Table 2 *Categories between which significant differences in AgNOR counts were observed ($p \leqslant 0.05$)*

Between	Normal	and	CIN III
Between	Normal	and	invasive carcinoma
Between	Atrophic	and	CIN II
Between	Atrophic	and	CIN III
Between	Atrophic	and	invasive carcinoma
Between	Chronic cervicitis	and	CIN III
Between	Chronic cervicitis	and	invasive carcinoma
Between	Metaplastic	and	CIN III
Between	Metaplastic	and	invasive carcinoma
Between	HPV	and	CIN III
Between	HPV	and	invasive carcinoma
Between	CIN I	and	invasive carcinoma

The difference between our findings and those of the previously published study suggests that there is a need for standardisation of counting procedures. It also suggests that there could be a high interobserver error. This is supported by the fact that we found a high inter- and intra-observer error in our study—9·4% and 8·8%, respectively.

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P MURRAY
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Reference

1 Rowlands DC. Nucleolar organising regions in cervical intraepithelial neoplasia. *J Clin Pathol* 1988;**41**:1200–2.

Dr Rowlands comments:

My study was limited to investigating the possible value of the AgNOR technique in the diagnosis of the different grades of cervical intraepithelial neoplasia (CIN). Ramsden *et al* have independently drawn the same conclusion that I did in my published study—that the NOR changes are small and do not add to the information gained by examining the morphological changes seen on sections stained routinely with haematoxylin and eosin.

Ramsden *et al*, however, seem very concerned that their numerical results of AgNOR counting differ from mine. Unfortunately, they do not give their numerical results, so it is difficult to make any specific comment, but some general remarks need to be made. Many different factors may affect the AgNOR count. It has been shown that different fixatives affect the results of the AgNOR stain[1] and variations in the processing of tissue may also have an effect. Variations in the thickness of sections will obviously affect the AgNOR count, higher counts being expected with increasing thickness of section. The length of time that sections are stained affects the visibility of AgNORs—longer staining shows up more small AgNORs but makes it more difficult to determine the number of AgNORs making up a larger clump.[2] It is also possible that differences in ambient temperature and in the reagents, particularly gelatin, used by different laboratories are important. Thus care must be taken when comparing absolute counts of similar lesions performed at different centres.

In fact, I suspect that none of these factors is important in explaining the differences between the two studies. Ramsden *et al* counted AgNORs at levels of the ectocervical epithelium which differed from those I studied. As AgNOR counts were observed to vary at certain levels of the epithelium, this difference in counting method probably explains the difference in counts that Ramsden *et al* observed.

Dr Ramsden and her colleagues call for the standardisation of the AgNOR counting procedures. This problem has been recently addressed by Crocker, Egan, and Boldy.[3] Difficulties in AgNOR counting, however, tend to occur in lesions where the AgNOR count is high. This was not the case in these two studies in which low AgNOR counts were found. More difficult still is the problem of standardisation of the staining technique and handling of specimens to be studied—this is currently being investigated.

The other problem raised by Dr Ramsden refers to the changes in AgNOR count as the grade of CIN increases. My study was intended to investigate the possible usefulness of the technique in diagnosis. I found a significant difference between CIN III and the lower grades of CIN and normal epithelium. Ramsden *et al* also showed a significant difference between CIN III and normal epithelium, but found intermediate counts for CIN I and II. This is supported by the study recently published by Egan *et al*.[4] If AgNOR counts are taken to reflect differences in proliferation then the change in AgNOR count with different grades of CIN is of interest, but as the present studies have only shown a small difference in AgNOR counts, a much larger study will be needed to assess the true difference among the various grades of CIN.

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3 Crocker J, Boldy DAR, Egan MJ. How should we count AgNORs? Proposals for a standardised approach. *J Pathol* (in press).
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Use of nucleolar organiser regions (NOR's) for diagnosing gynaecological neoplasia

I read with interest the article by DC Rowlands[1] as I have recently applied this technique to a wide variety of gynaecological conditions. The material and methods used were identical to those used by Rowlands. Some of my findings are summarised in table 1.

I studied some 25 cases comprising eight normal junctional cervices, three CIN I, eight CIN II, and eight CIN III. Although both investigations show a similar trend in terms of AgNOR number, the results differed considerably in magnitude, most importantly in the cases of normal tissue and CIN III (table 2). This perhaps serves to emphasise the degree of ambiguity which can arise during the counting procedure, particularly with respect to the large number of AgNOR aggregates present in CIN III. Having established normality, the "analysis of variance technique" indicated a significant difference between normal tissue and cases of CIN and between CIN III and the other two CIN grades. The AgNOR technique as used here, however, cannot be used to distinguish between CIN I and II. The important finding is that on the basis of Agnor counts I was able to distinguish cases of CIN from normal junctional cervix and to show very effectively cases of CIN III.

