and the arterial homografts, possible alternative materials are discussed.

We would like to thank Professor Charles Rob for permission to include some of his patients who were treated at St. Mary's Hospital. All the long-term cases fall into this category.

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TUBELESS GASTRIC ANALYSIS WITH AZURE A AND MAXIMAL HISTAMINE STIMULATION

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In an effort to avoid the unpleasant procedure of obtaining gastric contents for analysis, Segal *et al.* (1950) devised a practical "tubeless" method 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 easy to estimate the quantity excreted.

The first exchange resin used, "diagnex," contained quinine hydrochloride, which requires an ether–sulphuric-acid extraction of the urine and is therefore rather inconvenient for routine use. More recently, Segal and Miller (1955) simplified their tubeless technique by combining an indicator dye, azure A, with the resin (azuresin; "diagnex blue"). The azure A released by the acid is absorbed by the small intestine and excreted in the urine: the amount of dye in the urine may be measured by spectrophotometry or, more simply, by comparison with colour standards. The results of the azuresin tubeless test have been checked previously against the results of intubation following caffeine (Segal *et al.*, 1957), submaximal histamine (Fentress and Sandweiss, 1957; Poliner *et al.*, 1957; Dior *et al.*, 1957), and maximal histamine stimulation (Denborough *et al.*, 1958; Marks and Shay, 1960; Bock and Witts, 1961). Patients with a positive azuresin test almost always secrete "free" acid when tested by one of the above intubation procedures, but a negative azuresin test does not necessarily imply that the patient tested is unable to secrete "free" acid. The high incidence of false-negative tests reported in the literature (Bock and Witts, 1961) suggests the need of certain changes. The fact that histamine is a potent gastric stimulant in man provided the opportunity to investigate the possibility of its superiority to caffeine or betazole hydrochloride ("histalog") as the gastric stimulant in the detection of achlorhydria by the tubeless gastric analysis technique.

The present study was undertaken to evaluate the azure A test with "maximal" histamine stimulation as a possible method for the quantitative assessment of acid secretion. In spite of involving two injections, it was very seldom necessary for the patients to lie flat. Above all, the method does not involve passing a tube, which is, in our opinion, the major obstacle to gastric analysis.

We were also interested in determining the value of azuresin with maximal histamine stimulation as a test of acid secretion in gastrectomized patients.

Material and Methods

We studied 100 patients, the majority of whom were in-patients. A high incidence of hyperchlorhydria was expected, for most of the patients selected were being investigated as part of a study of the augmented histamine test in peptic ulcer. The series was divided into two groups. The first group included subjects with duodenal ulcer (40 patients) and gastric ulcer (10 patients), the remainder being patients with gastric cancer, gastritis, pernicious anaemia, and other miscellaneous disorders. The second, a selected group, was made up of 20 patients who had undergone subtotal gastrectomy for duodenal ulcer.

The azuresin test was performed as described by Segal and Miller (1955) with two important modifications: (1) histamine was used as the gastric stimulant, in the dose proposed by Kay (1953) for the augmented histamine test, and (2) azuresin was given 30 minutes after the injection of histamine, following our own observations that the output of hydrochloric acid in response to the augmented histamine dose is maximal during the 20 to 40 minutes after stimulation (Carneiro de Moura, 1961).

Test Procedure.—After an overnight fast the bladder was emptied, the urine was discarded, and the patients were given an intramuscular injection of 200 mg. of antazoline ("antistin") followed 15 minutes later by a subcutaneous injection of 0.04 mg. of histamine acid phosphate per kg. body weight. The control specimen of urine was collected half an hour later, and the patients were then given 2 g. of azuresin granules with a glass of water. The total volume of urine passed during the two hours following the administration of the resin was kept for the measurement of azure A. All medication was discontinued at least 12 hours before the test.

