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A qualitative test for the determination of isoniazid acetylator phenotype

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A qualitative test procedure for phenotyping isoniazid acetylators is described. It is based on a colour reaction which depends on the free sulphadimidine content in the total urine excreted over any one-hour period between 21 and 26 hours following a dose of sulphadimidine 1.0 or 1.5 g. depending on body-weight. The test correctly classified 96 per cent of 109 slow and 96 per cent of 68 rapid inactivators. Storage of urine samples at room-temperature up to 14 days did not affect the accuracy of the results.

Isoniazid and sulphadimidine are acetylated in the liver by similar enzymatic processes and the rate of acetylation of isoniazid closely parallels that of sulphadimidine[1]. Based on this observation, quantitative procedures employing sulphadimidine have been developed for phenotyping isoniazid acetylators[2,3]. A procedure based on a purely qualitative examination of urine would have obvious practical advantages, especially under field conditions. This communication describes such a procedure, using a qualitative test for free sulphadimidine in urine, and assesses its efficiency in relation to a standard quantitative method. The test was evolved from the observations in a pilot study that 33 of the 35 rapid inactivators excreted less than 2000 µg. sulphadimidine in a one-hour total urine collection obtained any time between 21 and 26 hours following a dose of sulphadimidine 30 mg./kg., while 33 of 35 slow inactivators excreted more than 3000 µg.

Material and Methods

Qualitative test for free sulphadimidine in urine : To 20 drops of diluted urine, 5 drops of 20 per cent trichloroacetic acid were added followed by 2 drops of 0.1 per cent sodium nitrite solution. The tubes were kept at room temperature for 3 minutes. Two drops of 0.5 per cent ammonium sulphamate solution were then added, followed 2 minutes later by 10 drops of 0.075 per cent N-(1-naphthyl) ethylenediaminedihydrochloride solution in 5 per cent ethanol, and the contents were left at room temperature for 10 minutes. The presence of sulphadimidine was indicated by the appearance of a pink colour.

Procedure for determining isoniazid acetylator phenotype : On the day of the test, the patient's urine was verified to be negative for acetylisoniazid by the test of Eidus and Hamilton[4], and also for sulphadimidine by the above

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qualitative test using a 1 in 5 diluted urine sample. The patient was then administered, under supervision, sulphadimidine 1.0 or 1.5 g. *(i.e.* 2 or 3 tablets) according to whether the body-weight was less than 40 kg., 40 kg. or more, and the total urine excreted over any one-hour period between 21 and 26 hours was collected. The volume was recorded and an aliquot was stored in the deep-freeze (–20°C) for periods not exceeding 2 days. Batches of about 10 samples were tested thrice a week. On the day of the test, the aliquot was diluted with water, the dilution corresponding to making up the volume of the total urine excreted to 2000 ml. (The effect of variations in volume on the content of free sulphadimidine per ml. is eliminated by this procedure). Thus, for example, if the volume of the urine was 200 ml., a 1 ml. aliquot was diluted to 10 ml. for convenience, a nomogram was used in practice. The specimens were coded and the qualitative test was performed independently by 2 persons, and the reaction colour was compared with that produced by sulphadimidine standard 1 µg./ml. in 1 in 10 diluted normal urine. (Since the total urine excreted is uniformly diluted to 2000 ml., a 1 µg./ml. standard would correspond to a total excretion of 2000 µg. of sulphadimidine). Solutions having a clearly greater colour intensity than the standard were reported as positive, those with clearly less intensity as negative, and others as doubtful. The last category was further classified as probably positive or probably negative. Patients with a positive or a probably positive results were classified as slow inactivators and those with a negative or a probably negative result as rapid inactivators.

Design of the studies : The above procedure was employed in 177 consecutive patients admitted to treatment. Simultaneously, the free sulphadimidine content in the total urine collection was also estimated, using a micro-method based on the technique of Bratton and Marshall[5]. These patients had their isoniazid inactivation status determined by a standard quantitative method also *viz.* the estimation of the ratio of acetyl isoniazid to isoniazid in a 3-4 hour urine collection following an intramuscular dose of isoniazid 3 mg./kg.; the analytical procedure of Venkataraman and associates[6] was followed in 56 patients and the modified procedure of Sarma and co-workers[7] in the remaining 121 patients.

