data produced by the medical staff differed in statistical significance. We believe that this reflects sample instability¹⁰ rather than simply operator or instrument performance and emphasises the need for careful planning of quality control measures adopted for equipment in use outside the main laboratory. Patients' plasma creatine kinase activities did not show such lability. Using correlation of results with the routine laboratory method as a practical measure of accuracy, both groups found similar positive Seralyzer bias with patients' plasma samples. This has not been observed before,⁸⁻⁹ but difference in calibration materials and the laboratory instruments used for comparisons may account for the finding. The Seralyzer proved reliable throughout, requiring little maintenance other than daily provision of control materials and occasional calibration.

Decentralised testing must make a measurable and economic contribution to patient care, as its use cannot be justified on the ground of technological progress alone. The instrument costs £2900 and creatine kinase test strips cost 44.8p each (both excluding VAT). In view of the time taken to produce controlled results, however, the logical role of the Seralyzer would not be to replace the routine enzyme service from the laboratory but to supplement that service for selected admissions. The ability of medical staff to identify those patients who were less likely to have their management altered by enzyme analysis on admission points to the probable success of such a selective policy and is consistent with the need to exercise discretion in requesting tests.¹¹ The added burden of analysis was thus small and well accepted as the doctors were motivated to produce results of immediate diagnostic utility. The substantial improvement in availability of creatine kinase estimations, particularly at weekends and on a bank holiday when the routine laboratory service was not available, contributed to overall economies, principally in the early discharge of patients from costly intensive care facilities to the general ward or even home.

In conclusion, we have shown that standards of precision and accuracy for Seralyzer creatine kinase analyses similar to those achieved by experienced analysts in the laboratory were attainable by doctors on a coronary care unit. The medical staff were able to produce rapid, reliable results by adhering to a simple analysis protocol after only minimal training in the use of the instrument. Such "real time" cardiac enzyme data aided earlier confirmation of diagnosis and facilitated appropriate patient management. Continuing success in the use of the Seralyzer, however, depends on day to day cooperation with the laboratory to ensure maintenance of performance quality and therefore maximum diagnostic reliability of results.

We are grateful to the Ames Division of Miles laboratories for the loan of the Seralyzer reflectance photometer for this study.

References

- 1 Zipp A. Development of dry reagent chemistry for the clinical laboratory. *Journal of Automatic Chemistry* 1981;3:71-5.
- 2 Curme HG, Columbus RL, Dappen GM, et al. Multilayer film elements for clinical analysis: general concepts. *Clin Chem* 1978;24:1335-42.
- 3 Weiner K. Pathology measurements closer to the patient? *J Clin Pathol* 1980;33:857-63.
- 4 Watson D. Analytical investigations closer to the patient. *Br Med J* 1980;281:31-5.
- 5 Marks V. Clinical biochemistry nearer the patient. *Br Med J* 1983;286:1166-7.
- 6 Drucker RF, Williams DRR, Price CP. Quality assessment of blood glucose monitors in use outside the hospital laboratory. *J Clin Pathol* 1983;36:948-53.
- 7 Evans SE, Buckley BM. Biochemists nearer the patient? *Br Med J* 1983;287:1399-1400.
- 8 Aguanno JJ, Finney M, Ritzmann SE. An evaluation of the Ames Seralyzer creatine kinase method. *Clin Chem* 1982;28:1618.
- 9 Stevens JF, Tsang W, Newall RG. Measurement of the enzymes lactate dehydrogenase and creatine kinase using reflectance spectroscopy and reagent strips. *J Clin Pathol* 1983;36:1371-6.
- 10 Perry B, Doumas B, Jendrzyszczak B. Effect of light and temperature on the stability of creatine kinase in human sera and controls. *Clin Chem* 1979;25:625-8.
- 11 Mitchell JRA, Wilcox RG, Hampton JR. Necessary tests or ritual dances? *Lancet* 1982;ii:990.

