Nucleolar organising regions in cervical intraepithelial neoplasia

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SUMMARY The variations in the numbers of nucleolar organising regions (NORs) among different grades of cervical intraepithelial neoplasia (CIN) were investigated using a silver staining technique. Twenty four biopsy specimens were studied (six normal and six of each of the three grades of CIN) by staining paraffin wax sections using a silver (AgNOR) method that stains the NORs as multiple black dots within nuclei (AgNORs). The number of AgNORs in the nuclei of cells in the basal half of the squamous epithelium was counted, and the average number of AgNORs in each cell calculated for each specimen (the AgNOR count). There was no difference in the number of AgNORs in the squamous epithelium of normal biopsy specimens and those showing CIN1 and CIN2, but there was a small but significant increase in the CIN3 group.

The 10 acrocentric chromosomes in man (pairs 13, 14, 15, 21, and 22) have nucleolar organising regions (NORs) on their short arms. These regions carry DNA that transcribes to ribosomal RNA (rRNA), and takes part in the formation of the nucleolus. The proteins of the NOR are argyrophilic, and this property was used to stain the NORs in metaphase chromosomes, the method known as the AgNOR technique. The amount of AgNOR staining reflects the activity of the rRNA genes. Ultrastructurally the silver is usually localised to the fibrillar centres and sometimes to the dense fibrillar component of the nucleoli. It is not the NOR itself that stains, but rather the acidic proteins associated with it, particularly nucleolin.

A similar silver staining technique has been used to stain NORs in interphase nucleoli in cell cultures and preparations for electron microscopy, and these studies have shown that there is a greater variation in AgNOR staining than in the chromosome preparations. Inactive cells (such as mature lymphocytes and hepatocytes in normal liver) showed less staining than tumour cell lines.

A recent paper described a one step procedure for staining AgNOR proteins that can be used on cell smears, plastic embedded cells, chromosomes, and paraffin wax sections of human histological material. This method is an adaptation of that described by Howell and Black, which uses gelatin as a protective colloid to control the silver staining. The number of silver dots visible in each cell is fewer with paraffin wax sections than with cell smears or chromosome preparations. Using this method on paraffin wax sections of human prostatic cancer tissue, however, more silver dots were seen on cancer cells than on cells of hyperplastic prostatic epithelium and lymphocytes.

The present study was carried out to investigate the value of the AgNOR technique on paraffin wax sections of cervical biopsy specimens that showed various grades of cervical intraepithelial neoplasia.

Material and methods

SPECIMENS Twenty four cervical biopsy specimens from different patients were examined. Six were normal on histological examination, and there were six of each of the three grades of CIN. The normal specimens did not show koilocytosis, and had been previously reported on sections stained with haematoxylin and eosin.

STAINING Sections were cut from routinely processed paraffin wax blocks. They were dewaxed in xylene and then
hydrated through ethanols to water. The freshly prepared AgNOR staining solution comprised a 2% solution of gelatin in 1% formic acid, to one volume of which two volumes of 50% silver nitrate solution were added. This was poured over the tissue sections and left for one hour in a dark cupboard at room temperature. The sections were then washed with deionised water, counterstained with Mayer's haemalum, dehydrated to xylene, and mounted in distance plasticiser xylol.

COUNTING PROCEDURE
I devised a simple counting procedure so that I could compare the numbers of positively stained AgNORs. The number of silver dots in the nuclei of 100 cells of the basal half of the squamous epithelium was counted. Only cells of the basal half were counted because little staining occurred in the nuclei of cells in the upper half of normal ectocervical epithelium. In the sections showing CIN the cells in the most dysplastic area were counted. In the normal specimens, cells in the non-metaplastic squamous epithelium adjacent to either endocervical or metaplastic squamous epithelium were counted. The mean number of AgNORs in each nucleus was calculated for each specimen. The significance of differences was calculated by Student's t test.

Results
The range in the number of AgNORs in each cell in normal ectocervical epithelium was 0-5 or 6 and there was a pronounced variation in the size of the AgNORs within each specimen (fig 2). Some large AgNORs seemed to represent an aggregate of NORs and this could introduce a counting error by reducing the NOR count. In the normal specimens there were fewer AgNORs in the basal cell layer of the squamous epithelium than in the cells in the parabasal layer, and those in the superficial cell layers were fewer and smaller. These variations did not occur in the biopsy specimens showing CIN, reflecting the changes in nuclear morphology. The AgNOR count did not vary among different areas of the squamous epithelium in normal specimens. The range among subjects was 1·92 to 2·44 (p < 0·001), a significant variation in the AgNOR count for ectocervical epithelium. There was a similar variation among subjects in the other three groups.

The results are summarised in fig 1. The mean (SD) AgNOR count for the normal specimens was 2·14 (0·209), for the CIN1 group 2·11 (0·182), and for the CIN2 group (2·27) (0·162); these differences are not significant. The count for the CIN3 specimens was 2·86 (0·259), which is significantly higher than in each of the other three groups (p < 0·001). The lowest AgNOR count in the CIN3 group was higher than the highest count in any of the other three groups.

![Image of AgNOR staining](http://jcp.bmj.com/)

**Fig 2** Basal half of normal ectocervical epithelium stained with silver technique for AgNORs. Most nuclei show one to four AgNORs.
the counting methods may not be comparable, the AgNOR counts of normal ectocervical cells in this study were greater than those reported for normal lymphocytes, low grade non-Hodgkin lymphomas, and benign melanocytic lesions. This is in keeping with the greater activity of normal epithelial cells. It seems that this method shows that there is little or no difference in the overall activity of cervical epithelia with mild degrees of CIN compared with normal epithelia. There may be a small biological difference, however, in ectocervical epithelia showing CIN3.

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References

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Discussion

Several recent papers have described the use of the AgNOR technique to investigate differences in NOR activity in various pathological lesions. There is a large difference in the number of AgNOR dots between cells of low and high grade non-Hodgkin lymphomas, which can reliably distinguish between them. It is also possible to distinguish between benign and malignant melanocytic lesions.

The present study was set up to investigate the pattern of AgNOR staining of ectocervical epithelium showing CIN. There was no difference between normal cervix and the lower grades of CIN. Though there were significantly more silver staining dots in the specimens showing CIN3 (fig 3), this difference was not large, and this technique does not seem to give more information about a cervical biopsy specimen than the morphological changes seen on a section stained with haematoxylin and eosin.

The degree of silver staining of NORs seems to reflect the activity of the rRNA genes. Active cells (such as normal follicle centre cells) have a larger number of AgNORs than cells that are comparatively inactive mitotically such as lymphocytes. Although Fig 3 Ectocervical epithelium with CIN 3, stained with silver techniques for AgNORs. AgNORs are more numerous than in fig 2.