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Classification Of Subjects As Slow Or Rapid Inactivators Of Isoniazid, Based On The Ratio Of The Urinary Excretion Of Acetylisoniazid To Isoniazid*

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Following an intramuscular injection of isoniazid 3 mg/kg body-weight, the urinary excretion of isoniazid and acetylisoniazid during the periods 0-1, 1-2, 2-3 and 3-4 h was determined for 124 patients with pulmonary tuberculosis. On the same occasion, the serum isoniazid concentration at 4½ h was determined by microbiologic assay. The ratios of acetylisoniazid to isoniazid in the urine collections at 2, 3 and 4 h. were bimodally distributed. Rules were derived from these. ratios for classifying subjects as slow or rapid inactivators of isoniazid. There was 100% agreement between the classification based on each of these ratios and that based on the serum isoniazid concentration at 4½ h.

Introduction

With the advent of intermittent regimens in the chemotherapy of tuberculosis, the rate of inactivation of isoniazid has attained considerable importance (Tuberculosis Chemotherapy Centre, Madras 1970). This rate is usually determined by estimating the concentration of isoniazid in plasma or serum after oral, intramuscular or intravenous administration of the drug. The present paper describes a simpler method requiring the collection of a single urine specimen after an intramuscular injection of isoniazid. The method is based on the finding that slow inactivators of isoniazid excrete a larger proportion of the dose in the free form and a smaller proportion as acetylisoniazid (and isonicotinic acid) in the urine, than do rapid inactivators (Armstrong and Peart 1960, Mukoyama **et al** 1963, Peters **et al** 1965, Short 1962, Tiitinen 1969). The efficiency of this (urine) method was assessed in parallel with that of a standard method based on microbiological assay of isoniazid in serum.

Material and Methods

Patients : In all, 124 patients with newly-diagnosed pulmonary tuberculosis, the latter part of an intake of 241 to a chemotherapy study, were included in this investigation.

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Collection of specimens : On the day of the test, a specimen of urine was collected from the patient and tested for acetylisoniazid by the method of Eidus and Hamilton (1964). If the result was negative, the patient was given an intramuscular injection of isoniazid, 3 mg/kg body-weight. Urine collections were made for the periods 0-l, 1-2, 2-3 and 3-4 h (and the volumes noted), and blood taken at 4½ h. Urine and serum specimens were then stored at –20°C for periods not exceeding one week.

Estimation of isoniazid in serum : The concentration of isoniazid in serum was estimated microbiologically, using a vertical diffusion method similar to that described by Lloyd and Mitchison (1964) (The lower limit for the sensitivity of this method is 0.2 µg/ml). It took 2 weeks for the result to become available.

Estimation of isoniazid aud acetylisoniazid in urine : Estimations of isoniazid and acetylisoniazid were undertaken on each urine collection. All the urine collections in each week were examined at a single session, after coding and rearranging them in a random order.

Isoniazid : Isoniazid was extracted by the method of Eidus and Little (1962) and was estimated by the method of Maher **et al** (1957) with minor modifications. In brief, 3 ml of urine was first mixed with 1 ml of 0.1 N sodium hydroxide solution and 3.2 g of powdered ammonium sulphate, and then shaken together with 30 ml of a solvent mixture containing chloroform and **n-** butanol (7 : 3) for 30 min in a rotary shaker. Next, 20 ml of the solvent extract was shaken for 15 min with 4 ml of 0.1N sulphuric acid. A 3 ml aliquot of the resulting acid extract was then treated with 0.3 ml of a 2 % vanillin solution in 25 % ethanol, and the colour intensity read at 380 mµ in a Unicam SP 600 spectrophotometer. The concentrations of isoniazid were read from a standard graph prepared with known concentrations of isoniazid.

Acetylisoniazid : Acetylisoniazid was estimated by the method of Venkataraman **et al** (1968), without treatment with potassium permanganate, and expressed as equivalent of isoniazid.

