Uraemic Waste Recovery II: 
*In Vitro* Studies

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It has been suggested that, in uraemic subjects, NPN compounds can be 
degraded, reconverted into non-toxic metabolites and partly reutilised by 
the organism. For this purpose appropriately selected non-pathogenic soil 
microorganisms are introduced into the gut of uraemic subjects (Setälä, 1970, 
1972; Setälä et al, 1971).

It was further suggested that NPN compounds do not represent valueless 
'wastes' which necessarily must be excreted. Only the surplus which is not 
needed, departs. NPN compounds are not 'end'-products but are potentially 
valuable intermediates which allow a shuttle-traffic in protein metabolism: 
synthesis $\cong$ degradation $\cong$ resynthesis.

To prevent an excess accumulation of untoward NPN compounds in the 
subjects of patients suffering from irreversible progressive renal diseases*, 
processes responsible for internal metabolism must be 'cycled backwards'. 
This is done as follows: (1) circumstances in the gut are changed so that 
biodegradation of NPN with subsequent biosynthetic events become possible; 
(2) when thus the concentration of a given NPN in the gut is decreased, the 
organism tends to achieve a new equilibrium following differences between 
the gradients. This results in a decrease in the total amount of this NPN in 
the body; (3) the intestinal excretion route is utilised instead of the occluded 
renal; and (4) a prerequisite is that the uraemic patient is held on an appro-
priately balanced diet.

Orienting tests on uraemic subjects with associated in vitro experiments 
have revealed that there are several saprophytic microorganisms in the 
human gut which are able to degrade NPN compounds such as uric acid, 
creatinine, and creatinine in a highly significant ($P < 0.001$) manner (op. 
cit.).

* From the viewpoint of general pathology it is erroneous to speak about 
'chronic' renal diseases as these conditions are irreversible and pro-
gressive in nature.

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MATERIAL AND METHODS

General and special experimental techniques have been presented earlier (Setälä, 1970, 1972; Setälä et al., 1971). The same applies to harvesting and isolation of the individual strains of the soil microorganisms. So far, NPN-degrading capacity has been examined in 729 different strains with altogether 60,000 - 70,000 cultures.

The following 12 NPN compounds (concentration expressed as g/l) have been used as a substrate added to the basic agar medium (Table I): urea (10.0), guanidine, ornithine, arginine, creatinine, creatine, glutaminic acid, histidine, allantoin and agmatine (1.0 and 1.5), and uric acid and xanthine (0.5 and 0.75). These compounds were selected because of their key positions in protein metabolism. Harvesting, basic cultivation and enrichment of the soil microorganism were performed at 28°C.

Table I. Distribution of 729 isolated strains of soil microorganisms according to their capacity to utilise 12 different NPN substances as a substrate

<table>
<thead>
<tr>
<th>NPN compound</th>
<th>NPN as the sole nitrogen source</th>
<th>NPN as nitrogen and carbon source</th>
<th>Simultaneous liberation of NH₃ when NPN is the sole nitrogen source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of strains</td>
<td>%</td>
<td>Number of strains</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------</td>
<td>---</td>
<td>----------------</td>
</tr>
<tr>
<td>Urea</td>
<td>521</td>
<td>71.1</td>
<td>Not tested</td>
</tr>
<tr>
<td>Guanidine</td>
<td>377</td>
<td>51.8</td>
<td>Not tested</td>
</tr>
<tr>
<td>Ornithine</td>
<td>637</td>
<td>87.3</td>
<td>302</td>
</tr>
<tr>
<td>Arginine</td>
<td>570</td>
<td>76.3</td>
<td>489</td>
</tr>
<tr>
<td>Creatinine</td>
<td>432</td>
<td>59.3</td>
<td>170</td>
</tr>
<tr>
<td>Creatine</td>
<td>322</td>
<td>41.4</td>
<td>171</td>
</tr>
<tr>
<td>Uric acid</td>
<td>595</td>
<td>81.6</td>
<td>448</td>
</tr>
<tr>
<td>Glutaminic acid</td>
<td>590</td>
<td>81.0</td>
<td>431</td>
</tr>
<tr>
<td>Histidine</td>
<td>631</td>
<td>87.9</td>
<td>517</td>
</tr>
<tr>
<td>Xanthine</td>
<td>478</td>
<td>65.6</td>
<td>279</td>
</tr>
<tr>
<td>Allantoin</td>
<td>657</td>
<td>90.0</td>
<td>417</td>
</tr>
<tr>
<td>Agmatine</td>
<td>620</td>
<td>86.0</td>
<td>504</td>
</tr>
</tbody>
</table>

For quantitative determination of the degree of degradation capacity of the individual strains conventional spectrophotometric techniques were used (for details, see references). Incubation was performed at 37°C. The concentrations of NPN compounds to be degraded was adjusted to correspond to those prevailing in the blood of uraemic patients.

