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Biochemistry

Enzyme regulation in neuroblastoma cells in a salts/glucose medium: Induction of ornithine decarboxylase by asparagine and glutamine

(cyclic AMP/actinomycin D/cycloheximide/protein half-life/cholera toxin)

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ABSTRACT L-Asparagine is necessary and sufficient for the maximal induction of ornithine decarboxylase (ODC) (L-ornithine carboxy-lyase, EC 4.1.1.17) activity in confluent N18 mouse neuroblastoma cells in a salts/glucose medium; L-asparagine also induces maximal ODC activity when added to a tissue culture medium. L-Glutamine is about one-half as effective as asparagine. Cholera toxin and agents that are known to raise intracellular cyclic AMP concentrations have no effect on the induction of ODC activity unless suboptimal concentrations of asparagine are present in the salts/glucose medium. Whereas actinomycin D does not inhibit induction of ODC activity by asparagine, it inhibits the induction of ODC activity in association with cyclic AMP. In the salts/glucose medium, the rate of loss of ODC activity following the inhibition of protein synthesis by cycloheximide or puromycin depends upon the presence or absence of asparagine; loss is rapid only in the absence of asparagine and does not appear to be related to the inhibition of protein synthesis. These results are discussed in the context that the overlay of the growth medium tends to mask the minimal requirements for enzyme induction, because the composition of the medium defines: (*a*) the requirements for the induction of ODC activity; (*b*) the effect, or lack of effect, of cyclic AMP (and of inducers of intracellular cyclic AMP) on the induction of ODC activity; (*c*) the effect, or lack of effect, of actinomycin D on the induction of ODC activity; and (*d*) the action of puromycin and of cycloheximide on the rate of loss of ODC activity. It will be interesting to determine whether these results are uniquely applicable to ODC, whether many of the reactions attributed to cyclic AMP in the literature may be mediated by asparagine and glutamine, and whether actinomycin D, cycloheximide, and puromycin can be relied upon to differentiate between transcriptional and post-transcriptional control.

Most studies on enzyme induction, including our own, especially in relation to ornithine decarboxylase (ODC) (L-ornithine carboxy-lyase, EC 4.1.1.17), have relied heavily on the use of cell cultures in incubation media of varying complexity or on the use of whole animals (1–3). Such studies tend to provide answers that are modified either by the overall physiology of the cell as affected by the complexity of the medium or by the inherent complexity of the animal as modified by any additional conditions of imposed environmental and experimental stress. For example, when enzyme induction by hormones is being determined in a cell culture, if the hormones have differential positive and negative effects on the transport of various substances, the effectiveness or noneffectiveness of a hormone in inducing an enzyme activity will be completely dependent upon the composition of the medium.

In order to minimize such extraneous influences, we induce ODC activity in confluent N18 mouse neuroblastoma cells in a minimal salts/glucose medium. Under these conditions, the addition of L-asparagine is necessary and, at optimal concentrations, sufficient to induce maximal ODC activity and to maintain it for at least 12 hr. When protein synthesis is inhibited by cycloheximide or puromycin in the presence of L-asparagine, the rate of loss of ODC activity is extremely slow ($t_{1/2}$ = 200–400 min). In contrast, removal of L-asparagine results in a decay of ODC activity with $t_{1/2}$ = 12–15 min. Prostaglandin E_1 plus isobutylmethylxanthine, dibutyryl cyclic AMP, or cholera toxin (an irreversible activator of adenylate cyclase) will induce ODC activity only in the presence of suboptimal concentrations of L-asparagine.

It is proposed that studies on the molecular mechanism of action of hormone control and of enzyme induction in intact cells would be greatly facilitated by the use of such minimal salts/glucose media. These media may provide a means of defining the factors that are essential to the phenomenon under study, as well as their mode of action. They should also help define the mechanism by which the progressively increased complexity of the medium brings into play additional reactions that modify the basal phenomenon.

MATERIALS AND METHODS

Materials. L-Asparagine, L-glutamine, Dulbecco's modified Eagle's minimal medium, and fetal calf serum were obtained from Gibco, Grant Island, NY. Dimethyl sulfoxide of spectral quality was from Matheson, Coleman and Bell, Norwood, OH. The L forms of all amino acids were used.

