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Alterations in the Growth Rate and Metabolism of Chinese Hamster Cells *In Vitro* (38530)

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The lifespan of animal cells in culture is characterized by a period of rapid cell proliferation (Phase II), followed by a period in which the fraction of cells undergoing division within the cell population gradually declines (Phase III), and the culture is lost or transformed to a cell line which can be propagated indefinitely. This phenomenon has been described for cell cultures derived from human (1), mouse (2, 3), chick (4, 5), *Potorous tridactylis* (6), rabbit (7), and tortoise tissues (8).

This report demonstrates that cell cultures derived from Chinese hamster lung tissue also undergo a gradual decline in proliferation (Phase III). In addition, cells in Phase III utilize more glucose/10^4 cells and produce more lactic acid/10^4 cells than do Phase II cell cultures during the growth cycle.

Materials and Methods. Chinese hamster (Don) lung cells, in the 28th passage, were obtained from the American Type Culture Collection (Rockville, MD; CCL 16). Cells were grown in antibiotic-free NCTC 135 medium supplemented with 10% newborn calf serum in a 5% CO_2-95% air atmosphere. Experimental and stock cultures were refed every 48 hr until the cultures reached confluency. At confluency, cells were subcultured using the method described by Pace and Aftonomas (9).

For growth experiments, 1.5–1.8 $\times$ 10^5 cells were inoculated into T-15 flasks. Twenty-four hours later, and each 48 hr thereafter, the spent medium of three cultures of young or old cells was pooled and frozen for glucose and lactate determinations; the cells were then harvested, counted, and the average cell population determined. Cell number was determined using a Coulter Counter.

Glucose utilization and lactate formation in the spent medium were analyzed colorimetrically every 48 hr. The method used for glucose determinations was that described by Hyvarinen and Nikkili (10). Lactic acid formation was determined using the method described by Scholz *et al.* (11).

Results. Results in Fig. 1 compare the growth rates of cells in Phase II and Phase III (a–d). Although the initial rate of cell proliferation after subcultivation (days 1–3) was similar in both Phase II and Phase III cultures (1.45 vs. 1.44 population doublings/48 hr respectively), the maximum number of cells at stationary phase in Phase III cultures declined as replicate experiments were conducted over a 1 mo period (a–d). For example, cells in Phase II reached a saturation density of approximately 1.1 $\times$ 10^6 cells/T-15 flask, whereas a maximum density of 0.27 $\times$ 10^6 cells/T-15 was attained in the last experiment in Phase III (d). Cells late in Phase III (d), reached stationary phase approximately 4–5 days after inoculation, whereas cells in Phase II typically entered stationary phase after 8 days of growth. In addition, approximately 30% fewer cells attached to the glass 24 hr after inoculation in late Phase III cultures (c–d). Cell cultures entered Phase III after approximately 110 population doublings or 7 mo in culture.

The rate of glucose utilization/10^4 cells during the growth cycle of cells in Phase II and Phase III (Table I) was greatest during the lag and log phases of cell growth (Phase II, 0–5 days; Phase III, 0–3 days) but reached a "steady-state" as cells approached and entered stationary phase (days 5–13). Cells in Phase III, however, consumed 1.2–2.2 as much glucose/10^4 cells as young cells throughout the entire growth cycle.

Lactic acid production in Phase II cells (Table II) was greatest during the early stages of cell growth (0–3 days) and reached a "steady-state" as cells approached and entered stationary phase. Phase III cells also produced a high amount of lactate

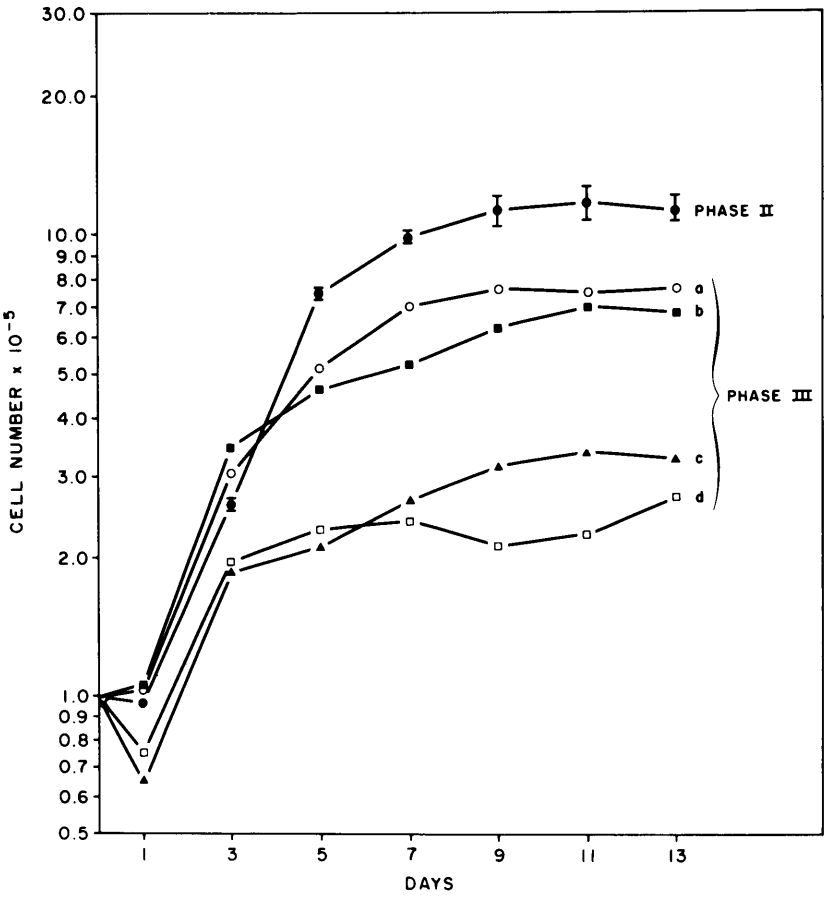

FIG. 1. The growth rate of Chinese hamster (Don) lung cells in Phase II and Phase III.