Side-by-side with the above determinations, screening experiments were carried out for visual examination of extracellular (and intracellular) fermentation. Here the colonies grew on a diagnostic medium at 37°C. The area or diameter of the developing translucent halos around the colonies were recognised by visual observation (as in Figures 1 and 2) or other signs rendered visible by appropriate subsequent treatment on the plate. This
Figure 1. Colonies of 3 different strains (72, 90, 94) of soil microorganisms growing on a diagnostic uric acid-agar medium. Translucent halos around colonies indicate enzyme production capable of degrading uric acid. Calculated uricase activity higher than 75 units (cf. reference plate in Figure 2).

Figure 2. Colonies of 3 different strains (92, 222, 332) of soil microorganisms growing on a diagnostic uric acid-agar medium. Diffuse fusion of halos of individual colonies of strain 92. Uricase activity higher than 100 units.
technique allows, in addition, an orientating semiquantitative study of the enzyme activity.

RESULTS

So far 60,000 - 70,000 cultures of soil microorganisms have been examined. Altogether 729 strains of them were able to grow on the very barren medium used and could utilise NPN compounds either as the sole nitrogen or as the nitrogen and carbon (energy) source (Table I). This signifies that the NPN compounds tested were biodegraded. It became further evident that among the strains there were about 30 which had the capacity to biodegrade all of the 12 NPN compounds used as a substrate either simultaneously or after an adaptation (induction) interval.

On the basis of the literature on the subject, it could be expected that urea can easily be biodegraded. Table I shows that 521 (71.1%) strains out of 729 utilised urea as the sole nitrogen source. The material comprised 7 particular strains (577-579, 588, 600, 677 and 722) the degradation capacity of which were remarkably high. On the other hand, if the urea-degrading capacity of a given microorganism was high, the capacity to biodegrade simultaneously other NPN compounds was in most instances significantly lower. However, strains 588 and 722 appeared rather versatile being able to degrade several different NPN compounds in addition to urea. Table II shows biodegradation of urea by some randomly-selected strains after 30 min incubation at 37°C. The material also comprised strains which degraded 85 mg (85%) of urea out of 100 mg under the same experimental circumstances.

Creatinine, the cyclic anhydride of creatine, is generally held as an 'end'-product in protein metabolism. However, there were numerous strains which rapidly biodegraded creatinine. Table I reveals that there are 432

<table>
<thead>
<tr>
<th>No of strain</th>
<th>Dry wt of cells (mg/ml)</th>
<th>Total quantity degraded (mg/ml)</th>
<th>Degree of urea degradation (μN of urea/mg dry wt of cells)</th>
<th>Simultaneous liberation of NH₃ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>3.6</td>
<td>33</td>
<td>5.46 · 10⁻²</td>
<td>± **</td>
</tr>
<tr>
<td>47</td>
<td>3.6</td>
<td>33</td>
<td>5.94 · 10⁻²</td>
<td>+ ***</td>
</tr>
<tr>
<td>50</td>
<td>3.6</td>
<td>57</td>
<td>4.44 · 10⁻²</td>
<td>+</td>
</tr>
<tr>
<td>577</td>
<td>3.6</td>
<td>57</td>
<td>9.56 · 10⁻²</td>
<td>+</td>
</tr>
<tr>
<td>578</td>
<td>3.5</td>
<td>61</td>
<td>12.53 · 10⁻²</td>
<td>+</td>
</tr>
<tr>
<td>579</td>
<td>3.7</td>
<td>90</td>
<td>9.96 · 10⁻²</td>
<td>+</td>
</tr>
<tr>
<td>677</td>
<td>1.6</td>
<td>75</td>
<td>30.57 · 10⁻²</td>
<td>+</td>
</tr>
</tbody>
</table>

* Results of preliminary tests. Presence of NH₃ transient as it is utilised quickly by other microorganisms
** ± presence of NH₃ uncertain (< 1 μg/ml)
*** + NH₃ concentration low (~ 1 μg/ml)

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(59.3%) strains which degrade this NPN compound utilising it as the sole nitrogen source, and 170 (23.4%) strains were able to utilise it both as nitrogen and carbon source. Quantitative determination using certain of the strains showed further that up to 8.9 mg (89%) of creatinine out of 10 mg were degraded in the course of 30 min incubation at 37°C (Table III).

Table III. Microbial degradation of creatinine (10 mg/100 ml) by 3 isolated strains of soil microorganisms selected at random. Incubation 30 min at 37°C

<table>
<thead>
<tr>
<th>No of strain</th>
<th>Dry wt of cells (mg/ml)</th>
<th>Degree of creatinine degradation</th>
<th>Simultaneous liberation of NH₃*</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1.2</td>
<td>2.1</td>
<td>5.89 · 10⁻⁴</td>
</tr>
<tr>
<td>591/a</td>
<td>1.2</td>
<td>3.6</td>
<td>6.60 · 10⁻⁴</td>
</tr>
<tr>
<td>591/b</td>
<td>4.3</td>
<td>8.9</td>
<td>9.25 · 10⁻⁴</td>
</tr>
<tr>
<td>656</td>
<td>3.8</td>
<td>4.3</td>
<td>4.47 · 10⁻⁴</td>
</tr>
</tbody>
</table>