To study the effect of storage, urine specimens (obtained as for the qualitative inactivation test) from 22 healthy volunteers (14 slow and 8 rapid) were stored up to 2 weeks at room temperature (35°C) after being covered with a layer of toluene; as controls, aliquots were stored in the deep-freeze (–20°C). The qualitative test was undertaken, before storage and at the end of 7 and 14 days of storage, independently by two persons, after coding the specimens. In parallel, free sulphadimidine estimations were also undertaken.

Results

The mean body-weight of the 177 patients was 43 kg. (range 28-63 kg.), and the mean dose of sulphadimidine administered was 31 mg./kg. (range 24-38 mg./kg.). The means were similar in slow and rapid inactivators.

The test results obtained by the two readers were identical in 172 (97 per cent) of the 177 samples. Further, very few

doubtful results were reported, *viz.* 3 by each reader, including one by both.

There was excellent agreement between the qualitative test and the standard method in the classification of patients as slow or rapid inactivators (Table). Thus 105 (96 per cent) of 109 slow inactivators and 65 (96 per cent) of 68 rapid inactivators were correctly classified by *both* the readers on the basis of the qualitative test results. Considering the two readers separately, one had the correct classification for 170 (96 per cent) patients and the other for 172 (97 per cent).

Considering the 7 patients (4 slow, 3 rapid) who were misclassified by one or other reader, all the four slow inactivators were misclassified by both the readers. Two of these had unusually small one-hour urine collections, *viz.* 10 and 13 ml. (no other patient in this study had a collection of less than 15 ml.), suggesting that a failure to collect the total urine excreted may have been the cause of the misclassification. Of the 3 rapid inactivators, 1 was misclassified by both readers and the other 2 by one reader only.

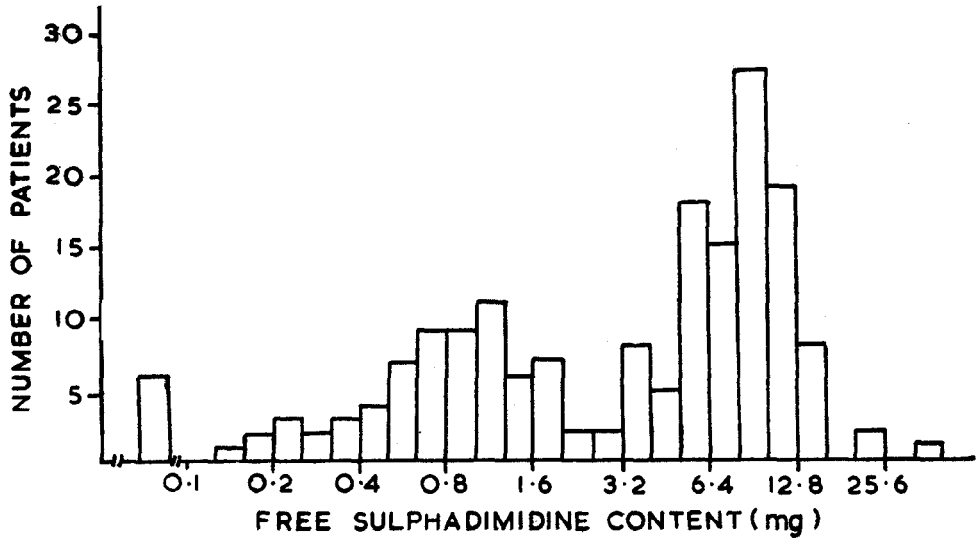
The above 7 patients had their rate of inactivation determined on another occasion by one of two other methods[8,9]. In 6 (3 slow, 3 rapid) of these, the classification by the standard method was confirmed. The 7th (slow) was labelled rapid, a classification in agreement with that made by both readers from the qualitative test result; this patient had a free sulphadimidine content of 229 µg. in. 31 ml. of urine.

The distribution of the 177 patients according to the free sulphadimidine content (on a logarithmic scale) in the one-hour total urine collection is presented as a histogram (Fig.). The shape of the distribution, especially the bimodality, suggests that the free sulphadimidine content is an efficient index for discriminating between slow and rapid inactivators of isoniazid, and that the critical value is approximately 2500 µg.