Methods of Estimation.—The control and two-hour urine collections were diluted to 300 ml. Two 10-ml. aliquots were taken from both specimens for estimation of diagnex-azure-A content of the urine. Each aliquot was acidified with two drops of 6N HCl and then placed in a boiling water-bath for 10 to 15 minutes. This procedure converted the dye (present in a colourless conjugated form) into blue azure A compound (Segal *et al.*, 1957). The aliquots were allowed to cool at room temperature for two to four hours and their colour was compared with two standards supplied by the manu-

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facturers (representing 0.3 and 0.6 mg. of azure A per 300 ml.). In order to obtain quantitative information, the reading was always made after a 24-hour waiting period, as suggested by Segal *et al.* (1957). In 57 cases the amount of azure A compound in the specimens was determined quantitatively with a Coleman photocolorimeter at a wavelength of 620 mμ.

Excretion of more than 0.6 mg. of azure A/two hours was regarded as positive, of less than 0.3 mg./two hours as negative, and of 0.3–0.6 mg./two hours as an equivocal or borderline result (Marks and Shay, 1960).

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) with minor modifications (Pinto Correia, 1961). The specimens were titrated with N/10 NaOH using Topfer's reagent and phenolphthalein as indicators for "free" and "total" acid respectively. The output of total acid during the period of one hour after the administration of histamine was regarded as the maximal acid output (M.A.O.) (Card and Marks, 1960).

Results

The 66 patients in group 1 who had a positive azuresin test secreted free acid after histamine stimulation. There were thus no false-positives among them (Table I). Ten out of 13 patients with a negative azuresin test did not secrete free acid when given the augmented

TABLE I.—*Comparison of Maximal Acid Output with Azure A Excretion*

Azuresin Test		Augmented Histamine Test					
		"Free" Acid Present (No. of Patients)			No "Free" Acid (No. of Patients)		
Azure A Excreted (mg./2 hr)	No. of Patients	Total	M.A.O. mEq/hr		Total	Lowest *p*H	
			>2·5	<2·5		3·5–6	>0·6
>0·6	66	66	66	—	—	—	—
0·3–0·6	1	1	—	1	—	—	—
<0·3	13	3	2	1	10	6	4

histamine test. There were thus three false-negative results among 11 patients. However, two of these false-negatives occurred in marked hyposecretors: 3.26 mEq total HCl/hour and 1.28 mEq total HCl/hour. The other false-negative (6.80 mEq total HCl/hour) was attributed to inadequate gastric emptying (pyloric stenosis). One patient showed borderline azure A excretion (0.3–0.6 mg. azure A/2 hours), the maximum acid output being low—1.10 mEq total HCl/hour.

Card and Sircus (1958) defined achlorhydria as "that state of gastric secretion in which under the conditions of the (augmented histamine) test the *p*H of the secretion fails to fall below 6.0 following stimulation." If these criteria are employed, all 66 patients with a positive azuresin test had evidence of acid secretion, but only 4 out of 13 patients with a negative test were achlorhydric (Table I). These findings show that, while a positive azuresin test can be regarded as a satisfactory indication of gastric acid secretion, a negative test is by no means indicative of achlorhydria. Our data also show that most of the false-negative results occur in patients with very low levels of secretion. Following the suggestion of Marks and Shay (1960), we decided to use the arbitrary M.A.O. of 2.5 mEq total HCl/hour to determine whether it may not provide a more suitable method of interpretating the results of the test (Table I).

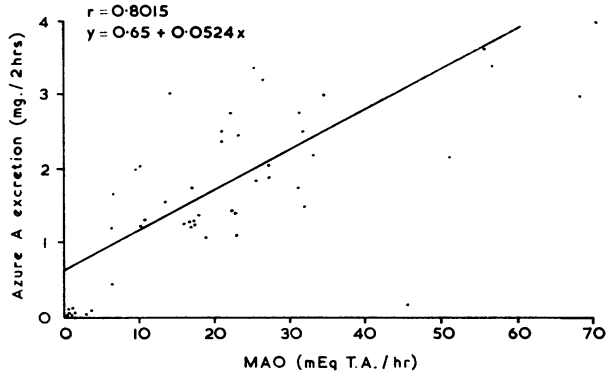
The 66 patients with a positive azuresin test had a M.A.O. >2.5 mEq total HCl/hour, and 12 out of 14 patients who secreted less than 0.6 mg. of azure A had an M.A.O. <2.5 mEq total HCl/hour.

