

Letters to the Editor

Value of factor VIII related antigen as a means of demonstrating extramedullary haemopoiesis

We have noted the comments of Dr AL Bloom and Dr JC Giddings¹ concerning our use of the localisation of factor VIII related antigen (F VIII RAg) in platelets and megakaryocytes.² These are, indeed, valid points and it is accepted that contaminants such as fibronectin may be responsible for weak activity of their corresponding antibodies in commercial antisera. It should be appreciated, however, that the staining obtained with such preparations is not only intense but appears to be found specifically in megakaryocytes and vascular endothelium.³ Moreover, histiocytes, which contain, for example, fibronectin, are not, in our hands, stained for F VIII RAg in tissue sections. Of additional value is the negative staining of Reed-Sternberg cells.^{2,3} Thus, regardless of impurities or minor contaminants, the value of staining for megakaryocytes with these commercial antisera is still considerable; the negative staining of other cell types serves as a practical negative control.

Dr Bloom and Dr Giddings also suggest that the use of monoclonal antibodies could overcome the problems suggested. Notwithstanding the current popularity of such preparations, however, the occurrence of shared epitopes often renders such antisera highly non-specific with regard to the staining of certain cell types and large molecules. For example, numerous and diverse unrelated cells or tissues have recently been shown to stain with monoclonal antisera to granulocyte⁴ and mast cell⁵ determinant molecules. Haematologists and histopathologists should, indeed, recognise this problem. Particular care should be taken to ensure that monoclonal antisera to F VIII RAg itself do not react with fragments of the molecule shared by other molecules. If megakaryocytes or endothelial cells are to be stained in tissue sections, commercial preparations of polyclonal antisera to F VIII RAg should, we believe, still be quite adequate for routine work.

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High incidence of group C streptococci isolated from throat swabs

We read with interest the letter by Dr M Millar¹ reporting the high incidence of group C streptococci isolated from throat cultures. We have also noticed an "apparent" increase in group C streptococcal isolates. In the last seven months 65 streptococci from throat cultures have been grouped by agglutination with protein A tagged streptococcal antisera.² Forty three (66%) belonged to group A, 15 (23%) to group C, and seven (11%) to group G. Of these 15 apparent group C streptococci, however, eight had atypical characteristics such as poor haemolysis, especially on aerobic incubation, small colonial appearance, reduced sensitivity to penicillin or the classic *Streptococcus milleri* smell. These eight strains were fully identified by the use of API-20 Strep galleries (API Code 2060) and found to be *Str milleri* (6), *Str mitis* (1), and *Str sanguis* (1). Unlike the findings from Leeds this increase in isolation of apparent group C streptococci has coincided with a change in laboratory procedure—the introduction of Columbia agar (Oxoid CM 331) as our base for horse blood agar plates.

Perhaps an explanation for the high incidence of group C streptococci isolated from throat swabs seen in Leeds is because many strains, as shown here, are not strictly group C streptococci but other "streptococci" which agglutinate with group C antisera.

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Pathological examination of cutaneous malignant melanomas

This laboratory deals with large numbers of cutaneous malignant melanomas and new primary cases are currently running at about 100 a year. In the past it has been our practice to assess the histological parameters required for microstaging by evaluating a single central transverse block through the melanoma. This technique shows maximum tumour thickness in 95% of melanomas.¹

This protocol, while almost certainly adequate for the assessment of thickness, has serious flaws with regard to the assessment of other features. This is particularly true of any regression which may be present in the lesion, since such regression is likely to be associated with areas significantly thinner than found maximally in the lesion. In consequence it is now our practice to block melanomas completely, using numerous transverse sections, generally about 2 mm in thickness. This technique, advocated by Kopf *et al.*,² shows striking variations within individual melanomas. Figs. 1 and 2 show significant differences in thickness and levels seen in two of four blocks, the central one showing maximum thickness, but not deepest extension. One of the two also shows an area of ulceration not seen in the other. The presence of areas of significant regression around the edges of nodular melanomas occurs quite regularly and areas of pronounced regression may be seen in one part of the tumour edge and not elsewhere. This regression, if involving more than three rete ridges from the edge of the nodule, indicates that the melanoma must have been originally a superficial spreading variant in which the development of the central nodule and its subsequent stimulation of the host immune response has caused the loss of the superficial spreading element.