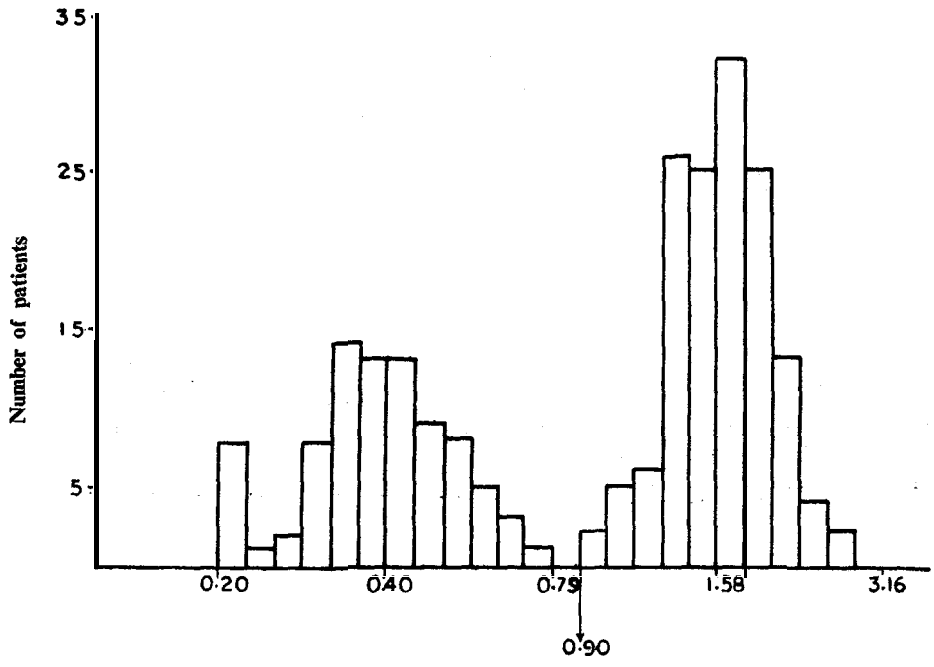
Results

Serum isoniazid concentrations : The distribution of patients according to the serum isoniazid concentration at 4½ h after an intramuscular dose of 3 mg/kg is set out in Graph 1. On the basis of this histogram, patients with a concentration of 0.90 µg/ml or more were classified as slow inactivators of isoniazid, and those with a concentration of 0.89 µg/ml or less as rapid inactivators. Thus, 67 (54 %) of the 124 patients in the present investigation were slow inactivators and 57 (46 %) were rapid inactivators.

Ratio of urinary excretion of acetylisoniazid to isoniazid : The ratios of the urinary excretion of acetylisoniazid to isoniazid at various intervals after an intramuscular injection of isoniazid 3 mg/kg are illustrated on a logarithmic scale in Graphs 2 and 3. The logarithmic transformation was employed, with success, to equalize the **error variance** (that is, the variance between duplicate estimations of the ratio) in the rapid inactivators and the slow inactivators.

Graph 1

Distribution of 241 patients according to the serum isoniazid concentration, 4½ h after an intramuscular dose of 3 mg/kg body-weight



