THE DISCOVERY AND PATHOLOGY

OF *H PYLORI*

Papers published by

JOHN ROBIN WARREN

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Married on 5 May, 1962
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GENERAL INTERESTS

Hobbies: Photography, Stamp collecting, Computer processing.

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Sports: (not active at present) Rifle shooting (Posttels small bore rifle club champion 1974 to 1977), Cycling, Badminton.

EDUCATION

St Peters College, St Peters, S.A. 1950 to 1954.
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University of Adelaide School of Medicine, 1955 to 1960, together with the Royal Adelaide Hospital, the Adelaide Children's Hospital and the Queen Victoria Maternity Hospital.

DISTINCTIONS

M.B., B.S.: Credit Second year.
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QUALIFICATIONS

M.B., B.S (University of Adelaide) 1961.

Fellowship of the Royal College of Pathologists of Australasia, 1967.

PAST APPOINTMENTS

Junior Resident Medical Officer, Queen Elizabeth Hospital, Woodville, S.A., 1961.

Registrar in Haematology and Clinical Pathology, Institute of Medical and Veterinary Science, Adelaide, 1962.

Temporary Lecturer in Pathology, University of Adelaide, and Honorary Clinical Assistant in Pathology, Royal Adelaide Hospital, 1963.

Registrar in Clinical Pathology, Royal Melbourne Hospital, 1964 to 1966.

Registrar in Pathology, Royal Melbourne Hospital, 1966 to 1968.

PRESENT APPOINTMENT

Pathologist, Royal Perth Hospital, since 1968.

PROFESSIONAL SOCIETIES

Royal College of Pathologists of Australasia, 1967.

Australian Society of Cytology, 1975.

International Academy of Pathology, 1975.

Australian Medical Association, 1960.

British Medical Association, 1960.

EXPERIENCE, SPECIAL INTERESTS

1961: General Medicine and Surgery.

1962 through 1967: All branches of Pathology studied prior to Membership (Fellowship) of the (Royal) College of Pathologists of Australia (Australasia).

1968 through 1996: Morbid Anatomy and Surgical Pathology. This includes some experience in Cytology and Electron Microscopy.

Since 1972 I have been interested in gastric pathology and since 1979 my work has centred around the study of curved bacilli (Campylobacter-like organisms, CLO, *Campylobacter pyloridis, C. pylori* or *Helicobacter pylori*) in the stomach and their relationship to gastritis and peptic ulceration.

Lymphoma Review Panel, Perth, 1983 to 1985.

Other pertinent interests include Mathematics, Statistics and Photography.

Course in Statistics, Murphy BP, Raine Medical Statistics Unit, School of Medicine, U. of W.A., August and September 1975 and June 1978.

Lymphoma studies, Robert J Lukes, Universities of Hawaii and Southern California, Honolulu, August 1976.

Tutorial on Haematopathology, Henry Rappaport, director, Pasadena, California, February 1981.

Introduction and Programming in dBASE II (data base program), Palmer JA and Fillery PJ, W.A.I.T. Business Systems Centre (Curtin University), June 1985.

ADDITIONAL AWARDS, PRIZES FOR MEDICINE.

Sixth International Workshop Campylobacter, Helicobacter and related organisms, 1991: Guest of honour.

Warren Alpert Foundation Prize 1994, Harvard Medical School, jointly with Dr. B. J. Marshall, "for research that has led to improved understanding and treatment of a specific disease: identifying *Helicobacter pylori* as a cause of peptic ulceration".

Australian Medical Association - Western Australian Branch: **1995 Award.**

Royal College of Pathologists of Australasia: Distinguished Fellows Award 1995, "for Distinguished Service to the Science and Practice of Pathology".

The First Western Pacific Helicobacter Congress, February 1996: Inaugural Award, "In recognition of his contribution to the advancement of medical science through the co-discovery of the gastric pathogen *Helicobacter pylori*".

The medal of the University of Hiroshima, September 1996.

The University of Adelaide Alumni Association: Distinguished Alumni Award, 24 October 1996, "in recognition of your contribution to the healing of peptic ulcers, to the relief of human suffering and to huge world wide economic savings."

The Paul Ehrlich and Ludwig Darmstaedter Award 1997, Paul Ehrlich Foundation, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany, jointly with Dr. B. J. Marshall, "for your discovery of *Helicobacter pylori* as cause for peptic ulcer".

Guest speaker at the Centenary Meeting of the German Society of Pathology, May 1997.

PUBLICATIONS

Warren JR. Cardiopulmonary resuscitation. (letter) Lancet 1966;ii:1078.

Warren JR. Insulin response in small vessel disease. (letter) Lancet 1972;i:956.

Lane GK, Warren JR, Cumpston GN. Successful removal of an intramural fibroma of the heart. Aust NZ J Med 1973;3:193-196.

Moses RG, Warren JR. Coumarin necrosis. Med J Aust 1973;2:76-77.

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The discovery and pathology of *H pylori*.

J. Robin Warren
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What are the early days? Many reports over the last 100 years described spiral bacteria in the stomach. Most of them were dismissed as incorrect or one off and of no importance. Freedburg saw several cases in 1940 and published a short article about them. Palmer in 1954 showed that no such bacteria existed. It has been standard medical teaching this century that no bacteria grow in a normal stomach and ingested microorganisms are rapidly killed by the acid environment. They grow in atrophic stomachs or in the necrotic debris in an ulcer, probably a secondary infection and often a fungus.

Before the 1970's gastric biopsies of good quality were unusual. Most stomachs we saw were surgical or, worse still, postmortem specimens, with autolysed mucosa. Any bacteria were long gone. The fine detail of the mucosa was rarely seen. Chronic gastritis was difficult to see and of little apparent importance relative to ulcers or carcinomata. I found the classification of gastritis used at the time of little practical significance. The only types of apparent importance were the gross atrophic gastritis related to pernicious anaemia, or some form of acute gastritis. Pernicious anaemia was one of the classics of the medical school, but rarely seen in clinical practice. Most biopsies showed chronic gastritis with variable partial atrophy, not considered clinically significant. This suddenly changed for me in the decade of the 70's.

The fibreoptic endoscope appeared and suddenly well-fixed fragments of tissue from the gastrointestinal tract became some of the commonest biopsies seen. In 1972 these were described and classified by Richard Whitehead. His classification appeared complex, but it actually divided the biopsies in a logical way, using features often present and easy to recognise and quantify, such as the position in the stomach, the depth, type (usually chronic) and severity of inflammation, atrophy of the gastric glands and intestinal metaplasia. Whitehead defined "activity", a commonly seen feature which had previously been ignored. This was based on specific changes in the superficial epithelium and focal epithelial leucocyte infiltration. I found Whitehead's classification easy to use and it gave consistent results.

In June 1979 the early days of *Helicobacter pylori* began for me. A biopsy showed severe active chronic gastritis and I saw an unusual blue line on the surface. With higher magnification I thought I could see numerous small bacilli, closely adherent to the epithelium. My colleagues did not agree until a Warthin Starry stain was very successful, and showed vast numbers of bacteria. Electron microscopy from the wax block was also of excellent quality and clearly showed the bacteria, which closely resembled Campylobacter. At last my immediate colleagues believed they were there, if of questionable significance.

I continued to examine all gastric biopsies for more of these bacteria, not really expecting to find them. To my amazement they appeared quite often. Usually the organisms were in smaller numbers or more patchy than the first case, but it soon became obvious that they were closely related to the chronic gastritis seen on histology, and often to Whitehead's so-called "active" gastritis. With experience, I found them in almost half our biopsies of the gastric antrum. By this stage they were being reported by everyone in our laboratory, although I don't think anyone really believed they were of any importance.

Most people suggested that the gastritis came first and the infection was just secondary. I could not get any help from the clinicians. They biopsied patients to show lesions, such as ulcers or tumours, so tissue came from anywhere in the stomach, usually related to other inflamed lesions and definitely not intended to show the bacteria or their effects. The idea of sending gastric biopsies for culture was ridiculous. The patients had to be considered first and it had been known for 100 years that no bacteria grew in the stomach. If they were really there or were of any importance, why had they not been reported before? I found this very hard to answer. I cannot understand myself why I did not see them.

I thought one useful investigation would be a negative control. How many of our old gastric biopsies filed as normal showed the bacteria? This was harder than I expected, because most of the so-called "normal" specimens were from the gastric body. These often showed only mild changes, while accompanying specimens from the antrum showed active gastritis with bacteria and were coded as such. I thought at first, incorrectly, that the bacteria only affected the antrum. Our file did not separate parts of the stomach, so it was difficult to find the cases in which the antrum had been coded normal. I eventually found 20 such cases. The bacteria were present in one of them, which also showed moderately severe gastritis and the reporting pathologist agreed that it had been incorrectly coded. Interesting, but hard for publishing! By this stage it appeared that the bacteria were present in almost half of the antral biopsies, all of them showing chronic gastritis, most with active changes, many with some degree of atrophy and some with focal intestinal metaplasia. And never when the antral histology was normal.

I was almost ready to publish my findings in 1981 when I met Barry Marshall, who asked to see my work. He was the gastroenterology registrar and was expected to publish a paper. Dr Marshall did not like one suggested project, so someone told him to see "that pathologist who was trying to make gastritis into a bacterial infection". Barry did not believe it, but was prepared to try a short series of special biopsies. I required a series of biopsies from the gastric antrum, from apparently intact mucosa, unaffected by ulcers or other macroscopic pathology. These confirmed to me that the gastritis and infection were not just secondary to nearby ulcers. Barry became very enthusiastic and has devoted his life to the bacteria ever since.

We set up a formal study of the next 100 patients referred for gastroscopy, with a detailed clinical protocol and standard antral biopsies for histology and culture. The results were unexpected. The bacteria and gastritis found on histology seemed almost unrelated to most clinical and gastroscopic features. Bad breath and burping were the only related symptoms. Most gastroscoped patients had epigastric pain, regardless of what was found. Gastroscopic gastritis was not related to histological gastritis. The bacteria resembled Campylobacter to me, so we cultured them as for Campylobacter, but without the antibiotics used for faecal samples. Cultures were all negative until the five day Easter weekend, after which a few more were positive. After completing the study we found the incubator had been leaking and, when we repaired it, culture became a very reliable diagnostic method. Finally, Barry reviewed the official gastroscopy reports. To our surprise, every patient with duodenal ulcer had *H pylori* in the stomach.

These findings were all very interesting, but they did not convince the clinicians. In 1983 I published a summary of my work as my letter in the Lancet, accompanied by Barry's letter describing our joint work. Then Barry reported our work to the Campylobacter

conference in Brussels and impressed Martin Skirrow, one of Britain's leading authorities on Campylobacter. This was a very lucky chance, because shortly afterwards we tried to publish our definitive paper in the Lancet. The paper was held up for months, because the editor could not get reviewers to believe it. This is the reward for publishing something which is known to be impossible. Finally we contacted Martin Skirrow, who got his laboratory to do a short series similar to ours and send the results to the Lancet. Our paper was published unaltered two weeks later, in June 1984.

The rest of the early days of *H pylori* covered three areas; diagnosis, treatment and proof. We investigated several methods of diagnosis, most of them suggested by Barry. These included pre-treatment serology, the breath test and the CLOtest, as well as histology, smears and culture. Treatment followed two main directions, the use of bismuth, which Barry read about in an old copy of William Osler's Textbook of Medicine and thought, correctly, might have worked by killing the bacteria, and the use of antibiotics such as amoxycillin, tetracycline, erythromycin and tinidazole. For proof, both Barry and Dr Arthur Morris in New Zealand used Koch's postulates, with varying success. Barry made himself very sick with acute gastritis and then cured himself easily. Dr Morris went to the other extreme, and I still have numerous biopsies he sent me showing chronic gastritis which he took years to cure.

Some of our first patients illustrate the different clinical groups involved. Non-steroidal anti-inflammatory drugs may cause duodenal ulcer. This does not mean that *H pylori* is not involved. A case in point is my wife, one of our first patients. She needed NSAIDs for arthritis and promptly got gastric pain instead. Stopping the drugs brought back the arthritis. And so on. So I sent her to Barry for investigation and treatment. The bacteria were there, and treatment for them allowed the use of the anti-inflammatory drugs. Then she noticed that I had bad breath, although I did not have any symptoms. I fitted into the vast majority of infected people, most asymptomatic, and the question is, what to do about them? This is a public health problem, being made worse because of the suggested relation to neoplasm. I took a course of treatment and my bad breath left.

We completed a double blind trial of antibacterial treatment for duodenal ulcer. The patients received antibiotic or placebo therapy for *H pylori* and we treated all ulcers. This successfully showed that ulcers recur with the bacteria, but rarely without. The study clearly showed the effect of the bacteria on histological gastritis. Eradicate *Helicobacter* and the active change immediately resolved. Other features disappeared more slowly and often incompletely. Anatomical changes, such as atrophy, metaplasia and fibrosis, showed little change. If *Helicobacter* remained, the gastritis was unchanged.

Since our studies, interest in *Helicobacter* has spread widely. Associated diseases include neoplasm and cardiovascular disease. Numerous new species have been found throughout the animal kingdom. The public health aspects are being seriously considered, including sources of infection, vaccines and the possibility of treating all infected individuals and eliminating the infection. Pharmaceutical companies spend large amounts on improved treatments. "Helicobacter" is now a Journal and *Helicobacter* has its own world congresses. It is one of the most widely published subjects of the last decade.

UNIDENTIFIED CURVED BACILLI ON GASTRIC EPITHELIUM IN ACTIVE CHRONIC GASTRITIS

SIR,—Gastric microbiology has been sadly neglected. Half the patients coming to gastroscopy and biopsy show bacterial colonisation of their stomachs, a colonisation remarkable for the constancy of both the bacteria involved and the associated histological changes. During the past three years I have observed small curved and S-shaped bacilli in 135 gastric biopsy specimens. The bacteria were closely associated with the surface epithelium, both within and between the gastric pits. Distribution was continuous, patchy, or focal. They were difficult to see with haematoxylin and eosin stain, but stained well by the Warthin-Starry silver method (figure).

I have classified gastric biopsy findings according to the type of inflammation, regardless of other features, as "no inflammation", "chronic gastritis" (CG), or "active chronic gastritis" (ACG). CG shows more small round cells than normal while ACG is characterised by an increase in polymorphonuclear neutrophil leucocytes, besides the features of CG. It was unusual to find no inflammation. CG usually showed superficial oedema of the mucosa. The leucocytes in ACG were usually focal and superficial, in and near the surface epithelium. In many cases they only infiltrated the necks of occasional gastric glands. The superficial epithelium was often irregular, with reduced mucinogenesis and a cobblestone surface.

When there was no inflammation bacteria were rare. Bacteria were often found in CG, but were rarely numerous. The curved bacilli were almost always present in ACG, often in large numbers and often growing between the cells of the surface epithelium (figure). The constant morphology of these bacteria and their intimate relationship with the mucosal architecture contrasted with the heterogeneous bacteria often seen in the surface debris. There was normally a layer of mucous secretion on the surface of the mucosa. When this layer was intact, the debris was spread over it, while the curved bacilli were on the epithelium beneath, closely spread over the surface (figure).

The curved bacilli and the associated histological changes may be present in any part of the stomach, but they were seen most consistently in the gastric antrum. Inflammation, with no bacteria, occurred in mucosa near focal lesions such as carcinoma or peptic ulcer. In such cases, the leucocytes were spread through the full thickness of the nearby mucosa, in contrast to the superficial infiltration associated with the bacteria. Both the bacteria and the typical histological changes were commonly found in mucosa unaffected by the focal lesion.

The extraordinary features of these bacteria are that they are almost unknown to clinicians and pathologists alike, that they are closely associated with granulocyte infiltration, and that they are present in about half of our routine gastric biopsy specimens in numbers large enough to see on routine histology. The only other organism I have found actively growing in the stomach is *Candida*, sometimes seen in the floor of peptic ulcers. These bacteria were not mentioned in two major studies of gastrointestinal microbiology[1,2] possibly because of their unusual atmospheric requirements and slow growth in culture (described by Dr B. Marshall in the accompanying letter). They were mentioned in passing by Fung et al.[3]

How the bacteria survive is uncertain. There is a pH gradient from acid in the gastric lumen to near neutral in the mucosal vessels. The bacteria grow in close contact with the epithelium, presumably near the neutral end of this gradient, and are protected by the overlying mucus.

The identification and clinical significance of this bacterium remain uncertain. By light microscopy it resembles *Campylobacter fetus* but cannot be classified by reference to *Bergey's Manual of*

Curved bacilli on gastric epithelium.

Section is cut at acute angle to show bacteria on surface, forming network between epithelial cells. (Warthin-Starry silver stain; bar = 10 μm.)

Determinative Bacteriology. The stomach must not be viewed as a sterile organ with no permanent flora. Bacteria in numbers sufficient to see by light microscopy are closely associated with an active form of gastritis, a cause of considerable morbidity (dyspeptic disease). These organisms should be recognised and their significance investigated.

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SIR,—The above description of S-shaped spiral bacteria in the gastric antrum, by my colleague Dr J. R. Warren, raises the following questions: why have they not been seen before; are they pathogens or merely commensals in a damaged mucosa; and are they campylobacters?

In 1938 Doenges[1] found "spirochaetes" in 43% of 242 stomachs at necropsy but drew no conclusions because autolysis had rendered most of the specimens unsuitable for pathological diagnosis. Freedburg and Barron[2] studied 35 partial gastrectomy specimens and found "spirochaetes" in 37%, after a long search. They concluded that the bacteria colonised the tissue near benign or malignant ulcers as non-pathogenic opportunists. When Palmer[3] examined 1140 gastric suction biopsy specimens he did not use silver stains, so, not surprisingly, he found "no structure which could reasonably be considered to be of a spirochaetal nature". He concluded that the gastric "spirochaetes" were oral contaminants which multiplied only in post mortem specimens or close to ulcers. Since that time, the spiral bacteria have rarely been mentioned, except as curiosities,[4] and the subject was not reopened with the

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Fung WP, Papadimitriou JM, Matz LR. Endoscopic, histological and ultrastructural correlations in chronic gastritis. *Am J Gastroenterol* 1979; 71: 269–79.

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2. Freedburg AS, Barron LE. The presence of spirochaetes in human gastric mucosa. *Am J Dig Dis* 1940; 7: 443–45.
3. Palmer ED. Investigation of the gastric spirochaetes of the human? *Gastroenterology* 1954; 27: 218–20.
4. Ito S. Anatomic structure of the gastric mucosa. In: Heidel US, Cody CF, eds. Handbook of physiology, section 6: Alimentary canal, vol II: Secretion. Washington, DC: American Physiological Society, 1967: 705–41.

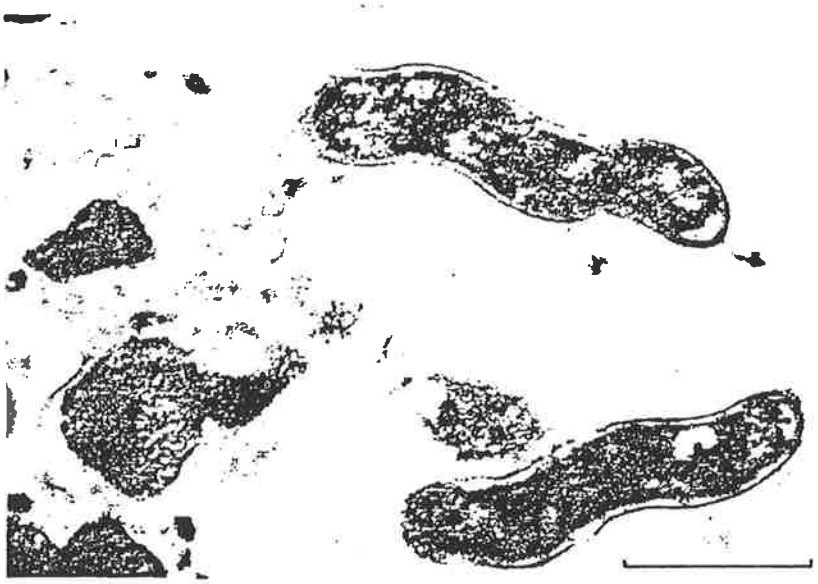

Fig 1—Thin-section micrograph showing spiral bacteria on surface of a mucous cell in gastric biopsy specimen. (Bar = 1 μm.)

advent of gastroscopic biopsy. Silver staining is not routine for mucosal biopsy specimens, and the bacteria have been overlooked.

In other mammals spiral gastric bacteria are well known and are thought to be commensals[5] (eg, Doenges[1] found them in all of forty-three monkeys). They usually have more than two spirals and inhabit the acid-secreting gastric fundus.[5] In cats they even occupy the canaliculi of the oxyntic cells, suggesting tolerance to acid.[6] The animal bacteria do not cause any inflammatory response, and no illness has ever been associated with them.

Investigation of gastric bacteria in man has been hampered by the false assumption that the bacteria were the same as those in animals and would therefore be acid-tolerant inhabitants of the fundus. Warren's bacteria are, however, shorter, with only one or two spirals and resemble campylobacters rather than spirochaetes. They live beneath the mucus of the gastric antrum well away from the acid-secreting cells.

We have cultured the bacteria from antral biopsy specimens, using *Campylobacter* isolation techniques. They are microaerophilic and grow on moist chocolate agar at 37°C, showing up in 3–4 days as a faint transparent layer. They are about 0·5 μm in diameter and 2·5 μm in length, appearing as short spirals with one or two wavelengths (fig 1). The bacteria have smooth coats with up to five sheathed flagellae arising from one end (fig 2). In some cells, including dividing forms, flagellae may be seen at both ends and in negative stain preparations they have bulbous tips, presumably an artefact.[7]

These bacteria do not fit any known species either morphologically or biochemically. Similar sheathed flagellae have been described in vibrios[7] but micro-aerophilic vibrios have now

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8. Shewan JM, Veron M. Genus I vibrio. In: Buchanan RE, Gibbons NE, eds. Bergey's manual of determinative microbiology, 8th ed. Baltimore: Williams & Wilkins, 1974: 341.

Fig 2—Negative stain micrograph of dividing bacterium from broth culture.

Multiple polar flagellae have terminal bulbs. (2% phosphotungstate, pH 6·8; bar = 1 μm.) Inset: detail showing sheathbed flagellum and basal disc associated with plasma membrane. (3% ammonium molybdate, pH 6·5; bar = 100 nm.)

n transferred to the family Spirillaceae genus *Campylobacter*.[8] mpylobacters however, have "a single polar flagellum at one or h ends of the cell" and the campylobacter flagellum is sheathed.[9] Warren's bacteria may be of the genus *Spirillum*.

The pathogenicity of these bacteria remains unproven but their ociation with polymorphonuclear infiltration in the human rum is highly suspicious. If these bacteria are truly associated h antral gastritis, as described by Warren, they may have a part to y in other poorly understood, gastritis associated diseases (ie, otic ulcer and gastric cancer).

thank Miss Helen Royce for microbiological assistance, Dr J. A. Armstrong electronmicroscopy, and Dr Warren for permission to use fig 1.

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BARRY MARSHALL

VASODILATOR PROSTANOIDS AND ACTH-DEPENDENT HYPERTENSION

SIR,—Dr Axelrod (April 23, p 904) proposes that the permissive ect of glucocorticoids on vascular tone is mediated via inhibition prostacyclin production and that this may contribute to the pertension of Cushing's syndrome. We became interested in this sibility following the suggestion by Rascher at al[1] that cocorticoids may produce hypertension as a result of inhibition phospholipase A_2 and a subsequent reduction in "vasodilator" ostaglandin synthesis. The demonstration by Weeks and Sutter[2] t prostacyclin (epoprostenol) infusion attenuated the elopment of DOCA (desoxycortone) induced hypertension in rat was also relevant. We have reviewed the evidence for such a othesis in relation to steroid and corticotropin (ACTH) pendent hypertension.[3] Our own studies have been concerned h the mechanism of ACTH induced hypertension in sheep, a m of experimental hypertension and features of glucocorticoid l mineralocorticoid excess but in which these two classes of enocortical steroid activity do not appear to account for more n about half of the hypertension.[3] On the basis of detailed eriments in conscious sheep we concluded that although sodilator" prostanoids such as prostacyclin appear to modulate ACTH induced rises in blood pressure they did not play a mary role in the development of the hypertension.

Although in sheep,[4] as in other species, indomethacin enhances oconstrictor responses to angiotensin II. ACTH treatment does alter pressor responsiveness to either angiotensin II, adrenaline, or arginine-vasopressin.[5–7] Also, indomethacin ng/kg daily for 3 days) had no effect on blood pressure in motensive sheep.[6] Further, pretreatment of sheep for 24 h with stacyclin at a dose which lowered total peripheral resistance but blood pressure did not alter the blood pressure response to

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UNIDENTIFIED CURVED BACILLI IN THE STOMACH OF PATIENTS WITH GASTRITIS AND PEPTIC ULCERATION*

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ummary Biopsy specimens were taken from intact areas of antral mucosa in 100 consecutive nsenting patients presenting for gastroscopy. Spiral or rved bacilli were demonstrated in specimens from 58 tients. Bacilli cultured from 11 of these biopsies were gram-gative, flagellate, and microaerophilic and appeared to be a w species related to the genus *Campylobacter*. The bacteria re present in almost all patients with active chronic stritis, duodenal ulcer, or gastric ulcer and thus may be an portant factor in the aetiology of these diseases.

