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Isoniazid Acetylator Phenotyping of Amharas in Ethiopia

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Summary. The rate of isoniazid acetylation was measured in eighty-seven members of the Amhara tribe using a simple dosage and urine analysis procedure. An extremely low proportion (17%) of phenotypically rapid acetylators was found.

Résumé. La vitesse d'acétylation de l'isoniazide a été mesurée chez quatre-vingt sept membres du tribu Amhara, en suivant des procédés posologiques et analytiques très simples. Une proportion très basse (17%) ont possédé le trait génétique qui comporte de l'acétylation rapide.

Among the varied peoples of Ethiopia the Amharas constitute an ethnically distinct group. They appear to be particularly susceptible to mycobacterial infections; for example, the incidence of leprosy may be as high as 3% of the population in parts of the Central Ethiopian Highlands, an area peopled almost entirely by Amharas (Dr E. W. Price, personal communication). Since the outcome of intermittent isoniazid chemotherapy for tuberculosis (and possibly of dapsone therapy for leprosy) relates to the individual patient's acetylator phenotype, we determined this phenotype in groups of Ethiopians, most of whom were Amharas.

SUBJECTS

The two groups of Ethiopians who were studied had volunteered after being advised that the results of the survey might help to improve antituberculosis treatment in their country.

Group A consisted originally of fifty-six adult male prisoners in Bhata Prison, Gondar. They were aged 13–53, averaging 32 years. The prison dressers certified that all were in good health and were receiving no medication. Fifty-five of these men were studied: fifty-one were Amharas, the majority from Beghemdir Province, and four were Tigres.

Group B consisted of seventy-one healthy male students at the Public Health College, Gondar, who were being trained as health officers or sanitarians. They were aged 17–30, averaging 21 years. Thirty-six were Amharas, from nine provinces, fifteen were Tigres, fourteen were Gallas, and six were of other tribes.

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METHODS

Subjects in Group A were paraded at 8 a.m. Name, age, province of origin and tribe were recorded, and an identification number was attached to each man's clothing. Each was observed to swallow one 100 mg tablet of isoniazid (Rimifon) at that time, at noon, and at 5 p.m. At 7 a.m. the next day a sample of the first morning urine was collected from each subject, into a container bearing his identification number.

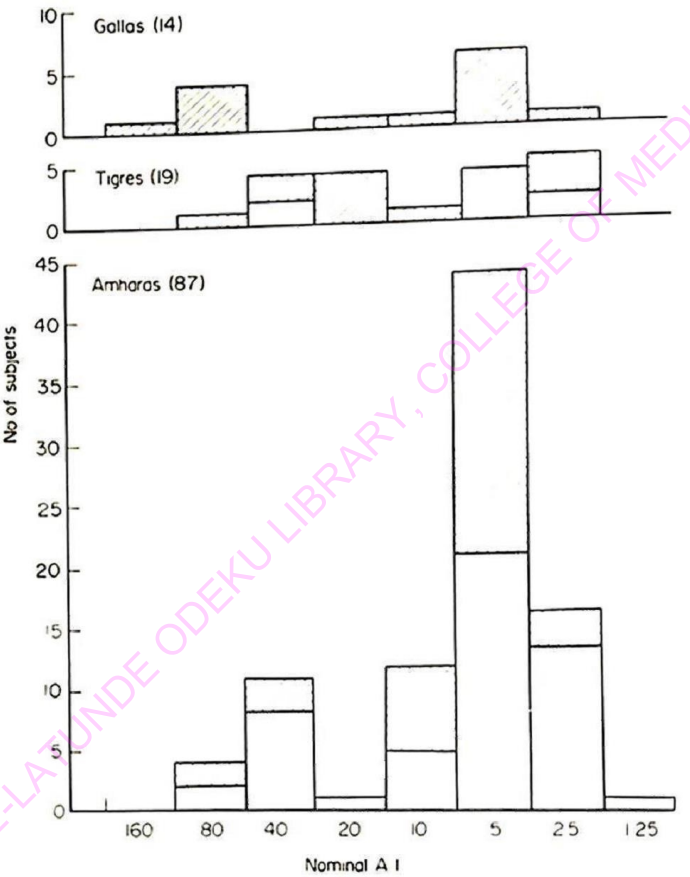

FIG. 1. Nominal steady-state urinary acetylisoniazid:isoniazid ratios in 120 healthy Ethiopian males. Stippled columns, Group A subjects; diagonally hatched columns, Group B subjects.

The men in Group B were similarly registered. Each was seen to swallow a 100 mg tablet of isoniazid after breakfast (7–8 a.m.), dinner (12–1 p.m.), and supper (6–7 p.m.). A numbered container was issued to each man after supper; he returned it the next day (7–8 a.m.) with a sample of the first morning urine.

