VOLATILE PHENOLS IN SERUM OF URAEMIC PATIENTS

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SUMMARY

1. The volatile phenols in sera of uraemic patients were analysed by gas-liquid chromatography. The concentrations of p-cresol, $1.17 \pm 0.58$ (mean $\pm$ SD), and phenol, $0.44 \pm 0.39$ mg/100 ml, were higher than the corresponding values ($0.23 \pm 0.17$ and $0.02 \pm 0.03$ mg/100 ml, respectively) in control subjects. Both compounds were recovered mainly as acid labile conjugates, probably sulphate esters. No unconjugated phenol, o-, m-, or p-cresol or conjugated derivatives of o- or m-cresol were detected.

2. When the standard hospital diet was replaced by an isocaloric low-protein diet, the concentration of p-cresol and urea in serum decreased in the two uraemic patients studied. The serum concentration of phenol was uninfluenced by this change of diet.

3. One female patient was studied during treatment with peritoneal dialysis, which on two out of three occasions appeared to result in decreased concentrations of phenol and p-cresol in serum.

Key words: uraemia, serum, phenols.

The blood concentrations of volatile phenols are reported to be abnormally high in uraemic patients, a finding that has been interpreted in various ways. Becker (1933) suggested that phenols, especially in the unconjugated form, were responsible for the uraemic symptoms. Dickes (1942) found evidence indicating that a high serum concentration of phenols is a grave prognostic sign. Mütting (1965) related the clinical symptoms of uraemia to the increase of toxic breakdown products, including the phenolic compounds. Dunn, Weinstein, Maxwell & Kleeman (1958), however, concluded that anaemia may develop in uraemic patients whether the blood concentrations of volatile phenols or phenolic acids are elevated or not.

The phenolic compounds include the volatile phenols (phenol and the cresol isomers) and a number of phenolic acids. The techniques previously used in the analysis of these products were usually based on quantitative determinations of groups of compounds, using spectrophotometric methods that are not particularly specific under such conditions (Jutzler, Kramer, Keller, Kramer & Doenecke, 1968). Wengle & Hellström (1971) measured the volatile phenols

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by gas–liquid chromatography (g.l.c.), a technique which, judging from mass spectrometric analyses of the gas effluents, appeared to be highly specific. The aim of the present investigation was to study the volatile phenols in the serum of uraemic patients.

MATERIALS AND METHODS

Patients

The study included ten female and six male patients, 23–90 years of age. They had various forms of renal diseases which had resulted in renal failure (Table 1). All patients were hospitalized during the investigation. The non-uraemic control subjects were patients and personnel of the ward and the laboratory. The daily dietary intake of protein was standardized in two experiments with subjects G.A. and J.J. These patients were initially fed the regular hospital diet [20% of the 6700–7600 kJ (1600–1800 kcal) per day were supplied as protein], which was replaced later by an isocaloric low-protein (20 g of protein/day) diet. One female patient (B.L.) was also studied while undergoing peritoneal dialysis. No patient or control subject was given antibiotics or chemotherapeutic agents during the study.

Methods

Samples of serum, obtained in the morning before breakfast, were diluted with HCl and water. The volatile phenols were isolated by distillation; g.l.c. was performed on trimethylsilyl ethers prepared from chloroform extracts of the distillates. Samples of serum from some of the patients were analysed for unconjugated volatile phenols, by a modification of the above method in which HCl was omitted (Wengle & Hellström, 1971). Standard statistical calculations were done (Snedecor, 1956).

RESULTS

The control subjects had normal renal function as evidenced by the concentration of urea and creatinine in serum (Table 1). All serum samples from these subjects contained p-cresol, (0.23±0.17 mg/100 ml, mean±SD). The concentration of phenol was generally low and in some instances below the detectable limit. None of the samples contained o- or m-cresol. There was no correlation between the age of the subject and the serum concentrations of p-cresol and phenol.

The concentration of p-cresol in serum was above normal in all uraemic patients, the mean (1.17±0.58 mg/100 ml) being significantly different from that of control subjects (P<0.001). Phenol was definitely above the normal range in twelve of the sixteen patients. No unconjugated volatile phenols or conjugated derivatives of o- or m-cresol were detected. There was no correlation between the serum concentration of p-cresol or phenol on the one hand and that of urea creatinine on the other.

