Isoniazid Acetylation Rates (Phenotypes) of Patients being Treated for Tuberculosis*

ELIZABETH MASON 1 & D. W. RUSSELL 2

Isoniazid therapy could be used more rationally if patients' isoniazid acetylation rates were known. To explore the clinical usefulness of a recently described simple method for acetylator phenotyping, acetylated and free isoniazid were measured semiquantitatively in morning urine specimens from sanatorium in-patients being treated with the drug. The distribution of acetylisoniazid: isoniazid ratios was bimodal. The ratios thus identify rapid and slow acetylators of isoniazid, without disturbing their routine or their therapy. Ratios measured in samples from control subjects who had received a single dose of isoniazid did not discriminate between these phenotypes, and urine from diabetic patients gave false reactions.

Human genetic polymorphism in the rate of acetylation of isoniazid (INH) and some other drugs is well known (Evans & White, 1964; Goedde et al., 1964). The speed of INH acetylation is of clinical interest, since this substance sometimes causes peripheral neuritis, in particular in patients who acetylate it slowly (Hughes et al., 1954); although neurological symptoms can be prevented by simultaneous administration of pyridoxine, the cost of this vitamin hinders its routine use (Kalow, 1962). Furthermore, INH interferes with the metabolism of phenytoin, resulting in toxic concentrations of the latter in the blood of slow acetylators receiving normal therapeutic doses of both drugs (Kutt et al., 1970).

Probably the most widely used techniques for acetylator phenotyping are the different variants of the serum half-life method of Jenne (1960), well exemplified by the work of Tiitinen (1969a). All drugs are withheld for up to 36 hours, at which time INH is injected intravenously; blood samples for analysis are taken before injection and at timed intervals subsequently. Clearly, a method involving oral administration, followed by urine analysis, would be more convenient. One such method has been described (White & Evans, 1968), using sulfadimidine as the test drug, based on the fact that acetylation of both INH and sulfadimidine appears to be controlled by the same hepatic enzyme system. More recently a method was reported in which INH itself was used as the orally administered test drug. Volunteers in a steady state in respect of INH excreted acetylisoniazid (AcINH) and INH in a ratio (A : I) that was bimodally distributed and that could be simply and rapidly determined (Russell, 1970).

Clinically, the persons most likely to require acetylator phenotype determinations are tuberculosis patients being treated with INH. Since these patients are in a steady state in respect of the drug, it should be possible to determine their phenotypes, without disturbing their routine or their therapy, by measuring their urinary A : I. This paper reports the results of such determinations in sanatorium patients. As was the case with the volunteers, the A : Is were bimodally distributed and individually reproducible.

METHODS

INH and AcINH analyses were performed, except where otherwise stated, by the methods described previously (Russell, 1970) and outlined for convenience below. Urine samples were analysed as soon as possible after collection.
Analysis for acetylisoniazid

The method used was adapted from that of Venkataraman et al. (1968). Urine (4 drops) placed in a test-tube of internal diameter 0.8 cm is treated with 10% w/w potassium cyanide (4 drops), followed after mixing by the addition of 10% w/w chloramine-T (9 drops). At 1 min the contents are again mixed: 1 min later 0.2 ml of acetone is added. After remixing, the concentration of AcINH is determined by comparison with its standards in normal drug-free urine (320, 160, 80, 40, 20, and 0 μg/ml) that have been treated in the same way. Each sample is allotted a nominal AcINH concentration equal to that of the standard that it matches or immediately exceeds in depth of colour—e.g., a sample intermediate in colour between the 80 and 160 μg/ml standards is allotted a nominal concentration of 80 μg/ml. The actual colour varies between pink and orange, depending on the urine concentration; comparison is made with respect to depth of colour rather than hue.

