B-Glucuronidase activity of gastric juice in gastric carcinoma

R. I. RUSSELL AND C. WATTS¹

From the University Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow, and the Department of Biochemistry, Western Infirmary, Glasgow

Carcinoma of the stomach is often not diagnosed until the disease has spread extensively. Attempts have thus been made to find additional methods which may be of value in earlier diagnosis. A test of gastric secretory function would be of great value if it were able to show distinctive changes in gastric carcinoma but so far this has not been achieved (Hitchcock and Aust, 1955; Fischermann and Koster, 1962; Gilbertsen and Knatterud, 1967). Electrophoresis of gastric juice proteins has shown minor quantitative changes which are of little diagnostic importance (Piper, Stiel, and Builder, 1963b).

More recently, several workers have turned their attention to the estimation of non-proteolytic enzymes in gastric juice and their relation to disease processes. Schenker (1959) measured lactic dehydrogenase in gastric juice and found raised levels in some cases of gastric carcinoma. These findings were confirmed by Levitan, Golub, and Zetzel (1960) and Smyrniotis, Schenker, O'Donnell, and Schiff (1962), although in the latter case, tests were performed only on patients exhibiting achlorhydria. Piper, Macoun, Builder, and Fenton (1963a) measured a series of non-proteolytic enzymes in the gastric juice, and found that for two of them, lactic dehydrogenase and B-glucuronidase, the levels were raised in some cases of carcinoma of stomach. The former enzyme was present in normal amounts in anaplastic tumours although elevated in most adenocarcinomas; thus attention was focused on the latter enzyme, B-glucuronidase, as being of possible diagnostic value in gastric carcinoma.

B-Glucuronidase is a lysosomal enzyme involved in the hydrolysis of B-D-glucosiduronic acid (de Duve, Pressman, Gianetto, Waftiaux, and Appelmans, 1955; de Duve, 1961). It is widely distributed throughout animal tissues. High levels of B-glucuronidase were found in homogenates of human gastric tumour tissue by Fishman and Anlyan (1947) and in several body fluids associated with

various tumours. The presence of increased amounts in gastric tumour tissue was shown by Fishman, Baker, and Borges in 1959.

The aims of this investigation were (1) to develop a simplified and reproducible technique for the collection of gastric juice for estimating B-glucuronidase. (2) To determine the gastric juice levels in patients with gastric carcinoma. (3) To assess the value of such an estimation as a possible diagnostic aid in gastric carcinoma, and (4) to compare this test with conventional methods of diagnosis.

CHEMICAL METHODS

The total soluble protein was measured by the method of Lowry, Rosebrough, Farr, and Randell (1951) and the results expressed as milligrams per 100 ml. B-Glucuronidase activity was measured by a modification of the method described by Talalay, Fishman, and Huggins (1946) and the results are expressed as units/100 ml. Gastric juice, 0.2 ml, was added to 0.8 ml of 0.1 M acetate buffer at pH of 4.5, and placed in a water bath for five minutes at 37°C. Of 0.5 M phenolphthalein-non-B-glucuronic acid solution at pH of 7.0, 0.1 ml was added and incubated for 24 hours. The reaction was then stopped by adding 4 ml of M-glycine buffer at pH 10.45. A control solution was prepared containing gastric juice and acetate buffer, and substrate was added after the glycine buffer. After centrifugation to remove any precipitated protein, the liberated phenolphthalein was assessed by comparison with the control in a spectrophotometer. The amount of phenolphthalein produced was calculated from a calibration curve using pure phenolphthalein standards. From this method a unit of B-glucuronidase activity was defined as the amount of enzyme which liberated 1 µg of phenolphthalein per hour at 37°C. The B-glucuronidase activity of the gastric juice was expressed as a specific activity relative to the protein content of the gastric juice sample, and measured as units/mg of soluble protein.

INITIAL STUDIES

Experiments were initially conducted to determine possible factors affecting the secretion and destruction of B-glucuronidase in the gastric juice. The factors

¹Present address: Department of Clinical Biochemistry, Glasgow University, and Veterinary Hospital, Bearsden, Glasgow.

investigated were salivary contamination, contamination with blood and bile, intragastric pH, parasympathomimetic stimulation, and repeated alkalization.

