Correspondence

Blue paraffin wax blocks: a solution to the problem

Thick slices of tissue can readily be stained with Mayer's haematoxylin. The ability to study tissue blocks 1000 to 3000 μ m deep after initial examination with conventional 5 μ m sections seems likely to increase the use of the method.¹ However, there remains a potential embarrassment for the practitioner of this technique, and possible frustration for those subsequently wishing to use the paraffin wax embedded material for the study of nuclear associated antigens. Return of tissue blocks to a referring pathologist with an unsightly dark blue hue, or overlaying of nuclei by retained haematoxylin, could prove counterproductive.

We have overcome both these problems with a simple procedure. The thick tissue slices are reprocessed with xylene and graded methanols from methyl salicylate or other clearing agents to isotonic 10% formaldehyde.¹⁻³ The haematoxylin staining is then removed by:

- (1) Immersion in running cold tap water for 30 minutes.
- (2) Soaking in 2% acid alcohol for two hours at room temperature.

(3) Immersion in cold tap water for a further 15 minutes.

(4) Brief storage in formaldehyde before processing for re-embedding in paraffin wax and later sectioning at conventional depth.

The tissue slices become completely decolourised macroscopically (thus avoiding the embarrassment of returning "blue blocks"), and the haematoxylin staining is entirely removed from the block and subsequent 5 μ m paraffin wax sections (figure), thereby allowing any future nuclear immunohistochemistry to be unhindered by the previous haematoxylin staining.¹

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Paraffin wax blocks (2 mm deep) in processing cassettes showing dark blue haematoxylin staining after thick section examination (left), which is entirely removed after the 2 hour acid alcohol treatment (right).

Benign familial hyperphosphatasemia

We were interested to read the paper of Rosalki *et al*¹ which describes in great detail the alkaline phosphatase isoenzyme findings in the largest published series of families with benign familial hyperphosphatasemia. In each family the proband had increased plasma intestinal alkaline phosphatase activity, although increases in the activities of the liver and bone isoenzymes were also found in some cases.

Rosalki *et al* do not state the ABO blood or secretor status of their patients. Inspection of the previously published reports summarised in their paper, however, indicates that in most cases where this information is given, patients were blood

group A. This is of interest because plasma intestinal alkaline phosphatase activity is normally lower in those with blood group A than in those with blood group O or B.² It is also lower in non-secretors of ABH blood group substance than in secretors^{3,4} although secretor status is rarely determined in studies of intestinal alkaline phosphatase activity.

We recently identified a family with benign familial hyperphosphatasemia. The proband was a 44 year old woman with irritable bowel syndrome, who had a very high serum intestinal alkaline phosphatase activity of 250 U/l (upper reference limit 12 U/l). Her sister had a similarly increased serum intestinal alkaline phosphatase activity of 290 U/l, and her son had a slightly

increased serum intestinal alkaline phosphatase activity of 46 U/l (although this was equivocal as he was not fasting when the blood sample was taken). Two daughters had normal serum intestinal alkaline phosphatase activity. The whole family were blood group A. The proband and her son were secretor positive, but her sister and daughters were secretor negative. The interesting feature of this family was the expression of hyperphosphatasemia in a blood group A non-secretor subject, and the finding that the serum intestinal alkaline phosphatase activity in this subject was as high as in her secretor positive sister.

The physiological basis of the relation between the ABO blood group system and serum intestinal alkaline phosphatase activity is uncertain. The genes determining ABO blood group encode glycosyltransferase enzymes, and there is a separate gene which determines whether the blood group antigens are secreted in the free form in body fluids.⁵ These glycosyltransferases might modify the carbohydrate moieties of the alkaline phosphatase molecule and therefore change the rate of clearance by carbohydrate binding receptors.⁶ Alternatively, there is evidence that blood group A erythrocytes bind intestinal alkaline phosphatase with high affinity and thereby enhance the rate of clearance from serum.⁷ Whatever the mechanism, blood group specific differences in serum intestinal alkaline phosphatase activity are probably due to differences in the rate of clearance rather than in the rate of synthesis and release of the enzyme. Our finding of benign familial hyperphosphatasemia with high serum intestinal alkaline phosphatase activity in a blood group A non-secretor subject (who would normally be expected to have very low serum intestinal alkaline phosphatase activity) lends further weight to the suggestion of Rosalki *et al* that the mechanism underlying this condition may be a mutation affecting a gene controlling the post-translational modification of alkaline phosphatase, or alternatively, that it might affect the function of the reticuloendothelial carbohydrate binding receptors which are responsible for the clearance of intestinal alkaline phosphatase.¹ It would be interesting to carry out further studies of the kinetics of clearance and erythrocyte binding properties of intestinal alkaline phosphatase in this group of patients.

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