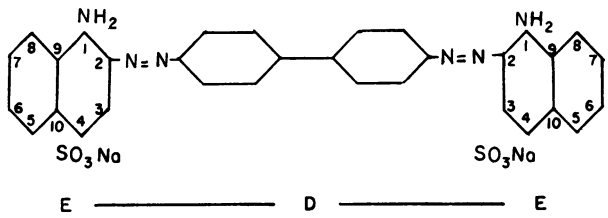

Fig. 1.—The structure of Congo red and the numbering of the naphthalene nucleus.

nucleus. Amino groupings were the dominant ones and substitution of either an amino or a hydroxyl group in position 1 caused loss of staining affinity.

With this information it was apparent that iodination of Congo red would give unsatisfactory results, since replacement by iodine occurs either in the benzidine portion or at position 1, with loss of the important amino group.

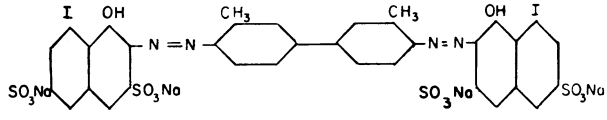

Fig. 2.—Iodinated trypan blue.

From among a number of potentially suitable compounds fulfilling the above criteria, trypan blue was selected for iodination at position 8, using the Sandmeyer reaction (Fig. 2). Excellent staining and radioautographs were obtained with the labelled material with relatively little uptake in normal splenic sections.

Metabolic studies using 10-200 μg. dye/kg. (specific activity 50-200 μc./mg.) in rabbits, dogs and ducks showed similar behaviour patterns. Activity fell rapidly in the blood and was close to the level of background activity by four days. Organ uptake was minimal except in the liver where it reached its peak of approximately 30-40% of the injected dose at four days. Thereafter this value slowly declined. Total uptake in the thyroid, heart, kidneys and spleen was less than 5% of the dose at this time.

The possible use of this material in the diagnosis of human amyloid disease is being explored.

REFERENCE

1. FRASER, J. G. AND KAYE, M.: *Canad. Med. Ass. J.*, 85: 967, 1961.

Specificity of the Isoniazid Drop Test for Control of Domiciliary Treatment of Tuberculosis

E. J. HAMILTON, B.Sc.,* A. G. JESSAMINE, M.D.† and L. EIDUS, M.D.,* *Ottawa, Ont.*

THE need for a reliable test to confirm that isoniazid is being ingested by home-treated tuberculous patients is increasing in importance with the expanding number of ambulatory patients on such domiciliary programs. The test serves not only as a control of isoniazid ingestion but also as a check on the co-operation of the patient in following the physician's instructions. Other antituberculous drugs have either toxic or gastrointestinal side effects, and the temporary discontinuance of these drugs by the patient may not be due to his lack of co-operation but rather to the side effects of the drugs.

A number of methods have been described[1-6] for the detection of isoniazid in the urine but these are complicated or lack precision. The method recently introduced by Kasik *et al.*[7] has been favourably received on this continent.[8] However, the preparation of the reagents for this test are laborious, and only isonicotinic acid, a cleavage product of isoniazid, can be detected by this technique.

ABSTRACT

A method for determining N-acetyl isoniazid in urine was found to be specific in a double-blind experiment on 1673 urine specimens of which 328 were positive and 1345 were negative. The urine specimens were obtained from patients in a sanatorium ((a) Tuberculosis Division, (b) Mental Health Division) and a general hospital. The patients in the Tuberculosis Division were receiving isoniazid and other antituberculosis drugs alone or in combination. The patients in the Mental Health Division and in the general hospital were receiving a wide variety of other drugs. After a single dose of 300 mg. of isoniazid, N-acetyl isoniazid could be detected in urines of 36 patients for at least 12 hours. In three 24-hour urine specimens trace amounts could still be demonstrated.

This test provides evidence that isoniazid is being ingested by home-treated tuberculous patients, and it can be performed by a doctor or nurse during routine visits to the home or clinic.

*Biologics Control Laboratory, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada.
†Royal Ottawa Sanatorium, Ottawa, Canada.

Canad. Med. Ass. J.
Mar. 14, 1964, vol. 90

We have reported a simple method employing easily prepared reagents.[9] This procedure detects N-acetyl isoniazid, the main metabolite of isoniazid.

METHOD

Reagents

1. A 10% solution of potassium cyanide (KCN) in distilled water.
2. A 10% solution of chloramine-T (sodium salt) (Eastman-Kodak Co., Rochester, N.Y.) in distilled water.

