

Total cell and lymphocyte recovery from peripheral blood by differential centrifugation over Ficoll-Paque under different conditions of separation

	(a)	(b)	(c)	(d)
Cell recovery %				
Mean (\pm SD)	52.5 (\pm 15.7)	92 (\pm 3.7)	69.2 (\pm 11.5)	85.5 (\pm 7.6)
Lymphocyte recovery %				
Mean (\pm SD)	38.5 (\pm 11.5)	52.8 (\pm 6.7)	51 (\pm 9.1)	65.8 (\pm 8.2)

(a) = Ficoll-Paque 4°C; centrifugation 35 min at 4°C

(b) = Ficoll-Paque 4°C; centrifugation 15 min at room temperature

(c) = Ficoll-Paque 4°C; centrifugation 35 min at room temperature

(d) = Ficoll-Paque room temperature; centrifugation 35 min at room temperature.

dark. While we have been storing it unopened at room temperature, once opened we keep it in a 4°C domestic refrigerator to minimise the possibility of bacterial growth. We believe that many laboratories will be following this standard bacteriological practice and that it is worthwhile to draw their attention to the detail of making sure that equilibration of the Ficoll-Paque with room temperature is allowed to occur.

It is interesting to note that Coulter Clone recommend that separation of mononuclear cells be performed at 4°C over Ficoll-Paque,¹ and they state that the procedure was provided by Drs Schlossman, EL Reinhertz, and LM Nadler. In our hands, separation at this temperature was significantly inferior to each other modification. Likewise, we did not find shortening of centrifugation time to 15 min helpful as have others²; the recovery of lymphocytes was less and that of monocytes and polymorphonuclear leucocytes greater.

In conclusion, we have improved our lymphocyte recovery rate by this simple step. Nevertheless, there is still a deficit of 44%. It seems that major lymphocyte subset proportions are relatively accurately portrayed in the 50% or so of cells which is routinely recovered. There is no guarantee, however, that minor sub-sets and sub-sets in altered states of activation are not either selectively lost or, alternatively, selectively recovered. Therefore interpretation of profiles delivered by sophisticated technology such as fluorescent activated cell sorters may be inexact. Equally, to confine analysis of minor sub-sets only to those samples with a high recovery may not produce a representative picture. Perhaps it is time to direct further effort towards improving cell recovery before results obtained from an incomplete census of the lymphocyte population in normal subjects are indelibly printed not only in bulk on paper but also in our minds.

D McKEATING
HD ALEXANDER
GERALDINE M MARKEY
TCM MORRIS
*Haematology Department,
Belfast City Hospital,
Belfast BT9 7AD*

References

- ¹ Coulter Clone. Monoclonal antibodies. *Coulter Immunology* 1983:1.
- ² Hicks MJ, Jones JF, Thies AC, Minnich LL. The effect of lymphocyte recovery on lymphocyte typing results. *Am J Clin Pathol* 1981;76:745-52.

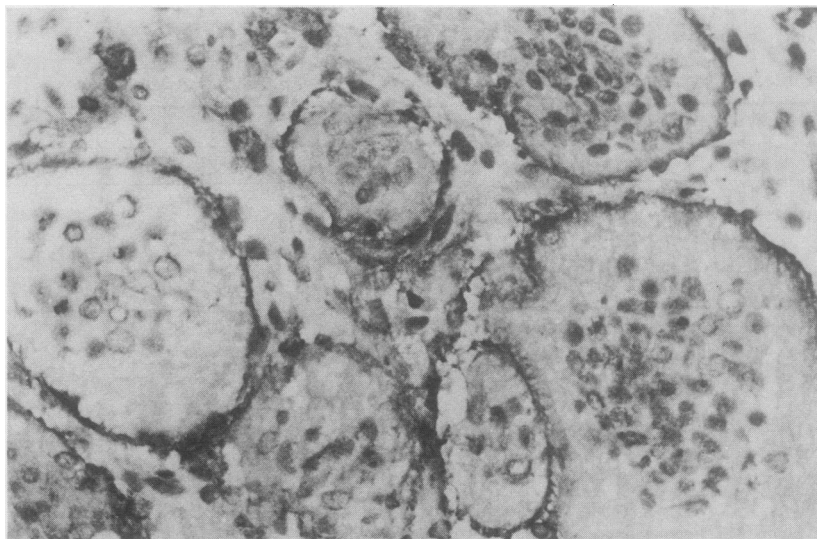
When is it an osteoclast?

We read with interest Dr Kaye's recent paper¹ on the problems encountered in the identification of osteoclasts in tissue (bone) sections when conventional histological

and cytochemical techniques are used. Dr Kaye correctly emphasises that cellular multinuclearity, characteristic of osteoclasts, can often be proved only on serial sectioning. Moreover, conventional methods cannot differentiate between osteoclasts and cells of the mononuclear phagocyte series and are incapable of positively identifying mononuclear osteoclasts.

Our approach to such problems of cell identification, and hence enumeration, is the use of monoclonal antibody techniques. We have recently derived a series of hybridomas against human giant cell tumours of bone (osteoclastomas) which secrete antibodies specific for osteoclasts; further, these antibodies fail to react with macrophages and thus effectively differentiate between these cell types (Horton *et al*; unpublished observations). A total of eight antibodies in six reactivity groups recognise osteoclast membrane antigens; a further two clones secrete antibodies to osteoclast restricted cytoplasmic determinants. The Figure illustrates typical specific membrane reactivity of monoclonal 23C6 (A4) on a frozen section of giant cell tumour, visualised by the immunoperoxidase technique.

