requiring neither treatment of tissue culture cells nor centrifuging of specimens. Briefly, specimens in 0-25 ml of transport medium\(^1\) 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\(^1\) 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 monolayered coverslips are examined by immunofluorescence technique for Chlamydia 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.

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

### Orcein staining for the demonstration of sulphomucins

Shousha and Boxer\(^1\) 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 diarnes are carcinogenic. However the authors have preceded the orcein staining by an oxidative step. According to Sipponen,\(^1\) 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 alcan blue for the separation of sulphated and non-sulphated acid mucins.


\(^{1}\) Sipponen P. Histochemical reactions of gastrointestinal mucosubstances with orcein, high iron diamine and alcan blue after prior oxidation of tissue sections. Histochemistry 1979;59:199-206.

### References


**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 cyto-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
Orcein staining for the demonstration of sulphomucins.

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