

Table 2 Detection of *C pyloridis* in gastric antrum, duodenum and oesophagus

Urease	Culture or Gram stain for <i>C pyloridis</i>		Total
	Positive	Negative	
Positive	44	17	61
Negative	30	108	138
Total	74	125	199

Table 3 Detecting *C pyloridis* in gastric antrum, duodenum and oesophagus (Brazilian study)

Urease	Culture or Gram stain for <i>C pyloridis</i>		Total
	Positive	Negative	
Positive	32	2	34
Negative	21	12	33
Total	53	14	67

86% and 60% for specificity sensitivity, respectively, obtained from our Brazilian study (table 3). Processing specimens immediately in the endoscopy unit and using the same specimen for the urease test seem to make no appreciable difference to the sensitivity and specificity of the test. The increase in false positive results may be explained by the fact that in the oesophagus and the duodenum the suppressive effect of gastric acid on the growth of contaminant microbial flora may not be as great as that in the gastric antrum.

On the basis of our results, we cannot recommend the biopsy urease test as a reliable and rapid test to assist in the diagnosis of *C pyloridis* infection, at least in sites other than in the gastric antrum. We also agree with Morris *et al*<sup>4</sup> that the test is really no faster than an adequate Gram stain, as most of our positive urease tests took longer than three hours to become positive.

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## Simple half-Gram stain for showing presence of *Campylobacter pyloridis* in sections

Like Gray *et al*,<sup>1</sup> we have abandoned the Warthin-Starry technique for identifying gastric *Campylobacter pyloridis* in tissue sections, because it is unpredictable, time consuming, and expensive. As an alternative to their modified Giemsa technique we can also recommend a simple half-Gram method that we have been using for the past six months, and which shows well the characteristic morphology of the organisms (figure).

Paraffin embedded sections are dewaxed, taken to water, and stained for 30 seconds in a 1/20 aqueous dilution of Hucker's stain (one part 10% alcoholic crystal violet plus four parts 1% ammonium oxalate). After a rinse in water they are treated with Lugol's iodine for 60 seconds, washed in tap water, then blotted and allowed to dry thoroughly before clearing in xylene and mounting in DPX.

Most of our patients investigated for the presence of *C pyloridis* have paired biopsy specimens taken, one for histology and one for culture. The biopsy specimen for culture is ground, and the suspension is plated on to 5% blood agar and also on to fastidious anaerobe agar (Lab M), containing nalidixic acid 10 mg/l, vancomycin 2.5 mg/l, and 5% horse blood. The plates are incubated for

seven days at 37°C in an atmosphere of nitrogen containing 5% oxygen and 6% carbon dioxide. Isolates are identified by colonial and morphological appearance, and by a rapid urease reaction.

The results of the histological half-Gram method correlated with those of culture in 91% of cases, which is similar to the experience of Marshall *et al*<sup>2</sup> using the Warthin-Starry stain. Of 102 paired biopsy specimens received, 45 were negative and 48 positive by both methods. Seven positive by the half-Gram method were culture negative, and in two cases culture was positive but no bacteria could be seen in half-Gram stained sections of the paired biopsy specimens.

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## Isolation of *Campylobacter*: what are we missing?

Numerous selective media have been described for the isolation of campylobacters, almost all containing several antibiotics as inhibitory agents. A method

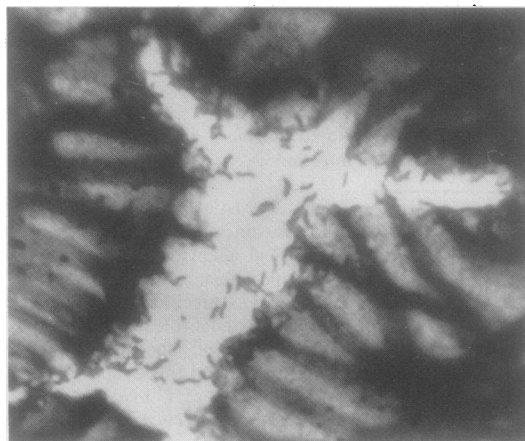


Figure  
Gastric pit. (Half-Gram).

that uses a non-selective medium in conjunction with a membrane technique has been advocated by Steele and McDermott<sup>1</sup> as it facilitates the isolation of campylobacters sensitive to antibiotics incorporated into several of the selective media. In an attempt to determine the extent of underrecording of *Campylobacter* isolations we examined diarrhoeal faeces from 1170 subjects and from two dogs using a selective agar (modified CCD agar),<sup>2</sup> which had been incubated microaerobically at 42°C for 48 hours, and the cellulose acetate membrane (0.45 µm pore diameter)—Columbia blood agar technique<sup>3</sup>; these culture plates were incubated microaerobically at 37°C for up to seven days.

