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TUBELESS GASTRIC ANALYSIS

BY

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During 1950–3 Segal and his colleagues (Segal, 1951 ; Segal, Miller, and Morton, 1950, 1952, 1953 ; Segal, Miller, Morton, and Young, 1950) have published the results of their investigations of the hydrochloric acid content of the gastric juice by an original method which does not require the passing of a Ryle's stomach tube. This elegant technique depends upon the power of the hydrogen cation (H^+) of the free hydrochloric acid of the gastric juice, at a *p*H of 3 or less, to displace the quininium cation (QH^+) from a quininium-resin indicator compound ; the quinine hydrochloride now formed is rapidly absorbed from the small intestine and quinine is excreted in the urine where it appears within 15 minutes of the ingestion of the resin ; the quinine is readily estimated quantitatively in the urine by a simple method based on its fluorescence in ultra-violet light.

Our interest in Segal's method arose from a brief note in the *Lancet* (Segal *et al.*, 1952) reporting the second national cancer conference in the U.S.A. This is apparently the only reference so far in the British literature. The details in the original papers suggested to us that the technique had such potential value that we decided to investigate the problem also.

Material and Methods

Segal's quininium-resin indicator is available in America as a commercial product under the trade name of " diagnex-QH " or " diagnex," but it is not yet sold in Britain. Although the method of preparation was not given completely in any of Segal's papers, one of us was able to prepare an equivalent product using the quinine hydrochloride and " amberlite XE–96 "* which we could obtain in this country.

The co-operation of the clinicians of this area was obtained for an investigation by this new method on every patient to whom the alcohol test-meal was given. In addition to this consecutive series, we included subjects who were likely to produce anomalous results: this is the chief reason why a large percentage of the results requires special consideration.

*Given to us by British Drug Houses and by Charles Lennig and Co.

Technique

A careful preparation of the patient is essential to yield reliable results. He should have taken no quinine (and hence no similar test) in the week before the test. In the two days immediately before the test he should have no treatment containing aluminium, barium, calcium, magnesium, kaolin, iron, or vitamins. An adequate state of hydration is ensured by the subject drinking about a pint of bland liquid in the evening before the test.

The patient fasts overnight. The test begins immediately on waking, or at a later hour, by the patient emptying the bladder completely and discarding the urine. The stomach (and kidneys) is stimulated by drinking a glass of water containing 250 mg. of caffein and sodium benzoate, 100 ml. of 7% alcohol, or a cup of tea or coffee without milk or sugar. About an hour later the bladder is again emptied and this urine is saved to act as a control for the absence of quinine and other fluorescent substances from the urine. The main test begins when the subject swallows 2 g. of quininium-resin indicator granules (=36 mg. quinine) suspended in several small volumes of water or alcohol (the granules are quite tasteless); 0.5 mg. of histamine should be injected subcutaneously at the same time if achlorhydria has to be proved. About one, and exactly two hours later, the bladder is emptied again and the urines collected in dark brown bottles: if micturition is not possible at the exact minute, a glass of water is given to drink and another attempt made in five minutes, making adjustments later in the calculations. The subject then resumes his usual mode of living.

The three urine samples are sent to the laboratory where the quinine content is estimated by a method based on the fluorescence in ultra-violet light. (Details of the method for measuring the quinine and for preparing the quininium-resin indicator are given in the technical appendix.)

We usually performed the tubeless analysis on the morning after the alcohol test-meal. If free hydro-

chloric acid was found in the alcohol test, the simple tubeless technique was used, but if achlorhydria had been found, histamine was usually added to the tubeless method. In a few instances we were able to combine the alcohol and the quininium-resin tests into a single examination.

Results

Two hundred and thirty-three tests were made on 200 subjects but 20 had to be discarded because one of the duplicate tests was unsatisfactory (Table I).

