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Determination of Acetylator Phenotype Based on the Ratio of Acetylisoniazid to Isoniazid in Urine Following an Oral Dose of Ordinary Isoniazid

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A simple method for classifying subjects as slow or rapid inactivators of isoniazid has been described. A uniform dose of 300 mg. of ordinary isoniazid was administered orally to 150 patients with pulmonary tuberculosis. The ratio of acetylisoniazid to isoniazid was determined in urine collected at hourly intervals from 4 to 8 h. At each hour the distribution of the ratios was clearly bimodal. The test based on the 5-6 h. urine collection is recommended for its convenience and excellent discrimination between slow and rapid inactivators. The agreement between this method and a 'standard method was of the order of 97 per cent.

Introduction

Phenotyping of tuberculous patients as slow or rapid inactivators of isoniazid is of considerable importance when patients are treated with once-weekly isoniazid-containing regimens (Tuberculosis Chemotherapy Centre, Madras, 1970, 1973; Tripathy, 1974). The methods usually employed for classification purposes involve the administration of an intramuscular or intravenous test-dose of isoniazid followed by the determination of the serum isoniazid concentration or the ratio of acetylisoniazid to isoniazid in urine (Tuberculosis Chemotherapy Centre, Madras, 1970, 1973; Eidus **et al.,** 1971; Venkataraman **et al,.,** 1972; Ellard **et al.,** 1973b). A test consisting of an oral dose that is independent of body-weight and requiring only urine collection would have obvious practical advantages, especially under field conditions. This paper reports on the efficiency of a classification procedure based on the ratio of acetylisoniazid to isoniazid (A/I ratio) in urine following a uniform dose of 300 mg. of isoniazid, and compares its discriminating ability with that of a standard procedure.

Material and Methods

All the investigations were undertaken in patients with pulmonary tuberculosis receiving ambulatory treatment at this Centre.

Pilot study: A pilot study was undertaken in 14 known slow and 11 known rapid inactivators. (Their isoniazid inactivation status had been determined earlier by administering an intramuscular dose of isoniazid 3 mg/kg. body weight and estimating the serum isoniazid concentration at 4½h. (Tuberculosis Chemotherapy Centre, Madras, 1973) and the A/I ratio in a 3-4 h. urine collection (Venkataraman **et al.,** 1972). (A uniform oral test-dose of 300 mg. of ordinary isoniazid was administered on an empty stomach, and the A/I ratios were determined in urine collected over the periods 2-3, 3-4, 4-5 and 5-6 h.

Main study : The main study with the oral test dose of isoniazid 300 mg., was undertaken in 150 consecutive patients admitted to treatment at this Centre. In the light of the findings in the pilot study, A/I ratios were determined in urine collected over the periods 4-5, 5-6, 6-7 and 7-8 h.

The inactivation status of these patients was also determined by an established method, namely estimation of the A/I ratio in a 3-4 h. urine collection following an intramuscular dose of isoniazid 3 mg./kg.; the analytical procedure of Venkataraman **et al.** (1972) was employed on the first 22 patients and that of Raghupati Sarma **et al.** (1974) in the remaining 128 patients.

Influence of food : In order to determine whether the A/I ratios in urine obtained after the oral test-dose of isoniazid are affected by the ingestion of food, a cross-over study was undertaken in 12 known slow inactivators and 12 known rapid inactivators. The classification was based on the serum isoniazid concentration at 4½ h. (Tuberculosis Chemotherapy Centre, Madras, 1973) and the A/I ratio in a 3-4 h. urine collection (Raghupati Sarma **et al.,** 1974) following an intramuscular test-dose of isoniazid 3 mg./kg.). These patients received an oral test-dose, together with breakfast on one occasion and on an empty stomach on another occasion (breakfast was given 2 h. later), the sequence being determined by random allocation. (Breakfast consisted of prepared cereal and provided approximately 15 g. of protein, 15 g. of fat and. 125 g. of carbohydrate with a total intake of about 700 cals.) The A/I ratios were determined in urine collected over the periods 2-3, 3-4, 4-5, 5-6, 6-7 and 7-8 h.

Conduct of the investigation : On the day of the test, a specimen of urine was tested for the presence of acetylisoniazid (Eidus and Hamilton, 1964). After verifying 'that the result was negative, the patient was administered, under supervision, 300 mg. of ordinary isoniazid. Urine collections were obtained over specified periods (see above), stored at −20°C. for periods not exceeding one week, then randomised and the A/I ratios determined (Raghupati Sarma **et al.,** 1974).

Results

Pilot study : The results of the pilot study are presented in Table I. At each period, there was a clear-cut gap between the A/I ratios for the known slow and the known rapid inactivators, and the. mean A/I ratio was substantially higher for the latter. There was some evidence that the index of discrimination increased slightly with the time of urine collection (last column).

Table I. Ratio of acetylisoniazid to isoniazid in urine following an oral dose of isoniazid 300 mg. in 14 known slow and 11 known rapid inactivators of isoniazid.

Period (h after test dose)	A/I ratio in known slow inactivators *		A/I ratio in known rapid inactivators*		Index of discrimination†
	Mean (geometric)	Range	Mean (geometric)	Range	
1 – 3	0.34	0.24 – 0.57	1.60	0.98 – 4.37	11.3
3 – 4	0.41	0.31 – 0.64	2.29	1.10 – 5.77	11.9
4 – 5	0.48	0.30 – 0.83	3.10	1.64 – 6.86	12.5
5 - 6	0.58	0.34 – 0.86	4.37	2.68 – 8.73	13.7

*Classification based on the ratio of acetylisoniazid to isoniazid in urine collected over the period 3-4 h. and the serum isoniazid, concentration at 4½ h., following an intramuscular test-dose of isoniazid 3 mg./kg. body weight.

†The difference between means in slow and rapid inactivators (after logarithmic transformation), divided by the standard error of the difference.

Main study : The distributions according to the A/I ratios in urine collected over various periods are illustrated on a logarithmic scale, in the form of histograms (Graph). All the distributions are clearly bimodal, indicating the presence of two distinct groups of patients namely, the slow and the rapid inactivators. On the basis of these histograms, the criterion for a rapid inactivator was chosen to be 1.60 or more with the 4-5 h. urine collection, 2.00 or more with the 5-6 h. collection and 2.50 or more with the 6-7 and 7-8 h. collections. Of the 150 patients, 95 were classified as slow inactivators and 53 as rapid inactivators by all four criteria. The remaining 2 patients were classified as slow inactivators by the A/I ratios at 4-5, 5-6 and 6-7 h. and as rapid by the ratio at 7-8 h.

As in the Pilot study, the mean ratio for the rapid inactivators was considerably higher than that for the slow inactivators at all 4 periods (Table II). Again, the discrimination appeared to be slightly better with the later hour collections (last column).

Further analyses showed that there was no association between the patient's body-weight and the A/I ratio in urine at any of the four periods. For example, considering them 5-6 h. collection, the mean ratios were 1.33, 1.76, 1.50, 1.40 and 1.39 in patients weighing less than 35.0 kg., 35.0-39.9, 40.0-44.9, 45.0-49.9 and 50.0 kg. or more, respectively ($P>0.2$).

Comparison with the standard method : The agreement between the oral test-dose method and the standard method in the classification of patients as slow or rapid inactivators was excellent (Table III). Thus, with the 4-5, 5-6 and 6-7 h. collections, both the oral test and the standard test classified 93 patients as slow and 52 as rapid, that is, there was agreement in 145 (97 per cent) of the 150 patients examined. With the 7-8 h. collection, the corresponding figure was 147 (98 per cent).

Table II. Ratio of acetylisoniazid to isoniazid in urine following a oral dose of isoniazid 300 mg. in 150 consecutive patients admitted to treatment.

Period (h.) after test-dose	A/I ratio criterion for rapid inactivator	Mean (geometric) A/I ratio		Index of discrimination†
		Slow inactivators*	Rapid inactivators*	
4 – 5	> 1.60	0.61	3.81	28.4
5 – 6	> 2.00	0·74	5.53	28.4
6 – 7	> 2.50	0·81	6.95	30.0
7 – 8	> 2.50	0·91	8.10	31.4

* Classification baaed on the findings with the oral dose of isoniazid, employing the criterion in the second column.

