Effects of Diamines on Ornithine Decarboxylase Activity in Control and Virally Transformed Mouse Fibroblasts

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1. The induction of ornithine decarboxylase activity in mouse 3T3 fibroblasts or an SV-40 transformed 3T3 cell line by serum was prevented by addition of the naturally occurring polyamines putrescine (butane-1,4-diamine) and spermidine. Much higher concentrations of these amines were required to fully suppress ornithine decarboxylase activity in the transformed SV-3T3 cells than in the 3T3 fibroblasts. 2. Synthetic $\alpha\omega$-diamines with 3–12 carbon atoms also prevented the increase in ornithine decarboxylase activity induced by serum in these cells. The longer chain diamines were somewhat more potent than propane-1,3-diamine in this effect, but the synthetic diamines were less active than putrescine in the 3T3 cells. There was little difference between the responses of 3T3 and SV-3T3 cells to the synthetic diamines propane-1,3-diamine and heptane-1,7-diamine. 3. These results are discussed in relation to the control of polyamine synthesis in mammalian cells.

Ornithine decarboxylase, which converts ornithine into putrescine, is a key enzyme in the biosynthesis of the polyamines spermidine and spermine (Pegg & Williams-Ashman, 1968; Tabor & Tabor, 1976; Jänne *et al.*, 1978). A number of studies have indicated that the activity of this enzyme is diminished when cultured cells or animal tissues *in vivo* are exposed to putrescine or the polyamines (Kay & Lindsay, 1973; Jänne & Hölttä, 1974; Clark & Fuller, 1975; Fong *et al.*, 1976; Heller *et al.*, 1976; Jefferson & Pegg, 1977; Pegg *et al.*, 1978). It has also been observed that administration of certain other diamines not normally present in living cells such as propane-1,3-diamine decreases ornithine decarboxylase activity in various rat tissues (Pösö *et al.*, 1977, 1978; Guha & Jänne, 1977; Jänne *et al.*, 1978; Pegg *et al.*, 1978), in rat hepatoma cells (McCann *et al.*, 1977; Heller *et al.*, 1978) and in Chinese-hamster ovary cells (Sunkara *et al.*, 1977). The decrease in ornithine decarboxylase activity in response to putrescine or polyamines may be an important mechanism for regulating polyamine biosynthesis and there is substantial evidence that polyamine synthesis is required for cell growth and is increased in response to growth-promoting stimuli (Tabor & Tabor, 1972, 1976; Raina & Jänne, 1975; Jänne *et al.*, 1978). Recently, it was postulated that some rapidly growing cells may have a decreased sensitivity to the control of ornithine decarboxylase activity by exogenous polyamines (Canellakis *et al.*,

1978). There is some evidence that polyamine concentrations in physiological fluids are increased in patients suffering from various forms of malignancy (Bachrach, 1976; Durie *et al.*, 1977; Rennert *et al.*, 1977; Jänne *et al.*, 1978) and many tumours have been found to contain higher polyamine concentrations than their normal counterparts. The ability to decrease polyamine synthesis by application of synthetic diamines may prove a useful tool both for investigating polyamine function and in selectively inhibiting the growth of certain cells (Pösö & Jänne, 1976; Kallio *et al.*, 1977*a*; Wiegand & Pegg, 1978; Piik *et al.*, 1978; Jänne *et al.*, 1978). A full evaluation of these possibilities requires more detailed knowledge of the mechanism by which the ornithine decarboxylase activity is altered by exogenous amines and of the relative sensitivities of different cell types to inhibitory amines.

In the present paper, the effects of spermidine and of a series of $\alpha\omega$-diamines on ornithine decarboxylase activity in mouse fibroblast cultures have been studied. It was found that all the diamines with from 3 to 12 carbon atoms were able to inhibit the increase in ornithine decarboxylase activity observed when cells were stimulated by serum. SV-transformed 3T3 cells were significantly less sensitive than 3T3 cells to the inhibitory effects of putrescine and spermidine, but there was much less difference in the responses of the normal and transformed cell lines to the synthetic diamines.

