TECHNICAL METHODS

A Dye-resin Test for Achlorhydria

G. M. G. BARTON AND A. H. FIRSTBROOK

*From the Biochemical Department, Salisbury Area
Pathological Service*

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The use of resins as a basis for "tubeless test meals" has made the unpleasant experience of aspiration of gastric contents unnecessary in many cases. A substance incorporated in the resin is released in the stomach only in the presence of free hydrochloric acid. This substance is then absorbed into the blood and excreted in the urine. Originally quinine was used in the test, and estimated fluorimetrically in the urine (Segal, Miller, and Morton, 1950; Harkness and Durant, 1953). Later, methylene blue was used instead of quinine, as it is more easily detected (Goldbloom, Feinstein, and Eiber, 1955; Segal, Miller, and Plumb, 1955).

Although different modifications describe varying degrees of quantitative exactness, the method is essentially a qualitative one. If no dye appears in the urine the patient is presumed to have achlorhydria. No measure of the level of gastric acidity is shown by the amount of dye in the urine. As a simple qualitative test for achlorhydria the dye-resin test can be substituted for a test-meal in many cases. When prepared in the laboratory the cost per test is between two and three pence instead of 4s., which is the cost of the proprietary preparation.

Method

The following requirements are sufficient for 40 to 50 tests.

- (1) Amberlite resin I R C 50 (H), B.D.H., 100 g.
- (2) Azure 1, B.D.H., 5 g.
- (3) Two-litre conical flask with side-arms.

The Dye Resin.—The dye is completely dissolved in a litre of distilled water with stirring. The solution is poured into a 2-litre conical flask fitted with a cork containing a piece of glass tubing reaching nearly to the bottom of the flask. The resin granules are added, and the contents of the flask well stirred, and then agitated overnight by attaching the side arm to a vacuum pump. Care must be taken that the flow of air is not so fast that the solution bubbles into the side-arm. On the following day the resin is allowed to settle, and the supernatant sucked off. About 800 ml. of distilled water is added, the solution well mixed, then allowed to stand, and the supernatant

again sucked off. This procedure is repeated six more times during the day, and then a final addition of water is mixed in and allowed to stand overnight. A completely colourless supernatant is not achieved, but the colour should be very pale. The granules are then filtered off by means of a large funnel and No. 1 Whatman filter paper, using more distilled water as required to wash all the granules out of the flask. The filter paper containing the granules is placed in a dish and dried in an oven at approximately 50° C. for two days. A paraffin oven at 50° C. as used in any histological department is suitable. Alternatively an incubator set at 37° C. for four days can be used. After drying, the navy-blue granules should lose no colour when shaken in water, but on shaking for a minute in N/10 hydrochloric acid should make the liquid pale blue. The dried granules can be placed in a screw-top jar, and dispensed as required. Although these quantities should theoretically produce sufficient for 50 tests, some granules are inevitably lost during the process, and elaborate precautions against this loss make the preparation tedious.

The Test

The patient receives no breakfast, but drinks of water are allowed. The early morning specimen of urine is discarded, and an hour later a specimen of urine is collected (specimen 1). Immediately after this 0.25 mg. of histamine is injected intramuscularly, and immediately after this 2 g. of dye resin is given in a glass of water, stirring well, so that all the granules are swallowed. Two hours later the urine is collected (specimen 2). Both specimens of urine are sent to the laboratory.

Results

Specimen 1 is used as a control, and compared with the colour of specimen 2. If the latter is obviously tinged green or blue there is free acid in the gastric juice. The shade varies not only with the amount of dye, but with the concentration of normal urinary pigments. If there is no obvious difference between the two specimens the reaction of both should be adjusted roughly to pH 3 with a drop of 20% HCl, and boiled. After cooling traces of dye may now be apparent. If there is still no difference between the two specimens it is assumed that the patient has achlorhydria. A quantitative report should not be given.

