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 hyperosteoitis." The confusing word is "hyperosteoitis." 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 hyperosteoitis 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 hyperosteoitis. It is an indication of mineralisation failure (in other words, histological osteomalacia).

CG WOODS
Nuffield Orthopaedic Centre, Headington, Oxford

References


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.

COLIN ANDERSON
Health Sciences Centre, Department of Pathology, University of Western Ontario, London, Canada N6A 5C1

References


Preservation of sections of unfixed undermineralised bone

Johnstone et al\(^1\) showed that adult, undermineralised, 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 material, 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 10 μm 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 Polyaprop 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, undermineralised 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.
This work has been supported by a grant from the Oliver Bird Fund of the Nuffield Foundation

NG STICKLEY
Division of Cellular Biology,
Kennedy Institute of Rheumatology,
Bute Gardens,
London W6 7DW

References

Myelofibrosis as a cause of pancytopenia in systemic lupus erythematosus

We read with interest the article by Dr Daly and Dr Scott describing the association between systemic lupus erythematosus and myelofibrosis. The association between systemic lupus erythematosus and myelofibrosis is rare and we report an additional patient showing this association.

CASE REPORT
A 28 year old white man had a four year history of polyarthritis, weight loss, and fatigue. Investigations showed that he had positive antinuclear factor and lupus erythematosus cells and systemic lupus erythematosus was diagnosed. Subsequent admissions were for several attacks of bilateral pleurisy and joint swelling (elbows, knees, ankles). Urinalysis also showed the presence of proteinuria (1-1 g/24 h). He received steroids in decreasing doses.

Bone marrow examination was performed two months before his final admission for investigation of progressive anaemia and thrombocytopenia. The peripheral blood picture at this time showed anaemia (haemoglobin 9-4 g/dl), leucopenia (white cell count 2-4 x 10^9/l), and thrombocytopenia (platelets 82 x 10^9/l). There was no history of exposure to irradiation or benzene. He had not received any immunosuppressive agents.

Bone marrow from the sternum and iliac crest was similar showing variable cellularity—hypocellular in some areas and hypercellular in others. Megakaryocytes were decreased in number; myeloid and erythroid activity was present. There were increased numbers of histiocytes and fibroblasts. No blast cells were seen. Masson trichrome stain revealed extensive fibrosis and Gomori stain showed a diffuse increase in reticulin.

On his final admission to hospital his major complaint was dyspnoea. Physical examination showed the following: pulse rate 100 beats/min, regular; blood pressure 120/70 mmHg; respiration rate 21 breaths/min. Chest examination showed an area of dullness over the right lower lobe with presence of bronchial sounds and crepitations. A pericardial friction rub was also present. Abdominal examination showed hepatosplenomegaly and ascites. There were several erythematous macular lesions on the skin.

Laboratory investigations gave the following results: haemoglobin 8-4 g/dl, leucocytes 2-1 x 10^9/l with 66% neutrophils, 18% stab cells, platelets 66 x 10^9/l. Serum electrolytes were normal. Urea nitrogen was 45 mg/100 ml and creatinine 1-8 mg/100 ml. Serum enzyme studies showed normal lactate dehydrogenase, serum aspartate transaminase, and alkaline phosphatase activities. Serum iron concentration was 18 µmol/l, total iron binding capacity 22 µmol/l. Direct and indirect Coombs' test was negative. Serum folate and B₁₂ were normal. Serum albumin concentration was decreased (18 g/l), γ globulins were increased (20 g/l). DNA binding was increased at 44%, antinuclear antibody positive at 1/40 dilution. Cold agglutinins were not present. Antibodies to platelets were not detected.

By the second day after admission the white cell count had fallen to 0-9 x 10^9/l and the subsequent course was marked by a rapid fall in haemoglobin, white cell count, and platelets. The patient also had fever, deterioration in renal function, increasing ascites, and hepatomegaly. All blood cultures obtained were negative. The day before he died his white cell count was 0-35 x 10^9/l and platelets were 1-0 x 10^9/l.

At necropsy the parietal pleurae of both right and left sides were thickened and covered with fibrinous exudate. The parietal pericardium was also thickened. There was massive ascites and a fibrinous exudate over the parietal peritoneum. The liver was considerably congested. The kidneys were mottled and diffusely granular.

Microscopy confirmed the presence of polyserositis. In the liver there was central fibrosis indicating chronic congestion. The kidneys showed changes consistent with systemic lupus erythematosus. The bone marrow sections confirmed the presence of myelofibrosis. There was no evidence of malignancy.

The above case report shows that myelofibrosis can be a cause of pancytopenia in systemic lupus erythematosus. The pathogenesis is not known but Dr Daly and Dr Scott have adequately reviewed the possible mechanisms by which myelofibrosis can occur in systemic lupus erythematosus. Unlike their patient, myelofibrosis was not reversed in our patient by corticosteroid treatment.

REFERENCES

Spiral organisms in endoscopic biopsies of the human stomach

We were particularly interested to read the article by Dr Rollason and his colleagues, in which they described spiral organisms, thought to be spirochaetes, in human gastric biopsies. We would like to draw attention to recent correspondence in the Lancet in which it is suggested that the Gram negative bacteria seen in gastric biopsies belong to the family Spirillaceae.

We are currently engaged in a prospective study of gastric biopsy material in an attempt to establish the nature and importance of the microflora in the normal and diseased human stomach. In our preliminary studies we have been able both to visualise and to isolate organisms which appear similar to those previously reported. We agree with Marshall on the basis of morphological and cultural characteristics, that the organisms are Gram negative bacteria of the family Spirillaceae rather than "spirochaetes." In fact, our results suggest that these organisms are members of the genus Campylobacter, although they cannot as yet be assigned to any of the currently recognised species.

Letters to the Editor

References

Spiral organisms in endoscopic biopsies of the human stomach

We were particularly interested to read the article by Dr Rollason and his colleagues, in which they described spiral organisms, thought to be spirochaetes, in human gastric biopsies. We would like to draw attention to recent correspondence in the Lancet in which it is suggested that the Gram negative bacteria seen in gastric biopsies belong to the family Spirillaceae.

We are currently engaged in a prospective study of gastric biopsy material in an attempt to establish the nature and importance of the microflora in the normal and diseased human stomach. In our preliminary studies we have been able both to visualise and to isolate organisms which appear similar to those previously reported. We agree with Marshall on the basis of morphological and cultural characteristics, that the organisms are Gram negative bacteria of the family Spirillaceae rather than "spirochaetes." In fact, our results suggest that these organisms are members of the genus Campylobacter, although they cannot as yet be assigned to any of the currently recognised species.

References