Materials and Methods

Materials

DL-[1-^{14}C]Ornithine (sp.radioactivity 45 mCi/mmol), [1,4-^{14}C]putrescine (sp. radioactivity 65 mCi/mmol) and [tetramethylene-1,4-^{14}C]spermidine (sp. radioactivity 55 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Iproniazid phosphate, NN'-diaminoguanidine and αω-diamines (except for putrescine and cadaverine) were purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. and tested for purity as previously described (Pegg et al., 1978). All other biochemicals were from Sigma Chemical Co., St. Louis, MO, U.S.A. The anti-mycotic, n-butyl p-hydroxybenzoate was purchased from Eastman–Kodak, Rochester, NY, U.S.A. All other media and reagents for tissue culture were products from Flow Laboratories, Rockville, MD, U.S.A.

Cell culture

Stock cultures of Swiss 3T3 and SV-40 virus-transformed 3T3 mouse embryo fibroblasts (Schuler et al., 1977) were maintained in Dulbecco's modified Eagle's medium with 10% foetal calf serum, 36 mM-NaHCO$_3$, penicillin (100 units/ml), streptomycin (100 mg/ml) and 2 μM-n-butyl p-hydroxybenzoate. Cells were grown in a humidified atmosphere of CO$_2$/air (1:9, v/v) at 37°C. Stocks were subcultured every 3 to 4 days by using a 0.5% (w/v) trypsin solution for transfer. Experimental and stock cultures were grown in tissue-culture dishes (100 mm × 20 mm). All plates were seeded with 4×10^5 cells.

For time-course studies, cultures of both cell lines were initiated as described above. 3T3 cells were grown for 7 days to ensure confluency. On day 7 the medium was aspirated, the cells were washed twice with phosphate-buffered saline (6.8 g of NaCl, 1.69 g of Na$_2$HPO$_4$ and 0.2 g of KH$_2$PO$_4$ in 1 litre) and placed in Dulbecco's medium containing no serum. The SV-3T3 cells were treated similarly on day 3. After incubation for 24 h in serum-free media, experiments were performed. Foetal calf serum was added to a final concentration of 10%. Stock solutions (usually 1.0 M) of the amines were made up and neutralized to a pH of 7 by addition of HCl when necessary. Amounts of these solutions were added to the cultures to give the concentrations shown. Control plates received only foetal calf serum or no additions. Cells were harvested as described below after incubating for 2–8 h and assayed for ornithine decarboxylase activity. Dose–response studies were carried out in a similar way. After serum-starving cells for 24 h, foetal calf serum was added to produce a 10% (v/v) final concentration and diamines or polyamines were added to final concentrations ranging from 0.1 μM to 10 mM. Cells were harvested

at the time shown. The osmolality of the medium before and after additions was determined by using an osmometer manufactured by Advanced Instruments, Newton Highlands, MA, U.S.A. Intracellular fluid space was measured by the method of Foster & Pardee (1969).

All samples were harvested by the following procedure. Medium was removed and cells washed twice with cold phosphate-buffered saline. Hypo-osmotic buffer (1 or 2 ml) containing 25 mM-Tris/HCl/0.1 mM-disodium EDTA/2.5 mM-dithiothreitol and having a pH of 7.5 was added and cells were allowed to swell at 4°C for 15 min. A rubber 'policeman' was used to scrape the cells from the plates. The cell mixture was freeze–thawed twice in liquid N$_2$ and the lysed-cell mixture centrifuged in a Sorvall RC5 refrigerated centrifuge at 17000 g for 30 min. The supernatant was removed and stored at −20°C for no more than 3 days before assaying for ornithine decarboxylase activity.

Measurement of ornithine decarboxylase activity

Ornithine decarboxylase activity was measured by studying the release of ^{14}CO$_2$ from [1-^{14}C]ornithine (Pegg & Williams-Ashman, 1968). The cell extract (0.2 ml) was incubated in a final volume of 0.25 ml containing 0.08 mM-pyridoxal phosphate/2.5 mM-dithiothreitol/60 mM-Tris/HCl (pH 7.5)/0.08 mM-disodium EDTA and 0.5 μCi (0.045 mM) of DL-[1-^{14}C]ornithine (sp. radioactivity 45 mCi/mmol). After incubation at 37°C for 30 min the reaction was stopped by the addition of 0.3 ml of 5 M-H$_2$SO$_4$, the ^{14}CO$_2$ released was trapped in 0.25 ml of Hyamine hydroxide and the radioactivity was determined as previously described (Pegg et al., 1978). Results were corrected for the small amount of radioactivity trapped when heat- or acid-inactivated tissue extracts were employed and expressed as nmol of ^{14}CO$_2$ released/mg of protein added. Protein was measured by the method of Lowry et al. (1951) with crystalline bovine serum albumin as standard. Samples were precipitated with 20% (w/v) trichloroacetic acid and dissolved in 0.1 M-NaOH as described by Jänne & Williams-Ashman (1971) to prevent interference in the assay by the high concentrations of dithiothreitol. In each experiment, ornithine decarboxylase activities were the mean for at least two separate estimations on separate samples that agreed within ±10%.