Introduction

GASTRIC spiral bacteria have been repeatedly observed, orted, and then forgotten for at least 45 years.[1-3] In 1940 edburg and Barron stated that "spirochaetes" could be nd in up to 37% of gastrectomy specimens,[4] but mination of gastric suction biopsy material failed to nfirm these findings.[5] The advent of fibreoptic biopsy hniques permitted biopsy of the antrum, and in 1975 Steer l Colin-Jones observed gram-negative bacilli in 80% of ients with gastric ulcer.[6] The curved bacilli they strated were said to be *Pseudomonas*, possibly a taminant, and the bacteria were once more forgotten. The eated demonstration of these bacteria in inflamed gastric ral mucosa[7] prompted us to do a pilot study in twenty ients. Typical curved bacilli were present in over half the psy specimens and the number of bacteria was closely ted to the severity of the gastritis. The present study was igned to confirm the association between antral gastritis the bacteria, to discover associated gastrointestinal ases, to culture and identify the bacteria, and to find ors predisposing to infection.

ed on paper read at Second International Workshop on Campylobacter tions (Brussels, 1983).

Patients and Methods

Patients

All patients referred for gastroscopy on clinical grounds were eligible for the study which continued until there were 100 participants who gave informed consent and in whom biopsy was considered to be safe. The study was approved by our hospital's human rights committee.

Questionnaire

Where possible patients completed a clinical questionnaire designed to detect a source of infection or show any relationship with "known" causes of gastritis or *Campylobacter* infection, rather than give a detailed account of each patient's history. The emphasis was on animal contact, travel, diet, dental hygiene, and drugs, rather than symptoms.

Endoscopy

The gastroscopies were done by colleagues at the Royal Perth Hospital. Participants fasted for at least 4 h before endoscopy. An Olympus GIF-K fibreoptic gastroduodenoscope was used. Routine biopsies were done when indicated. For the study two extra specimens were taken from an area of intact antral mucosa, at a distance from any focal lesion such as an antral ulcer. When the mucosa appeared inflamed the specimens were taken from a red area, otherwise any part of the antrum was used. One biopsy was immediately fixed in phosphate-buffered formalin for histological examination, the other was placed in chilled anaerobic transport medium and taken to the microbiology laboratory within 1 h. In a few cases an extra specimen was taken for ultrastructural examination.

The gastroenterologist dictated his report soon after the endoscopy. We had not planned to analyse these reports so a standard terminology was not used and no special attention was paid to minor endoscopic lesions. Findings of doubtful clinical significance, such as mild endoscopic gastritis or duodenogastric bile reflux, may thus have been under-reported. (Hereafter the term "gastritis" refers to a histological grade of chonic gastritis unless stated otherwise.) Before we analysed the data, the endoscopy reports were coded for the major diagnoses.

Histopathology

Sections were stained with haematoxylin and eosin (H & E) and graded for gastritis (by J. R. W.) as 0 (normal), inflammatory cells rarely seen; 1 (normal), lymphoid cells present but within normal limits and with no other evidence of inflammation (see below); 2 (chronic), chronic gastritis; or 3 (active), active chronic gastritis.

Gradings were based solely on the type of inflammatory cells. Other types of mucosal change, such as gland atrophy or intestinal metaplasia, were noted separately, but were not used as evidence of inflammation. "Chronic gastritis" indicated inflammation with no increase in polymorphonuclear leucocytes (PMNs). There were either increased numbers of lymphoid cells or normal cell numbers with other evidence of inflammation such as oedema, congestion, or cell damage. The term "active" was used to indicate an increase in PMNs.[8] The gastritis was considered active if a few PMNs infiltrated one gland neck or pit, if occasional PMNs were scattered throughout the superficial epithelium, or if there was an obvious increase in PMNs in the lamina propria.

Later, sections stained with Warthin-Starry silver stain were examined for small curved bacilli on the surface epithelium. Numbers of bacteria were graded as 0, no characteristic bacteria; 1, occasional spiral bacteria found after searching; 2, scattered bacteria in most high-power fields or occasional groups of numerous bacteria; or 3, numerous bacteria in most high-power fields.

Microbiology

Tissue smears were Gram stained and examined for curved bacilli resembling *Campylobacter*. The remaining tissue was minced, plated on non-selective blood and chocolate agar, and cultured at 37°C under microaerophilic conditions as used for *Campylobacter* isolation.[9] At first plates were discarded after 2 days but when the first positive plate was noted after it had been left in the incubator for 6 days during the Easter holiday, cultures were done for 4 days.

Analysis of Results

Questionnaires, gastroscopy reports, and histopathology and microbiology results were coded independently in separate departments. Complete results for individual patients were not known until the statistician had received all the data. The findings were tested for positive correlation with the presence of either bacteria or gastritis, by the chi-squared method. Fisher's exact test of significance was used for all the 2 × 2 tables in this paper.

Results

In 12 weeks 184 patients were examined by the gastroenterology unit. Of the 84 patients excluded, 5 refused consent, 4 had contraindications to biopsy, and 75 patients, mostly unbooked cases, could not be invited to participate. These patients closely matched the study group for age, sex, and incidence of peptic ulcers (table I).

Questionnaires

99 patients completed the questionnaires. The only symptom which correlated with gastritis or bacteria was "burping" which was more common in patients with bacteria ($p = 0{\cdot}03$) or gastritis ($p = 0{\cdot}007$). This association remained when patients with peptic ulcer were excluded. None of the other questionnaire responses showed any relationship to the presence of gastric bacteria or gastritis.

Endoscopy

There was a very close correlation between both gastric ulcer and duodenal ulcer and the presence of the bacteria (table II). Most patients with peptic ulcer also had gastritis (29/31; $p = 0{\cdot}0002$).

TABLE I—COMPARISON OF PARTICIPANTS WITH EXCLUDED PATIENTS

—	Study group (n = 100)	Exclusions (n = 84)
Mean age (range)	55 (20–88) yr	57 (18–88) yr
Males	63 (*63%*)	55 (*65%*)
Females	37 (*37%*)	29 (*35%*)
Gastric ulcer	22 (*22%*)	19 (*23%*)
Duodenal ulcer	13 (*13%*)	8 (*10%*)

TABLE II—ASSOCIATION OF BACTERIA WITH ENDOSCOPIC DIAGNOSES

Endoscopic appearance*	Total	With bacteria	p
Gastric ulcer	22	18 (*77%*)	0·0086
Duodenal ulcer	13	13 (*100%*)	0·00044
All ulcers	31	27 (*87%*)	0·00005
Oesophagus abnormal	34	14 (*41%*)	0·996
Gastritis†	42	23 (*55%*)	0·78
Duodenitis†	17	9 (*53%*)	0·77
Bile in stomach	12	7 (*58%*)	0·62
Normal	16	8 (*50%*)	0·84
Total	100	58 (*58%*)	

*More than one description applies to several patients (eg, 4 patients had both gastric and duodenal ulcers).
†Refers to endoscopic appearance, not histological inflammation.

TABLE III—HISTOLOGICAL GRADING OF GASTRITIS AND BACTERIA

Gastritis	Bacterial grade				
	Nil	1+	2+	3+	Total
Normal*	29	2	0	0	31
Chronic	12†	9	7	1	29
Active	2	5	15	18	40
Total	43	16	22	19	100

*Gastritis grades 0 and 1 normal.
†1 case showed bacteria on gram stained smear.

TABLE IV—RELATION BETWEEN GASTRITIS AND BACTERIA IN PATIENTS WITHOUT PEPTIC ULCER

Gastritis	Bacteria		
	No	Yes	Total
Normal	28	1	29
Chronic	8	12	20
Active	2	18	20
Total	38	31	69

Histopathology

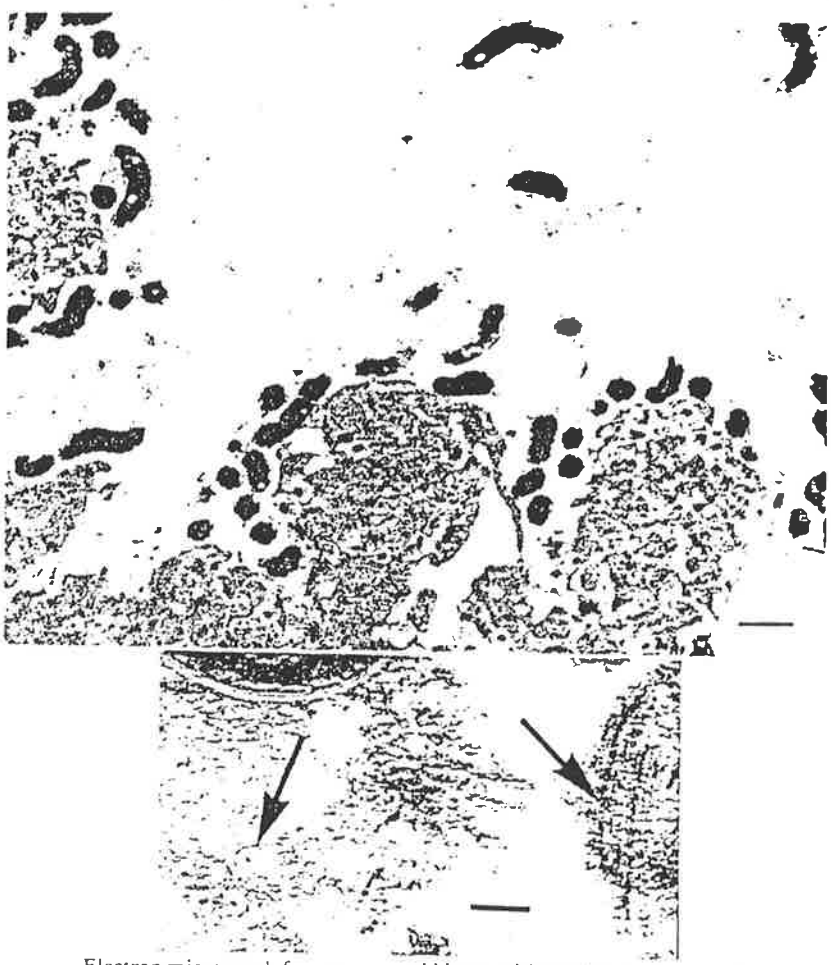
Gastritis could usually be graded with confidence at low magnification. There was some difficulty with about 25 cases where the changes were mild or the specimens were small, superficial, or distorted. To ensure that gradings were reliable, single H & E sections from the last 40 cases were examined "blind" by another pathologist who agreed with the presence or absence of gastritis in 36 cases (90%), and gave an identical grading in 32.

Gradings for bacteria by silver staining were more straightforward. The bacteria stained well and were easily differentiated from contaminant bacteria or debris. Silver staining was the most sensitive method of detecting the spiral bacteria. Silver stained sections and Gram stained smears were both done in 96 cases and spiral bacteria were seen in 56 of them; 32 with both stains, 23 with silver alone, and 1 case with the Gram stain alone.

The correlation between gastritis and bacteria, defined by Gram and/or by silver staining, was remarkable (table III). Gastritis was present in 55/57 biopsy specimens with bacteria ($p = 2 \times 10^{-12}$). When the 31 patients with peptic ulcer were excluded, the correlation persisted, implying that the presence of bacteria was not secondary to an ulcer crater (table IV).

Microbiology

Specimens for culture were received from 96 patients and 11 were culture positive, all being seen with Gram and silver staining also. No spiral bacteria were grown from the first 34 cases, probably because the cultures were discarded too soon.



Electron micrograph from a mucosal biopsy with active chronic gastritis.

Upper: many profiles of sectioned pyloric campylobacter are located on the luminal aspect of mucus-secreting epithelial cells; plasma membranes are intact, but indented and almost devoid of microvilli (bar = 1 μm).

Lower: at higher magnification groups of transversely and longitudinally cut sheathed flagella are visible (arrows; bar = 100 nm).

The bacteria were S-shaped or curved gram-negative rods, 3 μm × 0·5 μm, with up to 1½ wavelengths. In electron micrographs they had smooth coats and there were usually four sheathed flagella arising from one end of the cell. They grew best in a microaerophilic atmosphere at 37°C; a campylobacter gas generating kit was sufficient (Oxoid BR56). Moist chocolate or blood agar was the preferred medium. Growth was evident in 3 days as 1 mm diameter non-pigmented colonies. In artificial media the bacteria were usually larger and less curved than those seen on Gram stains of fresh tissue. They formed coccoid bodies in old cultures. The bacteria were oxidase +, catalase +, H_2S +, indole −, urease −, nitrate −, and did not ferment glucose. They were sensitive to tetracycline, erythromycin, kanamycin, gentamicin and penicillin, and resistant to nalidixic acid. DNA base analysis gave a guanine + cytosine content of 36 mol%, a value in the range for campylobacters.

Sources of Bias

The patient sample was from a defined population with gastric symptoms expected to have some gastroenterological abnormality. The biopsy tissue studied was from apparently intact mucosa—ie, not the sort of specimen a pathologist usually sees. We attempted to limit bias by making the study consecutive and blind, and were partly successful. The study was not strictly consecutive since 84 patients had to be excluded. However, gastroscopy reports and laboratory investigations were completed serially and usually independently ("blind") except that clinically relevant material was sent (to J. R. W.) with study biopsies, mainly from cases of gastric ulcer. However, an independent blind assessment of gastritis in 40 cases matched the study results well.

Discussion

The spiral bacteria of the human gastric antrum have never been cultured before, and their association with active chronic gastritis has not been described. They are a new species closely resembling campylobacters morphologically and in respect of atmospheric requirements and DNA base composition, but their flagellar morphology is not that of the genus *Campylobacter*.[9] Campylobacters have a single unsheathed flagellum at one or both ends of the cell whereas the new organism has four sheathed flagella at one end.[7,10] If it is premature to talk of "*Campylobacter pyloridis*"[11] perhaps the name "pyloric campylobacter" will do to define the site where these organisms are commonly found and to indicate the similarity to known *Campylobacter* spp.

There was no well-defined clinical syndrome associated with pyloric campylobacter. Only "burping" was significantly associated. Others have described this symptom in patients with non-ulcer dyspepsia and PMN infiltration of the antrum is also common in such patients.[12,13] We expected abdominal pain to correlate with pyloric campylobacter or gastritis, but it did not. Perhaps, since most patients undergoing gastroscopy have pain (75% in our study) the question "Do you have abdominal pain—yes or no?" was too general.

Much of the questionnaire was designed to select likely sources or causes of pyloric campylobacter infection. For example, bacteria might have colonised patients who already had gastritis and were taking antacids, milk, or cimetidine, thus impairing their "gastric acid barrier" and predisposing them to infection.[14] Animal contact and carious teeth were also considered as sources of infection. Campylobacters are commensals of domestic and farm animals (*C coli, C jejuni*), and they also inhabit the human mouth (*C sputorum* ss *sputorum*).[15] We found no evidence that any of these factors predisposed to the infection.

The absence of a relation between "known causes" of gastritis and the presence of histological gastritis has been noted by others. For example, analgesic abusers often have no gastritis, even when a gastric ulcer is present;[16] alcohol consumption is not clearly related to gastritis;[17] the quantity of bile in the stomach (duodenogastric reflux) is not obviously related to the state of gastric mucosa;[18] autoimmune disease is an unlikely cause, since gastric autoantibodies are uncommon except in pernicious anaemia, where the main histological changes are in the body of the stomach, not the antrum.[19] Gastric ulcer seems an unlikely primary cause of antral gastritis because the gastritis remains after successful treatment of the ulcer with cimetidine or carbenoxolone, and gastritis is just as common in patients with duodenal ulcer as with gastric ulcer.[6,20–23] Thus, the aetiology of chronic gastritis remains uncertain.

We have found a close association between pyloric campylobacter and antral gastritis. When PMN infiltrated the mucosa the bacteria were almost always present (38/40). In the absence of inflammation they were rare (2/31), suggesting that they are not commensals. The bacteria were not cultured unless the patient had histological evidence of both gastritis and pyloric campylobacter. We know of no other disease state where, in the absence of complicating factors such as ulceration (table IV), bacteria and PMNs are so intimately related without the bacteria being pathogenic.

How does pyloric campylobacter survive? The bacteria were usually in close contact with the mucosa, often in grooves between cells, within acinus-like infoldings of the epithelium or within the mucosal pits (figure). The surface mucus coating was superficial to the bacteria and any foreign material or organisms from the oral flora were present above the mucus, rarely mixed with it, and not beneath it: the mucus appeared to form a stable layer over the spiral bacteria. The antrum secretes mainly mucus, and the deeper levels of the surface mucus coating are slightly alkaline.[24] Thus pyloric campylobacter grows in a near-neutral environment, in close contact with the mucosa and protected from the bactericidal gastric juice. The absence of these bacteria from past reports of gastric microbiology may be because only gastric juice was cultured.[25,26] Even salmonellae cannot survive the low intragastric pH for more than a few minutes.[14] Where gastric biopsy material has been cultured,[6,27,28] microaerophilic techniques were not used and pyloric campylobacter did not grow.

Peptic ulcer was the only endoscopic finding associated with histological gastritis and pyloric campylobacter. This was surprising since the bacteria were not prominent on gastric ulcer borders and in duodenal ulcer no correlation would be expected. Perhaps the mucus coating is deficient or unstable near ulcer borders, thus allowing damage to the bacteria as well as the mucosa. Within a few millimetres of an ulcer, both pyloric campylobacter and gastritis were usually present. Other studies have shown continuing gastritis after ulcer healing with cimetidine and we have observed the persistence of pyloric campylobacter colonisation in such patients. The failure of the H_2 receptor antagonists to prevent ulcer relapse is attributed to an underlying ulcer diathesis which is unaffected by therapy. A bacterial aetiology, with continuing gastritis, could be the explanation. The diathesis may be a myth. Of ulcer-healing agents the only one thought to improve relapse rates is tripotassium dicitratobismuthate.[29] This compound is bactericidal to pyloric campylobacter and in patients treated with it the gastritis improved and the bacteria disappeared.[30]

The aetiology of peptic ulceration is unknown but until now a bacterial cause has not really been considered. We have found colonisation of the gastric antrum with pyloric campylobacter in over half of a series of cases at routine endoscopy. The bacteria were present almost exclusively in patients with chronic antral gastritis and were also common in those with peptic ulceration of the stomach or duodenum. Although cause-and-effect cannot be proved in a study of this kind, we believe that pyloric campylobacter is aetiologically related to chronic antral gastritis and, probably, to peptic ulceration also.

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Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa

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SUMMARY One hundred and three gastroscopic biopsies from 80 patients were cultured for *Campylobacter pyloridis* and studied histologically. Active chronic gastritis, as shown by the presence of polymorphonuclear leucocytes, was diagnosed in 51 biopsies and *C pyloridis* was found in 47. Sixteen gastric biopsies showed normal histology (no inflammation); *C pyloridis* was detected in only one of these, and a second biopsy taken from this patient at the same time showed active gastritis. Biopsies could be kept at 4°C for five hours without loss of viability of *C pyloridis*. An inoculum made by grinding the biopsy in a ground glass grinder consistently gave a much heavier growth of *C pyloridis* than one made by mincing the specimen. The campylobacter supplement ferrous sulphate, sodium metabisulphite, sodium pyruvate (FBP) (Oxoid) was inhibitory for some isolates; the inhibitory component was found to be sodium metabisulphite. Contaminants, but not *C pyloridis*, were inhibited by the incorporation of vancomycin 6 mg/l, nalidixic acid 20 mg/l, and amphotericin 2 mg/l, but higher concentrations inhibited *C pyloridis*. Undried plates kept in a plastic container at room temperature for up to two weeks were as satisfactory as freshly poured plates for the isolation of *C pyloridis*.

Spiral bacteria were cultured from endoscopic biopsies of gastric antral mucosa at this hospital during 1982.[1] The name *Campylobacter pyloridis* has been proposed for these bacteria,[2] which may be important in the aetiology of gastritis and peptic ulcer. During the study spiral bacteria were seen histologically in 58 of 100 biopsies but were cultured from only 11. In 1984 McNulty and Watson, working in a specialist laboratory, reported the presence of spiral bacteria in great abundance in Gram stained material from endoscopic biopsies. They were, however, "struck by the paucity of colonies that appeared on the culture plates." Since 1982 we have improved our culture techniques, and colony densities now closely match the number of bacteria seen on microscopy. This study was carried out in 1984 to evaluate a range of culture techniques for isolating *C pyloridis* from endoscopic biopsies.

Material and methods

All patients referred to the gastroenterological unit at this hospital and from whom an endoscopic biopsy had been taken from the upper gastrointestinal tract were eligible for the study. Between March and August 1984, 103 matching pairs of biopsies were obtained for culture and histology were obtained from 80 patients. The group comprised 41 men aged 26–90 (mean 59) and 39 women aged 23–85 (mean 62). Ninety pairs of biopsies were obtained from the stomach, 81 from the antrum and nine from the body. In addition, eight biopsies were taken from the oesophagus and five from the duodenum. In the antrum the biopsies were taken at a distance from any focal lesion such as a peptic ulcer.

The first 19 specimens for microbiological culture were transported to the laboratory in 2 ml isotonic saline. As such fluid could have caused dissolution of the mucus layer under which the bacteria are in close contact with the mucosa, with subsequent loss of the bacteria into the fluid, a small amount of hypotonic fluid (0·5 ml of 20% glucose) was used as the transport medium for the remaining specimens. The biopsies were kept at 4°C until they were inoculated on to solid media. The time until inoculation

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was recorded. Ten biopsies were divided, and one part was immediately processed for culture while the other part was kept at 4°C for varying lengths of time to determine the effect of delay on the isolation of *C pyloridis*.

PREPARATION OF GRAM STAIN AND INOCULUM

A portion of each biopsy was used to make a smear which was subsequently stained by Gram's method. The remainder was cultured. The first 31 specimens were minced with two sterile knives to prepare the inoculum. The other specimens were ground in 0·3 ml 20% glucose with a ground glass grinder. Three drops of suspension were obtained, and one drop was used as the inoculum. To determine which method yielded the largest number of colonies 10 specimens were divided; one half was processed by mincing and the other half by grinding, with inoculation on to brain-heart infusion agar with 10% horse serum, 0·25% yeast extract, and 0·4% tetrazolium chloride to visualise the colony densities.

COMPOSITION OF SOLID MEDIA

Before this study we had found that abundant growth of *C pyloridis* was obtained on freshly poured brain-heart infusion agar base (Oxoid) containing 7% horse blood and Isovitalex 1% (BBL Microbiology systems), with vancomycin 3 mg/l and nalidixic acid 10 mg/l. All specimens were inoculated on to this standard medium. Various other media were inoculated in parallel to answer the following questions concerning primary isolation of *C pyloridis:* must the medium be fresh; is lysed blood better than whole blood; is Campylobacter FBP (Oxoid) supplement useful; and what are the best concentrations of inhibitory antibiotics? Plates kept at room temperature in a closed plastic box for six to 19 days were used for 36 specimens. Lysed blood medium was used for 30 specimens and whole blood with FBP supplement for a further 20 specimens; when failures occurred the individual components of FBP were tested to find which inhibited *C pyloridis*. Medium without antibiotics was inoculated in parallel, and, in addition, higher concentrations of antibiotics were used in various media.

INCUBATION ATMOSPHERES

One plate from each specimen was incubated in a "preferred" atmosphere obtained by evacuation of an anaerobic jar to 220 mm Hg and replacement with an anaerobic gas mixture (10% carbon dioxide, 10% hydrogen, and 80% nitrogen) giving an atmosphere of 5% oxygen, 7% carbon dioxide, 8% hydrogen, and 80% nitrogen. Two other atmospheres were tested in parallel: a standard campylobacter gas mixture (8% carbon dioxide, 7% oxygen, and 85% nitrogen) for 46 specimens and a carbon dioxide incubator (7% carbon dioxide, 20% oxygen, and 73% nitrogen) for 33 specimens; water was placed in the bottom shelf, giving a humidity of 98% when measured by a Vaisala machine. We had previously determined that *C pyloridis* failed to grow in another carbon dioxide incubator with similar percentages of carbon dioxide and oxygen, but with a humidity between 94 and 98%, suggesting that for primary isolation *C pyloridis* required a constant atmosphere of at least 98% humidity. Sections were stained with haematoxylin and eosin and Warthin-Starry stain for spiral bacteria and were graded for gastritis as described by Marshall and Warren.[4] Gradings were based on the type of inflammatory cells present. Grades 0 and 1 signified normal. Grade 2, or "chronic gastritis," indicated inflammation with no increase in polymorphonuclear leucocytes. Grade 3, or "active gastritis," was used to indicate an increase in polymorphonuclear leucocytes, usually an intraepithelial infiltration.[4]

Results

DISTRIBUTION OF *C PYLORIDIS* IN THE GASTRIC MUCOSA

Most histological sections showed an uneven distribution of bacteria, often quite patchy. From 21 patients in whom bacteria were found, two or more biopsies had been taken from the stomach; 13 patients (62%) showed a different concentration of spiral bacteria on the two specimens, and in two cases the bacteria were not seen on one or more specimens. One of these patients showed chronic gastritis with gross atrophy and intestinal metaplasia in two biopsies, although no bacteria were seen, but a third biopsy, taken from an area adjacent to a gastric ulcer, showed active gastritis, no metaplasia, and numerous spiral bacilli. The second patient showed normal antral mucosa with occasional bacteria. Three gastric polyps from this patient were also biopsied; all showed active gastritis, although bacteria were not seen on two but were numerous on the third. Biopsies for histology and culture were taken as matching pairs, yet in 11 cases *C pyloridis* was found in only one of the pair, possibly due to the irregular distribution.

