CELL GROWTH AS A FUNCTION OF CELL DENSITY

H. RUBIN
UNIVERSITY OF CALIFORNIA, BERKELEY

1. Introduction

It is a common observation that while large numbers of cells from vertebrate animals grow in tissue culture exponentially without delay, small numbers grow poorly if at all. It is the purpose of this paper to set out the numerical and physiological parameters of this restriction on growth initiation, and to relate it briefly to the malignant transformation of normal into cancer cells.

The cells used in these experiments were obtained from 10 day old chicken embryos and ingredients of the medium are:

Nutrient mixture 199	83%
Tryptose phosphate broth	10%
Calf serum	4%
Chicken serum	1%
2.8% $NaHCO_3$	2%.

The experiments on cell growth were done in circular polystyrene plastic petri dishes. The test cells were suspensions of individual cells made by treating three to seven day old primary cultures with trypsin. The cells were counted in a Coulter Electronic Counter.

2. The number of cells required for sustained growth

When the population of cells is about 5×10^4 or greater per 21 cm^2 dish, the cells multiply exponentially without lag with a generation time varying from 16 to 24 hours. At concentrations much below 5×10^4 cells per dish, the growth is much slower, and there may occur a delay of three to four days before any growth is apparent. At concentrations below 10^4 cells per dish, only a small fraction of the cells may sustain their growth sufficiently to form a colony.

The question arises whether the growth of animal cells is dependent on the number of cells per milliliter of nutrient medium or on the density of the cell population on the floor of the dish. It should be noted that normal vertebrate cells in general must attach to a surface and spread thereon before they will grow efficiently. Experiments were carried out using differing volumes of medium, differing surface areas of the culture dish and differing numbers of cells, as follows: