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

Barry J. Marshall, MB.BS., F.R.A.C.P., J. Robin Warren, MB.BS., F.R.C.P.A., Graham J. Francis, B.S., Simon R. Langton, B.Sc., F.A.A.C.B., C. Stewart Goodwin, M.A., M.D., B.Chir., F.R.C.Path, Dip.Bact., F.R.C.P.A., Elizabeth D. Blincow

Gastroenterology Department, Royal Perth Hospital, Perth, Western Australia, 6001; and Fremantle Hospital, Fremantle, Western Australia, 6160

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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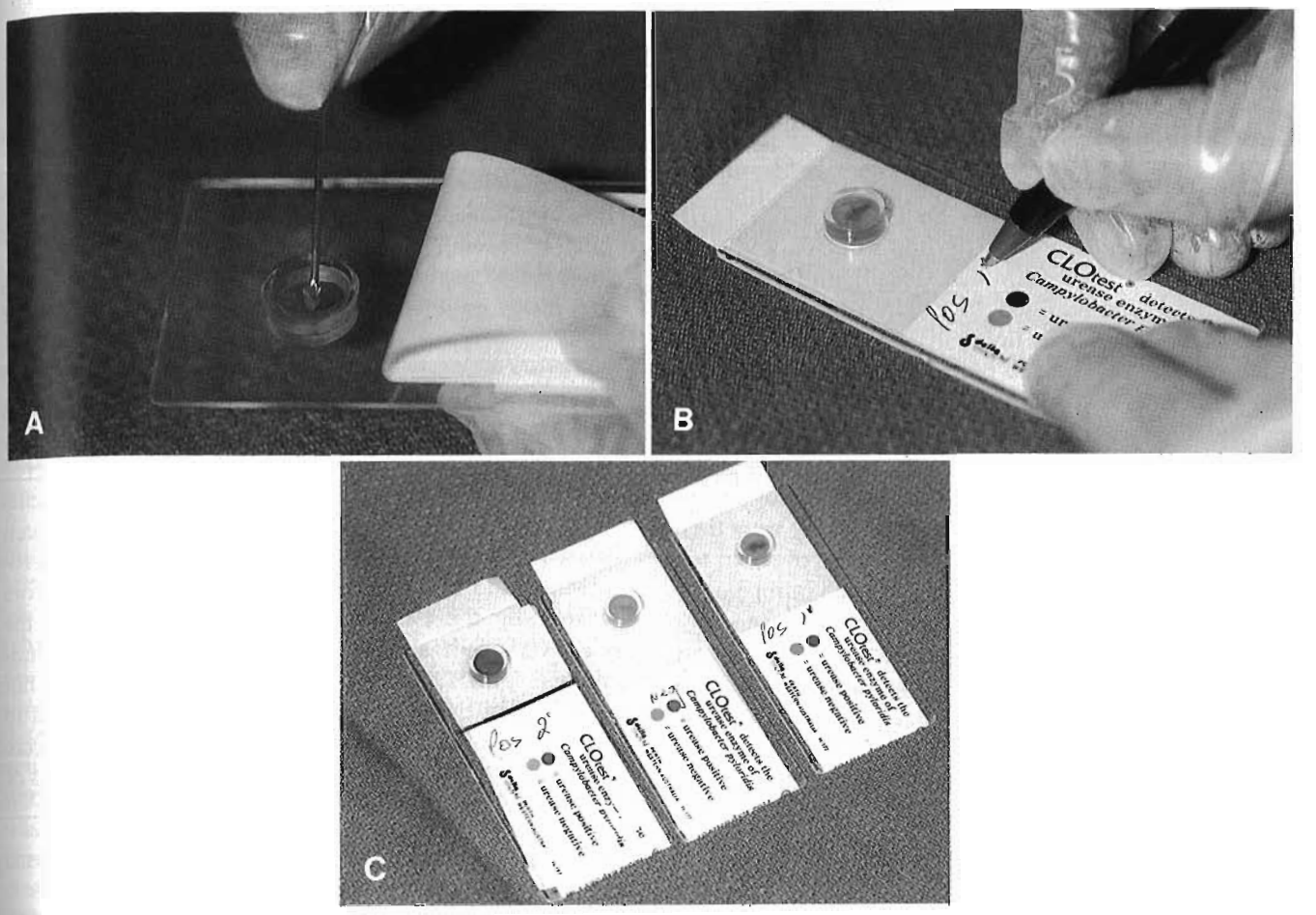