The enzyme is widely distributed in tissues and secretions, including saliva (Talalay *et al*, 1946; Fishman and Anlyan, 1947). The level of B-glucuronidase was measured in the saliva in a series of control patients, together with patients suffering from gastric and duodenal ulcers, proven gastric carcinomas, and miscellaneous tumours (lung and colon). The results of this study are shown in Table I. They confirm that saliva does contain a quantity of the enzyme but that there is no significant difference between the disease groups. However, it was concluded that steps should be taken to avoid salivary contamination of gastric juice. The patients were simply asked to expectorate saliva during the collection of gastric juice rather than salivary secretion being suppressed by the use of drugs such as atropine, in the absence of accurate information as to the possible effect of such drugs on the gastric production of B-glucuronidase (Piper *et al*, 1963a).

Specimens which contained appreciable amounts of blood or bile were not accepted, as B-glucuronidase activity has been shown to be inhibited by the presence of red blood cells and by bile (Piper *et al*, 1963a). In addition, bloodstained specimens produce high values for protein content which influence the B-glucuronidase values expressed as specific activity. Specimens from 10 (7.5%) of the total number of cases tested were discarded due to bile or blood contamination. Of these 10 patients, two suffered from gastric carcinoma, two from carcinoma of the colon, and the remainder from duodenal ulcer.

Initial studies designed to find the relationship between the acidity of the gastric juice and B-glucuronidase activity showed that acid denaturation of the enzyme occurred at pH 3.8 and has been found to be irreversible. The enzyme could therefore be measured either only on those basal samples of gastric juice which have a pH above this level, or alternatively the pH of the gastric juice could be raised by intragastric neutralization. As the former method may exclude up to 30% of gastric carcinomas (Bockus, 1963; Gilbertsen and Knatterud, 1967), a technique of intragastric neutralization using sodium bicarbonate modified from the method developed by Piper *et al* (1963a) was utilized.

The effect of repeated alkalization of gastric juice using sodium bicarbonate was studied, and reproducible results were obtained after two successive neutralizations. Any further alkalization did not alter enzyme levels.

The effect of parasympathomimetic stimulation using carbachol was studied in a number of patients and no effect on enzyme levels was detected.

DESIGN OF CLINICAL INVESTIGATION

The following clinical method was devised as a result of these initial studies. After an overnight fast, a nasogastric tube was passed, the tip being confirmed radiologically to be in the lower part of the stomach. The patient was asked to expectorate saliva throughout the test. After the collection of one basal five-minute sample, 80 ml of 26.8 m-equiv/litre sodium bicarbonate was introduced through the nasogastric tube. One further five-minute sample was collected but discarded, as the volume of this sample was such as to dilute the enzyme, thus precluding accurate measurement. Two five-minute samples of gastric juice were then collected and retained. After further alkalization with the same amount of sodium bicarbonate, two further five-minute samples of gastric juice were obtained. By this method, four post-alkalinization samples were collected for testing.

The volume and pH of each specimen were measured immediately after withdrawal and the specimens stored at 4°C until analysis of protein and B-glucuronidase activity could be performed at a later convenient time. Any very viscous specimens were homogenized.

The patients studied included a series of controls in order to establish the normal range of enzyme activity, 30 patients suffering from gastric carcinoma diagnosed by conventional methods of radiology or endoscopy, 30 suffering from peptic ulcer (16 with gastric ulcer and 14 with duodenal ulcer), 11 with newly diagnosed pernicious anaemia, and 21 with non-gastric malignant disease including carcinoma of colon, pancreas, and lung.