The assay conditions used in the present experiments were not saturating for the ornithine substrate. For this reason, the activities measured were less than those reported by Lembach (1974) and Clark & Fuller (1975). Although manyfold increases in ornithine decarboxylase activity were observed in all experiments when cells were stimulated with serum, the absolute values of the ornithine decarboxylase

activity varied significantly from experiment to experiment. This variability prevented the averaging of experiments unless the results were expressed as percentages of the control activity. Therefore, Figs. 2–4 represent the mean for three separate experiments in which the data were expressed as percentages of control activity and Tables 1–3 and Fig. 1 show representative examples from experiments that were repeated several times.

Toxicity of amines

The lack of toxicity of the amines used towards the cells was checked by cell counts and Trypan Blue staining. Normal cell growth and viability was observed over a 48h period in the presence of 5mM of all of the aliphatic diamines having a chain length of 7 carbon atoms or less. Octane-1,8-diamine and nonane-1,9-diamine were non-toxic at a concentration of 2.5mM and dodecane-1,12-diamine was non-toxic at 0.05mM.

Stability of amines during exposure to serum

The possible oxidation of added amines by the foetal calf serum was tested in two ways. Firstly, after addition of 1mM-putrescine or propane-1,3-diamine to the culture medium and incubation for up to 8h, samples were taken and analysed for the diamines by quantitative ninhydrin staining after electrophoretic separation (Pegg et al., 1978). No decrease in the diamine concentration was observed over the 8h period. Secondly, oxidation of spermidine and putrescine was tested by addition of 20μM-[^{14}C]spermidine or putrescine to the culture medium and after incubation for up to 48h the amines present were extracted, separated by high-voltage electrophoresis as described above and the radioactivity present measured. Only 10% of the putrescine was lost in 24h and 37% in 48h. However, 44% of the spermidine was lost in 24h and 82% in 48h. This loss in recoverable radioactive polyamines was completely prevented by the addition of 1mM-iproniazid phosphate or 1mM-NN'-diaminoguanidine monohydrochloride.

Putrescine uptake

This was measured as described by Foster & Pardee (1969). The cells were grown on coverslips (11mm × 22mm) for 3 days (SV-3T3 cells) or 7 days (3T3 cells). The cover slips were then rinsed in phosphate-buffered saline containing 0.1% (w/v) glucose and then incubated at 37°C in medium containing 0.05mM-[1,4-^{14}C]putrescine (sp. radioactivity 2mCi/mmol). After incubation for various times, the cells were rinsed rapidly in ice-cold phosphate-buffered saline containing 0.1% (w/v) glucose, solubilized in 0.6ml of 0.2M-NaOH and samples taken for determination of protein and assay of radioactivity.

