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AgNOR technique in relation to colorectal neoplasia

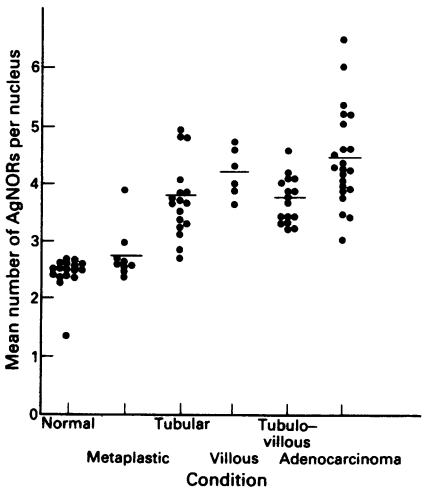
We read with interest the recent paper by Beer *et al*[1] because we have also applied this technique to intestinal tissue. This paper is one of a selection that has been published on this topic in recent years in which authors express varied enthusiasm for the method.[2-5]

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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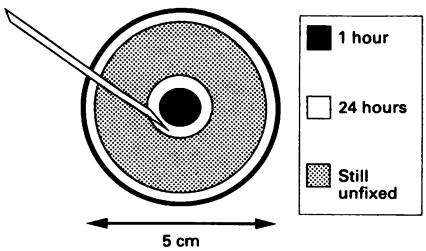

Penetration of 10% neutral buffered formalin into an unsliced 5 cm diameter lump after a single 1 ml injection of fixative.

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