(Accepted 31 January 1985)

Epidemic hypochlorhydria

T GLEDHILL, R J LEICESTER, B ADDIS, N LIGHTFOOT, J BARNARD, N VINEY, D DARKIN, RICHARD H HUNT

Abstract

During a study of gastric secretion four out of six previously healthy subjects developed hypochlorhydria after a transient illness with nausea, vomiting, and abdominal pain. Mean basal and peak acid outputs were 0 and 2.3 mmol (84 mg)/h one month after the onset of illness and 1.5 and 27.0 mmol/h (55 and 984 mg/h) at eight months' follow up. Two of the subjects were followed up at 18 months, when mean basal and peak acid outputs were 3.9 and 33.5 mmol/h (142 and 1221 mg/h).

Royal Naval Hospital, Haslar, Gosport, Hampshire

T GLEDHILL, CHM, FRCS, research fellow
R J LEICESTER, MB, FRCS, consultant surgeon
B ADDIS, MRCPATH, DCP, consultant pathologist
N LIGHTFOOT, MB, MRCPATH, consultant microbiologist
RICHARD H HUNT, MB, FRCP, consultant physician

Smith Kline and French Research Ltd, Welwyn, Hertfordshire

J BARNARD, MB, MRCP, senior medical adviser
N VINEY, BSC, chemist
D DARKIN, BSC, senior chemist

Correspondence to: Professor Richard H Hunt, Division of Gastroenterology, McMaster University Medical Centre, Hamilton, Ontario, Canada.

No endoscopic abnormality was seen at one and eight months, but biopsies showed active superficial gastritis, which resolved in one subject and became chronic in two. Schilling tests performed in three subjects at eight months showed diminished retention of vitamin B₁₂. During hypochlorhydria a 24 hour intragastric analysis was performed for total and nitrate reducing bacteria, pH, and concentrations of nitrite and total and stable N-nitroso compounds. Of the 48 samples of gastric juice examined, 47 had bacterial growth of more than 10⁶ organisms/ml and 46 had growth of nitrate reducing bacteria of more than 10⁵ organisms/ml. Mean intragastric nitrite concentrations were 10 times higher than in a group of eight healthy controls. Both mean total and mean stable N-nitroso compound concentrations, however, were not appreciably different from those in controls.

Although community transmission was a possibility, serological screening and electron microscopy of gastric biopsy specimens failed to show an infective cause. Transmission of an unidentified enteric pathogen via a contaminated pH electrode was therefore suspected. Thus gastric juice should not be returned to the stomach after contact with a contaminated glass electrode as this is a possible cause of atrophic gastritis.

Introduction

A sudden decrease in the secretion of gastric acid is a recognised but rarely recorded event.¹⁻³ During a study of gastric secretion four out of six previously healthy subjects developed hypochlorhydria. The original study was designed to test whether a new formulation of antacid should be administered according to the recommendation of Fordtran *et al.*⁴—that is, that antacids should be given one and three hours after food for maximum buffering effect. Six subjects with no clinical evidence of previous disease gave their informed consent, and the study was approved by the ethical committee of this hospital.

Subjects were investigated over six periods of six hours each separated by at least one week. On each occasion a standardised meal was administered and subjects took various combinations of antacid or placebo hourly throughout the study. They were admitted after an overnight fast. A 10 French gauge nasogastric tube was inserted and its position checked by water aspiration. Subjects then took a standardised meal of 400 ml Clinifed 400 (Roussel). Samples of 5 ml gastric juice were aspirated every 10 minutes for six hours and the pH measured with a glass electrode calibrated with buffers of pH 4 and 7 before each batch of measurements. After estimation of the pH the juice was returned to the stomach via the nasogastric tube. Every hour after the meal either antacid or placebo was administered for a total of four doses.

All patients secreted acid on the first study day as shown by a gradual fall in pH to values below pH 2.0 in every subject. On the second study day one subject reported that she had experienced a mild illness with nausea, vomiting, and periumbilical pain, which had lasted three days. Her subsequent intragastric pH in response to food (>6.4 in every sample of gastric juice) suggested that no acid was being secreted. Over the next four weeks a further three of the six subjects experienced similar symptoms, after which no acid response to food was observed.

