in an attempt to distinguish between benign and malignant tumours. This seems to have been successful in some cases, but not in all. As a recent review has indicated, however, if the AgNOR method is to have a place in routine tumour diagnosis it must do more than simply reflect what is obvious morphologically. Consequently, we attempted to use this method in a situation where morphological features may not be prognostically reliable—that is, in the prediction of recurrence of meningioma.

Study of 111 meningiomas resected over seven years from 99 patients showed that in 10 cases the tumour subsequently recurred within five years. A further 10 meningiomas from the 22 patients presenting in the first two years of the study period were selected by age-matching: scrutiny of patients' records excluded recurrences within five years. In each case we subjected 3 μm sections from paraffin wax embedded blocks of the original tumour to a standard AgNOR technique. A mean AgNOR number per cell was calculated after examining 200 cells using a ×100 oil immersion lens. Both groups comprised four histologically “typical” and six “atypical” tumours (the latter defined as those showing a high mitotic rate and at least one of the following: necrosis; atypical mitoses; high cellularity; poor differentiation). They were also matched to include the same number of syncytial and transitional meningiomas. Neither group contained tumours of fibroblastic or angioelastic morphology. Six of the recurrent, but only one of the non-recurrent meningiomas, were incomplete surgical resections.

Results were expressed graphically (figure). There was no significant difference in mean total AgNOR count between recurrent and non-recurrent groups. Comparison of histologically “typical” and “atypical” meningiomas, however, showed significant differences (Student's t test = 3.63; p < 0.01). It was noted that in the “typical” cases AgNOR dots were largely confined to nucleoli; “atypical” tumours contained greater numbers of intranuclear dots and also dissociated extranuclear AgNORs.

Site of origin and degree of resectability are generally regarded as the most important prognostic factors in predicting recurrence of histologically benign meningiomas. This was found to be the case in our series. Morphology proved to be a less reliable predictor of behaviour. Our results show that the AgNOR method is of no value in identifying those meningiomas that would eventually recur. Although the technique could distinguish “atypical” from cytologically bland meningiomas, there was an overlap between groups, limiting its value in classification of any individual tumour. We did not attempt to relate AgNOR score to individual “atypical” features or to evidence of invasion, but a larger study might have identified a positive correlation with one or more of these. For practical diagnostic purposes, however, equivalent information could be obtained from conventionally stained sections.

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References

Inability of AgNOR counts to differentiate between bronchial carcinoid tumours and small cell carcinoma of the bronchus

AgNORs are dark dots seen in cell nuclei after certain silver staining techniques have been applied. They are believed to be the result of binding to argyrophilic proteins associated with the nucleolar organiser regions of DNA. After a recent study which showed that AgNOR counts may be able to distinguish between hyperplasia and carcinoma in the prostate gland, enormous interest resulted in the possible value of AgNOR counts in a range of diagnostic problems in histopathology. It has already been shown that AgNOR counts may help distinguish small cell carcinoma of the bronchus from lymphomatous infiltrates; we attempted to use the technique to distinguish small cell carcinomas from carcinoid tumours.

We were able to find paraffin wax embedded material from 13 cases that had been originally diagnosed as oat cell carcinoma or small cell carcinoma, where there was plentiful untraumatised material for further study. Nine resection specimens with an

Figure Scattergrams showing mean AgNOR counts in meningiomas grouped according to behaviour and histological appearance.
original diagnosis of carcinoid tumour were also retrieved. Many of these diagnoses had been made before the concept of atypical carcinoid had been defined, and so each case was examined to permit reclassification, using established criteria. Four of the cases originally designated carcinoid tumour were found to be typical; five were atypical. Four of the cases also diagnosed as small cell carcinoma seemed to be examples of atypical carcinoid. New sections were cut and stained with a modification of Ploton's technique developed in this laboratory. AgNORs are seen as well defined, dark dots in each nucleus. This modification uses higher temperatures for shorter times than the usual histological methods, but requires glycine preincubation to suppress background staining.

The number of AgNORs in 200 nuclei was counted in each section, and a mean AgNOR count calculated for each case. The counts for the various diagnoses show a very large overlap (figure): that for the typical carcinoids was 5-10 (range 1.26-7.12), that for atypical carcinoids was 4.60 (1.72-8.98), and that for small cell carcinoma was 5.67 (2.67-7.25). This last value compares closely with the value of 5.7 (4.2-7.3) obtained for small cell carcinomas by Crocker et al. This similarity is important because doubt has been expressed about the reproducibility of AgNOR methods; in particular, it shows that reproducibility survived our technical modifications. Unfortunately, the overlapping ranges indicate that such methods are quite useless as an aid to differential diagnosis of neuroendocrine bronchial tumours. Furthermore, because these values are not absolute counts, it is impossible to come to any conclusions about the biology of these tumours. Advances on that front will only be made when cell imprints or smears can be studied.

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References

Simple carbol-fuchsin staining for showing C pylori and other spiral bacteria in gastric mucosa

After the discovery of Campylobacter pylori on gastric mucosa of patients with gastritis and peptic ulcer, several simple and good methods have been used to show the presence of this bacterium in gastric tissue. We propose here another simple method which we have been using very successfully for over a year, and which clearly shows the characteristic morphology of the microorganism. This method uses just a dilute carbol-fuchsin stain.

To check on the accuracy of the carbol-fuchsin stain in identifying C pylori we compared its results with culture and peroxidase anti-peroxidase (PAP) method in 30 patients with sympotms associated with the upper gastrointestinal tract. Two endoscopic antral biopsy specimens were obtained from each patient. The first was used for C pylori culture and the second was fixed in 4% neutral formaldehyde for histology, PAP, and carbol-fuchsin staining. Dewaxed tissue sections were taken to water and stained for five minutes in carbol-fuchsin solution prepared as follows: 0.4 g basic fuchsin; 2 g phenol crystals; 4 ml absolute alcohol and 100 ml distilled water. After rinsing in tap water the sections were briefly decolourised with acetone. The slides did not need to be mounted and they were examined under an oil immersion lens.

Histopathological study of the biopsy specimen sections showed chronic gastritis in 29 of 30 (97%) patients. Biopsy specimens from 25 (83%) patients were shown by culture to harbour C pylori, of which 23 (77%) were positive by the carbol-fuchsin and 24 (80%) by the PAP method. All those positive by culture showed chronic gastritis. The biopsy specimens which were C pylori negative by culture were also negative by PAP and carbol-fuchsin staining. The sensitivity, specificity, and positive predictive value of carbol-fuchsin compared with culture were 92%, 100% and 100%, respectively.

Fig 1 (a) Campylobacter-like bacteria (arrow) in a gastric antral biopsy specimen. (b) Large spiral bacterium (arrow) in lumen of antral gland.

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