Our data suggest that the azuresin test may be a useful method of separating patients with marked hypochlorhydria from those with an M.A.O.>2.5 mEq total HCl/hour.

In 47 patients in this group we studied the quantitative relationship between azure A excretion (mg. azure A/2 hours) and acid secretion (as mEq free HCl/hour and mEq total HCl/hour—MA.O.) as determined by the two tests. A significant correlation ($P<0.001$) was found between the maximum acid output (as mEq free HCl/hour) and the amount of azure A excreted ($r=0.8084$) and also between the M.A.O. and the amount of azure A excreted ($r=0.8015$). There was no significant difference between the two coefficients of correlation ($0.7>P>0.6$).

The Chart shows the M.A.O. plotted against the amount of azure A excreted. The regression line which gives the best fit is shown by:

$y=0.65+0.0524\ x$ (standard deviation from regression 0.67)



Relationship between azure A excretion during the azuresin test and the maximal acid output.

Despite the significant quantitative relationship between the two variables, the large error of the estimate (about 59%) indicates that the quantitative measure of azure A excretion was not a reliable index of the maximum acid output.

The second group consisted of 20 patients. It was found that nine patients who had a positive azuresin test secreted free acid after histamine stimulation. Table II shows that seven out of eight patients with a negative azuresin test did not secrete free acid when checked by intubation. The false-negative result occurred in a patient with a low M.A.O.—2.0 mEq HCl/hour (the *p*H of the gastric contents was, in three samples, 4.6, 3.5, and 6.0 respectively). In three patients equivocal results were obtained—two secreted free acid (M.A.O. 1.72 and 3.75 mEq total/hour), the other had an M.A.O. of 0.26 mEq total HCl/hour (no free acid).

TABLE II.—*Comparison of Maximal Acid Output (MAO) with Azure A Excretion (Patients with Partial Gastrectomy)*

Azuresin Test		Augmented Histamine Test					
		"Free" Acid Present (No. of Patients)			No "Free" Acid (No. of Patients)		
Azure A Excreted (mg./2 hr)	No. of Patients	Total	M.A.O. mEq/hr		Total	Lowest *p*H	
			>2·5	<2·5		3·5–6	>0·6
>0·6	9	9	9	—	—	—	—
0·3–0·6	3	2	1	1	1	—	1
<0·3	8	1	—	1	7	4	3

Using the arbitrary M.A.O. of 2.5 mEq total HCl/hour, we have nine patients with a positive azuresin test and nine with an M.A.O.>2.5; of all patients who excreted less than 0.6 mg. of azure A/2 hours only one had an M.A.O.>2.5 mEq.

Discussion

A review of the available literature on azure A (Carneiro de Moura, 1961) indicates that 144 false-negative results (9.8%) and only 28 false-positive results (1.9%) were detected in 1,462 tubeless analyses which were checked by gastric intubation. The most likely cause of this high proportion of false-negative tests seems to be inefficient gastric stimulation by caffeine benzoate (Poliner *et al.*, 1957; Denborough *et al.*, 1958). Segal *et al.* (1959a, 1959b), after the successful clinical trials by Kirsner and Ford (1955), replaced caffeine with the histamine isomer betazole hydrochloride as the gastric stimulator in tubeless analysis. Betazole hydrochloride is a better stimulant than caffeine benzoate (Segal *et al.*, 1959b; Bock and Witts, 1961); the incidence of false-negative results is 6% in the series reported. We attempted to reduce the incidence of false-negative results and to evaluate the azure A test as a possible quantitative test of acid secretion, using maximal histamine stimulation, in both the tube and the tubeless method of gastric analysis. This stimulation upsets the patients much less than the tube, and can be used in the out-patient department of any gastroenterology unit with trained nurses.

In a group of 80 patients (group 1) we registered no false-positive tests and only three false-negative results (less than 4%). These false-negative tests occurred in patients with very low output (<10 mEq CHl total/hour). We think that this low incidence of false results can be due to the use of maximal histamine as gastric stimulant in the tubeless method. From the evidence presented the azuresin test with maximal histamine stimulation may be regarded as a satisfactory method of differentiating patients with achlorhydria and marked hyposecretion from patients with an M.A.O.> 2.5 mEq total HCl/hour. We agree with Marks and Shay (1960) that it is necessary to employ the augmented histamine test to establish the presence of achlorhydria among these patients with a negative or equivocal azuresin test. Our data indicate that a positive test excludes the diagnosis of pernicious anaemia and that a negative test is strong evidence against the diagnosis of duodenal ulcer.

