination was made of post-mortem material from a case with a large urinary excretion of tyrosine, p-hydroxyphenyl pyruvic, lactic, and acetic acids, renal tubular defects, rachitis, cirrhosis of the liver, and an increased amount of tyrosine in serum. Samples of liver and kidney were obtained within nine hours after death and compared with liver and kidney material from an adult who died suddenly.

Para-hydroxyphenyl-pyruvate oxidase activity was absent in the liver and kidney from the tyrosinosis case.

The tyrosine-transaminase activity, as well as that of the homogentisic-acid oxidase, was present.

The phenylpyruvate oxidase activity was also lacking in the liver in spite of normal amounts of 0-hydroxyphenylacetic acid in the urine.

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References