Two of the six subjects who took part in the study were unaffected and produced an acid response to food on each day of the study. The following description applies to the remaining four subjects.

Method

All subjects underwent haematological and biochemical screenings before the study and one week after developing the illness. Blood was taken for viral serology two days and two weeks after the condition was recognised. Complement fixation tests were performed against the following antigens: influenza A, influenza B, adenovirus, cytomegalovirus, and *Mycoplasma pneumoniae*. Parietal cell antibodies were measured one week and one year after the onset of illness, and radioactive Schilling tests were performed on three subjects. Basal and peak acid outputs were measured before and after an intravenous infusion of promidine 10 $\mu\text{g kg h}$. In one subject peak acid output was also measured in response to pentagastrin 6 $\mu\text{g kg h}$. Estimations of peak acid output were repeated at eight months in three subjects and at 18 months in two.

All subjects underwent upper gastrointestinal endoscopy with biopsy of the body and antrum of the stomach between one and four weeks after the onset of hypochlorhydria. Three specimens from each area were taken: one for routine histology, one for electron microscopy, and one for virus culture. The following cell lines were inoculated: baboon kidney, HEP₂, and MRC5. Endoscopy with biopsy was repeated at eight months in all subjects and at 18 months in two.

One month after the onset of hypochlorhydria we admitted the subjects for 24 hours to study intragastric acidity, bacterial counts, and concentrations of nitrite and *N*-nitroso compound. They were admitted to a special study area after an overnight fast. A size 10 French gauge nasogastric tube was inserted and its position checked by aspiration. Gastric juice was sampled half hourly during the day (0900-2400) and hourly overnight (2400-0800). The pH of each sample was measured by a combined glass electrode (Radiometer, Copenhagen), and nitrite and *N*-nitroso compound concentrations were determined. Samples were taken at the following times and

examined microbiologically: 0900 (initial fasting sample); 1300, 1400, 1500, and 1600 (just before and after lunch); and 0200, 0300, 0400, 0500, 0600, 0700, and 0800 (overnight period, pH usually low).

Bacteriology—Gastric juice was aspirated with a sterile syringe and transferred to a universal container sterilised with gas; mucus and food were broken up and bacteria dispensed by vortex mixing. Samples were placed inside an anaerobic chamber (Programmed Anaerobic Controlled Environment, Labline Ltd), where all subsequent manipulations were performed. The gastric juice was diluted (10^1 to 10^{12}) in prereduced brain heart infusion broth and added to indole nitrite broth (Baltimore Biological Laboratories) in microtitre plates. The total numbers of bacteria and nitrate reducing bacteria were estimated by the most probable number method after anaerobic incubation at 36.5°C for five days. In addition, one sample from each patient was examined to identify the individual bacterial species present.

Nitrite—Each sample was immediately adjusted to over pH 7.0 by adding borax to prevent the destruction of nitrite and stored at 20°C for later estimation by polarography.

***N*-nitroso compound concentrations**—After the addition of saturated hydrazine sulphate solution to destroy any nitrite present each sample was titrated to pH 4.0 and divided into two. One aliquot was added to hydrogen bromide and acetic acid in ethyl acetate, and the nitric oxide that evolved was measured by chemoluminescence to give the total *N*-nitroso compound concentration.¹⁰ The remaining aliquot was stored at room temperature in an amber container for five to seven days, after which the stable *N*-nitroso compound concentration was estimated by the same method. This technique measures both total and stable *N*-nitroso compounds, including nitrosoureas and nitrosamines. The results of this 24 hour study were compared with those obtained during a previous investigation of eight healthy subjects following an identical 24 hour protocol and receiving an identical diet.¹¹

Results

The haematological and biochemical screenings performed before and two weeks after the onset of illness gave unchanged results. Virus serology detected no rise in titres to influenza A, influenza B, adenovirus, cytomegalovirus, or *Mycoplasma pneumoniae*.