FIG. 1. *A*, insertion of 2 mm pinch biopsy into the CLOtest gel. A 19G needle is preferred. *B*, the CLOtest is resealed and the time for a pink color to appear is recorded on the label (in this case 1 min). Ideally, CLOtests should be incubated at 30–33°C but it is often more convenient to keep them 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 old; 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 that in *A* and *B*, 3 min after insertion of the biopsy.

urea

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

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

To be of practical use it was decided that the CLOtest should be read before the patient left the endoscopy room (nominally 20 min after biopsy) so that therapy could be prescribed by the endoscopist. As day-cases remained in the hospital for 3 h after endoscopy, it was convenient to re-examine the tests at this time and diagnose further patients before they left hospital. After being left on the shelf overnight the CLOtests could be reexamined 24 h after biopsy. Infected patients detected at 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-

cation for endoscopy was with atropine and pethidine intramuscularly, with additional intravenous diazepam immediately before the procedure. No oral medication was permitted on the day of the test. Simethicone was not given, but patients sucked a lignocaine jube for throat anesthesia.

The instrument used was an Olympus GIFXQ10 fiberoptic panendoscope. After the stomach and duodenal cap had been examined visually and suspicious lesions had been biopsied, four extra mucosal biopsies were taken from areas uninvolved by any local lesion such as an ulcer. The first two biopsies were taken from the greater curve of the stomach about 5 cm from the pylorus. One was placed in the CLOtest gel. The second biopsy was placed in a drop of sterile 10% glucose, refrigerated, and transferred to the microbiology laboratory within 3 h. Two biopsies for histology were then taken within 5 cm of the pylorus, at angles of about ten o'clock and four o'clock. These were fixed immediately in buffered formol-saline. CLOtest results were not mentioned on the histology or microbiology request forms, but normal clinical information was recorded.

Pilot study

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

Accurately timed study (141 CLOtests)

This was a prospective study on consecutive patients in whom the CLOtest was carefully observed. When the biopsy was inserted into the CLOtest, the time was recorded on the package. The CLOtest was kept at 30°C for the next 3 h and after that at room temperature (20–25°C). The test was examined as soon as the endoscopy had been completed (2–5 min), at 5-min intervals during the first h, and at 30-min intervals until the patient was discharged from the hospital or 3 h had elapsed. If a definite pink color was observed in the gel surrounding the biopsy, a positive result was recorded and the time written on the label. Negative CLOtests were reexamined when the patients were discharged and again at 24 h. If the CLOtest had changed to orange or pink within this time, the result was recorded as positive.

By the time the data had been collected and analyzed, the *C. pyloridis* status of another 130 patients had been determined by CLOtest, histology, and microbiology. This group was not used in the analysis except to provide extra data in the graph of the CLOtest reaction time (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

polymorphs was always recorded as "activity," and "active chronic gastritis" was the usual diagnosis when polymorphs were seen. The term "chronic gastritis" was used if lymphocytes or plasma cells were present in excess, but polymorphs were difficult to find or totally absent. Other abnormalities such as intestinal metaplasia, atrophy, edema, and fibrosis were described but were not considered indicators of gastritis unless lymphocytes, plasma cells, or polymorphs were also present in excess. Typical histological appearances are shown in Fig. 2.

For ease of analysis, a coding scheme was adopted similar to that used in the study of Marshall and Warren (2). The method has been described in detail elsewhere (11) and has been used by McNulty (12) for the serial assessment of gastritis. In this method, the most severe form is gastritis with polymorphonuclear and mononuclear cells present (active chronic gastritis), and a lesser degree of gastritis is that which has no polymorphonuclear but an excess of mononuclear cells only (chronic gastritis). We gave a score of 0 for a completely normal biopsy, 1 for a biopsy showing minor abnormalities without significant inflammatory cell infiltrate, 2 for chronic gastritis as described above, and 3 for active chronic gastritis. For analysis of patients who were biopsied more than once in this series, grade 2 was further arbitrarily divided into mild and moderate chronic gastritis according to the number of mononuclear cells present (severe chronic inflammation never occurred without activity also being present), and grade 3 was divided into mild, moderate, and severe degrees of active chronic gastritis according to the number of polymorphs present.

