row showed erythrobastopenia (less than 1% of erythroblasts). The presence of serum anti-HPV IgM (radioimmunoassay) suggested a recent HPV infection. Packed red cells (600 ml) were transfused. Over the next few days the symptoms disappeared and haemoglobin concentrations remained stable. Ten days after admission reticulocyte count was 150 × 10^9/l. Eighteen months later, the patient was quite well and all haematological investigations yielded normal results blood count, haemoglobin electrophoresis, erythrocytic enzymes (glucose-6-phosphate-deshydrogenase, glucose-phosphogluconate-deshydrogenase, hexokinase, glucose-isomerase-phosphate, glucose-pyruvate, glutathione reductase, acetyl-cholinesterase, pyrimidine-5'-nucleotidase), osmotic resistance and autohaemolysis.

Our observation of HPV infection associated with aplastic crisis but without haemolysis differs from the transient erythroblastopenia seen in childhood, which often affects younger children (1 to 4 years) and occurs without HPV infection.[3]

Aplastic crisis associated with HPV infection has hitherto only been described in hereditary[2,4-6] or acquired[7] haemolytic anaemias. It seems that the erythroblastopenic effect of HPV is constant but goes unnoticed if the red cell life span is normal. A shortened red cell survival (haemolysis) is necessary to cause acute anaemia. Acute anaemia occurring without haemolysis due to an HPV infection is difficult to explain. In our patient only isotopic labelling of his erythrocytes could have completely excluded underlying haemolysis. Nonetheless, we wanted to record the experience to encourage doctors to ask for parvovirus serology in similar clinical circumstances.

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Long term freeze storage of *Campylobacter pyloridis*

The letter by Westblom *et al*[1] prompted us to review our technique for storing cultures of *Campylobacter pyloridis*.

For the past year we have been isolating *C pyloridis* from gastric biopsy specimens by inoculating the tissue on to chocolated blood agar containing 3 μg/ml amphotericin B and 10 μg/ml vancomycin. Plates were incubated in an anaerobic jar containing 90% nitrogen and 10% carbon dioxide for seven days at 37°C. The identity of the organisms was confirmed by colonial and Gram morphology and their ability to split urea very rapidly. Initially such organisms were harvested in to tryptone soy broth containing 15% glycerol and stored in a deep freeze at −70°C. Following Westblom *et al's* letter we retrieved some of these cultures, thawed them, and inoculated them on to chocolated agar plates as described above. Three cultures frozen seven and a half, seven and a half, and five and a half months previously yielded profuse growths and one frozen 10½ months previously still contained viable organisms although in small numbers. More recently cultures have been stored on "beads in cryopreservative fluid", supplied with the Protect Bacterial Preserver system (Technical Service Consultants Ltd PO Box 31, Bury BL9 5RA). A profuse growth was obtained from one of these which had been frozen three months previously.

Large numbers of strains will need to be stored for longer periods to confirm our observations, but in contrast to the experience of Westblom *et al*, we have not found freeze storage of *C pyloridis* in conventional media to be a problem.

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Comparative sensitivities to antimicrobial agents of *Campylobacter pylori* and the gastric campylobacter like organism from the ferret

Increasing evidence supports the association of *Campylobacter pylori* with antral gastritis and peptic disease, notably duodenal ulceration, in man.[1] Results of antimicrobial sensitivity tests on 110 isolates of *C pylori* from Australia, the United Kingdom, and France have shown good agreement,[2-4] and limited clinical trials have shown that treatment with certain antibacterial drugs clears *C pylori* from the gastric mucosa.

The isolation of campylobacter like organisms from the gastric mucosa of ferrets was first reported from Boston, USA[5,6]; this organism, with morphological similarities to *C pylori*, was isolated from about half of the animals examined. Histological studies suggested a possible association between the presence of the campylobacter and gastric inflammation.

In contrast, Rathbone *et al*[7] isolated a campylobacter like organism from the gastric tissue of all of the 17 ferrets that they examined, but the organism was associated with neither histological inflammation nor ulceration.

We have compared the sensitivities to antimicrobial and antiulcer drugs of gastric campylobacter like organisms (GCLO) isolated from 14 ferrets with those of 11 isolates of *C pylori*. Comparative studies, including enzyme, protein, and isoprenoid quinone composition, will be reported later.

