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Intracellular Levels of Ornithine Decarboxylase, Its Half-Life, and a Hypothesis Relating Polyamine-Sensitive Membrane Receptors to Growth

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In recent years, interest in the study of ornithine decarboxylase (ODC), the rate-limiting enzyme that catalyzes the synthesis of the diamine putrescine (29) from which the polyamines spermidine and spermine are synthesized, has greatly increased. The reasons for this interest in ODC are supplied in the two excellent recent books by Cohen (9) and Bachrach (2), which detail much of the work and have helped more recent arrivals to the field with concise summaries; in addition, Russell's report relating urinary polyamine levels to cancer (35) has greatly expanded the spectrum of scientific interest in ODC. Prominent among the newer developments are the cellular mechanisms that control ODC activity. The increasing rate of progress in this field makes evaluation of its present status worthwhile.

Extensive investigations from a large number of laboratories have emphasized that increases in the activity of an enzyme are most often accompanied by corresponding increases in the amount of enzyme (for a review, see ref. 34). Consequently, it would not be surprising if the large increases in ODC activity that are noted under a variety of conditions of induction are also accompanied by corresponding increases in the amount of ODC protein. However, recent results from our laboratory have emphasized that the activity of ODC can be diminished by a small-molecular-weight noncompetitive protein inhibitor whose synthesis is induced by the end-products of the ODC reaction. Because of the uniqueness of this mechanism for the control of enzyme activity (20), we have named this protein the ODC antizyme (15). The presence of such an inhibitory protein makes it plausible that the activity of ODC may be regulated by the ODC antizyme. In our laboratory, we are also interested in establishing whether there exist other proteins that activate ODC.

These newer developments make it very important to be absolutely certain that variations in ODC activity correspond to variations in the amounts of ODC, as contrasted to variations in associated protein activators or inhibitors of ODC. We, therefore, wish to summarize the evidence available to date on

the intracellular levels of ODC and the methods of determination and to define potential pitfalls.

In addition, we wish to consider the half-life of ODC, to present some recent data on the regulation of ODC activity by polyamine-sensitive membrane receptors, and to relate this control mechanism to the interaction between cells of different growth rates within the whole animal.

THE ODC ANTIBODIES

Early attempts at purification of rat liver ODC emphasized the instability of the enzyme (33) and provided relatively low purification factors. An attempt to purify this enzyme by Friedman et al. (12) yielded a single band on polyacrylamide gel electrophoresis (PAGE) and a purification factor of about 175-fold. When rabbit antibody was prepared to this preparation, it was shown to yield only a single precipitin line on Ouchterlony assays. Antibody precipitin assays of ODC indicated that the enzyme activity was lost and that a precipitate was formed. However, this enzyme preparation raised certain questions in our minds, prominent among which was the low purification factor of the ODC used as an antigen; this implied that about 0.4% to 0.6% of the soluble protein of regenerating rat liver was ODC.

Extensive purification of ODC from rat livers induced by dexamethasone was then undertaken in this laboratory (J. S. Heller) and resulted in the scheme indicated in Table 1. This unpublished method permitted a 3,100-fold purification of rat liver ODC from dexamethasone-induced rat livers, indicating that only 0.03% of the protein in the S-100 fraction could be accounted for by ODC.

Our experience with many antisera prepared both in rabbits and in a sheep against this 3,100-fold purified fraction has been: (a) not all the ODC activity of the S-100 fraction of rat liver could be inactivated, (b) antisera could be

TABLE 1. *Purification of rat liver ODC*

Step	Total protein (mg)	Specific activity (nmole/mg/30 min)	Relative purification	Total units[a]
Rat liver supernatant	41,000	0.701	1	28,700
Acid precipitation	4,080	6.6	9	27,000
DEAE-cellulose Chromatography	410	42.5	60	17,400
G-150 Sephadex chromatography	45	199	284	9,000
DEAE-Sephadex chromatography	6.5	605	865	3,930
Preparative acrylamide gel electrophoresis	0.4	2,200	3,100	880

[a]Total units based on 80 rats, approx. 5 g/liver.

obtained, that inactivated ODC but did not show precipitin lines in Ouchterlony plates, and (c) the titer of the various antisera was invariably low. Because of these discrepancies, the antiserum work was abandoned.