RESULTS

In all subjects within each disease group, the four post-alkalinization samples had greater specific activity than the basal samples, and by separating the results on each patient into four individual groups after neutralization, there was found to be no significant difference in enzyme activity between these groups. It was thus deemed justifiable to calculate the mean value for the four post-neutralization samples and the levels taken for each patient are the means of these four samples. From the group of control patients, the mean normal level of enzyme activity was 94 units/100 ml (range 32-279 units/100 ml), protein 189/100 ml, and specific activity 0.54 units/mg of protein (range 0.08-0.90 units/mg protein). The upper limits of normal were therefore taken as 300 units/100 ml for enzyme

TABLE I
B-GLUCURONIDASE LEVELS IN SALIVA

Condition	No. of Subjects	B-glucuronidase (units/100 ml)	Protein (mg/100 ml)	Specific Activity (units/mg P)
Normals	18	489	630	0.78
Peptic ulcers	18	742	703	0.99
Gastric carcinoma	7	476	595	0.79
Miscellaneous cancers	7	285	616	0.44

TABLE II

SUMMARY OF MEAN RESULTS IN DISEASE GROUPS

	Controls	Peptic Ulcer	Pernicious Anaemia	Miscellaneous Cancer	Gastric Cancer
B-glucuronidase (units/100 ml)	95.0	111.0	245.0	115.0	509.0
Protein (mg/100 ml)	189.0	178.0	281.0	323.0	262.0
Specific activity (units/mgP)	0.54	0.69	0.74	0.54	1.92

activity, 200 mg/100 ml for protein, and 1.0 units/mg protein for specific activity.

Table II shows that the mean enzyme levels in the gastric carcinoma group were almost five times greater than the normal range, and 28 of the 30 patients with gastric carcinoma (93.3%) had levels greater than 1.0 unit/mg protein (Fig. 1). Sixteen of the gastric tumours were in the pyloric region, 12 in the body, and two in the cardia. There was no significant difference in the levels of B-glucuronidase obtained from these three areas of the stomach. There was also no relationship between the degree of enzyme activity and either the histology or the size of the tumour, a small lesion such as a malignant ulcer being just as likely to have a raised B-glucuronidase level as a large adenocarcinoma. Normal enzyme results were obtained in two of the 30 cases of gastric carcinoma, a false negative level of 6.7%.

In a prospective study, B-glucuronidase activity was determined in 26 patients suspected on clinical grounds of suffering from gastric carcinoma. In 23 patients (88.5%) the result indicated accurately

the presence or absence of tumour. Two of the three erroneous results were obtained from the 16 patients of the group subsequently shown to have gastric carcinoma and one was shown to have a benign condition. Two of the 11 patients (18.2%) with newly diagnosed pernicious anaemia had raised levels of B-glucuronidase specific activity, the remainder being within the normal range. There was no significant difference between the results in the gastric and duodenal ulcer groups. One patient with gastric ulcer had a raised level of enzyme activity, giving a false positive percentage of 3.3. Two of the 21 cases in the miscellaneous tumour groups had false high levels (9.5%). One of these patients had carcinoma of the pancreas with blockage of the venous drainage from the stomach (this was demonstrated at operation) and the other suffered from carcinoma of the colon. Three further patients had high levels; one of these had had a gastric carcinoma resected three years previously but had no evidence of recurrence; the remaining two had symptoms suggestive of gastric carcinoma,

FIG. 1. Specific activity of B-glucuronidase activity measured in units/mg protein in controls and various pathological conditions.

but all investigations proved negative apart from a high B-glucuronidase result. The total false positive level in the series was 10.8%.

In the proven cases of gastric carcinoma, the efficacy of the test as a potential diagnostic aid was assessed by comparison with other diagnostic methods. The approximate percentage accuracy of each method compared with B-glucuronidase estimation in gastric juice is shown in Table III. Enzyme measurement would thus appear to have about the same order of accuracy as radiology or endoscopy.

TABLE III
COMPARISON OF DIAGNOSTIC AIDS IN GASTRIC CARCINOMA

	Present Series		Diagnostic Accuracy in Reported Series (%)
	No. of Cases	Diagnostic Accuracy (%)	
Radiology	26	93.3	80-85
Endoscopy	15	78.0	75-85
B-glucuronidase	26	88.5	—

DISCUSSION

This study has shown that a high proportion (93.3%) of patients with gastric carcinoma have raised levels of B-glucuronidase activity in the gastric juice. No specific pointers have been obtained as to the possible reason for this finding. Increased cellular breakdown as an explanation seems unlikely, as there is no correlation between DNA output in the gastric juice and enzyme activity (Piper, Griffith, Irving, and Fenton, 1966). It has been shown that many cancer cells exhibit increased permeability at their surface membranes and this abnormality could possibly explain the findings of the study (Wu, 1959; Abercrombie and Ambrose, 1962). Alternatively, there may be an increased content of enzyme within the tumour cells and this could be due to the absence of inhibitor substances within the abnormal cell. As yet, however, no specific evidence of these in gastric carcinoma is available.