C pyloridis was cultured from 48 of the 103 biopsies, and in another six specimens spiral bacteria were seen with the Gram stain but not on culture. Spiral bacteria were seen in 55 biopsies, and in another five biopsies *C pyloridis* was either seen in the Gram stain or grew on culture. There was no significant difference between the density of growth obtained from immediate or delayed culture after biopsies had been stored at 4°C for up to five hours.

There was a remarkable correlation between active chronic gastritis and the presence of spiral bacteria. These bacteria were found, either histologically or microbiologically, in 60 of 103 biopsies (44 of 80 patients): in 47 of 51 biopsies showing active chronic gastritis; eight of 23 showing chronic gastritis; one of 16 with "normal" mucosa; and four of 13 duodenal or oesophageal biopsies, all four of which showed gastric metaplasia and active inflammation. The negativity of the four biopsies from patients with active gastritis may have been related to the use of cimetidine. Nine biopsies from 26 patients with gastric ulcer showed *C pyloridis* and an identical number of biopsies showed the organism in 12 patients with duodenal ulcer.

DENSITY OF GROWTH

The Figure shows the growth of *C pyloridis* from one biopsy, which had been divided in half and one half minced and the other half ground. Seven other positive biopsies were similarly treated, and five gave noticeably more colonies after the specimen had been ground than after it had been minced.

Our culture methods yielded an abundant or moderate growth of *C pyloridis* in 62% of positive specimens. In four specimens bacteria were cultured when they were not seen on Gram staining.

VARIATIONS IN SOLID MEDIA

Plates were kept for six to 19 days at room temperature (24°C) in a closed plastic box in which the relative humidity was 92%. The 15 positive specimens plated in parallel on freshly poured and older plates gave the same density of growth on both.

Specimens plated in parallel on whole blood agar with 1% Isovitalex or FBP supplement, yielded *C pyloridis* on 11 occasions with Isovitalex but only seven with FBP supplement, six of which yielded a lighter culture. Incorporation of the components of FBP supplement into solid media indicated that sodium metabisulphite inhibited *C pyloridis* in solid media.

VARIATIONS IN CONCENTRATIONS OF ANTIBIOTICS

The standard medium contained vancomycin 3 mg/l and nalidixic acid 10 mg/l. When a medium without antibiotics was plated in parallel with this the standard medium yielded 29 isolates of *C pyloridis* whereas the matching plates without antibiotics gave only 24 cultures. The five other matching plates all showed a moderate or heavy growth of contaminants, which occurred in 83% of the plates without antibiotics. On the standard medium a moderate or heavy growth of contaminants occurred in 28%, and in two of these, although the Gram stain showed spiral bacteria, *C pyloridis* was not cultured. Medium containing vancomycin 6 mg/l, nalidixic acid 20 mg/l, and amphotericin 2 mg/l was tested in parallel, and 22 isolates of *C pyloridis* were obtained on this medium and the standard medium; the density of growth of *C pyloridis* on both media was the same. With the higher concentration of antibiotics, contaminants were scanty and on only 18% of the plates. Whenever spiral bacteria were seen in the Gram stain *C pyloridis* was cultured. An even higher concentration of antibiotics: (vancomycin 20 mg/l, nalidixic acid 30 mg/l, and amphotericin 4 mg/l) inhibited all contaminants, but four of nine isolates were also inhibited. In three of the five positive cultures the growth was heavier on the standard medium than on the medium with these higher concentrations of antibiotics. We concluded that the most satisfactory medium contained vancomycin 6 mg/l, nalidixic acid 20 mg/l, and amphotericin 2 mg/l, which allowed contaminants to be inhibited but not *C pyloridis*.

The density of growth on lysed blood agar and the size of the colonies were less than on whole blood agar for four of the 10 isolates of *C pyloridis* grown on plates inoculated in parallel.

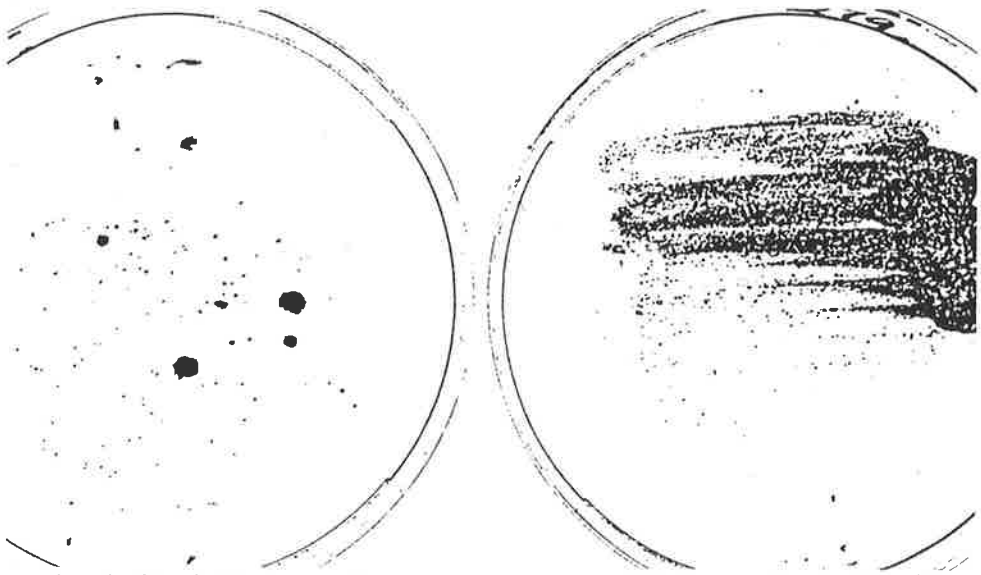
INCUBATION ATMOSPHERES

The preferred atmosphere, obtained by evacuation to 220 mm Hg and replacement with anaerobic gas mixture, was tested in parallel with the campylobacter gas mixture. The inoculum obtained from 33 ground specimens gave 15 positive cultures with each atmosphere, all from matching pairs, but showed a scantier growth with two biopsies in the campylobacter gas mixture. For the 13 minced specimens the six positive cultures grew equally well in both atmospheres.

Specimens cultured in parallel in the carbon dioxide incubator and the anaerobic jar mixture gave 16 positive cultures in the anaerobic jar mixture but only 13 in the carbon dioxide incubator: eight of these 13 cultures produced a scantier growth in the carbon dioxide incubator than in the anaerobic jar mixture.

CONTAMINATION OF ENDOSCOPE WITH *Campylobacter sputorum*

In one of our patients *C pyloridis* was isolated from the antrum, but another biopsy from the oesophagus also yielded a growth of spiral bacteria, although none was seen with Gram stain. On subculture growth appeared after only one day of incubation, and biochemical tests gave a heterogeneous result that was clarified only by extensive subculturing. The isolate from the oesophagus was finally confirmed as *C sputorum*, and we suspect that the



Growth of C pyloridis from one biopsy on serum-yeast agar containing tetrazolium and inoculum prepared by (a) mincing or (b) grinding.

original isolate had been contaminated by *C pyloridis*.

Discussion

Grinding the biopsy tissues with a ground glass grinder usually yielded a heavier, more uniform culture than mincing the tissue with knives (Figure). Such uniformity would be unlikely for the method described by Jones *et al*,[5] who "rubbed" the biopsy on the surface of the blood agar plate. With a consistently uniform inoculum primary isolation of *C pyloridis* on different media should be the most sensitive method of comparing media and incubation atmospheres. Provided biopsies were kept at 4°C, delay for up to five hours did not seem to affect the isolation of *C pyloridis*.

We were impressed by the fact that *C pyloridis* seems to be found in the gastric mucosa in a patchy distribution. Biopsies taken close together sometimes show an absence of spiral bacteria in one but an abundance in the other. Most biopsies showed a somewhat patchy distribution of bacteria. This may partly explain the instances when either culture or histology, but not both, was positive.

The most satisfactory medium was brain-heart infusion agar with 7% horse blood, 1·0% Isovitalex, vancomycin 6 mg/l, nalidixic acid 20 mg/l, and amphotericin 2 mg/l. Isolation of *C pyloridis* was excellent, and contaminants were inhibited almost completely. Concentrations of antibiotics higher than these inhibited *C pyloridis*. FBP supplement inhibited some isolates of *C pyloridis*, and we determined that the component responsible was sodium metabisulphite. Incubation in a carbon dioxide incubator is not recommended for primary isolation; this contradicts a previous report.[2]

Cimetidine may have a useful antibacterial action against *C pyloridis*. In a separate study, to be published elsewhere, on the susceptibility of *C pyloridis* to antibiotics and antiulcer agents we found that the minimum inhibitory concentration of cimetidine against *C pyloridis* was 4 mg/l. Isolation of *C pyloridis* from patients receiving cimetidine in the present study was low. From 35 biopsies from patients taking cimetidine there were 11 positive cultures (31%) (from nine of 25 patients), and from the 68 other biopsies there were 37 positive cultures (54%) (29 of 55 patients) ($p = 0·022$, Fisher's exact method). Three of the four patients with active chronic gastritis but with no apparent bacteria were receiving cimetidine. Eight of 11 of the positive cultures from patients receiving cimetidine, however, gave quite a heavy growth. The clinical importance of these findings is uncertain, and further investigation is required.

This study confirms previous observations[1] that *pyloridis* is closely associated with active chronic gastritis; in our study *C pyloridis* was detected on only one biopsy that appeared normal histologically, and the patient showed active chronic gastritis in a second specimen. Jones *et al*[5] found a close association between *C pyloridis* and both types of gastritis which they described as "superficial" or "atrophic"

Other studies have also reported a close association of *C pyloridis* with gastritis.[3,6] The clinical importance of *C pyloridis* will be clarified if histopathologists use similar nomenclature to describe varying forms of gastritis. Most histopathologists do not seem to differentiate between "chronic" and "active chronic" gastritis, conditions which have been clearly defined by Whitehead[4] and Marshall and Warren.[1] We found a pronounced difference between the detection of *C pyloridis* in active chronic gastritis (92%) and chronic gastritis (35%).

Finally, we must mention that although we have proposed the name *C pyloridis* for these spiral bacteria from the stomach, other studies of ours on the fatty acids and ultrastructure of *C pyloridis*[7] and the protein patterns reported by Pearson *et al*[8] suggest that these bacteria do not belong to the genus *Campylobacter*.

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Enzyme-Linked Immunosorbent Assay for *Campylobacter pyloridis:* Correlation with Presence of *C. pyloridis* in the Gastric Mucosa

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Antibody to *Campylobacter pyloridis* was measured by ELISA in the sera of 160 patients from whom gastric biopsy specimens were also obtained. The antigen was an acid-glycine extract of *C. pyloridis*, and titers ranged from 80 to 22,000 ELISA units (EU). Of 117 patients in whom *C. pyloridis* was detected microbiologically or histologically, 87 (74%) had a titer ≥300 EU, and only one had a titer <150 EU. Of 43 patients in whom *C. pyloridis* was not detected, only two (5%) had a titer >300 EU. Thus, for a titer of 300 EU the ELISA test had a specificity of 97% and a sensitivity of 81%. At 150 EU the specificity was 78%, and the sensitivity was 99%. Histological diagnosis of active chronic gastritis was associated with a high median ELISA titer (485 E), chronic gastritis with a much lower titer (150 EU), and normal histology with a titer of 110 EU. Discriminating use of this serological test could be of assistance to detect *C. pyloridis* in the gastric mucosa.

The name *Campylobacter pyloridis* has now been validated [1] for the campylobacter-like spiral bacteria first cultured at Royal Perth Hospital in 1982 from specimens of the gastric antral mucosa obtained by endoscopic biopsy [2, 3]. This new organism may be the etiologic agent in gastritis-associated dyspeptic disease and most cases of duodenal ulcer [4], and possible pathogenic mechanisms have been delineated [5]. Thus in patients with nonulcer dyspepsia, when the presence of *C. pyloridis* is indicated by detecting specific antibody, an attempt at curative antibacterial therapy may be justified. A discriminatory serological test could replace the difficult and expensive procedures such as upper gastrointestinal endoscopy and biopsy, which are presently required to demonstrate the presence of *C. pyloridis* in the stomach. However, as Svedheim [6] has stated, "the antigen is crucial in diagnostic serology." For *Campylobacter jejuni*, an acid-glycine extract in an ELISA is the most satisfactory preparation [6–8], but for *C. pyloridis* the antigens for serological tests in published reports were sonicated whole bacteria [9–11] or bacteria killed with formalin [12, 13]. Some healthy volunteers had histological gastritis and *C. pyloridis* in their stomachs [14]. In this study, serological results were obtained with an acid-glycine extract of *C. pyloridis* in an ELISA assay, and from every patient, a gastric biopsy specimen was obtained and examined microbiologically and histologically.

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Materials and Methods

Bacterial strain and antigen preparation. The antigen was prepared from a pool of 11 isolates of *C. pyloridis* obtained from the gastric mucosa and included strains 11637 and 11638 from the National Collection of Type Cultures (London). The preparation of the antigen and the ELISA technique were similar to those described for *C. jejuni* by Blaser and Duncan [8]. Isolates were grown on heated blood agar with IsoVitaleX® 1% (BBL Microbiology, Cockeysville, Md) for three days at 37 C in a *Campylobacter* gas mixture [15]. Bacterial cells were harvested in sterile distilled water, washed twice in sterile distilled water, and suspended in 0.2 *M* glycine-hydrochloride buffer (pH 2.2) at a concentration of 0.1 g (wet weight) of cells to 2.5 ml of buffer. Suspensions were stirred at 25 C for 15 min and centrifuged at 11,000 *g* for 15 min at 4 C. The supernatant was retained, and the pH was neutralized with sodium hydroxide. The supernatant was dialyzed against sterile distilled water for 24 hr at 4 C. The protein was filtered by using the Amicon Diafiltration system membrane type UM10 (Amicon, Dan-

vers, Mass), and protein concentrations were determined by the Lowry technique [16]. Preparations were stored at −20 C until use. Before coating the ELISA trays, we diluted the concentrated antigen preparation in 0.5 *M* carbonate buffer (pH 9.6) to give a final concentration of 2.5 μg of protein/ml. Polyvinyl chloride, "high activity" microtiter, flat-bottomed plates (Flow Laboratories, McLean, Va) were coated with the diluted antigen preparation by adding 0.2 ml to each well. The plates were covered and incubated for 24 hr at 4 C. Each well was then aspirated dry and refilled with 0.3 ml of PBS containing thimersol–Tween® 20 plus gelatin (1 mg/ml). The plates were kept at 4 C until use.

Proteins in the antigen. Protein profiles of the acid-glycine preparation were examined by discontinuous SDS-PAGE, as described by Laemmli [17]. After centrifugation at 6,000 *g* for 15 min, the supernatant was heated at 100 C for 5 min with a disintegration buffer that gave a final concentration of 50 nmol Tris hydrochloride (pH 6.8), 5% beta-mercaptoethanol (vol/vol), 2% SDS (wt/vol), 10% glycerol (vol/vol), and 0.01% bromophenol blue. The proteins were separated on a SDS-PAGE gel system [17] that consisted of a 3% stacking gel and a 6%–18% gradient gel. Electrophoresis was performed at 500 V for 4 hr with cooling, and the gels were stained with coomassie blue. The molecular weights of the peptides resolved were calculated on the basis of a calibration curve of marker proteins.

Patients, biopsy specimens, and sera. The patients were consecutive referrals to the Gastroenterology Unit at Royal Perth Hospital from September 1984 to August 1985, from whom endoscopic biopsy specimens were obtained. Patients who had been treated with antibiotics or antacid were excluded from the study. One hundred sixty patients were entered in the trial, including 101 men and 59 women with an age range of 20–85 years. The mean age for the men was 53 years, and mean age for the women was 48 years. Endoscopic biopsy specimens were obtained and cultured for *C. pyloridis* on a selective medium in a microaerophilic environment, as previously described [15]. One specimen was taken for microbiological culture and one specimen for histological diagnosis and detection of spiral bacteria. Serum specimens were stored at −20 C, but 66 specimens were studied unfrozen for the presence of IgM antibodies to *C. pyloridis* and were then studied again after being frozen at −20 C.

ELISA. For the ELISA, optimal dilutions of all reagents were determined by checkerboard titration. Antigen-labeled plates were removed from 4 C and brought to room temperature (~23C) before use. Wells were washed three times with PBS containing thimersol–Tween 20. Each test serum was diluted 1:100 in serum diluent (PBS containing thimersol–Tween 20 with 5 mg of bovine gammaglobulin/ml and 1mg of gelatin/ml). A 100-μl sample of each test serum dilution was added in triplicate to the microtiter plate and incubated for 90 min at 25 C in an incubator to maintain a stable temperature. Wells were aspirated and washed three times with 0.3 ml of PBS containing thimersol–Tween 20. Peroxidase-labeled goat antibody to human IgA, IgG, and IgM (heavy- and light-chain specific) was used at a dilution of 1:16,000, and specific conjugates were used as follows: IgG, 1:50,000; IgM, 1:4,000. All conjugates were obtained from Kirkegaard-Perry Labs, Maryland. Conjugates were appropriately diluted in PBS containing thimersol with 20μg of bovine serum albumin/ml and 1.0 mg of gelatin/ml, and 100 μl was added to each well and was placed in an incubator for 90 min at 25 C. Wells were washed three times with PBS containing thimersol–Tween 20, and then twice with PBS containing only thimersol. A 100 μl sample of substrate consisting of 2.2'-azino-di-(3-ethyl-benzthiazoline) sulfonate with 0.005% hydrogen peroxide was added to each well and incubated at 25 C for 15 min. The reaction was stopped with 50 μl of 0.001% wt/vol sodium azide in 0.1 *M* citric acid. Plates were read within 2 hr on a Titertek Uniscan® (Flow Laboratories, Sydney, Australia) at 405 nm. This machine allowed a single blank well to be used to make a baseline measurement.

Each plate contained dilutions of three control sera that had to meet stringent requirements for the plate to be accepted. One control was the calibration serum consisting of a pool of 11 positive sera with high titers of antibody. This was diluted from 1:50 in doubling dilutions to 1:12,800, which allowed the construction of a standard curve as described under analysis of data below. The second control was a pool of 14 negative sera that was put on each plate in duplicate at a dilution of 1:100, and the reading of these wells had to be <0.1 absorbance units for the plate to be accepted. The third control was a "weakly positive" serum that was diluted at 1:400 and assayed in three wells. The results were analyzed as described below. Before the procedure was accepted, the weakly positive serum was tested in 18 plates to ensure reproducibility.

Inhibition assay. To asess the specificity of our test, we incubated a pool of sera with high positive titers in four aliquots at 37 C for 30 min—one with 10μg of the *C. pyloridis* extract and the three others with an acid-glycine extract of *C. jejuni*, *Campylobacter fetus*, or *Escherichia coli*. After incubation, 200-μl samples of the serum were doubly diluted from 1:10 to 1:320 and placed in triplicate wells; the ELISA was performed as described above.

Calculation of ELISA. The absorbance readings obtained for each of the dilutions of the calibration serum were used to construct a standard curve in the following manner. The highest dilution of the serum, 12,800, was arbitrarily designated as being equal to one ELISA unit (EU), and with the dilution factor of 100, this gave a result of 100 EU. The results were plotted as absorbance versus dilution of serum (figure 1). The 12 results usually covered a range of absorbances from 0.1 to 1.2 and were entered onto a programmed calculator. The plate was accepted if the correlation coefficient of this standard curve was >0.980. For each test serum done in triplicate the results were calculated from the standard curve by linear regression analysis and expressed in EU [18]. The mean of the weak positive control serum was 359 EU, with a standard error of 85.5 EU. For the sera of patients without *C. pyloridis*, the upper limit of two standard deviations from the mean was 300 EU.

Results

Inhibition assay. The effect of different acid-glycine extracts on the titer of *C. pyloridis* antibody in a highly positive serum is shown in figure 2. First, it can be seen that the *C. pyloridis* acid-glycine extract adsorbed out antibody in the positive control serum. Second, at 1:20 and 1:40 there was a greater than fourfold difference in absorbance between *C. pyloridis* and the other three extracts. These results also indicate, however, a degree of cross-reactivity between antibodies to *C. pyloridis* and extracts from the other bacterial species tested.

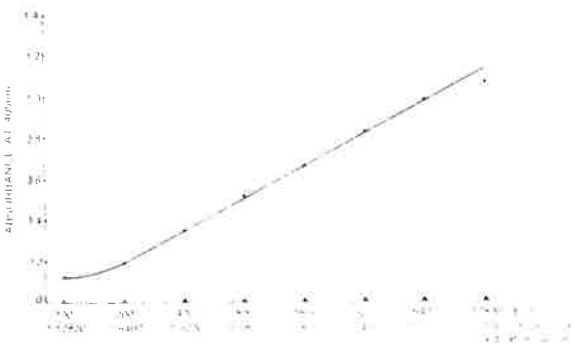

Figure 1. Standard curve of calibration serum used in the ELISA test.

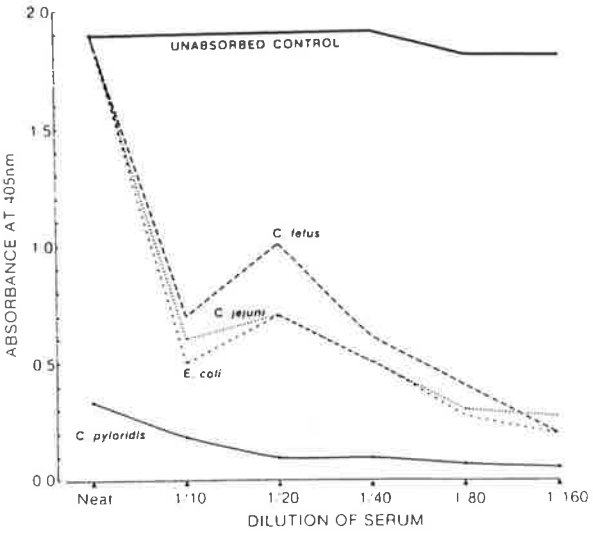

Figure 2. The effect of adsorption by acid-glycine extracts of four different bacteria on optical densities of serum that contained antibodies to *Campylobacter pyloridis*.

Protein bands in the ELISA antigen. The SDS-PAGE profile of the acid-glycine extact of *C. pyoridis* is shown in figure 3. A major triplet of protein bands was observed at 57 kDa, 62 kDa, and 64 kDa, with lesser bands at 24.5 kDa, 28 kDa, 33 kDa, and 84 kDa.

Detection of C. pyloridis by culture and histology, and histological diagnosis. For the 160 patients in the study, *C. pyloridis* was detected by culture or histology in 117 (73%) of the patients. In one patient, histology failed to reveal the organism, but *C. pyloridis* was cultured from the biopsy specimen (table 1). *C. pyloridis* was not detected in 43 (27%) of the patients. The histological diagnosis of active chronic gastritis, as defined by Whitehead [19] and Marshall and Warren [2], was made in 107 patients, and *C. pyloridis* was detected in all of these patients. Chronic gastritis without activity was present in 31 patients, and in 10 (32%) of these, *C. pyloridis* was detected. There were 22 patients with normal histology of the gastric mucosa, and in none of these was *C. pyloridis* detected.

ELISA results. In our polyclonal ELISA test,

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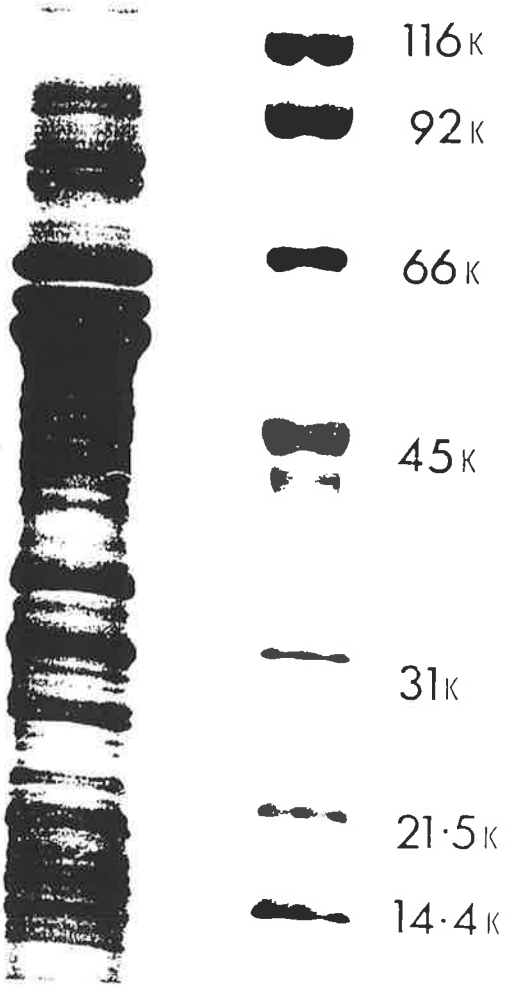

Figure 3. SDS-PAGE profiles of the acid-washed preparation used in the ELISA.

there was a linear relation between the reciprocal serial dilutions of the highly positive pooled serum and absorbance (figure 1) in the region from 200- to 6,400-fold serum dilutions. We found that serum IgA was not detected by our antigen; sera sent to us by Dr. Kaldor (Fairfield Hospital for Communicable Diseases, Melbourne), which apparently containing high titers of IgM antibody, had low titers, whether tested unfrozen or frozen.

