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AgNOR technique in relation to colorectal neoplasia

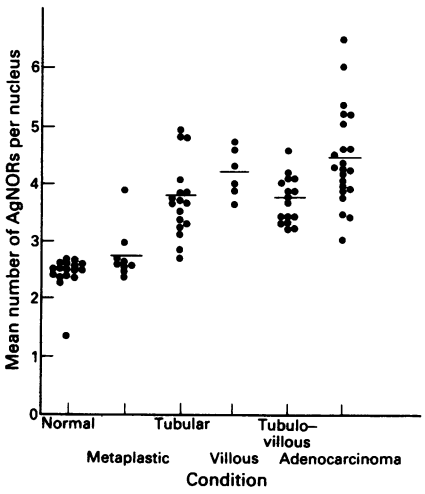
We read with interest the recent paper by Beer *et al*[1] because we have also applied this technique to intestinal tissue. This paper is one of a selection that has been published on this topic in recent years in which authors express varied enthusiasm for the method.[2-5]

We examined 91 surgical resection specimens of large bowel comprising normal mucosa (n = 10); tubular (n = 18), villous (n = 6) and tubulo-villous (n = 16) adenomas; and moderately differentiated adenocarcinomas (n = 22). The batch of malignant tumours comprised five Dukes' A, six B, and 11 C. The method used was as described by Smith and Crocker,[6] except that the staining time was one hour; 100 cells were counted.

The results are shown in the table. An unpaired *t* test was applied to the data and a highly significant difference (p = 0·001) was found between normal mucosa and both the adenomatous polyps and the adenocarcinomas. No statistical difference existed between normal and metaplastic, or between benign and malignant conditions. AgNOR numbers varied enormously in all but the normal and metaplastic states, this being most pronounced in malignancy (figure). No correlation was observed between the AgNOR number and the Dukes' stage, although we acknowledge that relatively few of each stage were examined.

Summary of results

Condition	Mean (SD) AgNOR count
Normal mucosa	2·37 (0·28)
Metaplastic polyp	2·71 (0·44)
Tubular adenoma	3·67 (0·64)
Villous adenoma	4·12 (0·43)
Tubulo-villous adenoma	3·62 (0·41)
Adenocarcinoma	4·34 (0·86)



In terms of diagnostic usefulness, our results for colorectal tissue agree with those of Beer *et al*[1] and Yu *et al*[5] for stromal tumours of the stomach and small intestine. This contrasts with the findings of Yang *et al*,[3] who discriminated colonic tubular and villous adenomas from adenocarcinomas. Surprisingly, Griffiths *et al*[2] could find no link between AgNOR number and neoplasia in large bowel tissue. Unlike us, Ofner *et al*[4] established a correlation between AgNOR number and Dukes' staging.

Clearly, AgNOR number is a reflection of increased cell proliferation and may be used to distinguish normal tissue from neoplastic. However, as an accurate discriminator of malignancy, this technique is inadequate when applied to intestinal tissues. We feel that it offers little more than the haematoxylin and eosin preparation in the area of large bowel diagnostic pathology.

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Breast biopsy specimen fixation

Further to the correspondence by Drs Start, Cross, and Smith[1] regarding the procedure of fixing breast biopsy specimens, we add our findings to this debate.

In our view the handling of this kind of specimen poses a dilemma: for best slicing and minimisation of distortion for assessment of resection margins and extent of lesion, the specimen should be fixed before slicing. To overcome this problem we suggest that the specimen should be injected with 10% neutral buffered formalin on receipt then left to fix for 24 hours before slicing.

We use a 10 ml syringe with a 21 gauge needle. The amount of formalin injected depends on the size of the specimen. The injection can be performed by technical staff, which means the specimen need not be sent dry and the pathologist does not have to be on hand when the specimen is received: this may often be the case in a district general hospital.

This technique offers adequate fixation of tissues deep within the specimen while allowing fixation of the outside which "hardens" the specimen, giving optimal slicing.