TABLE I
RESULTS OF 233 TESTS

Duplicate Tests Requested	No. of Subjects	No. of Tests
Free hydrochloric acid detected in the gastric juice by the usual alcohol or alcohol plus histamine test-meals	115	134
Achlorhydria detected by the usual alcohol or alcohol plus histamine test-meals	65	79
Tests which had to be excluded because of failure in one test:		
(i) Unsatisfactory alcohol test-meal	13	13
(ii) Unsatisfactory quininium-resin indicator tubeless analysis	7	7
Totals	200	233

The failures of the alcohol test-meal were more numerous and more difficult of correction as the fault lay in the subject (gastric haemorrhage, persistent vomiting, refusal to swallow tube, etc.). The tubeless analysis, by contrast, had four failures arising in nursing errors (administration of resin, collection of urine), and these tests were repeated successfully at a later date. In three patients, however, difficulties in the control of micturition made the test impossible.

In the group whose gastric juices contained free hydrochloric acid according to the alcohol or alcohol plus histamine test-meal the corresponding quininium-resin indicator test, with histamine if necessary, showed a urinary excretion in the first two hours of 27 μg. or more of quinine (Fig. 1 and Table II). Seven patients did not excrete so much

TABLE II
QUININIUM-RESIN TUBELESS ANALYSIS TESTS IN SUBJECTS IN WHOM ALCOHOL TEST-MEAL INDICATED PRESENCE OF FREE HYDROCHLORIC ACID

Total No.	Subjects	Tests	Quinine Excreted	
			First Test (μg.)	Second Test (μg)
	115	134		
Agreement obtained with initial test (i.e., >27 μg.)	99	108		
Disagreement (i.e., low quinine excretion)			9	35
(i) Discrepancies of known cause:	106		13	9
Discrepancy removed by repeating tubeless analysis with addition of histamine	7	14	18	63
			20	55
			20	91
			22	42
			22	30
Inadequate renal function to excrete the absorbed quinine	3	4	10	
			2	6
(ii) Discrepancies where cause can be suggested:			20	
Tests without histamine where there was no opportunity to repeat as in (i) above ..	2	2	20	
Patient had periods of achlorhydria as found also by repeated tests with alcohol plus histamine	1	1	15 20	
(iii) Discrepancies for no apparent reason:				
Agreement obtained with second tubeless analysis without histamine ..	1	2	15	29
Agreement obtained with second tubeless analysis, both tests including histamine	1	-	22	140
Remaining discrepancies ..	1	1	11	

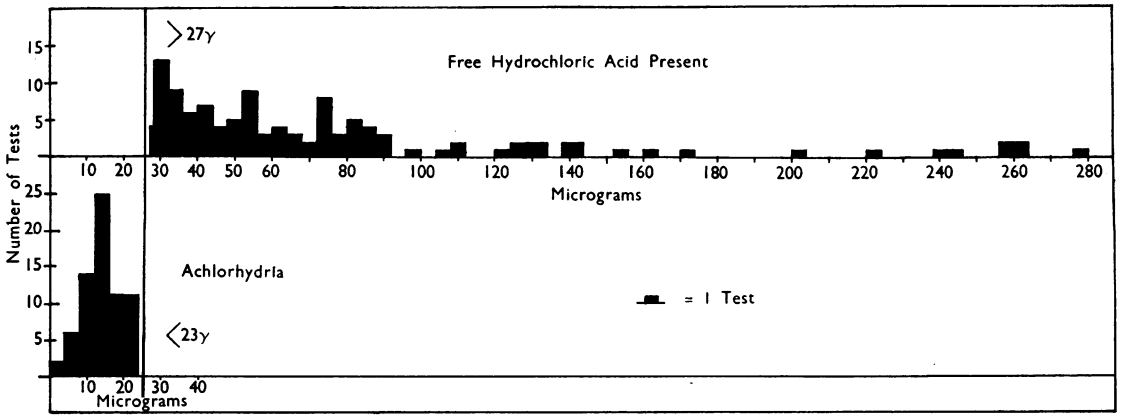

FIG 1.—The incidence of the amounts of quinine excreted in the two-hour period of the tubeless gastric analysis test which were regarded as satisfactory, classified according to the alcohol test-meal results.

quinine with the simple test but did so when the test was repeated with an injection of histamine; a further two patients had their operation performed before the test could be repeated with histamine. Two others had a satisfactory excretion at a second test, with no indication why the first test was low. Four low excretions were attributed to defective renal function. Another low excretion occurred in a patient who showed achlorhydria in some of her alcohol test-meals. The remaining low excretion was not explained.

