

# A sensitive urine-test method for monitoring the ingestion of isoniazid

G. A. ELLARD AND C. GREENFIELD

*From the MRC Unit for Laboratory Studies of Tuberculosis, Royal Postgraduate Medical School, London, W12 and the Medical School, University of Newcastle-upon-Tyne*

**SUMMARY** A method is described for monitoring the ingestion of isoniazid based on detecting its metabolites, isonicotinic acid, and isonicotinylglycine in the urine. The sensitivity of the method is high so that reliably positive results were obtained up to about 24 hours after the ingestion of 100 mg isoniazid. The method should facilitate monitoring the taking of isoniazid by tuberculosis patients and the use of isoniazid as a marker for assessing the regularity with which other drugs prescribed for self-administration are actually ingested.

Studies carried out in several countries have demonstrated that drug regimens that are highly successful in the treatment of pulmonary tuberculosis in controlled clinical trials often fail when used routinely in mass treatment because patients fail to self-administer their drugs regularly (Fox, 1968, 1972; Kent *et al.*, 1970). Several simple qualitative urine-test methods have been described to enable the ingestion of isoniazid, the most effective and widely used antituberculosis drug, to be monitored. The most satisfactory method at present available is that described by Eidus and Hamilton (1964) for detecting the metabolite acetylisoniazid in the urine. This method has been used to monitor the ingestion of isoniazid in several controlled clinical trials (East African/British Medical Research Council Fifth Thiacetazone Investigation, 1970; Tuberculosis Chemotherapy Centre, Madras, 1970; Singapore Tuberculosis Services/Brompton Hospital/British Medical Research Council Investigation, 1971; WHO Collaborating Centre for Tuberculosis Chemotherapy, Prague, 1971; British Medical Research Council Co-operative Study, 1973). In two studies patients with higher proportions of negative urine tests were shown to have had a poorer response to chemotherapy (Hong Kong Tuberculosis Treatment Services/British Medical Research Council, 1972; Tuberculosis Chemotherapy Centre, Madras, 1973).

The sensitivity of the Eidus and Hamilton (1964) acetylisoniazid method is such that reliably positive results are obtained for only about 12 hours after

the ingestion of doses of 200-400 mg isoniazid (Rao *et al.*, 1967; Venkataraman *et al.*, 1965). This restricts the application of the method. Thus, in order to obtain reliable evidence of the regularity of the self-administration of isoniazid by tuberculosis outpatients in East Africa, it was found necessary to restrict the application of the acetylisoniazid method to urine samples collected from two to 10 hours of the stated times of isoniazid ingestion (personal communication, Dr J. F. Heffernan). This report describes a more sensitive method for monitoring isoniazid ingestion which should enable it to be applied to urine samples collected from about one to 24 hours from patients self-administering daily therapeutic doses of isoniazid (300 mg). It might also permit the use of substantially smaller doses of isoniazid to be used as a marker to monitor the self-administration of other drugs.

## Methods

The new urine-test, hereafter referred to as the 'isonicotinic acid method', is based on detecting the isoniazid metabolites isonicotinic acid and isonicotinylglycine by a procedure simplified from that previously described for their quantitative determination (Ellard *et al.*, 1972). Urine samples (0.5 ml), which had been preserved by the addition of a crystal of thymol, were pipetted into small test tubes together with 0.2 ml 4 M pH 5.0 acetate buffer and reacted by the addition at 15-second intervals of 0.1 ml 10% aqueous potassium cyanide, 0.1 ml 10% aqueous chloramine-T, and 0.5 ml 1% barbituric acid in acetone/water (1:1 by volume). A positive

result was indicated by the appearance of a blue colour within 30 minutes. All reagents were freshly prepared. The whole procedure was carried out in a fume-cabinet and, by using automatic pipettes, samples could be reacted in batches of up to 16.

In order to evaluate the sensitivity of the isonicotinic acid method and to compare it with the acetylisoniazid method of Eidus and Hamilton (1964), urine samples were collected from 56 volunteers before ingestion of 50 mg isoniazid and further samples were then collected 23-24 hours and 47-48 hours later. The subjects then swallowed a 100 mg dose of isoniazid and further urine samples were collected 23-24 hours and 47-48 hours later.