Results

As previously reported by others (Lembach, 1974; Clark, 1974), ornithine decarboxylase activity was substantially enhanced when 3T3 cells that had been maintained at confluence for 24h in a serum-free medium were stimulated by addition of serum. This increase in activity reached a peak at 6h after stimulation (Fig. 1). When putrescine, spermidine or a variety of other diamines were added at the same time as the serum, this rise in activity could be prevented (Fig. 1 and Table 1). All the aliphatic $\alpha\omega$-diamines with from 3 to 12 carbon atoms were able to prevent the increase in activity when added at concentrations of 1mM or greater (Table 1). These concentrations of the shorter chain diamines did not produce any obvious cytotoxicity. However, the longer chain diamines, particularly dodecane-1,12-diamine, were more toxic. The latter diamine was therefore tested at a lower concentration and found to be active in preventing ornithine decarboxylase activity at 0.05mM, but showed no cytotoxic effects at this concentration. In contrast with these results, 5mM-histamine had no inhibitory effect on the increase in ornithine decarboxylase activity. This finding supports the conclusion that the decreased activity of the enzyme occurs as a specific response to the added diamines and was not due to a change in the
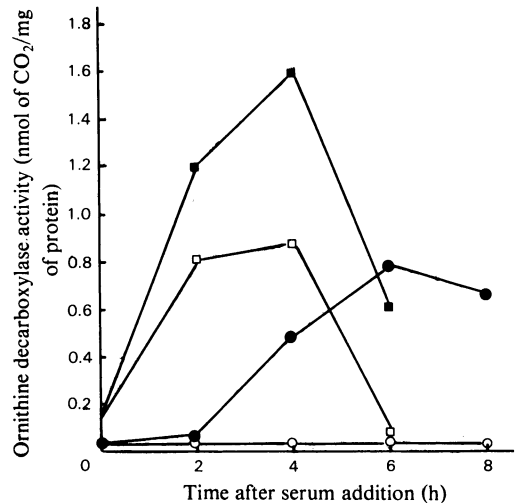

Fig. 1. *Time course of the stimulation of ornithine decarboxylase activity of serum-depleted cells by addition of serum*

At the times shown after addition of foetal calf serum to a final concentration of 10%, cells were harvested and ornithine decarboxylase activity was assayed. Results are shown for 3T3 cells exposed to serum in the absence (●) and presence (○) of 5mM-propane-1,3-diamine and for SV-3T3 cells exposed to serum in the absence (■) and presence (□) of 5mM-putrescine.

Table 1. *Inhibition of the serum-mediated increase in ornithine decarboxylase activity of 3T3 cells by diamines*
Ornithine decarboxylase activity was measured 6h after addition of foetal calf serum (to a final concentration of 10%)
and the diamines shown.

Diamine added	Ornithine decarboxylase activity (pmol of CO_2/mg of protein per 30min)
None, no serum	22
None	786
5 mM-Propane-1,3-diamine	16
5 mM-Putrescine	25
5 mM-Pentane-1,5-diamine	9
5 mM-Hexane-1,6-Diamine	10
1 mM-Heptane-1,7-diamine	11
1 mM-Octane-1,8-diamine	13
1 mM-Nonane-1,9-diamine	9
0.05 mM-Dodecane-1,2-diamine	28
5 mM-Histamine	745

ionic strength or cation composition of the medium. Although ornithine decarboxylase activity of certain cultured cells has been shown to be very sensitive to changes in the osmolality of the medium (Munro *et al.*, 1975; Chen *et al.*, 1976; Friedman *et al.*, 1977) the changes produced by even the highest concentrations of additions used in the present paper were found to be small (less than 1% of the total osmolality of the medium). Addition of an equivalent amount of NaCl did not alter the ornithine decarboxylase activity.

When SV-transformed 3T3 cells were stimulated by addition of serum in a similar way, ornithine decarboxylase activity was increased more rapidly and to a greater extent, reaching a peak within 4h (Fig. 1). The presence of putrescine decreased the magnitude of this increase, but as shown in Fig. 1, a substantial increase still occurred even in the presence of 5 mM-putrescine. This finding suggested that there may be a difference in the sensitivity of the transformed cells and the non-transformed line to a decrease of ornithine decarboxylase activity by diamines and polyamines. Therefore, comparisons were made of the inhibition of the serum-induced increase in ornithine decarboxylase activity in 3T3 and SV-3T3 cells as a function of the amine concentration with a number of different amines (Figs. 2–4). In these experiments the ornithine decarboxylase activity when the amines were present at the concentrations shown was expressed as a percentage of that present in serum-stimulated cells not exposed to the exogenous amines. Measurements of activity were made 6h after stimulation of 3T3 cells (the time at which a peak of activity was observed in Figs. 1) and 4h and 6h after stimulation of SV-3T3 cells, which showed a peak of activity at 4h (Fig. 1). It was therefore possible to compare activity both at the same time and at the time at which the different cell lines showed a peak of activity. It can be seen from Fig. 2 that ornithine decarboxylase activity in 3T3 cells was completely lost on exposure to concen-
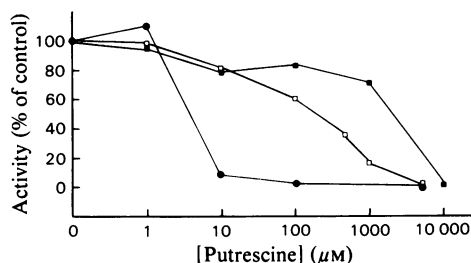

