Ultraviolet-absorbing Urinary Components of Mentally Retarded Children and Schizophrenic Adults

Adam W. Lis, Roman Bijan, Elaine W. Lis, and Konrad F. de Hackbeil

Urinary excretion of ultraviolet-light-absorbing end products of metabolism was studied in mentally retarded children and schizophrenic adults. Excretion of the major end product of pyrimidine metabolism, pseudouridine (5-ribosyluracil), and one of the catabolic derivatives of nicotinic acid, N-methyl-2-pyridone-5-carboxamide, were both found to be depressed in more than half the mentally retarded children and all the schizophrenic adults studied. In addition, a number of previously unidentified components have been found in both types of mental abnormality, as well as in control subjects, some of which components appear to be present in abnormal quantities.

Irrespective of true mechanisms involved in the function of the brain, a considerable amount of evidence has now been accumulated suggesting that the metabolism of ribonucleic acid (RNA) may be intimately related to, or is the actual biochemical seat of, the storage and retrieval of information. As a possible approach to investigation of this relationship, analyses of ultraviolet (UV) absorbing compounds in urine specimens, many of which have their origin in the nucleic acids, were carried out on normal and mentally retarded children, as well as on adult schizophrenic patients.

Pseudouridine (5-β-D-ribosyluracil, 5-RU) is a constituent nucleoside of RNA, particularly soluble RNA. The soluble RNA is capable of accepting and transferring activated amino acids, hence t-RNA. At the present time, all t-RNA’s have been found to have 2 or 3 nucleotide residues of 5-RU. As a result of nucleic degradation, 5-RU appears as

From the Nucleic Acids Laboratory, Department of Pathology, and Multiple Discipline Clinic, Crippled Children’s Division, University of Oregon Medical School, Portland, Ore. 97201; in cooperation with the Mental Health Division, Oregon Fairview Home, Salem, Ore., Dr. J. M. Pomeroy, Superintendent.

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the free nucleoside in mammalian urine (man, mouse, rat, dog), while uridine does not. Since free 5-RU is not utilized for biosynthesis of RNA in mammalian systems it may be used as a suitable index of RNA metabolism and turnover.

It has been reviewed and shown by Weissman (1) that in many diseases associated with elevated turnover of nucleic acids—such as psoriasis, gout, chronic myelogenous leukemia, hemolytic anemias, and the after effects, of ionizing radiation—there is an increase in the rates of excretion of 5-RU and uric acid. We have reported previously (2) that in comparison to normal children, mentally retarded children show depressed excretion of 5-RU. The data reported here are an extension of this research and include the finding of other urinary metabolites in abnormal amounts, some of which are as yet unidentified.

### Experimental Procedures

#### Collection of Urine Specimens

In view of the obvious difficulties associated with the clinical problems of mentally retarded and the 24-hr. urine collections, and in order to minimize some of the more obvious experimental complexities, a special study was devoted to the problem of specimen collection. There is considerable question as to the validity of using creatinine excretion as a reference in interpreting excretion of other metabolites (3). Urinary creatinine values vary appreciably from day to day in any individual owing to dietary variations and the fact that creatinine not cleared from the urinary collecting system on one day is added to that of the following day or days. For these reasons collection of random samples and determination of creatinine as a reference were discarded, and it was decided to obtain urine collected over a known period of time. A series of preliminary experiments were carried out in order to determine the time period for collection of urine which would be most convenient and during which it would be least affected by dietary variations. Collections were made overnight and every 4 hr. during the day for 2 different 72-hr. periods from 6 adults and 4 children. All samples were analyzed for 5-RU and N-methyl-2-pyridone-5-carboxamide (2-PY); in all cases, the overnight collection was found to contain the most consistent values for both metabolites. Although some variation does exist within an individual, the data indicate that the overnight 8- to 10-hr. urine collection represents what might be termed "basal output." It was also found to be most easily obtained without hospitalization, provided the child did not have nocturnal enuresis. Thus, all data represent urine samples collected as follows: The mother was
told to have the child urinate as usual just before bedtime, record the time, but make no collection. In the morning, the child urinated in the collection bottle and again the time was recorded. Precautions were taken to ensure that no urine was lost during collection. Thus, a complete volume of urine collected over a known period of time was obtained, and all determinations could be calculated as output per hour.