ELISA titers of serum antibody. The ranges of titers in patients in whom *C. pyloridis* was or was not detected are shown in figure 4. There is a highly significant statistical difference between these two

Table 1. Histological and endoscopic diagnoses related to detection of *C. pyloridis* and ELISA titers

Diagnoses	No. in group	No. with *C. pyloridis* detected	Median titer (EU)
Active chronic gastritis			
Duodenal ulcer	61	61	415
Gastric ulcer	20	20	685
No ulcer	26	26	965
Chronic gastritis	31*	10	150
Normal histology	22†	0	110

NOTE. Among patients with active chronic gastritis and a duodenal ulcer, none had <150 EU and 41 had >300 EU. Among patients with active chronic gastritis and a gastric ulcer, none had <150 EU and 15 had >300 EU. Among patients with active chronic gastritis and no ulcer, none had <150 EU and 21 had >300 EU. Among patients with normal histology, 15 had <150 EU and two had >300 EU.

* Four of these patients had ulcers.

† Two of these patients had ulcers.

groups (Student's $t = 3.04$, $P < .002$). In the 43 patients in whom *C. pyloridis* was not detected, only two (5%) had a titer >300 EU, and 28 (65%) had a titer <150 EU; only three had a titer >250 EU. Of the 117 patients in whom *C. pyloridis* was detected, 86 (74%) had a titer >300 EU, 94 (80%) had a titer >250 EU, three a titer of 150 EU, and one patient had a titer of 100 EU. Thus, at a cut-off point of

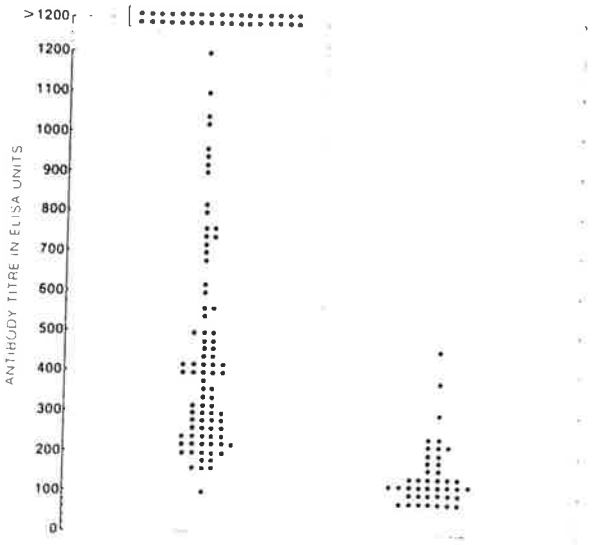

Figure 4. Serum titers of antibody to *Campylobacter pyloridis* in patients from whom a gastric antral biopsy specimen was examined for *C. pyloridis*. *Left*, titers when *C. pyloridis* was detected; *right*, titers when *C. pyloridis* was not detected.

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300 EU, our assay produced very few false-positive results. At 300 EU our test had a specificity of 97%, a sensitivity of 81%, a predictive positive value of 98%, a predictive negative value of 57%, and an efficiency of 86%. At a cut-off point of 150 EU our assay produced very few false-negative results. At 150 EU our test had a sensitivity of 99%, a specificity of 78%, a predictive positive value of 91%, a predictive negative value of 98%, and efficiency of 62%. Thus at 300 EU, our ELISA test showed good specificity, and at 150 EU the test showed good sensitivity.

Relation between ELISA titers and the histological and endoscopic diagnoses. Among the 107 patients with active chronic gastritis, 61 had a duodenal ulcer, 20 had a gastric ulcer, and 26 did not have an ulcer. Among 31 patients with chronic gastritis, only four had an ulcer; among 22 patients with normal histology, only two had an ulcer. The median ELISA titers are shown in table 1. In the patients with chronic gastritis the median titer was 150 EU, which was much lower than the median titer of those with active chronic gastritis regardless of whether the patient had a duodenal ulcer, (median, 415 EU), gastric ulcer (median, 685 EU), or no ulcer (median, 965 EU). Patients with normal histology had the lowest median titer, 110 EU. There were only five titers >6,000 EU, and to avoid weighting the statistical analysis, we estimated these titers to be 6,000 EU; the titers of those with active chronic gastritis were very significantly higher than those with chronic gastritis ($t = 3.13$, $P < .002$). The titers of patients with duodenal ulcers and active chronic gastritis were significantly lower than those with active chronic gastritis but no ulcer ($t = 2.36$, $P < .02$).

Among the 64 patients with a duodenal ulcer, only one had normal histology, and two had chronic gastritis; the titers of these three patients ranged from 80 to 220 EU. Among the 23 patients with gastric ulcers, only one had normal histology, and two had chronic gastritis; the titers of these patients ranged from 110 to 555 EU.

Discussion

A serological method to detect the presence of *C. pyloridis* should not give a high-positive result when *C. pyloridis* cannot be detected. Before a serological method is used for surveys it should be checked by the means we have used in this study, namely the analysis of gastric biopsy specimens for the presence of *C. pyloridis*. Apparently healthy volunteers have been found to have histological gastritis in the stomach, and *C. pyloridis* has been detected [14]. Therefore, the definition of a healthy individual must be made on histological and microbiological grounds and not on the absence of symptoms. Patients with nonulcer dyspepsia [5] have seen a doctor and been assured that they have no organic disease, but frequently are found to have gastritis and *C. pyloridis* infection.

Among the patients in whom *C. pyloridis* was not detected, the upper limit of two standard deviations above the mean of their titers was 300 EU; in patients with a titer >300 EU, *C. pyloridis* was nearly always found in the gastric antrum. When the titer was <150 EU, *C. pyloridis* was rarely found. With regard to titers between 150 and 300 EU, some patients may have falling titers because of the natural healing process [20], or healing may have been aided by undeclared antibacterial treatment such as bismuth tablets (bismuth is effective against *C. pyloridis* [4]). Some patients may have a relatively low titer if their infection has only just begun. In this study, there were nine patients with titers between 250 EU and 300 EU, and in only one of these was *C. pyloridis* not detected. Thirty-four (21%) of our 160 patients had a titer between 150 and 250 EU, and in 24 (71%) of these thirty-four patients, *C. pyloridis* was detected. There were two patients with a titer >300 EU in whom we could not detect *C. pyloridis*; they were each a close relative of a patient with peptic ulcer and *C. pyloridis* in the stomach. Among the patients who were excluded from the study because of prior antibacterial or antipeptic ulcer treatment, there were two patients who had apparently received cimetidine or sucralfate. In the patient who received cimetidine, *C. pyloridis* was not detected, but he had an antibody titer of 5,750 EU. In the patient who recieved sucralfate, *C. pyloridis* was also not detected, and the antibody titer was 1,030 EU.

We investigated the possibility that the higher ELISA titers in our patients would be associated with the most-severe inflammation histologically. However, among the 107 patients with active chronic gastritis, severe inflammation was found in the biopsy specimens of patients with low ELISA titers, between 200 EU and 300 EU, and some patients with high titers had only mild or moderately severe inflammation in their biopsy specimens.

An acid-glycine extract of *C. jejuni* has been found to be more reliable than are other antigenic preparations for the serological diagnosis of infection with

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that organism [6–8]. Despite the evidence that antibody to *C. pyloridis*, as measured in our ELISA, did show some evidence of cross-reactivity with the extracts of *C. jejuni*, *C. fetus*, and *E. coli*, there were nevertheless very few false-positive ELISA results.

Other antigens and methods have been reported for serological tests for *C. pyloridis*. A passive hemagglutination test with sonicated *C. pyloridis* as the antigen has been reported [10], but we have found that unsensitized erythrocytes hemagglutinated as readily as sensitized erythrocytes. An ELISA method with formalin-killed whole bacteria as the antigen [12, 13] detected high titers in apparently healthy contacts [12]. A CF method was used with an antigen that was "a thick suspension of organisms" harvested from a three-day culture [11]; a CF method was also used with sonicated *C. pyloridis* as antigen, but 12 of 78 patients negative for *C. pyloridis* were found to have a high titer of antibody [21]. The antibodies to *C. pyloridis* detected in healthy contacts of patients with peptic ulcer may be protective antibodies; immunoblot analysis may reveal whether different antigens are involved in the serological response of healthy contacts compared with patients with *C. pyloridis*.

A serological test can be made more reliable by using stringent controls. We used an additional control to those of Blaser and Duncan [8], namely a calibration serum on each plate, and for the plate to be accepted, a correlation coefficient >0.980 was required. This serum allowed us to record our results in a standardized fashion rather than directly as absorbances (OD).

The ELISA titers among our patients mirrored closely the histological diagnosis of active chronic gastritis, chronic gastritis, or normal histology. Table 1 shows that among the 31 patients with chronic gastritis the median titer was only 150 EU, whereas among the three groups with active chronic gastritis the median titers were 415 EU, 685 EU, and 965 EU. Among those with normal histology the median titer was only 110 EU. We regard these results as confirmation of the significance of the histological diagnosis of active chronic gastritis in relation to *C. pyloridis* infection. Price et al. [22] stated that in their study the incidence of *C. pyloridis* was similar in patients with chronic or active chronic gastritis. We question this observation and suggest that these authors have set their normal range for neutrophil infiltration too high, so that they relegated patients with active chronic gastritis to their chronic gastritis group. When active gastritis is absent in the gastric mucosa, neutrophils are almost completely absent. These authors may not have obtained biopsy specimens from sites similar to ours, or their selection of patients may have been different.

In addition to the presence of specific serum antibody in patients in whom *C. pyloridis* can be detected, the presence of local antibody in biopsy specimens would confirm that *C. pyloridis* is a genuine pathogen and not merely a commensal. Wyatt et al. [23] have reported that in vivo coating of *C. pyloridis* by host IgA was present in all 83 cases of active gastritis, and coating of bacteria with IgG or IgM was significantly correlated with activity of the gastritis and was rarely seen in the absence of neutrophil infiltrate.

Our conclusions from this study are that symptomatic patients who have a titer <150 EU will be highly unlikely to have *C. pyloridis* in the stomach, and patients with a titer >300 EU are very likely to have the organism in the stomach. As with any serological test there will be patients with intermediate results, and it is particularly these patients who will need an endoscopic examination to determine whether *C. pyloridis* is present.

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Rapid Urease Test in the Management of *Campylobacter pyloridis*-Associated Gastritis

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Campylobacter pyloridis **colonization of the stomach may be an etiological factor in gastritis and peptic ulceration. *Campylobacter pyloridis* produces large amounts of urease, and the presence of this enzyme in gastric mucosa usually indicates infection with the organism. In this paper we describe the use of a rapid urease test (CLOtest) to detect *C. pyloridis* infection in gastric mucosal biopsies. In 141 consecutive endoscopy cases, antral biopsies were taken for culture and histology, and an extra biopsy was inserted into the CLOtest gel. There were 79 patients infected with *C. pyloridis*, 78 of whom were detected by CLOtest: 75% were positive at 20 min, 92% at 3 h, and 98% at 24 h. There were no false positive results. Eighteen infected patients were rebiopsied after a course of amoxycillin and bismuth subcitrate. Active chronic gastritis resolved in eight of nine who were cleared of the organism, but histological gastritis was unchanged in nine patients who were still infected. CLOtest is a simple, sensitive, and highly specific test that enables the endoscopist to diagnose *C. pyloridis* infection in the endoscopy room. A negative test after antibiotic therapy correlates with clearance of the bacteria and healing of active gastritis.**

INTRODUCTION

The presence of *Campylobacter pyloridis* in the stomach of patients with gastritis, and the possible etiologic role of the organism in dyspeptic disease, has been discussed elsewhere (1–5). The bacterium is the most likely cause of type B (antral) gastritis and is possibly an etiologic agent for peptic ulceration associated with active chronic gastritis (6).

Until now, the study of *C. pyloridis* has needed the cooperation of a microbiologist and pathologist. Culture took 3 days, and histopathology at least 24 h. The most rapid way of diagnosing the infection was by microscopy of the smeared gastric mucus, but even in a research setting this procedure took at least 1 h and was very expensive in terms of the labor involved. We saw the need for a simple, fast, and reliable test that the endoscopist could perform to diagnose the infection in the endoscopy room.

The large amount of preformed urease enzyme produced by *C. pyloridis* afforded a means of detecting the organism without culture. Urease is not produced by mammalian cells (7), so if it is detected bacteria must be present. Herein we describe the evaluation of a rapid urease test (CLOtest) suitable for use by endoscopists. The effect of treating gastritis with antibacterial agents is also described, and the use of the test to monitor such therapy is suggested.

DESCRIPTION OF THE TEST

CLOtest is a mounted gel pellet containing urea, phenol red (a pH indicator), and a bacteriostatic agent. During manufacture, the gel is buffered to an acid pH which gives it a bright yellow color. The color of the gel changes to pink if the pH rises above 6.0. This color change should only occur when urea in the gel is hydrolyzed to release ammonia, *i.e.*, urease enzyme is present. *Campylobacter pyloridis* is the only bacterium inhabiting the gastric mucosa that contains enough preformed urease to be detected in the rapid urease test. CLOtest detects only preformed urease enzyme. Further production of urease by *C. pyloridis* or other contaminating organisms is prevented by a bacteriostat in the gel.

Before an endoscopy session, the CLOtests are warmed to 30°C in an incubator or in the endoscopist's pocket. At endoscopy, a 2-mm pinch biopsy is taken from the prepyloric mucosa, and the tissue is pushed beneath the surface of the CLOtest gel (Fig. 1*A*). The stomach contents are usually acid and the pH of gastric tissue approximately 6.0, so there is normally no color change in the gel. When *C. pyloridis* is present in the gastric biopsy, urea in the gel is hydrolyzed, ammonium is formed with the incorporation of hydrogen ion, and a rise in pH occurs (8).

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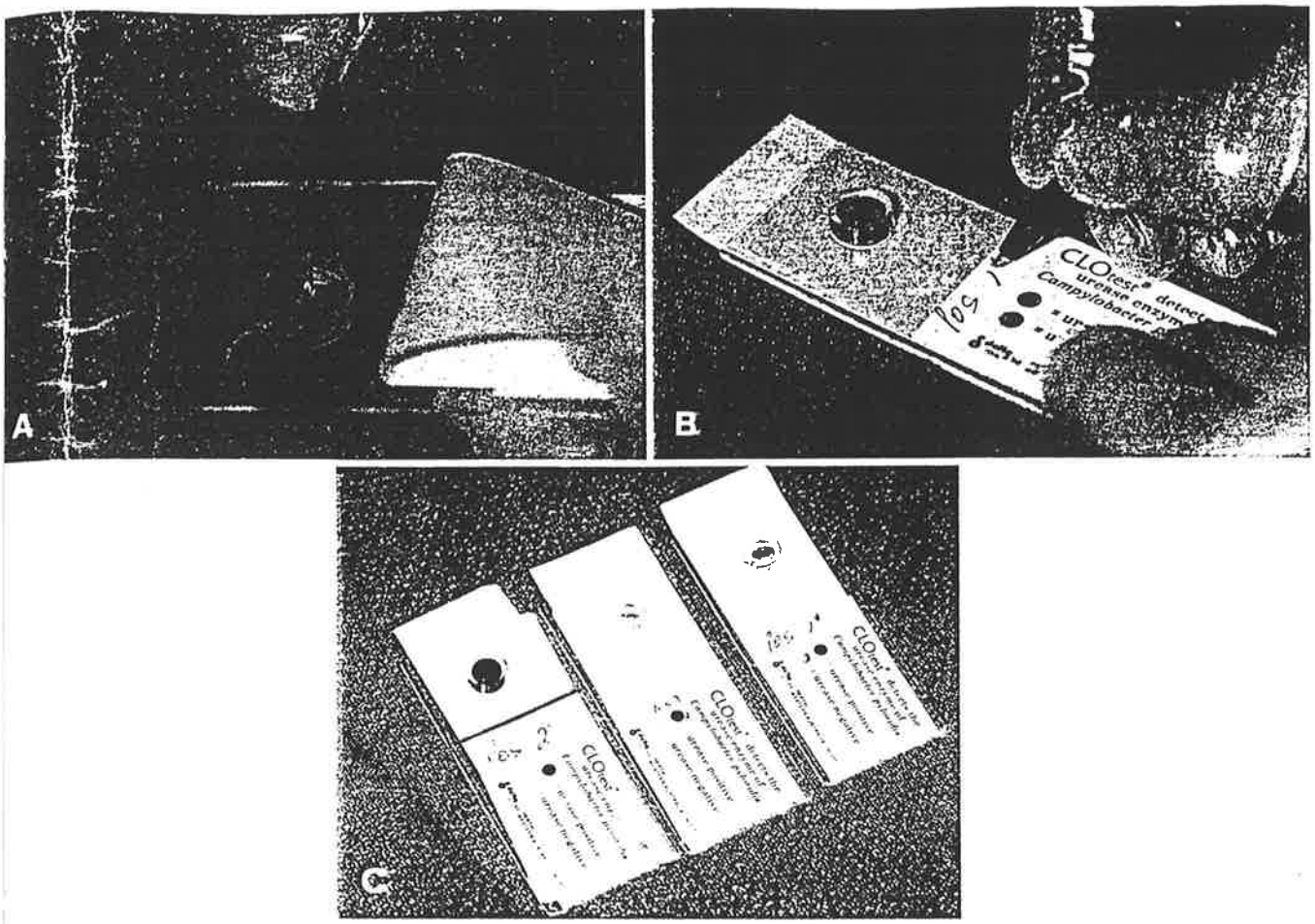

FIG. ... *A*, insertion of 2 mm pinch biopsy into the CLOtest gel. A 19G needle is preferred. *B*, the CLOtest is resealed and the time for a pink olor to appear is recorded on the label (in this case 1 min). Ideally, CLOtests should be incubated at 30–35°C but it is often more convenient to eep them in the endoscopist's pocket (also approximately 30°C). *C*, three CLOtests. The one on the *left* was positive at 2 min and is now 2 h ld; no further color change will occur. The center test from a CP− patient is 24 h old and remains yellow. The test on the right is the same as hat in *A* and *B*, 3 min after insertion of the biopsy.

rea

$$\begin{array}{l} NH_2 \\ | \\ C{=}O + 2H_2O + H^+ \xrightarrow{\text{urease}} \underset{2NH_4^+ + HCO3^-}{\text{ammonium bicarbonate}} \\ | \\ NH_2 \end{array}$$

In positive cases a red tinge develops around the iopsy, usually before the patient leaves the endoscopy oom (Fig. 1*B*). A color change does not occur if *C. yloridis* is absent (Fig. 1*C*).

To be of practical use it was decided that the CLOtest hould be read before the patient left the endoscopy oom (nominally 20 min after biopsy) so that therapy ould be prescribed by the endoscopist. As day-cases emained in the hospital for 3 h after endoscopy, it was onvenient to re-examine the tests at this time and iagnose further patients before they left hospital. After eing left on the shelf overnight the CLOtests could be eexamined 24 h after biopsy. Infected patients detected it this time could be telephoned to return for medication.

METHODS

Patients

The patients attended a dyspepsia research clinic. Every clinic patient underwent upper gastrointestinal endoscopy at which multiple biopsies were taken to detect *C. pyloridis* infection. Where possible, these investigations were repeated after antibacterial therapy. CLOtest was performed on every patient endoscoped between 1 August and 31 December 1985 to give the present series.

Endoscopy and biopsy

Patients were fasted overnight or for at least 4 h before endoscopy. If elective patients were taking H_2 receptor antagonists, antibiotics, or bismuth containing drugs, these medications were ceased and the endoscopy postponed for 14 days when possible. Patients were asked to drink a glass of milk the night before the endoscopy in the belief that this produced a white top on ulcer craters, which were photographed. Premedi-

i for endoscopy was with atropine and pethidine nuscularly, with additional intravenous diazepam diately before the procedure. No oral medication ermitted on the day of the test. Simethicone was iven, but patients sucked a lignocaine jube for t anesthesia.

e instrument used was an Olympus GIFXQ10 ptic panendoscope. After the stomach and duo- cap had been examined visually and suspicious is had been biopsied, four extra mucosal biopsies taken from areas uninvolved by any local lesion as an ulcer. The first two biopsies were taken from reater curve of the stomach about 5 cm from the us. One was placed in the CLOtest gel. The second y was placed in a drop of sterile 10% glucose. erated, and transferred to the microbiology labo- y within 3 h. Two biopsies for histology were then i within 5 cm of the pylorus, at angles of about ten ck and four o'clock. These were fixed immediately uffered formol-saline. CLOtest results were not tioned on the histology or microbiology request s, but normal clinical information was recorded.

study

ie first 52 patients were tested as a pilot study to rmine the best time to read the CLOtest. The color recorded on the endoscopy report, about 20 min the biopsy had been taken. The CLOtests were examined for further color change at 3 h and then e daily for 1 wk.

rately timed study (141 CLOtests)

his was a prospective study on consecutive patients hom the CLOtest was carefully observed. When biopsy was inserted into the CLOtest, the time was rded on the package. The CLOtest was kept at 30°C he next 3 h and after that at room temperature 25°C). The test was examined as soon as the en- opy had been completed (2–5 min), at 5-min in- als during the first h, and at 30-min intervals until patient was discharged from the hospital or 3 h had sed. If a definite pink color was observed in the gel ounding the biopsy, a positive result was recorded the time written on the label. Negative CLOtests e reexamined when the patients were discharged again at 24 h. If the CLOtest had changed to orange ink within this time, the result was recorded as tive.

y the time the data had been collected and analyzed, *C. pyloridis* status of another 130 patients had been rmined by CLOtest, histology, and microbiology. s group was not used in the analysis except to vide extra data in the graph of the CLOtest reaction e (see "Results").

CLOtests read by the microbiologist

These patients were undergoing endoscopic follow-up in a double-blind treatment study in which the endoscopist was not permitted to know if *C. pyloridis* infection was still present. In this group CLOtests were immediately sealed in an opaque envelope by the endoscopy nurse and sent to the research microbiology laboratory with the specimen for culture. The microbiologist recorded the CLOtest as positive or negative at 3 h (before the Gram stain had been seen) and at 24 h.

This was a select group of patients with duodenal ulcer disease and previously diagnosed *C. pyloridis* infection. In addition to standard ulcer therapy, they had received either antibiotic or placebo, administered double blind. As a result, about half of these patients were negative for *C. pyloridis*. All medication had been ceased 2 wk before follow-up endoscopy. The microbiologist did not know their *C. pyloridis* status before examining the CLOtest, but of course he knew the CLOtest result at the time he examined the Gram-stained smears. Correlation with the histology was not obtained in this group because the patients are still in a double-blind trial. Data from these patients were included to see if CLOtest could indicate the success or failure of antibacterial therapy.

MICROBIOLOGY

Microbiology specimens were processed in the routine laboratory except for the 64 cases mentioned above that were processed in the research laboratory. The tissue was ground, Gram stained and, cultured in a manner previously described (9). The number of spiral bacteria present on the Gram-stained smear was graded from zero (no bacteria seen) to three (many bacteria), and the culture result was recorded as positive or negative.

HISTOLOGY

For histological examination, formalin-fixed paraffin-embedded sections were stained with hematoxylin and eosin for grading gastritis and with a May Grunwald Giemsa method to show bacteria. Sections were treated as routine hospital specimens and reported by one of four pathologists according to the method of Whitehead (10) as modified by Warren and Marshall (1, 2). Only essential clinical information was made available to the pathologist on the request form; recent antibacterial therapy and the CLOtest result were not recorded, and examination of the histological specimens was consecutive and blind.

The four pathologists used slightly differing terminology when reporting the biopsies, but the presence of

norphs was always recorded as "activity," and ve chronic gastritis" was the usual diagnosis when norphs were seen. The term "chronic gastritis" was if lymphocytes or plasma cells were present in s, but polymorphs were difficult to find or totally it. Other abnormalities such as intestinal metaplatrophy, edema, and fibrosis were described but not considered indicators of gastritis unless lymytes, plasma cells, or polymorphs were also present cess. Typical histological appearances are shown g. 2.

r ease of analysis, a coding scheme was adopted ar to that used in the study of Marshall and Warren The method has been described in detail elsewhere and has been used by McNulty (12) for the serial sment of gastritis. In this method, the most severe is gastritis with polymorphonuclear and monoear cells present (active chronic gastritis), and a r degree of gastritis is that which has no polymornuclear but an excess of mononuclear cells only onic gastritis). We gave a score of 0 for a completely nal biopsy, 1 for a biopsy showing minor abnorties without significant inflammatory cell infiltrate, r chronic gastritis as described above, and 3 for ve chronic gastritis. For analysis of patients who biopsied more than once in this series, grade 2 further arbitrarily divided into mild and moderate onic gastritis according to the number of mononur cells present (severe chronic inflammation never urred without activity also being present), and grade as divided into mild, moderate, and severe degrees ctive chronic gastritis according to the number of morphs present.

DEFINITION OF BACTERIA POSITIVE AND NEGATIVE PATIENTS

o evaluate the CLOtest result, a *C. pyloridis* positive was defined as a patient who had spiral bacteria ical of *C. pyloridis* detected on Gram stain or culture istological sections. When the histology and micrology results disagreed, the histological sections were xamined, and a consensus was obtained. For CP+ ients histological active chronic gastritis was usually sent.

or patients participating in the double-blind study clinical data was provided, and for other patients y significant endoscopic findings were mentioned the laboratory request forms. At Royal Perth Hosal the diagnosis of *C. pyloridis* infection by histology culture is not in contention. *Campylobacter*-like anisms are routinely reported on gastric mucosal psies and have been diagnosed in 910 patients since 79. In addition, *C. pyloridis* has been cultured from r 300 patients since 1982.

