Simplified Method for Determining Acetylator Phenotype

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Summary

A chemical method has been developed for estimating the acetylator phenotype without the use of special equipment. One urine specimen taken after ingestion of sulphadimidine, sulphapyridine, or sulphasalazine is required. The acetylator phenotype was assessed correctly in 150 urine specimens from 100 healthy subjects. Between 15 and 25 specimens can be determined hourly. Urine specimens for the test can be stored for two weeks at 37°C without any loss of drugs or their metabolites.

Introduction

Some drugs are polymorphically acetylated in man, some persons being genetically constituted as slow acetylators (Mendelian recessives) and others as rapid acetylators (dominants). Examples of drugs acetylated by this enzymatic system are isoniazid, sulphadimidine, hydrallazine (Evans and White, 1964), dapsone (Gelber *et al.*, 1971), and sulphapyridine (Schröder and Evans, 1972a). The determination of the acetylator phenotype has in certain cases become of practical importance. In particular, with the advent of once-weekly treatment regimens for tuberculosis with isoniazid consideration must be given to the fact that the response of the rapid acetylators is substantially inferior to that of slow acetylators (Tuberculosis Chemotherapy Centre, Madras, 1970). Slow acetylators using diphenylhydantoin (phenytoin) together with isoniazid and para-aminosalicylic acid are more prone to be intoxicated by phenytoin and they should thus be given a lower dose of phenytoin (Kutt *et al.*, 1970). It has been suggested that the adverse effects recorded during therapy with sulphasalazine are caused by its primary metabolite sulphapyridine and that they are influenced by the polymorphic acetylation (Schroder and Evans, 1972b).

Methods for determining acetylator phenotype have been proposed (Evans, 1969; Tiitinen, 1969; Rao *et al.*, 1970; Russell, 1970; Eze and Evans, 1972), but they all need instrumentation such as photometers and centrifuges. Simpler methods suitable for routine screening are still required.

In the method described here one urine specimen is required after ingestion of a single dose of sulphadimidine or sulphapyridine (Schröder and Evans, 1972a). The chemical analysis is simple. A portion of the urine is hydrolysed and after reaction the colour developed is compared visually with that of the reference sample, which consists of an unhydrolysed portion of the same specimen. In this study 100 subjects were phenotyped both by quantitati.e methods and by the simplified procedure. The results were compared.

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Subjects and Methods

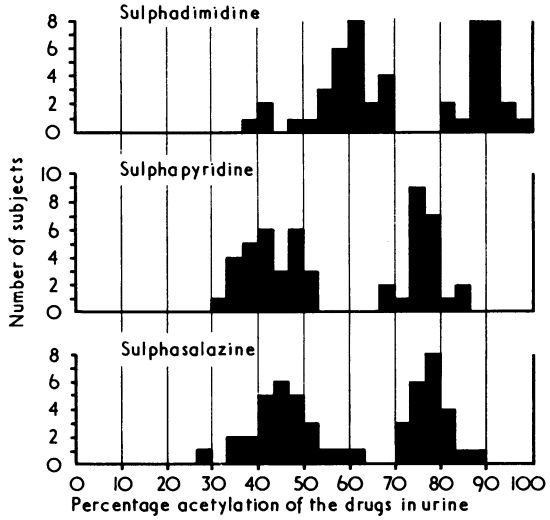
CLASSIFICATION OF PHENOTYPE

Fifty healthy subjects were phenotyped as slow or rapid acetylators of sulphadimidine according to the procedure described by Evans (1969) (series 1). Sulphadimidine in a dose of about 10 mg/kg body weight was given by mouth. Urine was collected for one hour only five to six hours after drug ingestion. One week later the procedure was repeated in the same subjects with about the same dose of sulphapyridine (series 2), and the urine formed between seven and eight hours later was collected. Urine specimens from a further 50 healthy subjects who had ingested 2-4 g sulphasalazine daily for at least five days were also tested (series 3). When patients on sulphasalazine therapy are tested urine can be collected at any time after the first day of therapy.

