AJH 1990; 3:148–150

Renin Production by Nephroblastoma Cells in Culture

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Nephroblastoma cells have been cultured for 19 days. During this time cell growth and active and inactive renin secretion were monitored. Inactive renin was found to be secreted in excess of active renin. The main secretory protein, precipitated with antirenin IgG was found to have a molecular mass of 52,000 daltons. Am J Hypertens 1990;3:148–150

Abnormally high concentrations of inactive renin have been identified in the plasma of patients with nephroblastoma.[1,2] However, it was not known whether this renin was secreted from the tumor or from the juxtaglomerular cells of the affected kidney. We have grown nephroblastoma cells *in vitro* and have monitored the secretion of renin into the growth medium over 19 days.

MATERIALS AND METHODS

Cell Culture Nephroblastoma cells were obtained from a patient with high plasma concentrations of active and inactive renin (2695 μUnits/mL total, 285 μUnits/mL active, 10% active renin). A primary culture was established by standard methods[3] and we obtained the cells at the third passage. The cells were cultured in 10 × 110 mm tissue culture tubes (Ambitube, Lux, Flow Laboratories, Herts., England) in either Dulbecco's Modified Eagle Medium (DMEM) or Medium 199 (M199). Both types of media were supplemented with 10% foetal calf serum; 2 mmol/L glutamine; 5 μg/mL Fungizone and 100 IU/mL penicillin/streptomycin (Gibco Biocult, Paisley, Scotland). The cultures were passaged at intervals of 3 to 4 weeks using 0.25% trypsin. Two groups of 21 tissue culture tubes, containing 1.5 mL of either DMEM or M199 growth medium, were inoculated with 1×10^4 cells per tube and incubated in an atmosphere containing 5% CO_2 at 37°C. Triplicate tubes from both groups were harvested with 0.25% trypsin 3, 4, 7, 10, 13, 16 and 19 days after the start of culture. The cell suspension was used to determine cell number. Active and inactive renin was assayed by radioimmunoassay.[4]

Molecular Weight Determination Nephroblastoma cells were cultured to confluence (6×10^6 cells) in M199 in a 75 cm^2 tissue culture flask. The growth medium was removed and the flask was washed with 2 × 20 mL of Earle's Minimal Essential Medium without methionine (EMEM). We placed 20 mL of EMEM containing 200 μCi S^{35}-methionine (Amersham International, England) into the flask and incubated at 37°C for 36 h. The medium was then removed and the cells were harvested with 0.25% trypsin and resuspended in 1 mL distilled H_2O. Renin was extracted from the culture media and cell suspension by double antibody precipitation. We incubated 100 μL antiserum, raised in rabbits against pure human active renin,[5] with 500 μL of growth medium or cell suspension in 400 μL phosphate buffered saline (PBS) pH 7.4 for 18 h at 4°C. The renin IgG was then precipitated from solution by incubation for 18 h at 4°C with 100 μL donkey antirabbit IgG. The precipitates were redissolved in 100 μL 72.5 mmol/L Tris-HCl, pH 6.8, containing 10% (w/v) (sodium dodecylsulfate (SDS), 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 0.002% (w/v) bromophenol blue, and electrophoresed at 3 mA per sample through 10% polyacrylamide column gels. After removal from the glass

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0895-7061/90/$3.50

supports, the gels were frozen and sectioned in 1 mm slices, solubilized with 200 μL 30% (v/v) H_2O_2 and counted in a scintillation counter in the presence of the scintillation cocktail NE 260 (Nuclear Enterprises, Scotland). The molecular weight of the main precipitated protein was calculated by reference to known molecular weight markers (Amersham International).

RESULTS

Growth Curves and Renin Secretion Nephroblastoma cells were observed to follow an exponential growth curve in the presence of M199, reaching a plateau after 16 days. Less growth was observed over the 19 day period in the presence of DMEM (Figure 1).

Active and inactive renin were detected in both media throughout the experiment. Inactive renin was secreted in excess of active renin in both media.

Molecular Weight Determination One major S^{35}-labelled protein peak was obtained from culture media and cell suspension (Rf = 0.5). By reference to another gel containing known protein molecular weight standards the antibody precipitated protein was calculated to have a molecular mass of 52,000 daltons. Two further peaks were detected using this procedure. A 59,500 dalton protein was detected in the culture medium. In the cell suspension samples a small peak, with an estimated molecular mass of 43,500 daltons was detected. This lower molecular mass protein is similar in size to active

