clinic. They either had diagnosed cervical chlamydial infection and were being seen at second visit, or were considered to be at risk for this infection because they had pelvic inflammatory disease or because they or their partner had gonorrhoea. Swabs were taken from the endocervix and, using a proctoscope, from the rectal mucosa avoiding gross faecal contamination. One of the swabs from the rectum was first used to make a smear for a direct immunofluorescence slide test (Imagen, NovoBiolabs) before being transferred to tissue culture transport medium. Specimens in their respective transport media were inoculated into McCoy cells treated with cycloheximide or were tested in a chlamydial antigen detecting ELISA (IDEIA, Mark 2; Boots-Celltech, Novo Biolabs). The latter specimens were also tested in the Mark 3 form of the test, modified to eliminate non-specific reactions with, for example, staphylococcal protein A.

None of the 84 women studied had overt proctitis. *C trachomatis* was isolated from the cervix of 39 [46·4%] and from the rectum of eight [9·5%] of the women, none of whom was in the group who admitted rectal intercourse. Positive rectal cultures were found only in those women who were also positive in the cervix, but cervical inclusion counts showed no correlation with rectal positivity, suggesting that factors in addition to autoinoculation, such as partiality of *C trachomatis* serovars for different sites,[3] may also be important. In all cases the rectal inclusion counts were less than 20, which leads us to suspect that we may be underdiagnosing rectal carriage, a situation which might be remedied by sonication of specimens before tissue culture inoculation. Nevertheless, the rate of rectal carriage found is significant and should be considered in determining the antibiotic regimens required to eradicate *C trachomatis* infection in women, particularly where pelvic infection is present, and in deciding which sites should be sampled in follow up.

Although chlamydial antigen detection tests are not recommended by manufacturers for diagnosis with specimens other than from the genital tract of adults, they have been and are being used for non-genital specimens, even in low risk groups.[4] Non-cultural methods of diagnosis were therefore included as a comparison with the direct culture method in non-genital specimens from this high risk group.

In the direct immunofluorescence test chlamydial elementary bodies were found in seven of the eight culture positive rectal specimens, although the numbers of elementary bodies bore little relation to the numbers of inclusions found in tissue culture. This result, however, has to be judged against the finding of 15% false positive smears from women who were culture negative in both cervix and rectum; and this, despite the rigorous application of criteria for numbers, size, shape, colour and intensity of fluorescence of elementary bodies.

The problem of specificity of the IDEIA Mark 2 when testing rectal/faecal specimens is well recognised,[5] so that finding 55% false positive results among rectal specimens from women who were culture negative in both cervix and rectum was not unexpected. Although this figure improved when the same specimens were tested in the modified IDEIA Mark 3, it was still too high at 20% to allow this test to be used in diagnosis of rectal chlamydial infection.

Under certain conditions the presence of *C trachomatis* in children indicates that sexual activity has occurred. If, in such low risk groups, cultural methods may result in underdiagnosis and antigen detection methods show high false positive rates,[4] the implications where sexual abuse is suspected may be serious.

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Cytomegalovirus and autoimmune liver disease

There are three major liver diseases for which no infectious or toxic causal agent has been identified, but in which the hepatic parenchyma is damaged: primary biliary cirrhosis (PBC); autoimmune (lupoid) hepatitis; and primary sclerosing cholangitis (PSC). Autoantibodies are common in PBC and lupoid hepatitis, suggesting an autoimmune process. This has been considered for PSC as well, but evidence is scanty. Among other possibilities, it has been suggested that occult viral infection might have a role in initiating these three diseases. Cytomegalovirus (CMV), which is common in the general population, is a potential candidate, having already been implicated in a paucity of intrahepatic bile ducts in neonates and after orthotopic liver transplantation.[1,2] CMV also causes acute hepatitis in immunocompromised patients and, occasionally, in immunocompetent hosts,[3] and like PBC, can have granulomatous features.[4] Moreover, CMV probably causes disease not only by lysing infected cells, but also by interacting with and changing the host immune system.[5,6] Ways in which it might do this and thereby induce an autoimmune process include perturbation of cell membranes of non-productively infected cells, and expression of viral proteins by such cells, leading to a T cell response or to stimulation of local interferon release.[6] In such circumstances productive viral replication would not necessarily occur and the classic cellular enlargement and inclusion bodies of productive infection would not be recognised. Yet, for CMV to be considered pathogenic, it must be identified at the affected site. We therefore used both immunoperoxidase and in situ hybridisation techniques to look for early viral antigens (which may be produced without complete viral replication) and viral nucleic acid, respectively.