An important difference between the method used by Rowlands and my own was that the former counted AgNOR's in the basal half of the squamous epithelium while I

Table 1 *Summary of results for gynaecological AgNOR study*

Tissue	*Pathology*	*Mean AgNOR count*	*SD*
Endocervix	Normal*	1·19	0·32
	Inflamed	2·13	0·24
	Adenocarcinoma	4·08	0·73
Junctional cervix	CIN I	2·43	0·22
	CIN II	2·98	0·53
	CIN III	5·19	0·68
	Carcinoma (WD)†	1·70	0·44
	Carcinoma (PD)‡	3·62	0·75
Endometrium	Normal	2·47	0·31
	Adenocarcinoma	3·12	0·60
Myometrium	Leiomyoma	1·08	0·27
	Leiomyosarcoma	2·20	0·52

*Same value applies for normal junctional cervix.
†Well differentiated.
‡Poorly differentiated.

Table 2 *Comparison of mean (SD) AgNOR counts for both CIN studies*

Pathology	*Rowlands study*	*Wood study*
Normal	2·14 (0·209)	1·19 (0·32)
CIN I	2·1 (0·182)	2·43 (0·22)
CIN II	2·27 (0·162)	2·98 (0·53)
CIN III	2·86 (0·259)	5·19 (0·68)

enumerated AgNOR's within nuclei below the level of cytoplasmic maturation.

Biochemical studies have shown the importance of NOR's in ribosome production,[2] and this unit underlies the process of cellular protein synthesis. This technique, however, relies on NOR-associated proteins for its success and it must be shown that these molecules have an equally vital role. Ultrastructural studies have shown that NOR-associated proteins are localised within the fibrillar centre (pars amorpha or pars fibrosa) and dense fibrillar zone. The degree of silver staining here greatly diminishes with decreased ribosomal RNA synthesis, suggesting an association between NOR-associated proteins and active ribosomal gene transcription.[1]

My findings indicate that the AgNOR number may correlate with the biological activity of the cell, and hence provide a useful index for use in diagnostic pathology. This phenomenon has been noted in non-Hodgkin's lymphomas[3] and in carcinomas of the skin[4] and breast.[5] Some caution however, is required as it is by no means a universal test for malignancy. The method has its peculiarities as exemplified by my own finding that tissue exhibiting CIN III appparently has a greater replicatory rate than does either type of carcinoma investigated.

Although little is known about the biochemistry associated with this method, its increasing use in histopathology has prompted important studies into factors which may prove detrimental to its performance, most notably the effect of various routine tissue fixatives.[6]

Probably the most important points to emerge from my investigation is that this technique does have a potential role in gynaecological pathology. Its use in the diagnosis of CIN may be questionable, but I believe it provides a useful companion to the haematoxylin and eosin stained section. The benefits of this method in other areas are more obvious than my results would indicate; the counting of AgNOR's rather than mitoses is a far more expedient way of diagnosing a leiomyosarcoma.

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Dr Rowlands comments:

It is very interesting that, in contrast to my study, Mr Wood was able to distinguish between normal cervical epithelium and epithelium showing CIN. In particular, he found a high AgNOR count in CIN III, making the technique very useful in assessing those biopsy specimens which are difficult to interpret using only sections routinely stained with haematoxylin and eosin. Mr Wood's results and conclusions concur with the study recently published by Egan *et al.*[1]

Mr Wood found significant differences between normal ectocervical epithelium and CIN grades I and II because he found newer AgNORs in normal epithelium than I did. He points out that the different results between the studies may be explained by our counting different areas of the ectocervical epithelium. This is particularly important in normal epithelium where the AgNORs are difficult to identify in the pyknotic superficial cells, and interestingly are more numerous in the parabasal cells than the cells of the basal layer. These variations have also been described by Egan *et al.*[1] I found that another problem in counting AgNORs of normal epithelium was the difficulty of obtaining biopsy specimens devoid of the common pathological changes that occur in this region, such as squamous metaplasia, koilocytosis, or mild inflammation, which probably affect the number of AgNORs. Any further studies or applications of this technique on cervical pathology need to take these factors into account.

I cannot explain the difference between the two studies of the AgNOR count observed for CIN III. Mr Wood refers to the difficulty in enumerating single AgNORs among larger clumps, a problem that has been addressed by Crocker *et al.*[2] I, however, did not observe clumps of AgNORs and do not consider this a problem in this situation. In my study I stained sections with the AgNOR solution for one hour, which may have been too long. It has been realised that shorter staining times make it easier to count the individual AgNORs where they occur together in clumps, and that longer staining times cause a clump of AgNORs to appear as a single large dot.[3] It may be that the ideal