Samples of gastric mucosa from the antrum, body, and fundus of 14 mature male ferrets obtained from one supplier were taken when the animals were killed after emisis protection experiments. On macroscopical examination one of the 14 ferrets

Table *Minimum inhibitory concentrations (MIC) (mg/l) of antimicrobial agents for C pylori and ferret gastric campylobacter like organisms (GCLO)*

Agent	MIC range		MIC_{90}		Control strains	
	*C pylori**	*Ferret GCLO†*	*C pylori*	*Ferret GCLO*	*Staphylococcus aureus NCTC 6571*	*C jejuni NCTC 11349*
Penicillin G	0·02 –0·1	0·8 –1·6	0·05	1·6	0·05	>6·4
Amoxycillin	0·01 –0·05	0·8	0·02	0·8	0·05	>6·4
Clavulanic acid	0·4 –3·2	6·4 –25·6	1·6	12·5	25·0	3·2
Cloxacillin	0·2 –3·2	12·8 –25·6	1·6	25·6	0·2	>6·4
Temocillin	3·2 –6·4	12·8 –51·2	6·4	30·0	>102·4	>6·4
Erythromycin	0·02 –0·05	0·02 –0·1	0·05	0·1	0·2	0·025
Furazolidone	0·0015–0·006	0·003–0·048	0·006	0·048	>0·048	0·012
Metronidazole	0·4 –0·8	1·6 –6·4	0·4	6·4	>12·8	0·4
Sulphathiazole	128 –256	128 –256	256	256	<16·0	256
Trimethoprim	512	128 –512	512	256	<16·0	>512
Tripotassium dictrato bismuthate‡	5 –10	2·5 –5	10	2·5	>10·0	5

*n = 11 (NCTC 11637, type strain, (Australian isolate) + 10 UK isolates).
†n = 14 (isolates from ferrets of one strain).
‡De-Nol tabs.

had small antral erosions, histological examination of which showed a small area of surface epithelial loss with vascular congestion and some re-epithelialisation at the lesion margin. Gastric campylobacter like organisms were especially abundant adjacent to the lesion.

All 14 ferrets showed evidence of low grade gastritis deep in the antral mucosa, which in some cases extended into the submucosa. Gastric campylobacter like organisms were seen in the gastric pits of all animals, but did not extend to the deep mucosa, where most of the pathological changes were seen.

Gastric campylobacter like organisms (GCLO), phenotypically resembling those previously reported,[6] were readily isolated from the gastric tissues (notably the antrums) of all ferrets on blood agar containing Skirrow's supplement. All the isolates of ferret GCLO grew microaerophilically with additional CO_2 at 37°C; none grew aerobically or anaerobically.

C pylori and the ferret GCLO were similarly non-fermentative, oxidase, and catalase positive, and rapidly hydrolysed urea. Three disc tests simply differentiated the strains, however, the ferret GCLO being resistant to cephalothin (30 μg) and the vibriostatic agent, 0129 (150 μg), but sensitive to nalidixic acid (30 μg). In agreement with a previous observation (McNulty CAM, Dent JE, abstract presented at XIV International Congress of Microbiology, 1986), leucine aminopeptidase was detected in all 11 isolates of *C pylori*. In contrast, none of the GCLO from ferrets produced this enzyme.

Minimum inhibitory concentrations were determined by incorporating the compounds in blood agar base no 2 (Oxoid) containing 5% defibrinated horse blood and inoculating with 10^4 colony forming units of a cell suspension harvested from the surface of three day blood agar cultures (Multipoint Inoculator, Denley Tec Limited, UK). All plates were incubated for three days at 37°C in an atmosphere of 5% O_2 and 10% CO_2.

The results show that the ferret GCLO was considerably more resistant than *C pylori* to certain compounds, notably the β lactam antibiotics (table); the activity of clavulanic acid against *C pylori* confirms the observations of Lambert *et al.*[4] Neither *C pylori* nor the ferret GCLO produced detectable β lactamase (Nitrocefin test), and combinations of clavulanic acid and amoxycillin were not synergistic.

Conversely, no appreciable differences between *C pylori* and the ferret GCLO were found in sensitivity to erythromycin or the bismuth salt or in their relative resistance to sulphathiazole or trimethoprim. *C pylori* and the ferret GCLO were similarly insensitive to agents which reduce gastric acid production (minimum inhibitory concentration (50%) for cimetidine and ranitidine >2000 mg/l; omeprazole 500–1000 mg/l.

