

## Letters

### Vitamin K and metabolic bone disease

The report by Vermeer and colleagues<sup>1</sup> that vitamin K may be important in the causation of metabolic bone disease is interesting, but there is an alternative hypothesis that could also explain some of the apparently vitamin K mediated bone effects. Vitamin B-6 is an essential cofactor for the enzyme ornithine decarboxylase which is the rate limiting enzyme in the formation of putrescine which regulates osteoblast glucose-6-phosphate dehydrogenase activity and thus osteoblast NADPH concentrations.<sup>2-5</sup> NADPH is essential for the vitamin K cycle in which the epoxide form of vitamin K is converted back to the naphthoquinone form which is required for  $\gamma$ -carboxylation of osteocalcin.<sup>6</sup> It is therefore possible that vitamin B-6 status could modulate the effects of vitamin K on bone metabolism.

Although the chain of events described above may seem excessively complicated there is evidence that vitamin B-6 deficiency in rats reduces bone healing<sup>7</sup> and that vitamin B-6, assayed by high performance liquid chromatography,<sup>8</sup> is lower in patients who spontaneously (traumatically) fracture their hips than in patients whose hip fracture is at the hands of an orthopaedic surgeon as an elective procedure.<sup>9</sup> Further research into the interaction of these two vitamins may be indicated.

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### Variability of immunohistochemical reactivity on stored paraffin slides

We read the paper by Berthau and colleagues<sup>1</sup> with interest. We have made similar observations in our laboratory while investigating the expression of the androgen receptors (AR) and epidermal growth factor receptors

(EGFR) in sections from formalin fixed, paraffin embedded archival prostatic specimens. Studies on the heterogeneity of androgen receptor expression in prostate cancer have focused exclusively on epithelial cells,<sup>2</sup> while reports on the EGFR content of similar tissues have produced conflicting results.<sup>3,4</sup> We aimed to determine the differential expression of androgen receptors in the stromal and epithelial compartments of prostate cancer glands, and also to scrutinise these neoplasms for the presence of a variant-type EGFR (EGFRvIII). As part of the initial tests to ascertain the validity of our results, we sought to exclude time related antigen degradation by investigating the effect of storage (at room temperature) on the intensity of staining in serial paraffin sections. During these tests we used five different antibodies which recognised antigens in the nucleus (anti-AR NCL-2F-AR, Novocastra), in the cytoplasm (anti-EGFRvIII; produced by Drs Moscatello and Wong, Kimmel Cancer Institute, Philadelphia, USA<sup>5</sup>), and on the cell membrane (anti-EGFR clone 31G7, Cambridge Bioscience; anti-prostatic specific antigen [PSA], Europath; and anti-prostatic acid phosphatase (PAP), Sigma). Three of these antibodies required pretreatment of the sections (microwave, anti-AR, and anti-EGFRvIII; or protease, anti-EGFR) for antigen retrieval, while the other two (anti-PSA and anti-PAP) did not.

In the androgen receptor studies, we found that the intensity of nuclear staining seen in serial sections from androgen receptor positive prostate cancer was dramatically reduced in sections stained after 10 days storage, and was completely lost after three weeks. On the other hand, the intensity of the immunoreactions to the anti-EGFR and anti-PSA were constant for three months but were reduced thereafter, though some staining was still visible in sections that were a year old. However, the intensity of both anti-EGFRvIII and anti-PAP staining remained virtually unchanged in sections that were stored for a year.

From these results we also concluded that there is a variable degree of antigen degradation in stored paraffin sections, and that this loss is proportional to the length of storage. The rapidity of decline in the intensity of staining is also dependent on the antigen, and seems to be highest in nuclear steroid receptors. Similarly, we found that the loss of immunoreactivity was not limited to antigens that require pretreatment for the exposure of the relevant epitopes. We would therefore agree with the authors that previous conflicting results from immunohistochemical studies on archival tissues could be caused in part by differences in the age of stored sections used. We also support their suggestion that the use of unstained paraffin sections that have been in long term storage should be avoided, and that immunohistochemical systems should be checked for age induced antigen degradation before carrying out both diagnostic and research studies, to ensure better standardisation of results. We have followed these protocols in our studies on archival material that have been done after these observations were made.<sup>6</sup>

We would like to thank Ms A Lycett, C Whiteley, and F Wright for their assistance with this project.

