with other colleagues, found that the most reliable, when checked for both false positive and false negative results by using simultaneous electronmicroscopical examination, was the phenol auramine technique; so far, every result has been confirmed by electron microscopy.

We report here that the phenol auramine method still works properly when faeces that have been previously fixed in 3% cacodylate buffered glutaraldehyde (pH 7.4) are examined. It may also be used to replace formalin in formol-ether concentrations, and the auramine staining is still excellent for cryptosporidium, even after secondary concentration for specimens with very low cyst content (Figs. 1 and 2). In general, we found that morphology was much better preserved using glutaraldehyde instead of formalin, both at light and electron microscopy levels for all these different techniques.

Safe and adequate fixation is achieved by using a 10:1 fixative: faecal mass mixture which is shaken and left for one hour before slow centrifugation to reform a mass for light or electron microscopy preparations.

Thus faeces from patients suspected of having AIDS or those likely to contain dangerous pathogens can be glutaraldehyde fixed and then stained for cryptosporidium by the phenol auramine method without loss of sensitivity, so avoiding the hazard of potential laboratory infection.

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Bacteraemia secondary to pseudomembranous colitis

In their recent paper Rampling et al described bacteraemia in nine neutropenic patients, out of a total of 17, who had Clostridium difficile infection. In the authors' experience none of the non-neutropenic patients developed this complication. We report a case of secondary bacteraemia that occurred after pseudomembranous colitis in a non-neutropenic patient.

An 80 year old woman was admitted to this hospital after a fall in which she fractured her left femur. She received Magnapen (fluoxacillin and ampicillin) 500 mg intramuscularly preoperatively, which was continued orally in the same dose four times a day for one week postoperatively. On admission her white cell count was 8.9 x 10^9/l. Ten days postoperatively she became feverish and confused. A midstream specimen of urine taken 48 hours previously had grown 10^6 organisms/ml of Klebsiella pneumoniae which were resistant to ampicillin. There were no pus cells. She was started on a course of cephalaxin 250 mg four times a day. After 72 hours of this treatment she developed abdominal pain and diarrhoea. The white cell count rose to 19.2 x 10^9/l with 90% polymorphs. Over the next four days her haemoglobin concentration fell from 13 g to 10 g and she became hypoalbuminaemic (23 g/l) and hypokalaemic (2.3 mmol/ml). Urea concentration rose to 13 mmol/l (78 mg/100 ml), but bilirubin concentrations remained within the normal range. Her condition deteriorated. She passed seven loose, greenish motions a day, took little orally, and was more confused and agitated. On examination her abdo-

References


Fig. 2 Same preparation further processed and sectioned for electron microscopy examination. Cysts are arrowed. X 3250.
men was distended, and tenderness and guarding were noted in both iliac fossae. Sigmoidoscopy was performed, and red, haemorrhagic rectal mucosa was noted. Visibility was hampered because of the diarrhoea. Subsequent histology was compatible with an "infective aetiology." *Clostridium difficile* toxin was detected by tissue culture of stools on the eighth day of her diarrhoeal illness. She was treated with oral metronidazole 500 mg three times a day and oral vancomycin 125 mg four times a day for one week. She made an impressive recovery; her confusion and disorientation improved within 48 hours, the diarrhoea was greatly reduced in quantity and frequency, and her abdominal distension and tenderness resolved.

Four days after starting the course of vancomycin and metronidazole she became febrile once more. She was confused again and for the first time during her admission was peripherally "shut down" and hypotensive (90/60 mm Hg). The white cell count was still raised at 18 × 10⁹/l. Her clinical condition was thought to be compatible with a Gram negative sepsicaemia. Blood cultures were taken, and she was started empirically on netilmicin 150 mg twice daily. Subsequent doses and frequency were determined by careful monitoring of her serum concentrations. She was alert and normotensive within 24 hours, and *Enterobacter cloacae* was isolated from her blood. This organism was resistant to ampicillin and cephalexin but sensitive to netilmicin, cefotaxime, and cefazidine. Her white cell count subsequently fell to 8.8 × 10⁹/l. After a prolonged convalescence she made a complete recovery, although *Clostridium difficile* toxin was detected in her stools for a further six weeks. Throughout this period she was nursed in a single room with enteric precautions that hampered her rehabilitation both psychologically and physically.

Two recent reports also highlighted sepsicaemia as a possible complication of pseudomembranous colitis, although the white cell count is not mentioned in either patient. Both these patients also had sepsicaemias of faecal organisms, like our patient and those reported on by Rampling et al. Interestingly, both patients reported on by Franson et al. and Spencer et al. our patient, and six of nine patients reported on by Rampling et al. had received a cephalosporin. Whether this represents a true association between cephalosporins and bacteraemia or merely reflects prescribing habits requires further study.

The action of *Clostridium difficile* enterotoxins A and B on colonic mucosa can presumably facilitate bacteraemia of gut origin. Although it seems that this complication is more likely in neutropenic patients, we think that this potential complication should also be considered in non-neutropenic patients with pseudomembranous colitis. This is especially true if their condition deteriorates or they become febrile once an initial response to oral vancomycin or metronidazole has been documented.

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References


Anaerobic media and pH changes during growth

In the last few years attention has been paid to the growth characteristics of anaerobes in different media, with special attention to the results when susceptibility to antibiotics is tested. Several problems, however, have arisen. Watt and Brown described a drop in surface pH of several media related to bacterial growth. The change in pH could not be controlled by the use of buffers, but new media with a stable pH had greatly inferior growth properties. This fall in pH might influence the outcome of the sensitivity testing of anaerobes to antibiotics such as erythromycin. Eley et al added to this problem by observing morphological changes in *Bacteroides*, including filamentation and formation of spheroplasts, after growth in Wilkins-Chalgren, thioglycollate, and Schaedler broths but not in brain-heart infusion broth. We also observed a fall in pH in liquid anaerobic media during growth of *B fragilis* and *B vulgatus*, which was accompanied by a sharp loss in viability. After 24 hours of incubation the percentages of viable cells in BM broth and thioglycollate broth were as low as 1-0 and 1-3 respectively; brain-heart infusion broth and Wilkins-Chalgren broth gave much better results (about 80% viability). The addition of a phosphate buffer to the BM medium and the omission of glucose resulted in a pH of 5.9 and 5.2, respectively, after 24 hours of incubation, whereas in combination the pH did not drop below 6.2. The viability in this last medium exceeded 50%.

We do not have any evidence of a change in the growth supporting properties of BM medium after these changes; and the morphological changes described by Eley et al were not observed. We believe that careful consideration of the concentration of fermentable sugars in relation to the buffering capacity of the media might reduce the fall in pH during growth. We agree with Watt and Brown that changes in pH in media and loss of viability are highly undesirable. More research on the growth characteristics of media designed for anaerobic bacteria, especially when testing susceptibility to antibiotics, is clearly required.

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References

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