Although our antibodies fail to react in formalin fixed tissue, they do for the first time allow an approach to the specific detection of osteoclasts in bone and in other pathological material. Thus, any cell showing reactivity with osteoclast specific antibodies, whether mono- or multinucleate, can be categorised as an osteoclast. We predict that these and other monoclonal



Frozen section of osteoclastoma stained by immunoperoxidase technique using monoclonal antibody 23C6 (A4) showing membrane reactivity on multinucleate cells. \times 390.

antibodies under development will prove to be of considerable value in skeletal pathology and in studies of the biology of bone cells.

MA HORTON*
JAS PRINGLE‡
TJ CHAMBERS†

Departments of *Haematology
and †Histopathology,
St Bartholomew's Hospital, London,
and ‡Department of Morbid Anatomy,
Royal National Orthopaedic Hospital,
Stanmore, Middlesex

Reference

† Kaye M. When is it an osteoclast? *J Clin Pathol* 1984;37:398-400.

Book reviews

Respiratory Distress Syndrome. Ed KO Raivio, N Hallman, K Kouvalainen, and I Välimäki. (Pp 421; £32.) Academic Press. 1984.

This volume contains the proceedings of the 8th Sigrid Juselius Foundation Symposium held in Helsinki in August 1982. The symposium, despite the title, dealt with respiratory distress syndrome (RDS) in the newborn only. There are six main sections—fetal and neonatal lung development, experimental models of RDS, clinical and pathophysiological features, treatment, complications, and prevention of RDS. Despite the two year gap between the symposium and publication, many of the contributors have updated their references.

The first quarter of the book deals with the normal fetal lung and aspects of its development. These chapters were generally clear concise reviews of the state of the art though I was disappointed that Professor Reid had not been able to include the important papers on lung growth by Thurlbeck (Thorax, 1982;37:564-83). Little histopathology was present in the symposium and this is rightly so since the death rate from "hyaline membrane disease" is falling. As with any multi-author book there was variation in style of the contributions but reading was generally easy. The editorial pen had not been heavily used since there were three chapters dealing with hormonal regulation of fetal lung maturation and two chapters on prostaglandins with obvious overlap.

This is a well set out and clearly illustrated book which will provide a useful summary of recent advances in RDS in the newborn at a reasonable price. The book will have an important place, not only on the neonatologist's shelf, but also anyone interested in the developing lung and its biochemistry.

PS HASLETON

Diagnostic Ultrastructural Pathology. A self-evaluation Manual. Feroze N. Ghadially. (Soft cover £9.95.) Butterworths. 1984.

The past few years have seen a number of books and treatises on the use of electron microscopy in diagnostic histopathology. Most of these books are on the large and expensive side and are likely to be used only by those actually engaged in using diagnostic electron microscopy. On the other hand, this little volume will be valuable to those trainees in histopathology who need to be aware of the possible uses of EM and to have a passing acquaintance with the ultrastructural appearances of various lesions but who do not intend to be personally involved in this technique.

The book is in the form of a diagnostic quiz, each page having an electron micrograph with structures to identify and questions to answer. The standard of micrographs is excellent and, unlike other similar books I have seen, the structures to be identified are clearly labelled and clearly visible at the magnification given. At £9.95 this book is very good value and would be useful to have in departments where junior pathologists are being trained.

JULIE CROW

Textbook of Immunology. 2nd ed. Emil R Unanue and Baruj Benacerraf. (Pp 324; soft cover £14.) Williams & Wilkins. 1984.

This is the second edition of a textbook originally based on lectures given as an introductory course in immunology to first year medical students at Harvard Medical School. It has been extensively revised and gives an up to date and comprehensive account of basic immunology. It is suitable for the preclinical medical student who wishes to master a relatively detailed understanding of the subject or the student

of biological sciences. References are provided at the end of each chapter and another useful feature is the glossary. The diagrams are very clearly presented and important points in the text are underlined. Unfortunately, some of the nomenclature used for describing micro-organisms is inaccurate and wrongly spelt (eg "*Diplococcus pneumoniae*" and "*Giardia lamblia*" para 2, p300). Since the main emphasis is on basic immunological phenomena, the book is less appropriate for those interested in the clinical applications of immunology.

RUTH MATTHEWS

Atlas of Breast Pathology. Vol 7 in the Current Histopathology Series. Rosemary R Millis. (£45.) MTP Press Limited. 1984.

This book is considerably more than an atlas. The text and the legends, though commendably brief, include the most important facts relating to their subjects and are clear. The photomicrographs are of a consistently high quality throughout and the colour reproduction is excellent. There are very few macroscopic photographs but the inclusion of several mammographic pictures should add perspective for the histopathologist.

The 24 chapters embrace, as well as the common lesions, very rare lesions—it is useful for example to see colour illustrations of a benign (pseudomalignant) osseous tumour of soft parts and a chondrolipoma of breast. Recognised areas of diagnostic difficulty such as the differential diagnosis between forms of epitheliosis and in situ carcinoma, and the problems of distinguishing between benign and malignant papillary lesions of the breast, are clearly expostulated and helpfully illustrated. I like the idea of separate chapters on axillary lymph nodes, and secondary tumours within the breast, and metastatic mammary carcinoma to other sites.

Most other books on breast pathology likely to be used by a trainee or consultant histopathologist are considerably less digestible than this volume. I consider that 99% of the diagnostic questions likely to be asked on breast pathology of a histopathologist could be answered from within the pages of an Atlas of Breast Pathology.

DA LEVISON