URINARY EXCRETION OF NICOTINIC ACID AND ITS DERIVATIVES BY NORMAL INDIVIDUALS*

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In the preceding paper (1) a study was made of the pyridine compounds found in urine in order to determine to what extent their excretion influences the chemically determined (2) urinary "nicotinic acid" values. Procedures were described for the estimation of nicotinuric acid and trigonelline in urine based upon the relative stabilities of these compounds to acid and alkaline hydrolysis. After prolonged acid hydrolysis nicotinuric acid is quantitatively converted to nicotinic acid and only after strong alkaline hydrolysis is trigonelline converted to a nicotinic acid-like reacting substance. The values obtained are reproducible and the procedures appear to be specific for these nicotinic acid derivatives. However, because of the admittedly semiquantitative character of the calculated figures from use of large conversion factors, only the total values obtained by direct chemical analyses are given in this paper.

Some pyridine compounds other than nicotinic acid and the above derivatives are included in the total urinary "nicotinic acid" values (1). Excretion of these pyridine compounds may mask differences between normal and deficient individuals with respect to the true nicotinic acid values of their urines. The evaluation of the extra excretion of nicotinic acid and its derivatives, after the

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‡ Upjohn Fellow in Clinical Research, 1938-40.
Normal Nicotinic Acid Excretion

oral administration of a test dose to subjects following a constant daily routine, offers a means for correcting for the initial lack of specificity of the chemical method. The present report deals with the range of values obtained in such studies conducted with normal individuals. Data are presented showing in what forms nicotinic acid is excreted in the urine, the prompt conversion of most of the nicotinic acid to these derivatives, and rapidity of excretion of the pyridine compounds after dosage. Differences in metabolic behavior of nicotinic acid and nicotinamide are also indicated.

EXPERIMENTAL

Response of Normal Individual to Oral Test Dose of Extra Nicotinic acid—Eleven adult subjects of varying size were used for this study. Selection of foods during the test period was left to the choice of the subject and represented what the individual generally ate. Smoking and coffee consumption were not restricted but were maintained constant during the test period.\(^1\) Three consecutive 24 hour urine samples were collected.\(^2\) Following the 24 hour basal period and just prior to the collection of the second sample, an aqueous solution of 500 mg. of nicotinic acid was taken orally at the completion of the largest meal of the day. The two subsequent 24 hour urine samples were collected. The results of the analyses of the three consecutive urine samples are given in Table I. Each sample was subject to three types of hydrolysis, as indicated. The increase in the nicotinic acid value after the prolonged period of acid hydrolysis is due to hydrolysis of nicotinuric acid and the increment after alkaline hydrolysis comes from the trigonelline fraction (1). The value for the extra urinary "nicotinic acid" excretion following the administration of the test dose was calculated by subtracting from the total figure, obtained for the first 24 hour period after dosage, the average of the values for the immediately preceding and following 24 hour periods.

Examination of the data in Table I shows that there is normally no excretion of nicotinuric acid in urine; this is indicated by no increase in the values obtained for the basal 24 hour urine samples.

\(^1\) This was necessary because of the influence of these factors upon the urinary "nicotinic acid" values (1).

\(^2\) The urine collection periods began in each case after dinner.
### TABLE I

**Urinary Excretion (in Mg.) of Nicotinic Acid and Its Derivatives by Normal Individuals before and after Oral Administration of 500 Mg. of Nicotinic Acid**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Weight</th>
<th>Height</th>
<th>Surface area</th>
<th>Urinary &quot;nicotinic acid&quot; values after hydrolysis</th>
<th>Excretion of oral dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>kg.</td>
<td>cm.</td>
<td>sq.m.</td>
<td>Per 24 hrs.</td>
<td>Per 1st 24 hrs.</td>
</tr>
<tr>
<td>E. W.</td>
<td>F.</td>
<td>53.5</td>
<td>161</td>
<td>1.55</td>
<td>2.3 2.3</td>
<td>18</td>
</tr>
<tr>
<td>O. B.§</td>
<td>M.</td>
<td>54</td>
<td>161</td>
<td>1.56</td>
<td>12.0 12.0</td>
<td>33</td>
</tr>
<tr>
<td>M. J.</td>
<td>F.</td>
<td>56</td>
<td>162</td>
<td>1.59</td>
<td>2.0 1.7</td>
<td>12</td>
</tr>
<tr>
<td>C. M.</td>
<td>M.</td>
<td>68</td>
<td>178</td>
<td>1.84</td>
<td>2.1 1.8</td>
<td>12</td>
</tr>
<tr>
<td>N. A.§</td>
<td>&quot;</td>
<td>71</td>
<td>174</td>
<td>1.85</td>
<td>16.4 16.5</td>
<td>29</td>
</tr>
<tr>
<td>A. M.</td>
<td>F.</td>
<td>72</td>
<td>169</td>
<td>1.82</td>
<td>2.0 1.9</td>
<td>3</td>
</tr>
<tr>
<td>W. R.§</td>
<td>M.</td>
<td>72.5</td>
<td>178</td>
<td>1.90</td>
<td>7.1 6.5</td>
<td>43</td>
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<tr>
<td>D. M.</td>
<td>&quot;</td>
<td>74</td>
<td>170</td>
<td>1.85</td>
<td>3.1 3.0</td>
<td>28</td>
</tr>
<tr>
<td>S. S.§</td>
<td>&quot;</td>
<td>75</td>
<td>178</td>
<td>1.91</td>
<td>5.1 4.4</td>
<td>30</td>
</tr>
<tr>
<td>H. H.§</td>
<td>&quot;</td>
<td>85</td>
<td>188</td>
<td>2.12</td>
<td>6.7 6.3</td>
<td>32</td>
</tr>
<tr>
<td>R. K.§</td>
<td>&quot;</td>
<td>86</td>
<td>187</td>
<td>2.12</td>
<td>29.3 28.1</td>
<td>71</td>
</tr>
</tbody>
</table>