EFFECT OF ERADICATION OF CP

Eighteen CP+ patients, not in the double-blind study, were rebiopsied after they had completed a course of antibiotic therapy. The therapy given was bismuth subcitrate tablets 1 qid with amoxycillin 500 mg qid concurrently for 14 days. These patients made a group in which analysis of the histology before and after treatment of *C. pyloridis* infection was possible. For analysis of this group, nine patients in whom therapy was unsuccessful (CLOtest still positive) were compared with nine in whom the bacteria had been cleared (CLOtest negative). In addition, the 18 patients described above were compared with six *C. pyloridis* negative patients who were biopsied twice and who did not receive antibacterial therapy or bismuth.

DATA ANALYSIS

To see if the CLOtest reaction time correlated with the number of bacteria seen on the biopsy, only CP+ patients with CLOtest reacting within 180 min were studied (*i.e.*, accurately timed). Data on CLOtest reaction times and numbers of bacteria were analyzed using one-way analysis of variance by means of the SPSS-X statistical package (13). Analysis of the trend between numbers of bacteria and CLOtest reaction time used polynomial regression. Comparison of means *a posteriori* utilized Scheffé's and Duncan's tests. Comparison of data on numbers of bacteria from microbiology and histology used McNemar's related samples χ^2 test. Because only CP+ patients were analyzed, bacteria numbers seen were grouped as 0–1, 2, and 3. An average grade for bacteria numbers was made by summing histology and Gram stain grades for each patient and grouping the result as 0–1 (category 1, low numbers of bacteria), 2–3 (category 2, moderate numbers), and 4–6 (category 3, many bacteria).

RESULTS

Pilot study in 52 patients

In this series there were 27 CP+ cases. Twenty two of these had a positive CLOtest result recorded at 20 min. Four more CLOtests became positive at 3 h and one failed to react. This single "false-negative" result was from a man with liver disease who was taking oral neomycin for hyperammonemia. CP were not seen on Gram stains and could not be cultured, but active chronic gastritis was present and occasional organisms were detected in silver-stained sections. The patient was therefore defined as CP+. All patients with positive CLOtest results (defined as a color change in under 24 h) had active chronic gastritis (Table 1).

There were four delayed CLOtest reactions (3–24 h)

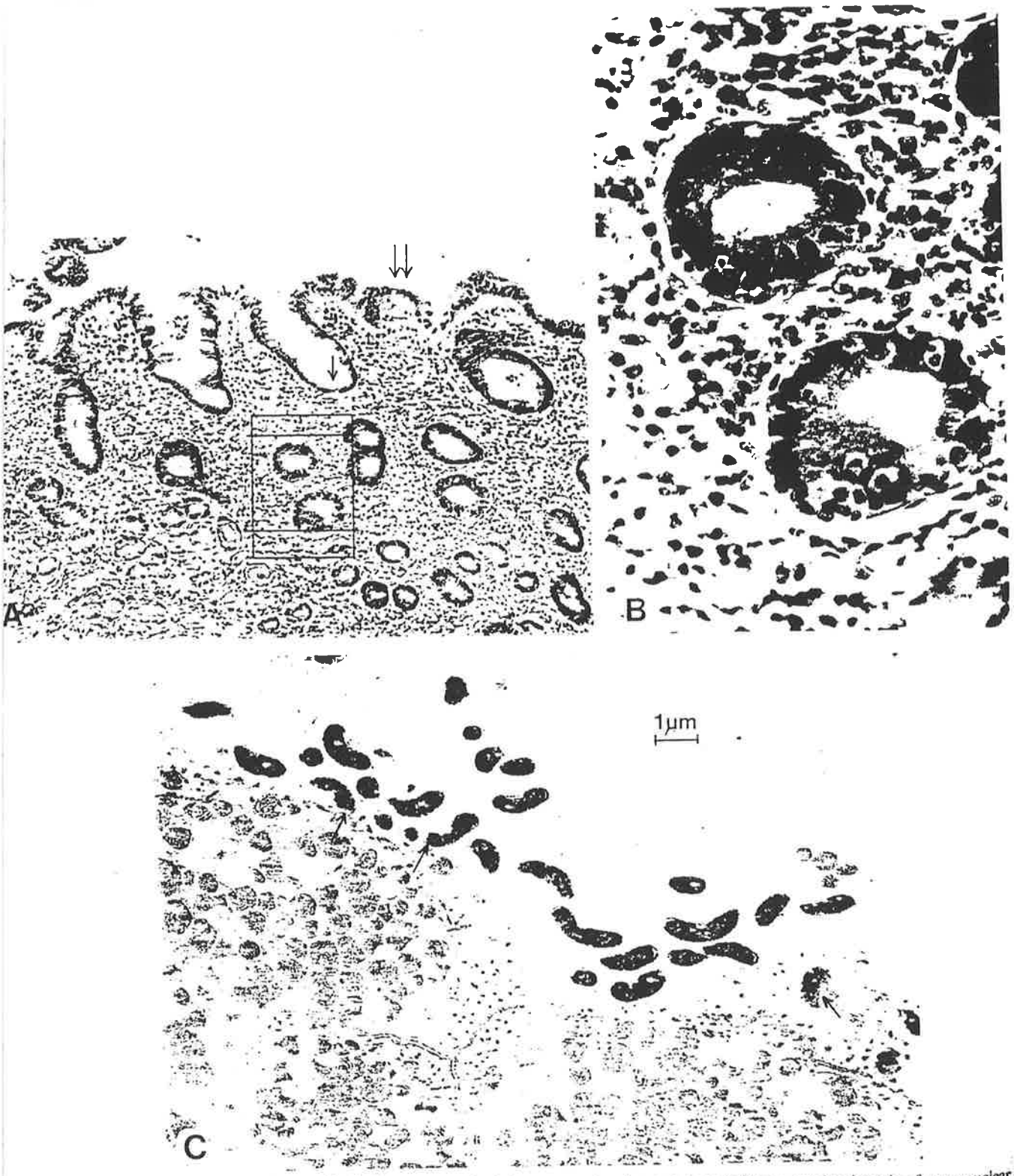

FIG. 2. *A* and *B*, severe active chronic gastritis (before treatment). *A*, low power view shows a dense infiltrate, predominantly of mononuclear cells, in the lamina propria. Luminal borders of the epithelial cells are indistinct and irregular (*single arrows*). Epithelial cells are stunted and show a severe reduction in intracellular mucus (*double arrow*) (hematoxylin and eosin, original magnification, ×120). *B*, higher power of tissue within the *box* shows many polymorphs within the lamina propria and infiltrating the epithelium of the gland necks. The lamina propria is heavily infiltrated with mononuclear cells, mainly plasma cells and lymphocytes (hematoxylin and eosin, original magnification, ×400). *C*, electron micrograph shows many *C. pyloridis* organisms in close proximity to the mucus-secreting gastric epithelial cells. Three of the organisms are intimately attached to the cell membrane (*arrows*), (original magnification, ×10,000).

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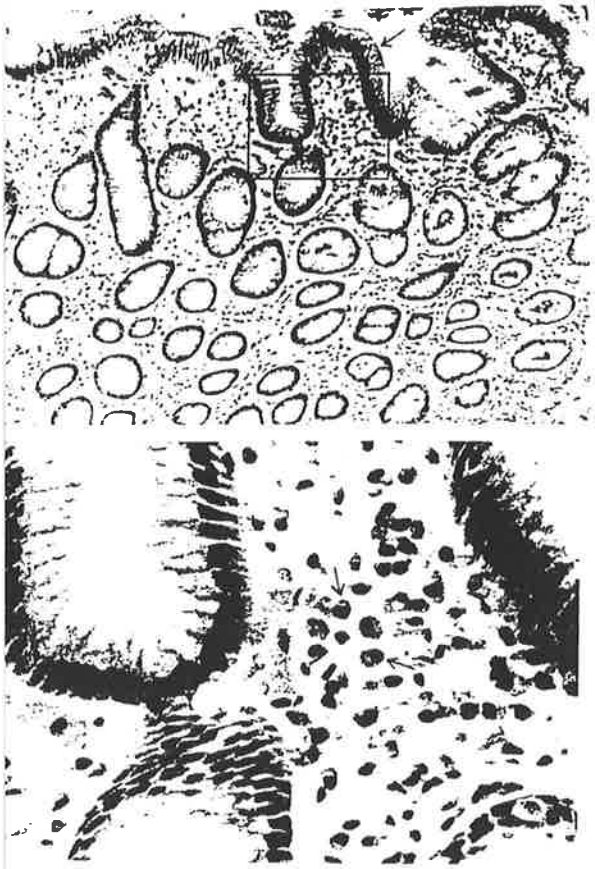

2. *D* and *E*, same patient 1 month after clearance of CP ms (now CLOtest negative). *D*, low power view after antibiotic shows normal appearance of the mucus-secreting epithelial rrow). They are now of regular height with parallel borders. ch has an intracellular cap of mucus equal to about 50% of depth (hematoxylin and eosin, original magnification, ×120). er power shows that the lamina propria still contains a slight of small round cells and plasma cells (*arrows*). This appearance be graded as either "minor changes only" or "mild chronic ." (hematoxylin and eoxin, original magnification, ×400).

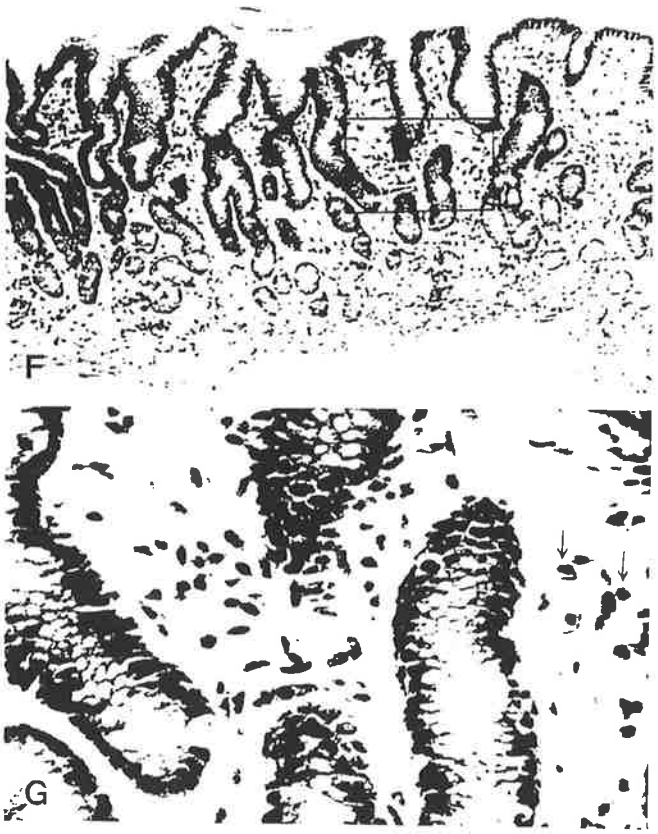

FIG. 2. *F* and *G*, example of normal antral mucosa. *F*, glands are plentiful. The lamina propria consists of loose connective tissue with few lymphocytes or plasma cells and virtually no polymorphs. The epithelial cells contain a thick intracellular mucus cap and the cells show the normal "picket fence" arrangement (also seen in *C* and *D*). (hematoxylin and eosin, original magnification, ×120). *G*, higher power of *F*. Polymorphs are absent although occasional plasma cells can be seen (*arrows*). (hematoxylin and eosin, original magnification, ×400).

re described in detail. In three of these CP were ed on Gram stain and culture, and in one case tology alone. All had low numbers of CP present histology sections. One patient was on no ther- ne was taking high dose antacids; one had ceased ycillin and bismuth subcitrate 7 days before en- py; and one had delayed gastric emptying and ken cimetidine 400 mg on the night before the copy [very high doses of cimetidine inhibit CP *in* 3)]. The CLOtest in these patients reached an color after 12–24 h and then progressed to red e cases by 48 h.

he 25 CP negative patients, only one case had a le positive CLOtest result. In this patient the st turned orange after 36 h. In this "false-posi- eaction, the biopsy was large and was not com- immersed in the gel, perhaps allowing urease- cing contaminant bacteria to grow. After the pilot results had been analyzed, all color changes oc- curring after 24 h were ignored. No CP negative patient in the pilot study had active chronic gastritis (Table 1).

141 CLOtests TIMED AND REPORTED BY THE ENDOSCOPIST

This series was obtained from 111 patients at 141 endoscopies: 18 patients were biopsied on two occasions and six patients on three occasions. The initial clinical and endoscopic findings of the 111 patients are summarized in Table 2.

Seventy nine of the 141 biopsies were from CP+ cases. The CLOtest was positive in 75% of these within 20 min, 92% within 3 h, and 98% at 24 h. All CLOtests reacting before 3 h gave a deep red color at 24 h. the five CLOtests that took 24 h to react only reached shades of orange at that time. CLOtests that had turned orange during the first 24 h progressed to pink or red by the third day. There were no false positive CLOtest results when color changes occurring after 24 h were

TABLE 1
Histological and Endoscopic Diagnoses, Pilot Study: CP+ Patients with Positive CLOtest at 20 min

n	Histology	Endoscopy
4	ACG	Duodenal ulcer [DU]
1	ACG	DU, gastric ulcer [GU]
2	ACG	DU, hiatus hernia [HH]
1	ACG	DU, esophagitis
2	ACG	DU, antral gastritis [AG]
1	ACG	GU, Barrett's esophagus, AG
1	ACG	GU, duodenitis
1	ACG	GU (healed), esophagitis, AG
1	ACG	AG, duodenitis
1	ACG	AG, esophagitis
4	ACG	AG
1	ACG	HH
2	ACG	Normal
CP+ patients with positive CLOtest at 24 h		
1	ACG	DU
1	ACG	DU, GU
2	ACG	DU (healed after antibiotics)
CP+ patient with negative CLOtest		
1	ACG	DU (on neomycin)
CP− patients with negative CLOtest (histology verbatim from path reports)		
3	Chronic atrophic gastritis	HH (2)
		Normal (1)
6	Mild chronic gastritis	Pyloroplasty (2)
		Esophagitis (1)
		Esophagitis, AG (1)
		Large gastric residue (2)
1	Quiescent gastritis	DU scar (1)
3	Past gastritis	DU, GU (taking aspirin) (1)
		AG (1)
		Normal endoscopy (1)
11	Normal histology, or minor changes only	Hiatus hernia (1)
		Barrett's esophagus (1)
		AG (3)
		Normal endoscopy (7)
CP− patient with "positive" CLOtest (at 36 h)		
1	Normal histology	Normal endoscopy

ignored. In the 141 consecutive cases tested there was only one CP+ patient who was not detected by the CLOtest. In this patient one of the histology specimens contained only intestinal epithelium, and it is possible that the biopsy put into the CLOtest was also intestinal and did not contain the bacteria. The curve in Fig. 3 was obtained by combining data from the 141 accurately timed CLOtests with the subsequent 130 in whom CP status was determined (see "Methods").

COMPARISON OF CLOtest, MICROBIOLOGY, AND HISTOLOGY

Gram stain and culture in the routine laboratory were not as sensitive as CLOtest. Seven biopsies with negative Gram stains and cultures, but showing *C. pyloridis* on histology, were CLOtest positive. One positive result reported by a junior pathologist had negative CLOtest and negative microbiology. The histological diagnosis was altered to CP− when the sections were reexamined.

Microbiology, histology, and CLOtest results for the 141 accurately timed tests are summarized in Table 3. Also included in Table 3 is the presence or absence of active chronic gastritis. It can be seen that active chronic gastritis was an extremely good predictor of the presence of *C. pyloridis*, and that the converse was also true; *i.e.*, in CP+ patients active chronic gastritis could be assumed to be present.

64 CLOtests REPORTED BY THE MICROBIOLOGIST

There were 32 CP+ and 32 CP− specimens as ascertained by subsequent Gram stain and culture. CLOtest correctly picked 29 of the positive cases at 3 h and the other three at 24 h (by which time the Gram stain result was also known to the technologist). Thus there was

TABLE 2
Patient Breakdown: CP+ (%)

	n	CP+	(%)
Sex			
Males	74	46	62
Females	37	18	49
Total	111	64	58
Endoscopic diagnosis			
Duodenal ulcer	36	33	92
Gastric ulcer	2	1	50
Gastritis or duodenitis	19	13	68
Esophagitis	8	3	38
Healed duodenal ulcer*	18	6	33
Healed gastric ulcer*	2	0	0
Normal endoscopy	28	8	29
Total	111	64	58

* CP+ ulcers were treated with bismuth plus antibiotics. It is not usual for CP to disappear when ulcers heal on other types of therapy.

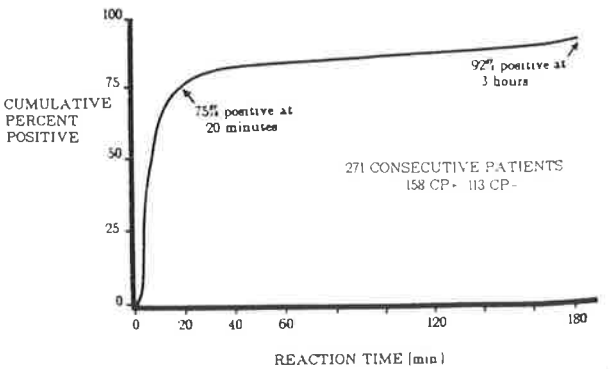

FIG. 3. Graph of CLOtest results *versus* time (0–180 min). The graph includes data from the 141 accurately timed patients plus 130 consecutive patients subsequently biopsied.

TABLE 3
Evaluation of CLOtest, Microbiology, Histology, and Gastritis, as Indicators of C. pyloridis Infection

	CLOtest			Micro-biology	Histology	Histology	
	20 min	3 h	24 h			ACG	Other
+ (79)	59	73	78	72	79	76	3
− (62)	62	62	62	62	61	2	60
se-negative	20	6	1	7			
se-positive					1		

0% concordance between the CLOtest and microbi-gy results. The histological data from this group uld not be studied to confirm the microbiological ults, but we know from other studies that the isola-n rate in the research laboratory approaches 100% infected cases (9).

These patients were participating in a double-blind al, and all had been CP+ before treatment. This ies is included to demonstrate that a negative CLO-t 2 wk after antibiotic therapy is evidence of clearance the bacteria.

EFFECT OF ERADICATION OF CP

Detailed analysis of the patients who were biopsied o or more times was undertaken. Active gastritis was bdivided into mild, moderate, and severe and chronic stritis into mild and moderate as described in "Meth-ds". There were 18 patients treated for *C. pyloridis* fection who were biopsied more than once; half of ese were cleared of the bacterium. Six CP− patients ere also rebiopsied and are also included as controls.

Active chronic gastritis resolved in eight of the nine atients in whom *C. pyloridis* was eradicated. ACG did ot resolve in any of the nine patients in whom the fection persisted (Fisher's exact test, $p = 0.0002$). The P negative patients had no active chronic gastritis itially, and no one developed it during the observation eriod (see Table 4).

CORRELATION BETWEEN CP NUMBERS AND CLOtest REACTION TIME

CP+ cases who had a CLOtest reaction time of 3 h r less were used to examine the correlation between eaction time and numbers of bacteria. In this group ie CLOtest had been accurately timed. Although a end appeared in the histology results the correlation as not significant ($p = 0.15$). There was no significant ssociation between the time it took the CLOtest to act and the numbers of bacteria seen by Gram stain Giemsa stain (see Table 4). However, when the CP umbers scored by the microbiologist and histopathol-gist were averaged there was a significant reduction ($p < 0.05$) in the reaction time for those with many bacteria (category 3) compared to those with few bacteria (categories 1 and 2 combined). Since the reduction was only significant with Duncan's test and not with Scheffé's test (the more conservative of the two) the association can only be regarded as tentative. These findings differ with those of McNulty and Wise (14) and Morris *et al.* (15) who reported good correlation between urease activity and *C. pyloridis* numbers in similar tests.

TABLE 4
CLOtest Reaction Time vs Bacteria Numbers (+ve CLOtests reacting within 180 min)

	n	Reaction Time (min)	(SD)	95% Confidence Interval
Gram stain				
0–1	19	21	41	1–40
2	21	26	33	11–41
3	33	10	12	6–15
Total	73			
Silver stain				
0–1	16	25	42	2.5–47
2	38	18	27	9–27
3	19	11	15	4–18
Total	73			

DISCUSSION

The first report of the isolation of *C. pyloridis* incorrectly stated that the organism was urease negative (16). The error arose because we had great difficulty propagating the new organism in 1982. The high urease activity of the bacterium was later noted by Langenberg *et al.* (17) and was subsequently used by Owen *et al.* (18) for rapid laboratory identification. The development of the rapid urease test followed our observation that the urea concentration was low in the gastric juice of patients infected with *C. pyloridis*, whereas gastric juice urea concentration in patients without gastritis approximated that of the blood (19).

In our first attempts at a biopsy test, Christenson's medium (a laboratory medium for detecting urease in bacterial cultures) was used. The gel changed color when infected gastric mucosa was inserted, but the color change usually took more than 1 h. We incorporated the reagents into a small gel pellet to obtain the necessary speed, but occasionally a false-positive reaction occurred, possibly due to contamination with alkaline bile or the growth of other urease producing bacteria. The addition of a bacteriostat and pH buffer enabled us to control these factors; in particular, there were no false-positive CLOtest results even in patients with duodenogastric bile reflux.

The population for the study was not entirely random. There were almost no emergency cases. Most of

ıe patients were referred from general practitioners. .ther than being hospital inpatients. Because of this it as usually possible to cease medications which may ıve interfered with detection of the bacteria, well ɛfore endoscopy. For instance, the procedure was post- ɔned for 10–14 days when the patient had been taking ısmuth containing drugs or antibiotics.

In this study we could not examine the possibility ıat patients with duodenal ulcer disease have more ıstric urease than, for instance, patients with nonulcer yspepsia. It is possible therefore that the excellent ɛsults obtained with CLOtest will not be repeated in ıore random samples of CP infected dyspeptic pa- ents. In addition, the routine ingestion of milk on the vening prior to endoscopy, may have enhanced the rease content of the mucosa (milk contains urea). To nable comparison of our patients with future studies, ve have included the endoscopic and histological find- ıgs of all patients in the pilot study (Table 1). All CP+ atients in the pilot study had histological active gastri- s. In these 27 patients, there were 15 patients with ctive ulcer disease, eight with antral gastritis and/or ther lesions and only four who were endoscopically ormal (includes two patients with "healed duodenal lcer"). In the 25 CP− patients, there were eight with a ormal endoscopy and six who had an esophageal ɛsion. The only duodenal ulcer in the bacteria-negative roup was in a patient who had taken nonsteroidal an- ıinflammatory drugs. Table 1 compares well with our ɔbservations in previous studies (2, 3) in which CP+ ɔatients tended to have abnormal endoscopic findings, ɔarticularly peptic ulceration ($p = 0.00004$, Fisher's ɛxact test).

Table 1 also demonstrates that a normal endoscopy loes not exclude the presence of gastritis. We are not he first to find that a normal endoscopy did not exclude he presence of histological gastritis (20). The corollary ɔf this observation has also been stated: the endoscopist nust perform a mucosal biopsy in every patient so that ıistological gastritis is not overlooked (21).

In the accurately timed series of 141 CLOtests 75% ɔf *C. pyloridis* infections were detected within 20 min- ıtes of biopsy (Fig. 3 and Table 3). In practice, when he CLOtest started to change color before 3 h, it always ɔntinued to deepen in color, reaching deep red or ɔurple at 24 h. Thus there were no equivocal readings ɔf CLOtests which had become positive in the first 3 h. Five CLOtests changed to orange at 24 h. These were ɛad as positive and all five proved to be from infected ɔatients. Although we recognize that the number of ıch slow reacting CLOtests was small, a report from ınother center (15) and subsequent experience at this ıospital indicates that CLOtests which turn orange at 24 h are CP+.

Our results are almost identical to those reported by Morris *et al.* (15) who found complete agreement between CLOtest and microbiology in a series of 70 patients, 48 of whom were infected with *C. pyloridis*. In our series the sensitivity of CLOtest was 98%, and the specificity was also 100%. [In order to refine these figures we biopsied 130 more patients (79 infected), after our detailed data were collected, and obtained identical results. The combined total of 271 accurately timed CLOtests is described in Figure 3. In this large consecutive series we did not see a false-positive result, and only three false-negative CLOtests occurred.]

We have included data on a series of 64 CLOtests read by the microbiologist because these were from patients taking part in a randomized double-blind trial of antibiotic therapy for duodenal ulcer. All these patients were CP+ initially and about half became CP− after therapy. The follow-up biopsies were always urease positive for CP+ patients (failed therapy) and negative for CP− patients (successful therapy). Although the test correlated with eradication of CP infection in all patients in this series, we have subsequently observed two cases in which false-negative results were obtained with CLOtest 2 wk after antibacterial therapy. Because of the decreased sensitivity of a urease test in these cases, we therefore recommend that after antibacterial therapy, 21 days should elapse before rebiopsy, and two biopsies taken from different areas of the antrum be tested.

In our study there was no significant association between bacterial numbers seen at microscopy and the rate at which the CLOtest changed color in infected patients (Table 4). Perhaps a slower test such as that used by McNulty *et al.* (11) would have given better resolution of actual reaction time. It is inconvenient to examine CLOtest before the endoscopy is complete so in many cases a very rapid reaction would have been noted only at 5 min. Another possibility is that orientation of the biopsy in the gel could affect the reaction time, a problem which would not be encountered in a liquid medium.

