

Prognostic importance of nucleolar organiser regions in embryonal rhabdomyosarcoma

The use of the silver colloid technique^{1,2} is of increasing interest to many pathologists and has been used in the investigation of a variety of human malignant tissues. The method seems to be useful in the discrimination between high and low grade non-Hodgkin's lymphomas,² in the identification of the three commonest round cell tumours of childhood,¹ in the differentiation between low grade fibrosarcoma and fibrous proliferation,³ in the differentiation between basal cell carcinomas and other basaloid skin tumours,⁴ in the diagnosis of melanocarcinoma,⁵ and in certain aspects of breast disease.⁶ In our experience the technique is of no use in a variety of endocrine neoplasms, in the grading of colonic and gastric dysplasia, and in certain fine needle aspiration specimens.

We applied the technique to 20 specimens of embryonal rhabdomyosarcoma which had been previously characterised by immunohistochemistry, light, and electron microscopy. The clinical and three year follow up details of these patients were known and the numbers of nucleolar organiser regions (AgNORs) produced were examined with respect to age, stage, recurrence and survival.

All tumours were either stage I or II and were from various sites including genitourinary and head and neck. The ages ranged from 4 months to 8 years (six patients were female and fourteen were male). There was no significant correlation between AgNORs and any factor examined. The combination of short follow up and limited disease may have obscured the clinical importance but it seems that, in common with Ewings' sarcoma,⁷ the method is of no use in the prediction of short term outcome. A long term follow up study is underway.

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Microsporidiosis in a British patient with AIDS

A jejunal biopsy specimen from a 34 year old human immunodeficiency virus (HIV) positive homosexual man with diarrhoea and malabsorption showed microsporidian spores within enterocytes. We believe that this is the first such case to be reported in the British literature.

Prior to the advent of acquired immune deficiency syndrome (AIDS) microsporans had rarely been found in man,^{1,2} although the organisms are widespread in animals.³ A small number of cases of infection by the parasite have been published from the United States of America^{4,5} and France,^{6,7} and a review of the species infecting mammals⁸ documents reports in primates, including man.

Our patient first presented in 1981 with generalised lymphadenopathy. In 1985 he developed hepatitis B and because of lymphopenia was tested for HIV antibody which proved positive. HIV antibody testing of serum stored from 1981 was also positive. By December 1985 he had a considerable degree of immunosuppression with a total T4 num-

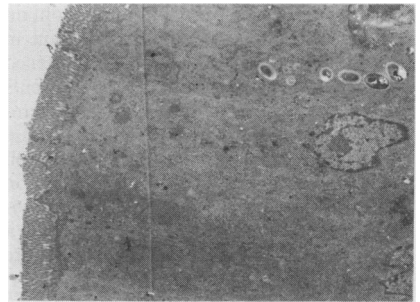


Fig 2 Low power of microvillous epithelium showing several microsporean spores within an enterocyte.

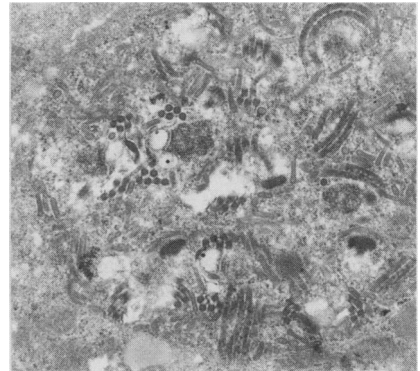


Fig 3 Sporoblast within an enterocyte showing many polar filaments and several nuclei.

ber of 337 and a reversed T4 (24%): T8 (56%) ratio with poor mitogen response. In 1986 he had *Pneumocystis carinii* pneumonia and recurrent varicella-zoster infection. In 1987 he developed watery diarrhoea and malabsorption and a jejunal biopsy was performed. No ova, cysts, or parasites were seen in the faeces. *Candida* and *Torulopsis glabrata* were isolated from the jejunal fluid.

The jejunal biopsy specimen was processed conventionally for light and transmission electron microscopy. Histologically there was preservation of villous architecture and no clinically important abnormalities were detected. Gram, Giemsa, Ziehl Neelson and Grocott stains failed to show identifiable organisms. Electron microscopic examination, however, showed microsporean spores with characteristic internal coiled filaments within some surface enterocytes (fig 1).