The table shows results. A total of 176 *Campylobacter* strains were isolated; 152 on the modified CCD agar, and 134 by the membrane technique. Both culture methods failed to isolate some strains of *C jejuni* and *C coli* detected by the alternative method. The 16 strains (14 of *C jejuni*, two of *C coli*), which were isolated by the membrane technique alone all grew when subcultured on to the modified CCD agar, confirming that these strains were not atypical and that the isolation results were probably due to the effect of sampling. In contrast, the membrane technique recovered a further eight strains of three *Campylobacter* spp, which were not isolated on the modified CCD agar and which failed to grow when subcultured on to this medium. Our findings are similar to those of Steele *et al*<sup>3</sup> and Megraud and

Bonnet,<sup>4</sup> who isolated catalase negative campylobacters (CNW) strains<sup>5</sup> and another group of similar organisms using the membrane technique but not on selective media. In addition, Steele *et al*<sup>3</sup> detected strains of *C jejuni* like organisms, which are nitrate negative, grow at 37°C, but grow either poorly or not at all at 42°C. We did not isolate any of these strains using the two culture methods.

In the present study and in the two previous reports<sup>3,4</sup> diarrhoea was the presenting symptom in patients from whom these unusual campylobacters were isolated. The results of the present survey, however, differed from the previous studies in the age groups of patients infected. The Australian<sup>3</sup> and French<sup>4</sup> workers isolated CNW stains mainly from children less than 9 years old, but in our study only one isolate was from a child (1 year old); the remaining human isolates were from adults aged 18 to 85 years. A CNW strain was also isolated from a dog with diarrhoea, but this was not unexpected because these strains were first recovered from dogs in a Swedish study.<sup>5</sup>

*C cinaedi* has been associated with enteric infections in homosexuals,<sup>6</sup> but in the present survey we isolated this organism from an adult dog with diarrhoea, and this may be the first report of *C cinaedi* from a non-human source. It is perhaps of epidemiological importance that a CNW strain and a *C cinaedi* strain were cultured from the faeces of dogs with diarrhoea. The two *C laridis* strains isolated only by the membrane

technique at 37°C were unusual because these organisms usually grow well on modified CCD agar at 42°C, and, in fact, when subcultured to this medium at 42°C, grew very well. Some *C laridis* strains may prefer a temperature below 42°C for primary isolation, and this could be a reason for under reporting of these strains.

Our findings suggest that conventional culture techniques for the isolation of campylobacters may require some revision, and our studies to establish the most appropriate techniques are continuing.

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#### Isolation of campylobacters using modified CCD agar and membrane technique

<i>Campylobacter</i> spp	Modified CCD agar at 42°C	Membrane technique at 37°C	Total (%)
<i>C jejuni</i>	146 (38)*	122 (14)*	160 (91)
<i>C coli</i>	5 (3)	4 (2)	7 (4)
<i>C laridis</i>	0	2 (2)	2 (1)
CNW†	1	6 (5)	6 (3.5)
<i>C cinaedi</i>	0	1 (1)	1 (0.5)

\*No of strains isolated by each method alone; †catalase negative campylobacters.

## Book reviews

**Key Facts in Pathology.** P Chandrasoma, CR Taylor. (Pp 445; soft cover £23.) ISBN 0-443-08270-7.

According to the preface the aim of this book is to supply the busy medical student with basic factual information, trimmed of the minutiae found in large pathology books. The entire text consists of lists of numbered statements in note form, subdivided under headings, the format being

similar to that commonly used in lecture handouts.

The style of this book will make it daunting to a student who uses it, as the authors intend, for primary learning rather than revision. Many of the lists are rather contrived and consist of related statements that would read better as free text. Contrary to their aim the authors succumb to the temptation to say something about everything, including details of lipid storage disorders, progeria, pinealomas, and even the Arminni-Ebstein lesion, which are hardly

"key facts". Many of the pictures are too dark or lack clarity and some seem to be incongruous in such an undergraduate text. For example, the only histological photograph in the lymphoid system illustrates Burkitt's lymphoma.

This type of book is probably more suitable for revision than as a main text. Even students who like the format, however, will find the price of £23 substantially higher than that of similar books which are available. It is difficult to recommend it.

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# Isolation of Campylobacter: what are we missing?

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