

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 al1 provides convincing objective evidence of vasculitis and parallels my experience2 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 al1 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.

I FRIEDMANN
Department of Histopathology, Northwick Park Hospital and Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

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 glass4 or thin card.5 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 alcohol and chloroform to paraffin wax in the usual way. The agar does not shrink appreciably during processing and is impregnated by wax just as if it were tissue. Paraffin sections are cut in the usual way, but both the agar and the biopsy are cut simultaneously. 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.

ES BLEWITT
T POGMORE
IC TALBOT

Departments of Histopathology, Leicester Royal Infirmary, Leicester General Hospital, & the University of Leicester, P O Box 65, Leicester LE2 7LX

References


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
Letters to the Editor


The accumulated expertise of the Bristol Bone Tumour Registry group in the application to bone tumours of histochimical methods has more recently been extended to cytological smears. In this book an excellent introductory chapter sets out the general principles of cytological diagnosis 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 in 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.

References
1 Sipponin P. Histochemical reactions of gastrointestinal mucosubstances with orcein, high iron diamine and alcian blue after prior oxidation of tissue sections. Histochemistry 1979;64:297-305

Orcin 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 diame are carcinogenic. However the authors have preceded the orcein staining by an oxidative step. According to Sipponen,2 they have not only demonstrated sulphonmmucins 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.

JA HARPER
L JOHNSON
RG THOMPSON
Public Health Laboratory
New Cross Hospital
Wolverhampton WV10 OQP

We are grateful for help from clinic and laboratory staff.

Reference
1 Richmond SJ. The isolation of Chlamydia sub group A (Chlamydia trachomatis) in irradiated McCoy cells. Med Lab Technol 1974;31:7-9.

Book reviews

Orcin 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 diame are carcinogenic. However the authors have preceded the orcein staining by an oxidative step. According to Sipponen,2 they have not only demonstrated sulphonmmucins 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.

References
2 Sipponen P. Histochemical reactions of gastrointestinal mucosubstances with orcein, high iron diamine and alcian blue after prior oxidation of tissue sections. Histochemistry 1979;59:199-206.

Drs Shousha and Boxer reply as follows: We agree with Dr Jass that according to Sipponen 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 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.

S SHOUSHA
GM BOXER
Department of Histopathology,
Charing Cross Hospital Medical School,
Fulham Road,
London W6 8RF

References
1 Sipponin P. Histochemical reactions of gastrointestinal mucosubstances with orcein, high iron diamine and alcian blue after prior oxidation of tissue sections. Histochemistry 1979;59:199-206.
2 Sipponin P. Histochemical reactions of gastrointestinal mucosubstances with high iron diamine after prior oxidation and methylation of tissue sections. Histochemistry 1979;64:297-305

requireing 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' balanced salt solution; tubes are kept at 37°C.) The tubes are examined by immunofluorescence technique for Chlamydia inclusions. Suspensions of cells prepared by centri- fuging for 10 minutes after each passage are inoculated into Eagles' MEM (Hanks' balanced salt solution; tubes are kept at 37°C.) After 2-3 days incubation at 36°C the infected culture medium is inoculated into Eagles' MEM (Hanks' balanced salt solution; tubes are kept at 37°C.) It is possible that the use of this technique will be encouraging for the isolation of Chlamydia. This is the basis for the technique described by Sipponin et al. (1979) for the isolation of Chlamydia.