TABLE. EXTENT OF AQREEMENT BETWEEN SULPHADIMIDINE QUALITATIVE TEST AND A STANDARD QUANTITATIVE METHOD.

Classification based on qualitative test	Classification based on standard quantitative method			
	Slow		Rapid	
	Number	Per cent	Number	Per cent
Slow, by both readers	105	96	1	**1**
Slow by one reader, and rapid by the other	0	0	2	3
Rapid, by both readers	4	4	65	96
Total	109	100	68	100

FIG. DISTRIBUTION OF 177 PATIENTS ACCORDING TO FREE SULPHADIMIDINE CONTENT IN TOTAL URINE EXCRETED OVER A ONE-HOUR PERIOD, ANY TIME BETWEEN 21 AND 26 HOURS AFTER A DOSE OF SULPHADIMIDINE 1 G. OR 1.5 G.



Storage study : Of the 22 urine samples collected, 14 were reported as clearly positive and 8 as clearly negative by both the readers on the day of the urine collection. Identical results were obtained on each sample (by both readers) after 7 and 14 days of storage, at room temperature (35°C) or in the deep-freeze (–20°C).

The free sulphadimidine content also appeared to be unaffected by storage at room temperature. Thus, in slow inactivators, the mean content was 10670 µg. after 14 days of storage at room-temperature as compared with 11176 µg. after storage for the same period in the deep-freeze (P=0.08). The corresponding values for rapid inactivators were 790 µg. and 824 µg., respectively (P=0.09).

Specificity of the qualitative test : The effect of sulpha drugs and anti tuberculosis drugs on the qualitative test for free sulphadimidine was investigated, using water standards. Sulpha drugs like phthalyl-sulphathiazole, sulphaphenazole, sulphamerazine, sulphadiazine and sulphaisodimidine gave, as may be expected, a positive reaction, following diazotisation and coupling with N-(1-naphthyl) ethylenediaminedihydrochloride. Anti-tuberculosis drugs like isoniazid, ethambutol, pyrazinamide, cycloserine, ethionamide and thiacetazone did not produce any colour, even in high concentrations. Para-aminosalicylic acid produced a pink colour when the reaction was carried out in the cold (–10°C); however, no colour was developed at room temperature.

Discussion

Earlier attempts at our Centre to phenotype isoniazid acetylators on the

basis of qualitative tests for isoniazid and acetylisoniazid were unsuccessful. In contrast, the sulphadimidine qualitative test reported in this paper was able to phenotype correctly 96 per cent of the patients, and the misclassifications were evenly divided among slow and rapid inactivators. These findings are highly encouraging.

The test procedure does not require any special equipment, not even a colorimeter. The dosage schedule is simple, being dependent only on whether the patient's weight is under 40 kg. or not. The test reaction is relatively easy to read; thus, very few results were called doubtful, and the agreement between duplicate readers was of the order of 97 per cent. Finally, the urine samples can be stored (after covering with a layer of toluene) at room temperature for up to 14 days without any effect on the test results.

In most of the quantitative methods[2,3,6-10], the patient is required to wait for 3-8 hours on the day of the test. Although this is not necessary in the-case of the present qualitative test, the patient has to be present for an hour the next day *i.e.* sometime between 21 and 26 hours after drug ingestion. Since the urine collection is a late one, instructions to the patient to avoid food before the test dose are unnecessary. However, all sulpha drugs and isoniazid because it might interfere with the acetylation of sulphadimidine must be avoided during the course of the test and for at least 24 hours prior to its start.

Most of the quantitative procedures employing urine are based on the ratio of the free drug (isoniazid or sulphadimidine) to its acetylated form, and it is therefore not vital to collect the total urine excreted over the specified period. However, this is not the case with the qualitative test, and some calculations undertaken suggest that incomplete collection would result in misclassification of some slow inactivators; for example, if only half the total urine is collected, about 10 to 15 per cent of the slow inactivators could be misclassified.

The sulphadimidine qualitative test reported here could be quite useful under field conditions; the importance of obtaining a total collection and the need to avoid sulpha drugs and isoniazid for at least 48 hours must, however, be stressed.

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