We did not set out to test for a correlation between CLOtest reaction time and bacterial numbers seen on Gram stain or histology so bacterial numbers were only estimated retrospectively from descriptions in the microbiology and pathology reports. We noted a statistically significant difference between numbers of bacteria reported by the two methods (McNemar's test, $p < 0.02$), the Gram stained smears producing higher counts. The poor correlation between CLOtest reaction time and bacterial numbers detected by Gram stain and/or histology suggests that the reported association between the two (14, 15) is weak and that *C. pyloridis* organisms, like gastritis, have a patchy distribution. Patchiness of bacterial colonization is especially likely in patients who have intestinal metaplasia of the stom-

40

ach, a type of epithelium not colonized by *C. pyloridis* (5).

Our study confirms the association between *C. pyloridis* infection and active chronic gastritis reported by ourselves and others (1–3, 22, 23). As shown in Table 3, the presence of polymorphs in a biopsy was an excellent predictor of *C. pyloridis* infection. Of patients with active chronic gastritis 97% had *C. pyloridis*.

The almost universal presence of active chronic gastritis in CP infected patients contradicts the findings of Jones *et al.* (24) who found that gastritis was associated with *C. pyloridis* infection, even when polymorphs were not increased. Our inclusion of two biopsies for histology enabled us to have at least one adequate biopsy specimen from every patient whereas other investigators may have had to compromise when only a single biopsy was examined by the pathologist. Perhaps in the study of Jones *et al.* (24) a mild increase in polymorph numbers was included as part of their normal range. Alternatively, our patient group may have differed from that of Jones *et al.* (24), perhaps containing a much higher proportion of patients with duodenal ulcer disease. Our histological findings are in agreement with a similar series reported by Ho *et al.* (22) who found ACC and CP infection in 98 and 99%, respectively, of 109 patients with duodenal ulcer.

We have included data on the healing of gastritis because it is not generally recognized that histological gastritis can be treated (25). Type B, or antral gastritis, is thought to be an aging process by some (26) or due to damaging agents such as bile by others (27). The association of gastritis with peptic ulcer disease is well known, but chronic gastritis alone has not been proven to cause dyspeptic symptoms.

One of the difficulties in the study of gastritis has been the complicated nomenclature, and the tendency of other authors to merge terms such as atrophy and intestinal metaplasia with those describing gastritis (23). To avoid this problem we have restricted the term "gastritis" to mean tissue which contains an excess of inflammatory cells, usually polymorphs and/or plasma cells and lymphocytes. In this scheme the most severe form is that containing polymorphs and mononuclear cells, "active chronic gastritis" which almost always transforms to a less severe grade of "mild chronic gastritis" after eradication of CP.

As shown in Figure 4, polymorph infiltrations disappeared in biopsies from eight of the nine patients in whom *C. pyloridis* was eliminated. This produced a striking change in the appearance of the mucosa. In the initial biopsy of a patient with severe active chronic gastritis a dense infiltrate of mononuclear cells and polymorphs was present (Fig. 2*A*), polymorphs invaded the necks of the glands (Fig. 2*B*), epithelial cells were damaged (Fig. 2*A*), and curved bacilli were present

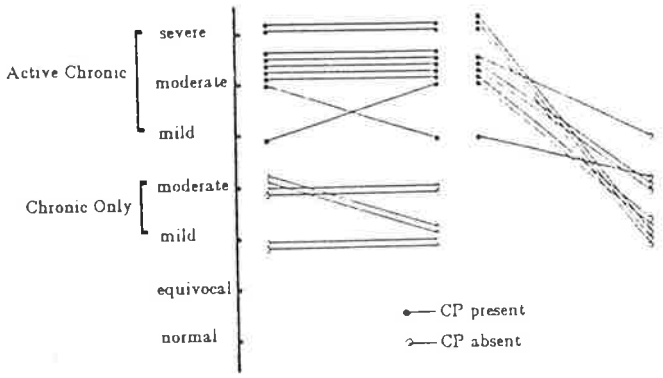

FIG. 4. Gastritis grades in patients who were rebiopsied after antibacterial therapy. The initial and final biopsies are included for 24 patients: 18 of whom showed *C. pyloridis* and six who did not. Nine of the CP+ cases were cleared of CP and the gastritis improved. In the nine who were not cleared of CP, active chronic gastritis remained. In the six patients who did not have CP, there was no change in the near normal appearance of the mucosa. Note that the lowest grade seen in any of these biopsies was "mild chronic gastritis:" none of the treated patients returned to complete normality.

(Fig. 2*C*). Two weeks after completing therapy, polymorphs had completely disappeared and mononuclear cells were greatly reduced in number in the lamina propria (Fig. 2*D*). The epithelial cells were then normal (Fig. 2*E*) but the biopsy still contained a slightly increased number of plasma cells as compared to a completely normal specimen (Figs. 2 *F* and *G*).

We emphasize that the changes described herein are not unusual. As demonstrated in Figure 4, which describes a subset of patients from the 141 accurately timed series of CLOtests, healing is the norm after successful antibacterial therapy. When the bacteria disappear, the histological gastritis shows a marked improvement. In contrast, active chronic gastritis continues in patients with persistent *C. pyloridis* infection.

Marshall *et al.* (28) were the first to report healing of active chronic gastritis and disappearance of *C. pyloridis* in patients treated with bismuth subcitrate (DeNol). That study could not exclude the possibility that the gastritis healed because of a "mucosal protective effect" of bismuth, unrelated to its antibacterial activity. Proponents of this view maintained that only inflamed mucosa was susceptible to colonization by *C. pyloridis* which was therefore an opportunistic invader rather than a primary pathogen (29). This controversy is addressed by the present study in which 18 patients were given 14 days of concurrent therapy with bismuth subcitrate and antibiotic. When they were rebiopsied 2 wk after ceasing therapy the gastritis had improved in all patients cleared of *C. pyloridis* but the histology did not change in patients who still had *C. pyloridis* infection (Fig. 4). As all patients received bismuth therapy the results suggest that bismuth has no antiinflammatory action except that which comes from its antibac-

terial action. Therapy for gastritis must therefore eradicate *C. pyloridis* to have a lasting effect.

We now use the rapid urease test to select dyspeptic patients who might benefit from antibacterial therapy. CLOtest positive patients are prescribed a 21-day course of colloidal bismuth subcitrate (De-Nol) tablets. Recent work by McNulty *et al.* (12) suggests that DeNol could be replaced by Pepto Bismol (bismuth subsalicylate) which is more widely available. As shown in Table 3 and Figure 3, therapy may be commenced immediately in the 92% of infected patients who are detected before leaving the hospital (3 h). After 10 days the patient's general practitioner telephones the hospital microbiology department for the antibiotic sensitivity results and gives the appropriate antibiotic concurrently with the De-Nol from days 11 to 21 of the course: (usually tinidazole 1 g daily, amoxycillin 2 g daily, or erythromycin 2 g daily for tinidazole-resistant organisms). CLOtest thus saves patients a second visit to the gastroenterologist in most cases, and enables the general practitioner to resume management of the patient immediately. As all isolates of *C. pyloridis* are sensitive to bismuth, all patients are immediately on effective antibacterial therapy and are not subjected to the risk of a useless antibiotic.

In conclusion, the rapid urease test is a sensitive, and highly specific indicator of *C. pyloridis* infection. Its routine use enables gastroenterologists without microbiological expertise to diagnose *C. pyloridis* infection. The test is a useful aid to the management of patients with gastritis.

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PROSPECTIVE DOUBLE-BLIND TRIAL OF DUODENAL ULCER RELAPSE AFTER ERADICATION OF CAMPYLOBACTER PYLORI

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Summary 100 consecutive patients with both duodenal ulcer and *Campylobacter pylori* infection were followed up to see whether eradication of *C pylori* affected ulcer healing or relapse. Patients were randomly assigned to 8 weeks of treatment with cimetidine or colloidal bismuth subcitrate (CBS), with tinidazole or placebo being given concurrently from days 1 to 10, inclusive. Endoscopy, biopsy, and culture were done at entry, in weeks 10, 22, 34, and 62, and whenever symptoms recurred. There was no maintenance therapy. *C pylori* persisted in all of the cimetidine-treated patients and in 95% of those treated with cimetidine/tinidazole, but was eradicated in 27% of the CBS/placebo group and 70% of the CBS/tinidazole group. When *C pylori* persisted, 61% of duodenal ulcers healed and 84% relapsed. When *C pylori* was cleared 92% of ulcers healed ($p < 0.001$) and only 21% relapsed during the 12 month follow-up period ($p < 0.0001$).

Introduction

THE association between peptic ulcer disease and antral gastritis has been well described and is especially strong for duodenal ulcers (DU).[1] When we observed that over 90% of our DU patients were colonised with *Campylobacter pylori*,[2,3] we suspected that the bacterium caused the disease. This view was strengthened by observations that colloidal bismuth subcitrate (CBS, ['De-Nol', Gist Brocades, Delft, Holland]), which inhibits the growth of *C pylori*, led to healing of duodenal ulcers as effectively as did the H_2-receptor antagonists and prevented duodenal ulcer relapse.[4,5]

In a pilot study, we observed long-term eradication of *C pylori* in some patients treated with CBS-antibiotic combinations.[6] Here we describe how we tested the hypothesis that persistence of *C pylori* after ulcer healing is related both to active chronic gastritis and ulcer relapse.

Methods

Patient Selection

Patients had to have a duodenal or pyloric canal ulcer at endoscopy of at least 3 mm diameter, aged 18 to 75 years and, apart from their DU, be in good health. Those known to have taken bismuth-containing drugs, antibiotics, non-steroidal anti-inflammatory drugs, or corticosteroids in the month before diagnosis were excluded, and these drugs were forbidden for the duration of the study, except as required for the treatment regimen. Patients who had undergone gastric surgery more extensive than a selective vagotomy and pyloroplasty, had gastric ulcers greater than 5 mm diameter, or had a contraindication to gastric biopsy were also excluded. Female patients were required to practise contraception during the treatment phase of the study. The study was approved by the Royal Perth Hospital Human Rights Committee in June, 1984, and conducted at the hospital between April, 1985, and August, 1987.

Endoscopy and Biopsy

Four biopsy specimens were taken from antral mucosa 5 cm proximal to the pylorus. The first specimen was for a rapid urease test ('CLOtest', Delta West, Perth), the second specimen was for microbiological examination, and the other two were for histological examination. Two biopsy specimens were also taken from the duodenal cap for histological examination—one from intact duodenal mucosa away from the ulcer, the other from the distal border of the ulcer.

If the CLOtest on the first antral biopsy specimen indicated the presence of urease (*C pylori* present) the patient was randomly assigned to one of the trial therapies. If the CLOtest was negative, the patient was given a consecutive study number but no therapy was assigned until proof of *C pylori* infection was obtained on histological examination or culture. If *C pylori* was not detected by histology or culture (7 patients), the patient was removed from the study and treated conventionally.

Doctors who managed the patients were blinded to the histology and microbiology findings except for those required for

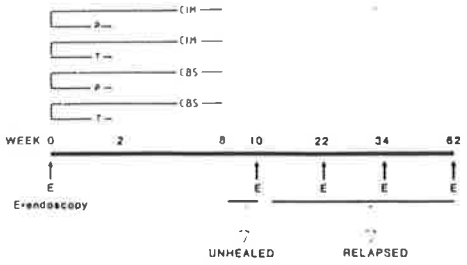

Fig 1—Study design.

randomisation. Endoscopic and laboratory findings were not revealed to patients unless they had completed, or were being removed from, the study.

Histology Methods

Pathology specimens were placed in buffered formol-saline, mounted in paraffin, and stained with haematoxylin and eosin (H&E) for general cytology and histology, and with Giemsa and also Warthin Starry (WS) stains to show the bacteria.

The H&E section was graded under low and medium power for gastritis, as previously described.[6] Polymorphonuclear neutrophil leucocytes (PMNs) and mononuclear leucocytes (monos) were scored 0 to 3. A gastritis "grade" of 0–6 was obtained by adding these scores.

All specimens were then examined for the presence of *Campylobacter*-like organisms (CLO) in the H&E, Giemsa, and WS stained sections.

For microbiological examination, a corner of the specimen was cut off with a sterile scalpel blade and a gram stain of that material was examined for CLO. The remainder was ground and cultured on selective and non-selective media for 6 days.[8] *C pylori* organisms were identified as gram-negative curved rods which produced catalase, urease, and oxidase. The susceptibility of each isolate to tinidazole was determined as reported elsewhere.[9]

Randomisation and Therapy

Patients were stratified for gender, age, smoking, and duration of ulcer disease. Within each group, the four therapies were assigned in random order. Patients received either cimetidine (CIM), 400 mg twice a day, or colloidal bismuth subcitrate (CBS,'De-Nol') one tablet four times a day (480 mg bismuth per day calculated as Bi_2O_3). CBS was given on an empty stomach, 30 min to 1 h before meals and at bedtime. Patients also received either tinidazole (T) 500 mg twice a day ('Fasigyn', Pfizer, Sydney), or an identical placebo (p) from the first to the tenth day of therapy. The four treatment groups were cimetidine·placebo (CIM p), cimetidine tinidazole (CIM T), CBS·placebo (CBS p), and CBS tinidazole (CBS T).

To blind the endoscopist to the temporary staining of the mouth and stools seen in some patients who take CBS, all therapy was withdrawn for 14 days before the second endoscopic examination at week 10. Patients with probable ulcer symptoms and/or ulcer craters were withdrawn from the study. Patients who were well and whose ulcers had healed were examined by endoscopy and biopsy 22, 34, and 62 weeks after entry (fig 1). There was no maintenance therapy, and patients were cautioned not to take antibiotics, antacids, or bismuth-containing drugs.

During therapy, problems with medication (eg, severe pain or possible side-effects) were managed over the telephone by the pharmacist who had usually not seen the patient but who did have access to the treatment codes. After therapy, if symptoms recurred, they also called the pharmacist (if available) or the investigators in an emergency. As far as possible there was no communication between patients and the chief investigator, so that the ulcer drug given remained investigator-blind.

Before each endoscopic examination the patient, with the aid of a research assistant, completed a symptom questionnaire and Zung depression scale.[10] Vital signs were then recorded, a brief physical examination was done, and a blood sample was drawn for *C pylori* serology.[11]

Completion Criteria

Patients completed the study if they had an ulcer (unhealed ulcer or relapse documented endoscopically) at any time after completing therapy; if their symptoms persisted during and after therapy, or recurred (symptomatic relapse); or if they remained ulcer-free and symptom-free and completed the follow-up period of 12 months (fig 1).

Study Design and Analysis

At each evaluation, *C pylori*-positive (CP +) patients were defined as those in whom the bacteria were detected by culture or histology. *C pylori*-negative (CP −) patients were those in whom *C pylori* was not detected by culture or histology.

For the statistical analysis and tables, patients were grouped as healed/unhealed at 10 weeks (second endoscopy), and relapsed/not relapsed at 62 weeks (1 year post-treatment). Patients with healed ulcers but persistent ulcer symptoms at the second endoscopy were therefore grouped as "healed DU" and "symptom relapse 0 weeks after healing".

For comparison of the treatment groups at baseline, a one-way analysis of variance was used for continuous variables and a χ^2 test was used for nominal or ordinal scale variables. Life-table analysis was done using the survival analysis available on the SAS package. The results obtained were consistent with significance values calculated with a Fisher's exact test, so for simplicity only the latter values are cited in the text. Histology grades were compared with Wilcoxon's test.

Results

Of 107 consecutive eligible patients with duodenal ulcer, 7 were withdrawn from the study because *C pylori* could not be detected on histological examination or culture. Thus 100 patients were randomised to therapy. There were no major differences between the four treatment groups (table I).

All except 2 patients completed all aspects of the study. 1 man did not attend for his second (week 10) endoscopy because he felt well and had taken a job in the outback. 3 weeks later, during an apparent ulcer relapse with vomiting episodes, he had a myocardial infarction. Barium meal revealed a duodenal ulcer crater 1·5 cm in diameter and *C pylori* was found at endoscopy in week 23. For the analysis he was classed as CP +, healed, with subsequent relapse in week 15. A second man left Australia, but before his departure in week 29 he was endoscopically and clinically normal with a CP − biopsy.

Effect of Therapy on C pylori *and Gastritis*

CIM p had little effect on *C pylori*. All 22 patients taking only cimetidine had the organism at the second and subsequent evaluations (table II). CIM T eradicated

TABLE I—BASELINE COMPARISON OF PATIENTS

Variable	CBS p n = 22	CBS T n = 27	CIM p n = 22	CIM T n = 29	All groups n = 100
Male sex (%)	16 (72%)	19 (70%)	17 (77%)	19 (66%)	
Smokers (%)	12 (55%)	15 (56%)	13 (59%)	15 (52%)	
Previous ulcer (%)	20 (90%)	25 (93%)	19 (86%)	24 (83%)	
Mean age (yr)	46·6	46·3	42·2	47	45·4
Mean duration of disease (yr)	8·5	14·0	10·8	12·6	11·5
Duration of current episode (wk)	3·6	4·6	6·5	2·9	4·53
Pain score (0–10) at entry	5·8	5·5	7·1	5·1	5·8
Ulcer diameter (mm)	11·8	13·2	12·0	9·2	11·5

Significance of difference between groups for pain score at entry 0·1 ≥ p > 0·2 for all other variables.

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TABLE II—HEALING AND RELAPSE DATA BY THERAPY AND BY C PYLORI STATUS AFTER THERAPY

Group (n)	Unhealed/ healed at 10 wk	Relapsed			Well (%)
		3 mo	6 mo	12 mo	
CIM/p					
CP + (22)	9/13	9	2	1	1 } 5%
CP − (0)	0	0	0	0	0 }
CIM/T					
CP + (28)	7/21	13	2	3	3 } 14%
CP − (1)	0/1	0	0	0	1 }
CBS/p					
CP + (15)	7/8	5	2	0	1 } 32%
CP − (5)	0/5	1	0	0	4 }
Recrudescent (1)	0/1	0	0	0	1
Reinfected (1)	0/1	0	0	0	1
CBS/T					
CP + (7)	5/2	0	0	0	2 } 56%
CP − (19)	2/17	2	1	1	13 }
Recrudescent (1)	0/1	0	1	0	0
All CP + cases (72)	28/44	27	6	4	*7–10%*
All CP − cases (25)	2/23	3	1	1	*18–72%*
Recrudescences (2)	0/2	0	1	0	1
Reinfection (1)	0/1	0	0	0	1
Column totals (100)	30	30	8	5	27

C pylori in only 1 of 29 patients (4%). The antibiotic failed because a tinidazole-resistant isolate emerged in nearly all cases in which CBS was not being taken concurrently[8] (see below).

CBS/p led to clearance of *C pylori* in 7 of 22 patients (32%). Recrudescence of infection occurred in 1 patient, so the eradication rate for CBS/p was 27% (6/22), which was significantly better than that obtained with CIM/T ($p = 0.02$, or CIM/p ($p = 0.01$). CBS/T cleared the infection in 20 of 27 patients (74%), and 19 of these patients remained CP − during follow-up—these findings were significantly better than the result obtained with cimetidine ($p < 0.0001$) or CBS alone ($p < 0.01$). When the initial *C pylori* isolate was sensitive to tinidazole in vitro, the bacterium was eradicated by the CBS/T combination in 85% of cases.[8]

Of the 28 patients cleared of *C pylori* at the 10-week biopsy, 3 were later found to have *C pylori*. 1 man given CBS/p was CP + at the 22-week study. He had oesophagitis with mild symptoms but did not have a relapse. A woman given CBS/T became CP + at 22 weeks and began to have symptoms at 25 weeks. We believe that these 2 patients had recrudescent infections and that the 10-week biopsy specimens, immediately after treatment, gave false-negative results. They were included in the healing analysis as CP − after therapy but were excluded from the relapse analysis. One reinfection occurred. A man given CBS/p was CP − at the 10- and 22-week endoscopies, but histological evidence of gastritis and *C pylori* were noted at the 34-week study. He remained well and completed the study. He was included in both the healing and relapse analyses and grouped with the CP − patients (ie, patients in whom *C pylori* was eradicated by therapy).

Clearance of *C pylori* organisms resulted in improvement in the histology. PMN scores decreased from 1·79 to zero in patients cleared of *C pylori* ($p < 0.0001$). In contrast, PMN scores were unchanged in patients with persistent infection (average grade after therapy 1·74 [SD 0·62]). Mononuclear cell scores also improved significantly, falling from a mean of 2·5 (0·5) to 1·6 (0·5) in patients cleared of *C pylori*, but not changing in patients with persistent infection ($p < 0.001$) (fig 2).

Ulcer Healing

Healing occurred in 92% of patients in whom *C pylori* was not detected at the 10-week endoscopy, whereas only 61% of patients with persistent *C pylori* healed (43/71) ($p = 0.001$).

Ulcer and Symptom Relapse

There were 70 patients with healed ulcers at the 10-week endoscopy, 35 of whom had received cimetidine. During the 12-month follow-up period, relapse occurred in 12 of 13 CIM/p patients (92%) and 18 of 22 CIM/T patients (82%) (fig 3). This difference was not significant. The relapse rate for all cimetidine-treated patients was thus 86% in 12 months.

In the CBS/p-treated group, 8 of 15 had a relapse (53%). The relapse rate was less than that in the combined cimetidine groups ($p = 0.027$). In this group, all 7 patients whose ulcers did not heal were found to be still CP + ($p = 0.037$). In those whose ulcers healed but were still CP +, relapse then occurred in 87% (7/8), whereas only 17% (1/6) of CP − cases relapsed ($p < 0.026$) (table II).

Of 20 patients whose ulcers healed with CBS/T only 5 went into relapse (25%). This was clearly better than the result in the combined cimetidine groups ($p < 0.0001$). Although maintenance of remission was commoner with CBS/T than with CBS/p, the difference was not significant ($p = 0.15$). However, if with unhealed ulcers and relapses patients were combined for analysis as "treatment failures", CBS/T therapy was superior to CBS/p (12/27 failed *vs* 17/22, $p = 0.04$).

The observed differences in relapse between the four treatment groups could be accounted for by *C pylori*. Excluding the 2 patients with recrudescent infection, relapse

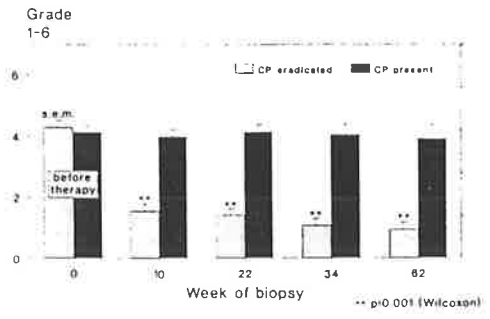

Fig 2—Effect of eradication of *C pylori* on gastritis grade.

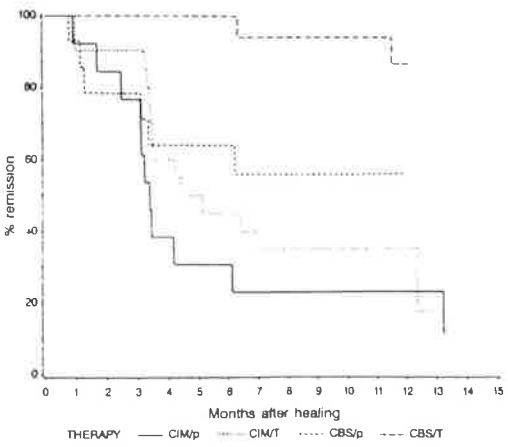

Fig 3—Effect of treatments on relapse.

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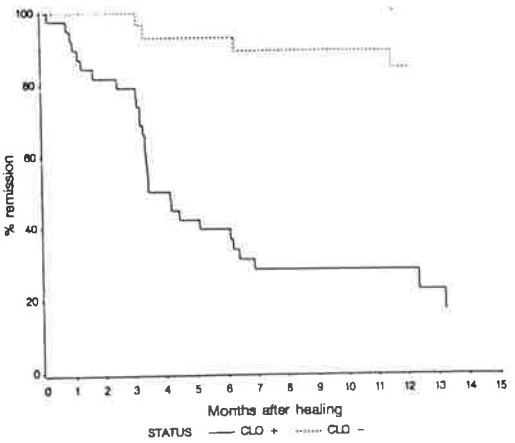

Fig 4—Differences between CP + and CP − groups on relapse.

occurred in 84% of CP + patients (37/44) but in only 21% (5/24) of the CP − patients ($p < 0.0001$) (fig 4).

The clinical picture at relapse was more acute and severe in CP + than in CP − patients. 5 CP − patients with relapses completed the study at the appointed follow-up endoscopy times; none of them had symptoms severe enough to warrant endoscopy. In contrast, 8 of the 38 CP + patients who went into relapse required an additional endoscopic examination because of severe ulcer symptoms ($p = 0.033$).

Of the 2 patients with recrudescent infection, 1 went into relapse. The second, and also the reinfected patient, completed the full 12-month follow-up period.

Sex, age, smoking, and history of a previous ulcer had no significant effect on relapse provided *C pylori* had been eradicated (table III). 28 patients with three or more of these risk factors did not relapse more often when *C pylori* had been eradicated. In contrast, of 16 CP + patients with three or more risk factors the relapse rate was 100% ($p = 0.04$). Relapse was also more common in CP + patients if they smoked ($p = 0.03$), but in this study male sex or increasing age was not a disadvantage in CP + patients.

