V is for von Kossa

A-Z of Staining - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.

CellPath
INNOVATION IN CELLULAR PATHOLOGY

Welcome to the ABC of Staining series where we continue with the letter 'V' for von Kossa.

In histology, silver nitrate is a key component of countless silver impregnation staining techniques that are aimed at visualizing diverse tissue components such as reticular fibres, nerve cells, bacteria and fungi. In the von Kossa technique, the chemical solution is used to detect calcium phosphate, a key component of bone mineral. The calcium deposits in tissues such as undecalcified bone are specifically processed so that their mineral content can be preserved. In this method, the silver cations in the silver nitrate solution react with the calcium ions in the bone to produce a temporary yellow colouration. When exposed to sunlight or artificial light source, the silver ions are reduced to black metallic silver. Any unreacted silver remaining in the undecalcified bone sections is removed by rinsing in a solution of sodium thiosulphate, leaving the black metallic silver deposits in situ.

Although counterstaining is optional, tissue sections can be stained with a nuclear dye such as neutral red which contrasts with the black staining of the calcium. Modifications using other counterstains such as van Gieson solution have been applied, which stains collagen and unmineralized bone red, and connective tissues yellow (Figure 1a). The use of MacNeal's tetrachrome (which consists of methylene blue, methylene azure A+B, and eosin) as a counterstain also allows a sharp distinction between mineralised and unmineralized bone (Figure 1b).

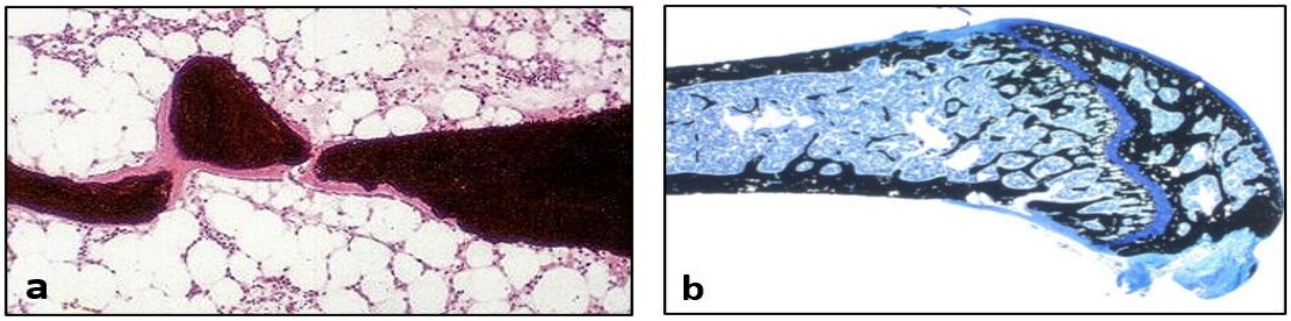

Figure 1. (a) Black bone and red osteoid following staining with von Kossa and Van Gieson. (b) Mouse femur stained with von Kossa and a tetrachrome counterstain

The combination of undecalcified bone sections with the von Kossa stain is a powerful tool for accurately studying the mineral component of bone. Undecalcified bone samples are typically sectioned from samples embedded in methyl methacrylate or epoxy resins. By visualizing the distribution and amount of mineralized bone matrix, histologists are able to understand the development of metabolic bone diseases such as osteoporosis and osteomalacia (rickets). Although layers of unmineralized bone matrix (osteoid) can be found on bone surfaces during formation, the rate of osteoid production and mineralization in healthy adult bones is balanced. However, large accumulations of osteoid in undecalcified bone indicates an imbalance as that seen in the disease osteomalacia. By preparing undecalcified bone sections and staining them with von Kossa, assessment of both unmineralized matrix (osteoid) and the mineralized tissue is possible (Figure 2).

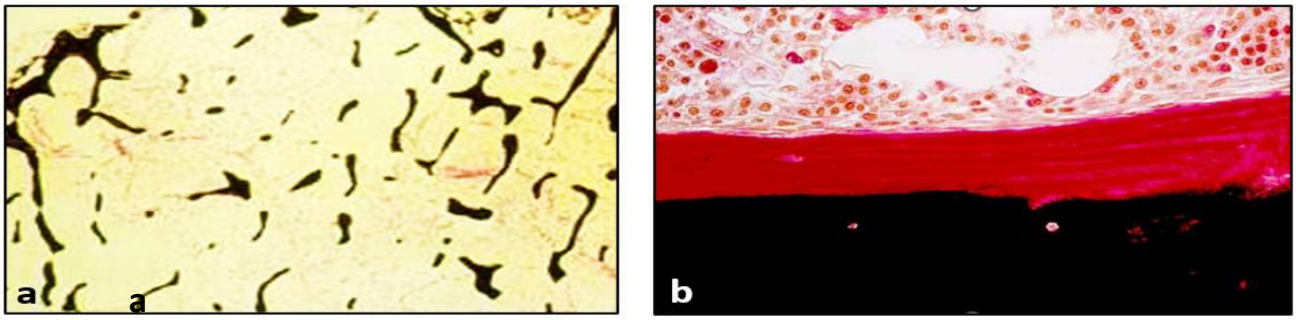

Figure 2. (a) Low bone density in a patient with osteoporosis. (b) The red osteoid seam contrasts with the black bone in a patient with osteomalacia (von Kossa / Van Gieson stain)

As previously described, undecalcified bone sections are thin slices of bone that have not been demineralized and are typically prepared by embedding the bone samples in a methacrylate or epoxy resin. Because the bone is dense, specialist microtomes designed for cutting resin-embedded blocks are often required. However, for laboratories who are unable to handle these harder samples, there is an alternative staining method that has the advantage of producing intact stained sections without the need for specialist embedding or cutting techniques. In the method of Tripp and Mackay, thin slices of undecalcified bone are immersed in a solution of silver nitrate for a period of 48 hours in the dark. Following washing in a sodium hypophosphite solution, the silver phosphate formed at the interface of osteoid and calcified bone is reduced to a black deposit. Following this stage, the bone slices are decalcified in a solution of formic acid and eventually processed through to paraffin wax. With the calcium now removed from the tissue, standard thickness sections can be cut and counterstained in Van Gieson. Microscopic examination of the stained sections show clear differentiation between osteoid and the outer limit of calcification in trabecular and cortical bone, allowing quantitative studies to be carried out.

Further reading

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