The highest urinary excretion of quinine in the satisfactory tests of the achlorhydric group was 23 μg. (Fig. 1 and Table III). Higher excretions were found where the preparation of the patient had been inadequate. A high output was given by a patient with a partial gastrectomy whose achlorhydric alcohol test-meal had not included histamine: another patient with a partial gastrectomy had two high quinine excretion tests and two alcohol tests plus histamine tests with achlorhydria.

TABLE III

QUININIUM-RESIN TUBELESS ANALYSIS TESTS ON SUBJECTS IN WHOM ALCOHOL TEST-MEAL INDICATED PRESENCE OF ACHLORHYDRIA

Total No.	Subjects	Tests	Quinine Excreted	
			First Test (μg)	Second Test (μg.)
	65	79		
Agreement obtained with initial test (i.e., <238)	56	62		
Disagreement (i.e., high quinine excretion):				
(i) Discrepancy due to interference by therapy which was continued to within 24 hours of the test:	6	12		
Aluminium silicate	1	2	37	14
Kaolin	1	2	180	18
Calcium, etc. ("casilan")	1	1	30	
Barium meal	1	2	32	23
Vitamin C+iron	1	2	35	18
Iron	1	2	35, 100	21
(ii) Discrepancy due to interference by quinine: Tubeless analysis test spoiled and second attempted two days later; error detected in laboratory by fluorescence of control urine	2	1	65	3
(iii) Discrepancies in partial gastrectomy patients: Alcohol test did not include histamine and may be the test in error	1	1	38	
Two alcohol tests with histamine indicated achlorhydria	1	2	37	28

Discussion

The criterion of the presence of free hydrochloric acid in a gastric juice is usually the colour change in congo red, thymol blue, or Topfer's reagent. The exact *p*H at which this change occurs is different for the three indicators but it is about *p*H 3.

The quininium-resin indicator consists of a resin (its composition has not been disclosed by the manufacturers but it is probably of the formula R-COOH where R is of acrylic nature) in which the quininium ion replaces the hydrogen of the original carboxylic acid group. At a sufficient concentration of hydrogen ions the quininium ion can be replaced by the hydrogen ion according to the equation:

$$R-\left(C\begin{matrix}O\\ O^-(QH)^+\end{matrix}\right)_n + nH^+Cl^- \rightarrow R-\left(C\begin{matrix}O\\ O^-H^+\end{matrix}\right)_n + n(QH)^+Cl^-$$

In vitro experiments in our own and Segal's laboratories have shown that a *p*H of 3 or less is required for the appreciable displacement of this equation from left to right. Thus the quininium-resin indicator will be affected by a gastric juice containing free hydrochloric acid by the usual standards of clinical biochemistry.

The only source of hydrogen ions other than hydrochloric acid likely to be found in gastric juice is lactic acid. Although lactic acid in aqueous solutions in concentrations such as found in gastric carcinoma may yield a sufficiently low *p*H, *in vitro* tests made by mixing the same amount of lactic acid with the gastric juice of patients with pernicious anaemia showed that the buffering power of the mucus and other gastric juice constituents was so great as to increase the *p*H values well above the critical level.

Nevertheless, even true achlorhydric subjects manage to excrete some quinine. This is due to the hydrogen ion being but one of several ions which can displace the quininium. These ions may be divided into two groups. There are the ions with a weak quininium-displacing action (e.g., sodium, potassium) which are normally present in the secretions of the stomach and small intestine and which are responsible for the small quinine excretions of the achlorhydrics. The other group consists of those ions with a displacing power greater than that of the hydrogen ion but which are not normally present in the diet and secretions in amounts sufficient to cause any error. The most important members of this group are aluminium, magnesium, calcium, barium, iron, and kaolin. It will be noted that these are frequently included in the medicines used in the treatment of patients with gastric disorder or anaemia who are the very subjects on whom the test will be most frequently applied. Our experience of anomalous results in achlorhydric subjects has shown that these medicines must be discontinued for at least two days before the test if this potential source of dangerous

