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 ($\equiv$ 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

level of the third lumbar vertebra. On the thirty-sixth day a third barium examination revealed the metal marker in roughly the same position, and it was thought highly probable that arrest had occurred at the duodeno-jejunal junction.

On the forty-third day it was decided that, as no progress had been made, the boy should have an exploratory laparotomy with a view to removal of the tube. Shortly before the date arranged for operation, on the fifty-third day, the boy was screened again, and this time the metal weight was clearly seen lying in the pelvis. Digital examination of the rectum confirmed the presence of the tube, which was hooked by the finger and withdrawn.

COMMENT

The interesting point in this case is the unusually long time (53 days) the tube remained in the alimentary canal; yet, despite this, the patient suffered no ill effects of any kind.

It seems probable from the radiological findings that the point of arrest was the duodeno-jejunal flexure, which must surely be unusual. Furthermore, after a delay there, known to have lasted 34 days and which was probably longer, the tube became dislodged and passed without further trouble.

It is reasonable to conclude, therefore, in dealing with cases of this kind, that, provided there are no symptoms, a delay of six to eight weeks is warranted before surgical removal is considered, and that, in addition, a failure of the tube to advance in the alimentary canal for as long as three to four weeks does not preclude the possibility of a natural passage.

I thank Dr. A. J. Moon for permission to publish this case.

WILLIAM WILSON, M.D.,
Lately Registrar, Pinewood Hospital, Wokingham.

Occupational Calcium Necrosis of the Skin

Necrosis of the skin due to calcium chloride solution appears to be a rarity in that only two cases have so far been published. Oppenheim (1935) described its occurrence in an ice-cream maker who accidentally allowed the concentrated solution of calcium chloride used as a freezing bath to come into contact with his skin. Heppleston (1946) described it in an operator using a 40% solution of calcium chloride as a dust-laying spray in a South Wales coal-mine. The following case, in a maker of anti-freeze solution, serves to confirm the existence of calcium chloride necrosis and to stress the need for protecting the skin from this substance.

CASE REPORT

A 29-year-old Turkish labourer was employed in making a 40% solution of calcium chloride. This was marketed as

FIG. 1.—Necrosis of friction areas on the legs due to calcium chloride solution.

an anti-freeze agent to add to water used for mixing mortar and concrete in cold weather. The manufacturing arrangements were primitive, and one day he splashed his trousers with the solution, but did not change them or wash his legs. That night he noticed considerable pain in the parts of his legs where the tops of his gum-boots had rubbed.

The next morning he reported to the industrial clinic and his legs appeared as in Fig. 1. On each was a thick black eschar surrounded by a thin border of inflammation. The appearance was similar to that of the coagulum produced by the old tannic acid treatment of burns. The eschar was obviously necrotic skin involving the full skin thickness.

With conservative treatment the coagulum began to separate along the edges on the 19th day, and was half separated on the 30th day. Healing was not complete until the 90th day.

A biopsy (Fig. 2) showed coagulative necrosis of the epidermis and underlying dermis. The latter was crowded with nuclear fragments and there was slight proliferation of fixed tissue cells in the surviving connective tissue. Von Kossa's reaction showed obvious impregnation with calcium.

FIG. 2.—Lesion of right leg, showing necrosis and calcium impregnation. Haematoxylin and eosin, Von Kossa.

COMMENT

The microscopical appearances in Heppleston's case were similar, except that in the less severe lesions there was necrosis and calcium impregnation of the dermis only. As in this case, severe lesions were present on those parts of the legs where friction from the tops of the gum-boots had occurred. Healing was prolonged, and it is for this reason that it is so important to protect the skin from calcium chloride solutions.

The cause of the lesions is probably the alkalinity of the solution combined with its necrosing effect. In the present case the pH of the solution was 9.4, and it was found by titration to have an alkalinity equivalent to N/50 NaOH. Calcium is a well-known necrosing agent, as shown by the dire results of its accidental injection subcutaneously or intramuscularly. The alkalinity would soften the horny layer of the skin so that penetration and subsequent necrosis could occur.

I would like to thank Dr. L. E. Glynn for kindly preparing and reporting on the section in this case.

PETER H. NASH, M.D., D.P.H., D.I.H.,
Formerly Senior Clinical Assistant,
Slough Industrial Health Service.

REFERENCES

- Heppleston, A. G. (1946). *Brit. J. Industr. Med.*, 3, 253.
Oppenheim, M. (1935). *Wien. klin. Wschr.*, 48, 207.