$N^6,O^{2'}$-Dibutyryl cyclic AMP (Bt_2cAMP), cycloheximide, actinomycin D (Act D), and puromycin were from Sigma, St. Louis, MO. 3-Isobutyl-1-methylxanthine (IBMX) was from Aldrich, Milwaukee, WI. DL-[*1*-^{14}C]Ornithine (32.2 Ci/mol) and L-[*U*-^{14}C]asparagine (0.15 Ci/mmol) were from New England Nuclear, Boston, MA. DL-[*4,5*-^{3}H]Leucine (50 Ci/mmol), [*6*-^{3}H]uridine (20 Ci/mmol), and [*methyl*-^{3}H]thymidine (15 Ci/mmol) were from Amersham/Searle, Arlington Heights, IL. Prostaglandin E_1 (PGE_1) was kindly provided by John Pike, Upjohn, Kalamazoo, MI. Cholera toxin was from Schwarz/Mann, Orangeburg, NY. All other chemicals were of reagent grade.

The salts/glucose medium was obtained from Gibco in sterile solution as Earle's balanced salt solution: 200 mg of $CaCl_2$, 400 mg of KCl, 200 mg of $MgSO_4 \cdot 7H_2O$, 6800 mg of NaCl, 2200 mg of $NaHCO_3$, 140 mg of $NaH_2PO_4 \cdot H_2O$, and 1000 mg of glucose in 1 liter of water. The corresponding sterile salts/glucose medium without Ca^{2+} and Mg^{2+} was used as phosphate-buffered saline.

Cell Lines. Mouse N18 neuroblastoma cells (4) were grown in 50-mm Falcon plastic dishes in 5 ml of Dulbecco's modified Eagle's minimal medium supplemented with 10% fetal calf serum at 37° in an air/CO_2 incubator. Cells reached the con-

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Abbreviations: ODC, ornithine decarboxylase; IBMX, 3-isobutyl-1-methylxanthine; PGE_1, prostaglandin E_1; Act D, actinomycin D; Bt_2cAMP, $N^6,O^{2'}$-dibutyryl adenosine 3′:5′-cyclic monophosphate; cAMP, adenosine 3′:5′-cyclic monophosphate.

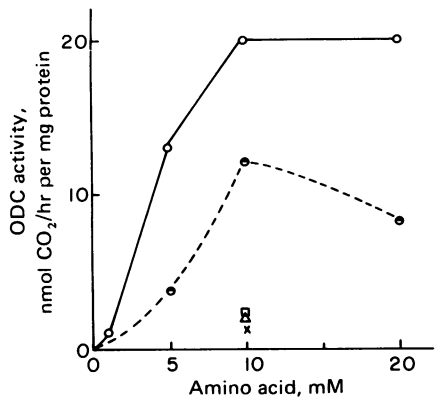

FIG. 1. Dose–response curve of the effects of asparagine (○) and glutamine (●) on the induction of ODC activity in N18 neuroblastoma cells in the salts/glucose medium; glycine (△), serine (×), and proline (□) were tested at 10 mM. ODC activity was measured at 4 hr.

fluent state 5 days after plating (1.2 to 1.5 × 10^6 cells per ml). They were then washed with two 5-ml portions of the salts/glucose medium at 37° and placed in 5 ml of the same test medium to which was added 10–20 μl of the various experimental additives, dissolved in saline (time zero). (PGE_1, IBMX, and Act D were dissolved in dimethyl sulfoxide; in these cases control samples with 1–2 μl of dimethyl sulfoxide were included and were invariably found not to affect ODC activity.)

ODC Assay. At the designated time after induction, the test medium was decanted and the cells were washed with ice-cold phosphate-buffered saline. They were scraped off the dish with a rubber policeman, suspended in 0.8 ml of assay buffer (0.1 mM EDTA/50 μM pyridoxal phosphate/5 mM dithiothreitol/50 mM Tris·HCl, pH 7.2 at 37°), freeze-thawed twice, and centrifuged at 12,000 × *g* for 10 min. ODC activity was determined in the supernatant fluid (5). Protein concentration was measured with bovine serum albumin as standard (6).

Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis. The crude enzyme extract for ODC assay was used for sodium dodecyl sulfate gel analysis. To 100 μl of extract was added 25 μl of sodium dodecyl sulfate solution which contained 10% sodium dodecyl sulfate, 40% (vol/vol) glycerol, 10% (vol/vol) 2-mercaptoethanol, and 0.005% pyronin T as tracking dye. Samples were heated at 100° for 3 min and applied to the slab gel. The linear gradient slab gel was made as previously described (7). After electrophoresis, the gel was stained overnight with 0.025% Coomassie blue in 25% (vol/vol) 2-propanol and 10% (vol/vol) acetic acid. Destaining was achieved by immersion in 0.0025% Coomassie blue/10% 2-propanol/10% acetic acid for 3 hr followed by 10% acetic acid. Tritiated polypeptide bands on the gel were detected by the fluorographic method of Bonner and Lasky (8).

RESULTS

The basal cellular ODC activity in confluent N18 neuroblastoma cells can be enhanced more than 500-fold by 10 mM L-asparagine in a salts/glucose medium (Fig. 1). Of the naturally occurring amino acids (alanine, arginine, aspartic acid, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, proline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine), all tested individually at 10 mM in this minimal medium, asparagine was the most significant inducer of ODC. L-Glutamine was about ½ as effective, while glycine, serine, and proline were about 1/10 to 1/20 as effective (Fig. 1). None of the other naturally occurring L amino acids induced ODC activity under these conditions. No ODC activity could be elicited subsequent to addition of cycloheximide (50 μg/ml) at zero time, in the presence of asparagine or glutamine. We have used asparagine predominantly in these studies; however, the results described below can also be reproduced qualitatively with glutamine, although it is a less effective inducer.

Most of our studies have been performed in a minimal medium, commercially available in sterile form as Earle's balanced salt solution. However, various experiments have indicated that the induction of ODC activity by L-asparagine can also be demonstrated, although to a lesser extent (to about ½–1/5), in glucose-supplemented phosphate-buffered saline.

Fig. 2*A* shows the time course of induction of ODC activity in the salts/glucose medium by addition of 10 mM asparagine. The induction generally achieved plateau values in 6 hr, remained at this level for another 6–8 hr, and then slowly declined. As can be seen from this figure, the presence of Bt_2cAMP, fetal calf serum, cholera toxin, or PGE_1+IBMX does not induce ODC activity in this medium in the absence of asparagine (or glutamine); consequently asparagine (or glutamine) is an essential inducer of ODC activity.