Basal acid output was 0 mmol/h in every subject during the first month of illness. Mean peak acid output was 2.32 mmol (84.5 mg)/h (range 0.8-8.0 mmol (0.292-292 mg)/h after promidine. Peak acid output in response to pentagastrin was 0.32 mmol (12 mg)/h within one month after the onset of hypochlorhydria in the one subject studied. The table shows basal and peak acid outputs at follow up.

Basal and peak acid outputs (mmol/h) at one, eight, and 18 months after onset of illness

Case No	Period	Months after onset of illness		
		One	Eight	18
1	Basal	0	2.6	2.6
	Peak	0.3	31.4	31.8
2	Basal	0	1.2	5.2
	Peak	8.8	29.4	35.2
3	Basal	0	0.8	*
	Peak	0	20.2	*
4	Basal	0	+	+
	Peak	1.2	+	+

*Refused further studies.

+Working overseas.

Conversion: SI to traditional units—Hydrochloric acid: 1 mmol/h $\approx$ 36.5 mg/h.

Initial endoscopy in all subjects after illness showed a macroscopically normal stomach. Histological examination (figs 1 and 2) showed active superficial gastritis in all subjects. The inflammatory response was most noticeable in the antrum, and in some subjects clusters of polymorphs were prominent within glands (fig 2). Cultures of gastric biopsy specimens in baboon kidney, HEP₂, and MRC5 cell lines did not show the presence of a virus. The cell lines would be expected to isolate a wide range of viruses. Electron microscopy did not disclose any viral inclusion bodies. After eight months endoscopy and biopsy were repeated in all subjects; results were normal in one (case 1), though the three other subjects had developed chronic gastritis with plasma cells and lymphocytes replacing the polymorphs

FIG 1—Mucosal biopsy specimen from gastric antrum, showing excess of inflammatory cells within mucosal lamina propria and groups of inflammatory cells within glands. Haematoxylin and eosin $\times 93$ (original magnification).

seen in earlier biopsy specimens. One patient (case 2) still had active chronic gastritis at 18 months' follow up. Although parietal cell antibodies were initially normal in all subjects, one (case 1) developed moderately raised concentrations after a year. At 18 months' follow up Schilling tests performed in three subjects (cases 1, 2, and 3) by the whole body counting method¹² showed retention of vitamin B₁₂ after one week of 37%, 54%, and 53%, respectively, compared with retention of vitamin B₁₂ bound to intrinsic factor after one week of 42%, 83%, and 100%.

Figure 3 shows the mean hourly pH for the four subjects over the study day compared with the eight controls studied earlier. All mean hourly pH values in the subjects throughout the study day were higher than those in the controls.

FIG 2—High power view of specimen in fig 1, showing groups of polymorphs infiltrating base of gland and mixed inflammatory infiltrate in lamina propria. Haematoxylin and eosin $\times 292$ (original magnification).

Figure 4 shows mean total and nitrate reducing bacterial counts in our subjects during the study day compared with those in eight controls. Forty seven samples from the subjects with hypochlorhydria had bacterial growth of more than 10^6 organisms/ml and 46 had growth of nitrate reducing bacteria of more than 10^5 organisms/ml. The differences between the controls and the subjects with hypochlorhydria was particularly noticeable overnight, when almost all samples of gastric juice from controls were sterile and almost all samples from the subjects with hypochlorhydria had more than 10^9 organisms/ml and more than 10^8 nitrate reducing organisms/ml. The bacteria isolated included *Neisseria*, *Corynebacterium*, *Streptococcus*, *Staphylococcus*, *Acinetobacter*, *Lactobacillus*, *Fusobacterium*, *Bacteroides*, *Veillonella*, and *Bifidobacterium*. These were all members

FIG 3—Mean hourly pH values in four subjects with hypochlorhydria (●—●) and in eight controls (○---○) receiving identical diets.

FIG 4—Mean hourly log bacterial counts/ml gastric juice in four subjects with hypochlorhydria (total bacteria ●—●; nitrate reducing bacteria ○---○) and six controls (total bacteria ■—■; nitrate reducing bacteria □---□).