Analyses

Equipment and supplies for the analyses were assembled in this laboratory; they fitted into one large suitcase. The methods used to determine steady-state nominal acetylisoniazid: isoniazid ratios (A:I) were as described previously (Russell, 1970), with modifications for

greater speed and precision. Thus, urine samples were measured using Eppendorf pipettes, and liquid reagents were delivered from Repette repetitive syringe dispensers. Also, the K_2HPO_4 solution was replaced by 100 mg K_2HPO_4 tablets which had been prepared to a special formulation developed by Dr B. Roufail in the Dalhousie College of Pharmacy; the tablets disintegrate rapidly (2–3 min) in urine. Each urine specimen was tested with Hema-Combistix for pH, protein, glucose and blood.

For isoniazid, 1 ml of urine was measured into a tube containing one K_2HPO_4 tablet. Urines were processed in batches of eighteen, so that by the time the 2,4,6-trinitrobenzene-sulphonic acid reagent (0·25 ml) was added, the tablet had disintegrated. The determination was completed as previously described. For acetylisoniazid, 0·2 ml of urine was treated with cyanide (0·2 ml) and chloramine T (0·45 ml) reagents and then with acetone (0·3 ml), the method being otherwise unaltered. Analyses were completed within 4 hr.

RESULTS

The urine of one Group A volunteer contained a moderate amount of glucose. Since the urine of diabetics in previous studies had given false-positive reactions for INH (Mason & Russell, 1971), this man was excluded from the study. All other specimens were normal by the Hema-Combistix test.

In the Amharas, rapid and slow acetylators were clearly distinguished (Fig. 1); the antimode was at A:I 20. Of the other tribes, only Tigres and Gallas were sufficiently numerous for an antimode to be distinguished: as shown in the figure, this was A:I 10 in the former and A:I 40 in the latter.

In assigning phenotype, a rapid acetylator was defined as one whose steady-state urinary A:I was greater than that of the antimode. On this basis, rapid acetylation occurred in fifteen (17%) of the Amharas, the 95% confidence limits being 10–27% (Mainland, Herrera & Sutcliffe, 1956), and in nine Tigres (47%), in whom the 95% confidence limits were 24–71%. The number of Gallas was too small for valid conclusions to be drawn.

DISCUSSION

In developing countries, particular interest attaches to the use of intermittent chemotherapeutic regimes, such as those used in antituberculosis treatment with isoniazid. The efficacy of weekly isoniazid treatment depends on the patient's acetylator phenotype (Menon, 1968; Tripathy, 1968), and recently it was reported that the acetylation characteristics of the antileprosy drug, dapsone, parallel those of isoniazid (Gelber *et al.*, 1971). Since a weekly dosage schedule for dapsone is widely used in these countries, it is important to determine whether the efficacy of this also is related to acetylator phenotype. Thus, a phenotyping procedure is needed that can be used even when laboratory facilities are not available.

The present study confirms that the urinary A:I ratio distinguishes clearly between the two acetylator phenotypes, and that the method used to determine it satisfies the criteria of cheapness, portability of equipment, and simplicity of dosage and analysis. One disadvantage is that standard dilutions of isoniazid and acetylisoniazid in the μg/ml range are needed for comparison. For this survey, suitable quantities of the two compounds were weighed into plastic vials in this laboratory and were used to prepare dilutions as required (it is hoped to develop a set of permanent standards). The solids required for preparing reagents can be weighed accurately enough on a portable dispensing balance.

Two features of the findings in Amharas are noteworthy. The first is the position of the antimodal A:I at 20: this ratio was 10 in previous studies of subjects of European origin (Russell, 1970; Mason & Russell, 1971). The difference is unlikely to be due to variations in technique, since the distribution for Tigres tested simultaneously showed a clear antimode at A:I 10. In the fifteen Gallas tested, the distribution was apparently bimodal, but with an antimode at A:I 40. The small number of observations on Tigres and Gallas restricts the usefulness of these additional findings, but the results suggest that the genetic basis of isoniazid acetylation polymorphism may be more complicated than was supposed. The point is clinically important: in determining a suitable isoniazid regime for any individual, the pertinent factor is not his apparent phenotype, but the absolute rate at which he acetylates the drug. Thus, in clinical application it may prove necessary to adopt an operational rather than a phenotypic definition of a rapid acetylator.

The second point of interest is the extremely low proportion (17%) of rapid acetylators among the Amharas: it is half that of the lowest previously recorded, namely 32% in a sample of 130 Swedes (Hanngren, Borgå & Sjöqvist, 1970). Apart from the ethnographic interest of this result, it appears that Amharas as a group (1) should respond well to once-weekly isoniazid treatment, as in the SHOW regime formulated by Menon (1968), and (2) are likely to suffer a high incidence of pyridoxine-deficiency symptoms (Hughes *et al.*, 1954) in association with isoniazid therapy, particularly since the majority of them consume a diet deficient in this vitamin (Mrs Pamela Miller, personal communication).

The relevance of these findings to intermittent dapsone therapy of leprosy merits further study.

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