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⁵ Quesenberry P, Levitt L. Haemopoietic stem cells. *N Engl J Med* 1979;301:755-9.

Methods of early detection of systemic or local vascular disease

There is critical need for useful methods for the early clinical detection of systemic (or local) vascular disease. The rectal biopsy technique described by Tribe *et al*¹ provides convincing objective evidence of vasculitis and parallels my experience^{2,3} in the study of nasal biopsies in systemic or local vascular diseases. The presence of vascular changes in nasal biopsies should alert the pathologist and surgeon and contribute to the early and correct diagnosis of the diseases forming the midfacial granuloma syndrome.^{4,5} Vasculitis provides the pathogenetic basis for linking under the same heading, idiopathic pleomorphic midfacial granuloma (Stewart's type) and giant cell Wegener's granuloma and granulomatosis. It is interesting to note in this context that the material examined by Tribe *et al*¹ has included rheumatoid vasculitis, polyarteritis nodosa and what the authors called "overlap syndromes" indicating the terminological difficulties when describing some of these often rare diseases.

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Double embedding in agar/paraffin wax as an aid to orientation of mucosal biopsies

Orientation of intestinal mucosal biopsies so that histological sections can be cut perpendicularly to the epithelial surface is of paramount importance to their histological examination and reporting.¹ Many biopsies of both small and large intestine yield little or no useful diagnostic information because correct orientation has not been achieved.

Orientation of strips of mucosa in the laboratory is greatly facilitated if they are allowed to adhere, flat, to a piece of frosted glass² or thin card.³ However, although such a procedure can be easily implemented in gastroenterological units, many intestinal biopsies, particularly rectal biopsies, are taken in diverse, non-specialised clinics and often reach the laboratory irregularly curled-up in formal saline and are a significant potential cause of wasted laboratory and clinical time and effort.

To increase the precision of orientation of all biopsies, regardless of their state on reaching the laboratory, we decided to use a method of embedding the tissue in the fixed but unprocessed state using a technique based on embedding in agar before processing into paraffin wax.⁴⁻⁶

The formalin-fixed biopsy fragments are embedded in molten 1% aqueous agar at 45°C, under dissecting microscopic control. The agar solution has several remarkable useful properties; although its melting point is 98°C, gelling does not occur until the temperature falls to 42°C. Solidification is fairly slow and correct orientation of a biopsy, even if curled, is easily achieved by gentle manipulation with a mounted needle during cooling. Moreover, the agar is colourless and transparent in both liquid and gel forms so that the process of orientation can be viewed through a dissecting microscope. Even photography of the dissecting microscopic appearance is possible at this stage. The embedding in agar can be conveniently performed in standard moulds, such as those used for preparing paraffin wax blocks—for example, Tissue Tek.

The block of solidified agar, with its embedded biopsy is trimmed with a scalpel or razor blade so that it presents a flat surface perpendicular to the epithelial surface and it is then processed through alcohols and chloroform to paraffin wax in the usual way. The agar does not shrink appreciably during processing and is im-

pregnated by wax just as if it were tissue. Paraffin sections are cut in the usual way. No technical difficulties have been experienced; the agar cuts just as if it were wax. On staining, the agar takes up a minimal amount of eosin and can just be seen as a very faint background stain but does not interfere with the histological appearances. Quantification and photomicrography are unimpeded.

This technique has been used in our laboratories now for three years. Although it is unnecessary for well presented, mounted biopsies or for tiny oesophageal and gastric biopsies it has proved invaluable for rendering small, unmounted, curled and apparently "unorientable" biopsies reportable.

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Culture of Chlamydia

There is currently much interest in the role and importance of Chlamydia as pathogens both in adults and neonates. Isolation is still a complicated procedure and we have devised a simplified method

requiring neither treatment of tissue culture cells nor centrifuging of specimens.