**Analyses**

Aliquots (2 ml.) of urine were filtered, partially dehydrated, and fractionated on a Dowex 1-formate, 2% DVB, 200–400 mesh column 50 cm. by 1 sq. cm. The elution was initiated with 1½ column volumes of water (Fig. 1) followed by 1 N formic acid gradient via 200 ml. water mixer. Peaks I, II, III, IV, and V were pooled and diluted with distilled water to 100 ml.; a 5-ml. aliquot was partially lyophilized and then applied to Whatman No. 1 filter paper. Whatman No. 1 filter paper contains large quantities of UV-absorbing materials which can be removed by layering the sheets of filter paper in 2 N acetic acid in a large, flat container and boiling for 1 hr. The paper is then washed several times in 95% ethyl alcohol and dried (4). Chromatograms were developed by ascending technic in n-butanol:water (86:14) solvent. In all instances the n-butanol:water solvent proved effective for the separation of 5-RU from other UV-absorbing components. Photographic records (Fig. 2) of the chromatograms were made by exposing the chromatograms over Scona reflex paper to an 8-w UV lamp with peak emission at 2537 Å. As is evident from UV spectrums of Compound

![Figure 1](image-url)
"V", the absorbance is near the UV region (2300 A), and thus it does not photograph well.

In order to resolve Compound A from creatinine, a double-layer exchange column was used without affecting the levels of 5-RU, 2-PY (Fig. 3), and uracil. A Dowex 1-formate, 2% DVB, 200–400 mesh column 4 cm. by 1 sq. cm. was packed in a lower layer, and a Dowex-50 cation-exchange column 4 cm. by 1 sq. cm. was packed directly on top of the Dowex 1 in the same manner as described in the preceding paragraph. Samples (2 ml.) of filtered and dehydrated urine were sorbed on the

Fig. 2. Paper chromatogram showing relative positions of unknown (V, Y, X, A, G) to known compounds and controls. Chromatogram was developed for 18 hr.
column, followed by 35 ml. of water. From the obtained eluate, 5-ml. aliquots were lyophilized and chromatographed as described. 5-RU (5) and 2-PY (6) were determined spectrophotometrically, whereas uric acid was analyzed on an AutoAnalyzer by Method N-13 b, adopted from a manual procedure (7).

Fig. 3. Ultraviolet absorption spectrums of N-methyl-2-pyridone-5-carboxamide.

Results and Discussion

Urine samples from 47 mentally retarded children, ages 5–10 and with IQ’s ranging from 10 to 79, were analyzed quantitatively for presence of 5-RU, uric acid, and 2-PY. In comparison with control values for 20 normal children in the same age range, in whom the 5-RU excretion averaged 50 µg./hr./lb. body weight, both boys and girls in the retarded group averaged 36 µg./hr. (Fig. 4). An interesting correlation between 5-RU and 2-PY was observed in that they paralleled each other in the level of decreased excretion (Fig. 5).

A variation in excretion of 5-RU was also observed when presented as a ratio of 5-RU to uric acid in some mentally retarded children (Fig. 6). It is possible that there might be an abnormality in renal filtration of this unusual nucleoside (5-RU), existence of an erroneous catabolic pathway for 5-RU, or a lack of C-C synthetase (8) or other enzyme responsible for the biosynthesis of 5-RU in t-RNA.
Fig. 4 (top). urinary excretion levels of pseudouridine compared to relative IQ values.

Fig. 5 (bottom). Urinary excretion levels of N-methyl-2-pyridone-carboxamide compared to relative IQ values.
During the course of another investigation being carried out in our laboratory in which urine samples of new born infants 3–6 days of age were analyzed, we observed complete absence of 2-PY in one male infant. It was subsequently discovered that the child suffered from a congenital condition associated with mental retardation caused by an abnormality in one of the chromosomes and referred to as the "cat cry" syndrome. Studies were interrupted owing to the death of the infant.

As a result of these findings, we investigated 10 adult patients diagnosed as schizophrenic at the Oregon Fairview Home. As may be seen in Table 1, output of both 5-RU and 2-PY is low, and they tend to parallel each other in the level of decreased excretion.

