Constant Protein Turnover in Mammalian Cells During Logarithmic Growth

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As a result of independent investigations both Eagle, Piez, Fleischman and Oyama (1959) and Jordan, Miller and Peters (1959) reported constant protein turnover in both resting and growing mammalian cell cultures. In a later publication King, Bensch and Hill (1960) reported no turnover of cell protein when L cells are maintained in logarithmic growth. King et al. based their contradictory conclusions on the finding that after labeling with L-leucine C^{14} the protein-bound radioactivity of L cells remained constant for the experimental period of 4 days. It seemed apparent to the present authors that the work of King et al. might have suffered from two errors in experimental design. First, in the isolation procedure, rinses with unlabeled carrier amino acid were not employed to reduce adsorption effects. Second, because of the very high cell concentrations employed, in the order of one million cells per ml., the pool of unlabeled leucine in the culture nutrient may have been inadequate to trap any labeled amino acids released. Repetition of the experiment of King et al., using adequate carrier technique, yielded no significantly different findings and need not be reported in detail here. When, however, the relative size of the unlabeled amino acid pool was increased approximately two hundred fold by doubling the leucine concentration of

the nutrient and decreasing the initial cell concentration to 10,000 cells per ml., strikingly different results were obtained. A constant decrease in protein-bound activity was observed, indicating protein turnover during logarithmic growth.

The cell proteins of a 20 ml. suspension culture of L cells were labeled by incubation for three days in a rotary incubator in Eagle's basal medium with the addition of .04 mg. of L-leucine C^{14} (sp. act. .05 mc per mg.). At the end of the labeling period the cells were washed three times in 5 mg. % L-leucine in Simms balanced salt solution. The cells were then suspended in 80 ml. Eagle's basal medium at twice the usual leucine concentration. After one day a 5 ml. sample was taken for cell counting with a standard hemocytometer and determination of cell protein C^{14} radioactivity. The cell count (45,000 cells per ml.) was adjusted to 10,000 cells per ml. by making 17.5 ml. of cell suspension to 80 ml. with Eagle's basal medium with double leucine. The added nutrient had been previously warmed and pH adjusted. The culture was then returned to the rotary incubator for 4 days. On each day a 5 ml. sample was taken for cell protein radioactivity.

Samples for cell protein determination were spun down and the cells were washed three times with 5 mg. % L-leucine in Simms balanced salt solution. Cell proteins were precipitated by the addition of 3 ml. 6% TCA; 1.0 ml. 2% L-leucine was added to minimize adsorption of labeled leucine and 0.1 ml. serum was added to provid additional protein precipitate for quantitative transfer. The precipitate was washed three times with 1% L-leucine in 6% TCA. The precipitate was then extracted twice with ethanol-ether at 48° C. for 45 minutes, once with 1% L-leucine in 6% TCA at 90° C. for one hour and finally was washed with ether. After drying, the total precipitate was taken up in one ml. of 1 M hydroxide of hyamine, which in turn was dissolved in 5 ml. toluene base scintil-

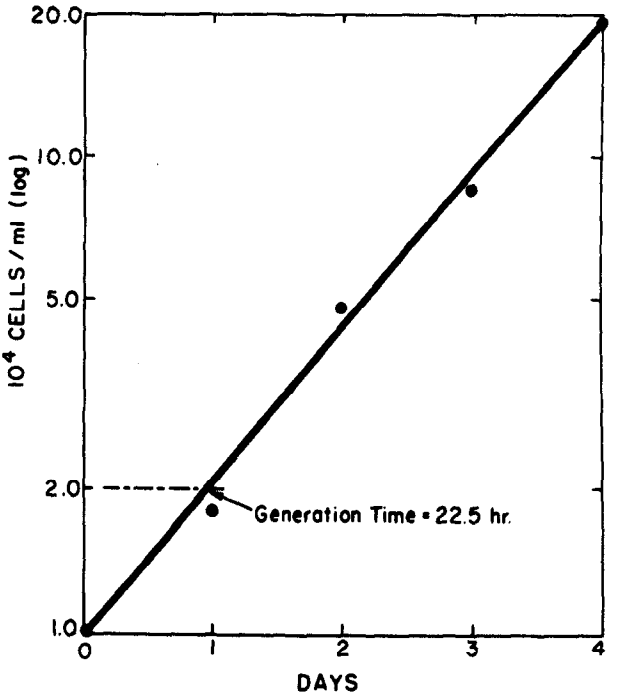

Fig. 1. Cell number vs. time.

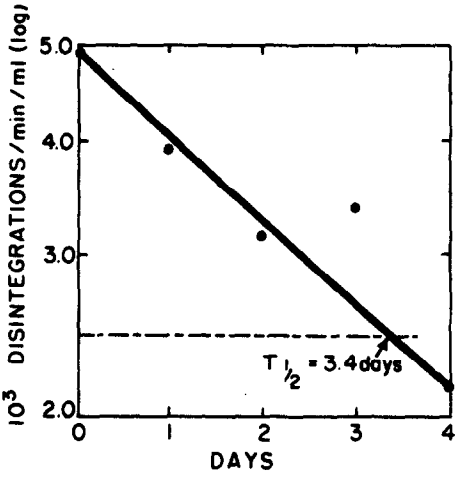

Fig. 2. Cell protein radioactivity vs. time.

lator for counting in a Packard Tri-Carb automatic scintillation counter. All samples were individually standardized against U.S. Bureau of Standards benzoic-C^{14} acid.

Cell counts and cell protein radioactivities are summarized in Figures 1 and 2. Cell growth is seen to be strictly logarithmic over the 4 day experimental period, with a generation time of 22.5 hours. Cell protein radioactivity declines in logarithmic fashion, with a biological half-life of 3.4 days. It should be pointed out that this half-life represents gross protein turnover and should not be directly compared with the half-life for protein catabolism, determined earlier by Jordan, Miller and Peters (1959), since turnover involves both protein catabolism and the transfer of whole proteins from the cells to the nutrient.

Prior to the work of King, et al., the authors pointed out the necessity of experimental demonstration of adequate pool size. Calculated pools do not necessarily represent intracellular concentrations. In experiments involving the release of labeled metabolites, particular care must be taken to assure adequate pools for the trapping of labeled compounds.

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