Known concentrations of isoniazid metabolites were also added to a duplicate set of portions of the 56 pretreatment urine samples. Since isonicotinic acid and isonicotinylglycine give purple and blue colours, respectively, when reacted by the isonicotinic acid method, and since similar amounts of both compounds are excreted in the urine after ingestion of isoniazid (Ellard and Gammon, 1976), equal amounts of the two compounds were added to the pretreatment urine samples so that the shades of the colour obtained would match those of the post-treatment urine samples as closely as possible. The final concentrations of added isonicotinic acid and isonicotinylglycine were 0.5, 0.75, 1, 1.5 or 2  $\mu\text{g/ml}$ . In order to evaluate the sensitivity of the acetylisoniazid method, acetylisoniazid was added to portions of the 56 pretreatment urine samples to give final concentrations of 10, 15, 20, 30 or 40  $\mu\text{g/ml}$ . All the 366 urine samples (56 pretreatment, 56 with added metabolites, and 224 post-treatment) were then coded and randomised before testing by both the isonicotinic acid and acetylisoniazid methods.

The reacted urine samples were read by six observers. Three of the observers read the first half of the reacted samples directly without reference to an appropriate standard, grading the results as either positive (blue for the isonicotinic acid method or pink for the acetylisoniazid method), negative, or doubtful. The observers then read the second half of the samples by comparison with reacted standards, samples being considered positive if their colour appeared to be deeper than that given by 0.5  $\mu\text{g/ml}$  isonicotinic acid plus 0.5  $\mu\text{g/ml}$  isonicotinylglycine, or 15  $\mu\text{g/ml}$  acetylisoniazid in normal urine, respectively. The reverse procedure was followed by the other three observers, the first half of the samples being read against a standard and the second half without. Thus for each of the 366 urine samples tested, six readings were obtained, three direct and three standardised. Urine samples were then classified positive if they were read as positive by at least

two of the three observers in any given procedure.

## Results

In order to obtain an estimate of the relative concentrations of the metabolites in the urine samples, pools were prepared by mixing equal volumes of the four sets of urine samples that had been collected 24 or 48 hours after dosage with 50 or 100 mg isoniazid. The concentrations of isonicotinic acid and isonicotinylglycine were then estimated colorimetrically, while acetylisoniazid was determined fluorimetrically (Ellard *et al.*, 1972). In the pool prepared from the urine samples collected 24 hours after dosage with 100 mg isoniazid the concentrations of these three metabolites were about 4, 4, and 7  $\mu\text{g/ml}$ , respectively. By 48 hours the concentrations of the three metabolites had fallen to about one-seventh of their 24-hour values. The concentrations of the metabolites excreted after dosage with 50 mg isoniazid averaged about 40% of those after 100 mg of the drug.

The percentages of urine samples classified as positive using the isonicotinic acid and acetylisoniazid methods are summarised in Tables 1 and 2. The specificity of each method was excellent, not a single false-positive result being obtained. Very similar results were obtained with the isonicotinic acid method whether the samples were read directly without a standard or by comparison with a standard containing 0.5  $\mu\text{g/ml}$  isonicotinic acid plus 0.5  $\mu\text{g/ml}$  isonicotinylglycine (Table 1). With the acetylisoniazid method a smaller proportion of the samples were classified as positive when read against a standard containing 15  $\mu\text{g/ml}$  acetylisoniazid. Whether or not a standard was employed, the sensitivity of the acetylisoniazid procedure was inadequate for monitoring the ingestion of either 50 or 100 mg isoniazid utilising urine samples collected at 24 hours. Thus only five (13%) of the samples obtained 24 hours after dosage with 100 mg isoniazid were classified as positive and by 48 hours all the samples were negative. By contrast, approximately 90% of the urine samples obtained 24 hours after ingestion of 50 mg isoniazid were classified as positive using the isonicotinic acid method, and after giving 100 mg isoniazid the proportion of positive results rose to 95%. Furthermore, about a third of the samples obtained 48 hours after ingestion of 100 mg isoniazid gave positive results.

The proportions of positive results given by the pretreatment urines to which various concentrations of metabolites had been added are summarised in Table 2. The results shown are those that were obtained when the tests were read directly without a standard for comparison. Using the isonicotinic acid

Table 1 Percentages of positive readings when urine samples were reacted by the isonicotinic acid and acetylisoniazid urine-test methods

Urine samples		Isonicotinic acid method		Acetylisoniazid method	
		Direct	Standardised	Direct	Standardised
Pretreatment		0	0	0	0
Isoniazid dose (mg)	Interval after dose (hr)				
50	23-24	88	93	2	0
	47-48	11	13	2	2
100	23-24	96	95	13	5
	47-48	34	30	0	0

Each group consisted of 56 samples.