It is recommended that fresh solutions be prepared in dark flasks each day. However, storage of the reagents for two to three days at 4° C. did not impair their effectiveness to a marked extent.

Drop Test

White porcelain plates with hemispherical depressions are used to allow a number of tests to be carried out simultaneously. To four drops of urine, four drops of the potassium cyanide solution, followed by 9-10 drops of chloramine-T, are added without shaking of the plate. Within minutes, a red colour develops. If only traces of N-acetyl isoniazid are present, a pinkish colour develops more slowly (two to three minutes). Negative urines remain yellow. A brownish decolouration of the urine, or the development of a pinkish shade on the addition of only potassium cyanide is not considered a positive result.

RESULTS

In order to demonstrate the specificity of the test, a double-blind experiment was conducted on urine specimens from patients at two Ottawa hospitals, viz. the Royal Ottawa Sanatorium (Tuberculosis Division, Mental Health Division), and the Ottawa Civic Hospital. The group of patients from the Tuberculosis Division of the Royal Ottawa Sanatorium were receiving the following antituberculous drugs alone or in combination: isoniazid, para-aminosalicylic acid (PAS), streptomycin, pyrazinamide, cycloserine, ethionamide, isoxyl, and pyridoxine. The patients from the Mental Health Division and those from the Ottawa Civic Hospital were receiving a wide variety of other drugs. All specimens were sent to a central agency and relayed to us in random order in coded uniform tubes.

Of the 1673 urine samples tested, 1345 were found to be negative and 328 were positive. Two of the samples classified as positive appeared to be false reactions (one from a female patient from the Ottawa Civic Hospital, and one from a male patient from the Sanatorium [Tuberculosis Division]). On investigation it was found that the patient at the hospital had brought isoniazid tablets to the hospital with her and was taking them along with the medication prescribed, thus accounting for the positive reaction in her urine. The other case could not be satisfactorily explained. This man was a chronic tuberculous patient with a tendency to take self-prescribed medication, and had ignored the physician's orders to take isoxyl and pyrazinamide. On collecting further urine specimens from him on that day and the following day, and testing them, the colour reaction became less and less intense and the next day at 12 noon was negative; on subsequent days specimens from this patient continued to be negative. He denied taking isoniazid, and thus we classify this case as a false-positive reaction.

One "false-negative" reaction was obtained. In the drop test the sample gave a weak positive reaction which was unstable and faded rapidly to a yellowish colour, and was classified as negative. The patient had received 100 mg. of isoniazid and 100 mg. of pyridoxine on the day of the test. These results are summarized in Table I.

TABLE I.—NUMBER OF SAMPLES: 1673

	Positive	*Negative*
	328	1345
Probable false positive	1	
False negative		1

The detection of the N-acetyl isoniazid in urine at various time intervals after a single dose of 300 mg. of isoniazid alone or in combination with other antituberculous drugs is illustrated in Table II. After this dose of isoniazid, the urine exhibited a positive reaction up to at least 12 hours, and in three 24-hour urine specimens traces of N-acetyl isoniazid were demonstrable. A less intense colour reaction was obtained in urines of patients to whom isoniazid had been administered simultaneously with pyridoxine. During a previous laboratory examination, we had found evidence of a slight interference from pyridoxine when that substance was present in twice the concentration of the isoniazid. This could also occur under physiological conditions. Urines from 30 patients from the Mental Health Division, Royal Ottawa Sanatorium, used in this experimental series as controls, gave negative results.

In the case of six patients who had received 100 mg. of isoniazid three times on the day prior to the collection of the specimens, the urines collected at 10 a.m. exhibited a positive reaction and traces of the metabolite were still detectable in the urine specimens collected at 2 p.m.

DISCUSSION

The simple test described for the determination of N-acetyl isoniazid in urine has proved to be specific. In a large number of samples no interference from other antituberculous drugs was obtained. Similarly, a large variety of drugs used in general hospital treatment did not result in false-positive reactions.

A single dose of 300 mg. of isoniazid gave positive urine reactions in 12-hour samples, and traces

TABLE II.—AVERAGE OF COLOUR INTENSITIES OBTAINED IN SPOT TEST AT DIFFERENT TIME INTERVALS FOLLOWING THE ADMINISTRATION OF THE DRUGS