A purification scheme for ODC similar to that described in Table 1 was published by Ono et al. (28) from thioacetamide-induced rat livers. This gave a 5,400-fold purification, which calculates to an ODC content of 0.018% in the S-100 fraction of these livers. The molecular weight of the native enzyme was reported as 100,000 daltons. No antibody work has been reported as yet with this preparation. These studies further indicated that the specific activity of the ODC in the original S-100 fraction was approximately the same whether ODC was induced by thioacetamide or by partial hepatectomy. In our laboratory we found this specific activity of ODC to vary in ratios of 0.5:1:1 when the enzyme is fully induced by dexamethasone, partial hepatectomy, or growth hormone, respectively.

A more extensive method of purification of ODC from rat livers induced with thioacetamide has been accomplished by Obenrader and Prouty (26,27). In this method, Sephadex G-150 fractionation provides good evidence for a single homogeneous peak of ODC with a molecular weight of 103,000 daltons and a purification factor of 5,300. When this peak fraction is eluted through an activated thiol-Sepharose 4B column, the ODC activity separates into a number of peaks. These have been collected into two fractions, designated Form I and Form II, with purification factors of 7,150 and 6,900, respectively; these purification factors indicate that ODC consists of 0.016% of the total protein of the S-100 fraction.

The extensive studies of Obenrader and Prouty with immunoglobulin G (IgG) prepared against this most highly purified ODC to date permit certain detailed correlations to be made: 1. This IgG does not inactivate all the ODC activity of the crude S-100 fraction of regenerating rat liver; however, it does inactivate all the ODC activity of more purified fractions. This indicates that at any one time the crude S-100 fraction contains a mixture of ODCs that differ in their immunological reactivity. 2. If, subsequent to induction of ODC activity by thioacetamide, the S-100 fractions of the 3-hr, 8-hr, and 18-hr time points are titrated with IgG, approximately twice as much IgG is required to inactivate the same total activity of ODC in the lower specific activity 3-hr fraction than in the higher specific activity 18-hr fraction; the 8-hr S-100 fraction is inactivated by an intermediate amount of ODC IgG. This indicates either that the ratios of different species of ODC change with the activity of the preparation or that the form of ODC used for the preparation of IgG is not representative of all ODCs; however, the statistical variations in these interesting results suggest a need for additional confirmation. 3. Following cycloheximide treatment the half-life of ODC as measured by the loss of activity is shorter than the half-life as measured by the amount of IgG necessary to inactivate 50% of the ODC. This shows that there exist at any one time forms of ODC that are enzymatically inactive but recognizable as antigens. 4. When Forms I and II are subjected

to PAGE, they reproducibly separate into a number of discrete peaks (see under "Multiple or Aggregate Forms of ODC and ODC Antizyme," later in this chapter).

These data indicate that more than one form of ODC exists, that these forms do not react equivalently with antiserum, and that they vary with the fluctuations in the overall activity of an induced preparation. Inherent in these findings is the conclusion that there can be no simple proportionality between the ODC activity and the amount of ODC in a given cell preparation after different times of induction as measured by this antiserum. This disproportionality would be further accentuated when ODC activities of different cell preparations are compared.

The complexity of the results obtained with the antiserum prepared against the most highly purified ODC preparation to date has raised uncertainty regarding the correspondence between ODC activity and the amount of ODC; note that this antibody was used to assay the *activity* of ODC and not the amount of ODC.

