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THE REVERSED ELECTROPHORESIS TECHNIQUE FOR ISOHORMONE PURIFICATION DOES NOT ALTER THE IMMUNOGENIC ACTIVITY OF THE HGH MOLECULE: APPLICATION OF A COMPUTERIZED 2X2 FACTORIAL BIOASSAY

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Classical polyacrylamide gel electrophoresis (PAGE) is a very flexible and rapid high resolution technique whose preparative application in our laboratory for isohormone purification has already been described. This technique was recently modified in order to obtain higher yields: the protein containing gel segment is sealed into the lower part of a dialysis tube supported on a bed of freshly polymerized gel and the protein eluted by current inversion (reversed electrophoresis).

Since some authors have reported a substantial decrease in hormone activity due to the application of analogous preparative PAGE techniques, our main concern in using it for isohormone purification for diagnostic use was to make sure that our conditions did not alter the biological potency of the whole hormone preparation. For this purpose, we used a heterogeneous hGH preparation, which was completely eluted from the gel under the conditions normally used for isohormone purification.

The retention of growth promoting activity was confirmed by carrying out a 2X2 factorial bioassay, injecting doses of 10 and 20 µg/day of the original (Std) and the electrophoretically eluted preparation (Unk) in four populations of 8 hypophysectomized rats, over a 10-day period. The data of this optimized bioassay design were processed through a computer program (BASSY) recently set up in our laboratory, giving the following parameters:
Relative Potency (of Unk.): 0.90. Fiducial Limits: 0.70-1.14. Index of precision: 0.132. Difference between preparations: not significant. Difference between doses: significant. Slope divergence: not significant.