# EFFECT OF GROWTH CONDITIONS ON THE ORNITHINE DECARBOXYLASE ACTIVITY OF RAT HEPATOMA CELLS.

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SUMMARY: Ornithine decarboxylase activity in rat hepatoma (HTC) cells in suspension culture declines progressively with increasing cell density and is barely detectable in stationary phase, high-density cultures. Dilution of these cells results in a large increase in ornithine decarboxylase activity, which reaches a maximum at about 4 hours, followed by a decline to a new steady-state level. This increase is abolished by cycloheximide but not by Actinomycin D, suggesting that, at least under some circumstances, the synthesis of this enzyme is under post-transcriptional control.

Ornithine decarboxylase (E.C. 4.1.1.17) catalyses the conversion of L-ornithine to putrescine, the first and perhaps rate-limiting step in the synthesis of polyamines in mammalian cells (1). A large increase in the activity of this enzyme has been observed soon after the transition of a number of cells from a non-growing to a growing state (2-4) or after stimulation of tissues by growth-promoting hormones (1, 5-9). In most cases where it has been studied this increase is inhibited by administration of Actinomycin D at the same time as the growth-stimulant (3-5).

Dilution of high-density suspension culture cells stimulates the incorporation of amino acids into protein, polyribosome formation, incorporation of uridine into RNA, and RNA polymerase and uridine kinase activity (10-13), all responses typical of the transition from the non-growing to the growing state. In this paper evidence is presented that dilution of high-density hepatoma cells results in a very large, but transient, increase in ornithine decarboxylase activity that is not abolished by Actinomycin D.

## METHODS

The rat hepatoma cell line (HTC) was kindly provided by Dr. G.M. Tomkins and was grown in suspension in modified Swim's 77 medium containing 5% foetal

calf and 5% calf serum as described (14). The population doubling time was about 24 hrs and cells reached a saturation density of approximately 7 x $10^5$ cells/ml. They were subcultured to a density of $<$ 1 x $10^5$/ml. For ornithine decarboxylase assays $\sim$8 x $10^6$ cells were harvested rapidly at 37°C, washed twice with phosphate-buffered saline at 4°C and disrupted by freezing and thawing in 0.1 mM EDTA, 0.05 mM pyridoxal phosphate, 5 mM DTT and 50 mM Tris pH 7.1. Reaction mixtures contained 0.5ml of a 10,000g supernatant fraction and 0.25 mM L-ornithine-1-$C^{14}$ (specific activity 1-2mC/mmole). $C^{14}O_2$ release, which was linear for at least an hour and was proportional to the amount of supernatant protein added, was measured after a 1 hr incubation at 37° as described (15). Control samples contained an equal volume of boiled supernatant fraction. Protein was measured by the method of Lowry (16), correcting for the presence of DTT in the reaction buffer. Tyrosine aminotransferase was assayed as described (14).

## RESULTS

Table 1 shows the ornithine decarboxylase activity of cells harvested from stock cultures of different densities over a period of several months. Activity declined progressively with increasing cell density and was barely detectable above the background in high-density, stationary phase cultures. The low activity of extracts from high-density cells was not due to the presence of an inhibitor since there was no increase after dialysis, and they did not inhibit low density cell extracts when mixed together. Tyrosine transaminase, another pyridoxal phosphate requiring enzyme, did not show any significant fluctuation in basal level with cell density.

When high-density ($\sim$6 x $10^5$ cells/ml) cultures were diluted to 2 x $10^5$ /ml with fresh medium a very large increase in ornithine decarboxylase activity was observed (Fig. 1). In most experiments (8/12) the activity reached a maximum at 3-4 hrs and then declined, until 8 and 9 hrs after dilution it plateaued at about 1500 μμ moles $CO_2$/mg protein/hr (Fig. 1, C). In two experiments enzyme activity increased rapidly over 1 hr to about 1700 μμ moles $CO_2$/mg protein/hr and remained at this level for the next four hours.

Table 1.

Effect of cell density on ornithine decarboxylase and tyrosine transaminase activity* of stock cultures of HTC cells.

| Cell density [cells/ml x $10^5$] | ornithine decarboxylase[a] | tyrosine transaminase[b] |
|---|---|---|
| 1 - 1.9 | 1858 ± 409 (6) | 9.8 ± 1.6 (4) |
| 2 - 2.9 | 904 ± 151 (8) | |
| 3 - 3.9 | 795 ± 154 (4) | 9.3 ± 1.7 (4) |
| 4 - 4.9 | 465 ± 125 (6) | |
| 5 - 5.9 | 140 ± 45 (3) | 7.8 ± 1.4 (4) |
| 6 - 6.9 | 16 ± 4 (5) | |

* Values are the mean ± S.E.M., with the number of samples taken from different cultures in parenthesis. Each assay was done in duplicate with an error of not usually more than 10%.

a μμmoles $CO_2$/mg protein/hr.
b (uninduced level) mμmoles p-hydroxyphenylpyruvate/mg protein/min.

As shown in Fig. 1, A an increase in ornithine decarboxylase activity was still seen upon dilution of high-density cells into medium containing 0.5 μg/ml Actinomycin D. At 4 hrs the activity attained was 62% of that of control cultures. In other experiments no significant difference between control cultures and those containing 0.5 μg or 5.0 μg/ml Actinomycin D was observed at 1 or 2 hrs after dilution but some inhibition was always observed at 3 hrs and later. Addition of 0.5 and 5.0 μg/ml Actinomycin D at 4 hrs after dilution did not prevent the subsequent decline in enzyme activity and produced a significant inhibition 6 hrs after dilution and later (Fig. 1, B and C). In these cells 0.5 μg/ml Actinomycin D was found to inhibit the incorporation of $H^3$-uridine into RNA by $>97\%$ over a 30 min incubation period.