† The difference between means in slow and rapid inactivators (after logarithmic transformation). divided by the standard error of the difference.

Table III. Classification of patients as slow or rapid inactivators of isoniazid by the oral test-dose method and a standard intramuscular test-dose method.

Classification based on standard method	Classification based on the oral test-dose method				Total patients
	4-5, 5-6 or 6-7 h. collection		7-8 h. collection		
	Slow	Rapid	Slow	Rapid	
Slow	93	1	93	1	94
Rapid	4	52	2	54	56
Total patients	97	53	95	55	150

For the comparison in terms of the index of discrimination, the analysis were restricted to 128 patients for whom the same methods of estimation of acetylisoniazid and isoniazid were employed with both the tests. The index of discrimination with the oral test-dose (26.6, 26.3, 27.5 and 27.9 with the 4-5, 5-6, 6-7 and 7-8 h. collections appeared to be better than the index with the standard method (22.6).

Influence of food : In rapid inactivators, the mean A/I ratio was consistently lower when the oral test-dose was administered with food than without (Table IV). The differences were appreciably larger with the later h. urine collections and attained statistical significance at 5-6 h. (P=0.05), 6-7h. (P=0.001) and 7-8 h. (P=0.01). As a result of this interference of food on the A/I ratio, one rapid inactivator was misclassified as slow at 4-5, 5-6, 6-7 and 7-8 h.

In slow inactivators also, the mean ratio with food was lower than without, but only with the 6-7 h. collection (P=0.01) and the 7-8 h. collection (P=0.2).

Graph

Distributions of 150 patients according to the ratio of acetylisoniazid to isoniazid in urine for the periods 4-5, 5-6, 6-7 and 7-8 h. after an oral dose of isoniazid 300 mg.

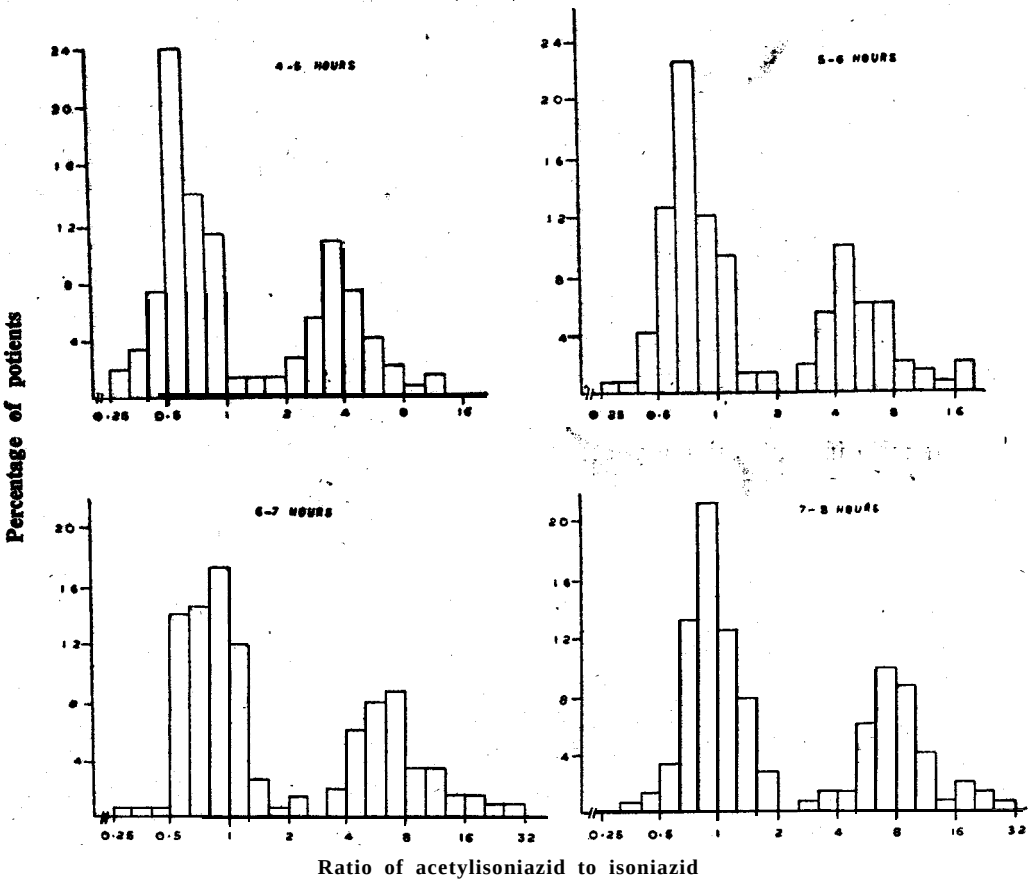

Table IV. Influence of food on the ratio of acetylisonlazid to isoniazid in urine following the oral test-dose of isoniazid 300 mg.

