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) 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.

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.

References

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, 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. 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. 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.

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.


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Parasites in faecoliths

Faecoliths are commonly found in resected appendices—up to 67% in one series. Many faecoliths are large enough to cause obstruction and therefore acute inflammation of the appendix. The formation of faecoliths 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 faecolith obstructing the lumen of the inflamed appendix. Subsequent appendicectomy confirmed the ultrasound findings (fig 1). After decalcification and histological examination, a large faecolith was confirmed and this was studied microscopically for evidence of parasites. The faecolith was examined using routine haematoxylin and eosin stains and showed numerous parasites, the organisms were not specific yet were similar to those described in other cases. A large faecolith was removed and submitted for examination. The faecolith contained many faecaliths or faecal concretions which showed numerous parasites. The faecoliths were reviewed using routine haematoxylin and eosin stains and showed numerous parasites, the organisms were not specific yet were similar to those described in other cases.
Cost effectiveness of routine necropsy renal histology

The recent work by Reid\(^1\) suggests that routine unselected necropsy histological examination is not cost effective for diagnostic purposes. I analysed the findings of the routine histological examination of kidneys in 50 consecutive necropsies performed in the department of pathology at this university. The results suggest a much lower level of cost effectiveness (as far as the kidneys are concerned) than was found by Reid.\(^1\)

In Hong Kong cases referred to the coroner are: sudden death of unconfirmed cause; death as a result of accidents; homicides; death while in custody; suspected neglect. The coroner usually orders necropsies to be performed. Coroners' cases were chosen for this study as their clinical histories were usually brief and they were more likely to yield incidental but clinically important findings on necropsy. The request forms and clinical histories were reviewed; the complete necropsy reports and all the slides had already been prepared.

The total number of blocks sampled in the 50 cases studied was 890 (average 17.8 per case), of which 100 (average two per case) were of kidney tissue. Eight necropsies were on patients with a definite clinical history, for which histological examination of the kidneys was indicated. These included four patients who had a long history of diabetes mellitus; one in chronic renal failure who had been receiving haemodialysis for three years; one with suspected massive rhabdomyolysis, and finally, two heroin addicts, one of whom died of an overdose of heroin and alcohol and the other of fulminant pulmonary tuberculosis. Gross focal lesions were observed in four additional patients; these included a medullary fibroma 0.3 cm in diameter, acute pyelonephritis in a hydronephrotic kidney, pyonephrosis complicated by perinephric abscess and papillary necrosis, and the incidental finding of a 3 cm diameter renal cell carcinoma in a 60 year old man.

Asymmetrical coarse cortical scars were described in a further five patients; the scars were associated with distortion of renal calyces in one; the final diagnoses were nephrosclerosis in four patients, with no formal diagnosis given in the final report for the other patient. Congestion was the only remark made on gross examination of the kidneys in six other patients. Benign nephrosclerosis was diagnosed in nine patients; the gross finding in the kidneys of these patients was generalised cortical thinning. A small number of cortical cysts and cortical thinning were described in an additional three patients. In the remaining 18 patients the kidneys were considered to be normal on gross and microscopic examinations.

In the present study the number of patients showing grossly normal or near normal kidneys was 33, according to the criteria suggested by Reid.\(^1\) Patients with grossly abnormal kidneys or a clinical history indicating histological examination numbered 17. The corresponding figures reported by Reid were 77 and 83, respectively. The result of the two studies differed at a level of p < 0.05 (\(\chi^2\)). While I included the five patients with asymmetrical coarse renal scars among the cases indicated for renal histological examination; little useful information was obtained from this examination. If the five cases were to be deleted from the 17 cases indicated for renal histological examination, the difference in the results between the two studies would become more significant.

The results of the present study not only support the findings of Reid,\(^1\) they further suggest that an even greater increase in the cost effectiveness of necropsy histology may be achieved by selectivity than has been suggested. It is worth noting that the policy of non-selective necropsy histology has been considered to have contributed to the recent decline in the number of necropsies performed.\(^3\)

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References