Analysis for free isoniazid

The method used was adapted from that of Dymond & Russell (1970) for determining INH in blood. The sample (20 drops) is treated with 50% (w/w) K₂HPO₄ (10 drops) followed by a solution (1.25%) of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in 4-methyl 2-pentanone (isobutyl methyl ketone) (0.25 ml). The contents are mixed vigorously for 10 seconds, allowed to stand for 2 min, mixed again vigorously for 20 seconds, and briefly centrifuged. The INH concentration in the sample is determined by comparison with standards of INH in normal drug-free urine (64, 32, 16, 8, 4, 2, and 0 μg/ml) that have been treated in the same way. The orange colour due to INH is in the upper phase; the lower phase is ignored. To each sample a nominal INH concentration is allotted in the same way as for AcINH.

Notes on the methods

Aqueous reagents are measured from Pasteur pipettes held at an angle of approximately 45°. One pipette is used for all urine samples and standards and one for each aqueous reagent. For organic liquids a graduated pipette is used. Mixing is done with a Vortex Genie mixer.¹ For each sample a “nominal A: I ratio” is calculated from the nominal concentrations.

¹ Scientific Industries, Springfield, Mass., USA.

Patients

The patients, most of whom were of European stock, were receiving 300 mg of INH daily, in a single dose after breakfast, in 2 doses (after breakfast and the evening meal), or in 3 doses after meals. These regimens will be referred to as 1-300, 2-150, and 3-100 respectively, with the suffix ‘P’ if 4-amino-salicylic acid (PAS) was administered concurrently. Once a week, nurses at the Nova Scotia Sanatorium collected morning specimens of urine from approximately 50 randomly selected patients, not necessarily the same ones each week.

Series A (Jan.–Mar. 1970). In this preliminary study, AcINH was estimated according to the method of Eidus & Hamilton (1964), by the sanatorium laboratory staff. Results were scored on an arbitrary scale from 0 (negative) to 4 (strongly positive). INH was estimated by us, simultaneously but independently, using the same scale. Altogether, 92 of about 120 patients were tested on at least two occasions.

Series B (Apr.–May, 1970) included 35 series-A patients. INH and AcINH analyses (Russell, 1970) were performed by us. A total of 72 patients were tested on three or more occasions.

Volunteers

Thirty healthy volunteers each ingested one 300-mg dose of INH at bedtime and collected their urine during the next 10 h.

Results

False reactions

The possibility of false reactions in the INH test was investigated in urine samples from 50 healthy adults and from 24 patients without tuberculosis. The latter samples, of pH 5–9, contained protein (0–1 000 mg per 100 ml), a substance which by reacting with and removing TNBS might give rise to false negative reactions.

False positive reactions. Only one sample gave a positive reaction, and the donor of this was found to be taking INH prophylactically.

False negative reactions. INH (4 μg/ml) was added, and the sample was compared with a standard solution of the same concentration in drug-free urine: this amount of INH was detected in all 74 samples. Many samples from sanatorium patients
reacted positively for AcINH but contained no detectable INH. To all of 10 such samples in one week’s batch, INH was added (4 \( \mu g/ml \)): this amount was detected in all cases, indicating that the negative results were valid.

In the presence of large amounts of PAS alone the upper phase became bright yellow, with no trace of the orange-brown colour characteristic of INH. When INH (4 \( \mu g/ml \)) was added, this amount was detected by the test in all 6 PAS-containing samples. False, faintly positive reactions were obtained with some specimens of urine from diabetic patients.

