biology in histopathology. Chichester: Wiley, 1994:1231–49.
4 Trerè D, Migaldi M, Trentini GP. Higher reproducibility of morphometric analysis over the counting method for interphase AgNOR quantification. *Anal Cell Pathol* 1995; in press.
5 Trerè D. Critical analysis of the methods commonly employed in the assessment of cell proliferation: advantages of the NOR silver-staining technique in routine cyto-histopathology. *Anal Cell Pathol* 1993;5:191–201.
6 Derenzini M, Sirri V, Trerè D. Nucleolar organizer regions in tumor cells. *J Cancer* 1994; 7:71–7.

Drs Barnes and Gillett comment:

We thank Dr Trerè for his interest in our editorial. He is obviously a keen proponent of the AgNOR technique; however, the value of AgNORs in pathology remains controversial, as denoted by the large number of conflicting publications. In response to his four areas of criticism of our review we would like to confirm our original points.

The accurate identification of AgNORs is highly dependent upon tissue preparation and methodological procedures. Excessive silver deposition and hence loss of NOR definition can occur as a result of variations in tissue thickness, the use of different fixatives and prolonged silver incubation times. These features also affect the amount of non-specific staining, which can cause problems with the accurate identification of AgNORs.

There is no universally accepted method of evaluating AgNORs. Counting methods have evolved in order to obtain as much information as possible about demonstrable NORs. Some studies have counted all discernible NORs, others have counted the number of NOR clusters and satellites, whilst further groups have incorporated the AgNOR distribution pattern into their assessment.[1] As we pointed out previously, there is marked variation in the numbers of cells assessed, 100–200 being the usual number, far less than would be evaluated when staining with a proliferation associated antibody such as MIB1, Ki67 or KiS1. By Dr Trerè's own admission these enumerative methods are "time-consuming and subjective". However, manual counting is the only method of evaluation open to most pathologists, who do not have the necessary equipment to carry out computer aided image analysis.

A number of studies have combined AgNOR scores with established methods of predicting prognosis and directly with clinical outcome. Whether AgNORs are associated with ploidy or with cell proliferation requires further clarification.[2 3]

There are studies which have shown AgNORs to be associated with patient prognosis but among these, the prognostic value of AgNORs is less than in the more established methods[4] and in some cases do not provide independent prognostic information when included in multivariate analyses.[5]

In conclusion, we stand by our previous statement that AgNORs were of great interest when they were one of the few methods of assessing proliferative activity in formalin fixed, paraffin wax embedded material. As a prognostic marker, AgNORs have now been superseded by other methods, in particular the development of the Ki67 associated antibodies, which are easy to use and are open to more standardised quantification.

1 Evans AT, Orrell JM, Grant A. Re-evaluating silver-stained nucleolar organizer regions (AgNORs) in problematic cutaneous melanocytic lesions: A study with quantitative and pattern analysis. *J Pathol* 1991;165:61–7.
2 Suresh UR, Chawner L, Buckley CH, Fox H. Do AgNOR counts reflect cellular ploidy or cellular proliferation? A study of trophoblastic tissue. *J Pathol* 1990;160:213–15.
3 Borgiani L, Cogorno P, Oliviero J, Toso F, Gambini G, Tunesi G, *et al.* AgNORs in ductal breast cancer: correlation with ploidy and S-phase fraction by DNA flow cytometry. *Eur J Histochem* 1994;38:171–6.
4 Eskelinen MJ, Lipponen PK, Collan Y, Syrjanen KJ. the role of nucleolar organiser regions as prognostic factors in breast cancer. *Eur J Cancer* 1991;27:989–92.
5 Aaltomaa S, Lipponen P, Syrjanen K. Nucleolar organizer regions related to morphometry, flow cytometry, sex steroid receptor content, tumour histology and prognosis in female breast cancer. *Pathol Res Pract* 1993;189:416–21.

Book reviews

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(The price and availability are occasionally subject to revision by the Publishers.)

Genetic Laboratory Investigations. 10th edn. T K Merten, R L Hammersmith. (Pp 277; £18.95). Prentice Hall. 1995. ISBN 0-02-380-601-X.