THE HALF-LIFE OF ENZYMES

The half-life of an enzyme has been postulated (38) to be related to the cellular requirements for rapid change in intracellular concentration and to the enzyme's role in intermediary metabolism (4,13). In this sense the short half-life of ODC has been attributed to its cellular function as a rate-limiting step of polyamine synthesis. Short half-lives have also been associated with the isoelectric point of proteins (14); with aberrant proteins, i.e., hemoglobin into which valine or lysine analogs have been incorporated (32); with incorporation of canavanine in proteins (30,37); or with proteins containing transcriptional errors (1). However, Johnson and Kenney (18) find no alteration of the rate of turnover of tyrosine aminotransferase containing fluorotryptophan. A correlation of shorter half-lives with large molecular weights has also been made (10), although more recently this correlation has been shown to relate better to the subunit size than to the multimer size (11,25).

The rate of degradation of a protein can also be altered by association with macromolecules within the cell (41) or be defined by group-specific proteinases such as the proteases found by Kominami et al. (21) to degrade enzymes containing pyridoxal phosphate, especially in their apo form.

Decreased rates of enzyme degradation can also be elicited by substrates or cofactors, as indicated by Schimke and co-workers (39,40). For instance, administration of tryptophan or α-methyltryptophan leads to increased levels of tryptophan oxygenase as a result of continued enzyme synthesis and decreased enzyme degradation (40). This finding has been extended by Li and Knox (23,24) for cell-free extracts, where they indicate that tryptophan reduces the rate of spontaneous degradation of typtophan oxygenase rather than the rate of proteolytic degradation.

However, the fact that the individual components of the complex formed by ODC and ODC antizyme can be reisolated, each retaining its respective activity, indicates that denaturation or spontaneous degradation of the protein may not be an essential first step in the inactivation of this enzyme (15).

THE HALF-LIFE OF ODC

The half-life of ODC has been determined chiefly by assaying the ODC activity (36) after inhibition of protein synthesis. However, it has been shown that cycloheximide and other antibiotic inhibitors of protein synthesis can also inhibit protein degradation (3,16,19). Most recently, Stiles et al. (42) have shown that cycloheximide can inhibit the turnover of mRNA for tyrosine aminotransferase. The half-life of ODC also varies greatly depending on a number of factors. When half-life is measured at various times during the ascending and descending portions of a glutamine induction curve of ODC in HeLa cells, it varies from 12 to 60 min (30). When induced by dilution of stationary HTC cells, it lengthens from 11 min to 60 min (17). The shorter half-life has been associated with low ODC activities; the longer has been associated with high ODC activities. However, in 3T3 cells, Clark's results (8) indicate that upon induction of high ODC activity the half-life can drop from 240 min to 40 min, indicating an opposite correlation.

The evidence to date indicates that synthesis of both ODC and ODC antizyme is inhibited by cycloheximide. If the ODC antizyme participates in the normal turnover of ODC, the half-life of ODC in the presence of cycloheximide is of limited biological significance because of the absence of the mechanisms that normally control its turnover. Consequently, the half-life of ODC should be studied under more selective conditions, this point is further emphasized in the discussions of the ODC antizyme later in this chapter.

ALTERNATE MEANS OF MEASUREMENT OF ODC LEVELS AND HALF-LIFE

The availability of the ODC antizyme presents a new approach to the study of enzyme turnover: (a) it provides a physiological enzyme antagonist that can be used for the quantitative assay of ODC and thus provides an alternative to the antibody assay of ODC; (b) it provides an opportunity to study a connecting step (ODC–ODC antizyme complex) between active enzyme and denatured enzyme; and (c) just as the ODC antizyme can be used to measure the level of ODC, ODC can be used to measure the level of ODC antizyme.

An antizyme-neutralization test provides a distinct advantage over the antibody assay. The rat liver antizyme neutralizes preparations of ODC from a variety of sources (15); it therefore can be used for the assay of ODC under the large variety of conditions of induction that occur in different cells. It also does not cross-react with a number of other pyridoxal phosphate enzymes. Should some conditions of induction produce more "antizyme-neutralizing" material

than can be accounted for by the increase in ODC activity, an important question can be raised: What is the relationship of these additional antizyme-neutralizing components to the induction of ODC and to the homeostatic mechanisms involved in the induction of ODC? The antizyme can thus be used as a means of recognizing new participating homeostatic controls.

