THE EXCRETION OF N-METHYL-2-PYRIDONE-5-CARBOXYLAMIDE BY MAN FOLLOWING INGESTION OF SEVERAL KNOWN OR POTENTIAL PRECURSORS*

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The development of a rapid method for the determination of N-methyl-2-pyridone-5-carboxamide (pyridone) in urine (1) has made it practical to attempt to confirm and extend the studies of Holman and de Lange (2) and others (3–5) concerning the excretion of the pyridone following the administration of various known or possible precursors to man. In the conversion of nicotinic acid to the pyridone it must be amidated, N'-methylated, and oxidized in the sixth position. All six possible derivatives of nicotinic acid involving these three modifications in the structure singly (6-hydroxynicotinic acid, nicotinamide, trigonelline) or in pairs (6-hydroxynicotinamide, N'-methylnicotinamide, N-methyl-2-pyridone 5 carboxylic acid) have now been administered to human subjects, and the results suggest that these reactions probably occur in the order indicated. In addition, the present studies determined the relationship between the dosages of the tested compounds and the extent to which they may be accounted for by increased excretion of the pyridone in the urine.

Materials and Methods

Subjects—Four male laboratory workers between the ages of 26 and 33 years were used for these studies. They were not known to have any chronic disease and had no history of serious metabolic, renal, or gastrointestinal disease.

Chemicals—The 6-hydroxynicotinic acid from commercial sources had a satisfactory melting point (6) and was used as obtained. Its acid chloride formed readily on refluxing with thionyl chloride for 2 hours. After removing the excess thionyl chloride by vacuum distillation, the pale yellow acid chloride was added slowly with stirring to an excess of cold concen-

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treated NH$_4$OH. After 3 hours at 0° the excess NH$_4$OH was removed at reduced pressure on a water bath at 35°. The light tan amide was collected by filtration and was recrystallized from water with decolorizing charcoal. The colorless 6-hydroxynicotinamide melted at 317.2–317.8° (uncorrected), while Bradlow and VanderWerf (6) reported a melting point of 313–314.4° (corrected).

To prepare N-methyl-2-pyridone-5-formamidoacetic acid, the powdered acid chloride of N-methyl-2-pyridone-5-carboxylic acid (7) was slowly added to the sodium salt of glycine, the aqueous solution being kept at pH 9 to 10 by addition of 2 N NaOH. The glycine conjugate precipitated on acidification and was recrystallized three times from hot water (yield 70 per cent). The product melted at 233–235° when heated at 1.5° per minute if placed in the block at 220°, and melted at 209° when mixed with N-methyl-2-pyridone-5-carboxylic acid. When chromatographed on paper with the system described by Dalgliesh (8), N-methyl-2-pyridone-5-formamidoacetic acid was revealed as a single dark spot (R$_f$ 0.58) on inspection with ultraviolet light, while N-methyl-2-pyridone-5-carboxylic acid appeared as a similar spot but with R$_f$ 0.75. With the same chromatographic system 6-hydroxynicotinic acid (R$_f$ 0.70) and 6-hydroxynicotinamide (R$_f$ 0.50) were also visible as absorbing spots.

Commercial quinolinic acid was recrystallized from 40 per cent glacial acetic acid. All other compounds were prepared as previously described (1) or were available commercially.

Pyridone Determinations—The daily excretion of the pyridone was determined as previously described (1). However, two points require further emphasis at this time. It has recently been noted that if the columns were run at a speed slower than that recommended (1) the recoveries of added pyridone were reduced in proportion to the length of time used. This suggested decomposition of the pyridone in the presence of the ion exchange resins. When a solution of the pyridone was shaken at 24° in a suspension of Dowex 50 (H$^+$) or Dowex 1 (OH$^-$) and aliquots of the suspension were analyzed at intervals for the pyridone, it was found that the pyridone slowly disappeared in the presence of Dowex 1 (OH$^-$). By 48 hours over 90 per cent of the pyridone had disappeared, but no significant loss occurred in the presence of Dowex 50 (H$^+$). In 1 hour there was no significant loss in the presence of Dowex 1 (OH$^-$); hence with proper operation of the columns this was no problem. Columns of 1.6 cm. outside diameter have been found to give results identical with those obtained with columns 1.2 cm. in diameter and have proved to be much easier to operate at the proper speed.