There are two possible hazards that need to be borne in mind when using this technique. The first is the danger of needlestick injury; the second concerns the splashback of formalin which can occur if too much pressure is applied, particularly when injecting firm areas of tissue. Accordingly, appropriate protective clothing should be worn and great care taken when performing this procedure.

We have found a definite improvement using this method in the quality of morphology in subsequent sections compared with those from specimens which were allowed to fix overnight before slicing and were not injected.

We propose that this method helps reduce the inevitable variation in fixation that occurs with these specimens, and thereby reduces the associated variation in mitotic counts which may affect grading.[2] It also improves assessment of resection margins and extent of lesions.

We accept that our findings are subjective and anecdotal, but feel that there is sufficient benefit to merit extending the use of this procedure from localisation biopsy specimens and wide local excision specimens to mastectomy specimens.

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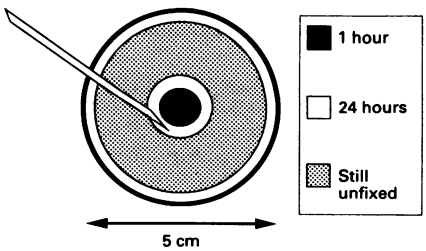

Penetration of 10% neutral buffered formalin into an unsliced 5 cm diameter lump after a single 1 ml injection of fixative.

Drs Start, Cross, and Smith comment:
We were most interested to read the letter from Dr Ruban *et al* and are receptive to suggestions for improving the efficiency of tissue fixation, but we have reservations regarding the injection of fixative into breast biopsy specimens.

The authors correctly refer to two potential hazards but we are more concerned by the potential effects on the subsequent histological interpretation of specimens treated in this way. The injection of even a small volume of fixative into excision biopsy specimens could produce tissue artefacts and irreversibly change the overall tissue morphology. Neoplastic lesions could be expanded by the fixative, leading to a false worsening prognostic index which is directly influenced by tumour diameter.[1] Alternatively, failure to inject the tumours would not prevent delayed fixation and the possible changes in tumour grade which could result from a reduction in the number of observable mitotic figures.[2] Fragments of tumour could be forced into vascular channels, simulating vascular invasion, or into breast ducts, simulating carcinoma in situ, and the distortion caused by injecting fixative into small localisation specimens containing peripheral lesions could complicate the assessment of adequacy of excision. The impact of a single 1 ml injection of 10% neutral buffered formalin into a solid 5 cm lump would be minimal (figure). The bolus of fixative would only penetrate 3·8 mm into the surrounding tissue in 24 hours,[3] and multiple injections would therefore be required for rapid and uniform fixation.

Although the authors report an improvement in tissue morphology, a more detailed appraisal of the effects of fixation injection is required before the method can be fully evaluated. An efficient specimen delivery service should allow the rapid assessment of specimens within routine laboratory hours and we would recommend that all breast specimens are described and sliced on receipt in the fresh state after the marking of appropriate resection margins.

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BOOK REVIEWS

All titles reviewed here are available from the BMJ Bookshop, PO Box 295, London WC1H 9TE. Prices include postage in the United Kingdom and for members of the British Forces Overseas, but overseas customers should add £2 per item for postage and packing. Payment can be made by cheque in sterling drawn on a United Kingdom bank, or by credit card (Mastercard, VISA or American Express) stating card number, expiry date, and your full name.

Interpretation of Protein and Isoenzyme Patterns in Body Fluids. T Sun. (Pp 225; $51.) Williams & Wilkins Ltd. 1991. ISBN 0–896420–202–9.

On my first perusual of this book I thought that it was just a repetition of similar books in this field. However, on settling down to review it a few weeks later I found myself wishing I had more time to spend studying it carefully.

The presentation appears simple, but in fact contains important selected information supported by carefully thought out tables, diagrams, and photographs. The first four chapters cover the clinical importance of plasma proteins, including the complement system and immunoglobulin abnormalities. The major part of the book is then devoted to the techniques of electrophoresis, immunoelectrophoresis, fixation and blotting, quantitation of heavy and light chains, lipoprotein analysis and isoenzymes. The valuable part is the discussion on the findings in disease.