Titration of ODC activity (induced by partial hepatectomy) in crude S-100 extracts of rat liver with 120-fold purified ODC antizyme gave the results presented in Fig. 1. The decrease in ODC activity when increasing amounts of antizyme are added to 0.4 unit of ODC activity is approximately linear up to 13 μg of antizyme. The addition of further amounts of antizyme does not eliminate all the ODC activity, indicating that there is about 10 to 15% of poorly reactive ODC (compare with our antisera results and Obenrader and Prouty's results, discussed earlier). After 26 μg of antizyme has been added, there should be an excess of 0.3 unit of antizyme based on the linear portion of curve A (13 μg of antizyme neutralizes 0.3 unit of ODC activity). At this point increasing amounts of ODC are added, and even after 0.48 unit of ODC activity has been added, there is no linear increase (curve C).

A further appreciation of the complexity of the reaction is indicated in Fig. 2. In this experiment 13 μg of ODC antizyme are neutralized with increasing amounts of ODC (the amounts of ODC added are represented by curve D). According to the estimates from the linear portion of curve A of Fig. 1, this amount of ODC antizyme should neutralize 0.30 unit of added ODC.

When ODC is added, in portions, to the ODC antizyme and the sample assayed for residual ODC activity after each addition, curve AB is obtained.

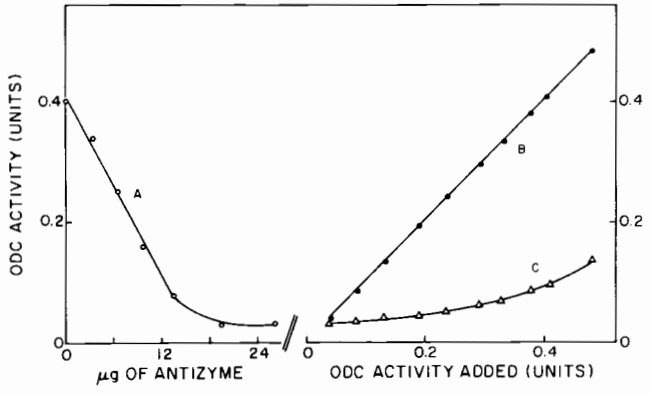

FIG. 1. Titration of ODC with ODC antizyme. ODC antizyme (120-fold purified) and ODC (S-100 fraction, i.e., the supernatant fraction obtained from homogenates of regenerating rat liver after centrifugation at 100,000 × *g*) (see text), were used. Increasing amounts of ODC antizyme were added to 0.4 unit of ODC (○——○). After 26 μg of ODC antizyme had been added, increasing amounts of ODC were added up to 0.48 unit (△——△). A control containing only an equivalent amount of ODC was also assayed (●——●). Assay was performed (15) in 60 μl.

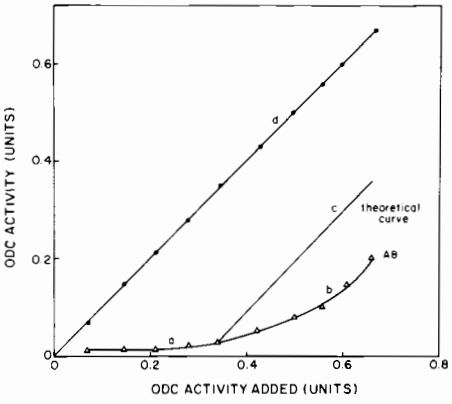

FIG. 2 Titration of ODC antizyme with ODC. Increasing amounts of ODC were added to 13 μg of ODC antizyme (△——△). Control reactions with the equivalent amounts of ODC minus antizyme were also assayed (●——●). For further details, see legend to Fig. 1.

The calculated theoretical curve is represented by section a of curve AB followed by section c; these calculations do not take into account the 10 to 15% of poorly reactive ODC. The difference between sections b and c may be caused by different modes of interaction of ODC and ODC antizyme; this results in a greater neutralization of ODC when the ODC is added to excess antizyme than when antizyme is added to excess ODC. The reasons for this discrepancy are probably inherent in the mode of interaction of these two proteins.