**Sanatorium patients : Series A**

Of 148 samples tested, 82 gave positive reactions to tests for both INH and AcINH, and 37 were

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Reason for exclusion</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Receiving PAS, not INH</td>
<td>Tests for AcINH and INH consistently negative</td>
</tr>
<tr>
<td>5</td>
<td>Receiving neither INH nor PAS</td>
<td>Tests for AcINH and INH consistently negative</td>
</tr>
<tr>
<td>3</td>
<td>Incomplete dosage information available</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Patients had diabetes mellitus</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Infant</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Series-B patients who were tested 3 times and whose test results were excluded.**

![Fig. 1. Distribution of group number in 87 series-A patients receiving INH. A group number of 3 corresponds to a urinary A:I of approximately 10; lower values indicate A:I < 10 (slow acetylators), and higher ones indicate A:I > 10 (rapid acetylators). The group number is the difference between the sum of the AcINH and INH scores on the first two occasions of testing; e.g., a patient who scored 4 for the AcINH test on both occasions, and 2 and 1 for the INH test, would be assigned to score group (4 + 4) - (2 + 1) = 5.](image1)

![Fig. 2. Distribution of mean nominal A:I ratios in 66 series-B patients receiving INH. A value of < 10 identifies a slow acetylator, and > 10 a rapid acetylator.](image2)
Table 2. Distribution of mean nominal A: I in relation to therapy in series-B patients

<table>
<thead>
<tr>
<th>Regimen code (see text 1)</th>
<th>Mean nominal A: I</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>1–300</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2–150</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3–100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>1–300P</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>2–150P</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3–100P</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>A:I&gt;10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30(45.5%)</td>
<td></td>
</tr>
</tbody>
</table>

Table showing the distribution of mean nominal A: I in relation to therapy in series-B patients.

positive for AcINH only. The latter samples were invariably from patients for whom, it was later learned, INH had been prescribed.

Taken in conjunction, the results of the two tests could be used to divide the patients according to the difference between the scores for AcINH and INH. At the time this was established, 92 patients had been tested more than once. Reactions in both tests were negative in five who, it was found, were not receiving INH: these patients were excluded from this part of the study. To each of the remaining 87 patients a group number was assigned, equal to the difference between the sum of the AcINH scores and that of the INH scores obtained on the first two occasions of testing: these group numbers were distributed bimodally (Fig. 1). Check analyses, and scoring by known concentrations of AcINH and INH in drug-free urine, established that the antimode, which occurred at group number 3, corresponded to an A:I of approximately 10.1

**Sanatorium patients: Series B**

For this series, the arbitrary scoring method used in Series A was discarded and concentrations were determined by comparison with standards (Russell, 1970). Details of INH and PAS dosage were obtained from the sanatorium authorities after completion of the tests.

Urine samples were received from 102 patients, 80 of whom were tested on three or more occasions; 14 were excluded from the study (Table 1), and nominal A:I ratios were calculated for the remaining 66. Negative INH test results were recorded as 1 μg/ml if AcINH was detected. Nominal ratios were averaged, using the last three values if more than three tests had been performed, and the averages were rounded to the nearest nominal A:I ratio in the series. Two mean values (3.75 and 30) were ambiguous: they were arbitrarily set at 5 and 40, respectively.

Mean nominal A:I ratios were distributed bimodally, as shown in Fig. 2. Details of all results obtained are shown in Table 2. The occurrence of an antimode at A:I = approximately 10 was confirmed. As shown in the table, the position of the antimode was unaffected by simultaneous administration of PAS and was independent of the division of the daily dose of 300 mg of INH.

The reproducibility of results in series B is shown in Fig. 3. Thirty-eight patients were tested twice in series A (January–March) and three times in series B (April–May) also. Comparison of the results obtained in the two series (Fig. 4) showed no change in apparent phenotype.

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1 Arbitrary scores were found to correspond very roughly to a logarithmic series of concentrations for both AcINH and INH. It follows that the difference between the AcINH and INH scores, irrespective of the actual values, was roughly proportional to the ratio of their concentrations.
Fig. 3. The A: I determined on the first occasion of testing each of 66 series-B patients is compared with that patient's mean nominal value from three successive tests. Dosage regimens are identified as follows: open circles, 1–300; solid circles, 1–300P; open triangles, 2–150; solid triangles, 2–150P; open squares, 3–100; solid squares, 3–100P.