A trace of green may be present in the urine after four hours in persons with achlorhydria. Only the two-hour result, therefore, is significant. The patient should be warned that he may pass blue urine for three days after the test.

Discussion

This test is quick, cheap, simple, and painless. It is adequate in most cases for the diagnosis of achlorhydria. The usefulness of test meals for diagnosing any other gastric abnormality is questionable.

The term "achlorhydria" is usually taken to mean that the gastric juice has a pH of more than 3, as shown by Topfer's reagent or thymol blue. It denotes a relative "an"-acidity rather than a complete absence of hydrochloric acid.

The results of the dye-resin test can be varied by altering the quantity of dye and resin and the time of urine collection. A standardized procedure, therefore, is necessary.

The conditions which may give misleading results in resin tests have been described by Harkness and Durant (1953). They include the administration in the two days before the test of such ions as aluminium, magnesium, calcium, barium, iron, and kaolin that may release dye from the resin. Pathological states which may interfere with excretion of the dye include pyloric stenosis, intestinal malabsorption, and diseases of the urinary tract.

The commercial preparation of the dye resin ("diagnex," Squibb) substitutes 500 mg. of caffeine sodium benzoate for histamine. Although caffeine is a powerful gastric stimulant, the meaning of achlorhydria is generally taken to be the absence of free acid after histamine stimulation. It is therefore preferable to use histamine instead of caffeine.

Summary

A simple and cheap method of preparing and using a dye-resin compound as a test of achlorhydria is described.

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Beginning with the issue of the *Journal of Clinical Pathology* of January, 1960, summaries of papers will be replaced by abstracts written by the author. These abstracts will precede the paper, and will be fuller than most summaries. For the guidance of contributors the following suggestions may be useful:

The abstract should always include (1) a statement of the purpose for which the investigation or review was undertaken; (2) the selection of material used; (3) the methods, procedures, or treatment employed; (4) the results; (5) discussion.—EDITOR.

A Jig for the Agla Micrometer Syringe for Automatic Quantitative Applications in Paper Electrophoresis

D. A. OSBORN AND D. BAWDEN

From the Department of Pathology, Institute of Laryngology and Otology, University of London

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The device described here is designed to achieve quantitative applications combined with an even distribution of material by means of continuous automatic expulsion from the micrometer syringe as the needle moves across the strip. The method is suitable for use with electrophoresis tanks of the horizontal type with the strips already in position. The inexpensive jig can be made quite easily from "perspex."

An "agla" syringe with a micrometer attachment is mounted by means of Terry clips (A in Fig. 1) on a laterally moving platform (B). When in position, the machined handgrip of the micrometer rests firmly against a rubber strip (C) supported on a fixed upright. When the locking screw (D) is released, movement of the platform causes the micrometer screw to turn. The platform is moved by hand and is limited by a removable check pin (E) capable of insertion at intervals corresponding to the width of the strip. The base is in two parts (F and G) sliding on each other when the locking lever (H) is released to allow lining up of the needle point with the application line on the strip. A long needle ("record" No. 20), filed level at the end, is attached to the syringe and bent at right angles to reach the strip through a narrow opening in the tank. The height of the needle is finally adjusted by means of the screw (I) operating against the flap (J). The front end of the base-plate (G) rests against a bevelled "perspex" bar on the lid of the tank fixed in such a position as to allow the needle to pass through the opening and at the same time permit both tilting and lateral movement of the jig on the lid. On the under surface of (G) is a strip of rubber (K) so that pressure of the hand causes the base-plate to grip the lid during movements of the platform (B).

The handgrip of the micrometer has a circumference of exactly 2 in., so that lateral movement of 1 in. results in the expulsion of 0.005 ml. of fluid. Obviously, the maximum number of consecutive applications depends on the width of the strip and the jig respectively. The apparatus is reset for further applications by locking the micrometer and pulling the platform across with slight upward pressure to clear the rubber strip (C).

Fig. 2 shows the complete apparatus with the platform (B) in mid position.