* The test dose of nicotinic acid in aqueous solution was taken immediately after the largest meal of the day. The three consecutive 24 hour periods, during which the urine samples were collected, began in each case after dinner.
† The significance of the different types of hydrolysis, indicating which of the nicotinic acid derivatives is excreted, is explained in the text.
‡ These figures were calculated by subtracting from the total values, obtained for the first 24 hour period subsequent to the administration of the test dose, the averages of the values for the immediately preceding and following 24 hours.
§ These individuals are habitual smokers, smoking from twenty to thirty cigarettes daily.
when subjected to prolonged acid hydrolysis. Trigonelline is a normal constituent of urine; in each case a definitely larger value for the urinary excretion of nicotinic acid is obtained following the period of alkaline hydrolysis of the sample.\(^3\) The presence of trigonelline in urine is due to both passive excretion of the betaine following its consumption as such in the diet\(^4\) and that arising, as a detoxication measure, when large doses of nicotine (1, 3), nicotinic acid, and possibly other pyridine compounds are taken. The average increased urinary “nicotinic acid” value following alkaline hydrolysis of the basal urine samples in the series presented is 20 mg. per 24 hours. This represents a daily excretion of approximately 60 mg. of trigonelline (1). Values for the basal 24 hour urinary “nicotinic acid” values following acid hydrolysis vary widely, from 1.7 to 29.3 mg. The three samples yielding very large values were from smokers who excreted nicotine principally as the unchanged alkaloid (1, 4). Studies in which volatilization of the pyridine compounds by steam distillation of the urine samples at various pH values was employed have indicated that free pyridine is also present in some to an appreciable extent. There is a definite tendency for the values obtained during the basal periods to be less following prolonged acid hydrolysis compared with those obtained after the short period of acid hydrolysis. Repeated recovery experiments have shown that the decrease is due to volatilization or destruction of a urinary pyridine compound other than those studied ((1) Table I). It is thus apparent that the “nicotinic acid” values for the basal periods include pyridine compounds other than nicotinic acid. The true urinary nicotinic acid is believed to be in the neighborhood of the lower normal range.

\(^3\) Only subject A. M., one of the non-smokers and non-coffee consumers, failed to show the very large increment in the urinary “nicotinic acid” value following alkaline hydrolysis. The small increment in this case, however, is real; recovery experiments with trigonelline added to ½ hour aliquots of the same urine sample gave the usual 33 per cent conversion of the betaine to the nicotinic acid-like reacting substance.

\(^4\) When one of the standardized subjects (D. M., Table II) took a post-prandial oral test dose of trigonelline, equivalent to 350 mg. of nicotinic acid, the increase in the urinary nicotinic acid value occurred only in the sample subjected to alkaline hydrolysis. The increase represented an extra excretion of 135 mg. of trigonelline, all within the first 24 hours after dosage. (The trigonelline was furnished by General Biochemicals, Inc.)
The oral administration of the test dose of extra nicotinic acid is followed by a rapid and marked increase in the urinary “nicotinic acid” value. Within 24 hours after dosage the values for nicotinic acid and its derivatives in the urine are back to normal; the urinary values during the following 24 hour period are practically the same as the basal figures. In each case there is a marked urinary excretion of nicotinuric acid after the oral administration of the test dose (increase in values following prolonged acid hydrolysis). It has been shown (1) that this increment in the urinary “nicotinic acid” values represents only one-third of the nicotinuric acid present. Thus, it is readily noted that after dosage most of the extra urinary nicotinic acid excretion, determined after prolonged acid hydrolysis of the samples, is due to the presence of nicotinuric acid. Some individuals, such as N. A. and H. H., excreted practically all the nicotinic acid, so determined, as the glycine conjugate. When the urinary “nicotinic acid” values are very large, due to the intake of a relatively large dose of the compound by the smaller individuals, such as E. W. and O. B., or when the test dose is taken by the fasting subject (see Fig. 2), appreciable amounts of free nicotinic acid (or amide) are excreted. In each case, listed in Table I, there is a marked but variable excretion of extra trigonelline (increase in values following alkaline hydrolysis) after dosage. This increment should be multiplied by 3 for the estimation of the absolute excretion of the betaine (1).