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### Sudden infant death syndrome, long QT interval, and *Helicobacter pylori*

In response to the recent editorial by Addison,<sup>1</sup> I would like to outline two potentially important theories on the aetiology of SIDS which, if true, may act in concert. First, long QT interval. Schwartz *et al* performed electrocardiography on 33 034 newborns at three to four days of age and subsequently found that of the 34 deaths in this group, 24 were due to SIDS.<sup>2</sup> Twelve infants who died of SIDS, but none who died from other causes, had prolonged QT intervals (p 0.01). Long QT interval is known to be a marker of cardiac electrical instability and is therefore a predisposition to fatal cardiac arrhythmia.<sup>3</sup> It is also known that the QT interval is increased from the second until the fourth month of life, returning to previous values by the sixth month.<sup>4</sup> This is also the period of peak incidence of SIDS. Therefore, it is hypothesised that a sudden increase in sympathetic activity may be the trigger which leads to fatal arrhythmia in these cases.<sup>2</sup>

Second, neonatal *Helicobacter pylori* infection. As reviewed by Pattison *et al*,<sup>5</sup> epidemiological aspects of SIDS and *H pylori* infection are similar; both are more common in males, poor populations, and large families; both SIDS and *H pylori* infection are associated with growth retardation and familial clustering; and *H pylori* infection has been documented in infants aged three to six months. In an *H pylori* infected neonate, vomiting or gastro-oesophageal reflux of gastric juice containing *H pylori* urease, combined with microaspiration may result in deposition of large amounts of urease in the alveolae, resulting in urea hydrolysis and ammonia supplied to the systemic circulation, with possible respiratory arrest due to ammonia toxicity. In addition, gastric inflammation due to *H pylori* infection results in synthesis of the cytokine interleukin-1 (IL-1), leading to fever, activation of the immune system, and increased deep sleep which, combined with a relatively minor additional infection, overwrapping, or prone sleep position, may lead to hyperthermia.<sup>5</sup>

These two theories are consistent with each other, in that IL-1 is known to activate the sympathetic nervous system in humans. Therefore, in neonates with cardiac electrical instability resulting from long QT interval

and who become infected with *H pylori*, IL-1 mediated activation of the sympathetic nervous system may provide the trigger for fatal arrhythmias leading to SIDS.

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### Epithelial-myoepithelial carcinoma?

In the July edition of this journal, McCluggage *et al* describe a myoepithelial carcinoma arising in a pleomorphic adenoma.<sup>1</sup> The report is interesting but raises the question as to whether the original tumour was in fact an epithelial-myoepithelial carcinoma, with the subsequent recurrences representing overgrowth of the malignant myoepithelial component.

In their description of the original tumour, McCluggage *et al* describe the epithelial element as being set in a hyalinised stroma with other areas showing myoepithelial cells in a myxoid stroma. It is not clear from the illustration, but could the former be the sclerotic pattern of epithelial-myoepithelial carcinoma as described by Simpson *et al*?<sup>2</sup> The encapsulated nature of the first tumour would not be against this diagnosis as this is an accepted feature of epithelial-myoepithelial carcinoma.<sup>3</sup> The subsequent recurrences could then be interpreted as overgrowth of the myoepithelial element, again a well recognised feature of epithelial-myoepithelial carcinoma<sup>2</sup>; this would be supported by the presence of ducts and tubules in the first recurrence.

Although I would not dispute that malignant transformation occurs within pleomorphic adenomas the slightly atypical appearance of the original tumour in this case should perhaps raise the possibility of an epithelial-myoepithelial carcinoma.

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#### Authors' response

We thank Dr Jones for his letter concerning our recent case report of myoepithelial carcinoma arising in a pleomorphic adenoma. We

are confident that the original diagnosis of a pleomorphic adenoma (albeit with some atypical features) was correct and that this neoplasm does not represent an epithelial-myoepithelial carcinoma. Epithelial-myoepithelial carcinoma is characterised by tubules with a double layer of inner epithelial and outer myoepithelial cells. The ducts and tubules in the original tumour and in the first recurrence in the case we describe had a single cell lining. These cells were morphologically and immunohistochemically (AE1/AE3, EMA, and CEA positive; S-100 protein,  $\alpha$  smooth muscle actin, and vimentin negative) characteristic of epithelial cells. We agree that from the illustration (fig 1A) it is not entirely clear whether the tubules have a single or double cell layer but we consider that the impression of more than one cell layer is due to plane of section artefact.