Fig. 4. Comparison of mean nominal A: I of 38 series-B patients with their group numbers previously obtained in series A.
Four series-A patients (three had been tested four times, and one had had identical INH and AcINH scores on two occasions) were phenotyped by the serum half-life method (Table 3) by Dr. L. Eidus, in Ottawa, without knowledge of our results. Individuals having values <110 min are classed as rapid acetylators (Tiitinen, 1969a). Two of the patients were later included in series B, after the conclusion of which the results obtained by Dr. Eidus were communicated to us.

Table 3. Nominal A : I ratios and acetylator phenotypes of four patients

<table>
<thead>
<tr>
<th>Half-life of INH in serum (min)</th>
<th>INH and AcINH test results in urine</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series A</td>
<td>Series B</td>
</tr>
<tr>
<td>Score group</td>
<td>Nominal A : I</td>
<td>&lt;10; &lt;10</td>
</tr>
<tr>
<td>166</td>
<td>1 : 2</td>
<td>&lt;10; &lt;10</td>
</tr>
<tr>
<td>105</td>
<td>6</td>
<td>&gt;10</td>
</tr>
<tr>
<td>246</td>
<td>2 : 1</td>
<td>&lt;10; &lt;10</td>
</tr>
<tr>
<td>98</td>
<td>5 : 7</td>
<td>&gt;10; &gt;10</td>
</tr>
</tbody>
</table>

*a* A serum half-life <110 min indicates a rapid acetylator.

*b* A urinary steady-state A : I >10 indicates a rapid acetylator.

There were four diabetics in series B. Three were taking INH, which was detected in their urine. The fourth was receiving neither PAS nor INH, but three tests gave a faint positive reaction for the latter; AcINH was not detected. A similarly false positive reaction occurred in one of two samples from the one diabetic patient in series A. Therefore, test results of these five patients were excluded.

Distribution of nominal A : I ratios was unimodal in the 10-h collections of urine from the 30 volunteers given one 300-mg dose of INH (Fig. 5).

**DISCUSSION**

Previously it was shown that, under defined INH-dosage conditions, an individual’s acetylator phenotype could be simply determined by semiquantitative analysis of a morning urine sample for AcINH and INH (Russell, 1970). A prerequisite for using the method is clearly that steady-state concentrations of the two compounds be attained or at least approached; for, in the present study, analysis of the urine excreted after a single dose of INH gave results that did not permit distinction between the two phenotypes.

Once a steady state has been achieved, individual differences in A : I are very marked. Even in series A, when concentrations of AcINH and INH were assessed subjectively, two groups were clearly distinguished. In no case were these results contradicted by the more objective results of series B, or by the results of independent acetylator phenotyping by an established method. A total of 115 individual patients were tested in the two series. For 6 of these the mean value of A : I fell at the antimode. Of the remaining 109, 51 (46.8%) had values greater than 10. This is very close to the proportion of rapid acetylators (45%) quoted recently (Hanngren et al., 1970) for 784 Caucasians in the USA and Canada. We conclude that even under sanatorium conditions, where many patients are receiving a variety of other drugs, the steady-state urinary A : I is a reliable indicator of acetylator phenotype. The phenotypic difference in this ratio is apparently so great as to obscure the reported effects of PAS on INH metabolism (Tiitinen, 1969b; Hanngren et al., 1970). These effects may have altered the true antimodal ratio, but not enough to affect the mean nominal value measured in this study.
The results reported here show that it is possible, with minimal apparatus and little disturbance of routine, to determine the acetylator phenotype of most patients who are taking INH regularly. The information may be useful clinically, to aid the choice of a rational dosage regimen for INH that would minimize its side-effects, to help avoid therapeutic incompatibilities such as phenytoin intoxication, and to identify those patients for whom pyridoxine supplements may be required. On general grounds it is preferable to carry out the determination before therapy with other drugs is begun, but as shown in the present study this restriction is not essential for identifying the phenotype. Where facilities are adequate, more strictly quantitative analytical methods, such as those of Venkataramanan et al. (1968) for AcINH and Russell (1971) for INH, may be preferred; again, the present results show that such a high degree of analytical precision is not essential.