Briefly, specimens in 0.25 ml of transport medium¹ are inoculated onto circular coverslips in flat-bottomed tubes, 30 to 60 minutes after each tube has been seeded at room temperature with 1 ml of suspension of McCoy cells, 200 000 per tube, in Eagles' MEM (Hanks based) containing 10% fetal calf serum, 0.5% glucose, 100 mcg of vancomycin and 50 mcg of streptomycin per ml. At the time of inoculation most cells have adhered to the coverslip and can be seen easily with an inverted microscope; tubes are rejected if this is not so. After 2-3 days incubation in 5% carbon dioxide at 37°C the now monolayered coverslips are examined by immunofluorescence technique for Chlamydial inclusions. Suspensions of cells prepared by orthodox methods for seeding tubes may be kept at 4°C for up to 24 hours before being used.

We have now examined 91 urethral specimens from males attending a sexually transmitted diseases clinic; 45 have yielded Chlamydia. This is an acceptable isolation rate. Isolates may be passed repeatedly in plastic flasks using this technique, and numerous inclusions can be produced making such a technique encouraging as a basis for high antigen-yielding systems.

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Orcein staining for the demonstration of sulphomucins

Shousha and Boxer¹ suggest that orcein staining may be used as an alternative to high iron diamine (HID) for the demonstration of sulphomucins. Whilst the HID method is the most sensitive and specific means of detecting sulphomucins, it would be desirable to replace it with another method for the reasons given by Shousha and Boxer and also because diamines are carcinogenic. However the authors have

preceded the orcein staining by an oxidative step. According to Sipponen,² they have not only demonstrated sulphomucins but also sulphonic acid residues resulting from the oxidation of disulphide groups. This would explain the unexpected positive results in small intestine and intestinal metaplasia of the stomach. Only when orcein is *not* preceded by an oxidative step can one expect a pattern of staining comparable to the HID method. It would be interesting to know if orcein can be combined effectively with alcian blue for the separation of sulphated and non-sulphated acid mucins.

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Drs Shousha and Boxer reply as follows: We agree with Dr Jass that according to Sipponin¹ the method we used demonstrates both acidic sulphated mucin and acidic mucins "with presumed sulphonic residues" as evidenced by the reaction noticed in the small intestine, and that our results are thus comparable to HID staining with, rather than without, oxidation.^{1,2} We have tried using orcein without prior oxidation but the results were weaker and less defined. We also tried to combine orcein staining, with and without oxidation, with an alcian blue technique at pH 2.5, both before and after the orcein staining, with no success.

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gastrointestinal mucosubstances with high iron diamine after prior oxidation and methylation of tissue sections. *Histochemistry* 1979;64:297-305

Book reviews

Cytology of Bone Tumours. A Colour Atlas with Text. NG Sanerlein and GM Jeffree. (Pp 168; 259 illustrations; £32.50. John Wright & Sons Ltd. 1981.

The accumulated expertise of the Bristol Bone Tumour Registry group in the application to bone tumours of histochemical methods has more recently been extended to cytological smears. In this book an excellent introductory chapter sets out the general principles of cytodiagnosis and emphasises the advantages of speedier and more accurate diagnosis when histology is complemented by cytology. The method of making smears and, in the appendix the staining techniques are so clearly described that worthwhile preparations can soon be obtained even by those of us inexperienced in this field, though skill in interpretation will clearly take longer to acquire.

The remainder of the book consists of chapters on the different types of primary bone tumours according to the predominant cell type as well as on metastatic carcinoma and a number of non-neoplastic lesions. In each chapter the important histological and cytological diagnostic features and the most helpful stains in differential diagnosis are described and well illustrated, mostly in colour. The careful matching of histological and cytological preparations increases their value.

The use of cytological techniques seems to this reviewer to be especially helpful in two spheres. Alkaline phosphatase staining of smears may resolve the notorious difficulty in differentiation of small biopsies between chondroblastic or fibroblastic osteosarcoma and true chondrosarcoma or fibrosarcoma. Similarly cytology may aid the important assessment of cell detail cartilage tumours. This book must be of interest to all pathologists concerned with the diagnosis