LETTERS TO THE EDITOR
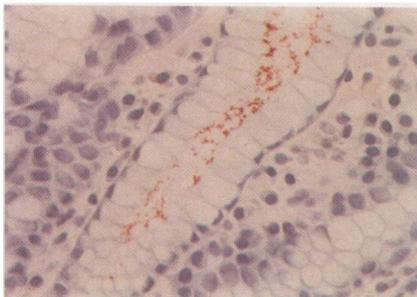
Immunocytochemical detection of Helicobacter pylori in formalin fixed tissue biopsy specimens

We have read with great interest the many excellent articles published in this journal concerning *Helicobacter pylori* infections.[1] Several of these reports have used histological examination as a reference standard for the comparison of other methods to identify patients with *H pylori* infections. Staining of tissue sections with haematoxylin and eosin may not prove sensitive in the identification of small numbers of *H pylori* organisms. Histochemical stains such as Giemsa and Warthin-Starry can prove difficult to interpret because of unwanted background staining. Additionally, these special histochemical stains do not distinguish *H pylori* from other spiral Gram negative organisms which have now been reported to occur in the stomach.[2]

Previous reports have shown the usefulness of immunocytochemical methods that use monoclonal antibodies in the identification of *H pylori*.[3 4] These antibodies however, are not commercially available nor do they seem to be applicable to formalin fixed, paraffin wax embedded tissue biopsy specimens. Preliminary studies performed in our laboratory using a commercially available monoclonal antibody (Bioproducts for Science, Inc., Indianapolis, USA) which reacts with a formalin resistant flagellar epitope on *Helicobacter* organisms produced promising results in identifying *H pylori* in formalin fixed, paraffin wax embedded tissues.[5]

We have now investigated the gastric biopsy specimens from 130 patients for the presence of *H pylori* with this monoclonal antibody. The use of a sensitive avidin-biotin immunoperoxidase labelling system allows the monoclonal antibody to be used at a dilution of 1/200000, making it very cost effective. Crisp, clean immunoreactivity is obtained which is easily recognised (figure). Immunolabelled specimens can be screened under low power magnification due to the high signal:noise ratio. No labelling of other Gram negative bacteria or normal tissue elements has been observed.

In a recently concluded study with Drs Scholes and Santogade at St Luke's/Roosevelt Hospital Center (New York) we

Immunoperoxidase labelling of Helicobacter pylori within a gastric foveola (formalin fixed, paraffin wax embedded gastric biopsy) specimen.

had the opportunity to apply our immunocytochemical technique to 115 biopsy specimens from 51 patients where tissue was also cultured for the presence of *H pylori*. All 24 patients with a positive tissue culture had positive immunoreactivity with Helicobacter monoclonal antibody. Immunoperoxidase labelling identified five additional positive specimens that were culture negative. There were no false positive results.

Immunoperoxidase labelling has now become the routine method for *H pylori* identification in tissue biopsy specimens at our institution. This method is attractive because all tissue fragments can be submitted to the surgical pathology laboratory in formalin fixative, thereby reducing potential sampling errors. The resulting immunoreactivity is easy to identify, making screening for *H pylori* less tedious. *H pylori* organisms can also be distinguished from contaminating bacteria. Based on our experience with this monoclonal antibody and immunoperoxidase labelling, we recommend immunocytochemistry for the sensitive and specific identification of *H pylori* in processed tissues.

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AgNOR counts in intraendometrial neoplasia

Many classifications of endometrial hyperplasia have been suggested, including that of intraendometrial neoplasia (IEN grades I-III).[1] Although this term is not in common use and has been criticised,[2] we have found it to be a simple and reproducible way of describing increasing grades of architectural, cellular, and nuclear atypism in dysplastic endometrial proliferation—that is, confined above the myometrium. Recently the enumeration of silver stained nucleolar organiser regions (AgNORs) in tumour pathology has yielded a wide range of conflicting results.[3 4] Consequently we performed the AgNOR technique on IEN lesions, as well as on normal and frankly malignant endometrial tissue.

This technique was applied to 56 endometrial curettage samples[3] which were
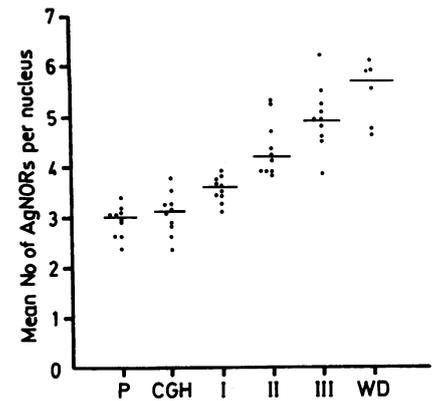
Mean number of AgNORs in each nucleus in proliferative endometrium (P), cystic glandular hyperplasia (CGH), IEN (I, II, and III) and well differentiated adenocarcinoma (WD). Bars = median values.

grouped by a separate observer into the following categories: normal proliferative (n = 10); cystic glandular hyperplasia (n = 10); IEN I-III (10 cases for each grade); and well differentiated adenocarcinoma (n = 6). Myometrial invasion was ruled out in IEN III by subsequent examination of the hysterectomy specimens. One hundred randomly selected cells were counted for each case using a × 100 oil immersion lens.

Statistical analysis was performed using the Mann-Whitney U test (figure).

The lowest AgNOR counts were seen in normal (median 2·99) and hyperplastic (median 3·12) tissue; intermediate counts in IEN I (median 3·58); II (median 4·19), and III (median 4·93); and highest counts were obtained in adenocarcinoma (median 5·72). There was considerable overlap among the groups. Significant differences (p ≤ 0·05) existed between the following groups: (1) benign endometrium (proliferative, cystic glandular hyperplasia) and IEN; (2) benign endometrium and adenocarcinoma; (3) IEN I and the two other IEN grades; (4) IEN I and adenocarcinoma; (5) IEN II and adenocarcinoma.

IEN denotes dysplastic intraendometrial growth including adenocarcinoma in situ (IEN III), which is considered to be a precursor to adenocarcinoma. Despite the lack of morphological criteria to predict the outcome of endometrial dysplastic lesions, morphometrical studies indicate that cases with larger and more pleomorphic nuclei progress to cancer.[5] Our results show that AgNOR counts may reflect on increase in nuclear activity with this increase in nuclear atypism and hence IEN grades.

Our conclusion is that despite significant differences, AgNOR counts are of no use for diagnosis of any single case in the studied groups because absolute differences between counts for the various categories were small.

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