Letters to the Editor

Histomorphometry of bone

The review article by Dr Revell[1] contains a sentence which is confusing and requires clarification. On p 1326, under the heading "Normal values," he makes the following statement: "Up to four such lamellae are present in normal bone, so that a greater number than this is an indicator of hyperosteoidosis."

The confusing word is "hyperosteoidosis." In 1961, at a time when osteomalacia was identified by measurement of "surface cover," "mean seam thickness," and "volume," Lichtwitz *et al*[2] pointed out that there were diseases in which the rate of new bone formation was greatly increased, to an extent that all the criteria then in use for the morphometric diagnosis of osteomalacia were fulfilled, but the osteoid was mineralising normally. Bone disease due to primary hyperparathyroidism and active Paget's disease are the most commonly encountered causes of this situation. The term hyperosteoidosis was used by those authors to distinguish greatly increased, but normal bone formation from mineralisation failure. If that use of the term is adhered to, then osteoid with more than four birefringent lamellae is *not* hyperosteoidosis. It is an indication of mineralisation failure (in other words, histological osteomalacia).

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[1] Revell PA. Histomorphometry of bone. *J Clin Pathol* 1983;**36**:1323–31.
[2] Lichwitz A, DeSèze S, Parlier R, Hioco D, Bordier PR. La parathyroide: Glande de protection du capital calcique. *Presse Med* 1961;**69**:1015.

Histomorphometry of bone

I read with interest the review article on histomorphometry of bone by Dr PA Revell.[1]

I would like to draw readers' attention to the fact that Messrs Carl Zeiss have developed a software package to be used with the Zeiss Videoplan which allows one to quantitate data from trabecular bone semiautomatically, and a software package which allows semiautomatic quantitative analysis of osteocytes and osteocyte lacuna. A software package for the quantitative analysis of cortical bone is in the final stages of development and will, I am told, be available in 1984.

Numerous bone histomorphometry centres in Europe and North America have been using these packages for the last two years or so, and it is likely that the Osteoplan system will be the yardstick for the foreseeable future. This has the added advantage that methods for the histomorphometric analysis of static and dynamic bone parameters is becoming standardised in an increasing number of laboratories where this examination is carried out.

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[2] Anderson C. *Manual for the examination of bone.* Boca Raton, Florida: CRC Press, 1982.
[3] Recker RR. *Bone histomorphometry: techniques and interpretation.* Boca Raton, Florida: CRC Press, 1982.
[4] Malluche HH, Sherman D, Meyer W, Massry SC. A new semiautomatic method for quantitative static and dynamic bone histology. *Calc Tiss Int* 1982;**34**:439–48.

Preservation of sections of unfixed undemineralised bone

Johnstone *et al*[1] showed that adult, undemineralised, fresh bone could be sectioned at normal thickness provided that a suitably heavy microtome, with a special tungsten-carbide tipped knife, was used at sufficiently low temperature in a cryostat. An obstacle to the routine use of such sections has been the difficulty of getting the sections, containing hard and soft matter, flat on the slide. Moreover, during processing there is a tendency for movement of the bone to distort, or even cause total loss of, the softer tissues, including cartilage associated with the bone.

The adhesion of sections to glass slides is normally achieved with glycerol-albumin. We have now found that a film of 5% polyvinyl alcohol (5 g of GO4/140 PVA in 100 ml of 0·05 M glycyl glycine buffer, pH 8·0; Wacker Chemicals, Sunbury on Thames) gives superior results. All sections were dried in air for 30 min before inspection and further treatment.

The study has been performed with sections cut at 10μm of the whole knee joints of mice. In these it was essential to retain the topographic distribution of even minor tissue components. In the first study, performed on sections of otherwise untreated joints, of 166 sections mounted on glycerol-albumin coated slides there was loss of material in 77. In contrast, of 86 sections picked up on PVA coated slides only three showed histological distortion.

A more detailed study was made to find out whether pretreatment of the joint by injecting it with a 40% (wt/vol) solution of Polypep 5115 (Sigma) improved the state of the sections and whether the processing, for histological staining or cytochemical reactions, would cause further deterioration. For this investigation, five knee joints were taken after injection and five with no pretreatment. Five sections were taken from each joint and mounted on slides coated with glycerol-albumin and another five on PVA coated slides. For the non-injected joints, of the 25 sections mounted on glycerol-albumin only 10 were histologically acceptable when examined dry; deterioration occurred in a further four after a cytochemical reaction. Of the 25 sections mounted on PVA coated slides, 22 were acceptable when examined dry and three showed further damage after the reaction. The injection of the joints before chilling made little difference to the glycerol-albumin results but slightly improved the PVA results. Thus of the 25 sections mounted on glycerol-albumin, 12 were histologically acceptable when examined dry and a further four became displaced during the reaction. Of the 25 sections mounted on PVA coated slides, all were retained when examined dry and after incubation.

PVA coated slides have now been used for sections of human unfixed, undemineralised bone, including cancellous bone, with a similar degree of effectiveness in maintaining the sections intact and flat on the slide. It therefore seems that the use of a thin film of tacky PVA allows the routine preservation of serial sections of bone. It may also be helpful for sections of other tissues which have to be exposed to potentially damaging histological or cytochemical reactions, including silver impregnation.