* Results of preliminary tests. Presence of NH₃ transient as it is utilised quickly by other microorganisms
** + NH₃ concentration low (~ μg/ml)
*** - NH₃ not present

Uric acid, also held as an 'end'-product of protein metabolism in man and anthropoid apes, was similarly degradable by a high number of soil microorganisms (Table I). Out of 729 strains 595 (81.6%) utilised uric acid as the sole nitrogen, and 446 (61.1%) both as nitrogen and carbon source. Figures 1 and 2 are drawn from experiments with visual examination of enzyme activity. These photographs illustrate the growth habit of 6 randomly-selected strains on a diagnostic uric acid-agar medium. The translucent halos which coalesce at several places, demonstrate production of extracellular (and intracellular) enzyme(s) capable to biodegrade uric acid.

Table I shows further that glutaminic acid, which partakes in several ways in protein metabolism particularly in formation of arginine, ornithine and urea, was degradable as well. Thus 590 (81.0%) strains utilised this compound as the sole nitrogen source, and 431 (59.1%) both as nitrogen and carbon source.

It became evident that ornithine, arginine and agmatine which partake in the biosynthesis of creatine and creatinine, were also easily degradable. Out of the 729 strains 637 (87.3%) utilised ornithine, 570 (78.3%) arginine, and 620 (86.0%) agmatine as the sole nitrogen source; while 302 (41.5%) were able to utilise ornithine, 489 (67.0%) arginine, and 504 (69.2%) agmatine both as nitrogen and carbon source.

Allantoin which is derived from uric acid by opening of the pyrimidine ring in the purine molecule, was biodegraded by 657 (90.0%) strains which utilised it as the sole nitrogen source; 517 (57.1%) strains utilised allantoin both as nitrogen and carbon source.
Xanthine, a deamination product of amino purines, a metabolite in the oxidation of purines to uric acid and an intermediate in the synthesis of purine rings, was biodegraded by 478 (65.6%) strains which utilised this compound as the sole nitrogen source, while 279 (38.3%) strains used xanthine both as the nitrogen and carbon source.

In biodegradation and biosynthesis of proteins, formation of an excess of ammonia needs particular consideration. Table I reveals that liberation of NH$_3$ as a result of biodegradation of NPN compounds is not an invariable. On the contrary, the present material comprised numerous strains of microorganisms which did not produce ammonia in detectable quantities. Accordingly, 103 (72.1%) strains out of 143 tested, biodegraded creatine, and 96 (58.1%) out of 165, creatinine without liberation of NH$_3$. Further, though eg strains 591/b and 656 (Table III) very rapidly (within 30 min) degraded 89 and 43% respectively, of creatinine, no NH$_3$ liberation was detectable. It was also found that the strains either did not produce ammonia at all, or as in the case of a transient NH$_3$ liberation, the same strain itself could utilise ammonia. There were further strains which utilised only NH$_3$ as their sole nitrogen source.

The strains of soil microorganisms had in general an optimal proliferation temperature of about 28°C. The majority of the strains either died at 37°C or did not grow at the human body temperature. However, the enzyme system(s) responsible for biodegradation of NPN compounds remained active.

**DISCUSSION AND CONCLUSIONS**

It has been demonstrated that there exist numerous strains of soil microorganisms which in vitro rapidly biodegrade significant quantities of just those NPN compounds which accumulate in the body of uraemic patients. Many of the strains had a very high biodegradative capacity and were able to decompose up to 90% of the NPN used as a substrate. Among the microorganisms there were about 30 different strains which were so versatile that they could decompose all of the 12 NPN compounds examined. The microorganisms utilised NPN either as their sole nitrogen, or both as nitrogen and carbon (energy) source (Table I).

Particularly important in the present context is the fact that the strains lived and could be enriched at a temperature of 28°C. The majority of the strains could not divide at human body temperature. Irrespective of this, the enzymes preserved their specific biodegrading capacity.

If an accumulation of NPN compounds in persons suffering from uraemia or preuraemia could be tackled along the principles outlined (Figure 3, see also references), it is likely that many of the symptoms and their causative factors would disappear. It is an easy procedure to select a whole series of strains of soil microorganisms with predetermined properties. The
enriched (eventually lyophilised) masses either of naturally-occurring or purposefully-'trained' microorganism could be given to the uraemic subjects by the oral route.

When the present technique has been improved to a desired level, it may be advantageous if the kidneys of uraemic patients are removed to prevent secretion of hormone like substance(s) by the damaged renal tissues which provoke untoward side effects, e.g. the development of arterial hypertension.

There are numerous suggestions in the literature of the possibility of preventing the accumulation of dangerous NPN metabolites within the bodies of uraemic patients.

The present in vitro observations were in perfect accordance with our previous in vitro and in vivo results.

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