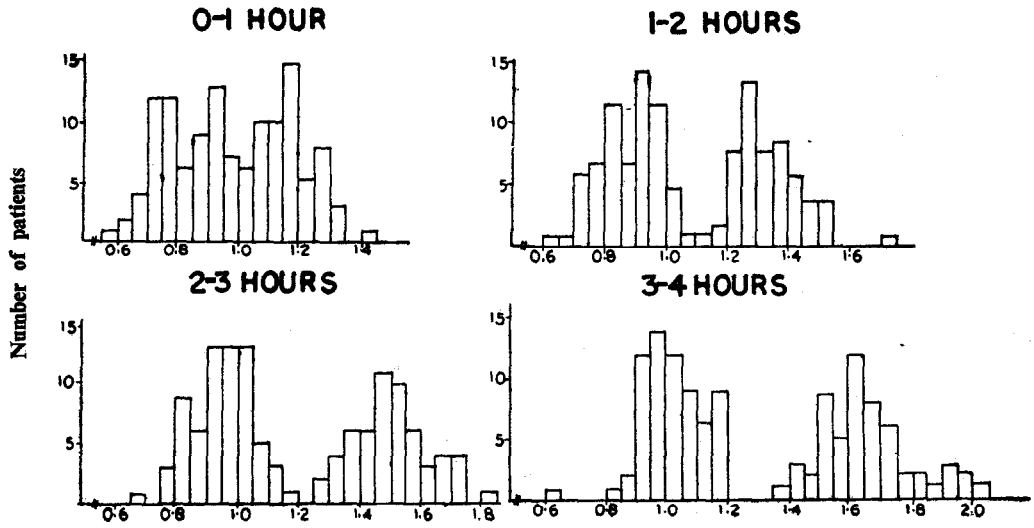
Isoniazid concentration (µg/ml)
The concentration was < 0.20 µg/ml in 16 patients

The ratio in the first hour collection failed to separate the patients into two distinct groups (Graph 2) and is therefore not considered further in this report. Considering the ratios for the periods 1-2, 2-3 and 3-4 h (Graph 2), and for O-2 h (Graph 3), the distributions clearly indicate the presence of two distinct groups of patients – namely, the slow inactivators (left side) and the rapid inactivators (right side). The geometric mean ratio and the range for the two groups of patients are presented in Table I, together with the criterion for classifying a patient as slow inactivator. It will be seen that the critical ratio increases with increasing period after the test dose. The classification based on each of the urinary ratios (1-2, 2-3, 3-4, 0-2 h) and that based on the standard microbiologic method employing serum were identical for every patient.

The last column of the Table provides an index of the efficiency with which each of the urinary ratios discriminates between slow and rapid inactivators of isoniazid –the larger the index, the more efficient is the discrimination. The indices for the ratios at 2-3 and 3-4 h are similar, and were of the same order as the index (untabulated) obtained with the standard microbiologic method on the same set of patients.

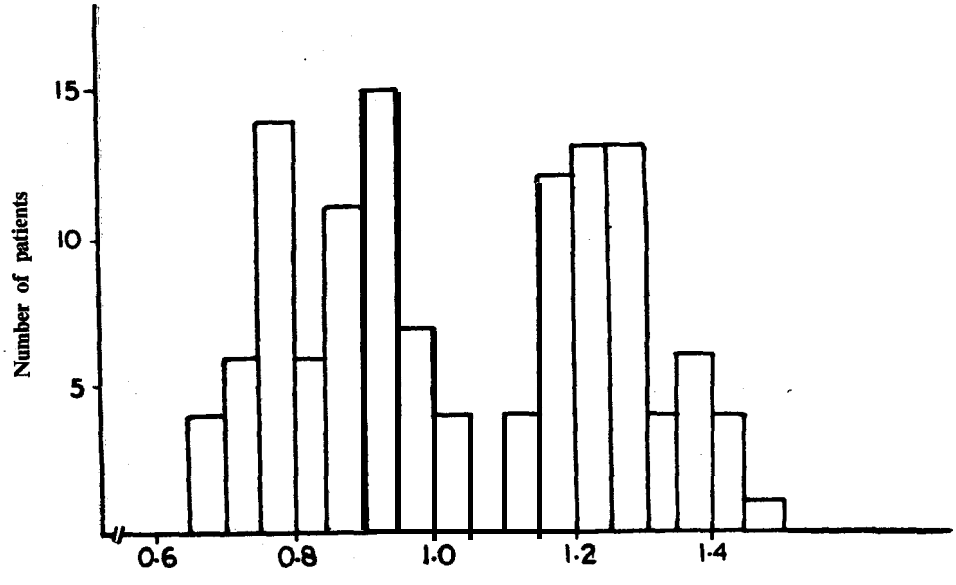
Graph 2

Distributions of patients according to the ratio of acetylisoniazid to isoniazid in urine for the periods 0-l, 1-2, 2-3 and 3-4 h



Graph 3

Distribution of patients according to the ratio of acetylisoniazid to isoniazid in urine for the period 0-2 h



It will also be noticed that the indices for the ratios at 1-2 and 0-2 h are slightly smaller than the indices for the ratios at 2-3 and 3-4 h.