Thus, in a significantly large number of cases of various types of mental abnormalities, a decrease in urinary 5-RU and 2-PY has been observed. The UV-absorbing peaks obtained from exchange columns were found to contain 5-RU in Peak II (Fig. 1); uracil, Peak III; 2-PY, Peak IV; thymine and xanthine, Peak VIII; and uric acid, Peak X. In addition, other unknown UV-absorbing constituents of low molecular weight were isolated. Creatinine was found in Peaks I and II, Compound
Fig. 7. Ultraviolet absorption spectrum of unknown urinary Compound G.

Fig. 8. Ultraviolet absorption spectrum of unknown urinary Compound X.
Table 1. Excretion of Pseudouridine and N-Methyl-2-Pyridone-5-Carboxamide in Adult Schizophrenic Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr.)</th>
<th>Sex</th>
<th>Pseudouridine</th>
<th>N-Methyl-2-pyridone-5-carboxamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.C.</td>
<td>36</td>
<td>M</td>
<td>1.54</td>
<td>0.01</td>
</tr>
<tr>
<td>A.R.</td>
<td>21</td>
<td>M</td>
<td>3.02</td>
<td>0.02</td>
</tr>
<tr>
<td>C.D.</td>
<td>35</td>
<td>M</td>
<td>1.67</td>
<td>0.01</td>
</tr>
<tr>
<td>D.C.</td>
<td>20</td>
<td>M</td>
<td>2.05</td>
<td>0.01</td>
</tr>
<tr>
<td>R.S.</td>
<td>31</td>
<td>M</td>
<td>3.01</td>
<td>0.02</td>
</tr>
<tr>
<td>J.H.</td>
<td>40</td>
<td>F</td>
<td>0.47</td>
<td>0.004</td>
</tr>
<tr>
<td>M.W.</td>
<td>19</td>
<td>F</td>
<td>0.70</td>
<td>0.006</td>
</tr>
<tr>
<td>C.W.</td>
<td>48</td>
<td>M</td>
<td>0.80</td>
<td>0.004</td>
</tr>
<tr>
<td>C.S.</td>
<td>17</td>
<td>M</td>
<td>0.14</td>
<td>0.02</td>
</tr>
</tbody>
</table>

A was predominant in Peak IV, G in Peak V, X in Peak IV, Y in Peak XIII, and V in Peak IV. Semiquantitative evaluation of the unknown compounds showed variation in excretion levels or their absence.

Compound G (Fig. 7) was immobile in butanol:water solvent, whereas in butanol:water with ammonia gas, it migrated with an Rf of uridine, which is indicative of a positive charge. In butanol:acetic acid:water (5:2:3) it had an Rf of pseudouridine. The compound was found in human, bear, and rat urines, and we also isolated it from the germ of the coffee bean.

Compounds X (Fig. 8) and Y (Fig. 9) were found only in some mentally retarded and in two control subjects in trace amounts. Compound V (Fig. 10) absorbed near the UV spectrum, was extremely mobile and moved with the solvent front in butanol:water (86:14).

Compound A (Fig. 11) can be easily resolved from creatinine by use of a double-exchange column, and creatinine is then retained on Dowex 50. It is evident from UV spectrums that there is a certain similarity between Compound A and 5-acetylamino-6-amino-3-methyluracil isolated from urine by Fink et al. (9). However, chromatographic mobilities of the two compounds and the stability of Compound A in 0.1 N HCl, at 100° for 1 hr., indicate Compound A to be a different urinary derivative.

The depletion of 5-RU excretion may be due to low pyrimidine contents of the total RNA, or to low production of 5-RU-rich t-RNA. It is of the brain, wherein the catabolic contribution of 5-RU may be below worth considering also the possibility that there is depressed activity normal. The basis of the decreased excretion of the nicotinic acid derivative, 2-PY, and its correlation with decreased 5-RU excretion remains
Fig. 9. Ultraviolet absorption spectrum of unknown urinary Compound Y.

Fig. 10. Ultraviolet absorption spectrum of unknown urinary Compound V.
obscure. This problem is under investigation at the present time. Urine analyses on over 150 mentally retarded children, as well as on control subjects in each age range will have been carried out in the near future.

The possibility of the variations being caused by differences in dietary intake can definitely be ruled out. A complete dietary evaluation was made on each child, and no correlation was found between dietary intake and urinary excretion values. Many of the controls were normal siblings of the retarded child; thus both were on the same dietary regimen.

**Fig. 11.** Ultraviolet absorption spectrum of unknown urinary Compound A.
References