Technical methods

glass slide and examined under a dissecting microscope to check orientation. The tissue was kept moist with the minimal amount of physiological saline if required. A suitable block was selected for histochemical study and rolled in talcum powder until evenly coated. Meanwhile, a small piece of aluminium foil was folded to provide a groove on to which was applied a small amount of Tissue-Tek II OCT embedding compound (Lab-Tek). The coated muscle tissue was then placed into the OCT compound at one end, care being taken to ensure that the muscle fibres were running in a parallel direction with the groove. The aluminium foil was held in a pair of forceps and immediately plunged into a Dewar of liquid nitrogen and vortex mixed.

When large bubbles stopped forming the aluminium foil, together with the frozen tissue, was transferred into the cryostat at −20°C. The frozen strip of OCT compound with the muscle block embedded in it was easily freed from the aluminium foil. Excessive OCT compound was then trimmed away using a precooled razor blade at the end opposite to the tissue to provide a flat base for embedding. A tissue holder was then placed on to the freezing stage of the cryostat and a small blob of OCT compound added. When it began to solidify the block of frozen muscle was carefully picked up with a pair of precooled fine forceps and immediately embedded flat end first into the OCT compound. Attachment was facilitated by a cryospray.

Results and discussion

This method allows well orientated transverse sections to be easily obtained, using the antroll device of the cryostat, although slight adjustment of the tissue holder may sometimes be required. Rapid freezing of the muscle tissue is essential to avoid ice crystal artefacts, and this can be achieved by using talcum powder.1 2 Ice crystals can still occur, however, if the frozen tissue is allowed to thaw again during the stage of embedding. With the technique described here only the previously frozen strip of OCT compound is fused with the newly applied OCT embedding medium, leaving the tissue practically untouched. Therefore, sections cut from this final preparation are essentially free of any ice crystal artefacts. Finally, although this technique is primarily designed for percutaneous needle biopsy specimens, it applies equally well to samples obtained at open surgical biopsy.

References


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Fig. 1 Concentrated faecal preparation after Glutaraldehyde fixation of cryptosporidium cysts, showing fluorescence after phenol auramine staining. × 3000.

Letters to the Editor

Safe method for identifying cryptosporidium cysts in the faeces of patients with suspected AIDS, or those infected with other serious concomitant pathogens

The first case of human cryptosporidiosis was reported in 1976.1 Since then this parasite has been associated with outbreaks of diarrhoea in children and occasionally adults and, recently, as a serious complication in some patients with the acquired immune deficiency syndrome (AIDS).2 Although faeces sent for examination from patients in these first two groups pose no special safety problems, specimens from patients with suspected AIDS need to be handled with special precautions.

Recent work suggested that the Ziehl-Neelsen (hot or cold) and phenol auramine fluorescence stains2a b are among the best methods for identifying cryptosporidium cysts in faecal smears.3 We tried these and other methods over a wide range of medical and veterinary specimens and,
with other colleagues, found that the most reliable, when checked for both false positive and false negative results by using simultaneous electromicroscopical 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 (fluoxacinil 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 10⁹/l. Ten days postoperatively she became feverish and confused. A midstream specimen of urine taken 48 hours previously had grown 10⁶ 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 10⁹/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/L). 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 abdomi-