This book provides a series of practical demonstrations, that may be undertaken by students themselves, to illustrate some of the fundamental concepts of genetics. It is aimed at first year undergraduates with questions and answers as well as references on each topic. The authors find themselves in the curious position of having to defend the use of practical laboratory work in courses on genetics which they do clearly and succinctly.

The book was first published in 1951 and is now in its 10th edition and therefore clearly has found a niche in this particular market. No doubt the book has evolved a great deal since the first edition but unfortunately some mutations have crept in over this period of time.

In the chapter on "Linkage and Crossing-over" somatic cell hybridisation is explained in great detail in the section on human gene mapping. Other approaches (for example, in situ hybridisation) are dealt with in one line—surely unacceptable in a genetic textbook published in 1995. In the chapter on "Human Chromosomes" the chromosome pairs 17 and 18 are transposed in one figure (Fig 11.3) and the ISCN karyotype for Klinefelter syndrome is incorrectly given as 47,XY, +X rather than 47,XXY. In the same chapter the cytogenetic consequences of the presence of the Philadelphia chromosome, observed in chronic myeloid leukaemia, are incorrectly described.

One is left with the impression that some parts of this book may have evolved faster than others. The book would appear to be a useful source for classic genetic experiments but care should be exercised in relying upon it as a source of instruction and information in those areas undergoing rapid development, such as human gene mapping, which will benefit from revision for the next edition.

J WATERS

Concepts of Genetics. WS Klug, MR Cummings. (Pp 779; £21·95.) Maxwell Macmillan International. 1994. ISBN 0-02-364801-5.

The era of the coffee-table science text is clearly with us! This book is beautifully produced and illustrated, with striking photographs, some enhanced by computer, and with tables and graphics which make understanding of data and processes both appealing and easy. To describe it as a coffee-table book does not in any way mean that it is not scientifically and academically a work of excellence. Few pathologists would until, say, five years ago, have picked up and read this work. However, the revolution in molecular genetics has now changed that position. What, then, is there here for us to use (and even enjoy!)?

Naturally, much of the background understanding of genetics is based on data which were obtained from studies of yeasts, *Drosophila*, *Xenopus*, and so on, but increasingly this information is being extended to humans. Thus, in medical science we should not baulk at the included information regarding "lower organisms".

What, then, do I feel are the limitations of this book? Very few. If I turn to my three main areas of personal interest, the results are good. Thus, the section on ribosomal RNA genes and nucleoli is unusually well covered and explained. Another part of the text close to me namely that related to viral oncogenesis is good but, very surprisingly, omits the extensive (albeit confusing) literature on Epstein–Barr virus in this field. The cell cycle is dealt with quite adequately and comprehensively. Finally, the book is a bargain.

J CROCKER

PCR in Situ Hybridization: Protocols and Applications. 2nd edn. GJ Nuovo. (Pp 276; $94.) Raven Press. 1994. ISBN 0–88167–940–2.

That this text is now in its second edition, despite the fact that PCR in situ hybridisation (PCR-ISH) is still in its infancy, probably reflects rapidly growing interest in the technique. This is not surprising, as PCR-ISH offers the high sensitivity in signal detection afforded by PCR and yet permits the investigation to localise that signal architecturally.

For the novice, the first two chapters provide useful introductions to the relevant molecular biology, then the theoretical basis for PCR and ISH is discussed. At this point and subsequently the chapters include practical protocols to lead the reader through a maze of techniques, variants and complex controls. Numerous photographic figures, some in colour, are used to illustrate examples of applications and results. It must be stated, however, that the quality of some of these figures is not always optimal, many being rather grey. Perhaps the most useful chapter is that describing "start-up" protocols for the beginner. This part of the book, combined with the contents of the appendices, are most helpful to those brave enough to attempt this notoriously fickle methodology.

The author's specialist interests are reflected by the inclusion of two chapters, one regarding the application of PCR-ISH to the detection of papilloma viruses, the other to the use of the method in the investigation of HIV related diseases.

Perhaps the greatest hope for the success of PCR-ISH is the use of reverse transcriptase methodology. This can eliminate problems of non-specificity in ISH caused by DNA repair. This is discussed clearly and practically in a further chapter.

In general, this is a well produced and organised volume and can be recommended to those about to step into this minefield of techniques and protocols. It is certainly of interest to speculate as to how many laboratories will be applying PCR-ISH by the time a third edition of this book is published!

J CROCKER