These preliminary results indicate: 1. The 120-fold purified ODC antizyme does not cross-react with all forms of ODC; yet we know from the *in vivo* studies that when we induce the production of the ODC antizyme by the addition of putrescine, all the cellular ODC activity can be neutralized. Therefore, there may be forms of antizyme that are specific for forms of ODC and that have been lost during the purification. 2. In the presence of excess ODC antizyme, a greater than equivalent amount of ODC is required to neutralize the antizyme. 3. The interaction of ODC with its antizyme is probably not a simple one-to-one interaction.

MULTIPLE OR AGGREGATE FORMS OF ODC AND ODC ANTIZYME

ODC antizyme of rat liver has been purified 120-fold in this laboratory (W. F. Fong, D. A. Kyriakidis, and E. S. Canellakis, *unpublished*) using column chromatography by elution with assay buffer from (a) DEAE-Sephadex A-25 in a gradient of 0.05 M KCl to 0.50 M KCl; (b) Sephadex G-75; (c) Fine Sephadex G-75; and (d) DEAE-Sephadex A-25. This provides a symmetrical peak (15) that, upon acrylamide gel electrofocusing (Fig. 3), indicates heterogeneity in the region of pH 5.5 to 6.5 with at least three peaks of ODC antizyme activity. This heterogeneity is also obtained with PAGE and should be compared with the results of Obenrader and Prouty (26,27), which show the heterogeneity of the activity of ODC after PAGE. It is possible that there exists a correspondence between these various forms of ODC and ODC antizyme or that both these

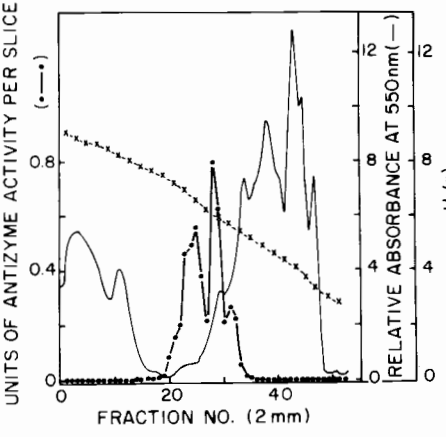

FIG. 3. Isoelectric focusing of partially purified ODC antizyme. Approximately 25–50 μg of ODC antizyme protein (see text) was electrophoresed at 4° C for 12 hr at 2 mA/tube (43). A Gilson gel slicer was used to cut 2-mm sections, which were extruded into Falcon tubes and assayed for ODC antizyme activity (15).

proteins tend to aggregate. However, it is still too early to make any definitive statements; we can only indicate the complexity of the problem.

THE SPERMINE EFFECT: POLYAMINE-SENSITIVE MEMBRANE RECEPTORS AND GROWTH

We have also been interested in how external factors can control the intracellular ODC activity. L1210 cells diluted from a stationary phase with fresh medium show a rapid induction of ODC activity. We have previously reported that low concentrations of cations, as well as microtubule and microfilament disruptive agents, will inhibit this induction of ODC (5,6).

Figure 4 shows how increasing concentrations of spermine affect the induction of ODC. Very low concentrations of spermine cause a precipitous fall in ODC activity. At intermediate spermine levels a stabilization of ODC activity occurs, and with higher concentrations of spermine, the activity of ODC falls to zero. Subsequently, ODC antizyme can be assayed for in free form.

In addition to putrescine, spermidine, and spermine, a series of α,ω-diamines of the general structure $NH_2(CH_2)_nNH_2$ (where $n = 2,3,5,7,8,10,12$) has been tested for these effects. Only putrescine, spermidine, and spermine showed this inhibitory effect on the induction of ODC activity at concentrations below 10^{-6} M. None of the others had any appreciable effect at concentrations below 10^{-5} M, with the exception of heptyl diamine ($n = 7$), which mimicked the spermine effect; interestingly enough octyl diamine ($n = 8$), the paraffin analog of spermidine, enhanced ODC activity (7).