Fig. 2. *Effect of putrescine concentration on inhibition of ornithine decarboxylase activity*
3T3 cells (●) or SV-3T3 cells (■, □) were exposed to serum as described in Table 1 and ornithine decarboxylase activity measured 4h (■) or 6h (●, □) later. Putrescine was added at the same time as the serum at the concentration shown. The activity observed for each cell line at the time considered when no putrescine was added was designated 100%. The absolute values of this activity were similar to those shown in Fig. 1.

trations of putrescine of 10 μM or more. The SV-3T3 cells required much higher concentrations of putrescine for a similar decrease in ornithine decarboxylase activity. Complete inhibition required the addition of 5 mM-putrescine when measured 6h after stimulation and 10 mM-putrescine when measured 4h after stimulation.

A similar effect was observed when spermidine was added to the cells instead of putrescine (Fig. 3). Almost complete prevention of the increase in ornithine decarboxylase activity was observed when 3T3 cells were exposed to 5 μM-spermidine. However, SV-transformed 3T3 cells required spermidine concentrations of 1 mM or greater to produce a similar inhibition. These higher concentrations were required whether activity was measured 3, 4 or 6h after serum addition (Fig. 3). These results obtained with the naturally occurring putrescine and spermidine
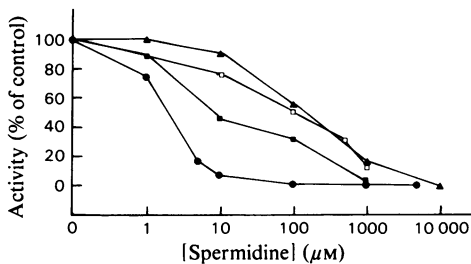
Fig. 3. *Effect of spermidine concentration on inhibition of ornithine decarboxylase activity*
3T3 cells (●) or SV-3T3 cells (■, □, ▲) were exposed to serum as described in Table 1 and ornithine decarboxylase activity was measured 4 h (■), 3 h (▲) or 6 h (●, □) later. Spermidine was added at the same time as the serum at the concentration shown. Other details were as described in the legend to Fig. 2.
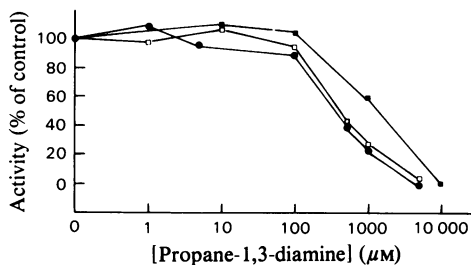

Fig. 4. *Effect of propane-1,3-diamine concentration on inhibition of ornithine decarboxylase activity*
3T3 cells (●) or SV-3T3 cells (■, □) were exposed to serum as described in Table 1 and ornithine decarboxylase activity was measured 4 h (■) or 6 h (●, □) later. Propane-1,3-diamine was added at the same time as the serum at the concentration shown. Other details were as described in the legend to Fig. 2.

suggest that the transformed cells may be less sensitive to inhibition of their ornithine decarboxylase activity than the 3T3 cells when exposed to exogenous concentrations of these amines. As shown in Table 1 a number of non-physiological $\alpha\omega$-diamines were also able to decrease the ornithine decarboxylase activity of serum-stimulated 3T3 cells. When these non-physiological diamines were tested for their abilities to diminish ornithine decarboxylase activity of 3T3 and SV-3T3 cells at various concentrations, it was found that the difference in sensitivity between the two cell lines was much decreased. Thus, as shown in Fig. 4, ornithine decarboxylase activity of 3T3 cells was not significantly decreased until concentrations of 0.5 mM were reached and 5 mM was required for complete loss of activity. When SV-3T3 cells were exposed to propane-1,3-diamine the decrease in ornithine decarboxylase activity 6 h after serum stimulation was similar to that in the 3T3 cells. When

activity was measured 4 h after stimulation it was somewhat less sensitive to propane-1,3-diamine in SV-3T3 cells than in the 3T3 cells (Fig. 4), but the difference in sensitivity was much less than that observed with putrescine.