	No. of patients	Treatment in mg.			Time in hours						
		Isoniazid	Other drugs		2	4	6	8	10	12	24
Sanatorium:											
Tuberculosis Division	36	300	———		++	+++	+++	+++	++	+	
		600	———		++	++	+++	++	+++	+++	
		300	Pyridoxine	100	+++	+++	+++	+	+	+	
		—	Pyridoxine	100	0	0	0	0	0	0	
		300	Ethionamide	500	+	+++	+++	++	++	+	
		—	Ethionamide	500	0	0	0	0	0	0	
		300	Pyrazinamide	1000	+++	+++	+++	++	++	+	
		—	Pyrazinamide	1000	0	0	0	0	0	0	
		300	Isoxyl	3000	+	++	+++	++	+	+	
	3	300	———		+++	++	—	—	—	—	±
Mental Health Divsion	30	Treated with chlordiazepoxide (Librium), phenobarbital, aminophylline, diphenhydramine hydrochloride (Benadryl), penicillin, sulfisoxazole (Gantrisin), etc.			0	0	0	0	0	0	

No colour development....	0
Trace of colour	±
Light colour	+
Moderate colour	++
Intense colour	+++

of N-acetyl isoniazid were still detectable in overnight samples. Similar results were obtained when, one day prior to the examination, 100 mg. of isoniazid three times per day, had been administered to the patients.

This drop test can be performed during the visit of a doctor or nurse to the patient's home. For this purpose a half-ounce amber dropping bottle, sealed with a screw cap, may be used. The bottles contain one gram of potassium cyanide and one gram of chloramine-T, respectively. Before use, 10 ml. of distilled water is added to each bottle and mixed thoroughly to dissolve the chemicals. These reagents are applied as indicated in the foregoing description of the method. The extremely low cost of the reagents permits a frequent renewal of the solutions.

SUMMARY

A method for determining the presence of N-acetyl isoniazid in the urine is described. This procedure was found to be specific for N-acetyl isoniazid in 1673 urine samples examined, of which 328 were positive, and 1345 negative. This test is of value in confirming that tuberculous patients, for whom isoniazid has been prescribed on a home-treatment basis, are in fact taking that medication.

REFERENCES

1. SHORT, E. I. AND CASE, E. M.: *Tubercle*, 38: 288, 1957.
2. GANGADHARAM, P. R. J. *et al.*: *Ibid.*, 39: 191, 1958.
3. CATTANEO, C., FANTOLI, U. AND BELASIO, L.: *Ann. Ist. Forlanini*, 20: 59, 1960.
4. GANGADHARAM, P. R. J. AND SUBBAIAH, T. V.: Abstracted *In*: *Excerpta Med.* [XV], 14: No. 2604, 672, 1961.
5. GANGADHARAM, P. R. J.: *Indian J. Tuberc.*, 9: 219, 1962.
6. HOBBY, G. L. AND DEUSCHLE, K. W.: *Amer. Rev. Resp. Dis.*, 80: 415, 1959.
7. KASIK, J. E. *et al.*: *Ibid.*, 85: 282, 1962.
8. HUEMPFNER, H. R. *et al.*: *Ibid.*, 87: 844, 1963.
9. EIDUS, L. AND HAMILTON, E. J.: *Ibid.*, In press.

PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

THE O.M.A. AND C.M.A.

Now that the [Canadian Medical] Association has become so firmly founded, and its necessity so obvious, that is, if there is to be an organic profession, growing as the country grows, rather than a congeries of conflicting interests, the time has come to examine the structure, to ascertain if there are any unnecessary stresses and strains which can be avoided.

In the outset it was considered advisable that certain of the rights inherent in the provincial associations should be either sacrificed or held in abeyance. To enable the large body to obtain a foothold each provincial association agreed to forgo the right of meeting on the year in which the larger association met in the province.

Upon the smaller provinces this self-denying ordinance entailed little hardship. They received an important assemblage in exchange for a more humble and local one, and a visit of all the leading physicians in Canada. In Quebec there was no sacrifice whatever, since there was no provincial association in that province to have its meetings interrupted.

Ontario stands in a different category. On account of the importance of that province in respect of size, population, cities, and medical schools, the annual meeting is bound to be held in Ontario once in four years rather than once in a decade, as in the case of the smaller and more remote provinces. Besides, the Ontario Medical Association ranks in size and quality next to the Canadian Association, and the suspension of a meeting is all the more hardly felt.

At the annual meeting in July the abrogation of this rule might well be made a matter for consideration. Meetings in Toronto of both associations, simultaneously or in succession, would enhance the value of each. Visiting members from outside of the province would doubtless be made welcome, as they are at present, and they would see much to instruct them. Most important, a substantial grievance would be removed; and as members from Ontario so largely predominate in the Association the matter is to that extent in their own hands. They may be assured of the sympathetic co-operation of members from the other provinces.—Editorial, *Canad. Med. Ass. J.*, 4: 310, 1914.