The microstaging of melanomas is a complex process requiring the assessment of many parameters. We currently assess: histological type, thickness, level, pigment production, cellular type, number of

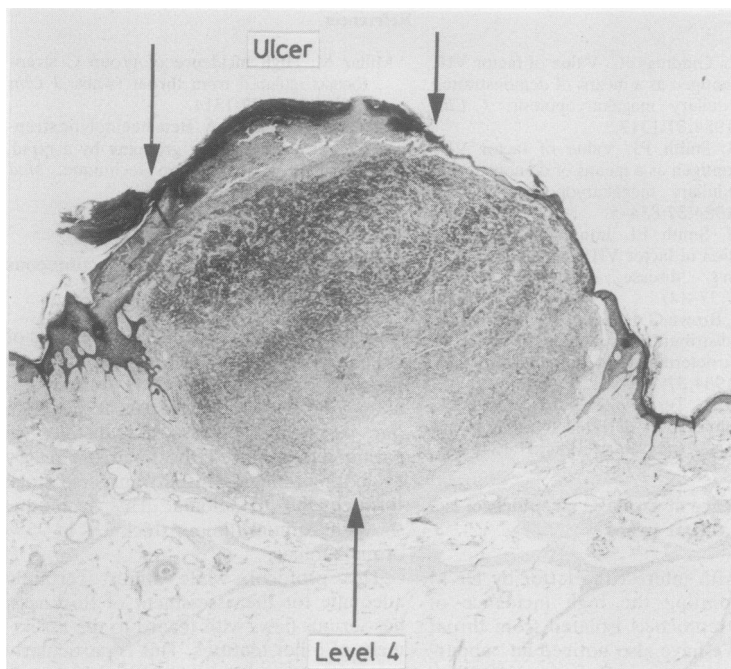
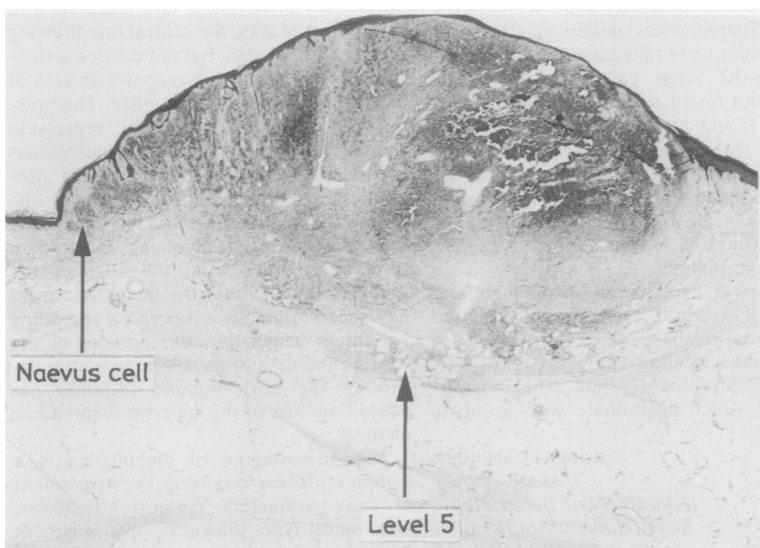


Fig. 1 Central transverse block of a melanoma. Level 4 invasion and an ulcerated area are shown.

Fig. 2 Another of the four transverse blocks showing no ulceration, but level 5 invasion. The thickness revealed is also different from that in Fig. 1.



mitoses/5 high power fields (= 1 sq mm on our microscopes), presence or absence of ulceration, degree of lymphocytic reaction to tumour, degree of regression, presence of dermal blood vessel/lymphatic invasion, evidence of a pre-existing cellular naevus, and adequacy of excision. These features can be so variable within a given melanoma that we believe that only by blocking the entire lesion in serial blocks can proper assessment be made. In those cases where the basic melanomatous nature of the lesion is uncertain at the time of cut up, not all the lesion is blocked; some is kept back for possible electron microscopy etc. When the melanoma is confirmed the reserve is then utilised to complete the paraffin section series. Such techniques do not add greatly to our workload, even with the number of new primary cases which we see.

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Book Reviews

Royal Microscopical Society Microscopy Handbooks. 04 **Histochemical Protein Staining Methods**. J James and J Tas (Pp 40; paperback £3.95.); 06 **Lipid Histochemistry**. Olga Bayliss High (Pp 68; paperback £4.50.); 11 **An Introduction to Immunocytochemistry**. Julia M Polak and Susan van Noorden (Pp 57; paperback £3.95.); Oxford University Press. 1984.

The Royal Microscopical Society has produced three handbooks that are of interest to pathologists. All are small, cheap paperbacks with enough staying power to survive for some time on the laboratory bench. Each gives fundamental information about the tissue components that are to be visualised and explains the rationale of the methods which are succinctly stated.

Protein Staining Methods is the slimmest, and is mainly composed of classical techni-