ESTIMATION OF ISONICOTINIC ACID HYDRAZIDE IN BLOOD SERUM AND CEREBROSPINAL FLUID

BY

G. HUNTER, M.A., D.Sc.

*Electro-Medical Research Unit, Medical Research Council,
Stoke Mandeville Hospital, Aylesbury, Bucks*

Cuthbertson and his colleagues (1954) described a method of estimating isonicotinic acid hydrazide (I.N.H., isoniazid) in blood plasma and urine that marked a considerable advance on previous methods. Their method, however, requires about 10 ml. of blood and is rather elaborate for routine purposes. Our interest in the subject has been confined to blood and cerebrospinal fluid (C.S.F.), for which it has been possible to shorten the procedure in a manner that may be of interest to others.

Method for Serum and C.S.F.

Reagents.—Acetic acid, 0.05N. Phosphate buffer pH 6.5: 3 vol. M/5 Na_2HPO_4 and 7 vol. M/5 KH_2PO_4 . Picryl chloride solution: 35 mg. (B.D.H.) in 10 ml. ethanol. Acetic acid, 5N. *n*-Butanol.

Protein Precipitation (in test-tube, 16 by 125 mm.).

Serum.—To 1.25 ml. of serum add 7.5 ml. of water and 1.25 ml. of 0.05N acetic acid.

C.S.F.—To 1.25 ml. of C.S.F. add 7.5 ml. of water and 1 ml. of 0.05N acetic acid. (When other amounts of serum or C.S.F. are used the above proportions of 0.05N acetic acid are strictly adhered to, and the volume is always made to 10 ml.) The tubes are immersed in a bath of boiling water for about three minutes to coagulate the protein. They are then centrifuged.

Colour Reaction.—To a test-tube (16 by 125 mm.) add 8 ml. of the supernatant fluid ($\approx$ 1 ml. of serum or C.S.F.), 2 ml. of buffer, and 0.25 ml. of picryl chloride solution. Heat the tube in a bath of boiling water for 20 minutes. Cool. Add 0.4 ml. of 5N acetic acid and 3 ml. of *n*-butanol, and shake vigorously for one minute. Spin, and remove the clear butanol layer into small tubes containing 2 drops of ethanol to prevent clouding. Transfer to a glass cuvette with 1-cm. light path and note the optical density at wavelength 500 $\text{m}\mu$.

Calibration Curve.—This was constructed from a series of readings made from solutions containing 8 ml. of water, 2 ml. of buffer, and 0.25 ml. of picryl chloride solution, and containing 0, 0.5, 1, 2.5, 5, and 10 μg . of I.N.H. Such solutions were heated as described above and the optical density of the butanol extracts was measured. When no I.N.H. was present there was a reading of about 0.01 optical density. When this was subtracted from all readings a strictly linear curve in the above range was found such that the observed optical density $\times 34.5 = \mu\text{g}$. I.N.H.

It may be noted here that the optical density from controls containing no I.N.H. increases rapidly with light of wavelength less than about 480 $\text{m}\mu$, whilst at great wavelengths the optical density from the reaction product of I.N.H. and picryl chloride decreases rapidly. At 500 $\text{m}\mu$ the blank value is almost negligible and the sacrifice in sensitivity is not more than about 20%.

Serum and C.S.F.—Controls.—Under the above conditions it has been found that sera from patients who have not received I.N.H. commonly give values, expressed as I.N.H., of 1 to 2 μg . per ml., and corresponding C.S.F.s about half such values. Such blank values are somewhat reduced but not eliminated by the extraction procedure of Cuthbertson *et al.*, so that control observations are always necessary for accurate estimation. However, as the

amount of serum or C.S.F. required is now moderate, proper control determinations are practicable with sample volumes ordinarily obtainable in hospital wards.

Recovery of I.N.H. Added to Serum.—The amount of I.N.H. recoverable from serum by the present method of analysis may be seen from the observations shown in the Table. With C.S.F., recovery values are rather better.

Recovery of I.N.H. Added to Serum

I.N.H. added, μg . / ml. serum	0	0.4	0.8	1.2	1.6	2.0
I.N.H. recovered, %	Range of blanks: 1.41–2.07 μg . as I.N.H./ml.	89	87	85	82	84
Range of recovery values, %		86–95	83–95	78–92	69–88	77–90

To seven sera, in six lots each, I.N.H. was added in the amounts noted. In each case 1.25 ml. of the serum was deproteinized as described and the equivalent of 1 ml. filtrate was treated as further described for the colour reaction. The range of blank values is inserted to indicate the necessity for control determinations.

Tungstic acid may be used, as described by Cuthbertson *et al.*, for deproteinization, but the above method gives more consistent results, is simpler, and indeed yields better filtrates with C.S.F.

Summary

A simplified method suitable for routine estimation of isonicotinic acid hydrazide in blood serum and cerebrospinal fluid is described.

I should like to express my appreciation to Dr. S. W. A. Kuper (Brompton Hospital, London) for details of the method of Cuthbertson *et al.* before publication; to Dr. F. D. Stott for designing the photoelectric colorimeter; and to Mr. G. J. Bowser for technical assistance.

REFERENCE

Cuthbertson, W. F. J., Ireland, D. M., Wolff, W., and Kuper, S. W. A. (1954). *British Medical Journal*, 1, 609.

Medical Memoranda

Delay in Passage of a Ryle Tube Ingested Accidentally

Many papers in the medical literature have dealt with ingested foreign bodies and complications therefrom, but, so far as can be determined, none has mentioned a Ryle stomach tube as the foreign body. Furthermore, none has described arrest occurring at the duodeno-jejunal flexure. For these two reasons the following case is worth recording.

CASE REPORT

On February 16, 1953, a youth aged 16 suffering from a left pleural effusion had a Ryle tube passed to obtain a specimen of his gastric washings for examination for tubercle bacilli. During the procedure he swallowed the tube accidentally. A strong saline emetic given immediately failed to return the tube by mouth, but it was thought that it would pass through the alimentary canal without incident.

The following day a radiograph of the abdomen showed the tube high in the abdominal cavity, probably in the stomach. Seven days later, as the tube had not passed, a second radiograph was taken and this showed that its position had not materially altered. In an attempt to locate it a barium meal was given nine days after the tube had been swallowed, and the radiograph revealed part of it emerging from the barium-filled pylorus and occupying the curve of the duodenum. Following this, regular screening demonstrated the metal weight in the end of the tube to maintain a fairly constant position on the left of the vertebral column, at the level of the third lumbar transverse process, corresponding to the position of the duodeno-jejunal flexure.

Sixteen days after the ingestion of the tube a second barium meal was given, and a lateral radiograph showed the metal weight still lying posteriorly to the stomach, at the