reference to other papers and reflects the confusion existing at the time of their publication. More recently published work, particularly by Current *et al*, has largely established the classification and life-cycle, although some areas of uncertainty remain.[1 3] Although it is true to say that most infections in man and in livestock mammals are with a single species, probably *Cryptosporidium parvum*, the parasite is no longer regarded as a single species genus, there being at least four distinct named species.[3] Shepherd *et al* refer to "sporocysts" and to "so called oocysts". The sporogenous developmental stages share some features of both oocysts and of sporocysts of other coccidia. The stage excreted in the stool, however, should correctly be referred to as an oocyst.[3]

Other studies on oocyst excretion were briefly reviewed and a claim made to this being the largest series (49 subjects) reported so far. Other excretion studies not cited include the first such detailed report on 33 patients in Liverpool,[4] on 50 patients in Finland,[5] and on 91 patients in north Wales.[2] Some of the differences in findings referred to by Shepherd *et al* probably result from the effects of statistically small sample sizes, variable sampling patterns, and to variations in laboratory methodology. Attention is drawn by Shepherd *et al* to the continuation of excretion of oocysts after diarrhoea has stopped. Lack of correlation between diarrhoea and oocyst excretion in some patients has been noted previously.[4] The finding of a continuation of symptoms beyond the last direct smear positives for oocysts is not uncommon and may be attributable to several possible factors, including continuing infection with intermittent or low level oocyst excretion, post-infective or residual damage to the intestinal mucosa, or extra-intestinal infection. The statement that "oocysts had cleared completely", which presumably refers to direct (unconcentrated) faecal smears, needs to be qualified. The modified Ziehl–Neelsen stain is not as sensitive as some other staining methods such as phenol-auramine. Concentration by an appropriate method or immunofluorescence, which are not mentioned, may continue to detect oocysts after Ziehl–Neelsen stained direct smears have become negative. The importance of symptoms other than diarrhoea is now well recognised. Although excretors without diarrhoea may provide a reservoir of infection, however, it may be argued that diarrhoea is the most important symptom in relation to the potential for transmission.

There is little reliable evidence on the incubation period and numerous confounding factors hinder attempts to arrive at an estimate in most cases, because of the complex epidemiology of the infection. The review paper cited to support speculation on the effect of age on the incubation period refers to experimental animals and not to patients. There is no evidence that the incubation period in man, as opposed to animals, is directly influenced by the age of the subject. The reasons for the apparent age dependency of susceptibility to infection in some animals but not in man are not yet clear. The differences in age specific incidence in man are probably related to exposure and hence to immune state. This may not be the case in some animals species as the phenomenon of decreasing susceptibility with increasing age has been detected experimentally in previously unexposed young gnotobiotic or specific pathogen free animals (Dr K W Angus, personal communication).

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Nucleolar organising regions in cervical squamous pathology

The variation in nucleolar organising regions (NORs) stained by a silver technique was investigated on the following types of squamous cervical tissue: normal, atrophy, chronic cervicitis, squamous metaplasia, papilloma virus infection (HPV), cervical intraepithelial neoplasia grades I-III, and invasive squamous carcinoma (table 1). Our

Table 1 *Mean AgNOR count in the various types of tissue studied*

Group	Mean AgNOR count
Normal	1·31
Atrophic	1·09
Chronic cervicitis	1·47
Immature squamous metaplasia	1·80
Koilocytosis only	1·54
CIN I	1·65
CIN II	2·10
CIN III	2·36
Invasive squamous carcinoma	2·46

findings did not agree with those of Rowlands, who performed a similar, though less extensive, study of AgNOR's in cervical intraepithelial neoplastic tissue.[1]

Unlike Dr Rowlands, who found a significant difference in AgNOR counts between normal, CIN I, and II biopsy specimens compared with those showing CIN III, we did not. In our study there was a gradual increase in AgNOR counts with increasing severity of CIN, but there was an overlap between each group, with a significant difference observed only between normal biopsy specimens and those showing CIN III.

In the nine groups of cervical tissue samples studied, 10 biopsy specimens from each group were examined with an extra five biopsy specimens in the invasive carcinoma group. We used the same standardised silver staining method for NORs as Dr Rowlands had used, but our counting procedure was different. Counts were made only on the basal cells in all groups except those showing CIN or invasive carcinoma; for these counts were made on the full thickness of the epithelium occupied by morphologically abnormal cells. All cases were counted by one observer and then two cases from each group were recounted by the same and a different observer. The AgNORs in 200 cells were counted in each case. This method of counting gave a lower mean AgNOR count in normal biopsy specimens and those showing CIN of all grades than was observed in the published study.[1] While counting AgNORs in CIN, we observed occasional pockets of increased counts, reflecting increased activity.

Scheff's test was applied to the results which showed significant differences among the groups (table 2).

Our study showed that the use of AgNOR counts in cervical (squamous) pathology is of limited value and gives no extra information over that gained by morphological examination of conventional, haematoxylin and eosin stained sections.

Table 2 *Categories between which significant differences in AgNOR counts were observed* (p ≤ 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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Reference

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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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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