Gastric Metaplasia and C pylori *in the Duodenum*

At the first endoscopic examination, adequate ulcer border and duodenal bulb biopsy specimens were obtained from 88 of the 100 CP + patients. Gastric metaplasia was more common in the ulcer border than in the adjacent bulb

TABLE III—SUCCESS OF THERAPY BY SEX, AGE, SMOKING, AND ULCER HISTORY

	All healed patients (n = 70)		CP eradicated healed (n = 24)	
Variable	Success	Relapse	Success	Relapse
Sex				
Female	8	12 60%	5	1 16%
Male	19	31 62%	14	4 14%
Age				
< 35	7	7 54%	5	0 0%
35–49	15	22 59%	10	2 17%
> 49	5	14 75%	4	3 43%
Smoking				
No	13	20 61%	6	3 33%
Yes	14	23 62%	13	2 13%
Previous ulcer				
No	2	6 75%	1	0 0%
Yes	25	37 60%	18	5 22%
No of risk factors				
0, 1, or 2	18	24 57%	10	2 17%
3 or 4	9	19 67%	9	3 25%

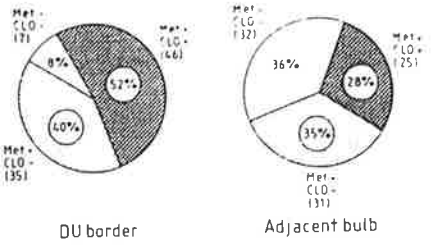

Fig 5—Gastric metaplasia in duodenal ulcer border and adjacent bulb.

(92% *vs* 63%, $p = 0.0001$), but *C pylori* was present in about half of the areas of metaplasia seen, irrespective of its location (fig 5).

Effect of Therapy on Symptoms

For patients whose ulcers healed and who remained in the study after the second endoscopic examination, there was no difference among the four treatment groups in response of symptoms to therapy. The Zung scale was significantly lower for patients cleared of *C pylori* (mean Zung score = 0·32) than for patients in whom *C pylori* was detected at the second endoscopic examination (mean Zung score = 0·38). The difference was significant even though patients whose ulcers remained unhealed or relapsed at 10 weeks were excluded from the analysis ($p = 0.014$, analysis of variance). The difference increased slightly during the follow-up period, with mean Zung values of 0·30 and 0·37 for the CP − and CP + patients, respectively, at the third endoscopic examination ($p = 0.003$, analysis of variance).

Side-Effects

There were more side-effects in patients who received tinidazole compared with those who did not, but the difference was not significant. 2 patients taking CBS T had severe diarrhoea but were able to continue therapy; and 2 others noticed more frequent stools. 2 patients complained of a temporary "burning anal irritation" while 3 others complained of eructation, flatulence, or bloating. Constipation was uncommon. 1 case of oral candidosis occurred in each of the cimetidine groups.

Discussion

In the early 1980s, interest in bismuth compounds was revived when it was noted that the relapse rate for duodenal ulcers fell when ulcers were healed with CBS.[4,5] The ability of CBS not only to heal but also to cure some people of the disease suggested to us that it had another action besides mucosal protection, an action directed at the underlying duodenal ulcer diathesis.

The isolation and culture of *C pylori* and its association with type B gastritis led to the hypothesis that this new bacterium was the cause of the gastroduodenal inflammation in patients with duodenal ulcer. The findings that *C pylori* was inhibited by some bismuth salts,[6] and that suppression of infection led to healing of gastritis,[7] lent further support to the thesis that the ulcer diathesis was closely related to *Campylobacter pylori* gastritis.

How bacterial infection in the antrum can lead to ulceration in the duodenal bulb is at present unknown. However, we found gastric metaplasia in over 90% of the duodenal ulcer borders; and adherent *C pylori* were also commonly found in this location, more so than elsewhere in the bulb. The known association between *C pylori* and

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active duodenitis with gastric metaplasia[12] suggests that the bacterium causes "gastritis" in the bulb, just as it does in the antrum. Other reported abnormalities such as deficient mucosal bicarbonate secretion[13] could be direct or indirect consequences of this action.

When the present ulcer trial was planned in 1984, the aim was permanent eradication of *C pylori*, in a controlled fashion, from patients with duodenal ulcer disease. We had no clinical experience with tinidazole in combination with cimetidine, but in-vitro studies suggested that tinidazole had high activity against *C pylori*. In this study, the development of tinidazole resistance by *C pylori* enabled the bacterium to survive in 28/29 patients treated with CIM/T. CIM/p had no effect on the presence of *C pylori*, as was expected.

The two cimetidine therapies produced very similar healing and relapse rates, and *C pylori* was not cleared in either group. CIM/p and CIM/T patients were therefore combined in the analysis to give one CP+ cimetidine-treated control group.

C pylori was cleared from 30% of those treated with CBS/p and 74% of those treated with CBS/T.

In 92% of patients cleared of *C pylori* the ulcers healed (26/28), but for those with detectable *C pylori* after therapy the healing rate was only 61% (44/72). These data support findings reported by Bayerdorffer et al,[14] who noted enhanced DU healing when the quinolone antibiotic ofloxacin was added to standard ranitidine therapy. In our study no individual therapy had superior healing properties. In the CBS groups, any advantage conferred by rendering patients CP− may have been diluted by slightly worse healing in patients who remained CP+. For example, in 50 patients who remained CP+ after cimetidine therapy, 34 had ulcers that healed (68%), but in 21 patients who remained CP+ after receiving CBS, only 9 had ulcers that healed (42%) ($p = 0.06$). The trend suggests that healing of ulcers with CBS is related more to its antibacterial action than to its "mucosal protective" action.[15]

The poor healing seen in our two cimetidine-treated groups may have been an artifact produced by our study design. In studies in which higher healing rates have been reported with H_2-receptor antagonists,[16,17] patients were assessed while still taking the drug. In our study some patients whose ulcers healed at 8 weeks could well have relapsed by the second endoscopic examination at 10 weeks. As a consequence, these patients completed the study as "unhealed" rather than as "relapsed".

Of the 28 patients who were CP− at the 10-week endoscopy, 2 had ulcers that had not healed and 2 had rapid recrudescence of the infection, leaving only 24 CP− patients who could be observed for ulcer relapse. Sequential biopsy of these patients over a 12-month period demonstrated that *C pylori* could be permanently eradicated and that reinfection is unusual in adults. Only 1 patient had reinfection as we defined it. Our data support those of Rauws et al,[18] who reported a similar incidence of *C pylori* reinfection—about 5% per annum.

Of the patients from whom *C pylori* was eradicated and whose ulcers healed, only 21% went into relapse. The statistical significance of the result in such a small sample means that eradication of *C pylori* had a considerable clinically useful benefit. In accord with this, when CBS p or CBS T did not eradicate *C pylori*, the relapse rate was no different from that in the cimetidine group. Thus, presence of the bacterium, not type of therapy, was the factor which determined relapse. The findings support those of Coghlan,[19] who noted that the benefit of bismuth therapy

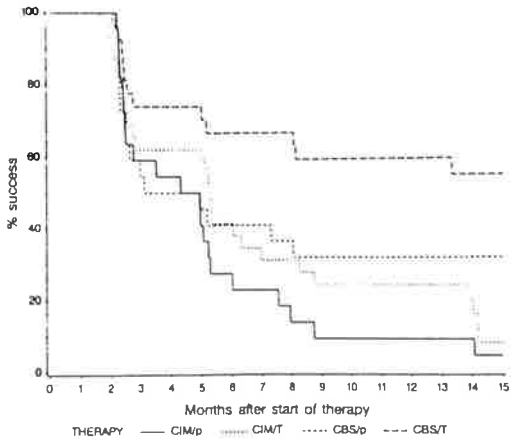

Fig 6—Differences between treatments by % success of therapy.

over H_2-blocker therapy was confined to those patients in whom *C pylori* had been eradicated.

In other studies of ulcer relapse, the importance of age, sex, and smoking have been emphasised,[20] perhaps because they were the only factors other than continuing ulcer therapy that seemed to make any prognostic difference. How important are they compared with *C pylori*? Apparently they are of secondary importance. Once *C pylori* had been eradicated the patient did well, even if he (or she) smoked, or had a previous history of severe relapsing disease, or had multiple adverse factors. On the other hand, patients with persistent *C pylori* infection had an adverse prognosis if they had multiple risk factors, or if they smoked.

A one-time therapy which both heals duodenal ulcers and stops relapse is, by definition, curative. In future studies the distinction between unhealed ulcers and ulcers which relapse within 12 months of therapy may be unnecessary since both outcomes are really treatment failures. Conversely, by defining treatment success as a patient whose ulcer heals and who remains well for 12 months without therapy, a striking difference is evident between conventional H_2-receptor antagonist therapy and our anti-*C pylori* therapy. Treatment success in patients treated with CIM/p was 5%, with CIM T 14%, with CBS p 32%, and with CBS T 56% (fig 6).

Our results imply that *C pylori* is the most important aetiological factor so far described for duodenal ulcer. We propose that detection of *C pylori* should be part of the routine management of patients with acid peptic disease and eradication of the bacterium a major therapeutic goal.

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We thank Mrs Dorothy Heys, Ms Ann McDermid, and Mrs Kim Marshall for their help with the endoscopies, Mrs Marcia Finney and Mrs Adrienne Marshall for reviewing the paper, and the many patients who volunteered for the study.

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DUODENAL ULCER RELAPSE AFTER ERADICATION OF CAMPYLOBACTER PYLORI

SIR,—Professor Lam (Feb 18 p 384) criticises our study. We wish to reply.

Eradication of *Campylobacter pylori* in our patients was associated with a much longer remission of healed duodenal ulcers and the apparent rate of healing was also higher. The apparent ulcer-healing rate at follow-up endoscopy was low in our patient group in which *C pylori* was not eradicated (CP + ve patients), irrespective of the therapy. Our Table II shows healing in 44/72 (61%) CP + ve patients. Since 27/44 (61%) of these healed ulcers relapsed before 3 months, we cannot see why it is difficult to understand our suggestion that others probably relapsed before the first follow-up 2 weeks after therapy ended. Even if the argument is not substantiated, it is not unreasonable. The failure rates at 12 months were the same for both cimetidine (46/50, 92%) and colloidal

ocitrate (CBS) (19/22, 86%). We agree that our patients as Lam says, since all patients are unique.

fort was taken to keep the trial blind. Follow-up was eks after therapy ceased (so that mouth-staining would by the endoscopist) and communication was avoided je clinician and the patient before gastroscopy. More the pathologist and microbiologist were independent; knowledge of the therapy and neither the clinician nor knew of the presence or absence of bacteria. Thus we do iat the title is misleading.

ntions that our definition of ulcer relapse includes "any of ulcer symptoms". This is almost correct—our re treated as in normal practice. If they complained of requiring therapy, they were considered to have relapsed (32/43, 74%) an ulcer was found on gastroscopy. We unreasonable to call such patients "successfully treated" a recurrence of symptoms but did not have an ulcer crater py.

ee that "bismuth not cimetidine is associated with f campylobacter", but if Lam really believes we did not against investigator or patient bias", we suggest he the following. There is no way to demonstate CBS histology or microbiology, unless Lam thinks that the f gastritis and campylobacter, only seen with CBS auses observer bias. All bias can thus be eliminated by g only the group of patients treated with CBS. This CP + ve patients, 10 (45%) healed at follow-up and 3 l healed at 12 months; compare this with 24 CP − ve 2 (92%) healed and 17 (71%) still healed at 12 months.

ole was not intended to "differentiate cimetidine and nor for that matter was our study. If Lam is interested in he should examine our results for CBS with or without which show an improvement with tinidazole. We were ng the effect of the eradication of *C pylori*, not the effect of , CBS, or tinidazole per se—our results should be viewed aspect.

tribution of campylobacter is patchy, as stated by Lam, it a microscopic level and, with improved methods, we e contradictory results. In this series no CP + ve patient tive histological findings and culture without treatment l patient gave two successive negative biopsy specimens y a *C pylori* positive culture (probably a true re-infection). includes about 450 biopsies on 100 patients. Under these nces we consider "eradication" a correct and justified

rk was designed to show the effect of the eradication of n duodenal ulcer relapse. It was never intended as a drug etidine is an ulcer-healing agent but will not eradicate Our results suggest that eradication of *C pylori* is associated matic reduction in the relapse of healed ulcers.

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...e ^{14}C-urea breath-test for the detection of gastric *...ampylobacter pylori* infection

(for editorial comment, see page 426; see also page 431)

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...TRACT A breath-test has been developed for the detection of gastric ...tion with *Campylobacter pylori*. Urea that is labelled with carbon 14 ...ministered to a fasting patient and the patient's breath is sampled for ...activity over the following 30 minutes. If *C. pylori* is present in the ...ent's stomach, urease activity causes hydrolysis of the urea and the ^{14}C ...sorbed as carbon dioxide. This carbon dioxide enters the patient's ...rbonate pool and eventually is excreted in the breath. The results are ...essed as a percentage of the administered dose/mmol carbon dioxide ...g body weight. Sixty-three patients who were undergoing endoscopy ...e studied. The radioactivity in exhaled breath which was sampled within ... minutes of ^{14}C-urea administration was attributed to the presence of ...se enzyme in mouth organisms and was discounted. The time–...oactivity curves for breath samples from five to 30 minutes after the ...inistration of ^{14}C-urea gave an excellent separation between subjects ...negative results of the examination of gastric-biopsy samples and ...ents with microbiological and histological evidence of infection with ...*ylori*. The area under the time–radioactivity curve at between five and ...minutes after the administration of ^{14}C-urea in 24 patients with negative ...robiological results was 6.9 ± 4.4 area units; in 35 of 39 patients with ...itive microbiological results, this area was greater than 40 area units. ...asured against the results of the microbiological examination of gastric-...psy samples, the sensitivity of breath-testing was 90% and the specificity ...100%. Measured against the results of histological examination for the ...sence of *C. pylori* infection, breath-testing had a sensitivity of 94% and ...pecificity of 93%. A positive breath-test result also correlated well ...0.0001) with the serological antibody test-result. The role of non-invasive ... — enzyme-linked immunosorbent assays and ^{14}C-urea breath-testing ...the management of gastritis and peptic ulcer disease is discussed. We ...sider that the ^{14}C-urea breath-test has an important role in the non-...asive confirmation of gastric infection with *C. pylori* and in the follow-... of patients after treatment. (Med J Aust 1989; 151: 435-439)

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It is accepted by many authors that *Campylobacter pylori* plays a significant role in the pathogenesis of type-B gastritis and peptic ulcer disease.[1-5] Marshall and Warren were the first to give prominence to the association between gastritis and the curved organisms which now are known as *C. pylori*[6] — an association that since has been observed world-wide. The strength of this association with type-B gastritis,[6-15] duodenal ulcers[6,8,13-15] and gastric ulcers[6,14,15] cannot be disputed.

Koch's postulates in relation to human infection with *C. pylori* partly have been verified by two independent volunteers.[16,17] Both subjects reported the development of histologically demonstrated gastritis after infection with the bacteria. Goodwin has suggested a mechanism whereby resistance to the action of acid and pepsin by gastric epithelium and/or gastric metaplasia in the duodenum is impaired in *C. pylori* gastritis.[18]

A consequence of the *C. pylori* hypothesis must be an increasing tendency to treat dyspeptic symptoms with antibiotic agents. Talley has warned of the potential dangers in the indiscriminate use of antibiotic agents for a symptom group as common and as non-specific as "dyspepsia".[19] The precise treatment of infection with *C. pylori* requires a relatively simple, efficient, inexpensive and safe diagnostic procedure that can be applied and used in the follow-up of a large number of patients.

Diagnosis by histological examination and microbiological culture is the "gold standard" by which all other diagnostic procedures must be calibrated. Obtaining material for pathological examination requires that patients undergo endoscopy. An endoscopic diagnosis may be made rapidly at the time of biopsy by the performance of a "CLOtest" with gastric mucosa.[20] The non-invasive diagnosis of infection with *C. pylori* can be achieved by serological examination.[21-26] The measurement of antibodies to *C. pylori* in plasma is of great value in epidemiological studies.

A breath-test that is based on the ingestion of labelled urea has been advocated as a useful test in the diagnosis of infection with *C. pylori*.[27] The principle of the test is identical to that of the

CLOtest". Urease enzyme is produced in large amounts by *C. pylori* organisms.[28] In cases of infection with *C. pylori*, the increased urease activity results in the rapid hydrolysis of urea in gastric juice producing ammonia and carbon dioxide. This is recognized in the CLOtest" by a pH change,[20] or in the carbon 14-labelled urea breath-test by labelled carbon dioxide which enters the carbon dioxide-bicarbonate pool and eventually is excreted in the breath. A similar test that uses urea which has been labelled with carbon 13 and mass spectroscopy has been described by Graham et al.[29] Recently, several centres have reported on the high accuracy of ^{14}C-urea breath-testing for the detection of infection with *C. pylori*.[30-35] We now have applied this breath-test to a further group of patients at Royal Perth Hospital and have correlated the results of breath-testing for infection with *C. pylori* with the findings of upper gastrointestinal endoscopy, so as better to define the utility and limitations of the ^{14}C-urea breath-test in the detection of active infection with *C. pylori*.

Patients and methods

Sixty-three adult patients, 33 men and 30 women, whose ages ranged from 16 to 87 years (mean ± standard deviation [SD], 58.8 ± 14.5 years of age), were examined. Patients were recruited consecutively from the routine list of names of patients for upper gastrointestinal endoscopy and were requested to give their informed consent to undergo a ^{14}C-urea breath-test according to protocols that had been approved by the Royal Perth Hospital Human Rights Committee. All patients complained of symptoms that were attributed to upper gastrointestinal disease; 13 (20.6%) of the 63 patients were undergoing follow-up endoscopic examination. A detailed retrospective review of all the patients' notes, which was supplemented by telephone calls to their general practitioners, was performed by one of us (C.S.G.) for possible antibiotic drug interactions with the tests.

At endoscopy at least two biopsy specimens were obtained from each patient. Histological sections were stained with haematoxylin and eosin and Giemsa stains and by the Warthin-Starry method and were examined for the presence of *C. pylori*. Specimen cultures were performed by the method of Goodwin et al.[36] A subjective visual assessment of bacterial growth was made after 72 hours of laboratory culture. This assessment was related to the amount of ^{14}C that was recovered in the patient's breath.

Serological tests were performed according to the method of Goodwin et al.[23] An antibody titre of less than 150 was considered to be a negative result, an antibody titre of between 150 and 250 was considered an equivocal result and a titre of more than 250 was considered to indicate infection.

The breath-test was performed according to the protocol of Marshall and Surveyor.[27] Patients fasted overnight and, to reduce contamination from urease-producing commensal flora in the mouth, brushed their teeth before the test. The water that was used for teeth-brushing was not swallowed.

The patients then provided a control specimen of breath by exhaling through a short tube. Breath was passed over a drying agent (calcium chloride granules) and then bubbled through a 2-mL solution of the basic amino acid, hyamine. (Hyamine solution is strongly caustic and may be dangerous if swallowed.) The molarity of the hyamine was measured by acid-base titration. The breath-sample collection was terminated when a pH indicator in the hyamine (thymolphthalein) changed from blue (alkaline) to colourless (acid). Most patients could effect this colour change in a single breath.

Breath samples were obtained at three, five, 10, 15, 20, 25 and 30 minutes after drinking 400 kBq of a ^{14}C-urea solution (Amersham International, Buckinghamshire, UK). Ten millilitres of liquid scintillant (United Technologies, Packard Instrument Company, Downers Grove, Illinois, USA) were added to both breath samples and a ^{14}C-urea counting standard. All liquid scintillation counting was performed to a 1% coefficient of variation.

The results at each sample point were calculated as the percentage of the administered dose/mmol carbon dioxide × kg body weight. The radioactive counts for the breath samples were multiplied by body weight so as to attempt to correct for the influence of endogenous carbon dioxide production on the ^{14}C specific activity of the breath.[37] Interpretation of the test was performed by plotting the radioactivity of the breath sample against the time of the sample. The area under the time-radioactivity curve from five minutes to the last collection at 30 minutes, as calculated by use of the trapezoid rule, provided a convenient summary of a patient's result.

A normal or "negative" group of subjects was defined as the group of subjects from whom *C. pylori* organisms were not grown and in whom organisms were not seen on either Gram-stain or histological examination. It was a requirement of this definition that biopsy specimens were obtained from both the gastric fundus and antrum for both culture and histological examination.

Standard statistical tests were used in the analysis of results.[38]

Results

In the ^{14}C-urea breath-test some patients with negative results for the presence of *C. pylori* infection demonstrated an early peak to their time-radioactivity curve which usually lasted for fewer than five minutes and always had returned to below the level of 0.6% of the administered dose/mmol carbon dioxide × kg body weight in the 10-minute sample. Figure 1 is a plot of the range of data-points that were obtained from 24 patients in whom culture and Gram-stain and histological examinations all gave negative results for the presence of *C. pylori* organisms.

From 10 minutes onwards, all breath samples for those patients with negative microbiological and histological results showed a radioactivity of less than 0.08% of the administered dose/mmol carbon dioxide × kg body weight. For the patients with negative microbiological results, the time integral (±SD) from five to 30 minutes was 6.9 ± 4.4 area units (95% confidence interval, 5.0–8.7 area units). In the patient group with negative culture results, the highest value for the five- to 30-minute integral was 16.25 area units; this did not overlap with the results of 35 of 39 patients with positive culture results, who all showed an integral of greater than 40 area units. Four patients were interpreted as having false-negative breath-test results.

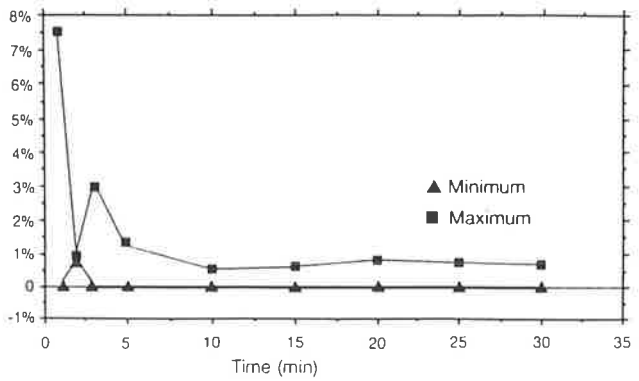

FIGURE 1: Time-radioactivity curve showing upper and lower range for radioactivity in breath as a percentage of the administered dose/mmol carbon dioxide × kg body weight for 24 patients with negative culture results of gastric-biopsy samples.

The correlation between the results of the microbiological examination and the histological examination of the gastric biopsy material is shown below.

	Microbiological results	
Histological results	*Positive*	*Negative*
Positive	32	0
Negative	3	24

Four cases were excluded from this analysis because specimen collection for the histological examination did not conform to the study protocol.

The correlation between the results of the breath-test and the microbiological examination is listed below.

	Microbiological results	
Breath-test results	*Positive**	*Negative*
Positive	35	0
Negative	4	24

*Includes two patients who had positive results by both Gram stain and culture for the presence of *C. pylori*.

The sensitivity of breath-testing was 90% (35 of 39 patients) and the specificity was 100%; the accuracy of breath-testing was 93.7%.

In the Table, the results of breath-testing are related to the presence of antibodies to *C. pylori* in plasma, as measured by an enzyme-linked immunosorbent (ELISA) assay; a highly significant

ABLE: Correlation between results for ^{14}C-urea breath-testing and antibody levels for *C. pylori* infection by ELISA

reath-test results	Antibody titre		
	Less than 150	150–250	More than 250
ositive	0	1	32
legative	8	6	14

$P = 0.0001$) χ^2 relationship was calculated.

The relationship between the results for the ^{14}C-urea breath-test nd the presence of organisms as detected by histological examination s shown below.

	Histological results	
Breath-test results	*Positive*	*Negative*
Positive	30	2
Negative	2	25

Measured against histological diagnosis, ^{14}C-urea breath-testing has a sensitivity of 94% (30 of 32 patients) and a specificity of 93% (25 of 27 patients).

After 72 hours the culture agar from both gastric fundus and antrum biopsy samples were examined visually and a subjective grading of no growth (24 cases), a light growth (five cases), a moderate growth (eight cases) or a heavy growth (19 cases) was assigned. The specimen with the highest grading was used in this analysis. Four cases were excluded from this analysis because the breath-test result was considered to be falsely negative when related to the microbiological examination result. Other exclusions were made because organisms were not cultured in spite of being seen both in Gram-stained and histological preparations or because cultures from the two biopsy sites were not obtained.

The mean time-radioactivity curves for the four microbiological groups are shown in Figure 2. There was a good correlation between the percentage of the administered dose of the ^{14}C-urea solution as detected by radioactivity measurements of the breath samples and the recovery of organisms from gastric-biopsy samples. The integral of the time-radioactivity curves between five and 30 minutes for the four groups was subjected to an analysis of variance test and a highly significant ($P = 0.0001$) difference among group means was demonstrated.

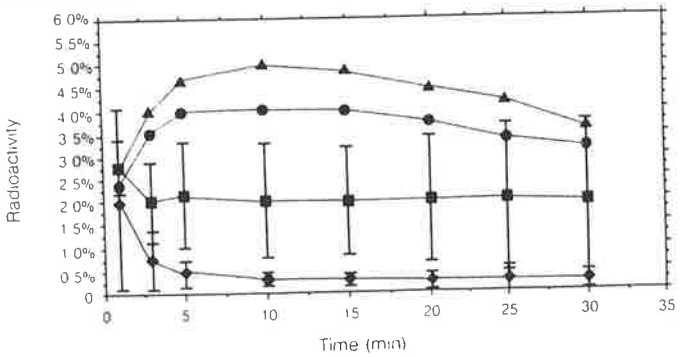

FIGURE 2: Time-radioactivity curves for radioactivity of breath as a percentage of the administered dose/mmol carbon dioxide × kg body weight. Curves show the mean values at data-points for 24 patients with negative culture results (◆), and for five patients with a light growth (■), eight patients with a moderate growth (●) and 19 patients with a heavy growth (▲) of C. pylori organisms as judged visually after 72 hours of culture. (For ease of reading, error bars have been omitted from the curves for moderate [●] growth and heavy [▲] growth.)