It should be emphasized that spermidine is a particularly effective inhibitor of the induction of ODC; at 10^{-8} M it produces a 6% inhibition (Fig. 6) and at 10^{-7} M a 50% inhibition. These extremely low concentrations of added polyamines (10^{-8}–10^{-6} M) are unlikely to raise the high intracellular polyamine concentration (putrescine, spermidine, spermine vary from 0.2 to 5×10^{-3} M (1)

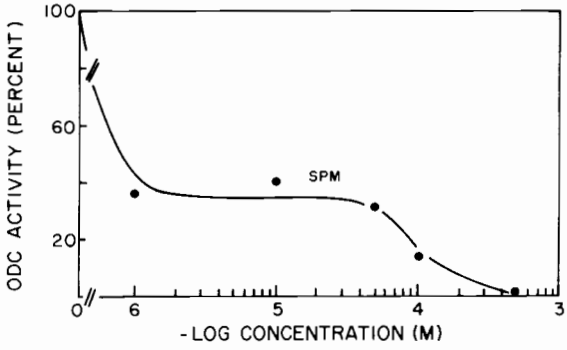

FIG. 4. Concentration curve of the effect of spermine (SPM) on the induction of intracellular ODC activity in L1210 cells. L1210 cells were grown as described previously (5,6). The cells were grown to the plateau phase at about 8–9 X 10^5 cells/ml and then diluted with fresh Fischer's medium plus 10% horse serum to about 3 X 10^5 cells/ml. The neutralized spermine was added to 15-ml aliquots of the diluted cells to give the final concentrations indicated in the figure. After 2 hr incubation at 37° C, the cells were harvested by centrifugation and washed twice with phosphate-buffered saline. The cell pellets were resuspended in 0.5 ml of assay buffer (50 mM Tris-HCl, 0.1 mM EDTA, 5 mM dithiothreitol, 0.05 mM pyridoxal phosphate, pH 7.2 at 37° C) and freeze-thawed twice. The cell extracts were dialyzed overnight against 10 mM Tris, 50 mM KCl, 0.1 mM EDTA, 0.05 mM pyridoxal phosphate, 5 mM dithiothreitol, pH 7.2 at 4° C. Little or no ODC activity was lost during dialysis. The reaction mixture contained 0.06 ml of the dialyzed fraction, 5 μl of substrate (0.75 μCi/0.1 μmol DL-1-^{14}C-ornithine), and 5μl of a 0.5 M Tris-HCl buffer so that the final pH at 37° C was 7.2. The reactions were assayed as described in legend to Fig. 1.

and do not inhibit protein synthesis (i.e., ^{14}C-leucine incorporation into protein is not affected; results not presented). We therefore postulate that these added polyamines are acting on membrane receptors that are responsive to these low extracellular polyamine concentrations; stimulation of these receptors results in a drop in ODC activity. However, under the conditions of the experiment, this fall in ODC activity occurs at an intracellular concentration of polyamines that is permissive to the continuous induction of ODC. Consequently, the fall in ODC is counteracted or buffered by an increase in ODC, resulting in a maintenance of a constant ODC activity over a large range of polyamine concentrations. As we continue to raise the polyamine concentrations, this results in a progressive production of antizyme that now neutralizes the ODC activity.

Figure 5 demonstrates our visualization of this aspect of the normal control of ODC activity by the cell. At all times this is defined by the level of polyamines. Low intracellular polyamine levels permit increases in ODC activity; however, as these polyamine levels are raised, polyamines diffuse out of the cell and, at very low extracellular levels, can inhibit ODC activity through the membrane on which are located polyamine receptors. Additional intracellular increases in polyamine levels act either on an additional intracellular site or on the same membrane receptors and produce large amounts of antizyme that neutralize the ODC; persistence of these high polyamine levels permits the production