It can be seen from the comparison of Figs. 2 and 4 that the 3T3 cells were considerably more sensitive to putrescine than to propane-1,3-diamine. The concentration of putrescine required for 50% inhibition was estimated at less than 10μM, whereas 500μM-propane-1,3-diamine was required for a similar inhibition. The SV-3T3 cells, on the other hand, responded almost equally to putrescine and to propane-1,3-diamine, requiring concentrations of greater than 500μM for 50% inhibition.

The longer chain $\alpha\omega$-diamines, which were also able to prevent the increase in ornithine decarboxylase activity produced by serum in 3T3 cells (Table 1), had similar effects at equivalent concentrations in SV-3T3 cells (results not shown). The longer chain diamines, although less potent than putrescine or spermidine, were rather more effective than propane-1,3-diamine in decreasing ornithine decarboxylase activity. For example, approx. 200μM-heptane-1,7-diamine or 100μM-nonane-1,9-diamine was required for 50% inhibition of activity.

The decrease in ornithine decarboxylase activity observed in the present experiments in response to various di- and poly-amines was not due to the metabolism of the added amines by the serum. Such metabolism could generate H_2O_2 and an aldehyde by the oxidation of the amines and these compounds might produce inhibitory effects. However, measurement of the concentration of putrescine and of propane-1,3-diamine in the medium over the course of the present experiments did not show any significant decline, suggesting that the foetal calf serum did not oxidize these diamines to any significant extent. This is consistent with other published reports (Heller *et al.*, 1978; Clark & Fuller, 1975). When radioactive spermidine was incubated with the medium and foetal calf serum, there was a significant loss suggesting that some spermidine oxidase activity was present. However, this oxidation could be prevented completely by the addition of 1 mM-iproniazid or -NN'-diaminoguanidine and such addition did not prevent the inhibition of ornithine decarboxylase activity by 10μM-spermidine (Table 2). Furthermore, the addition of small amounts of H_2O_2 to the plates did not alter the response to serum or the effects of spermidine and the addition of horseradish peroxidase did not influence the effects (Table 2). The addition of peroxidase to the cell cultures was shown to cause rapid decomposition of 10μM-H_2O_2. In some experiments the addition of H_2O_2 at concentrations of 20μM caused a significant increase in ornithine decarboxylase activity. The explanation for such an increase is at present unclear and this phenomenon

Table 2. *Effect of spermidine on ornithine decarboxylase activity of 3T3 cells in the presence of oxidase inhibitors or H_2O_2*

The experiment was carried out as described in Table 1 with the additions shown being made at the time of serum addition. One unit of peroxidase is the amount of enzyme required to produce 1 mg of purpurogallin in 20s at 20°C.

Addition	Ornithine decarboxylase activity (pmol of CO_2/mg of protein per 30 min)
Serum	1325
Serum+10μM-spermidine	39
Serum+10μM-spermidine+1 mM-NN'-diaminoguanidine	47
Serum+10μM-spermidine+1 mM-iproniazid	32
Serum+10μM-spermidine+1 unit of peroxidase	17
Serum+10μM-H_2O_2	1354
No serum	25

Table 3. *Uptake of labelled putrescine into 3T3 and SV-3T3 cells*

Uptake was measured as described in the Materials and Methods section and expressed as nmol of putrescine/mg of protein assuming that all the radioactivity was present as putrescine.

Time of exposure (min)	Putrescine uptake (nmol/mg of protein)	
	3T3 cells	SV-3T3 cells
2	0.58	0.17
4	1.32	0.24
6	1.33	0.33
10	1.62	0.58
20	1.75	1.08
30	2.40	1.06
60	2.71	1.90

was not studied further. However, the possibility that addition of readily oxidized polyamines to cultured cells might have complex effects resulting from the addition of an inhibitory effect due to the amine and a stimulation from any peroxide produced during its oxidation should be considered. This possibility could provide an explanation for the complex effects on ornithine decarboxylase activity in L1210 cells observed by Heller *et al.* (1978) and Canellakis *et al.* (1978).

