referred to by Dequeker and Verstraeten. However, so has the absolute parameter 'cortical area', which they advocate. Meema and Meema have recently pointed out that this is not a sensitive measure of endosteal bone resorption, because the calculation of cortical area (D^2 - d^2) is more dependent on the external width (D) than the marrow cavity width (d).

![Graph](image)

**Fig. 1** Mean values for total metacarpal width (D) and medullary width (d) in normal women (solid lines) and in osteoarthritic women (dotted lines).

As it happens, our own deductions were not materially affected by the application of any single parameter. Whether expressed in terms of relative bone mass (as in our paper) or absolute bone mass (cross-sectional cortical area), the results in our osteoarthritic patients showed the same trend: a slight increase above the normal means for males and no significant increase in females. This is predictable from straightforward values for the external width (D) and the medullary width (d), with the representation of cortical thickness as shown in Fig. 1.

A point ignored by Dequeker and Verstraeten is that there are considerable differences in the 'normal' curves presented in various studies. Our own female population—a true random sample obtained in field studies of socially active suburban families—has a mean bone mass and bone density greater than those reported in several European studies, where the elderly 'normals' were selected from hospitals or other institutions. This could account for the fact that our osteoarthritic patients do not show the remarkable increase above 'normal' bone density reported by Dequeker and others.

University of Witwatersrand, Johannesburg.

L. Solomon

**Reference**


**Identification of antibodies to acidic antigens by counterimmunoelectrophoresis**

Sir, Counterimmunoelectrophoresis (CIE) is a rapid and sensitive method for the detection of precipitating antibodies to a variety of nuclear and cytoplasmic antigens, such as Sm, RNP, Ro, and La. Usually these precipitins are then identified by Ouchterlony immunodiffusion, although Kurata and Tan described a modified form of CIE for this purpose. In our laboratory we employ an alternative modification to the standard method of CIE that has enabled us to identify over a dozen recurring precipitin systems in SLE and autoimmune liver disease.

10 ml of 1% agarose in 0.05 M barbitone/sodium barbitone buffer pH 8.4 is poured on to 80 x 80 mm glass....

**Fig. 1** Area of glass plate showing arrangement of wells and trough. Human spleen extract (HSE) has been used as the source of antigens. Precipitin lines merge or cross according to the identity or nonidentity of antibodies in adjacent wells.
plated. Sixteen wells 3 mm in diameter and 1.5 mm apart are cut in a row, and a trough 3 mm wide is cut parallel to the wells 3 mm from their cathodal side. Heat inactivated sera (8 µl) are added to the wells, and a tissue extract containing soluble antigens (150 µl) is placed in the trough. We use human spleen extract or rabbit thymus extract at a final protein concentration of 5 mg/ml. 1 Electrophoresis is carried out at 12 mA/slide for 1 hour in barbital buffer, after which the slides are washed in saline and stained in 0-1% Coomassie blue. Precipitins are identified by the formation of lines of identity with reference sera in adjacent wells (Fig. 1).

CIE carried out in this way permits the detection and immunological identification of precipitating antibodies by a single technique. Staining the plates enhances the appearance of weaker precipitin lines and provides a permanent record. CIE is more sensitive than Ouchterlonny immunodiffusion and far more economical in the use of reference sera and antigen extract. Furthermore the need to perform enzyme digestion of the antigens is largely eliminated because of the greater separation and sharpness of precipitin lines produced by CIE. The virtue of the method is its simplicity, making it suitable for the identification of antibodies to acidic antigens in most laboratories.

Rheumatology Unit, R. M. Bernstein
Royal Postgraduate Medical School, C. C. Bunn
Hammersmith Hospital, G. R. V. Hughes
Du Cane Road,
London W12

References

Use of visual analogue scales

Sir, We have previously studied the reproducibility and errors involved in the use of vertical visual analogue scales (VAS) and showed that reproducibility varies along the length of the scale. 1 We have now extended this study both to horizontal scales and to the serial use of visual analogue scales, which better mimics the clinical situation.

Reference VASs were prepared by drawing out 13 10-cm scales and crossing them at 0-0. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 cm respectively. 0.1 and 0.2 cm were included, as these distances correspond to the golden section, which we have previously shown to be important. 1

Correspondence 555

The 13 vertical reference scales were presented in a random order to 30 normal volunteers, and immediately after viewing a given scale the volunteer attempted to reproduce the line on a separate blank 10 cm vertical

![Graph](attachment://graph.png)

(a) Variation in reproducibility along the length of (a) horizontal and (b) vertical VAS (n=30).
Identification of antibodies to acidic antigens by counterimmunoelectrophoresis.

R M Bernstein, C C Bunn and G R Hughes

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