PART VII

METABOLIC AND CLINICAL PROBLEMS

Chairmen: G Schütterle
           E Bartels
Indolic Tryptophan Metabolism in Uraemia

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It is known that abnormalities in tryptophan (Try) metabolism occur in persons with renal failure. The exact relationship between these aberrations in Try metabolism and uraemia is not known. Plasma Try has been found to be both elevated Müting (1965) and decreased Gulyassy (1970). Urinary excretion of Try is reported to be above normal Gulyassy (1970). Plasma indican (Ludwig et al 1968; Müting 1965) and indolacetic acid (IAA) (Müting et al 1965) are increased. Some metabolites have been investigated in dialysis fluid (Aviram et al 1971, Hicks et al, 1962; Ludwig et al, 1968). These data were obtained with paper chromatography counter current distribution and in situ colorimetry. We are investigating Try and its indolic metabolites in urine, plasma and dialysate of uraemic patients, using two chromatographic methods of higher resolutions, thin-layer (TLC) and high pressure liquid (HPLC) chromatography. With these tools we were able to detect a wider range of Try metabolites which includes some compounds not previously reported in uraemia.

MATERIAL AND METHODS

Ten subjects with advanced chronic renal failure were examined. Of these six were on intermittent haemodialysis (Gambro, Lundia 13.5 μ). In four subjects in the pre-dialysis stage, 24 hour urines were analysed before and for 48 hours after an oral load of 25 mg/kg L-Try given in the fasting state.

Dialysate from the first hour of dialysis in one subject (nephrectomised, intermittent dialysis) and from the first and seventh hour in another (intermittent dialysis) was concentrated from 50 L to 5 L. One L portions were worked up. Plasma samples (1-2 ml) from four other dialysed patients were examined immediately before dialysis. Urine and concentrated dialysate were extracted with ether. The extracts were resolved on TLC and the stained spots were quantitated by in
TABLE I. Excretion rates (mg/24h) of tryptophan and some indolic metabolites in four patients with uraemia. B = basal; L<sub>1</sub>, L<sub>2</sub> = 1st and 2nd 24h after oral load of 25 mg/kg L-Try; O = not detected.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Patient</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>L&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>6.3</td>
<td>4.8</td>
</tr>
<tr>
<td>(0.96)</td>
<td>(2.3-10.2)</td>
<td>(0-6.8)</td>
</tr>
<tr>
<td>Indole-3-carboxylic A</td>
<td>1.8</td>
<td>4.6</td>
</tr>
<tr>
<td>N-acetylcarboxylic A</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>N-acetylindole-3-carboxylic A</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>N-acetyltryptophan acid</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>(0-10.0)</td>
<td>(0-20.5)</td>
<td>(0-8.8)</td>
</tr>
</tbody>
</table>

| Age (yrs)  | 10 | 9 | 15 | 8 |   |
| Diagnosis  | Nephronophthisis | Oligomegane-phonias | Nephronophthisis | Segmental hypoplasia | healthy |
| C<sub>CR</sub> ml/min/1.73 m<sup>2</sup> | 16 | 8 | 10 | 4 |   |
situ colorimetry as previously described (Byrd et al., 1974). Denatured plasma was worked up in a similar manner and analysed by both TLC and HPLC (Trefz et al., 1976).

RESULTS AND DISCUSSION

As seen in Table I, basal urinary Try appeared to be decreased in three uraemic patients while in one it was essentially normal. The lack of a definite increase in the rate of excretion of Try in the patients after the load is surprising. These results agree with those of Mütting (1965) who reported a decrease in urinary Try. On the other hand Gulyassy et al. (1970) reported that the renal clearance of Try and some other amino acids increases as renal function decreases and as the tubular reabsorption of these amino acids becomes less effective.

Two metabolites of tryptophan which have not been previously reported in uraemia are indole-3-carboxylic acid (I-3CA), a normal metabolite, and indole-3-carbaldehyde (I-3Ald). The basal excretion of I-3CA was elevated in two of the patients. In only one subject was I-3CA clearly increased after the Try load.

I-3Ald has not been found in controls. The formation of both of these metabolites from Try in man has been proved in tracer experiments with 14 C-Try (Byrd et al., 1974; Kochen and Byrd, 1973). I-3CA does not appear to be a product of bacterial break down of Try (Byrd et al., 1975). Of particular interest was the detection of N-acetyltryptophan (Try-N,Ac) in the urine of uraemic patients. To our knowledge this compound has not been reported previously as a component of uraemic urine. To date we have not been able to see this metabol-

![Figure 1. Indolic tryptophan metabolites in dialysate of uraemic patient (No. VI)](image)

A = 1st hour of dialysis  
B = 7th hour of dialysis

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ite on chromatograms of normal urines (Aviram et al, 1971). The Try load apparently increased the excretion rate of Try-N,Ac in three subjects.

The Try metabolite indolelactic acid (ILA) was detected in three of the urines. However, the comparison with the control groups is rather ambiguous. While the excretion rates are comparable to the mean excretion rates in the controls few of the controls actually excrete this substance. Thus we think that although ILA is found in the urine of some clinically healthy persons, it appears to be an unusual urinary excretion product. We were able to quantitate 1-3CA, IAA, Try-N,Ac, Ind, ILA and I-3Ald in dialysate solution at the beginning and end of dialysis in one patient. Try was not detected. There were differences in the behaviour of these metabolites during dialysis in that ILA, Ind and I-3Ald could not be detected in the sample from the seventh hour and the concentration of I-3CA was reduced. The concentration of IAA was unchanged at the end of dialysis. The presence of these same compounds in the dialysate of the first hour of a second, bilaterally nephrectomised, patient is interesting as this proves that the kidney is not essential for their formation in man.

In addition to these metabolites 13 as yet uncharacterised amine compounds in high concentration were observed on the chromatograms from dialysis fluid.

Table II presents the results of analysis of Try metabolites in plasma of seven controls and four uraemic patients. While only traces of IAA were detected in the plasma of one of the seven controls, three of the patients had levels of this

<table>
<thead>
<tr>
<th>Controls (n = 7)</th>
<th>IAA</th>
<th>ILA</th>
<th>Try-N,Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>trace</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.03</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Uraemic patients (no)+</th>
<th>IAA</th>
<th>ILA</th>
<th>Try-N,Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>VII</td>
<td>0.06</td>
<td>0.15</td>
<td>2.11</td>
</tr>
<tr>
<td>VII</td>
<td>–</td>
<td>–</td>
<td>1.25</td>
</tr>
<tr>
<td>VIII</td>
<td>0.25</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>IX</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>X</td>
<td>0</td>
<td>0.06</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE II. Indolic tryptophan metabolites in plasma in four patients with uraemia and controls (mg %)

0) = not detected; –) = not investigated
+) = all patients on intermittent dialysis

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Figure 2. High-pressure liquid chromatogram of plasma extract from uraemic patient
(No.VII, Table II)
Siemens S 200; Column: Lichromsorb S 100, 5 μm, 50 x 0.6 cm; Solvent: organic phase
from CHCl₃/C₂H₅OH/H₂O, 1770/150/80 (V/V) acidified with 1% AcOH;
Flow: 3 ml/min, R.T. F₂ = isolated sample of N-acetyltryptophan

MASS SPECTRA
of above) authentic N-acetyltryptophan and below) sample from uraemic plasma

Figure 3. Mass spectra of a) auth. N-acetyltryptophan and b) sample isolated from plasma
of uraemic patient. Du Pont 21-492, 70eV, solid inlet; source temp: 180°C
metabolite comparable to those reported by Ludwig and co-workers (Ludwig et al., 1968).

ILA was quantitated in the plasma of two controls. The average amount of this metabolite in three of the patients was almost four times higher than in the controls. The plasma concentrations of Try-N,Ac in one patient was over twice the normal concentration of Try. This patient had been on dialysis for one year.

Endogeneous Try-N,Ac was isolated from plasma by HPLC (Figure 2) and its mass spectrum was determined as shown in Figure 3. The typical fragmentation pattern and abundance of fragments in the spectrum of the isolated sample were identical to those of Try-N,Ac. To our knowledge, this is the first report of endogeneous Try-N,Ac in human plasma.

Our findings that urine, dialysis fluid and especially the plasma of uraemic patients contain significant amounts of ILA and Try-N,Ac suggest the presence of two definite abnormalities in Try metabolism in terminal renal failure: enhanced transamination of Try and an impairment of renal amino acid acylase.

![Diagram](image)

Figure 4. Some pathways of indolic tryptophan metabolism in man.

ILA is formed by the transamination of Try by Try aminotransferase and subsequent reduction of the intermediate indole pyruvic acid (Figure 4). The first abnormality of Try metabolism in uraemia appears to be an enhanced transamination of Try similar to that described in patients with phenylketonuria who also uniformly excrete excess ILA in the urine (Armstrong & Robinson 1954) and have elevated levels of ILA in plasma (Byrd et al., 1975). It should also be noted
that phenylalanine and its transamination product phenylpyruvic acid (Giordano et al, 1969), are also increased in uraemia.

The data on Try-N,Ac suggest a possible impairment of renal amino acid acylase in uraemia or an increase in the rate of hepatic N-acetylation or both. There is evidence that Try and other amino acids such as phenylalanine and histidine are acetylated by liver enzymes. On the other hand, the normal kidney is known to have a high titre of amino acid acylase which hydrolyses acylamino acids to the free amino acid (Byrd et al, 1974). The increased formation of the N-acetyl-derivatives of phenylalanine and histidine appear to be employed in the detoxification of phenylalanine in phenylketonuria (Goldstein, 1970) and of histidine in histidinaemia (Wadman et al, 1971). While our data represent only a limited number of patients we feel that the transamination and N-acetylation of Try and other aromatic amino acids in uraemia deserve further investigation.

SUMMARY

Tryptophan and some indolic metabolites were studied in urine, plasma and dialysate of uraemic patients using thin-layer- and high-pressure liquid chromatography. Some new metabolites: indole-3-carboxylic acid, indole-3-carbaldehyde, indolelactic acid and N-acetyltryptophan were detected. Levels of the latter two metabolites in urine, dialysate and especially plasma suggest increased transamination of tryptophan and a possible defect in renal amino acid acylase in uraemia.

References

Armstrong, M D and K S Robinson (1954) Archives of Biochemistry and Biophysics, 52, 287
Goldstein, F B (1970) Biochimica et Biophysica Acta, 71, 855
Gulyassy, P F, A Aviram and J H Peters (1970) Archives of Internal Medicine, 126, 855

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Open Discussion

FINE (Glasgow) I wonder are your results compatible with increased hepatic acetylation in uraemia as we demonstrated with sulphadimidine and presented at EDTA last year?

BYRD Possibly. We examined acetyl tryptophan in phenylketonuria (PKU) patients and found that they also have higher blood concentration of this metabolite, perhaps due to an increase in hepatic acetylation in PKU. We think that kidney acetylase may be damaged somehow in uraemia rather than that an increase of the liver enzyme has occurred.

FINE On the other hand, Burke and colleagues in Dublin have shown by liver perfusion experiments in uraemic rats that they have increased hepatic acetylation.

BYRD I am not familiar with that data.