Short Communication

Human Growth Hormone-Anti-(Growth Hormone) Immunoprecipitates in the Presence of Dextran

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Heterogeneity of many seemingly pure proteins is observed when they are examined by electrophoretic techniques. For example, several components in various purified human growth hormone preparations have been found by Ferguson & Wallace (1961), Lewis (1962), Reisfeld, Lewis, Bring & Steelman (1962), Barrett, Friesen & Astwood (1962), Fevold, Gemzell, Bates & Porath (1964), Chen & Wilhelmi (1964), Meisinger, Cirillo, Davis & Reisfeld (1964), Lewis & Cheever (1965), Saxena & Henneman (1966), Fønss-Bech (1966), Hanson, Roos & Rymo (1966) and Peckham (1967). This heterogeneity may stem, among other things, from contaminants, fragmentation caused by enzymes, deamination or aggregate formation, or any combination of these.

It was decided to test whether the various human growth hormone components seen on polyacrylamide-gel electrophoresis exhibit immunological activity. The double-diffusion method and immunoelectrophoretic method usually show more or less homogeneous single immunoprecipitin bands of purified substances that are shown to be heterogeneous by disc electrophoresis on polyacrylamide gels. A new immunoelectrophoretic method was developed (Ceska, 1968*a,b*; Ceska & Grossmüller, 1968) that allows the separation of immunoprecipitin bands into distinct bands by using a dextran infusion technique. As antigen, a highly purified human growth hormone preparation (a gift from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md., U.S.A.) was used. Goat anti-(human growth hormone) was purchased from Mann Research Laboratories Inc., New York, N.Y., U.S.A. Dextran 250 and Dextran 2000 (of average molecular weight 250 000 and 2 000 000 respectively) are products of Pharmacia AB, Uppsala, Sweden. The immunoelectrophoresis, as well as dextran infusion, was performed on microscope slides in an LKB immunoelectrophoresis apparatus. To summarize, the slides were coated with a 1.5% (w/v) agar solution made up in sodium veronal buffer, pH 8.6 and *I* 0.03. The electrophoretic separation was run in sodium veronal buffer,

pH 8.6, and *I* 0.1 in a cold-room at 250 v and 27 ma (4.5 ma/strip). About 2 μ l. of growth hormone solution was added to the circular well. The time of electrophoresis was 60 min. Troughs were cut into the agar gel and about 0.25 ml. of anti-(growth hormone) serum was added. After 3 hr. the troughs were filled with an 8% (w/v) solution of the appropriate dextran. The microscope slides were then placed in a moisture chamber and incubated for 2 days at 37° in an air-incubator. During this incubation the dextran solutions were added to the troughs twice. After 2 days at 37° the slides were placed in a cold-room for an additional 3 days of incubation. Immunoelectrophoretic slides were stained with Amido Black 10B.

Disc electrophoresis of 50 μ l. of human growth hormone (1 mg./ml.) was performed in a Buchler analytical apparatus on 10% (w/v) polyacrylamide gel. (The buffer in the upper chamber was tris-glycine buffer, pH 9.64, and that in the lower chamber was tris-HCl buffer, pH 8.84.) The staining was performed with Coomassie Blue in acetic acid solution.

The electrophoretic separation of human growth hormone preparation on polyacrylamide gel revealed four protein bands (Plate 1*a*). When human growth hormone was subjected to immunoelectrophoretic analysis two rather diffused bands were observed (Plate 1*b*). Dextran 250 infusion not only intensified the first two bands located nearer the trough, but in addition separation of immunoprecipitates into five immunoprecipitin bands was observed (Plate 1*c*). Infusion of Dextran 2000 gave similar results (Plate 1*d*), except for the structure and the number of immunoprecipitin bands. Now the most intense immunoprecipitin bands were located close to the circular well and the total number of bands was four. By visual comparison the total intensity of the various immunoprecipitin bands in the presence of Dextran 250 or Dextran 2000 is in excess of that seen in the control experiment (Plate 1*b*).

Very similar results were also observed with human growth hormone prepared by Dr P. Roos and a preparation obtained by Dr C. H. Li. Also,

different sources of anti-(growth hormone) serum were used and gave essentially identical results. Rabbit sera obtained from zero-time bleedings failed to show any precipitin bands with human growth hormone.

Thus the number of immunoprecipitin bands of the human growth hormone-anti-(growth hormone) system obtained by the dextran infusion technique is related to the number of growth hormone components seen on polyacrylamide-gel electrophoresis. Whether the various components seen in both techniques are identical remains to be determined.

Barrett, R. J., Friesen, H. & Astwood, E. B. (1962). *J. biol. Chem.* **237**, 432.

Ceska, M. (1968a). *Nature, Lond.*, **217**, 356.

Ceska, M. (1968b). *Immunology*, **15**, 837.

Ceska, M. & Grossmüller, F. (1968). *Experientia*, **24**, 391.

Chen, H. C. & Wilhelmi, A. E. (1964). *Abstr. Endocrine Soc. Meet., San Francisco*, p. 80.

Ferguson, K. A. & Wallace, A. L. A. (1961). *Nature, Lond.*, **190**, 632.

Fevold, H. R., Gemzell, C. A., Bates, R. W. & Porath, J. O. (1964). *Fed. Proc.* **23**, 820.

Fønss-Bech, P. (1966). *Biochem. J.* **101**, 43c.

Hanson, L. Å., Roos, P. & Rymo, L. (1966). *Nature, Lond.*, **212**, 948.

Lewis, U. J. (1962). *J. biol. Chem.* **237**, 3141.

Lewis, U. J. & Cheever, E. V. (1965). *J. biol. Chem.* **240**, 247.

Meisinger, M. A. P., Cirillo, V. J., Davis, G. E. & Reisfeld, R. A. (1964). *Nature, Lond.*, **201**, 820.

Peckham, W. D. (1967). *J. biol. Chem.* **242**, 190.

Reisfeld, R. A., Lewis, U. J., Bring, N. G. & Steelman, S. L. (1962). *Endocrinology*, **71**, 559.

Saxena, B. B. & Henneman, P. H. (1966). *Biochem. J.* **100**, 711.

EXPLANATION OF PLATE 1

(a) Disc electrophoresis of human growth hormone on polyacrylamide gel. (b) Immunoelectrophoretic run of human growth hormone and anti-(human growth hormone) (control experiment). (c) Immunoelectrophoretic run of human growth hormone and anti-(human growth hormone) (infusion with Dextran 250). (d) Immunoelectrophoretic run of human growth hormone and anti-(human growth hormone) (infusion with Dextran 2000).