Secondly, Hunter and Handler (9) observed that precipitation of pro-

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teins from homogenates with trichloroacetic acid resulted in hydrolysis of the N-methyl-2-pyridone-5-carboxamide present to the corresponding acid. When protein was removed from urine with trichloroacetic acid, there was no detectable loss of pyridone (and therefore no hydrolysis since the corresponding acid would be retained by the analytical columns) (1). Further studies have confirmed these previous observations (1). Furthermore, it has been possible to recover 98 to 100 per cent of the pyridone when the pure compound was allowed to stand in 5 or 10 per cent trichloroacetic acid at 23–26° for as long as 1 week. Therefore, it appears unlikely that the difficulties experienced by Hunter and Handler (9) apply to the removal of protein from urine as previously described (1).

**EXPERIMENTAL**

The subjects were allowed free choice of diet, except that they were instructed to avoid ingestion of foods especially high in niacin, such as liver and nuts. Every practical effort was made to avoid dietary excesses or irregularities.

Each subject collected two or three 24 hour urines (1) and then ingested the compound under test as a single dose. The urine was then saved for four or five more 24 hour periods. At least two subjects were used for each dose of each compound.

With compounds which have not been studied with respect to acute or chronic toxicity, the larger doses were omitted, especially if the lower levels yielded conclusive results.

**Results**

The data obtained are summarized in Table I. When increasing amounts of nicotinamide were administered, there was an increased excretion of the pyridone, but the per cent of the administered dose accounted for as urinary pyridone gradually declined. Similar results were obtained with nicotinic acid, but in general the per cent conversion to the pyridone was lower, except at the smallest dosage. As the dose increased, longer periods were required for complete elimination of the additional pyridone, but even with the largest doses the daily pyridone excretion returned to normal levels after 3 or 4 days.

Administration of the pyridone itself resulted in essentially complete recovery of the administered dose except for the smallest one.

$N^1$-Methylnicotinamide administration was followed by increased excretion of the pyridone, but the larger doses caused no further increase. When 0.82 mmole was taken over 12 hours in twenty-four equal doses, the pyridone excretion increased considerably above the level observed when it was administered as a single dose.
TABLE I
Daily Urinary Excretion of Pyridone by Human Males before and after Administration of Several Known or Potential Precursors

The results represent the average data from the number of experiments listed in the first column. The average daily excretion of the pyridone for the 2 or 3 days before the supplementation was subtracted from the daily excretion following supplementation, and the total difference was recorded as the millimoles of supplement accounted for as urinary pyridone. This column and the last one were based on the assumption that an increased pyridone excretion was the result of conversion of the supplement to the pyridone and that no net increase in urinary pyridone was due to failure of conversion.

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Supplement*</th>
<th>Mg. pyridone excreted per 24 hrs.</th>
<th>mmoles supplement accounted for as urinary pyridone</th>
<th>Per cent converted to pyridone</th>
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<tr>
<td></td>
<td></td>
<td>No. of days before supplementation</td>
<td>No. of days after supplementation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<td>0.113</td>
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<td></td>
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<td>0.82</td>
<td>0.90</td>
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* MPC, N-methyl-2-pyridone-5-carboxylic acid; MPF, N-methyl-2-pyridone-5-formamidoacetic acid; DPN, diphosphopyridine nucleotide.
† Taken in twenty-four equal portions over a 12 hour period.
Nicotinuric acid administration was followed by a slight, but definite, increase in pyridone excretion. However, $N$-methyl-2-pyridone-5-carboxylic acid, its glycine conjugate, quinolinic acid, trigonelline, 6-hydroxynicotinic acid, and 6-hydroxynicotinamide did not lead to increased pyridone excretion.

Diphosphopyridine nucleotide was administered at only one dosage level to conserve material. It appeared to be about as effective as nicotinamide in increasing the pyridone excretion.

With the compounds that did not appear to give rise to increased pyridone excretion, only the results of the largest doses administered are recorded in Table I. In all cases, however, the smaller doses gave essentially the same negative results.