After changing from the standard hospital to the isocaloric low-protein diet, both subjects’ concentration of p-cresol and urea in serum decreased by approx. 50% within 10 days (Figs. 1 and 2). The serum concentration of phenol and creatinine remained almost constant throughout the experimental period.

Subject B.L. was submitted to peritoneal dialysis on four occasions (Fig. 3). Although the blood samples were obtained somewhat irregularly, the data demonstrate that the marked
### Table 1. \( p \)-Cresol and phenol in the serum of uraemic patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>( p )-Cresol (mg/100 ml)</th>
<th>Phenol (mg/100 ml)</th>
<th>Urea (mg/100 ml)</th>
<th>Creatinine (mg/100 ml)</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.H.</td>
<td>52</td>
<td>F</td>
<td>0.45</td>
<td>1.02</td>
<td>117</td>
<td>3.4</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>M.V.</td>
<td>80</td>
<td>F</td>
<td>0.60</td>
<td>0.04</td>
<td>136</td>
<td>6.0</td>
<td>Chronic glomerular nephritis</td>
</tr>
<tr>
<td>S.H.</td>
<td>61</td>
<td>F</td>
<td>0.65</td>
<td>0.85</td>
<td>242</td>
<td>10.2</td>
<td>Chronic glomerular nephritis</td>
</tr>
<tr>
<td>G.S.</td>
<td>67</td>
<td>F</td>
<td>0.72</td>
<td>0.03</td>
<td>—</td>
<td>1.7</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>B.H.</td>
<td>45</td>
<td>F</td>
<td>0.73</td>
<td>0.81</td>
<td>205</td>
<td>9.8</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>B.L.</td>
<td>26</td>
<td>F</td>
<td>0.93</td>
<td>1.21</td>
<td>460</td>
<td>24.0</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>L.A.</td>
<td>24</td>
<td>M</td>
<td>1.03</td>
<td>0.20</td>
<td>156</td>
<td>5.2</td>
<td>Chronic glomerular nephritis</td>
</tr>
<tr>
<td>A.J.</td>
<td>56</td>
<td>M</td>
<td>1.04</td>
<td>0.63</td>
<td>140</td>
<td>14.8</td>
<td>Polycystic kidneys</td>
</tr>
<tr>
<td>B.B.</td>
<td>50</td>
<td>F</td>
<td>1.05</td>
<td>0.15</td>
<td>242</td>
<td>2.6</td>
<td>Nephropathia diabetica</td>
</tr>
<tr>
<td>E.H.</td>
<td>57</td>
<td>M</td>
<td>1.08</td>
<td>0.76</td>
<td>224</td>
<td>12.4</td>
<td>Polycystic kidneys</td>
</tr>
<tr>
<td>E.S.</td>
<td>79</td>
<td>F</td>
<td>1.22</td>
<td>0.04</td>
<td>78</td>
<td>6.8</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>G.A.</td>
<td>58</td>
<td>M</td>
<td>1.26</td>
<td>0.22</td>
<td>250</td>
<td>8.6</td>
<td>Chronic glomerular nephritis</td>
</tr>
<tr>
<td>B.F.</td>
<td>23</td>
<td>F</td>
<td>1.47</td>
<td>0.30</td>
<td>180</td>
<td>2.3</td>
<td>Chronic glomerular nephritis</td>
</tr>
<tr>
<td>A.S.</td>
<td>90</td>
<td>M</td>
<td>1.86</td>
<td>0.04</td>
<td>—</td>
<td>2.0</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>F.J.</td>
<td>79</td>
<td>M</td>
<td>1.95</td>
<td>0.42</td>
<td>440</td>
<td>11.0</td>
<td>Chronic pyelic nephritis</td>
</tr>
<tr>
<td>J.J.</td>
<td>65</td>
<td>M</td>
<td>2.68</td>
<td>0.27</td>
<td>287</td>
<td>17.0</td>
<td>Chronic glomerular nephritis</td>
</tr>
</tbody>
</table>

Mean ± SD 57 ± 20 1.17 ± 0.58 0.44 ± 0.39 225.5 ± 111.7 8.6 ± 6.3

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls (n=23)</th>
<th>Difference to patients</th>
<th>( P &lt; 0.001 )</th>
<th>( P &lt; 0.001 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.S.</td>
<td>48 ± 20</td>
<td>0.25 ± 0.19</td>
<td>0.01 ± 0.02</td>
<td>( \leq 40 )</td>
</tr>
</tbody>
</table>

N.S., not significant.