Donovan and Tighe (1955) have reported that tubeless gastric analysis could be a quantitative one. They used the original quinine carbacrylic resin and found a good correlation between the two-hour urinary quinine excretion and the hydrochloric acid aspirated in the conventional gastric analysis. It should be pointed out, however, that the data used in their correlation were pooled, in that mean values for ranges of free acid were plotted against mean concentration values for urinary quinine. Galambos and Kirsner (1955), using the azure A resin with betazole as a stimulus, could find no correlation between the quantity of azure A in the urine and the quantity of hydrochloric acid in the gastric content. Marks and Shay (1960) compared the azuresin test with the augmented histamine test in 85 patients and reported a significant correlation (P<0.001) between the maximum histamine response (M.H.R.=mEq total HCl/30 minutes) and the amount of azure A excreted. In our series of 47 patients the coefficient of correlation was 0.802 and the error of estimate 59%; these constants being significantly different from the constants of Mark's and Shay's series, r=0.68 and CV=76%. For us these differences can be largely attributed to the use of the same stimulant in both tests and to the use of M.A.O. as a correlation parameter instead of M.H.R. Nevertheless, the still large error of estimate indicates that no one test can be used to predict satisfactorily the results of the other test in the majority of patients.

Various authors have reported that tubeless gastric analysis cannot be considered reliable in gastrectomized patients, since both false-positive and false-negative results may occur (Shay *et al.*, 1954; Poliner *et al.*, 1957; Denborough *et al.*, 1958; Mortimer, 1959; Duret and Jacobs, 1959; de Rubertis *et al.*, 1960). We investigated 20 patients gastrectomized for duodenal ulcer; in these there were no false positives and only two false negatives (5%); in this case the M.A.O. was 2.0 mEq total HCl/hour. In three cases of jejunal ulcer after gastrectomy the excretion of azure A was higher than 0.6 mg./2 hours in three consecutive azuresin tests carried out for each patient. Although the number of cases studied is insufficient for statistical evaluation, our data indicate that the azure A test with maximal histamine stimulation may prove a practical and valuable method for the study of acid secretion in gastrectomized patients whenever it is necessary to obtain repeated information about gastric acid secretion and it is not possible to carry out an intubation.

Summary

The results of 100 tubeless gastric analyses using the dye azure A as indicator are reported. In these tests "maximal" histamine stimulation (0.04 mg. of histamine acid phosphate per kg. body weight) was used instead of caffeine benzoate or betazole hydrochloride. The data of the tubeless test were compared with the findings after augmented histamine stimulation. No false-positive tests were detected; the few false-negative results occurred in patients with a low maximal acid output.

Statistical examination of the results of both tests in a group of 47 patients showed a significant positive linear correlation between the M.A.O. and the amount of azure A excreted. However, the large error of the estimate (59% as coefficient of variation) indicates that the results of the azuresin test cannot be used to predict the M.A.O. satisfactorily in any given individual.

The tubeless method of gastric analysis, using azure A maximal histamine stimulation, appears to be of particular value in assessing acid secretion when it is impossible to carry out the intubation procedure. In these instances, azuresin with maximal histamine stimulation may prove more informative and accurate than the usual test with caffeine or betazole as gastric stimulant.

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THIRTY-EIGHT CASES OF THE GUILLAIN-BARRÉ SYNDROME: AN IMMUNOLOGICAL STUDY

BY

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The Guillain–Barré syndrome (Guillain, Barré, and Strohl, 1916) has numerous alternative names—for example, acute peripheral neuritis, acute infective or post-infective polyneuritis, acute polyradiculitis, acute encephalo-myeloradiculitis, etc. This confusion of nomenclature reflects our ignorance of the aetiology of this condition or group of conditions, and justifies the continued use of the eponymous title.