I strongly recommend that this book be bought for the department library. Trainees—both scientific and medical—will find it not only informative but easily digestible.

BRENDA SLAVIN

Surgical Pathology of the Mediastinum. 2nd ed. AM Marchevsky, M Kaneko. (Pp 351; $124.00.) Raven Press. 1992. ISBN 0-88167-818-X

There has been an increasing trend in the pathology literature towards writing texts based on the pathology of specific anatomical regions. This approach provides a ready reminder of the potential diagnoses at a given site, but there is a tendency towards brevity in the pathological descriptions. Although devoted to the mediastinum, the authors have deliberately, and understandably, excluded the heart and great vessels from their brief. One third of the text is devoted to the thymus with the remainder covering miscellaneous inflammatory conditions and cysts, neuroendocrine tumours, germ cell neoplasia, soft tissue tumours and lymphomas.

In the section on thymus it is regrettable that little coverage is given to Müller-Hermelinck's prognostically relevant classification of thymoma. This aside, the overall description of thymomas is good, although some of the low power figures are of poor quality.

The chapter on lymphoma is a little unsatisfactory. For example, the special problems of diagnosing Hodgkin's disease in the mediastinum are not addressed, and primary thymic disease is only briefly described in an earlier chapter. The coverage of sclerosing mediastial B cell lymphoma is equally unhelpful without consulting the references. The computed tomogram scan purported to be of such a case shows a posterior mediastinal mass.

By contrast, the chapter on mediastinal cysts contains careful descriptions which are well illustrated with clinical and pathological photographs. The other sections of the book steer a midway course but I doubt whether the soft tissue chapter will be used by anyone with a serious interest in the field.

Overall, however, the book performed rather better than I had anticipated. In places it does tend to lapse into a gazetteer of conditions spotted in the region, but this is offset by sections in which truly diagnostically useful descriptions are given. This certainly provides a preferable alternative to the aging AFIP fascicle.

AJ NORTON

Melanocytic Tumors of the Skin. Atlas of Tumor Pathology. DE Elder, GF Murphy. (Pp 216; $40.) Armed Forces Institute of Pathology. 1991. ISBN 0160-6344.

This is volume 2 in the (confusingly) renumbered AFIP fascicles on tumour pathology. This volume is devoted entirely to various melanocytic tumours and only those occurring in the skin are considered. The reason for pointing this out is that the closest rival is the recent volume in the "Biopsy Pathology" Series (Vol 17: *Biopsy pathology of Melanocytic disorders* by Mooi and Krausz £59·50) which covers a similar field, costs a similar price (allowing for postage, VAT etc), but contains sections on extracutaneous melanocytic lesions, and melanocytic cytology.

David Elder is a well known authority in the field of melanoma studies and all who have seen him in public debate with Bernard Ackerman on the subject of dysplastic naevi will know that he is a cogent, reasoning pathologist whose views are well researched and lucidly presented; exactly the same can be said of this book. The illustrations of histopathology are in black and white and are excellent, there are also some colour photographs of the gross pathology which are also uniformly good. The references are wide ranging; in the section on benign melanocytic tumours (which includes dysplastic naevi) there are 128 references about 10 of which come from non-American sources. I can find no references to AgNOR studies, although there are several in the literature, and I can find no references to Ackerman as first author on dysplastic naevi, although I know that he has written on the subject (with considerable scepticism). Several other papers sceptical of the importance of sporadic dysplastic naevi are also omitted (they are to be found in the relevant chapters in biopsy pathology of melanocytic disorders).

In spite of these criticisms this remains an extremely well produced book, well up to the high standards of the AFIP fascicle series, and will be a very useful bench book in a very difficult area.

D COTTON

NOTICE

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The Association of Clinical Pathologists runs a locum bureau for consultant pathologists.

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