Two of the 30 proven cases of gastric carcinoma had normal levels of B-glucuronidase activity. This gives a false negative level of 6.7%, which compares favourably with the 24% of false negatives reported by Piper *et al* (1966) but is similar to the figures reported by Kim and Plunt (1965). In this latter study, however, positive results included cases showing raised levels of enzyme activity itself but not of specific activity. It is important that the enzyme activity be related to the protein content of the gastric juice for accurate standardization. The

most likely explanation for the false negative results is inadequate neutralization of the gastric juice. Small pools of gastric juice may have remained unmixed by the sodium bicarbonate. This is also a point in favour of two neutralizations, as performed in this study. Alternatively, differences in the cellular content of B-glucuronidase or permeability to enzymes may occur in certain tumours.

In this series, there was a false positive level of 10.8%. This compares with 6% in the series reported by Piper *et al* (1966) and 5% in that of Kim and Plunt (1965). Taking only gastric ulcers into account, as in Piper's series, our false positive level was 6.25%. It is possible that some of these false positive results could be due to salivary contamination of the gastric juice. Alternatively, some increase in the B-glucuronidase content of gastric mucosal cells may be associated with malignancy elsewhere in the gastrointestinal tract. Increased levels may be indicative of a pre-malignant or potentially pre-malignant state. The high percentage of false positive levels in cases of pernicious anaemia is of particular interest, in view of the widely accepted association of pernicious anaemia with gastric carcinoma (Jenner, 1939) and may be associated with cellular changes due to the gastric atrophy of pernicious anaemia. Raised B-glucuronidase levels in pernicious anaemia were also reported by Kim and Plunt (1965). Repeat estimations in several of the patients with pernicious anaemia showed no significant difference in B-glucuronidase activity.

Measurement of B-glucuronidase activity does seem to be of value as a reasonably accurate prediction of the presence or absence of gastric carcinoma. The results of this test are comparable with those obtained from radiology and endoscopy, and are superior to the reported results of exfoliative cytology (Cabre-Fiol, Olo-Garcia, and Vilardell, 1959; Burnett, Macfarlane, Park, and Kay, 1960; Brandborg, Taniguchi, and Rubin, 1961). The test is easy to perform clinically and entails little discomfort to the patient. Laboratory analysis of the gastric juice presents no difficulties. The evidence so far available does suggest, however, that the raised levels of enzymes may be present in association with early malignant lesions. Thus the test may be of value as a screening procedure in patients considered at risk of developing gastric carcinoma, such as those with a family history of the disease, or with pernicious anaemia.

SUMMARY

After initial studies of the effect of various forms of stimulation on the level of B-glucuronidase activity in gastric juice, a simplified test was devised to

collect gastric juice and measure the enzyme. The test is easily performed using standard biochemical methods and is acceptable to the great majority of patients.

Normal levels of B-glucuronidase activity in gastric juice were established, and it was found that 93.3% of cases of gastric carcinoma had high enzyme levels and a prospective study indicated an accuracy of diagnosis comparable with that of radiology and endoscopy.

A small number of false positive (10.8%) and false negative results were obtained and the possible reasons for these results are discussed.

We have pleasure in thanking Professor A. W. Kay for his advice and encouragement throughout the study, and for the use of the facilities of the Department of Surgery, Western Infirmary, Glasgow, Professor G. M. Wilson for helpful advice on reading the manuscript, Mr I. E. Gillespie for his support and valuable criticism, and Professor D. W. Piper for advice at the beginning of the study.

We also wish to thank Dr E. B. Hendry and Mr R. A. Macallister for technical help, and the many physicians and surgeons of the Western Infirmary, Glasgow, for referring patients to us.