Table 2 Proportions of positive readings given by pretreatment urine samples containing added isonicotinic acid plus isonicotinylglycine or acetylisoniazid

No. of samples	Isonicotinic acid method			Acetylisoniazid method		
	Added metabolites ( $\mu\text{g/ml}$ )	Positive	%	Added metabolites ( $\mu\text{g/ml}$ )	Positive	%
56	0	0	0	0	0	0
12	0.5	11	92	10	3	25
11	0.75	11	100	15	7	64
11	1.0	11	100	20	8	73
11	1.5	11	100	30	9	82
11	2.0	11	100	40	10	91

method positive readings were given by all but one of the samples containing  $0.5 \mu\text{g/ml}$  isonicotinic acid plus  $0.5 \mu\text{g/ml}$  isonicotinylglycine, and by all of the samples containing  $0.75 \mu\text{g/ml}$  or more of these two metabolites. By contrast, using the acetylisoniazid method only about a quarter of the samples containing  $10 \mu\text{g/ml}$  acetylisoniazid were classified as positive and relatively reliably positive results were obtained only with samples containing 30 or 40  $\mu\text{g/ml}$  acetylisoniazid.

The observers found the colours obtained using the isonicotinic acid method with moderately or weakly positive urine samples (blue/green) easier to distinguish than the orange/pink colours given by the acetylisoniazid method. Differences were noted between the proportions of urine samples designated as positive by the six different observers, and these differences were somewhat reduced when the results were read against the reacted isonicotinic acid plus isonicotinylglycine or acetylisoniazid standards. However, there did not appear to be significant differences between the observers in their ability to discriminate between pretreatment urine samples and those containing isoniazid metabolites. It was therefore concluded that the benefit gained by reading test samples against a reacted standard would be outweighed in normal practice by the complication of having to set up the standard, particularly since isonicotinylglycine is not readily available. Furthermore, in most situations a single observer

would be likely to read all the urine-tests from a given investigation.

## Discussion

Although the new isonicotinic acid method is slightly more elaborate than the Eidus and Hamilton (1964) acetylisoniazid procedure, a single person could use it to test between two and three hundred urine samples per day. A comparison of the results set out in Table 2 indicated that the new isonicotinic acid method could detect concentrations of isonicotinic acid and isonicotinylglycine about 80 times less than the acetylisoniazid concentrations detected by the Eidus and Hamilton method. From the relative amounts of isonicotinic acid, isonicotinylglycine, and acetylisoniazid excreted after ingestion of isoniazid in the current study it was calculated that the isonicotinic acid method should be capable of detecting the ingestion of isoniazid doses approximately 40 times smaller than those detectable by the acetylisoniazid method. The new isonicotinic acid method, which reliably detected concentrations of  $0.75 \mu\text{g/ml}$  isonicotinic acid and isonicotinylglycine in the urine, is also much more sensitive than the former method of Kasik *et al.* (1962) which was only capable of detecting concentrations down to  $10\text{--}25 \mu\text{g/ml}$  isonicotinic acid.

The sensitivity of the new isonicotinic acid method should therefore permit greater flexibility in the

collection of urine samples for monitoring the regularity with which tuberculosis patients self-administer therapeutic doses of isoniazid (about 300 mg a day), since urine samples should give reliably positive results for up to 24 hours or more with the new method as compared to only about 12 hours with the acetylisoniazid method.

A recent investigation has demonstrated the suitability of using isoniazid as a marker to monitor the regularity of the self-administration of other drugs (Stark *et al.*, 1975). In this investigation volunteers were allocated to five weeks' self-administered treatment with a synthetic isoquinoline compound or a matching placebo in a trial of chemoprophylaxis against natural influenza infection. Five marker tablets containing isoniazid (150 mg) were incorporated into each regimen and their ingestion was monitored by testing for acetylisoniazid in the urine by the Eidus and Hamilton (1964) procedure. The increased sensitivity of the isonicotinic acid method for monitoring isoniazid ingestion should permit the use of smaller doses of isoniazid for monitoring the self-administration of other medicaments. Thus evidence from the amounts of isonicotinic acid and isonicotinylglycine excreted during the period immediately after dosage with 50 mg isoniazid indicated that positive results would be reliably obtained with the isonicotinic acid method on urine samples voided within one hour of ingestion (Ellard and Gammon, 1976). Furthermore, from the relationship demonstrated in the current study between the positivity of the isonicotinic acid method and the mean urinary concentrations of isonicotinic acid and isonicotinylglycine, it was concluded that 12 hours after dosage with 50 mg isoniazid at least 95% of urine samples should still give positive results.