The values listed for the extra excretion of nicotinic acid and its derivatives after the administration of the test dose show a tendency of the smaller individuals to excrete more of the pyridine compounds. However, individual variation in the handling of the test dose makes this correlation poor. The average values obtained in this study are 40.9 mg. of extra “nicotinic acid” following the short period of acid hydrolysis, 54.2 mg. after prolonged acid hydrolysis, and 73 mg. following alkaline hydrolysis. Calculations, based upon the fundamental concepts set forth in the preceding paper (1), indicate a total extra excretion of 110 mg.

This is due to the fact that nicotinuric acid, although not appreciably hydrolyzed during the short period of acid hydrolysis, reacts directly with the reagents to give a color the intensity of which is equal to 62 per cent of that obtained with an equivalent amount of nicotinic acid. The value obtained after prolonged acid hydrolysis, however, does include all the voided nicotinuric acid expressed as free nicotinic acid.
of nicotinic acid and derivatives, or 22 per cent of the test dose. 51 per cent of the increased urinary excretion of nicotinic acid is in the form of trigonelline, 36 per cent is in the form of nicotinuric acid, and only 13 per cent in the form of free nicotinic acid or amide.

Reproducibility of Values for 24 Hour Urinary Excretion of Nicotinic Acid and Derivatives before and after Administration of

<table>
<thead>
<tr>
<th>Values obtained after hydrolysis†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
</tr>
<tr>
<td>1 hr., 4 N HCl</td>
</tr>
<tr>
<td>2.1–3.4</td>
</tr>
<tr>
<td>1.6–3.9</td>
</tr>
<tr>
<td>2.4–5.5</td>
</tr>
<tr>
<td>5.3–8.6</td>
</tr>
<tr>
<td>24.3–30.2</td>
</tr>
</tbody>
</table>

* These were obtained when the diet furnished the sole intake of nicotinic acid and its derivatives.
† These samples were collected over a period of 6 months and were spaced at least 1 week apart.
‡ The significance of the different types of hydrolysis, indicating which of the nicotinic acid derivatives is excreted, is explained in the text.
§ These individuals are habitual smokers, smoking from twenty to thirty cigarettes daily.

Test Dose of Extra Nicotinic Acid—In Table II are summarized the results of the analyses of 53 basal 24 hour urine samples from five normal individuals. Each sample was subjected to the three types of hydrolysis, as indicated. The values, though extending over an appreciable range, are within definite limits characteristic for each of the subjects. Much of the variation noted is undoubtedly due to no attempt having been made by these individuals to follow a constant dietary régime during the urinary collection
periods. When the diet and daily routine are maintained constant, the urinary "nicotinic acid" values are reproducible (1). The average basal excretion values listed in Table II have been used to correct the total figures obtained when the same individuals received test doses of nicotinic acid and nicotinamide. The results have been plotted (Figs. 1 to 3) as extra urinary "nicotinic acid" excreted.

In Fig. 2 are presented the results for repeated determinations of the extra urinary excretion of nicotinic acid and its derivatives when the normal subject takes the 500 mg. test dose of nicotinic acid after eating. The average deviation of the two values in a set from its average figure is ±9 per cent, with a maximal deviation of ±18 per cent. These results indicate that the values obtained are sufficiently reproducible to make the data in Table I valid and allow significance to be attached to the differences in the urinary excretion of nicotinic acid when varying test doses are taken (Fig. 1), when the test dose is taken by the subject fasting rather than after eating, and when nicotinamide is taken in place of the free acid (Figs. 2 and 3).

Correlation between Urinary "Nicotinic Acid" Values and Size of Test Dose—The first 24 hour urine samples were collected from two normal subjects after they had received varying oral test doses of nicotinic acid after the largest meal of the day.6 The extra urinary excretions of nicotinic acid and derivatives are indicated in Fig. 1. Only small amounts of these pyridine compounds are excreted when the dose of extra nicotinic acid is 3.4 mg. per kilo of body weight. In these tests the extra urinary "nicotinic acid" values (with calculations made to convert the results obtained after hydrolysis into absolute quantities of the nicotinic acid derivatives) account for only 3 and 5 per cent of the test doses. With larger test doses there is not only an absolute increase in these urinary pyridine compounds but also a marked augmentation in the percentage of the test doses excreted. Thus, when the dose of extra nicotinic acid was 9.4 mg. per kilo of body weight, the urine values accounted for 25 and 14 per cent of the test dose.