DEFINITION OF BACTERIA POSITIVE AND NEGATIVE PATIENTS

To evaluate the CLOtest result, a *C. pyloridis* positive case was defined as a patient who had spiral bacteria typical of *C. pyloridis* detected on Gram stain or culture or histological sections. When the histology and microbiology results disagreed, the histological sections were reexamined, and a consensus was obtained. For CP+ patients histological active chronic gastritis was usually present.

For patients participating in the double-blind study no clinical data was provided, and for other patients only significant endoscopic findings were mentioned on the laboratory request forms. At Royal Perth Hospital the diagnosis of *C. pyloridis* infection by histology or culture is not in contention. *Campylobacter*-like organisms are routinely reported on gastric mucosal biopsies and have been diagnosed in 910 patients since 1979. In addition, *C. pyloridis* has been cultured from over 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)

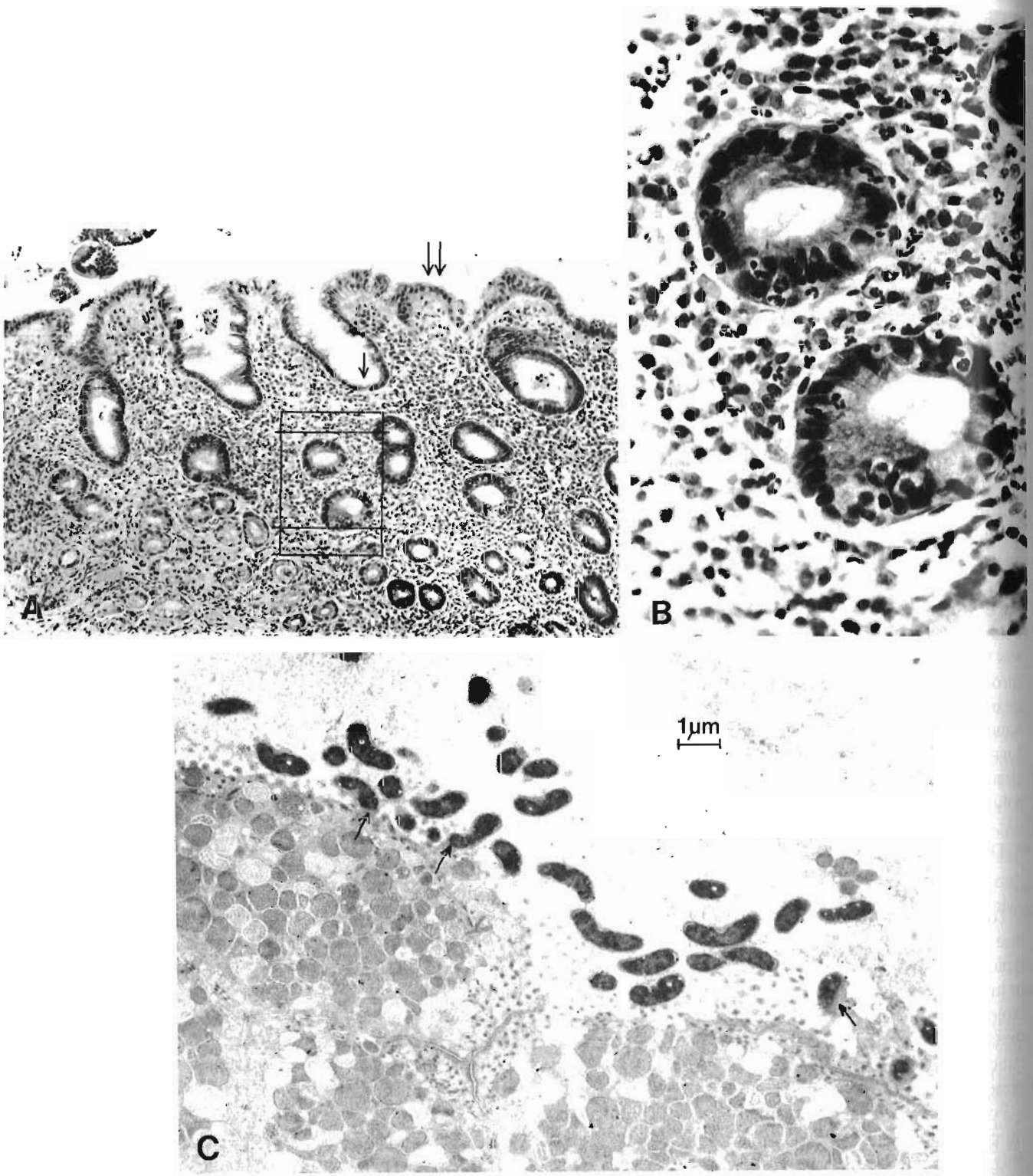

FIG. 2. *A* and *B*, severe active chronic gastritis (before treatment). *A*, low power view shows a dense infiltrate, predominantly of mononuclear cells, in the lamina propria. Luminal borders of the epithelial cells are indistinct and irregular (*single arrows*). Epithelial cells are stunted and show 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).

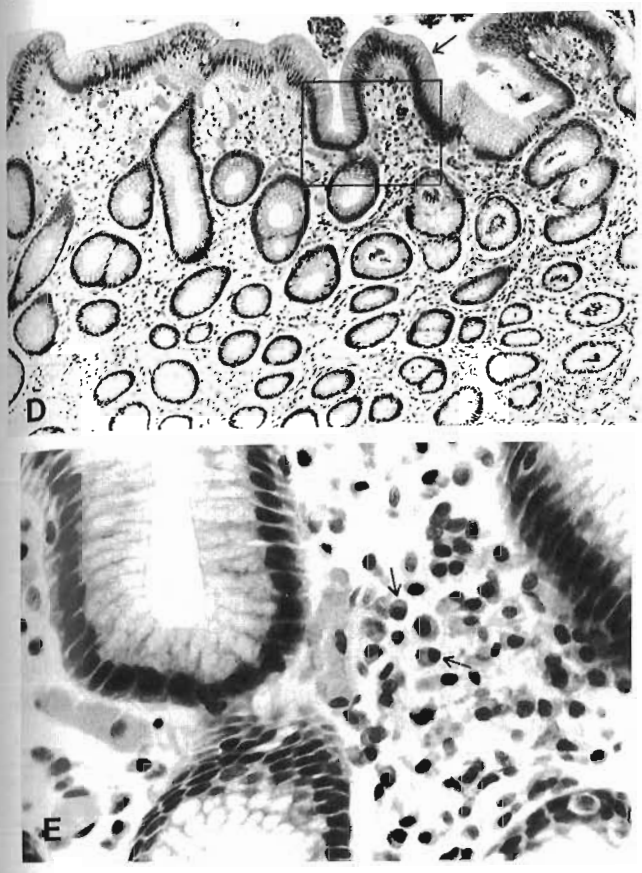

FIG. 2. *D* and *E*, same patient 1 month after clearance of CP organisms (now CLOtest negative). *D*, low power view after antibiotic therapy shows normal appearance of the mucus-secreting epithelial cells (*arrow*). They are now of regular height with parallel borders, and each has an intracellular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