FIG 5—Mean hourly nitrite concentrations in three subjects with hypochlorhydria (●—●) and eight controls (○---○).

Conversion: SI to traditional units—Nitrite: 1 mmol/l $\approx$ 46 mg/100 ml.

of the normal oral flora and had presumably been swallowed. Coliforms were not isolated.

Nitrite concentrations were measured in three subjects, and figure 5 shows the mean hourly values compared with those in the eight controls. Nitrite concentrations were roughly 10 times higher in the subjects with hypochlorhydria than in the controls. The mean total *N*-nitroso compound concentration was 1.82 (SEM 0.17) nmol/l (83.7 (7.8) ng/100 ml) in the subjects with hypochlorhydria, which was not appreciably different from the concentration of 1.77 (1.17) nmol/l (81.4 (53.8) ng/100 ml) in the controls. The mean stable *N*-nitroso compound concentration, however, was 0.37 (0.08) nmol/l (17.0 (3.7) ng/100 ml) in the subjects with hypochlorhydria, which was lower than but not appreciably different from the concentration of 0.66 (0.08) nmol/l (30.3 (3.7) ng/100 ml) in the controls.

FIG 6—Mean hourly total and stable *N*-nitroso compound concentrations in four subjects with hypochlorhydria (total $\circ$ — $\cdots$ — $\circ$; stable $\square$ — $\cdots$ — $\square$) and eight controls (total $\bullet$ — $\bullet$; stable $\blacksquare$ — $\blacksquare$).

Conversion: SI to traditional units—*N*-nitroso compound: 1 mmol/l $\approx$ 46 mg/100 ml.

Discussion

Spontaneous hypochlorhydria is a recognised but rarely recorded event,¹⁻⁸ and only one other report has described this phenomenon in a cluster of patients. Ramsey *et al* found hypochlorhydria in 17 of 37 healthy volunteers participating in studies of gastric secretion.⁸ These investigators measured gastric acid secretion after stimulation by food and returned gastric juice to the stomach after estimating the pH. As the pH electrode was not sterilised between measurements their suggestion that an infective agent was transmitted via a contaminated glass electrode seems likely, though no specific agent was identified. We also returned gastric juice to the stomach after estimating the pH, considering this to be essential to avoid aspirating antacid, which might have resulted in loss of buffering effect. Although no transmissible agent was identified and community transmission was possible, we also think that an infective agent transmitted via a contaminated pH electrode was the most likely mechanism. We therefore recommend that if during studies of gastric secretion juice must be returned to the stomach after estimation of the pH then the electrode should be adequately sterilised before each measurement or a separate electrode used for each subject.

All of our subjects had superficial gastritis, which became chronic in three. The prevalence of atrophic gastritis in the general population has been reported as 28% in those aged 16-65 and 54% in those aged over 50.¹³ Although the mucosal inflammation may subsequently have resolved in all subjects,

one developed moderate titres of parietal cell antibodies after the illness, which suggests that this condition could progress to atrophic gastritis.

Hypochlorhydria has been observed after cholera but is considered to be a predisposing factor rather than a cause.¹⁴ Our study suggests that mild gastrointestinal upsets can lead to hypochlorhydria and may be more common than is realised. Documentation would be unlikely, however, unless gastric secretion was studied in patients who experienced such mild gastrointestinal symptoms as nausea and vomiting.