Table I. Ratio of urinary excretion of acetylisonlazid to isoniazid after an intramuscular dose of isoniazid 3 mg/kg body-weight

Period (hours) after test dose	Slow inactivators		Rapid inactivators		Criterion for slow inactivator	Index of discrimination*
	Mean	Range	Mean	Range		
1 - 2	0.74	0.44–1.11	2.19	1.36–5.45	<1.26	24.1
2 - 3	0.89	0.47–1.40	3.16	1.82–6.32	<1.58	27.4
3 - 4	1.02	0.43–1.56	4.47	2.48–10.76	<2.00	27.8
0 - 2	0.71	0.45–1.09	1.78	1.30–2.94	< 1.12	24.2

*The difference in means between slow and rapid inactivators, divided by the standard error of the difference

Heterogeneity among rapid inactivators : Graph 4 correlates the serum isoniazid concentration at 4½ h with the ratio of the urinary excretion of acetylisoniazid to isoniazid for the period 3-4 h. The scattergram demonstrates a clear distinction between the slow inactivators and the rapid inactivators. Also, within the rapid inactivators, it is possible to identify 8 patients (marked as x), all of whom had very low serum isoniazid concentrations (<0.2 µg/ml) **and** very high urinary ratios (> 6.3). All eight patients are included in the group of 9 patients (also marked as x) who can be identified from Graph 5 as having a high ratio for the period 2-3 h (>4.0) **and** for the period 3-4 h (>6.3). Finally, the ratios for the period 1-2 h for these eight patients were all higher than the corresponding ratios for the remaining patients. These findings demonstrate that there was heterogeneity among the rapid inactivators, and that there were probably 8 or 9 homozygous rapid inactivators in the population examined in the present study.

Stability of isoniazid and acetylisoniazid in urine : From each of 27 patients (14 slow inactivators, 13 rapid inactivators), collections were made of the total urine excreted during the period 24-26 h after an oral dose of a slow-release preparation of isoniazid (35 mg/kg) or soluble isoniazid (15 mg/kg), and estimations of isoniazid and acetylisoniazid were undertaken. After adding a crystal of thymol as a preservative, aliquots of the 27 specimens were stored at 37°C and the estimations repeated at 2, 4, 7, 10 and 14 days. The mean isoniazid content for the 27 patients showed a significant linear decline ($P< 10^{-5}$), the reduction being 8 % by 7 days and 15 % by 14 days (Table II). In contrast, the mean acetylisoniazid content did not appear to be affected by the storage. Further analyses, not tabulated here, showed that the classification as slow or rapid inactivator was not altered for any patient as a result of the loss in isoniazid content from storage.

Graph 4

Correlation between serum isoniazid concentration at 4½ h and ratio of acetylisoniazid to isoniazid in urine for the period 3-4 h

