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Jan. 5, 1990

Dear Sirs:

Enclosed find our interim report for the period ending Jan. 1, 1990 for AID Grant No. DPE-5544-G-SS-6046.

Sincerely,

M. Shemesh

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Progress Report

AID Grant No. DPE-5544-G-SS-6046-00

Jan. 1, 1990

Title: Isolation of a lutotropic substance produced by the blastocyst and early pregnancy diagnosis in cattle.

Objective: Purification of the protein by isoelectric focusing.

Methods: Preparative isoelectric focusing was carried out using a flat-bed LKB system (Multiphor). A circulation cooling bath maintained the cooling place at a temperature of 4°C. A pH gradient of pH 3-10 was prepared using ampholytes purchased from Pharmacia (Uppsala, Sweden), on a support of Ultradex (LKB). Samples to be subjected to preparative isoelectric focusing (300 mg protein) were mixed with an ampholyte/Ultradex slurry and added to the flat-bed plate. Isoelectric focusing was then carried out as described in LKB Application Note 198. After isoelectric focusing, a grid was applied to the bed, dividing it into 15 fractions. Each section was removed, mixed in a tube with 1 ml of deionized water and poured into a 5x100 mm column (Econo-column, Bio-Rad, Richmond, CA). The gel was then eluted with 5 ml of deionized water. The pH of each eluate was determined; the eluates were then dialyzed against deionized water to remove ampholytes and 0.1 ml aliquots of the fraction were examined for binding in the radioreceptor assay.

Results: The 40% ammonium sulfate protein precipitate that was separated on the gel yielded two principal peaks of hCG-like activity as determined by RRA, one at pH 4.5 and one at pH 9.5. Fig. 1 represents the results of 13 separate determinations.

Objectives for the last six months:

The Costa Rican partner, Dr. Comacho will come to Israel to complete the injections of the protein into rabbits to produce antibody to the luteotrophic substance.

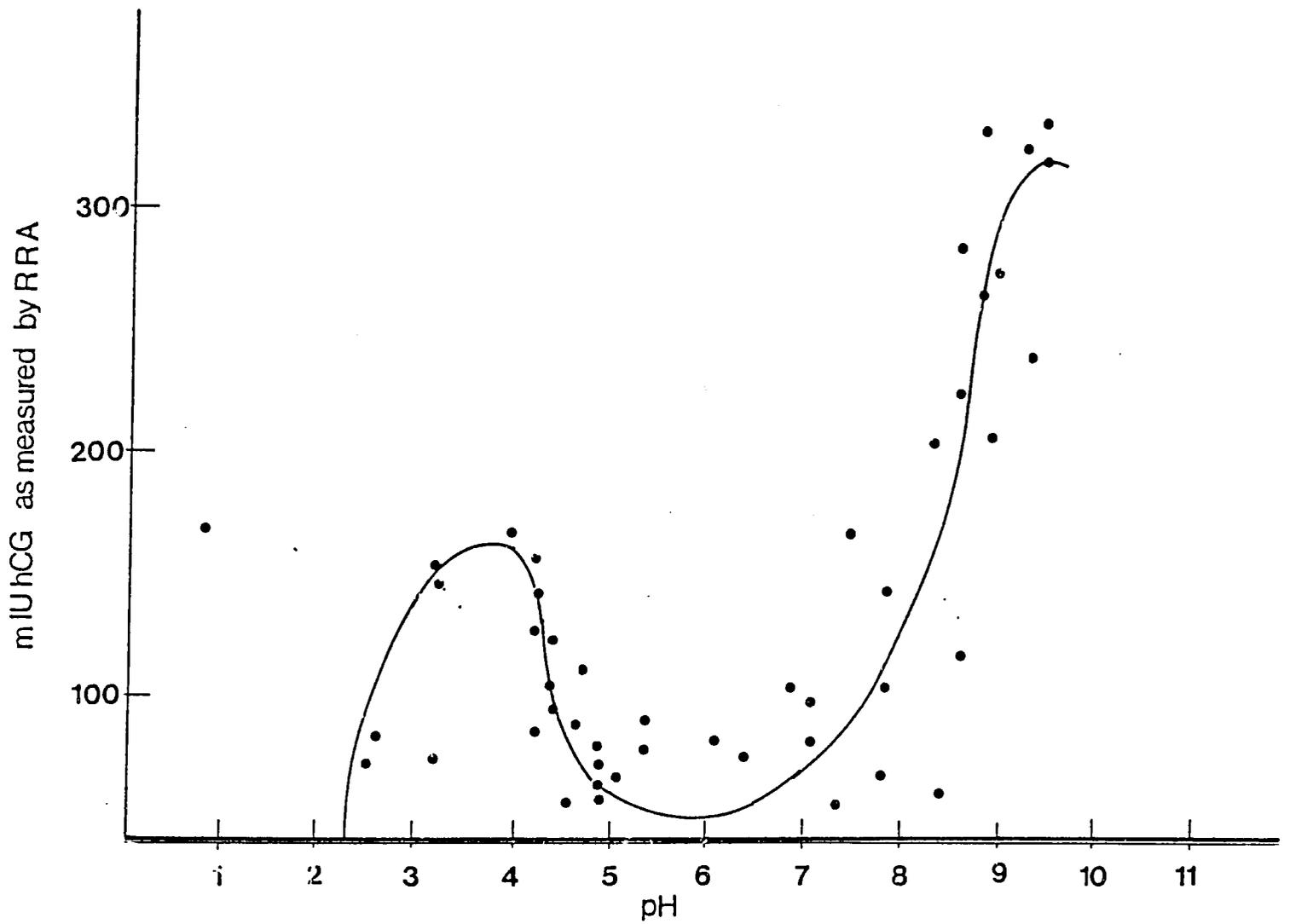


Fig. 1. hCG-like activity as measured in extracts of gel electrofocused fractions of ammonium sulfate precipitate of fetal cotyledons from 50-150 days of gestation.

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