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### Is the practice of haematology evidence based?

For the record, serum ferritin is measured in  $\mu\text{g/litre}$ . Storage iron deficiency may be assessed indirectly by this index when iron metabolism is in equilibrium. For functional iron deficiency the percentage of hypochromic cells is the most direct yardstick, irrespective of the serum ferritin level.

Haematology used to be a laboratory based discipline. Because no result or interpretation could be better than the method by which it was produced it was the haematologist's job to worry about the details so that the clinician could interpret each result with confidence. The increasing emphasis on clinical haematology has meant that some things have had to be left behind and the chief of these is the involvement in the laboratory. Indeed, it is likely that nowadays the most informed opinion on the diagnosis and treatment of iron deficiency may be found within renal units.

Yes, I cavil at the details.

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#### Authors' response

We thank Dr Cavill for his letter, which we read with a wry grin. The data used for serum ferritin were reproduced as they appeared in the text we referred to in our paper. We agree we should have pointed out the inaccuracy of the units used in the original source material but somehow, in our enthusiasm to make our point, we slipped. Sorry!

Dr Cavill's slightly tongue-in-cheek letter does not, however, mask his disappointment at the shrinking laboratory role of many haematologists. We share that disappointment. We agree that on appointment most haematologists undertake both a clinical and a laboratory role. It is one of the consequences of, for example, the reduction in junior doctors' hours that haematologists have now had to dedicate more of their time to clinical activities at the expense of the laboratory work. The 1997 haematology report from the Clinical Benchmarking Company<sup>1</sup> showed that the majority of haematologists spent 45% of their time in activities related to laboratory work. For those laboratories who par-

ticipated in both the 1996 and 1997 clinical benchmarking study, there was a reduction in consultants' laboratory sessions which equated to a 17.5% drop in consultant input into laboratories. This probably reflects the increased commitment haematologists have to clinical duties.

It is clear from our own experience that clinicians who use laboratory services may not interpret the results of diagnostic tests in the same way that an experienced haematologist would. This can result in additional testing and unnecessary referral. The haematologist is, of course, ideally placed to aid clinicians' interpretation of diagnostic tests in haematology. If likelihood ratios are to be used as ways of aiding clinicians, it is difficult to see how anyone other than haematologists can lead this process. We recognise that current manpower limitations might impede changes, even if we do wish to move in this direction. Perhaps this is an area which the British Society for Haematology, the Royal College of Pathologists, and the Association of Clinical Pathologists may wish to review.

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- 1 Clinical Benchmarking Company Haematology Feedback Report 1997. London: CBC, 1998.

## CD-ROM

**Peripheral Blood—Tutor (CD-ROM).** University of Washington. (£450.00.) Lippincott-Raven, 1995. ISBN: 0397553773.

The advent of computer graphics has led to an explosion in material available for teaching, and this is another potential candidate for the list. How does this CD-ROM fair? Does it fulfil its promise of helping you make the right call when looking at peripheral smears?

It is aimed mainly at the laboratory technical staff and possibly junior doctors. Illustrations and concise relevant text give a good overall flavour of morphology but it is not of sufficient depth to be a particularly useful aid to higher specialist training. An accompanying atlas is available but was not reviewed.

A general introduction covers sampling and film preparation, with useful examples of preparation artefacts. Sections on cell morphology and disease associations follow. It is easy to use; however, the menu based format is at times a little cumbersome. A quiz and two final examinations provide an interactive approach. The answers, as promised, provide a rationale for the correct response but the lack of a differential diagnosis detracts from the potential educational value. As with all such products the computer graphics are one of the main limiting factors; some of these images are excellent but others lack clarity, with poorer resolution occurring with image enlargement.

There is no doubt that a CD-ROM is a useful educational tool; given the cost, however, this addition does not offer any