Biopsy specimens from eight patients with PSC, seven with autoimmune hepatitis, and 15 with PBC were examined. Two of the patients with PBC had two biopsy specimens each. Thus 32 formalin fixed, paraffin wax-embedded biopsy specimens were studied. Sections were examined using commercially available primary antibodies to early and late viral antigens (Dako Inc.) and DNA probes (Enzo Inc.). Biopsy specimens from patients with CMV oesophagitis were used as positive controls. These specimens had been processed in the same way as those from our study cases and had typical cytomegalic cells present in all levels as well as infected cells identifiable only by the special staining techniques. Appropriate method controls

were also used. The immunoperoxidase staining was performed according to the manufacturer's recommendations, modified by the substitution of CMV negative human serum for the manufacturer's horse derived blocking serum. This modification was made to eliminate possible non-specific binding of antibodies to the Fc receptor produced by the virus. The in situ hybridisation process was modified by carrying out the denaturation step at 80°C, thereby minimising non-specific background staining due to endogenous biotin.

None of the specimens showed positive staining for components of cytomegalovirus with either technique. We have thus not been able to support our hypothesis. The possibility remains, however, that CMV might act as an initiator in primary biliary cirrhosis, primary sclerosing cholangitis, or autoimmune hepatitis, but by the time the advanced disease process has been recognised the virus has already been eliminated.

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AgNORs in diagnosis of serous and mucinous ovarian tumours

In view of the recent interest in AgNORs as a tool in diagnostic histopathology and their recent application to problematic epithelial tumours[1 2] we examined their potential as a diagnostic aid in serous and mucinous ovarian tumours.

The following tumours were selected from formalin fixed, paraffin wax embedded archival material: mucinous cystadenomas (n = 10); borderline tumours (n = 10); cystadenocarcinomas (n = 7); serous cystadenomas (n = 10); borderline tumours (n = 10); and cystadenocarcinomas (n = 10). Silver staining was performed according to the method of Crocker and Nar.[3] The silver solution was in contact with the slide for one hour. Two hundred nuclei were examined in each tumour. In most preparations the nuclei contained large homogeneously staining structures resembling nucleoli, some of which showed denser areas suggestive of internal structures. We followed a similar policy to Howat *et al*,[4] counting each such structure as one AgNOR, and did not attempt to count intranucleolar structures which, in formalin fixed tissue, are difficult to resolve. Tumours with higher AgNOR counts also have variable numbers of small dispersed intranuclear AgNORs which are a feature of malignant cells.[2]

The figure shows the distribution of AgNORs in the six types of tumour. Mucinous cystadenomas had significantly lower counts than borderline tumours and cystadenocarcinomas ($p \leqslant 0{\cdot}01$), but no significant difference was found between mucinous borderline tumours and cystadenocarcinomas. Serous tumours showed no significant difference between cystadenomas and borderline tumours, but cystadenocarcinomas had significantly higher AgNOR counts than borderline tumours.

AgNOR counts may therefore be useful in distinguishing borderline serous tumours from serous cystadenocarcinomas, but do not seem to be useful in mucinous tumours. The full potential of the AgNOR method may not be achieved in formalin fixed tissue because of the tendency of a variable proportion of the nucleoli to stain homogeneously, obscuring the internal structure of arrays of dot-like NOR-associated proteins seen in air dried and alcohol fixed smears.[5] This raises the question of whether the pathologist should attempt to count partially fused intranucleolar dots, a process which is both tedious and subjective or, as we have done in this study, count only clearly discrete structures. Howat *et al* have examined this problem in detail.[4] In studies where the nucleolus stains homogenously[1 2] and is therefore counted as one AgNOR it may conceal many dot-like NOR-associated proteins. One nucleolus may also be comprised of several loops of chromosomal rDNA (NORs) as several NOR-containing chromosomes may contribute to forming one nucleolus. Thus counting one nucleolus as one AgNOR gives no information about the activity of NORs either at chromosomal or transcriptional levels.

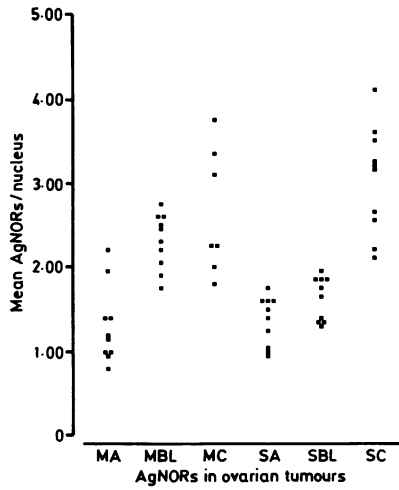

Figure *Mean counts of AgNORs per 200 nuclei in ovarian tumours: mucinous (MA), borderline tumour (MBL), and cystadenocarcinoma (MC); serous adenoma (SA), borderline tumour (SBL), and cystadenocarcinoma (SC).*

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