On the basis of similarities between the man and the ferret in gastric anatomy and physiology and the isolation of a similar gastric campylobacter from both, Cave *et al*[5] suggested the ferret as a useful model of gastric campylobacter infection. Our findings on the relative resistance of the ferret campylobacter to certain antibacterial agents would appear to limit the value of this animal model for experimental chemotherapy. Moreover, the question of whether gastric mucosal infection with (or colonisation by) GCLO from the ferret has the potential to progress to peptic ulceration, with similar pathology to *C pylori* infection in man, remains to be determined.

We thank Miss A Hassan, Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex, for providing the clinical isolates of *C pylori*.

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Diagnosis of peritonitis

Diagnosis of peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD) is important in terms of patient management and effective chemotherapy. Various methods have been introduced to improve isolation rates such as filtration, pour plates, and broth enrichment culture.[1–4] Peritoneal dialysis effluents (PDE) may contain very small numbers of organisms.[1] It has been suggested that bacteria may reside inside phagocytes in some cases of apparently culture negative peritonitis.[5] To investigate this possibility further we compared the results of PDE cultures before and after cell lysis with saponin.

Diagnostic samples of PDE were obtained from 33 consecutive CAPD patients presenting with peritonitis—that is, a cloudy PDE and one or more of the following: (i) symptoms of peritonitis; (ii) PDE white cell count (>50%) neutrophils); (iii) positive dialysate culture. After centrifugation a Gram stain was performed on the spun deposit which was then divided into two aliquots. The first aliquot was inoculated by placing one drop of the deposit on to aerobic and anaerobic blood agar plates, a McConkey agar plate, and a diagnostic sensitivity test plate for sensitivity testing. To the second aliquot 4 drops of 10% saponin were added and the mixture allowed to stand at room temperature for five minutes. Culture plates were inoculated in an identical manner to that of the first aliquot. Plates were examined for growth after 18 hours of incubation, then reincubated for a further 24 hours. All organisms were identified by standard methods.

The table compares the isolation rates by the two methods. Saponin lysis of the cells in the deposit resulted in the isolation of organisms from three specimens in which the conventional culture was negative. Thus saponin lysis added 9% to the specimen positivity rate (64% to 73%). Secondly, additional organisms were detected in two specimens using saponin, one of these was an anaerobe and of particular importance.

Table *Comparison of cultural results on 33 consecutive diagnostic peritoneal dialysis effluents*

		Results of culture techniques	
Category	*No (%)*	*Conventional*	*With saponin*
Growth on only one medium (MAC) by conventional methods; growth on both media after saponin	6 (18·2)	*S epidermidis (3)* *Bacillus* sp *Enterobacter cloacae* *S aureus*	*S epidermidis (3)* *Bacillus* sp *Enterobacter cloacae* *S aureus*
Growth enhanced by saponin treatment	13 (39·4)	*S epidermidis (7)* *S aureus (2)* *S saprophyticus* *S faecalis* *S bovis* *C freundii*	*S epidermidis (7)* *S aureus (2)* *S saprophyticus* *S faecalis* *S bovis* *C freundii*
Growth after saponin; treatment no growth by conventional methods	3 (9·1)		*S saprophyticus* *S aureus* Diphtheroids
Additional organisms after saponin treatment	2 (6·1)	*Klebsiella aerogenes* *Ps maltophilia*	*Klebsiella aerogenes* plus *B fragilis* *Ps maltophilia* plus *Enterobacter cloacae*

Of the remaining nine specimens, eight (24·2%) were sterile by both methods of culture and 1 (3%) grew diphtheroids in identical numbers by both techniques.
Key: *S epidermidis = Staphylococcus epidermidis; S saprophyticus = Staphylococcus saprophyticus; Ps maltophilia = Pseudomonas maltophilia; B fragilis = Bacteroides fragilis; C freundii = Citrobacter freundii.*

We found that when identical organisms were cultured by both methods, the number of organisms was always higher in the material treated with saponin. In many cases these cultures showed confluent growth on the droplet area whereas conventional culture yielded three to four colonies. The increased numbers of organisms permitted more rapid and reliable direct sensitivity testing.

Our results strongly suggest that significant numbers of bacteria reside intracellularly in specimens of PDE and that they can be released for culture by lysis of the cells. Although our culture positive rate is lower than some centres, particularly those using broth enrichment methods, use of saponin allows a culture and sensitivity result to be obtained within 18 hours of receiving the specimen, considerably faster than when using broth enrichment. The use of larger volumes of PDE fluid may further improve the positivity rate. The use of lytic agents may have applications for the culture of other purulent body fluids.

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