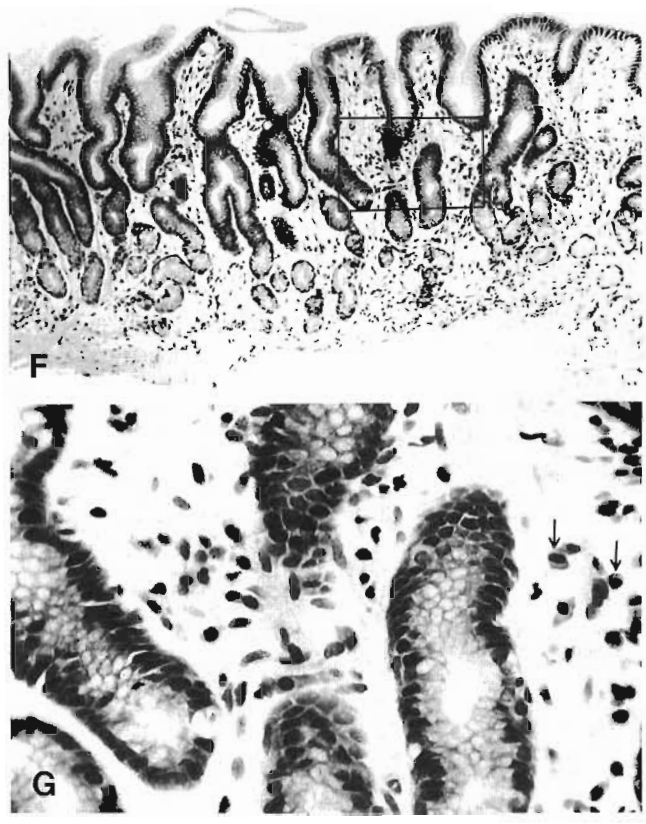

FIG. 2. *F* and *G*, example of normal antral mucosa. *F*, glands are plentiful. The lamina propria consists of loose connective tissue with few lymphocytes or plasma cells and virtually no polymorphs. The epithelial cells contain a thick intracellular mucus cap and the cells show the normal "picket 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).

that are described in detail. In three of these CP were detected on Gram stain and culture, and in one case by histology alone. All had low numbers of CP present on the histology sections. One patient was on no therapy; one was taking high dose antacids; one had ceased amoxycillin and bismuth subcitrate 7 days before endoscopy; and one had delayed gastric emptying and had taken cimetidine 400 mg on the night before the endoscopy [very high doses of cimetidine inhibit CP *in vitro* (3)]. The CLOtest in these patients reached an orange color after 12–24 h and then progressed to red in three cases by 48 h.

In the 25 CP negative patients, only one case had a possible positive CLOtest result. In this patient the CLOtest turned orange after 36 h. In this "false-positive" reaction, the biopsy was large and was not completely immersed in the gel, perhaps allowing urease-producing contaminant bacteria to grow. After the pilot study results had been analyzed, all color changes occurring 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 Positve 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.

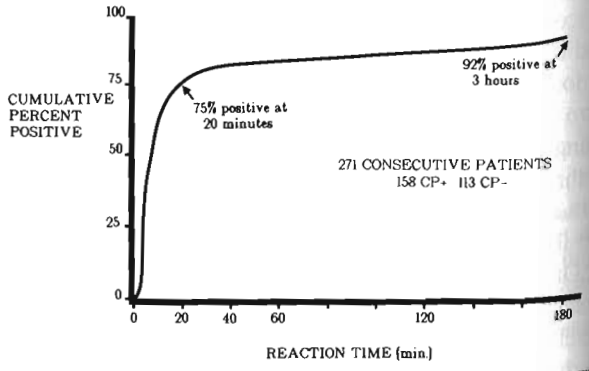

FIG. 3. Graph of CLOtest results *versus* time (0–180 min). The graph includes data from the 141 accurately timed patients plus 130 consecutive patients subsequently biopsied.

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
CP+ (79)	59	73	78	72	79	76	3
CP− (62)	62	62	62	62	61	2	60
False-negative	20	6	1	7			
False-positive					1		

100% concordance between the CLOtest and microbiology results. The histological data from this group could not be studied to confirm the microbiological results, but we know from other studies that the isolation rate in the research laboratory approaches 100% for infected cases (9).

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

EFFECT OF ERADICATION OF CP

Detailed analysis of the patients who were biopsied two or more times was undertaken. Active gastritis was subdivided into mild, moderate, and severe and chronic gastritis into mild and moderate as described in "Methods". There were 18 patients treated for *C. pyloridis* infection who were biopsied more than once; half of these were cleared of the bacterium. Six CP− patients were also rebiopsied and are also included as controls.