The loss of ornithine decarboxylase activity observed in the present experiments in response to diamines and spermidine was not due to a direct inhibition of enzyme activity by any amine present in the cell extracts. Firstly, the activities observed were proportional to the amount of protein added and unaffected by dilution of the extract. Secondly, the direct inhibition of ornithine decarboxylase activity by putrescine and spermidine is very weak (Pegg & Williams-Ashman, 1968), requiring concentrations much higher than those likely to be present in the extracts after the dilution provided by the homogenizing medium. Other $\alpha\omega$-diamines have little or no direct effect on ornithine decarboxylase activity (Pösö *et al.*, 1977; Pegg *et al.*, 1978). Direct measurement of the radioactivity taken up by the

cells when exposed to 50μM-[^{14}C]putrescine revealed that the fibroblasts may concentrate the amine to a significant extent (Table 3). By 60min the apparent putrescine uptake in 3T3 cells amounted to 2.9nmol/ mg of protein. Measurements of the intracellular fluid space of the cells indicated that the average cell volume was 2.4μl/10^6 cells [in reasonable agreement with the estimates of Clark & Fuller (1975) and Foster & Pardee (1969)]. Since 10^6 cells were equivalent to 0.7mg of protein, the concentration of intracellular putrescine due to the uptake may have been as high as 0.85mM. However, it is likely that this value is a substantial overestimate since the radioactivity measured may be partly in metabolites of putrescine and bound to intracellular polyanions. It is also possible that some of the putrescine is not actually taken up, but adsorbed on the cells and not removed by the washing procedure. Such adsorption has been described for *Escherichia coli* by Tabor & Tabor (1966). Therefore, it is premature to conclude that there is substantial accumulation of the putrescine against a concentration gradient even though the calculated intracellular concentration is an order of magnitude higher than the external concentration. As shown in Table 3, uptake of the [^{14}C]putrescine was somewhat more rapid in the 3T3 cells than in the SV-3T3 cells, but the difference did not appear to be sufficient to account for the substantial difference in sensitivity of the ornithine decarboxylase between the two cell lines.

Discussion

In our experiments, the serum-induced increase in ornithine decarboxylase activity of 3T3 cells was quite sensitive to inhibition by putrescine and spermidine. A 50% decrease in activity was produced in 6h by less than 10μM-putrescine and less than 5μM-spermidine. This is in good agreement with the data of Clark & Fuller (1975) who reported a value that was 50% of control activity 3h after exposure to 1μM-spermidine or 10μM-putrescine. However, our results differ from those reported by Heller *et al.* (1978) and Canellakis *et al.* (1978) who

found that ornithine decarboxylase activity in 3T3 cells was not completely lost even with 1mM-putrescine. The explanation for this discrepancy is not clear, but may be related to differences in the culture conditions or the source of the fibroblasts.

The present experiments indicate that loss of ornithine decarboxylase activity in response to putrescine is not a specific effect, but can also be produced by many other $\alpha\omega$-diamines including those with 9 or 12 carbon atoms. These findings extend previous reports from this and other laboratories that propane-1,3-diamine, pentane-1,5-diamine and hexane-1,6-diamine cause a loss of ornithine decarboxylase activity in mammalian tissues (Pösö et al., 1977; Kallio et al., 1977a; Guha & Jänne, 1977; McCann et al., 1977; Pegg et al., 1978). Recently, Heller et al. (1978) have also reported loss of ornithine decarboxylase activity in response to longer chain aliphatic $\alpha\omega$-diamines.