Up to six spores measuring 1.5 to 1.7 µm in length and 0.9 to 1 µm in diameter were found in thin sections of individual cells (fig 2). The walled spores contained a single nucleus, five complete turns of the polar filament, and a multilamellar polaroplast.



Fig 1 Microsporean spore showing characteristic cross sections of internal coiled polar filament.

Other enterocytes were found to contain spherical sporoblasts, about 4 to 5 µm in size, containing a number of nuclei, flattened vesicles, and a large number of filaments 65 nm in diameter, almost randomly arranged within the cytoplasm (fig 3). These sporoblasts sometimes indented the host cell nucleus. Sporoblasts with more electron dense cytoplasm (2.5 × 3.5 µm) also contained several polaroplast bodies, suggesting a stage just prior to division and final spore formation.

Three genera of microsporans have been reported in man—*Nosema*,⁹ *Encephalitozoon*,¹⁰ and *Enterocytozoon*.⁶ The microsporans described here does not have diplo-karyotic nuclei like *Nosema*, nor does it develop within a parasitophorous vacuole like *Encephalitozoon*, nor are the spores of a similar size to those of the species *Enterocytozoon bienewisi* (1.5 × 0.5 µm). The specific classification of this microsporan will need further investigation, but it is more closely related to *E bienewisi*⁶ than to the other two genera.

Electron microscopy was essential for the diagnosis of this parasite, and it is suggested that all intestinal biopsy specimens from patients with AIDS should be investigated by this technique so that we may learn more about this hitherto little known parasite.

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Use of photocopier for recording pathological specimens

For those who are working in a surgical pathology laboratory, there is often a demand for photographic recording of gross pathological specimens. Polaroid photography gives good results but is expensive. Conventional photography is less expensive and gives prints of the best quality but some delay in getting the prints is inevitable. There are times when the requirement for the quality of reproduction is not critical—for example, when several blocks are taken from excised skin lesions or slices of large tumours and solid organs such as the liver, lungs, spleen, kidneys and pancreas, it is helpful to have a reasonably accurate pictorial representation of the gross specimen to mark the sites from which those blocks are sampled. A similar situation occurs when a stomach or a segment of the large bowel shows several mucosal lesions which are individually sampled. A rapid, cheap, and reliable way of producing a photographic print of acceptable quality is by the use of a photocopying machine. The slice of organ or tumour or the opened viscera, sandwiched between two plastic sheets, can be laid on the machine. Copying is then performed in the usual way. It is also convenient to use a photocopier to record the dermatoglyphics of abnormal fetuses. Apart from its low cost, an additional advantage is that the prints are on ordinary papers which can be easily filed with the other records. With the photocopier, the labour of putting in a scale when taking photographs of a specimen becomes unnecessary unless the machine is set to perform size enlargement or reduction.

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Role of immunocytochemistry in diagnostic pathology: information from necrotic tissue

The paper by Mason and Gatter¹ is a valuable summary of many aspects of the diagnostic applications of immunocytochemistry. I would just like to expand on its potential for gathering useful information from sub-optimal biopsy specimens. Their paper illustrates the preservation of immunoreactivity in crushed and distorted specimens. Another problem with tiny biopsy specimens is when the whole sample is necrotic. Reticulin staining will often show tissue architecture in these circumstances and permit a useful conclusion to be drawn. There is also often preservation of reactivity with antibodies against cytokeratins (such as CAM 5.2) or the leucocyte common antigen (CD45). Though great care must be exercised in reaching conclusions from necrotic samples, it is sometimes possible to separate lymphoma from carcinoma with more confidence than would be possible without antibody studies. Some bronchial biopsy specimens that have only shown necrotic material have also been shown to be composed of disorderly sheets of epithelial cells, quite consistent with carcinoma. In the appropriate clinical setting it has been possible to proceed without recourse to a repeat biopsy. Not every necrotic sample will react, but it is worth trying.

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Protein-bound vitamin B₁₂ absorption test

Dr Chanarin makes the unreferenced statement that the protein-bound B₁₂ absorption test has been interpreted as detecting a lack of intrinsic factor at a stage when the standard B₁₂ absorption test is normal.¹ Although this possibility was considered,² subsequent investigations have shown that the addition of intrinsic factor does not