TECHNIQUE OF SEPARATION OF THE COMPLEX ANTIGEN-ANTIBODY FROM THE FREE ANTIGEN ON POLYACRYLAMIDE GEL ELECTROPHORESIS. ITS APPLICATION IN RADIOIMMUNOASSAY AND IN THE DETECTION OF ANTIGENICITY DURING AND AFTER TREATMENT WITH HUMAN GROWTH HORMONE.

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Several methods have been tried for the separation of the complex antigen-antibody (B) formed after incubation, from the free labelled antigen (F). As it is well known this technique is a necessary tool in the determination of an antibody titre and its optimal dilution to be used with a certain labelled antigen before setting up a radioimmunoassay, and also in the detection of antibody occurrence, titre and binding capacity, before, during and after hormone therapy.

Polyacrylamide gel electrophoresis (PAGE) was preferred to other techniques (electrophoresis on paper and cellulose acetate, chromatoelectrophoresis, adsorpion on activated charcoal, gel filtration) because, even being more laborius, it gives a complete separation of the two peaks, presents minimal absorption phenomena, it is a quali- and quantitative system and providing the way of calculating independently both B and F, opens the way to a complete separation between the bound and the damaged antigen, not possible with other techniques.

The original method of Davis had to be adapted, especially for what concerned the application of the sample, that had to be polymerized on top of the stacking gel or of the separation gel directly, to avoid back diffusion of the inevitably large volume of incubate that had to be applied. So the original solutions had to be changed in volume and concentration in order to obtain the polymerization of this gel. The best concentration of the sample gel was found to be at 2% acrylamide. In this way the damaged antigen remained trapped in this gel, while the complex entered stacking gel and the separation gel, still keeping well separated from the free antigen that runs close to the tracking dye.

The application of the present technique to a radioimmunoassay of human growth hormone (HGH) provides a method that does not need a second antibody, reaches a sensitivity of 0,1 ng/ml. (5 pg in absolute!) and allows to visualize the type of complex formed. In this respect, running an experiment at different antigen-antibody ratios, it was obtained that a decrease in this ratio (that is an increased antibody concentration) meant a decrease in Rm, confirming the hypothesis of the formation of more highly polymerized complexes, up to the formation of the lattice.

This same technique has been successfully applied to the detection and measurement of naturally formed antibodies in patients treated with different HGH extracts, representing to this purpose a better tool than the traditionally used paper electrophoresis or chromatoelectrophoresis. It permits in fact a higher number of assays to be run simultaneously, which is very useful especially in the determination of titre and binding capacity.

APPLICATION OF THE POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) TECHNIQUE TO MOLECULAR WEIGHT DETERMINATION; DEGREE OF PURIFICATION AND AGGREGATION OF DIFFERENT PREPARATIONS OF HUMAN GROWTH HORMONE: NIH, KABI IEA.

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After having first controlled the molecular weight of our HGH preparation throung the gel-filtration technique on Sephadex G-100 (P. Andrews), obtaining a value of about 22,000, the PAGE technique was applied following the method of J. L. Hedrick.

It is known that relative mobilites (Rm) of a certain protein band present decreasing values when plotted against increasing concentration of acrylamide in gel. This plot originates a family of parallel lines when the bands are relative to "charge isomers", because migration for molecules having same weight and different charge is independent of the sieving action of the polyacrylamide. On the contrary it produces a family of non-parallel lines extrapolating to a common point in the vicinity of 0% gel concentration, when the bands are relative to "size isomers", which are molecules having the same charge and different molecular weight. The last case provides a method for the determination of molecular weight based on plotting the slopes of these last curves versus the correspondent molecular weights.

Our runs were perfomed at gel concentration of 7,10,12,14% in acrylamide. This gave for HGH-IEA a value around 21.500 and for Bovine Growth Hormone (WHO) a higher value, both in good agreement with those reported by E.V. Cheever.

Very interesting was also the presence of minor bands due to charge and size isomers of the fundamental HGH molecule. In this respect we compared the electrophoretical behaviour of HGH-IEA to similar preparations from NIH and KABI.

NIH—HGH presented a major band whose slope corresponded to a M.W. of 22,000 and only a very faint faster band, presumably due to a deamidated form.