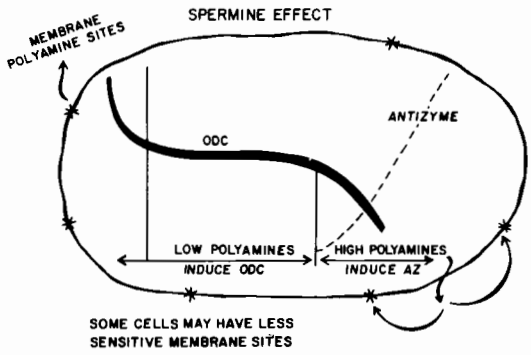

FIG. 5. Schematic representation of the regulation of polyamine synthesis by the cell. The membrane contains receptors sensitive to low concentrations of polyamines. Upon stimulation, these receptors inhibit ODC activity. Consequently, the polyamine concentration of the serum will determine the level of polyamine synthesis. Intracellularly, at low polyamine levels, the fall in ODC activity is counteracted by an increase in ODC activity, and a "buffering" level of ODC is maintained over a large range of concentrations. As the level of polyamines increases, either intracellularly or extracellularly, beyond certain limits, the ODC activity is further inhibited until free ODC antizyme can be detected to increase. We do not know at what concentration of polyamines the ODC antizyme is produced. This remains to be established. Some tumor cells, whose membrane receptors have lost their sensitivity to polyamines will make unusually large amounts of polyamines. In such cells the inhibition of polyamine synthesis is dependent on the ODC antizyme. They tend to be excretors of polyamines and produce an excessive inhibition of ODC activity in other cells, thereby modifying the host's pattern of polyamine production. The curve is representative of the effect of spermine on the ODC activity of L1210 cells; it is also representative, with some minor differences, for putrescine and spermidine.

of free antizyme. It is conceivable that all these effects of polyamines are mediated by the membrane receptors in response to the polyamine that has been synthesized intracellularly and has leaked into the medium. We obviously do not know whether these membrane receptors are related to the putrescine molecules that Quash et al. (31) have visualized on the surface of embryo cells; however, it is an interesting possibility to consider.

We, therefore, postulate that cells, at various stages of development, have membrane receptors that are differentially responsive to the extracellular polyamine concentration. The more sensitive these receptors are, the lower the intracellular ODC activity and, consequently, the level of intracellular polyamines. Within this spectrum of cells, some tumors or other rapidly growing cells may have membrane receptors that are poorly responsive to polyamines. Such cells will maintain high intracellular ODC and polyamine levels and become excretors of polyamines.

This differential sensitivity of the ODC activity of different cells to putrescine is shown in Fig. 6, which compares the effect of putrescine and spermidine on the induction of ODC in 3T3 and L1210 cells. In L1210 cells the I_{50} for spermidine is 7×10^{-8} M. The I_{50} for putrescine is 2.5×10^{-6} M for L1210 cells and 5×10^{-3} M for 3T3 cells.

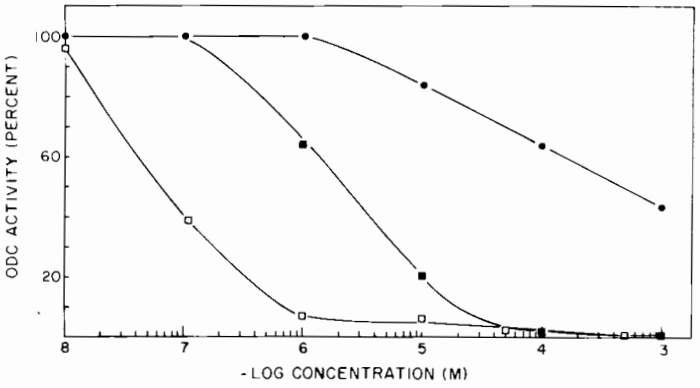

FIG. 6. Concentration curve of the effect of putrescine and spermidine on the induction of ODC in 3T3 and L1210 cells. L1210 cells were induced as described in the legend to Fig. 4, except putrescine or spermidine was added to the inducing medium. 3T3 cells (CCL 92 from the ATCC cell repository) were grown as described by Clark (8), the ODC activity induced by starving for fetal calf serum overnight and then refeeding with fetal calf serum (22). The cells were harvested 6 hr later with trypsin as described by Lemback (22), freeze-thawed, and assayed according to the legends of Figs. 1 and 4. L1210 cells plus putrescine, ■——■; L1210 cells plus spermidine, □——□; 3T3 cells plus putrescine, ●——●.