When Waksman and Adams (1955, 1956,), by the inoculation into rabbits, guinea-pigs, and mice of homologous or heterologous peripheral nerve tissue with suitable adjuvant, produced "experimental allergic neuritis" an experimental model became available which to some extent resembles the Guillain–Barré syndrome. These authors suggested, therefore, that the possibility of this syndrome being an "autoimmune disease" should be investigated.

Patients and Methods

Guillain–Barré Syndrome.—Thirty-eight cases have been studied (22 males and 16 females). With one exception (a patient who died before the commencement of this investigation but whose clinical history and post-mortem findings were typical) all of them were interrogated and examined personally; many had electromyographic and nerve-conduction studies, and a few had motor-point muscle biopsies (Coers and Woolf, 1959). The diagnostic criteria comprised an afebrile, acute, or subacute neurological illness which resulted in flaccid weakness, sensory impairment, and absence of deep reflexes. The findings were usually symmetrical in all four limbs, and cranial-nerve palsies were also present in some cases. A less constant feature was an antecedent infective illness, usually of "influenzal" type. Some patients had a period of apparent well-being between the initial illness and the onset of the neurological syndrome. Each diagnosis was confirmed by one or more of a panel of consultant neurologists. In 30 cases there was the characteristic finding of a raised protein level in the cerebrospinal fluid (more than 50 mg./100 ml.) with a normal cell count (albumino-cytological dissociation). Reasons are given later for making the diagnosis of Guillain–Barré syndrome in eight other cases who differed from the main group only in having cerebrospinal fluids that were entirely normal to routine laboratory testing.

Other Types of Neuropathy (56 cases).—Details of these, together with those of the Guillain–Barré syndrome, are given in Tables I and II. The "chronic" neuropathies were characterized by a more insidious onset of symptoms and by either getting no better or even worse after an arbitrary period of six months. With the exception of the diabetic neuropathies all these cases were also personally examined. The pathogenesis of diabetic neuropathy is by no means fully established, and for the purpose of this survey one symptom—usually pain or sensory disturbance in the legs—and one sign—for example, loss of ankle-jerks, or some sensory loss, together with the diabetes—were regarded as minimal diagnostic criteria.

TABLE I.—*Incidence of Antibodies to Nervous Tissue in Different Types of Peripheral Neuropathy*

Type of Neuropathy	C.F. Titres		
	Negative	1 : 8	>1 : 8
Gullain-Barré syndrome	19	7	12
Fully recovered cases of G.-B. syndrome	8	0	0
Chronic	4	2	4
Recurrent	4	0	0
In "collagen disease"	1	2	1
Carcinomatous	3	0	1
Diabetic	17	1	1
Miscellaneous (see Table II)	4	2	1
Total	41	7	8

TABLE II.—*Antibodies to Nervous Tissue in Various Neuropathies*

Diagnosis	No. of Cases Studied	C.F.T.
Alcoholic polyneuritis	1	Negative
Steatorrhoea polyneuritis	1	,,
Peroneal muscular atrophy	1	,,
Leprosy	1	,,
Haemochromatosis	1	1 : 16
Hypertrophic polyneuritis	1	1 : 8
Sensory neuropathy	1	1 : 32

Other Conditions.—A total of 1,218 other subjects were examined serologically, but as it was not possible to examine all these patients personally, they were classified as follows, mainly according to the clinical notes provided with the specimens: (*a*) 183 normal subjects: healthy medical and laboratory staff and blood donors; (*b*) 608 patients whose sera were referred for routine virological (or bacteriological) testing; (*c*) 59 general medical cases—for example, ischaemic heart diseases, cardiac arhythmias—in whose aetiology "allergic" mechanisms were not suspected; (*d*) 198 cases of neurological disease, specifically excluding the demyelinating diseases of the central nervous system (cases of suspected or proved poliomyelitis and viral meningitis were included in this group); (*e*) 86 cases of demyelinating disease, consisting of disseminated sclerosis (17 cases), retrobulbar neuritis (11 cases), and acute encephalitis (58 cases): apart from some cases of "acute encephalitis" alternative diagnosis had been as

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