**DISCUSSION**

If one accepts these results in the broadest sense, namely, that increased excretion of the pyridone following the administration of a known or possible precursor indicates conversion to the pyridone and the absence of an increase indicates no metabolic conversion, these results suggest that in man there may be only one metabolic pathway from nicotinic acid to the pyridone. This conversion requires amidation, methylation, and oxidation, apparently in that order. Thus nicotinamide was converted to the pyridone, but methylated or oxidized nicotinic acids apparently were not so converted. Of the three possible derivatives of nicotinic acid, including combinations of two of these structural changes, only the $N'$-methylated amide led to an increase in pyridone excretion, while $N$-methyl-2-pyridone-5-carboxylic acid and 6-hydroxynicotinamide were inactive. The fact that $N'\text{-methyl}n\text{icotinamide was much less effective than nicotinamide as a precursor of the pyridone in vivo may have been due in part to rapid excretion of this metabolite. It has been shown by Beyer et al. (10) that there is a mechanism for rapid tubular elimination of $N'\text{-methyl}n\text{icotinamide in the dog. The increased excretion of pyridone which followed ingestion of the compound in small portions over a 12 hour period, compared with a single dose of the same quantity, would be compatible with such an explanation for the present results. Similar complications in the absorption or excretion of some of the other compounds may account for the results obtained in these studies.**

The nearly physiological doses of nicotinic acid and nicotinamide were about 80 per cent accounted for as urinary pyridone, while, with 20 times that dose, only 40 to 50 per cent was accounted for as this metabolite. These observations were in agreement with the results of Lin and Johnson (11) and Huff and Perlzweig (12) that very large doses of these compounds led to the urinary excretion of metabolites which could not be regarded as significant metabolites under normal conditions.
The percentage of precursor accounted for as urinary pyridone with the largest doses administered (3.28 mmoles of pyridone equal 500 mg.) was in excellent agreement with the data of Holman and de Lange (2) who administered 500 mg. doses of some of these compounds. In the average of two experiments by Holman and de Lange the following were accounted for to the extent of the percentage indicated: pyridone 77, nicotinic acid 40, nicotinamide 52, trigonelline 1, N'-methylnicotinamide 12, and N-methyl-2-pyridone-5-carboxylic acid 0. Holman (4) and Sarett (5) found very slight if any increase in pyridone excretion following ingestion of 1.73 to 23.1 mmoles of quinolinic acid.

The average basal values for urinary pyridone ranged from 18 to 20 mg. for the four subjects studied. These levels of pyridone excretion would indicate that all subjects were ingesting considerably more niacin than the minimal daily requirement. According to Goldsmith et al. (13), in a human subject receiving about 7 mg. of nicotinamide daily on a low tryptophan diet there was no detectable pyridone in the urine, but signs of niacin deficiency failed to develop. The addition of another 30 mg. of nicotinamide to the daily intake raised the pyridone excretion to 15.2 mg. daily, which was in the range observed in the present study. On the basis of the careful studies by Goldsmith et al. (13, 14) it seems safe to assume that the subjects used in this study had an adequate dietary intake of niacin and that any administered supplement represented an excess. This would probably reduce the tendency for retention of administered supplements.

The pyridone excretion returned to basal values in about the same period of time after the ingestion of nicotinamide, nicotinic acid, or the pyridone. This suggests that it was the rate of excretion of the pyridone and not its rate of formation that was the limiting factor in the return to the normal levels of excretion.

**SUMMARY**

1. The daily excretion of N-methyl-2-pyridone-5-carboxamide (pyridone) by human males before and after oral administration of several known or possible precursors has been determined.

2. The increased excretion of the pyridone following administration of 0.164 mmmoles of nicotinamide (20.0 mg.) or nicotinic acid accounted for about 80 per cent of the administered dose. Increasing the dose by 5, 10, or 20 times resulted in increased absolute amounts, but lowered the percentage of the dose accounted for as pyridone. Diphosphopyridine nucleotide appeared to be about as effective as nicotinamide in increasing the pyridone excretion.

3. The pyridone itself appeared to be excreted almost quantitatively.

4. Owing probably to rapid renal excretion, doses of 0.164, 0.41, or 0.82
mmole of \(N^1\)-methylnicotinamide all resulted in equal increases in pyridone excretion, while dividing the 0.82 mmole dose over a 12 hour period resulted in a 3-fold increase in per cent accounted for.

5. Nicotinuric acid was accounted for to the extent of 13 per cent as urinary pyridone.

6. N-Methyl-2-pyridone-5-carboxylic acid, its glycine conjugate, quinolinic acid, trigonelline, 6-hydroxynicotinic acid, and 6-hydroxynicotinamide failed to increase the excretion of the pyridone.

7. The data indicate that in its conversion to the pyridone nicotinic acid is probably amidated, methylated, and oxidized, in that order, since all the possible intermediates for other metabolic sequences of these three reactions failed to increase the pyridone excretion.

**BIBLIOGRAPHY**

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C. J. Walters, R. R. Brown, Masako Kaihara and J. M. Price