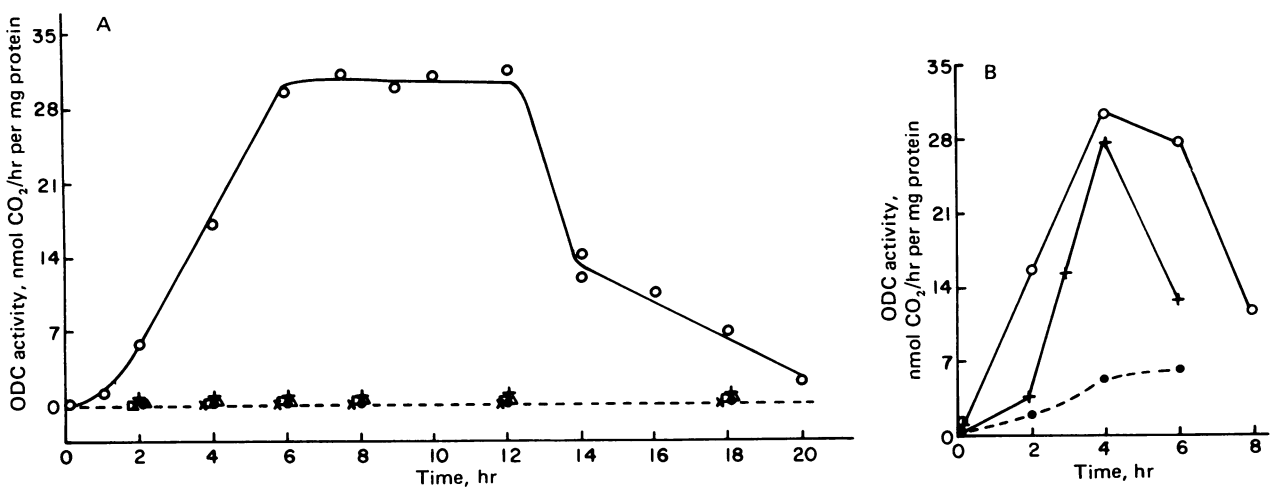

FIG. 2. (*A*) Time course of the induction of ODC activity in the salts/glucose medium. ODC activity of N18 neuroblastoma cells was induced in the salts/glucose medium by adding 10 mM asparagine (○), 10% fetal calf serum (×), 0.5 mM IBMX + 10 μM PGE_1 (△), 50 μM Bt_2cAMP (□), cholera toxin at 2 μg/ml (+), or nothing (●). (*B*) Time course of the induction of ODC activity in Dulbecco's medium. ODC activity of neuroblastoma cells was induced in Dulbecco's medium by adding cholera toxin at 2 μg/ml (+), 10 mM asparagine (○), or nothing (●).

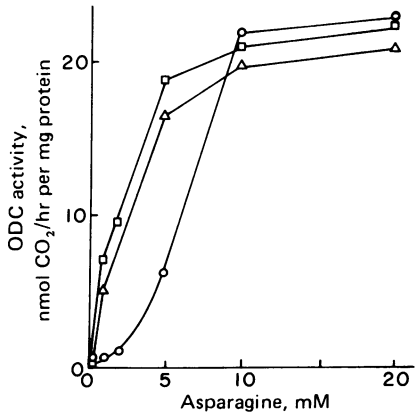

FIG. 3. The effect of Bt_2cAMP and of PGE_1+IBMX on the induction of ODC activity in the presence of various concentrations of asparagine. ODC activity in N18 neuroblastoma cells was induced in the salts/glucose medium by adding asparagine (○), asparagine plus 50 μM Bt_2cAMP (□), or asparagine plus 0.5 mM IBMX + 10 μM PGE_1 (△). ODC activity was measured 4 hr after the additions were made.

These results can be contrasted to those shown in Fig. 2*B*. These indicate that the low level of ODC activity that is induced by Dulbecco's medium with its complex composition (it also contains 3.3 mM glutamine, which only partially induces ODC activity in a salts/glucose medium; Fig. 1), can be stimulated by cholera toxin to levels of total ODC activity equivalent to the level obtained when 10 mM asparagine is added to the salts/glucose medium (Fig. 2*A*) or to Dulbecco's medium (Fig. 2*B*). Fig. 2*B* also shows that the induction of ODC activity by asparagine or by cholera toxin in Dulbecco's medium yields curves that emphasize a rapid decay of ODC activity after maximal induction, in contrast to the sustained ODC activity induced by asparagine in the salts/glucose medium. We have verified Bachrach's observation (9) that a similar level of ODC activity can be induced in Dulbecco's medium if fetal calf serum or PGE_1+IBMX are added. We have also found that addition of 10 mM glutamine (in the absence of PGE_1+IBMX) to Dulbecco's medium induces ODC activity to a level comparable to that obtained when PGE_1+IBMX are added to Dulbecco's medium (results not shown).

The ODC activity of neuroblastoma cell N18 cells maintained in a salts/glucose medium remains inducible by 10 mM asparagine, at rates identical to the rate shown in Fig. 2, for periods up to at least 10 hr. A series of curves, parallel to the curve shown at zero time in Fig. 2*A*, is obtained under these conditions; these curves are not presented for lack of space.

Fig. 3 indicates that in the salts/glucose medium: (*a*) the induction of ODC activity by Bt_2cAMP or by PGE_1+IBMX is completely dependent upon the presence of asparagine; (*b*) in the presence of limiting concentrations of asparagine, Bt_2cAMP or PGE_1+IBMX have a synergistic effect on the induction of ODC activity; (*c*) in the presence of optimal concentrations of asparagine, Bt_2cAMP or PGE_1 + IBMX do not further induce ODC activity. Qualitatively similar results have been obtained (but are not shown) with cholera toxin in the presence of various concentrations of L-asparagine. In accord with the curve of ODC induction in the salts/glucose medium, the enzyme assays were performed at 4 hr after the additions were made; however, similar results were obtained at 2 and 3 hr. Consequently, we may conclude that these stimulatory effects of cAMP on the induction of ODC activity are asparagine-dependent and that cAMP is completely replaceable by high concentrations of asparagine; furthermore, the effects establish a *distinctive* role of asparagine (and of glutamine) in the induction of ODC in this system. The effects of cAMP therefore appear to be related to a "facilitation" of ODC induction by an asparagine-dependent reaction.

