

Letters to the Editor

Prognostic importance of nucleolar organiser regions in Ewing's sarcoma of childhood

Nucleolar organiser regions are loops of DNA which occur in cells that possess ribosomal RNA genes. They are of importance with respect to the ultimate synthesis of protein and may maintain the extended configuration of DNA or regulate transcription. They may represent ploidy or act as a replicatory marker.

Whatever their precise nature, they have been observed for over a decade and are best visualised using a silver colloid technique. They have previously been the province of cytogeneticists until recent modifications enabled a one step method to be applied to paraffin sections at 20°C. Recently the technique has been used to investigate certain malignant tissues including non-Hodgkin's lymphomas, melanocarcinoma, and naevocellular naevi, breast lesions, skin tumours, and small round cell tumours of childhood. The enumeration of nucleolar organiser regions in these studies was found to be diagnostically useful.

This silver colloid technique for nucleolar organiser regions (AgNORs)¹ was applied to 20 specimens of Ewing's sarcoma of bone from the same number of patients aged 2 to 12 years. The tumours examined were from various sites including ribs, femur, and pelvis, and included diffuse, lobular, filigree and trabecular types. The clinical details and outcome of these patients were known and the mean number of AgNORs/200 cells for each case was calculated and compared to determine their clinical importance. In all cases the specimens had been characterised using the existing battery of special diagnostic procedures, including immunohistochemistry and electron microscopy.²

The mean number of AgNOR/cell was 9.66 and the range was 7-10.7. There was no significant correlation between the number of AgNORs and survival, sex, site, age or histological type.

This investigation found no prognostic importance in the mean number of AgNORs in Ewing's sarcoma of childhood. This simple technique, which may be of value in investigating certain human malignant tissues, has no apparent prognostic value in Ewing's sarcoma of childhood.

M EGAN,
F RAAFAT,
J CROCKER,
D WILLIAMS

Department of Pathology,
The Royal Hospital,
Wolverhampton WV2 1BT

References

- 1 Crocker J, Nar P. Nucleolar organizer regions in lymphomas. *J Pathol* 1987;151:111-8.
- 2 Variend S. Small cell tumours in childhood—a review. *J Pathol* 1985;145:1-25.

Improved visualisation of mucus penetration by *Campylobacter pylori* using a Brown-Hopps stain

When Warren first described the finding of *Campylobacter pylori* in active chronic gastritis,¹ he used the Warthin-Starry stain to visualise the organism. This stain has been adopted by many other investigators but has several drawbacks. Even though it shows the organism very well, it is time consuming and costly. We have also noted that interbatch variation can be a problem if the technique is not used on a regular basis by the same technician.

Other investigators have looked for alternative stains such as Giemsa.² We have also used the Giemsa stain and found it to be very good for identifying the organism. Because of lack of counter staining with the Giemsa technique, however, some information about the surrounding tissues is lost or not readily apparent. In particular, we have been disappointed with both Giemsa and Warthin-Starry stains when it comes to visualising the intraluminal mucus layer which the organisms often inhabit.

The Brown-Hopps staining technique has been used to visualise bacteria in tissue.³ It uses crystal violet and Gram's iodine solution for the initial staining, but differentiation is done using Cellosolve. Counter staining is done with basic fuchsin and tartrazine. The basic fuchsin is differentiated and fixed using Gallego's differentiating solution. This staining technique can be used on tissues fixed in either formalin, glutaraldehyde, or formol-Zenker's fluid.³

Because the Brown-Hopps stain promised to give better results of the surrounding tissues, we recently used it on our gastric biopsy specimens to compare its properties with the Giemsa stain. We were pleased to find that not only did we have a 100% correlation with Giemsa, when it comes to identifying the organism, but we achieved a striking improvement in the amount of information we could read from the tissue sections. Most notably, the mucus layer itself

was easily identified (staining yellow) and gave us the opportunity to assess fully mucus penetration of *C pylori*, which stains purple. We can now measure the density of organisms inside and outside the mucus as well as the thickness of the mucus layer itself. We find this to be very valuable information, particularly in light of proposed theories by some investigators that the organism may change the characteristics of the mucus once it starts colonising it.

These striking features of the Brown-Hopps stain convinced us that it is superior to both Warthin-Starry and Giemsa, and we plan to use it as our primary stain in the future.

TU WESTBLOM,

*E MADAN,

*JUDITH KEMP,

MA SUBIK,

JENNY TSENG

Departments of Medicine and *Pathology,
Marshall University School of Medicine,
Huntington, West Virginia 25701-5490
USA

References

- 1 Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273.
- 2 Gray SF, Wyatt JI, Rathbone BJ. Simplified techniques for identifying *Campylobacter pyloridis*. *J Clin Pathol* 1986;39:1280.
- 3 Luna LG. Methods for bacteria, fungi and inclusion bodies. In: *Manual of histologic staining methods of the armed forces institute of pathology*. New York: McGraw Hill, 1968: 224-5.

Parasites in faecaliths

Faecaliths are commonly found in resected appendices—up to 67% in one series.¹ Many faecaliths are large enough to cause obstruction and therefore acute inflammation of the appendix. The formation of faecaliths is thought to be due to slow deposition of faeces around a nidus of foreign matter such as food particles, and possibly dead parasites or their ova.

In a recent case in which a 35 year old man presented with signs and symptoms of acute appendicitis, an ultrasonogram of the appendiceal mass showed a 2 cm faecalith obstructing the lumen of the inflamed appendix. Subsequent appendectomy confirmed the ultrasound findings (fig 1). After decalcifica