Although some of our subjects had a daytime pH response to food during the study day one month after the onset of illness, all were still anacidic overnight. These differences in pH may well have accounted for the large variations in bacterial counts noted between the subjects with hypochlorhydria and controls overnight. The high concentrations of nitrate reducing bacteria in the subjects with hypochlorhydria may have accounted for the high nitrite concentrations. Total *N*-nitroso compound concentrations, on the other hand, did not increase, and the mean stable *N*-nitroso compound concentration was lower in the subjects with hypochlorhydria than in the controls. This agrees with our previous observations in healthy volunteers, in whom reduced gastric acidity did not result in raised intragastric concentrations of *N*-nitroso compound.¹¹ These findings and those of others observing lesser changes in intragastric pH¹¹⁻¹⁵ do not support the hypothesis that increased intragastric pH increases the risk of gastric cancer as a result of an increase in *N*-nitroso compound concentrations.¹⁶ Increased pH, however, does result in increased total and nitrate reducing bacterial counts and intragastric nitrite concentrations, either of which may in itself be a risk factor in carcinogenesis. Bacteria alone could have caused the gastritis we observed, and this factor may be responsible for the increased risk of carcinoma in patients with atrophic gastritis, as has been observed with other chronic inflammatory processes. Ramsey *et al* reported that inflammation of the gastric mucosa was associated with hypochlorhydria and suggested several hypothetical arguments.⁸ Gastritis may, however, be a response to the presence of bacteria that are allowed to colonise the stomach with a high intragastric pH.

References

- 1 Spiro HM, Schwartz RDL. Superficial gastritis: a cause of temporary achlorhydria and hyperpepsinogenemia. *N Engl J Med* 1958;259:682-4.
- 2 Waterfall WE. Spontaneous decrease in gastric secretory response to humoral stimuli. *Br Med J* 1969;iv:459-61.
- 3 Desai HG, Zaveri MP, Anita FP. Spontaneous and persistent decrease in maximal acid output. *Br Med J* 1971;iii:313-5.
- 4 Lawrie RS, Williamson AWR, Hunt JN. Zollinger Ellison syndrome treated with poldine methyl methosulphate. *Lancet* 1962;ii:1002-4.
- 5 Desai HG, Anita FP. Spontaneous achlorhydria with atrophic gastritis in the Zollinger Ellison syndrome. *Gut* 1959;10:935-9.
- 6 Wiersinga WM, Tytgat GN. Clinical recovery due to target parietal cell failure in a patient with Zollinger Ellison syndrome. *Gastroenterology* 1977;73:1413-7.
- 7 Hirschowitz BI, Streeten DHP, London JA, Pollard HM. A steroid induced gastric ulcer. *Lancet* 1956;ii:1081-3.
- 8 Ramsey EJ, Carey KV, Peterson WL, *et al*. Epidemic gastritis with hypochlorhydria. *Gastroenterology* 1979;76:1449-57.
- 9 Fordtran JS, Collens JAH. Antacid pharmacology in duodenal ulcer. *N Engl J Med* 1966;274:921-7.
- 10 Bavin PMG, Darkin DW, Viney NJ. Total nitroso compounds in gastric juice. In: Batch H, O'Neill IK, Castegnoro M, Okada M, Davies W, eds. *N-nitroso compounds: occurrence and biological effects*. Lyons: International Agency for Research on Cancer, 1982:337-44. (Scientific publication No 41.)
- 11 Milton-Thompson GJ, Lightfoot NF, Ahmet Z, *et al*. Intragastric acidity, bacteria, nitrite and *N*-nitroso compounds, before, during and after cimetidine treatment. *Lancet* 1982;ii:1091-5.
- 12 Findlayson NDC, Simpson JD, Tothill P, Samson RR, Girdwood RH, Shearman DJC. Application of whole body counting to the measurement of vitamin B₁₂ absorption with reference to achlorhydria. *Scand J Gastroenterol* 1968;3:211-23.
- 13 Suirala M, Isokoski M, Vars K, Kekki M. Prevalence of gastritis in a rural population. *Scand J Gastroenterol* 1968;3:211-23.
- 14 Nalin DR, Levine RJ, Levine MM, *et al*. Cholera, non-vibria cholera and stomach acid. *Lancet* 1978;ii:856-9.
- 15 Keighley MRB, Morris D, Paxon V, *et al*. *N*-nitroso compounds after operation for duodenal ulcer. *Gut* 1982;23:A905.
- 16 Ruddell WSJ, Axon ATR, Findlay JM, Bartholomew BA, Hill MJ. Effect of cimetidine on the gastric bacterial flora. *Lancet* 1980;ii:672-4.

(Accepted 6 February 1985)