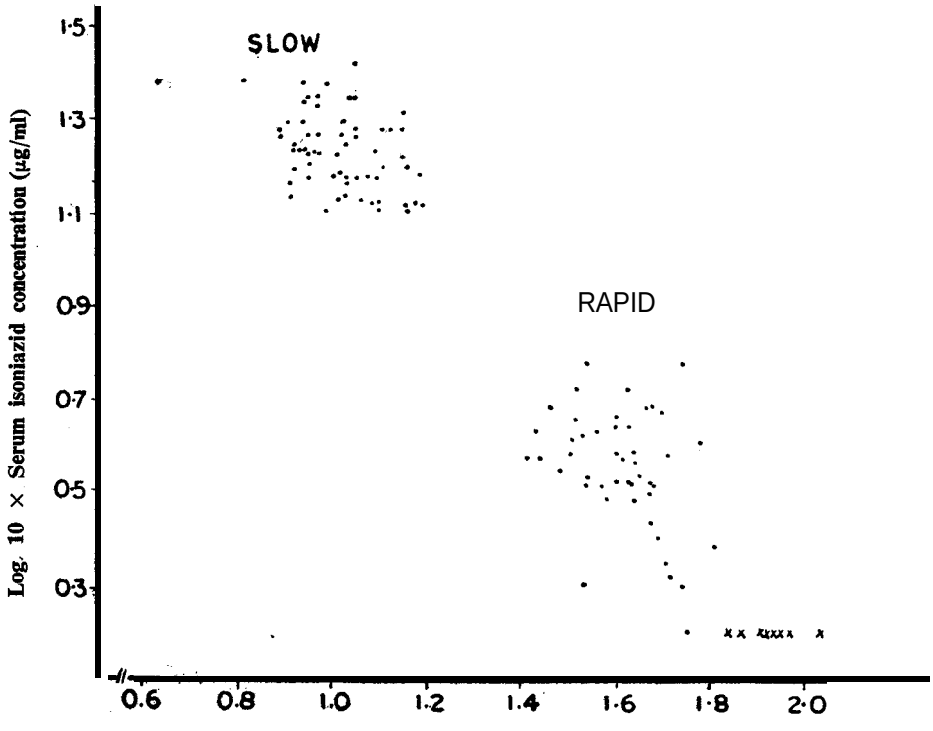


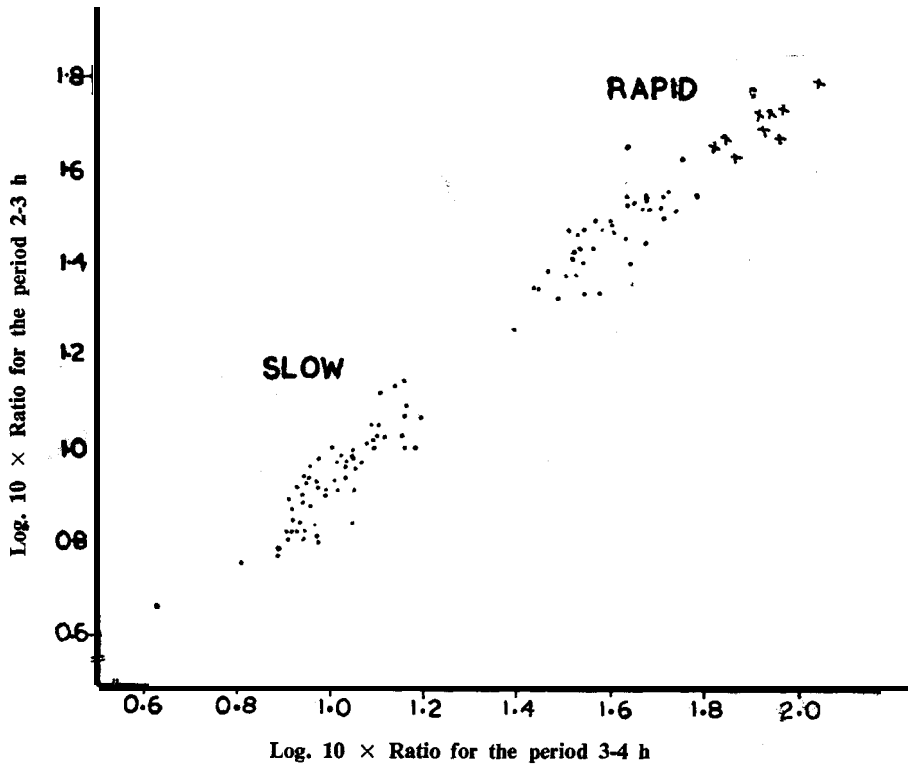
Table II. Effect of storage at 37°C on isoniazid and acetylisoniazid in urine*