To account for the weak false positive INH results obtained with urine from diabetics, we must assume that they excrete some unidentified amine or hydrazine derivative in abnormally large amounts. It is not known whether this material is normally present in the urine of diabetics or whether in these cases it was a drug metabolite. The INH method cannot be recommended at present for use in cases of diabetes mellitus.

The results obtained in series A show that the acetylator phenotype can be determined without using INH and AcINH standards. However, if an arbitrary scoring method is used, the position of the antimode must be separately established by each observer. We therefore strongly recommend that standards be used as described.

ACKNOWLEDGEMENTS

Much of this work was carried out at the Nova Scotia Sanatorium, Kentville, N.S.: we are greatly indebted to Dr H. N. Holden, medical director, Dr G. A. Kloss, staff physician, and Miss Helen Morse and her laboratory staff. We thank Dr L. Eidus of the National Reference Centre for Tuberculosis, Ottawa, for performing the serum half-life determinations; Miss M. Jakin of this laboratory, for technical assistance; and Mr C. L. Cousins of the Nova Scotia Provincial Pathology Institute for selecting pathological urine specimens.

RÉSUMÉ

TAUX D'ACÉTYLATION DE L'ISONIAZIDE (PHÉNOTYPES) CHEZ DES MALADES TRAITÉS POUR TUBERCULOSE

Une méthode simple de dosage semi-quantitatif de l'isoniazide (INH) dans l'urine, basée sur l'emploi de l'acide trinitro-2, 4, 6 benzénesulfonique, a fait l'objet de contrôles destinés à déceler d'éventuelles fausses réactions. On n'a constaté aucune réaction faussement positive avec 49 échantillons d'urine recueillis chez des volontaires bien portants, 24 échantillons fournis par des malades non tuberculeux et 6 échantillons (provenant de sujets normaux) auxquels on avait ajouté 3 mg/ml d'acide para-aminosalicylique. Après addition à ces divers échantillons de 4 µg/ml d'INH, la méthode a permis de retrouver la quantité de produit introduite dans tous les cas.

Ce test, et un autre permettant de doser l'acétylie-soniazide (AcINH), ont été appliqués sur des échantillons d'urine recueillis chez des patients hospitalisés pour tuberculose. Les échantillons fournis par des malades non traités par l'INH ont donné des résultats négatifs dans les deux épreuves, à l'exception de deux d'entre eux, provenant de malades diabétiques, où l'on a observé une réaction faussement positive pour l'INH. Les échantillons fournis par des malades traités par l'INH ont donné des résultats positifs dans les deux épreuves, à l'exception de deux d'entre eux, provenant de malades diabétiques, où l'on a observé une réaction faussement positive pour l'INH.
tillons d'urine recueillis chez 30 volontaires bien portants après absorption d'une dose unique d'INH.

Chez 6 de 115 malades recevant de l'INH et étudiés dans les deux séries, le rapport A : I était égal à 10. Chez les autres, on mesurait dans 46,8 % des cas un rapport A : I > 10 (acétylateurs rapides) et dans 53,2 % des cas un rapport A : I < 10 (acétylateurs lents). Le phénotype a été confirmé chez 4 malades par détermination de la demi-vie de l'INH dans le sérum.

L'application de ces deux épreuves permet de calculer les rapports A : I chez des malades non diabétiques traités par l'INH avec une précision suffisante pour distinguer, dans la plupart des cas, les acétylateurs rapides et les acétylateurs lents. Les tests n'exigent qu'un appareillage sommaire et ne provoquent aucun désagrément pour les malades. Ce procédé simple de détermination du phénotype pourrait permettre une approche plus rationnelle du traitement de la tuberculose par l'INH.

REFERENCES


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