Discussion

The requirement for a reliable, non-invasive test for the detection of infection of gastric mucous membranes with *C. pylori* has become an urgent priority as the hypothesis of a causal relationship between the organism and gastritis and, by reasonable extrapolation, peptic ulcer disease, becomes more accepted in the medical community.

The ^{14}C-urea breath-test and serological tests are strong candidates for this non-invasive role. Although the importance of serological examination has been stressed by Mitchell et al.,[24] there are problems with the interpretation of the results, for instance, cross-reaction with other organisms, especially *Campylobacter jejuni*. Mitchell et al. noted that five of 65 cases with normal histological results showed raised serum antibody titres. It was observed by Vaira et al. that antibody levels may decrease after treatment with colloidal bismuth,[25] but it is by no means assured that, after successful treatment, the antibody levels will fall to the values that are obtained for control subjects.

It is clear from an examination of the time-radioactivity curves for breath-testing that data for breath samples that are obtained earlier than five minutes after the ingestion of ^{14}C-urea should be ignored. This early peak in radioactivity is believed to be a result of contamination with commensal flora of the mouth and, therefore, is discounted in our interpretation. The data-points that were obtained from the patients in this series with negative culture and histological results were very similar to those of our previously reported series.[27]

Only three patients in the group with negative culture results exceeded a radioactivity of 0.5% of the administered dose/mmol of carbon dioxide × kg body weight and only by a very small amount, namely, 0.56%, 0.58% and 0.59%, respectively. It was noted that for most patients with positive culture results a peak occurred in their time-radioactivity curves at between 10 and 20 minutes after the administration of the ^{14}C urea; in the occasional patient, the radioactivity continued to increase during the 30-minute collection period.

In this series, the time-radioactivity curves for all the patients with positive culture results exceeded 1.5% of the administered dose/mmol of carbon dioxide × kg body weight at more than one data-point in the time interval of 10–30 minutes. However, we would recommend that if the time-radioactivity curve for any patient were to fall within the range of 0.5%–1.5%, the test-result should be termed equivocal and the test repeated. Excellent discrimination could be obtained between infected and non-infected cases when the time integrals of the time-radioactivity curves at between five and 30 minutes were calculated.

It is not clear how long an interval should be observed between the cessation of a course of colloidal bismuth or antibiotic treatment and undergoing the ^{14}C-urea breath-test. In three of the four patients with false-negative breath-test results when compared to the results of microbiological examination, these results could be related to such treatment — with colloidal bismuth in two cases and with amphotericin lozenges that were prescribed for a monilial oesophagitis in the third case. (Laboratory observations have confirmed that amphotericin can inhibit the growth of *C. pylori*.) We suggest that false-negative results may be obtained if a breath-test is performed within the first month after the cessation of antibiotic or colloidal bismuth therapy.

A good correlation was found between the results of the serological tests and those of the breath-test, but of those cases with a high antibody titre, only 32 (69.6%) of 46 cases showed positive breath-test results. It is not known for how long the levels of antibodies to *C. pylori* will remain elevated after the organism has been treated successfully.

In the correlation between the results of histological examination and microbiological testing for the presence of *C. pylori* infection, there was agreement in 56 of 59 cases. When the results of histological examination were compared with those of breath-testing, there were two false-positive results and two false-negative results for the breath-test. This could be explained by the irregular distribution of bacteria throughout the stomach mucosa. Hazell et al. have emphasized the need to obtain samples from both the gastric fundus and antrum if a confident histological diagnosis is to be made.[11] Marshall and Warren have commented on the difficulties of pathological interpretation from small and perhaps "mechanically distorted" specimens.[6]

The evidence from these data together with data from other reports[27,29–35] confirm the high sensitivity and specificity that is obtained with breath-testing. From Figure 2 it can be inferred that the patient response to labelled urea, as measured by the level of

ivity of the breath, is proportional to the extent of gastric tion with *C. pylori* organisms.

am et al. used urea that had been labelled with the non-ive nuclide ^{13}C.[29] This has the obvious advantage of not to the body's burden of radiation, but the assaying of ^{13}C quires a gas-isotope mass-spectrometer. On the other hand, be assayed in a liquid scintillation counter, a general-purpose us which is available readily in all major medical centres. a is not expensive and, from the purchase of one freeze-dried e of 9.25 MBq, 22 patient doses may be dispensed.

adiation dose of a ^{14}C-urea breath-test has been estimated shall and Surveyor as 44 μGy in bone, 180 μGy in the lungs, / in the gonads and 70 μGy in the urinary bladder.[27] It is ed that any ^{14}C atoms which are not excreted by either the the pulmonary route enter the natural body pool of ^{14}C and e indistinguishable from the other radioactive carbon atoms constitute part of the radiological burden of all living ms.

ided that the patient has not been taking antibiotic agents bidal bismuth in the four weeks before testing, the ^{14}C-urea test has a high over-all accuracy of 93.7%. The test is ly simple and inexpensive to perform and provides an accurate of detecting gastric infection with *C. pylori*.

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Duodenal ulcer treated with *Helicobacter pylori* eradication: seven-year follow-up

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Summary

The long-term benefits of *Helicobacter pylori*-eradication treatment (HET) in *H pylori*-associated duodenal ulcer are unclear. We followed up patients with duodenal ulcers from a trial of *H pylori* eradication in 1985–86.

63 of 78 patients (81%) were reviewed clinically and had upper gastrointestinal endoscopy with gastric antral biopsy. Of 35 patients previously rendered *H pylori* negative, 32 (92%) remained *H pylori* negative after 7·1 years (mean). All patients initially *H pylori* positive remained infected, unless HET was given in the interim. Duodenal ulceration was found in 20% (5 out of 25) of patients remaining *H pylori*-positive, compared with 3% (1 of 38) of *H pylori*-negative patients ($p < 0·05$).

The reduction of duodenal ulcer relapse obtained from *H pylori* eradicaton in *H pylori*-associated duodenal ulcer extends to at least 7 years after treatment, and is likely to be due to freedom from *H pylori* infection. However, duodenal ulcer may recur in patients rendered *H pylori* negative, due to factors other than reinfection with *H pylori*.

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Introduction

The association between gastric *Helicobacter pylori* colonisation and duodenal ulcer (DU) is well established,[1] and many studies have shown that eradication of *H pylori* reduces DU recurrence.[2,3] Most of these studies have followed patients for 2 years or less, and little is known of the outcome over longer periods. Borody's group found that 97% of patients rendered *H pylori* negative remained negative by C^{14}-urea breath test after 4 years of follow up;[4] earlier work suggested that patients remaining *H pylori* negative were free from DU relapse for up to 4 years, though small numbers were involved.[5] Although eradication of *H pylori* is clearly important in the short term, *H pylori*-eradication treatment (HET) would be more valuable if its effect was shown to extend over longer periods. We determined the long-term outcome of HET in a cohort of patients who took part in the earliest controlled trial of HET in duodenal ulcer,[6] after which some were *H pylori* positive and others negative.

Patients and methods

100 patients were enrolled in a prospective double-blind trial of HET in 1985 and 1986, in which endoscopic appearances and *H pylori* status were recorded over 12 months.[6] At the end of the trial, patients returned to the care of their general practitioner. These patients were traced through hospital records, their general practitioner, or by telephone. Patients who were contactable were approached to undergo clinical examination and upper-gastrointestinal endoscopy with antral biopsy.

Medical attendances for DU-related symptoms and results of relevant investigations were recorded and corroborated from case

Initial *H p* status	Current *H p* status	
	Positive	Negative
Positive (n = 28)	23	5*
Negative (n = 35)	2	33†

*All 5 received *H pylori* eradication therapy.
†One patient had documented *H pylori* reinfection at 5 years, received *H pylori* eradication therapy, and is now *H p* negative.

Table 1: **Current *H pylori* (*H p*) status compared with *H p* status at the end of the initial study**

records. Upper gastrointestinal endoscopy was done by a gastroenterologist blinded to details of the patient's past medical history and 3 gastric antral biopsies were taken.

Relapse of DU was defined as current—active DU at follow-up endoscopy; proven—relapse proven by endoscopy or barium meal in the period between studies; and clinical—relapse during the intervening period as judged by the patient's history (symptoms identical to previous DU symptoms and which were considered consistent with DU disease), whether confirmed by investigation or not, and excluding asymptomatic relapses detected at follow-up endoscopy.

Formalin-fixed paraffin-embedded sections of gastric antral biopsies were examined for the presence of inflammation and *H pylori* with haematoxylin and eosin, and Giemsa stains. Sections were examined by an experienced histopathologist blinded to the patient's details. The *H pylori* status of the patient was defined histologically as *H p* + or *H p* −. The study was approved by the Ethics Committee of Royal Perth Hospital.

Results

Of 100 patients in the original study,[6] 78 were available for follow-up and 63 (81%) agreed to clinical review and endoscopy 5·1–7·6 years (mean 6·5) later. There were 44 males and 19 females (mean age of 53, range 24–83). Of the remaining 22 from the original study, 14 were uncontactable, 4 had died, and 3 were unfit for endoscopy. 1 patient had undergone total gastrectomy for Zollinger Ellison syndrome.

Initial *H pylori* status of the 63 patients is compared with current *H pylori* status in table 1. Patients who were initially *H p* − include a group of patients who completed the initial study by Marshall et al[6] and a group who failed treatment, were withdrawn from the study and after subsequent HET were shown to be *H p* − during the following 12 months. 32 of 35 patients (92%) initially *H p* − remained *H p* − up to 7·1 years (mean) (range 6·1–7·9) follow-up after eradication of *H pylori*. 1 patient initially *H p* − was found to have *H p* + chronic antral gastritis without DU after 5 years at a time of clinical relapse; he received HET and was *H p* − at follow-up endoscopy 2 years later. 2 other patients initially *H p* − became *H p* +, and both had documented DU relapse, although one was associated with non-steroidal anti-inflammatory drug (NSAID) use. 28 patients were *H p* + at the end of the initial trial. 23 of these remain *H p* + and 5, all of whom received further HET in the interim, have been rendered *H p* −.

At follow-up endoscopy (table 2), active DUs were present in 5 of 25 (20%) patients currently *H p* + in contrast to 1 of 38 (3%) *H p* − patients (χ^2 5·28; $p < 0·05$) (current relapse). Proven relapse occurred in 9 of 26 (35%) *H p* + patients and 3 of 37 (8%) *H p* − patients (χ^2 6·97; $p < 0·01$). One patient who was *H p* + at the end of the initial study had an endoscopically-proven DU accompanied by gastric antral *H pylori* after 5 years, received HET, and is currently *H p* −. He is included in the *H p* + group for analysis of proven and clinical relapse, despite being currently *H p* −. Of patients with proven relapse, 9 had DUs documented endoscopically and 3 by barium meal. Clinical relapse occurred in 11 of 26 (42%) *H p* + patients compared with 8 of 37 (22%) patients who were *H p* − at follow up (χ^2 3·1; $p < 0·10$).

H pylori status	Current DU relapse	Proven DU relapse	Clinical DU relapse
H p +	5 (n = 25)	9 (n = 26)	11 (n = 26)
H p −	1 (n = 38)	3 (n = 37)	8 (n = 37)
p	< 0·05	< 0·01	< 0·1

DU = duodenal ulcer. For definitions of relapses see text.
One patient currently *H p* − had proven DU relapse accompanied by persistence of *H pylori* 4·8 years after the initial study; hence he was *H p* − in the proven and clinical groups for statistical analysis. "n" = number with specified *H pylori* status.

Table 2: **Incidence of duodenal ulcer relapse compared with *H pylori* status at time of relapse**

3 patients received maintenance H2-receptor antagonists during the intervening years (2 currently *H p* + and the *H p* − patient who had an active DU at follow-up endoscopy). 6 patients received further HET when symptoms of DU recurred after completion of the initial study. Only 2 of these 6 patients had DU confirmed by endoscopy or barium meal. Use of NSAIDs accompanied clinical DU relapse in 3 patients (2 currently *H p* + and 1 *H p* −).

Discussion

This is the first long-term study of the natural history of *H pylori* infection and DU after *H pylori* eradication. Others have shown, either in small numbers or in studies without endoscopy, that reinfection is uncommon up to 4 years post-eradication;[4,5] our data confirm this and extend the period of observation further. Given that 3 of 35 previously *H p* − patients became reinfected over a total of 248 post-eradication patient years, this gives an annual reinfection rate of 1·2% (95% confidence intervals 0–4·8%), which is slightly higher than previously-reported estimates of up to 0·64%.[4,5,8] The absence of spontaneous loss of *H pylori* over this time is also consistent with other reports.[5] A criticism of our study might be that 37 of the 100 patients treated in the original trial could not be followed up and that this might bias our results; however, 21 of these 37 were *H p* − at the end of the initial study, a similar proportion to that in the remaining 63 patients who were followed up.

A major benefit of HET is the reduction in DU recurrence; this has been shown over a 2-year follow up,[2] and in small numbers of patients up to 4 years.[5] The value of HET would be greater still if the risk of DU relapse was reduced indefinitely. This propostion is supported by our study which shows a significantly lower long-term relapse rate in patients rendered *H p* − compared with those who remain *H p* +. Inaccuracies exist in documenting relapse rates in the ways described in our study. In particular, there may be underestimates because of clinically-silent DU disease. Indeed, of the 6 patients found to have an active DU at follow up endoscopy, 2 had no symptoms. The relatively low incidence of proven DU relapse in *H p* + patients in our study (35% over 6·5 years) presumably reflects the absence of surveillance endoscopy over that time period. Clinical relapse as defined in our study, although subject to inaccuracies of diagnosis, is of importance when examining the cost effectiveness of HET. Clinical relapse occurring in the intervening period between studies occurred in 42% of *H p* + patients compared with 22% of *H p* − patients ($p < 0·1$). Some of these relapses may have been due to non-ulcer dyspepsia, reflux, or other diseases. When a patient has frequent relapses of DU, traditional practice is to choose between

repeated courses or long-term maintenance with H2-receptor antagonists. Our findings suggest that HET is an alternative which may result in reduced costs and be used increasingly as less complex HET regimens with fewer side effects are developed.

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HELICOBACTER PYLORI

Long-term follow-up of gastric histology after *Helicobacter pylori* eradication

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Abstract *Helicobacter pylori* causes chronic active gastritis and is thought to be associated with the development of gastric atrophy, intestinal metaplasia and carcinoma. As the effect of *H. pylori* eradication on this process is poorly understood, we sought to determine the long-term effects of *H. pylori* eradication on gastric histology. Fifty-four patients with duodenal ulceration associated with *H. pylori* infection received *H. pylori* eradication therapy in 1985/86 and either remained infected ($n = 22$) or had the infection eradicated ($n = 32$); patients were followed up by endoscopy with gastric antral biopsy for 7.1 years (mean). Histopathological analysis of gastric antral mucosa from patients rendered *H. pylori*-negative revealed a marked decrease in both inflammatory cells within the lamina propria and intra-epithelial neutrophils and an increase in epithelial mucinogenesis. Gland atrophy remained unchanged in both *H. pylori*-positive and -negative patients. When examined for the presence and severity of intestinal metaplasia, there was neither a difference between the two patient groups nor a change with time. These data demonstrate that significant long-term improvements in gastric histology accompany *H. pylori* eradication when compared with histology in patients with persistent infection. Whether this confers a protective effect by reducing the risk of gastric carcinoma remains unknown.

Keywords: eradication, follow-up, gastric atrophy, gastric carcinoma, *Helicobacter pylori*, histology, metaplasia.

RODUCTION

obacter pylori causes chronic active gastritis and is gly associated with the development of duodenal gastric ulceration. Numerous studies have nstrated that eradication of *H. pylori* reduces the rence rate of duodenal ulcer over a 1 and 2 year w-up period; more recently, this protective effect been shown to extend to 7 years after treatment.[1] benefit of *H. pylori* eradication therapy in patients gastric ulceration probably exists,[2,3] but is less well lished. Of greater controversy is the relationship een *H. pylori* infection and gastric carcinoma and, e, the role of eradication therapy in protecting ast carcinoma.

licobacter pylori infection is an independent risk r for the development of gastric carcinoma,[4] and large multinational study suggested that this risk is old when compared with subjects not infected by *ylori*.[5] The putative mechanisms linking primary *ylori* infection ultimately to gastric carcinoma are unproven, but may relate to the development of chronic gastritis, mucosal atrophy and intestinal metaplasia[6–8] as precursor lesions to malignancy.[9–12] Eradication of *H. pylori* is accompanied by resolution or improvement in the histological severity of gastritis in the short term,[13] but the effects of *H. pylori* eradication on gastric mucosal histology are unknown for periods greater than 2 years.[14] In the present study we examined the long-term histological outcome of *H. pylori* eradication and compared this with patients who remained infected.

METHODS

One hundred patients with duodenal ulceration associated with gastric antral *H. pylori* infection were enrolled in a double-blind trial of *H. pylori* eradication therapy in 1985/86, and were prospectively followed by endoscopy with gastric antral biopsy for up to 12 months.[15] Of 78 patients available in 1992/93 for

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DISCUSSION

The present study has shown that successful eradication of *H. pylori* is accompanied by long-term improvement in the histological abnormalities that occur with this infection. In patients rendered *H. pylori*-negative there was a marked reduction in the lamina propria inflammatory cell infiltrate, almost complete loss of intraepithelial neutrophils and an improvement in the amount of epithelial mucinogenesis. By contrast, in patients who remained *H. pylori*-positive, there was no improvement in any of these histological features.

In neither *H. pylori*-positive nor *H. pylori*-negative patients did the amount of glandular atrophy change over the 7.1 year follow up. Furthermore, none of the *H. pylori*-positive patients developed histological features of 'gastric atrophy': the combination of severe gland atrophy, extensive metaplasia and negligible inflammation.[17] Proposals that *H. pylori* infection leads ultimately to gastric atrophy stem from longitudinal population based studies,[11] which suggest that chronic gastritis eventually progresses to atrophic gastritis. Further support for this sequence of events comes from a recent 5.5 year follow-up study of a group of untreated *H. pylori*-positive patients who developed atrophic gastritis and intestinal metaplasia more frequently than did a group of patients never infected.[6] This was an important observation in light of the association between gastric atrophy and carcinoma.[12] Intestinal metaplasia is also thought to be a precursor lesion for gastric carcinoma.[10,11] If chronic *H. pylori* infection does lead to gland atrophy, intestinal metaplasia and ultimately, in certain individuals, gastric carcinoma, this process is likely to occur over several decades. Hence, longer duration of follow up of our patients may be needed to detect progression of potentially premalignant histological abnormalities.

A further limitation of our study is that only three gastric antral biopsies were taken at each endoscopy, thus creating the possibility of histological sampling error. Overall, intestinal metaplasia was generally mild or absent, both in patients who remained *H. pylori*-positive throughout the study period and those in whom *H. pylori* was eradicated. Our preliminary histological studies[18] were not undertaken using the combined periodic acid-Schiff and alcian blue stain to examine for intestinal metaplasia. This resulted in an overestimation of the presence of intestinal metaplasia in our initial studies, especially when persistent *H. pylori* infection was present, and illustrates the importance of this tissue examination.

Future long-term studies of this type may be difficult to undertake in view of the link between *H. pylori* and gastric carcinoma[19,20] and the recent World Health Organisation classification of *H. pylori* as a class I carcinogen. It is unknown whether successful *H. pylori* eradication therapy results in lessening of the gastric cancer risk to levels seen in subjects who have never been infected. Previous follow-up studies of up to 2 years suggested that eradication of *H. pylori* results in incomplete[21] or no improvement in intestinal metaplasia and gland atrophy.[14] These observations and the present study suggest that if *H. pylori* eradication is associated with a lessened risk for gastric carcinoma, this reduced risk may not be to the level seen in subjects never infected.

In conclusion, previous data suggest that *H. pylori* infection results in a progressive form of chronic gastritis, which may predispose to gastric cancer.[6,8–12] Our data indicate that *H. pylori* eradication is accompanied by long-term improvement in the histological severity of gastritis, but whether this is reflected in clinical practice by a reduced incidence of gastric carcinoma is unknown and may be difficult to show.

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and endoscopic follow-up, 63 (81%) agreed; the of that study have been reported previously.[1]

ne time of each endoscopy, three gastric antral s were taken for histological examination, which dertaken by a single experienced histopathologist blinded to the clinical details of each patient and t reference to previous pathological specimens. lin-fixed paraffin-embedded sections stained with toxylin and eosin, Giemsa and combined periodic chiff and alcian blue were examined for the fol- features, derived from the Whitehead classifica- of gastritis:[16] (i) lamina propria inflammatory cell te; (ii) neutrophil infiltration of epithelium; (iii) ion in epithelial mucinogenesis; (iv) gland atro- nd (v) intestinal metaplasia.

s was performed for biopsies taken at entry to the 36 study (time = 0), upon completion of that study t = 0.8 years) and at the time of the 1992/93 (mean t = 7.1 years). These histological parame- ere graded semiquantitatively according to a 10 grading system as outlined in Table 1; the results tients who were initially rendered *H. pylori* nega- nd remained so over the 7.1 years of follow-up compared with those patients who remained ed throughout. Statistical analysis of the difference stological grade from t = 0 to 7.1 years between *lori*-positive and -negative patient groups was per- ed using the Mann-Whitney *U*-test.

ULTS

3 patients followed between 1985 and 1993, nine excluded from histological study because they r received *H. pylori* eradication therapy ($n = 6$) or became reinfected with *H. pylori* ($n = 3$) during the val period between studies. Twenty-two patients remained *H. pylori*-positive (15 male, seven female; n age 52 years; range 30–83 years) and 32 patients remained *H. pylori*-negative (23 male, nine female; n age 52 years; range 24–78 years) had a mean logical follow-up over 7.1 years (range 6.0–7.9).

Table 1 Grading of the five histological parameters

Lamina propria inflammatory cell infiltrate/reduction in epithelial mucinogenesis/ gland atrophy		Neutrophil infiltration of epithelium/intestinal metaplasia	
0–3	Normal	0	None
4–5	Mild	1–3	Mild
6–7	Moderate	4–6	Moderate
8–9	Marked	7–9	Marked

Six (27%) *H. pylori*-positive patients and five (16%) *H. pylori*-negative patients ($\chi^2 = 1.09$; NS) received histamine H_2-receptor antagonists during follow-up (continuously in three patients: two *H. pylori*-positive and one *H. pylori*-negative). No patient received proton pump inhibitors.

The histological results are summarized in Table 2. Before *H. pylori* eradication was attempted, both groups had similar histological features with the exception of gland atrophy, which was more marked in the group for whom eradication failed ('*H. pylori*-positive' group; $P = 0.006$, Mann-Whitney *U*-test). However, this difference was no longer present at the 0.8 year (mean) follow-up. In patients who remained *H. pylori*-positive over the next 7.1 years, there was no change in the grade of inflammatory cell infiltrate in the lamina propria, neutrophil infiltration of the epithelium, intestinal metaplasia, gland atrophy or epithelial mucinogenesis.

By contrast, patients who remained *H. pylori*-negative showed a marked reduction in both inflammatory cells in the lamina propria and intraepithelial neutrophils at 0.8 years and this persisted to follow-up at 7.1 years. There was some return of epithelial mucinogenesis, but no change in gland atrophy, which remained mild to moderate in severity in the *H. pylori*-negative group. Overall, the grade of intestinal metaplasia was mild and there was no difference in severity between *H. pylori*-positive and -negative patients.

le 2 Histological grading of patients who remained *H. pylori*-positive compared with those rendered *H. pylori*-negative

	LP infiltrate	Epithelial PMN	Metaplasia	Atrophy	Mucin
+ patients					
= 0	7.6 ± 1.0	5.0 ± 1.4	0.7 ± 1.4	5.0 ± 1.5	6.0 ± 1.7
= 0.8	6.8 ± 1.4	4.6 ± 1.7	0.7 ± 1.3	4.5 ± 1.3	6.9 ± 1.6
= 7.1	7.0 ± 0.8	4.9 ± 1.6	0.9 ± 1.3	4.7 ± 1.0	5.6 ± 1.7
– patients					
= 0	7.3 ± 1.3	5.3 ± 1.6	0.8 ± 1.3	3.8 ± 1.4	5.7 ± 1.2
= 0.8	3.7 ± 1.5	0.3 ± 0.5	0.5 ± 1.2	4.4 ± 1.8	4.6 ± 1.5
= 7.1	1.9 ± 0.8	0.3 ± 1.1	0.7 ± 1.4	3.8 ± 1.6	4.0 ± 1.5
alue	< 0.0001	< 0.0001	NS	NS	0.0004

ll patients were *H. pylori*-positive at t = 0; *H. pylori*-negative patients were all negative for *H. pylori* at 0.8 and 7.1 years.
LP infiltrate, the lamina propria inflammatory cell infiltrate; epithelial PMN, neutrophil infiltration of the epithelium; taplasia, intestinal metaplasia; atrophy, gland atrophy; mucin, reduction in epithelial mucinogenesis; NS, not significant; alue, analysis of the difference in histological grade from 0 to 7.1 years between Hp + and Hp – patient groups.

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