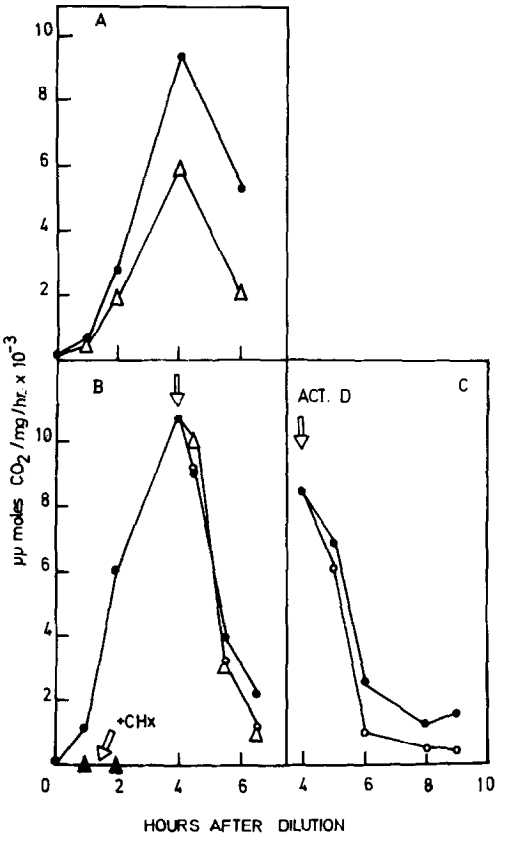


Fig. 1: Effect of culture dilution on ornithine decarboxylase activity of HTC cells.

Cells at the following concentrations (A, 6.9 x $10^5$/ml; B, 6 x $10^5$/ml; C, 5 x $10^5$/ml) were diluted at 0 time to 2 x $10^5$/ml with fresh medium containing serum. In A Actinomycin D was added at the time of dilution in B and C the drug was added 4 hours after dilution.

control; + 0.5 μg/ml Actinomycin D;
5 μg/ml Actinomycin D; 20 μg/ml cycloheximide.

The activity of the cells before dilution was 28 μμmoles $CO_2$/mg/hr in B and was not significantly above background in A.

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Addition of 20 μg/ml cycloheximide at the time of dilution completely inhibited the increase in enzyme activity (Fig. 1, B) and reduced the basal activity to undetectable levels. This is consistent with the very short half-life of ornithine decarboxylase activity (about 10 mins) observed in the presence of inhibitors of protein synthesis in a variety of tissues, including HTC cells (3-5, 17).

## DISCUSSION

The exact magnitude of the very large and rapid increase in ornithine decarboxylase activity in HTC cells after dilution is difficult to estimate because the pre-dilution level is often near the limits of sensitivity of the assay, but it may reach several hundred-fold (Fig. 1, B). Among the factors that may be involved in producing this increase are, (i) changes in substrate and inhibitor concentrations, (ii) increased synthesis, and (iii) decreased degradation of the enzyme. Although changes in substrate and inhibitor concentrations may play a role in the fine regulation of enzyme activity *in vivo* (1) it is unlikely that they could account for such large increases as those observed in Fig. 1. Moreover, dialysis and mixing experiments failed to provide evidence for an inhibitor of ornithine decarboxylase in high-density cell extracts.

The observation that cycloheximide inhibits the increase in enzyme activity upon dilution suggests that the synthesis of new enzyme molecules is involved, rather than the activation of precursors, but until specific immunological techniques are available this conclusion is, of course, open to question. In HTC and other cells (3-5, 17) ornithine decarboxylase has a very short half-life of activity in the presence of inhibitors of protein synthesis, implying that it is rapidly degraded. Under these circumstances the pool of enzyme molecules in cells in which the rate of protein synthesis is low would be small, and any increase in protein synthesis would produce a rapid rise in ornithine decarboxylase relative to other enzymes with longer turnover times (18). Cells grown to a high density are probably starved both for amino acids and serum growth-factors, and dilution of such cells has been shown to stimulate the general rate of protein synthesis (10, 13) and polyribosome formation (12). Such effects may, at least in part, account for the increase in ornithine decarboxylase after dilution of hepatoma cells. The fact that this increase is largely resistant to Actinomycin D suggests that the messenger for this enzyme continues to be synthesised, or is stored, in

stationary-phase, high-density HTC cells and is available for more frequent translation when conditions for protein synthesis improve. In tissues such as liver and lymphocytes (3-5) the increase in enzyme activity after stimulation by growth-promoting agents is inhibited by simultaneous doses of Actinomycin D that completely prevent RNA synthesis. It is therefore possible that in different tissues, or in the same tissue under different circumstances, the synthesis of ornithine decarboxylase is limited either by the supply of messenger RNA or its rate of translation.

In addition to an effect on messenger RNA translation, dilution of high-density cells may result in a decrease in the rate of degradation of ornithine decarboxylase. Degradation of general cell protein and tyrosine aminotransferase increases when HTC cells are shifted into medium lacking serum or amino acids (19), although the mechanism of this effect is not known. Experiments to measure the turnover of ornithine decarboxylase under different conditions are in progress.

The physiological significance of the rapid fluctuations in ornithine decarboxylase activity is not clear (for review see 20). Among other things, it has been implied that an increase in this enzyme is linked to an increase in the synthesis of ribosomal RNA (4). The observation that a large stimulation of ornithine decarboxylase can occur in the absence of RNA synthesis suggests that such a coupling, if it exists, is not an obligatory one.

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