REFERENCES

- Abercrombie, M., and Ambrose, E. J. (1962). The surface properties of cancer cells (Review). *Cancer Res.*, **22**, 525-548.
- Brandborg, L. L., Taniguchi, L., and Rubin, C. E. (1961). Is exfoliative cytology practical for more general use in the diagnosis of gastric cancer? A simplified chymotrypsin technique. *Cancer (Philad.)*, **14**, 1074-1080.
- Burnett, W., Macfarlane, P. S., Park, S. D. S., and Kay, A. W. (1960). Cancer of the stomach: an evaluation of diagnostic methods including exfoliative cytology. *Brit. med. J.*, **1**, 753-755.
- Bockus, H. L. (1963). *Gastroenterology*, 2nd ed, vol. 1. X-Ray Examination. pp. 760-776. Saunders, Philadelphia and London.
- Cabre-Fiol, V., Olo-Garcia, R., and Vilardell, F. (1959). Five years of cytologic diagnosis of gastric cancer by exfoliative biopsy. *Proc. Wld Congr. Gastroent.*, **2**, 1006-1008.
- de Duve, C. (1961). Lysosomes and chemotherapy. In *Biological Approaches to Cancer Chemotherapy*, edited by R. J. C. Harris. Academic Press, New York. pp. 101-122.
- , Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955). Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.*, **60**, 604-617.
- Fischermann, K., and Koster, K. H. (1962). The augmented histamine test in the differential diagnosis between ulcer and cancer of stomach. *Gut*, **3**, 211-218.
- Fishman, W. H., and Anlyan, A. J. (1947). β -Glucuronidase activity in human tissues. Some correlations with processes of malignant growth and with the physiology of reproduction. *Cancer Res.*, **7**, 808-817.
- , Baker, J. R., and Borges, P. R. F. (1959). Localisation of β -glucuronidase in some human tumors. *Cancer (Philad.)*, **12**, 240-245.
- Gilbertsen, V. A., and Knatterud, G. L. (1967). Gastric analysis as a screening measure for cancer of the stomach. *Ibid.*, **20**, 127-133.
- Hitchcock, C. R., and Aust, J. B. (1955). Diagnosis of asymptomatic malignant disease; a report of six and one-half year's operation of Cancer Detection Center, University of Minnesota. *Postgrad. Med.*, **17**, 145-156.
- Jenner, A. W. F. (1939). Perniziöse Anämie und magenkarzinom. *Acta med. scand.*, **102**, 529-590.
- Kim, Y. S., and Plunt, A. G. (1965). β -glucuronidase studies in gastric secretions from patients with gastric cancer. *Gastroenterology*, **49**, 50-57.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randell, R. J. (1951). Protein measurement with the Folin-Phenol reagent. *J. biol. Chem.*, **193**, 265-275.
- Levitan, R., Golub, M., and Zetzel, L. (1960). Lactic dehydrogenase activity in saliva, bile, gastric and duodenal contents. *Amer. J. Dig. Dis.*, **5**, 458-465.
- Piper, D. W., Griffith, E. M. M., Irving, L. G., and Fenton, B. H. (1966). Value of β -glucuronidase activity in gastric juice in the diagnosis of gastric carcinoma. *Gastroenterology*, **51**, 172-179.
- , Macoun, M. L., Builder, J. E., and Fenton, B. H. (1963a). Nonproteolytic enzymes in gastric juice. *Amer. J. dig. Dis.*, **8**, 701-708.
- , Stiel, M. C., and Builder, J. E. (1963b). The electrophoretic pattern of normal human gastric juice and of the gastric juice of patients with gastric ulcer and gastric cancer. *Gut*, **4**, 236-242.
- Schenker, S. (1959). Lactic dehydrogenase activity in gastric juice in the diagnosis of gastric cancer: a preliminary report. *Amer. J. dig. Dis.*, **4**, 412-418.
- Smyrniotis, F., Schenker, S., O'Donnell, J., and Schiff, L. (1962). Lactic dehydrogenase activity in gastric juice for the diagnosis of gastric cancer. *Ibid.*, **7**, 712-726.
- Talalay, P., Fishman, W. H., and Huggins, C. (1946). Chromogenic substrates. 11. Phenolphthalein glucuronic acid as substrate for the assay of glucuronidase. *J. biol. Chem.*, **166**, 757-772.
- Wu, R. (1959). Leakage of enzymes from ascites tumor cells. *Cancer Res.*, **19**, 1217-1222.