We are grateful to LEPRO for financial assistance and to the observers for their help in reading the urine-tests.

#### References

- British Medical Research Council Co-operative Study (1973). Co-operative controlled trial of a standard regimen of streptomycin, PAS and isoniazid and three alternative regimens of chemotherapy in Britain. *Tubercle (Lond.)*, **54**, 99-129.
- East African/British Medical Research Council Fifth Thiacetazone Investigation (1970). Isoniazid with thiacetazone (thioacetazone) in the treatment of pulmonary tuberculosis in East Africa—Fifth Investigation. *Tubercle (Lond.)*, **51**, 123-151.
- Eidus, L. and Hamilton, E. J. (1964). A new method for the determination of N-acetylisoniazid in urine of ambulatory patients. *Amer. Rev. resp. Dis.*, **89**, 587-588.
- Ellard, G. A. and Gammon, P. T. (1976). Pharmacokinetics of isoniazid metabolism in man. *J. Pharmacokin. Biopharm.*, **4**, 83-133.
- Ellard, G. A., Gammon, P. T., and Wallace, S. M. (1972). The determination of isoniazid and its metabolites acetylisoniazid, monoacetylhydrazine, diacetylhydrazine, isonicotinic acid and isonicotinylglycine in serum and urine. *Biochem. J.*, **126**, 449-458.
- Fox, W. (1968). Organisational and administrative considerations in the diagnosis and treatment of pulmonary tuberculosis in the developing countries. *Tubercle (Lond.)*, **49**, 332-346.
- Fox, W. (1972). General considerations in the choice and management of regimens of chemotherapy for pulmonary tuberculosis. *Bull. Int. Un. Tuberc.*, **47**, 49-67.
- Hong Kong Tuberculosis Treatment Services/British Medical Research Council Investigation (1972). A study in Hong Kong to evaluate the role of pretreatment susceptibility tests in the selection of regimens of chemotherapy for pulmonary tuberculosis. *Amer. Rev. resp. Dis.*, **106**, 1-22.
- Kasik, J. E., Heller, A., Lester, W., and Barclay, W. R. (1962). A simple spot test to determine whether a patient is taking isoniazid. *Amer. Rev. resp. Dis.*, **85**, 282-284.
- Kent, P. W., Fox, W., Miller, A. B., Nunn, A. J., Tall, R., and Mitchison, D. A. (1970). The therapy of pulmonary tuberculosis in Kenya; a comparison of the results achieved in controlled clinical trials with those achieved by the routine treatment services. *Tubercle (Lond.)*, **51**, 24-38.
- Rao, K. V. N., Eidus, L., Jacob, C. V., Radhakrishnan, S., and Tripathy, S. P. (1967). Sodium nitroprusside test for the detection of isoniazid and acetylisoniazid in urine. *Tubercle (Lond.)*, **48**, 45-50.
- Singapore Tuberculosis Services/Brompton Hospital/British Medical Research Council Investigation (1971). A controlled clinical trial of the role of thiacetazone-containing regimens in the treatment of pulmonary tuberculosis in Singapore. *Tubercle (Lond.)*, **52**, 88-116.
- Stark, J. E., Ellard, G. A., Gammon, P. T., and Fox, W. (1975). The use of isoniazid as a marker to monitor the self-administration of medicaments. *Brit. J. clin. Pharmacol.*, **2**, 355-358.
- Tuberculosis Chemotherapy Centre, Madras (1970). A controlled comparison of a twice-weekly and three once-weekly regimens in the initial treatment of pulmonary tuberculosis. *Bull. Wld Hlth Org.*, **43**, 143-206.
- Tuberculosis Chemotherapy Centre, Madras (1973). Controlled comparison of oral twice-weekly and oral daily isoniazid plus PAS in newly diagnosed pulmonary tuberculosis. *Brit. med. J.*, **2**, 7-11.
- Venkataraman, P., Eidus, L., Ramachandran, K., and Tripathy, S. P. (1965). A comparison of various methods for the detection of isoniazid and its metabolites in urine. *Tubercle (Lond.)*, **46**, 262-269.
- WHO Collaborating Centre for Tuberculosis Chemotherapy, Prague (1971). A comparative study of daily and twice-weekly continuation regimens of tuberculosis chemotherapy, including a comparison of two durations of sanatorium treatment. *Bull. Wld Hlth Org.*, **45**, 573-591.