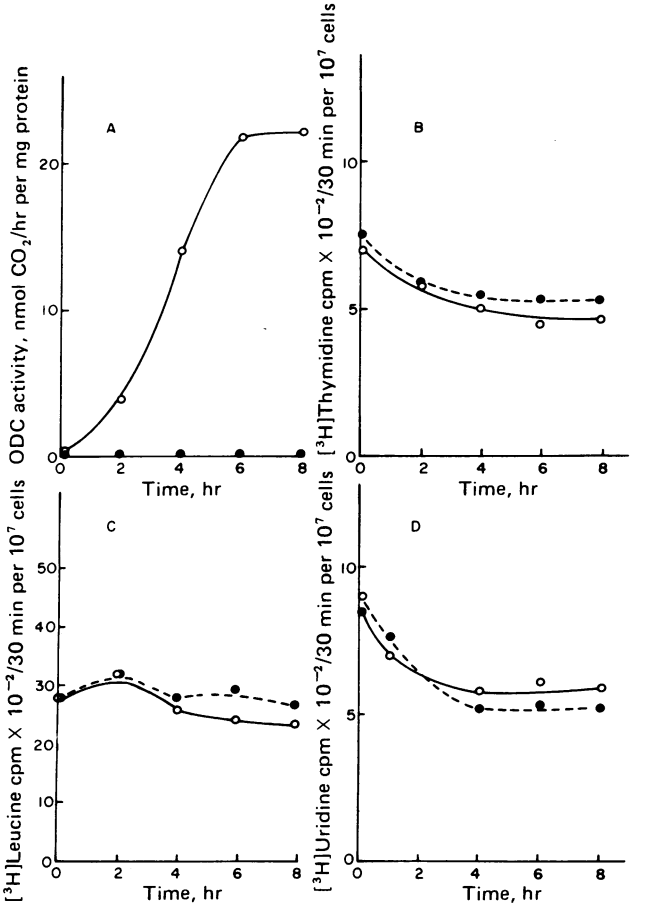

FIG. 4. Rates of incorporation of [^{3}H]thymidine, [^{3}H]leucine, and of [^{3}H]uridine into the DNA, protein, and RNA, respectively, of N18 neuroblastoma cells maintained in the salts/glucose medium in the presence or absence of 10 mM asparagine. The cells were maintained in the salts/glucose medium in the presence of 10 mM asparagine (○) or absence of asparagine (●), for 8 hr. At the designated times, [^{3}H]thymidine, [^{3}H]leucine, or [^{3}H]uridine at 0.4 μCi/ml was added and the incubation was continued for an additional 30 min. (*A*) ODC activity; (*B*) [^{3}H]thymidine incorporation; (*C*) [^{3}H]leucine incorporation; (*D*) [^{3}H]uridine incorporation. The cells were washed and suspended in 0.8 ml of ice-cold ODC assay buffer; one half was used for the incorporation assay (6) and one half was used for the ODC assay.

Fig. 4A shows the typical induction curve of ODC activity that is obtained in the presence of 10 mM asparagine, and its control, showing the lack of any ODC activity in the absence of asparagine. Samples removed during various times, under these two experimental conditions, were assayed for the rate of DNA, RNA, and protein synthesis. The general conclusions are: (*a*) in the salts/glucose medium, neuroblastoma cells are metabolically active for at least 8 hr; (*b*) the induction of ODC is not associated with an increased rate of incorporation of precursors into DNA, RNA, and protein (*c*) the rates of precursor incorporation into RNA and DNA, after an initial fall up to 2 hr, appear to remain constant for the ensuing 6 hr. In addition, cell viability, as determined by the trypan blue exclusion test, indicated that more than 90% of cells were viable after 14 hr in the salts/glucose plus 10 mM asparagine medium, while in the salts/glucose medium (minus asparagine) cell viability was about 80% after 14 hr. The variety of polypeptides in which [^{3}H]leucine is incorporated into the cytoplasmic