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**Fig. 1.** Concentration of volatile phenols in relation to the dietary intake of protein (subject G.A.).
(a) Urea; (b) \( p \)-cresol; (c) creatinine (\( x \)) and phenol (\( o \)).
decrease of serum urea observed after the first and fourth dialyses was associated with a decreased amount of $p$-cresol and phenol in serum. Such an effect was not observed after the second dialysis.

**DISCUSSION**

The volatile phenols excreted in the urine of mammals are considered to be formed in the gut by bacterial degradation of aromatic amino acids derived from ingested proteins (Rogers, Burdick & Burnett, 1955; Bernhart & Zilliken, 1959). In the human most phenols are excreted as conjugates with sulphuric or glucuronic acid. Conjugation probably occurs mainly in the liver but may occur in other organs. Phenol sulphokinase has been detected in several human tissues, including the kidney and mucosa of the gut (Boström & Wengle, 1967).

Studies of the volatile phenols in blood have been invalidated by the absence of specific analytical methods. With the gas-chromatographic technique used in the present study (Wengle
Volatile phenols in renal failure

it is possible, however, to determine the phenol and the individual cresol isomers. Serum from all healthy subjects investigated was found to contain an acid-labile conjugate of p-cresol, probably the sulphate ester. Such a conjugate of phenol was recovered in serum of seven of the fifteen subjects studied. No serum samples contained detectable amounts of unconjugated phenol or p-cresol, or free or conjugated derivatives of o- and m-cresol. Other analyses after incubation of serum samples with β-glucuronidase indicated that most of the circulating phenol and p-cresol in healthy subjects were sulphate conjugates.

The volatile phenols in serum have been determined previously as a group by spectrophotometric methods (Jutzler et al., 1968). Using such a technique, Dunn et al. (1958) found the same concentration of free phenols in serum from control and uraemic subjects. Dunn et al. (1958) also concluded that renal failure was associated with an elevation of the conjugated volatile phenols in serum. Mütting (1965) studied the volatile phenols in serum of uraemic patients by means of paper chromatography and reported the presence of phenol, p- and m-cresol.

![Fig. 3. Effect of peritoneal dialysis (bar) on the concentration of volatile phenols in serum of a subject (B.L.) with chronic pyelonephritis. (a) Urea; (b) p-cresol; (c) creatinine (×) and phenol (○).](image-url)
In the present study the serum of all patients was found to contain abnormally high concentrations of an acid-labile conjugate of p-cresol. The concentration of such a conjugate of phenol was within normal limits in some of the patients but elevated in others. In all instances p-cresol was the dominant compound. Phenyl glucuronides are not split under the conditions used for the hydrolysis and most of the analysed compounds should be sulphate esters. The small amounts of glucuronide-conjugated p-cresol that may be found in the serum of uraemic patients (Wengle & Hellström, 1971) were not analysed in the present study. In contrast to the reports of Dunn et al. (1958) and Mütting (1965), there was no evidence either of unconjugated phenol or of m-cresol in the serum of uraemic patients.

When the standard hospital diet was replaced by a low-protein diet, the concentration of urea and p-cresol in serum decreased markedly, whereas that of phenol remained constant. This phenomenon, which is well known as far as urea is concerned, supports the view that p-cresol is produced to a considerable extent from ingested proteins, most probably by the action of intestinal micro-organisms. These experiments also indicate that phenol is formed by mechanisms that are not influenced by the dietary intake of proteins.

It is not known whether increased concentrations of phenol and p-cresol may contribute to the development of the uraemic syndrome. However, the treatment commonly used in uraemia, i.e. restriction of dietary proteins and peritoneal dialysis, does seem to decrease the concentrations of these potentially toxic substances in parallel with that of urea.

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