The mechanism by which exogenous diamines and polyamines suppress the expression of ornithine decarboxylase activity is not at present well understood. Evidence has been obtained that this effect may be mediated through a decline in the synthesis of ornithine decarboxylase at the post-transcriptional steps (Clark & Fuller, 1975; Kallio et al., 1977b,c). On the other hand, high concentrations of physiological and synthetic diamines lead to the appearance of a macromolecular inhibitor of ornithine decarboxylase that has been referred to as an 'antizyme' (Heller et al., 1976, 1978; Fong et al., 1976; McCann et al., 1977; Jefferson & Pegg, 1977; Pegg et al., 1978). The results obtained in the present study support the hypothesis that both mechanisms might occur within certain cells with low concentrations of physiological amines producing a decline in the synthesis of the enzyme and higher concentrations of natural and synthetic diamines decreasing activity by means of the induction of the macromolecular inhibitor (McCann et al., 1977; Pegg et al., 1978; Heller et al., 1978; Pösö et al., 1978). Our finding with 3T3 cells showing that ornithine decarboxylase was much more sensitive to putrescine than to propane-1,3-diamine is similar to that of McCann et al. (1977) in rat hepatoma cells and of Heller et al. (1978) in L1210 cells. It is possible, therefore, that the particular sensitivity of the 3T3 cells to putrescine and spermidine rather than to the synthetic diamines may be due to the greater abilities of the former to decrease the rate of synthesis of the enzyme. It appears that SV-transformed 3T3 cells are much more resistant to the inhibitory effects of putrescine or spermidine and this may be related to the absence or decreased activity of this inhibitory site. The loss of ornithine decarboxylase activity in both cell lines with exogenous diamine concentrations of 500 μM or greater may be due to the appearance of the putative 'antizyme'.

It is of interest that the experiment shown in Table 3 may indicate that SV-transformed 3T3 cells take up putrescine less rapidly than normal 3T3 cells. This result is the opposite of the well known enhanced transport of certain amino acids and sugars in virally transformed cells (Foster & Pardee, 1969; Isselbacher, 1972; Weber et al., 1976; Dubrow et al., 1978). However, it should be stressed that the uptake of radioactivity measured in the present experiments merely indicates the maximal rate of net uptake of the added putrescine and may not reflect a true rate of transport because of complications that may arise from further metabolism, a difference in the starting intracellular putrescine concentration, and the possibility that some of the putrescine is merely tightly adsorbed to the cell surface. Similarly, although the data suggest that putrescine might be concentrated within the cells against a concentration gradient, this cannot be proven by the present results. Pohjanpelto (1976) provided more convincing evidence that putrescine was substantially concentrated by human fibroblasts when added to the medium at a concentration of 0.1 μM and reported a K_m for uptake of 1.1 μM and a $V_{max.}$ of 0.92 nmol/10^6 cells per h, which is in reasonable agreement with the results shown in the present paper. Clark & Fuller (1975) also reported carrier-mediated transport of putrescine in 3T3 cells with a K_m of 4.6 μM, but their reported $V_{max.}$ was only 3.4 pmol/mg of protein per h, which is much less than that observed in the present study.

Heller et al. (1978) and Canellakis et al. (1978) have suggested that a critical regulatory site for ornithine decarboxylase may reside in the cell membrane. There is, as yet, no conclusive evidence that extracellular di- and poly-amines can exert effects on ornithine decarboxylase at such a site because, as shown in the present paper and by others (Clark & Fuller, 1975; Pohjanpelto, 1976; Kano & Oka, 1976; Heller et al., 1978), these amines may be taken up into the cell by an active-transport system. However, the very low extracellular concentrations at which these exert effects on ornithine decarboxylase (which are substantially less than the normal intracellular concentrations, unless most of the intracellular polyamine is bound to macromolecular anions) renders this an attractive hypothesis. It is known that viral transformation of mouse fibroblasts results in the alteration of membrane characteristics including changes in the content of glycolipids, glycoproteins and surface proteins as well as changes in the transport of sugars and amino acids (Van Nest & Grimes, 1977; Weber et al., 1976; Isselbacher, 1972; Dubrow et al., 1978). It is possible that a change in the receptor site for putrescine or spermidine might be responsible for the lack of sensitivity of the SV-transformed cells. In this way the transformed cells might be able to maintain a substantial

rate of polyamine synthesis even in the presence of extracellular polyamine concentrations, that would suppress polyamine production in other cells. These observations therefore, and those of Isom & Backstrom (1979), may provide an example of the possibility suggested by Canellakis *et al.* (1978) in which malignant cells continue to synthesize and excrete polyamines even in the presence of polyamine concentrations that would normally inhibit putrescine production. Such excretion might result in the increase in serum and urinary polyamine concentrations that is seen in malignancy (Bachrach, 1976; Durie *et al.*, 1977; Rennert *et al.*, 1977; Jänne *et al.*, 1978). However, whether normal concentrations of polyamines in physiological fluids, which have been estimated to be as low as 0.1 μM for spermidine (Bartos *et al.*, 1978), are sufficient to suppress ornithine decarboxylase in many normal cells remains an open question.

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