Duration of storage (days)	Isoniazid (µg/ml)	Acetylisoniazid (µg/ml)
0	22.8	119.1
2	23.2	116.3
4	22.5	109.9
7	21.0	109.9
10	19.8	117.7
14	19.4	116.1

*Mean of the values for 27 samples

Graph 5

Correlation between the value-s of the ratio of acetylisoniazid to isoniazid in urine for the periods 2-3 and 3-4 h



Discussion

The methods commonly used for determining the rate of inactivation of isoniazid consist in measuring the plasma/serum concentrations of the drug 6 h after oral dosage (Evans **et al** 1960, 1961, Mukoyama **et al** 1963, Sunahara **et al** 1961), the serum ealf-life of the drug after intravenous or intramuscular injection (Jenne 1960, Scott **et al** 1969, Tiitinen 1969), or the serum isoniazid concentration 4½ h after intramuscular injection (Gangadharam **et al** 1961, Tuberculosis Chemotherapy Centre, Madras 1970). The isoniazid inactivation rate can also be determined by measuring the percentage of acetylated sulphadimidine in plasma/blood or urine 6-8 h after an oral dose of sulphadimidine (Evans 1969, Rao **et al** 1970).

This paper describes a method for classifying subjects as slow or rapid inactivators by measuring the ratio of the **urinary** excretion of acetylisoniazid to isoniazid for the periods 0-2, 1-2, 2-3 or 3-4 h after an intramuscular dose of isoniazid 3 mg/kg body-weight. There was 100 % agreement between the classification based on each of the

above ratios and that based on a standard microbiologic method employing serum. A further study of the efficiency of each of these ratios in discriminating between slow and rapid inactivators (Table I) suggested that a collection of urine over the period 0-2 h would be very satisfactory ; also, that no major extra benefit would be derived *(a)* by asking the subject to empty his bladder at 1 h and collecting only the urine excreted during 1-2 h, or *(b)* by collecting the urine excreted during 2-3 or 3-4 h.

The above method of classification, based on the ratio of acetylisoniazid to isoniazid in a 0-2 h collection of urine, has several advantages. First, it does not require the collection of blood, unlike many of the earlier methods. Secondly, the period of waiting is reduced to 2 h for the subject, and the test result can be made available the same day. Lastly, since the classification is based on a ratio, the accuracy of the method is unlikely to be affected by the failure to obtain a complete urine collection. A disadvantage of the method, however, is that the test dose has to be administered by intramuscular injection. Another limitation, especially under field conditions, is the instability of isoniazid in urine. In the present study, storage at 37°C reduced the isoniazid content by 8 % in 7 days and by 15 % in 14 days, despite the addition of a crystal of thymol as a preservative. However, this did not alter the classification (as slow or rapid) in any instance.

It is difficult to differentiate clearly between homozygous and heterozygous rapid inactivators of isoniazid on the basis of the isoniazid half-life after an oral or intramuscular dose of the drug (Scott **et al** 1969). In the present study, scattergrams relating the ratio of acetylisoniazid to isoniazid in urine for the period 3-4 h with the corresponding ratio for the period 2-3 h and with the serum isoniazid concentration at 4½ h suggested the presence of a group of 8 or 9 homozygous rapid inactivators. It is of interest that, according to the Hardy-Weinberg law, the expected number of homozygous rapid inactivators in the population under study is 8.

Since the completion of this study, a method has been described by Russell (1970) for classifying subjects as slow or rapid inactivators by measuring, semi-quantitatively, the ratio of acetylisoniazid to isoniazid in a specimen of morning urine obtained after oral dosage with 300 mg of isoniazid in 3 divided doses (after meals) on the previous day. Although such a method might be technically convenient, it is unlikely to be very practicable under field conditions. More recently, Eidus **et al** (1971) have described a method similar to ours, but collecting the urine over the period 6-8 h after an intramuscular test dose of isoniazid 8 mg/kg.

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