TABLE I. MICROMOLES GLUCOSE UTILIZED PER 10^4 CELLS/48 HR DURING THE GROWTH CYCLE OF CHINESE HAMSTER (DON) LUNG CELLS DURING PHASE II AND PHASE III.[a]

Days	Phase II	Phase III[b]	Phase III / Phase II
0–1	0.084 ± 0.02 (4)	0.147 ± 0.02 (4)	1.75
1–3	0.075 ± 0.02 (5)	0.121 ± 0.04 (4)	1.61
3–5	0.061 ± 0.007 (5)	0.076 ± 0.007 (3)	1.24
5–7	0.041 ± 0.008 (5)	0.074 ± 0.02 (4)	1.8
7–9	0.037 ± 0.007 (5)	0.083 ± 0.01 (3)	2.2
9–11	0.044 ± 0.005 (5)	0.092 ± 0.02 (3)	2.09
11–13	0.047 ± 0.008 (5)	0.106 ± 0.03 (3)	2.25

[a] The values show the mean ± standard error of the mean followed by the number of determinations in parentheses. Each determination was done in triplicate.

[b] All values are significantly different from Phase II values; $P < 0.05$.

during the early stages of cell growth (0–3 days), but maintained a high lactate production throughout the remainder of the growth cycle. Also, Phase III cells produced 1.3–2.1 as much lactate throughout the growth cycle as did phase II cells.

TABLE II. MICROMOLES LACTIC ACID PRODUCED PER 10^4 CELLS/48 HR DURING THE GROWTH CYCLE OF CHINESE HAMSTER (DON) LUNG CELLS DURING PHASE II AND PHASE III.[a]

Days	Phase II	Phase III[b]	Phase III / Phase II
0–1	0.133 ± 0.03 (5)	0.183 ± 0.02 (3)	1.38
1–3	0.083 ± 0.01 (4)	0.163 ± 0.02 (2)	1.96
3–5	0.066 ± 0.01 (4)	0.092 ± 0.02 (3)	1.39
5–7	0.063 ± 0.006 (5)	0.120 ± 0.02 (3)	1.90
7–9	0.058 ± 0.006 (5)	0.125 ± 0.02 (3)	2.15
9–11	0.065 ± 0.01 (5)	0.137 ± 0.05 (3)	2.10
11–13	0.080 ± 0.007 (4)	0.153 ± 0.04 (4)	1.91

[a] The values show the mean ± standard error of the mean followed by the number of determinations in parentheses. Each determination was done in triplicate.

[b] All values are significantly different from Phase II values; $P < 0.005$.

The amount of lactic acid produced/μg glucose consumed did not vary significantly in Phase II and III cultures (Table III).

Discussion. The data presented demonstrate that Chinese hamster (Don) lung cells, after several months of serial subcultivation, undergo a gradual decline in proliferative capacity which has characterized the finite lifespan of cells derived from other animal species (1–8).

The high rate of glucose utilization of Chinese hamster cells during a single growth cycle confirms earlier reports concerning glucose consumption of tissue culture cells (12, 13). However, the increased rate of glucose utilization of cells in Phase III appears to differ from earlier reports by Cristofalo and Kritchevsky for human cells (12). They reported no difference in the rate of glucose consumption per milligram dry weight in Phase II and Phase III human cells. This apparent difference may be due to inherent differences in the glucose consumption of the two cell types, or the manner in which the data is expressed. For example, it has been shown that the cell volume (14) and protein content per cell (15) increase in Phase III human and *Potorous tridactylis* cells. It is likely that glucose consumption/cell would increase if the cell mass increased and would not be compensated for if glucose consumption and lactic acid formation are expressed on a per cell basis. In this regard, Goldstein and Trieman (16) have shown that Phase III human cells utilize more glucose than cells in Phase II if consumption is expressed on a per cell basis.

TABLE III. MICROGRAM LACTIC ACID PRODUCED PER MICROGRAM GLUCOSE CONSUMED DURING THE GROWTH CYCLE OF CHINESE HAMSTER (DON) CELLS DURING PHASE II AND PHASE III.

Days	Phase II	Phase III
0–1	1.58	1.24
1–3	1.11	1.35
3–5	1.1	1.21
5–7	1.54	1.60
7–9	1.50	1.51
9–11	1.48	1.49
11–13	1.70	1.44
Mean	1.44	1.41

The increased rate of lactic acid production in Phase III cells complements the increased consumption of glucose in that greater glucose consumption should result in increased production of lactic acid. This data, coupled with the observation that no significant change occurs in the glucose per lactate ratio of Phase III cells, suggests that the increased amount of glucose consumed by these cells is being utilized through the glycolytic pathway in the same proportion as in Phase II cells.

If the same proportion of a larger amount of glucose being consumed is utilized through the glycolytic pathway, then, in turn, an increased amount of glucose should also be directed into other metabolic pathways. In this regard, increased amounts of glyco-

gen, lipid, protein and RNA have been noted in Phase III human cells (15). Whether these parameters are altered similarly in Chinese hamster cells in Phase III awaits further experiments.

Summary. Chinese hamster (Don) lung cells undergo a gradual decline in proliferation (Phase III) during their lifespan in culture. In addition, cells in Phase III utilize more glucose per 10^4 cells and produce more lactic acid per 10^4 cells than do Phase II cell cultures.

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