interference is to be removed ; discontinuation for a single day is not enough. Another source of error related to this problem is the surreptitious taking of these sedatives by the patient who has been treated with them for so long that he is afraid to stop the medication for even the two days' interval essential for the test. One anomalous result of a high quinine excretion puzzled us for some time until we discovered that the patient had evaded the ward restrictions to obtain a single dose of aluminium silicate on the day before the test, and we have assumed that there was sufficient aluminium remaining in the stomach on the following morning to separate the quininium from the resin. Luckily this is the only type of error found in achlorhydrics.

There are several reasons why a subject with free hydrochloric acid in the gastric juice may fail to excrete a large amount of quinine (i.e., over 27 μg.). No subject should be diagnosed as achlorhydric unless an adequate gastric response has been assured by an injection of histamine: this is equally true of the alcohol test-meal. Theoretically a diagnostic error could arise if delayed pyloric opening or defective absorption from the small intestine prevented the adequate absorption of the quininium which had been adequately separated, but we have not encountered any case within our series with an error of this type. We did find three patients in whom a defective renal function prevented an adequate excretion of quinine although the urine volumes were within normal limits. Following our early experiences of this error we examined patients with raised blood urea values, but we have been unable to determine a critical level for the blood urea or renal function beyond which the test is bound to fail: we have been hindered by the very high incidence of achlorhydria (to the alcohol and histamine test) among patients with raised blood urea values. Finally, we have seen three patients in whom the test could not be attempted because of an inability to pass urine on demand. A Ryle's tube is still to be preferred to the tubeless gastric analysis plus a catheter.

The absence of intubation is not the only benefit to the patient. If he is considered sufficiently trustworthy to adhere to the restrictions imposed by the proper preparation for the test and sufficiently intelligent to follow the printed instructions for the actual test, there is no reason why he should not carry out the whole procedure in the privacy of his own home at a time which will cause him no loss by absence from his employment. If a high quinine content is found in the urines by the laboratory, the test is concluded. If the quinine level is not high enough to indicate the presence of free hydrochloric acid the test must be repeated with histamine after a week. Even then he can carry out the first part of the test at home, arriving at the laboratory at the end of the first hour to pass the urine specimen, swallow the granules, and receive the injection of histamine. The urine passed after another hour can be tested within five minutes: if the quinine content is 20 μg. or over, the presence of free hydrochloric acid is confirmed and the patient may be dismissed, but if the quinine content is below 20 μg., the other urine specimen will be necessary.

The laboratory benefits too. The estimation of the quinine requires about eight minutes' working time. This is longer than needed to test a series of bottles with Topfer's reagent and to perform a Gunzberg test, but for the alcohol technique one must also take into consideration the time and labour for the preparation of the Ryle's tube, syringe, collecting bottles, etc., and the technician's time for the aspiration of the multiple specimens when the laboratory undertakes this test on out-patients.

Future modifications of the tubeless technique may simplify the laboratory procedures by the incorporation of a coloured or radio-active group in place of the quininium.

It is to be regretted that the test cannot indicate the amount of free acid, i.e., milliequivalents per minute, present in the stomach juice. High quinine excretions (over 100 μg. in two hours) were encountered only in patients who had a high acid curve in the alcohol test, yet other patients with identical high acid curves had quinine excretions as low as 30 μg.

Complete agreement between the results of the alcohol and the quininium-resin techniques should not be expected. Watkinson and James (1951) found with their 24-hour gastric analysis that while the patients with pernicious anaemia maintained a complete achlorhydria, free hydrochloric acid was detected in no less than 10 out of 12 patients with peptic ulcer who had an apparent achlorhydria to the usual alcohol and histamine test-meal, including one patient who had such an achlorhydria in seven tests. They attributed this difference partly to the nearer approach by their method to physiological conditions. Similarly, we feel that a fasting patient who faces a cup of tea instead of three feet of Ryle's tubing is more likely to be in a physiological state and therefore liable to secrete free hydrochloric acid. Thus, in the two patients with partial gastrectomy with an alcohol achlorhydria and a high quinine output, we cannot con-

firm the resin test to be in error until there have been further investigations.

Provided that a subject has been prepared properly to exclude the recognized causes of discrepancy and error, that histamine has been injected, and that errors due to faulty absorption and excretion can be excluded, the quininium-resin indicator tubeless gastric analysis is capable of indicating the presence or absence of free hydrochloric acid in the gastric juice in the large majority of individuals. Using the criteria that an excretion of 27 μg. or over indicates the presence of free hydrochloric acid, and 23 μg. or less indicates achlorhydria, in a series of 233 tests on 200 persons there was an inexplicable discrepancy from the results of the alcohol test-meal in only six instances, and even this small number includes three tests on two partial gastrectomy patients where we cannot be certain that the quininium test did not, in fact, yield the true result. The corresponding critical levels in Segal's papers were 25 and 15 μg. respectively.

Conclusions

The quininium-resin indicator technique for the detection of the presence or absence of achlorhydria, which does not subject the patient to intubation, has proved to be a reliable method of investigation.

Errors may arise from several sources. Their nature has been indicated so that precautions may be taken by which the majority may be excluded.

The technique cannot measure the amount of free hydrochloric acid which is present.

The test will find its greatest use as a screening test in the investigation of possible achlorhydric subjects among patients with anaemia and suspected gastric carcinoma.

TECHNICAL APPENDIX

Preparation of the Quininium-resin Indicator*

Rubber and plastic materials must be excluded as they may contain fluorescent substances.

The following quantities make a convenient batch size, amounting to 20 test doses.

"Amberlite XE-96" (B.D.H.), 40 g., is placed in a 2-litre flask, 800 ml. of 0.1 *N* NaOH solution added, stoppered, shaken vigorously for a few minutes, and allowed to stand overnight. The granules are collected on a large No. 1 Whatman filter paper and washed with 2 litres of distilled water. The filter tip is pierced and the granules are washed into another flask with 800 ml. of 0.1% aqueous solution of quinine hydrochloride. The solution is shaken repeatedly and allowed to stand overnight. The satisfactory product is reached when the supernatant fluid has only a slight fluorescence. It is filtered on to a No. 1 Whatman filter paper, washed with 2 litres of distilled water, blotted gently with filter paper, and then dried in air at a temperature not exceeding 30° C. The resin is stored in a dark brown bottle.

Each 2 g. dose of resin contains 36 mg. of quinine.

* Since submitting this paper "diagnex" has become available from Messrs. E. R. Squibb and Sons, 17-18 Old Bond Street, London, W.1.

Estimation of the Quinine in Urine

Each specimen of urine is diluted to 300 ml. with tap water, and 30 ml. transferred to a separating funnel and made alkaline with 0.5 ml. of *N* NaOH solution. Ether, 15 ml., is added, the funnel stoppered and shaken vigorously for 15 seconds. Any emulsion may be cleared by adding a few drops of 95% alcohol and shaking for a further five seconds. Then 8.2 ml. of the ether is transferred to another funnel containing 5 ml. of 0.1 *N* sulphuric acid, shaken vigorously for 15 seconds, the layers allowed to separate, and the lower acid run into a tube of standard diameter. The fluorescence in ultra-violet light is compared with that of similar tubes containing known concentrations of quinine.

The equivalent to half of the ether, to compensate for the 90% extraction of quinine, is taken as 8.2 ml.

Segal has designed a cylindrical separating funnel graduated at 8.2, 30.0, and 45.5 ml., which allows all the extractions to be performed in a single funnel. We can confirm that it simplifies the procedures.

A stock standard of quinine (10 mg. per 100 ml.) is prepared by dissolving 12 mg. of quinine sulphate, $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$, in 100 ml. of 0.1 *N* sulphuric acid. This standard keeps indefinitely. Working standards are prepared every few days by further dilutions with 0.1 *N* sulphuric acid. Standards should be chosen at intervals small enough to allow the worker to be certan of his reseults to within 1 μg. in the range below 20 μg. The values chosen in this Laboratory were 0, 5, 7, 10, 12, 15, and 20 μg.

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