The information obtained by the endoscopist depends directly on his experience, and his knowledge of the limitations, indications, and contraindications of peritoneoscopy.

For the evaluation of hepatic disease we prefer needle aspiration biopsy under peritoneoscopic guidance to biopsy with biting forceps and electrocoagulation.

A hospital is considered to be not completely equipped if it lacks a peritoneoscope and a peritoneoscopist.

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TUBELESS GASTRIC ANALYSIS WITH AZURE A AND BETAZOLE HYDROCHLORIDE

BY

O. A. A. BOCK, M.A., B.M., B.Sc.

AND

L. J. WITTS, C.B.E., M.D., F.R.C.P.

*Nuffield Department of Clinical Medicine, the Radcliffe
Infirmary, Oxford*

The ability of the stomach to secrete hydrochloric acid (Prout, 1824) is often a crucial observation in the differential diagnosis of diseases affecting the alimentary tract. In an attempt to overcome the unpleasant necessity for intubation, Segal *et al.* (1950a, 1950b) introduced the method of "tubeless gastric analysis" which depends on the dissociation of a cation exchange resin by the hydrochloric acid in the stomach. This cation is absorbed and appears in the urine, where it is simple to estimate the quantity of cation excreted.

The first exchange resin used contained quininium ("diagnex"), which requires an ether-sulphuric-acid extraction of the urine and is thus rather inconvenient for routine use. More satisfactory is the dye azure A, which is present in considerable quantities in commercially available methylene blue (Segal *et al.*, 1955). This is used in diagnex blue ("azuresin"). Dilute hydrochloric acid displaces the dye from its carbacrylic exchange resin "amberlite RXE-96," and examination of the urine in a comparator block indicates the quantity of the dye excreted, release of which both *in vivo* and *in vitro* starts at pH 3.2 and is maximal at pH 1.5. Segal *et al.* (1950a, 1950b, 1955) had used 500 mg. of caffeine benzoate as parietal-cell stimulant, but after the successful clinical trials by Kirsner and Ford (1955) of the histamine isomer betazole hydrochloride [3-(2- β -aminoethyl) pyrazole] ("histalog") as gastric stimulant, Segal *et al.* (1959a, 1959b) replaced the caffeine with 50 mg. of betazole hydrochloride given orally or subcutaneously.

We are now in agreement with the extensive American experience which suggests that the azure A test combined with betazole hydrochloride is a useful and reliable procedure for indicating the presence of acid in the stomach and deserves wider clinical application. In this paper we record our further experiences with this test, which we use routinely in screening patients for achlorhydria. In many instances the results of the tubeless analyses were confirmed by comparison with the augmented histamine test of Kay (1953).

Present Study

Selection of Patients.—We studied 182 patients, of whom 50 were out-patients. A high incidence of achlorhydria was expected, for most of the patients selected suffered from diseases thought to be more commonly associated with achlorhydria, such as pernicious anaemia, iron-deficiency anaemia, rheumatoid arthritis, thyrotoxicosis, myxoedema, and polycythaemia vera.

Test Procedure.—This was essentially the same as that reported earlier from this department (Denborough *et al.*, 1958). After an overnight fast the bladder was emptied, the urine was discarded, and the patients were given either 500 mg. of caffeine or 50 mg. of betazole hydrochloride diluted in a glass of water. One hour later the bladder was emptied again and the "control" specimens were collected. Immediately afterwards 2 g. of the granules containing the dye azure A were given by mouth and the patients instructed not to have anything else until two hours later, when the bladder was emptied and the "test" specimens were obtained.

Method of Estimation.—Like Gilbert *et al.* (1957), we accepted as indicating the presence of acid only those tests in which the colour of the urine matched or was darker than the 0.6-mg. standard; less colour indicated achlorhydria. It was found that the estimation could be simplified in two ways without lowering the accuracy of the interpretation. Dilution of the urine specimens to 300 ml. was not necessary. Instead of heating aliquots suspected of containing reduced dye in a water-bath for 10 minutes, the specimens, to which one drop of Benedict's reagent and one drop of 6 N hydrochloric acid had been added, were heated gently over an open flame until the maximum colour had developed. They were then cooled under running water and compared with the standards supplied by the manufacturers.

Test of Comparison.—The results of the tubeless tests were correlated with the augmented histamine test carried out in the manner described by Kay (1953), but we used only 50 mg. of mepyramine maleate as the antihistamine and we collected specimens for one hour after the injection of histamine. Titration was carried out with decinormal NaOH to pH 7.4 in a direct-reading pH meter, and the results were expressed as the total milli-equivalents of acid secreted in one hour (mEq/hr.) after the injection of histamine (Bock, 1961a). The normal range for men would appear to be 15–30 mEq/hr. (mean 22.4 mEq/hr.) and for women about 5 mEq/hr. less (mean 14.6 mEq/hr.) (Bruce *et al.*, 1959).