	Mode of administration of oral test-dose	Mean (geometric) A/I ratio in urine collected over the following periods					
		2-3 h.	3-4 h.	4-5 h.	5-6 h.	6-7 h.	7-8 h.
Rapid inactivators	With food	1.78	2.40	3.34	4.63	5.27	5.97
	On an empty stomach*	2.03	2.52	3.62	5.26	6.75	7.84
Slow inactivators	With food.	0.35	0.39	0.49	0.59	0.61	0.66
	On an empty stomach	0.35	0.40	0.47	0.59	0.69	0.72

*Food was given 2 h. after the test-dose.

Discussion

The findings in this paper demonstrate that the test using an oral dose of 300 mg. of ordinary isoniazid, regardless of body-weight, and subsequent determination of the A/I ratio in urine offers a simple and satisfactory method for classifying patients as slow or rapid inactivators. This method compares favourably with an established procedure based on intramuscular administration of a weight-graded dose of isoniazid. It was also observed that there-was no association between the A/I ratio with the oral test-dose and the body-weight of the patient in the weight range tested (25-55 kg.). However, when the test-dose was administered with food, the A/I ratios obtained in the later hour urine. collections were lowered, probably due to the delayed absorption of a major portion of the drug. This could lead to misclassification of a few rapid inactivators as slow inactivators, and hence it would be advisable to administer the drug on an empty stomach. As regards-the timing of urine collection, a 5-6 h. collection would probably be optimal, taking into account both discrimination (Graph) and convenience,

A procedure has been recently evolved at this Centre for the determination of the A/I ratio in urine using a photo-electric calorimeter (Raghupati Sarma **et al.,** in preparation). Coupling oral administration of a uniform dose of ordinary isoniazid 300 mg. with this modified procedure would offer an even-simpler method for determining the rate of inactivation of isoniazid even under the conditions of tuberculosis control programmes in developing countries.

Russel (1970) and, more recently, Eidus and Hodgkin (1973) have described simple tests involving oral administration of isoniazid and determination of the A/I ratio in urine for phenotyping patients as slow or rapid inactivators of isoniazid. In the procedure recommended by Russel (1970), the onus is on the patient to self-administer isoniazid in divided doses- during the previous day (the last dose to be taken at least 2 h. before bed time), to empty the bladder before retiring and to provide a specimen of urine voided on waking up. There would be serious difficulties in applying this method under conditions that exist in developing countries. The method of Eidus and Hodgkin (1973) involved oral administration of a weight-related dose of isoniazid (10 mg./kg.) and collection of urine over the period 6-8 h.

Oral administration of sulphadimidine and subsequent determination of the percentage of acetylated sulphadimidine (Rao **et al.,** 1970) or the ratio of acetylsulphadimidine to free sulphadimidine (Viznerova **et al.,** 1973) in urine has been shown to yield excellent results in the classification of patients as slow or rapid inactivators of isoniazid. Sulphadimidine was given in the form of a suspension and the dosages were weight dependent; however, the method has the advantage that both sulphadimidine and acetylsulphadimidine are heat stable and the estimations are simple.

A highly satisfactory method for classifying patients as slow or rapid inactivators from the A/I ratio in urine at 24-26 h. following the oral administration of a slow-release ‘preparation of isoniazid, matrix isoniazid, has been reported by Ellard **et al.,**

(1973a) and Kailasam et al. (1975). While slow-release preparations of isoniazid are not available commercially and the dosages employed were rather large, tests using these preparations have the advantage that the results are not affected by the influence of food on the rates of absorption of isoniazid from gastrointestinal tract.

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