Urinary Excretion of Nicotinic Acid and Its Derivatives When Test Dose Is Taken by Fasting Subject. Difference in Urinary

6 Approximately 10 days were allowed to lapse between each of the periods when the test doses were taken.
Excretion Values When Nicotinamide Is Taken—Test doses of nicotinic acid were administered orally to three of the normal subjects, but this time the solutions were taken 12 hours after dinner and 3 hours prior to breakfast. The results are plotted in Fig. 2. For comparative purposes the values obtained when these same subjects received the same test doses after eating are also presented. It will be observed that there is a marked increase in the extra urinary "nicotinic acid" values (from 100 to 300 per cent, when calculations are made to convert the values obtained after hydrolysis into absolute quantities of the nicotinic acid derivatives). The "flooding" effects, resulting from taking the test dose while fasting, are also apparent from the values
calculated to yield the per cent of total pyridines excreted as free nicotinic acid (or amide); 40 per cent in the present series compared with only 13 per cent when the test dose is taken after dinner.

When nicotinamide is taken by the same subjects under the same experimental conditions, the extra urinary "nicotinic acid" values are generally very much less than those obtained when

There is one exception in the series presented. In the case of D. M., the extra urinary "nicotinic acid" value, when calculations were made to convert the results obtained after hydrolysis into absolute quantities of the nicotinic acid derivatives, was appreciably greater following the use of nicotinamide as the postprandial test dose. This was due to the relatively much greater excretion of trigonelline.
nicotinic acid constitutes the test dose (Fig. 2). From 80 to 90 per cent of the extra nicotinic acid in the urine is calculated to be present as trigonelline. No appreciable difference in the urine values is obtained when the test dose of nicotinamide is taken by the subject after eating or while fasting.

Rapidity of Conversion of Nicotinic Acid into Nicotinuric Acid and Trigonelline Following Its Oral Administration, and Prompt Excretion of These Compounds—Fractional urine samples were collected during the first 24 hours after the oral administration of test doses of nicotinic acid or nicotinamide to the normal subject, W. R. (Fig. 2), and analyzed separately. The results are presented in Fig. 3. When the 500 mg. sample of nicotinic acid was taken orally by the subject, either before eating or while fasting, there was a prompt increase in the urinary "nicotinic acid" values. Practically all the excreted pyridines are voided within 4 hours after administration of the test dose; the maximal excretion is within the 1st hour. When the same quantity of nicotinamide is
taken as the test dose, a more gradual and much smaller excretion of nicotinic acid and its derivatives is observed. In all cases there occurs a prompt and continuous conversion of nicotinic acid to nicotinuric acid and trigonelline. The blood values associated with these urinary "nicotinic acid" figures are presented in Fig. 1 of the following paper (5). The only curve of urinary excretion which fails to parallel the blood values is that obtained following the administration of the oral test dose after eating. The reason for this is discussed elsewhere (5).

SUMMARY

Trigonelline is a normal constituent of urine; nicotinuric acid is not. The basal 24 hour urinary "nicotinic acid" values obtained in a study with eleven well nourished individuals varied from 1.7 to 29.3 mg. Because of the demonstrated non-specificity of the method the true nicotinic acid values are believed to be in the neighborhood of the lower normal range. In addition, the average excretion of nicotinic acid as trigonelline amounts to approximately 60 mg. per 24 hours. The oral postprandial administration of the test dose of 500 mg. of extra nicotinic acid is followed by a rapid and marked increase in the urinary "nicotinic acid" values, almost all of the excreted pyridine being voided within the first 4 hours. The maximal excretion occurs during the 1st hour after the test dose is taken. On the average 110 mg. (22 per cent) of the test dose of nicotinic acid are excreted, 51 per cent in the form of trigonelline, 36 per cent as nicotinuric acid, and 13 per cent as free nicotinic acid or amide. The percentage excretion of the test dose varies directly with the size of the dose. There is an increase of from 100 to 300 per cent in the extra urinary excretion of nicotinic acid and derivatives over that following postprandial doses when the nicotinic acid is taken while fasting. In either case there is a prompt and continuous conversion of nicotinic acid to nicotinuric acid and trigonelline.

When nicotinamide constitutes the test dose, the extra urinary "nicotinic acid" values are generally very much less and indicate no appreciable difference arising from the relation of dosage to the meal. Also, the rate of excretion of the nicotinic acid and derivatives is much more gradual, with trigonelline accounting for from 80 to 90 per cent of the excreted pyridine. The values before
and after the administration of the test doses are sufficiently reproducible to justify the conclusions drawn.

BIBLIOGRAPHY

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