Results

In the 77 subjects given caffeine as gastric stimulant the azure A test indicated the presence of acid (positive result) in 51 and apparent achlorhydria (negative result) in the other 26 (Table I). None of the positive results

was checked by intubation and 9 of the negative results were not checked. Of the remaining 17 patients with apparent achlorhydria 11 were found to secrete acid on augmented histamine stimulation and in five this was in excess of 11 mEq/hr.

TABLE I.—Comparison of Azure A Results With Augmented Histamine Tests (A.H.T.)

Stimulant	Total	Azure A Results	No.	A.H.T. Result		Not Checked
				Acid	No. Acid	
Caffeine	77	Acid	51	—	—	51
		No Acid	26	11	6	9
Betazole hydrochloride	105	Acid	68	27	2	39
		No Acid	37	3	18	16
Total ..	182		182	41	26	115

Betazole hydrochloride was used as a stimulant in 105 patients and acid was detected in 68. The augmented histamine test was carried out in 29 of the 68 patients and the presence of acid was confirmed in 27. Seven of them secreted 5 mEq/hr. or less, the lowest amount being 1.21 mEq/hr. This group included three patients with megaloblastic anaemia who had been referred to this department with achlorhydria, which had been established by "routine fractional analyses"; in each case acid was secreted in response to augmented histamine stimulation. Two false-positive results using betazole hydrochloride were noted in this series. In both, subsequent inquiry revealed that collection of the test specimens had been delayed. Repeat tests, which included collections at the same intervals as in the first test, showed very little dye in the urine at the correct time but sufficient colour in the subsequent specimens to warrant classifying them as positive.

Of 21 patients whose azure A tests after betazole hydrochloride were negative, augmented histamine stimulation revealed achlorhydria in 18; the other three secreted 0.21, 2.01, and 8.0 mEq/hr. respectively. This comparison was not undertaken in the remaining 16 cases of apparent achlorhydria. Betazole hydrochloride was a better stimulant than caffeine benzoate, since acid was detected in only three of the 21 negative tests when this drug was used, whereas 11 of the 17 patients in whom achlorhydria was suspected after caffeine benzoate secreted acid. Betazole hydrochloride was also a safe drug to use; only twice were unpleasant side-effects noted in the 105 tests. A 45-year-old woman developed a generalized urticarial eruption which responded to an antihistamine preparation, while an 18-year-old girl vomited. A few patients complained of mild headache.

Discussion

A review of the available literature indicates that 146 false-negative results (7.1%) and only 27 false-positive results (1.3%) were detected in 2,054 tubeless analyses which were checked by gastric intubation (Table II) (Segal *et al.*, 1950b, 1955; Malach and Banks,

TABLE II.—Comparison of Tubeless Analyses With Findings on Intubation

Indicator Used	Tubeless Result	Intubation Result		Total	Total Checked
		Acid	No Acid		
Quininium	Acid	476	11	487	682
	No Acid	18	177	195	
Azure A	Acid	831	16	847	1,372
	No Acid	128	397	525	
Total ..		1,453	601	2,054	2,054

1952; Becker and Maslon, 1953; Conway and Meikle, 1953; Flood *et al.*, 1953; Harkness and Durant, 1953; Sharp *et al.*, 1954; Shay *et al.*, 1954; Pollard *et al.*, 1954; Fentress and Sandweiss, 1955, 1957; Galambos and Kirsner, 1955; Goldbloom *et al.*, 1955; Segal and Miller, 1955; Sievers and Gieselman, 1956; Bolt *et al.*, 1957; Gilbert *et al.*, 1957; Poliner *et al.*, 1957; Rechtshaffen *et al.*, 1957; Sievers and Fischer, 1957; Denborough *et al.*, 1958; Rodman *et al.*, 1958; Rosenthal and Buscaglia, 1958; Glass *et al.*, 1960; Marks and Shay, 1960.

Although more false-negative results occurred with azure A, 9.4%, against 2.2% using quininium, some of this difference may be accounted for by the better techniques used for comparison in the azure A studies. Betazole hydrochloride is a better stimulant than caffeine benzoate, since acid was detected after betazole hydrochloride stimulation in 51% of 149 patients in whom caffeine indicated achlorhydria (Segal *et al.*, 1959b). We did not attempt to evaluate the azure A-betazole hydrochloride test as a possible method for the quantitative assessment of acid secretion, but we found no false-negative results among those patients who secreted 10 mEq/hr. or more after augmented histamine stimulation. This suggests that a negative azure A-betazole hydrochloride test would be strong evidence against the diagnosis of duodenal ulceration (Marks and Shay, 1960).

