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A simple method for the application of two samples to polyacrylamide gel for comparative electrophoresis

A method for the separation of histone fractions by electrophoresis in polyacrylamide gel has been described[1], which enables the various fractions to be determined quantitatively.

It was anticipated that the mobilities of the fractions would be slightly variable from one gel to another since small differences in polymerisation and equilibration of the gels, time of electrophoresis, and voltage applied are difficult to avoid. However, it was also demonstrated that the relative mobilities of the fractions, with respect to a marker protein (bovine plasma albumin) also varied slightly, and that as a range of relative mobilities was obtained for each fraction the method only had a limited use for qualitative work for identifying individual fractions and mixtures of fractions.

To overcome this difficulty and to enable valid comparisons to be made between two different samples, the following technique was developed for applying two samples to the same gel.

The electrophoretic method and the apparatus used have been described previously[1], and modifications to the destaining procedure have also been given[2].

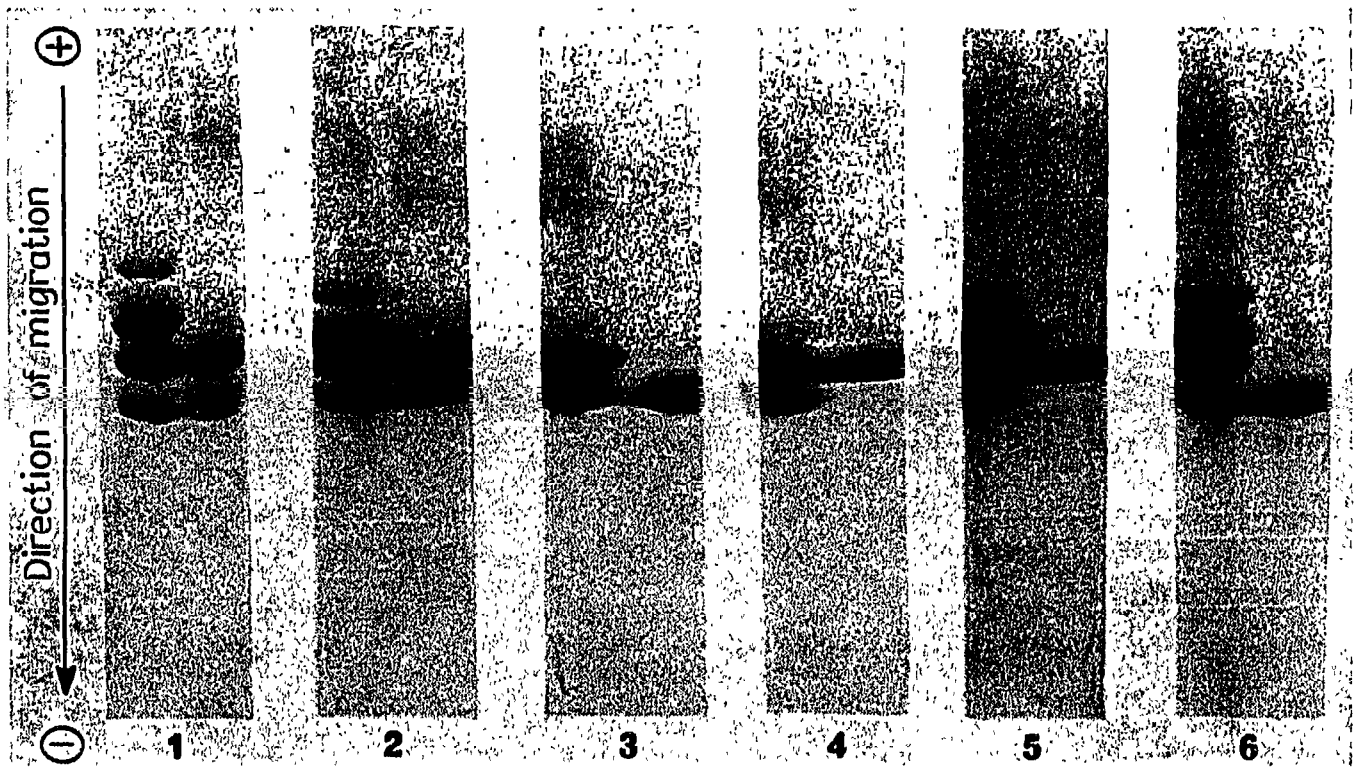

Fig. 1. A comparison of whole histone and histone fractions in polyacrylamide gel. The bands obtained using whole histone correspond to the following histone fractions in order of increasing mobility, F1, F2B + F3, F2A2 and F2A1 (ref. 1). For further information regarding the histone fractions see ref. 3. 1 = whole histone (L), fraction F2A (R); 2 = whole histone (L), fraction F2A (R); 3 = fraction F2A (L), sub-fraction F2A1 (R); 4 = fraction F2A (L), sub-fraction F2A2 (R); 5 = whole histone (L), sub-fraction F2A2 (R); 6 = whole histone (L), sub-fraction F2A1 (R).

After equilibration the tubes containing the gel were removed from the apparatus and the upper surface of the gel dried carefully using a paper tissue. The samples for comparison were dissolved in the sample solvent (0.5–2.0 mg/ml of 1 *M* sucrose in 2 m*N* acetic acid, depending on the complexity expected) and applied to opposite sides of a paper disc (7 mm diameter, cut from Whatman filter paper No. 1), but were prevented from diffusing into contact with each other by a line of Silicone Repelcote water-repellant (Hopkin and Williams Ltd., Chadwell Heath, Essex, Great Britain) previously applied across the diameter of the paper disc using a fine glass capillary. The paper disc containing the samples was then dropped on to the dried surface of the gel and immediately covered with 1 ml of sample solvent (1 *M* sucrose in 2 m*N* acetic acid). Buffer solution was then quickly overlayered on to the denser sucrose layer until the upper part of the tube was full. The tube was then replaced in the apparatus and the electrophoresis, staining and destaining carried out in the usual manner. Using this method it is possible to make valid comparisons between different samples on the same gel. The results obtained are shown in Fig. 1.

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