All specimens were analysed for free and acetylated sulphapyridine according to methods given by Schröder and Evans (1972a) (series 1 and 2) and by Hansson and Sandberg (1972) (series 3). In series 3 the subjects acetylating less than 65% of the excreted sulphapyridine were classified as slow acetylators and those acetylating more than 65% as rapid acetylators. All determinations were performed in duplicate.

SIMPLIFIED ASSAY

The urine specimens taken during the three series of tests were assayed by the simplified procedure. The method used was based on the Bratton-Marshall technique. A 10-µl. aliquot of urine was added to test-tube A and 50 µl. to test-tube B in series 1 (sulphadimidine). In series 2 and 3 (sulphapyridine) the aliquots were 20 µl. to test-tube A and 50 µl. to test-tube B. After the addition of 1 ml of 6 M hydrochloric acid (1 part concentrated hydrochloric acid and 1 part water) to sample A



Results of acetylator phenotype determination.

the contents were hydrolysed (16 hours at 25°C or 3 hours at 37°C or 30 minutes at 60°C or 5 minutes at more than 80°C). After the hydrolysis of sample A was completed 1 ml of 6 M hydrochloric acid was added to B. Both samples were then treated with 1 ml of 0·12% sodium nitrite in water. Two minutes later 1 ml of 0·8% ammonium sulphamate in water was added. Finally, 1 ml of 0·8% *N*-1-naphthylenediamine dihydrochloride in water was added to each tube. The red-purple colour in the two test-tubes was compared after at least one minute. If the colour in A was weaker than that in B the subject was classified as a slow acetylator but if the colour in A was stronger than that in B the subject was classified as a rapid acetylator. Single determinations were performed throughout.

The test-tubes used can be made either of glass or of transparent plastic and they must be of identical shape to permit correct assessment of intensities. Solutions of sodium nitrite and ammonium sulphamate can be stored in a refrigerator for one week. *N*-1-Naphthylethylenediammonium dihydrochloride should be freshly prepared. The colour developed in the two test-tubes is relatively stable for at least 24 hours. If the colours developed in A and B are too strong the samples should be diluted with the same volume of water up to five times. If, however, the colour is too faint the determination should be repeated with fourfold aliquots of urine in both A and B.

Results

The distribution of percentage acetylation of the drugs in urine as found by the quantitative determinations are given in the Chart. There is a clear separation of the acetylator phenotypes in all three series.

Three persons who were not acquainted with the simplified procedure and were unaware of the acetylator phenotype of the samples each analysed one series of 50 urine specimens. The final assessment of intensity in each series was then performed independently by two additional persons. They were asked to compare the colour in test-tube A with that in test-tube B. The results are shown in the Table. All assessments were correct. In series 1, however, one observer found the assessment of one specimen to be difficult because the difference between A and B was small. In series 2 one specimen was considered difficult to assess by all three observers and another specimen by two observers because of the similarity in intensity. All the three specimens found difficult to assess belonged to slow acetylators.

Aliquots of urine from one slow and one rapid acetylator from each of the three series were stored for 14 days at 37°C, room temperature (23°C), and 4°C without adding any preservative. Quantitative analysis for free and acetylated sulphonamide showed that storage did not affect the concentrations of drug metabolites.

Results of Simplified Phenotyping Test

Series	Phenotyping Test after Ingestion of:	No. of Urine Specimens	No. of Observers	No. of Assessments	Assessment of Phenotype		
					Correct		Wrong
					Simple	Difficult	
1	Sulphadimidine	50	3	150	149	1	0
2	Sulphapyridine	50	3	150	145	5*	0
3	Sulphasalazine	50	3	150	150	0	0

*Refers to two urine specimens.

Discussion

This communication shows that the acetylator phenotype can be determined by a simple colour test on one urine specimen. The advantage of the present method over those previously published (Evans, 1969; Tiitinen, 1969; Rao *et al.*, 1970; Russell, 1970; Eze and Evans, 1972) is that instrumentation is not required. A technician can analyse between 15 and 25 specimens each hour. This procedure would be of great value for instituting more rational and successful once-weekly dosage regimens against tuberculosis. Slow acetylators who require a lower dosage of phenytoin when they concomitantly are on isoniazid and para-aminosalicylic acid could also be identified (Kutt *et al.*, 1970).

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