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The identification of the five main histone fractions by comparative electrophoresis in polyacrylamide gel

The histones, the basic proteins associated with DNA in the nuclei of most multicellular organisms are thought to control transcription in some way. Five main fractions have been isolated and characterised from calf thymus, and designated F1, F2B, F2A2, F2A1 and F3 (ref. 1). It is now known that these five fractions also occur in bird[2], fish[3] and plant[4] somatic cells.

Methods for their preparation in large quantities[5,6] and for their characterisation by amino acid analyses[7] and polyacrylamide gel electrophoresis[8] have also been described. More recently we have described a simple method for running two proteins on one gel for comparative electrophoresis[9] and this paper shows how this method can be used for the identification of each of the five main histone fractions.

Polyacrylamide gel electrophoresis was carried out essentially as described previously[8,10] but with the addition of urea to the gel to a final concentration of 2 *M*. The application of two samples to one gel was also carried out as described previously[9].

The results obtained are shown in Fig. 1. It can be seen that each histone fraction can be identified with a corresponding band in the whole histone pattern. Frac-

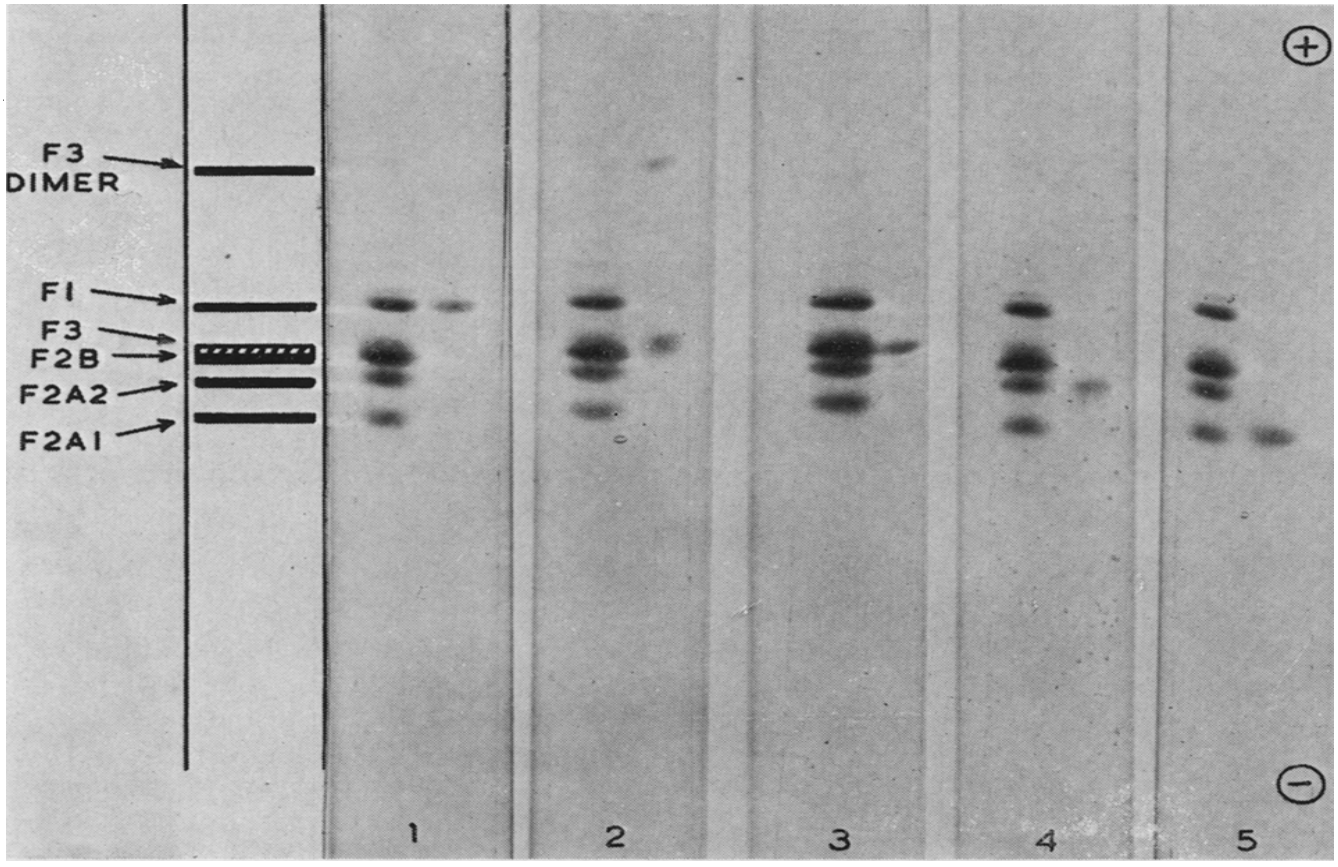

Fig. 1. A comparison of whole histone and the five main fractions by electrophoresis in polyacrylamide gel at pH 2.4. The fractions are on the right-hand side of the gels and are compared with whole histone on the left-hand side. 1 = F1; 2 = F3; 3 = F2B; 4 = F2A2; 5 = F2A1.

tions F3 and F2B run close together but are easily differentiated because of the unique dimer formation of F3 (ref. 11). F3 is the only histone fraction to contain cysteine (1 residue per mole) and partial dimer formation occurs by oxidation under these conditions giving a band of approximately half the mobility of F3. This does not occur with any other histone fraction. F3 and F2B can of course be better separated by extending the length of migration.

Using this method, any histone fraction or mixture of fractions can be identified and characterised, and if quantitative methods are subsequently employed[8] the relative amounts of each fraction can be determined.

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Electrophoretic heterogeneity of crystalline insulin: A comparison of methods

The heterogeneity of crystalline insulin has been demonstrated by a number of methods. On paper[1] or cellulose acetate[2] electrophoresis with high concentrations of urea present to dissociate aggregated proteins, about six fractions may be separated, which apparently correspond to a series of deamidated insulins. MIRSKY AND KAWAMURA[3] studied crystalline insulins from eleven species by polyacrylamide gel electrophoresis, and found marked heterogeneity in each case, all the separated fractions having the biological properties of insulin. The presence of 8 *M* urea, however, had no effect on the electrophoretic pattern, so aggregated insulin molecules may have been absent. Other separation techniques have nevertheless shown the presence of molecules very different in size from insulin: gel filtration of bovine insulin on Sephadex G-50 resulted in the separation of several fractions one of which, with a molecular