Intubation is uncomfortable and, particularly in older people, often difficult or impossible. Unless the tube is screened and its position localized in the stomach, the tip, according to Callender *et al.* (1960), can be in the pharynx, oesophagus, fundus of the stomach, or duodenum in 44% of cases. The reaction of the gastric juice can be alkaline because the unpleasant psychological influences consequent upon the anticipation and passing of the tube have inhibited acid secretion (Grossman *et al.*, 1945; Wolf, 1959). Some patients who secreted less than normal amounts of acid on augmented histamine stimulation, but in whom azure A-betazole hydrochloride tests were positive, had no detectable acid in the basal secretion (Bock, 1961b). The augmented histamine test is a somewhat arduous procedure. The tubeless method of gastric analysis overcomes these difficulties at the expense of a small proportion of false-negative results.

The azure A-betazole hydrochloride method of analysis is not infallible as a screening test for the detection of acid in the stomach. In such a screening test false-negative results are not a source of anxiety provided they are not too frequent, as negative results will usually be confirmed by intubation of the stomach. False-positive results are a good deal more worrying, as they denote a failure in the screening procedure. Although false-positive results are possible, they appear to be extremely infrequent when the test is properly performed. Interpretation of the test should be cautious in patients who have pyloric stenosis, malabsorption syndrome, severe diarrhoea, or renal disease (Poliner *et al.*, 1957; Bolt *et al.*, 1957; Rodman *et al.*, 1958).

Summary

The results of 182 tubeless gastric analyses using the dye azure A (diagnex blue) as indicator are reported. In 105 of these tests 50 mg. of betazole hydrochloride ("histalog") was used as the stimulant instead of 500 mg. of caffeine benzoate.

In 67 instances the results of the tubeless test were compared with the findings after augmented histamine stimulation. Only two false-positive results were detected, and these were explained by errors in technique. Fewer false-negative results occurred with betazole hydrochloride than when caffeine benzoate was used as the stimulant.

The tubeless method of gastric analysis, using the dye azure A and betazole hydrochloride, appears to be a reliable method for the screening of patients for achlorhydria.

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FAECAL P.V.P. EXCRETION IN HYPOALBUMINAEMIA AND GASTRO- INTESTINAL DISEASE

BY

A. M. DAWSON, M.D., M.R.C.P.

Lecturer, Department of Medicine

ROGER WILLIAMS, M.D., M.R.C.P.

Lecturer, Department of Medicine

AND

H. S. WILLIAMS, M.Sc.

Chief Hospital Physicist, Physics Department

The Royal Free Hospital, London

Until recently hypoproteinaemia in disease has been ascribed to poor intake, impaired absorption, or faulty metabolism of such protein precursors as amino-acids or peptides. Apart from the nephrotic syndrome, excessive catabolism or loss from the body has only rarely been invoked. Nevertheless recent reports indicate that excessive catabolism due to loss of protein into the intestinal tract is predominant in a variety of disorders associated with low serum proteins (Citrin *et al.*, 1957; Gordon, 1959a; Schwartz and Jarnum; 1959; Steinfeld *et al.*, 1960; Jarnum and Schwartz, 1960). Hypoproteinaemia results because the liver has only a limited capacity to synthesize albumin, and breakdown soon outstrips synthesis (Schwartz and Jarnum, 1959; Steinfeld *et al.*, 1960).

Intestinal loss is difficult to demonstrate because the gut lumen is relatively inaccessible and any exuded protein is broken down by digestive enzymes and the products are reabsorbed so that the faecal nitrogen is not necessarily raised. Similarly, if plasma albumin labelled with ^{131}I is used as a tracer, it enters the gut lumen, is broken down and the radioactive products are reabsorbed, and the faecal radioactivity may be normal. The ingenious technique of Gordon (1959a) of using polyvinylpyrrolidone (P.V.P.) has partially overcome the problem. P.V.P. labelled with ^{131}I is of similar molecular size to albumin, and so, when injected intravenously, can leak through sites that are permeable to the albumin molecule. In contrast to albumin it is relatively unchanged by the action of digestive enzymes and colon bacteria, and therefore much of it can reach the faeces, where it can be assayed by counting the radioactivity of the iodine label. This paper reports the results of this test obtained in a group of patients with a variety of disorders associated with hypoproteinaemia.

Methods

The P.V.P., which was obtained from May and Baker Limited, had an average molecular weight of about 40,000. It was labelled with ^{131}I as described by Gordon (1958) and dialysed in a Visking tube against running water to constant activity. This usually took three days. The starting polymer was slightly different from that used by Gordon and the product probably resembled that produced by Ravin *et al.* (1952). When fed by mouth approximately 40% was recovered in the faeces, less being recovered if the patient was constipated or the preparation was old. Even so, this preparation has proved to be satisfactory for the intravenous test when used within one week of preparation. The material was

A new £75,000 training centre for rural medical aids in Tanganyika was formally opened at Ifakara on July 22. The centre is staffed by three doctors, two biologists, and three technicians, and is sponsored and financed by a Swiss organization, the Basle Foundation for Aid to Developing Countries.