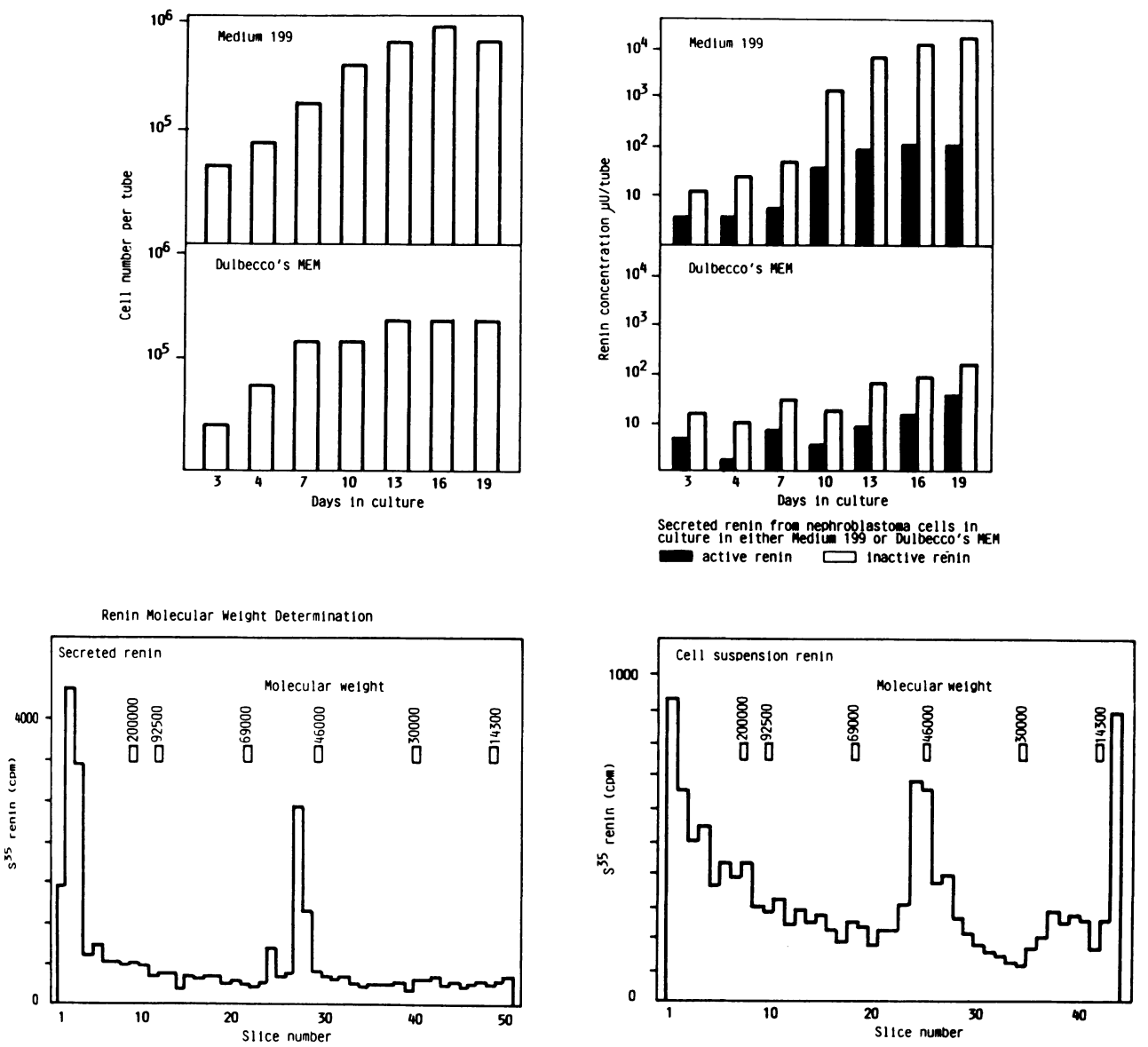

FIGURE 1. Top left: *Growth curves of nephroblastoma cells grown in either Medium 199 or Dulbecco's Modified Eagle Medium.* Top right: *Concentrations of active and inactive renin found throughout the 19 day growth period.* Bottom: *Polyacrylamide gel electrophoresis of molecular weight markers and renin obtained from either 199 Growth Medium* (left) *or extracted from the cell suspension* (right).

renin and is present in approximately the same proportions to inactive renin as in the patient's plasma. In addition, some very high molecular mass material (> 200,000 daltons) was detected in both preparations. This may be labelled protein fragments, nonspecifically associated with the donkey antirabbit IgG complex.

DISCUSSION

We report that human nephroblastoma cells can be cultured in Medium 199 supplemented with 10% foetal calf serum. During 19 days of culture, the cell number increases exponentially to a maximum after 16 days. Inactive renin and to a lesser extent active renin is secreted throughout the incubation period.

Double antibody precipitation of a nephroblastoma cell suspension and culture medium produced an S^{35}-labelled protein of molecular mass 52,000 daltons. The molecular mass of pure, normal inactive renin was previously shown to be 48,000 daltons by SDS gel electrophoresis and 51,000 daltons by gel filtration.[6] These differences may not be significant. The ratio of active to inactive renin secreted by the tumor cells is similar to those found in the patient's plasma and confirms that the main secreted product is inactive renin (prorenin). The very high molecular mass labelled protein (> 200,000 daltons) found in both gels is probably S^{35}-labelled double antibody complexes (the molecular mass of each IgG molecule is ~ 150,000 daltons). The low molecular mass contaminant, found in both preparations but only depicted in the cell suspension graph, is likely to be free S^{35}-methionine.

Renin secreting cell cultures have been described before, Pinet et al[7] established a human juxtaglomerular cell line and Chansel[8] described a human mesangial cell culture. Both types of culture secrete mainly inactive renin. The nephroblastoma cell line described here may be a suitable model for investigating the secretion of human renin and its precursors.

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