To the extent that such polyamine-insensitive tumors or fetal cells or other rapidly growing cells can effectively increase the serum polyamine concentration, this higher serum polyamine concentration may reach a level that, according to our scheme, would stimulate the polyamine-sensitive membrane receptors of the cells of the large mass of body tissues and inhibit their ODC. This would tend to decrease the synthesis of polyamines by these tissues. This interplay between high polyamine producers and the large mass of body tissues should, therefore, result in fluctuations in serum and urinary polyamine concentrations that could become very complex and possibly show time-related variations that differ from normal diurnal variations.

These thoughts are intended to stimulate our thinking about the body as a whole and to point out that the serum or urinary polyamine level may not necessarily be indicative of rapidly growing or malignant tissues alone. We believe that the serum or urinary polyamine level in a variety of states of rapid growth is probably representative of a delicate interplay between the rapidly growing tissue and the large mass of normal tissue of the host.

ANTIZYMES TO OTHER ENZYMES?

We think the antizyme may constitute part of a normal control mechanism that defines the level of ODC activity. However, it is a noncompetitive protein inhibitor and cannot be detected in free form while there is measurable ODC activity. Consequently, proof of the participation of the antizyme in the normal

control of ODC will have to await the development of methodology that will permit isolation of the ODC–ODC antizyme complex, separation of the complex into the component parts, and assay of their respective activities. The methodology described previously (15) is cumbersome to use considering the large number of samples that must be processed, and we are currently developing new techniques to solve this aspect of the problem.

We do know that the cell will produce free antizyme in the presence of high diamine or polyamine concentrations. Under these conditions, the tissue culture cells are in a relatively "unhappy" state; in the rat, the amount of putrescine required to elicit high levels of antizyme makes the animal extremely sick. Consequently, in the search for antizymes to other enzymes, we believe the following criteria should be kept in mind:

1. The product should be added in high enough concentrations so that no enzyme activity can be detected
2. The inhibition should be maintained over long enough periods of time to maximize the amount of antizyme

We believe that under these extreme conditions the cell is responding to the excess product by what may be its last available defense mechanism: producing additional noncompetitive inhibitory proteins to the enzyme in order to lower the level of the product.

CONCLUSIONS

The cellular mechanisms that control ODC activity in many eukaryote cells are beginning to be elucidated. These appear to be very complex, especially as a multiplicity of aggregate forms of ODC and ODC antizyme appear to be involved, and purification may tend to favor some forms over others. These control mechanisms may prove unique to ODC; alternatively, they may be indicative of the existence of similar control mechanisms for other enzymes. Great care should be exercised in relating ODC activity to the amount of ODC under various conditions of induction until more is known about this enzyme system.

Our experimental findings suggest that ODC activity may be controlled by specific membrane receptor sites responsive to polyamines. We present the hypothesis that some rapidly growing cells may either have lost the polyamine-sensitive membrane receptor sites or have a reduced sensitivity to control of ODC activity by the level of polyamines at these sites. We therefore suggest that serum and urinary polyamine levels are probably an expression of a delicate interplay between the rapidly growing tissue and the large mass of normal host tissue; consequently, they may not always mirror the anticipated increases of polyamine-producing tumors.

Recent results (Chen, K. Y. and Canellakis, E. S. (1977): Enzyme induction in neuroblastoma cells in a salt-glucose medium: The induction of ornithine

decarboxylase by asparagine and glutamine. *Proc. Natl. Acad. Sci. USA (in press)*] emphasize the dependence of the half-life of ODC on asparagine.

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