Active chronic gastritis resolved in eight of the nine patients in whom *C. pyloridis* was eradicated. ACG did not resolve in any of the nine patients in whom the infection persisted (Fisher's exact test, $p = 0.0002$). The CP negative patients had no active chronic gastritis initially, and no one developed it during the observation period (see Table 4).

CORRELATION BETWEEN CP NUMBERS AND CLOtest REACTION TIME

CP+ cases who had a CLOtest reaction time of 3 h or less were used to examine the correlation between reaction time and numbers of bacteria. In this group the CLOtest had been accurately timed. Although a trend appeared in the histology results the correlation was not significant ($p = 0.15$). There was no significant association between the time it took the CLOtest to react and the numbers of bacteria seen by Gram stain or Giemsa stain (see Table 4). However, when the CP numbers scored by the microbiologist and histopathologist 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

the patients were referred from general practitioners, rather than being hospital inpatients. Because of this it was usually possible to cease medications which may have interfered with detection of the bacteria, well before endoscopy. For instance, the procedure was postponed for 10–14 days when the patient had been taking bismuth containing drugs or antibiotics.

In this study we could not examine the possibility that patients with duodenal ulcer disease have more gastric urease than, for instance, patients with nonulcer dyspepsia. It is possible therefore that the excellent results obtained with CLOtest will not be repeated in more random samples of CP infected dyspeptic patients. In addition, the routine ingestion of milk on the evening prior to endoscopy, may have enhanced the urease content of the mucosa (milk contains urea). To enable comparison of our patients with future studies, we have included the endoscopic and histological findings of all patients in the pilot study (Table 1). All CP+ patients in the pilot study had histological active gastritis. In these 27 patients, there were 15 patients with active ulcer disease, eight with antral gastritis and/or other lesions and only four who were endoscopically normal (includes two patients with "healed duodenal ulcer"). In the 25 CP− patients, there were eight with a normal endoscopy and six who had an esophageal lesion. The only duodenal ulcer in the bacteria-negative group was in a patient who had taken nonsteroidal antiinflammatory drugs. Table 1 compares well with our observations in previous studies (2, 3) in which CP+ patients tended to have abnormal endoscopic findings, particularly peptic ulceration ($p = 0.00004$, Fisher's exact test).

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

In the accurately timed series of 141 CLOtests 75% of *C. pyloridis* infections were detected within 20 minutes of biopsy (Fig. 3 and Table 3). In practice, when the CLOtest started to change color before 3 h, it always continued to deepen in color, reaching deep red or purple at 24 h. Thus there were no equivocal readings of CLOtests which had become positive in the first 3 h. Five CLOtests changed to orange at 24 h. These were read as positive and all five proved to be from infected patients. Although we recognize that the number of such slow reacting CLOtests was small, a report from another center (15) and subsequent experience at this hospital 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-

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 ACG 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

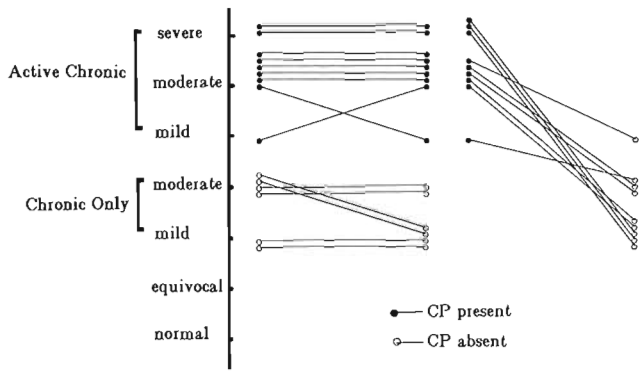

FIG. 4. Gastritis grades in patients who were rebiopsied after antibacterial therapy. The initial and final biopsies are included for 24 patients; 18 of whom showed *C. pyloridis* and six who did not. Nine of the CP+ cases were cleared of CP and the gastritis improved. In the nine who were not cleared 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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Reprint requests: Barry J. Marshall, M.D., Postdoctoral Research Fellow, Division of Gastroenterology, Box 145, University of Virginia Hospital, Charlottesville, VA 22901.

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