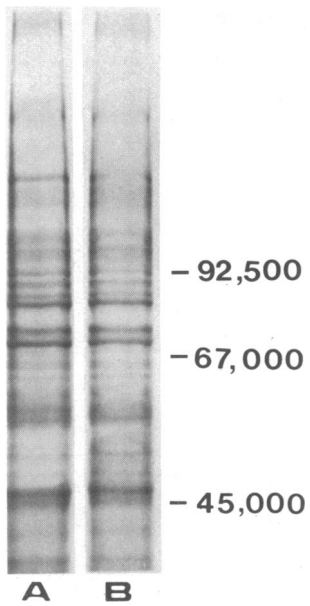

FIG. 5. Fluorogram of the [^{3}H]leucine-labeled supernatant fluid of N18 neuroblastoma cells maintained in salts/glucose medium in (*A*) the presence of 10 mM asparagine or (*B*) the absence of asparagine. The confluent cells were exposed for 2 hr to [^{3}H]leucine at 5.0 μCi/ml (from zero time to 2 hr). Positions of marker proteins of known molecular weight are given.

fraction of N18 cells in salts/glucose medium under basal conditions is emphasized by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (Fig. 5). Addition of 10 mM asparagine retains the variety of incorporation; detailed investigation of the labeling of these bands does not indicate any gross changes in the intensity of labeling of any bands.

Table 1 shows that when actinomycin D is added to the salts/glucose medium, it does not inhibit the induction of ODC activity for 5–6 hr when asparagine or asparagine + PGE_1+IBMX is used as an inducer. Neither does actinomycin D inhibit the induction of ODC activity when asparagine alone is used as an inducer in Dulbecco's medium. However, actinomycin D completely inhibits the induction of ODC activity by PGE_1+IBMX or by asparagine + PGE_1+IBMX in Dulbecco's medium. The dependence of the inhibitory effect of actinomycin D on the composition of the medium and on the inducing agent may explain the conflicting data published regarding the effects of actinomycin D on the induction of ODC activity (3, 10, 12).

Table 2 shows that the short $t_{1/2}$ of ODC (in the order of 12–15 min) is only obtained when asparagine is removed from the medium. This short $t_{1/2}$ is unaffected by the presence of cycloheximide or puromycin if these are added to an asparagine-free medium. However, if asparagine is retained in the medium, cycloheximide or puromycin gives $t_{1/2}$ values of the order of 400 min after 4 hr of induction and of 200 min after 8 hr of induction. We find the $t_{1/2}$ of ODC activity in Dulbecco's growth medium in the presence of cycloheximide to be 60 min; a previously reported value was 66 min (13).

As noted, when cycloheximide is added with asparagine at zero time; it inhibits the induction of ODC activity. In keeping with this and the results of Table 1, we may therefore conclude: (*a*) in the presence of cycloheximide (or puromycin), the *further* induction of ODC activity by asparagine is inhibited; (*b*) protein synthesis is not required for the *maintenance* of the existing level of ODC activity, but maintenance is more related to the presence of asparagine; (*c*) the $t_{1/2}$ of ODC as normally defined, by the decay in enzyme activity subsequent to the inhibition of protein synthesis, will vary depending upon the composition of the medium.

Table 1. Effect of actinomycin D on the induction of ODC activity in N18 neuroblastoma cells

Inducer	ODC activity* Salts/glucose medium −Act D	Salts/glucose medium +Act D	Dulbecco's medium −Act D	Dulbecco's medium +Act D
Asparagine (10 mM)	18	16	21	21
PGE_1 (10 μM) + IBMX (0.5 mM)	NI	NI	18	2
Asparagine (10 mM) + PGE_1 (10 μM) + IBMX (0.5 mM)	20	17	24	1

NI, activity was not induced under these conditions (see Fig. 2).
* ODC activity is the 4-hr time point taken from the time activity curves of induction of ODC activity under various conditions. In the salts/glucose medium, Act D slightly enhances ODC activity at 2 hr and in general does not inhibit ODC activity for a period of at least 5–6 hr under conditions where uridine incorporation is more than 97% inhibited. ODC activity is expressed in nmol CO_2/hr per mg of protein; each number in the table represents the averages of at least two experiments with duplicate samples. Act D added was 5 μg/ml.

Cycloheximide does not destroy the ability of these cells to be induced by asparagine. If cells are exposed to cycloheximide of 40 μg/ml for 1 hr and the cycloheximide is then removed, they still can be induced with 10 mM asparagine to normal levels of ODC activity (9200 cpm versus control 9500 cpm). Consequently, the effects of cycloheximide are reversible when the cells are exposed for short periods of time to cycloheximide.

With longer periods of exposure to cycloheximide, the $t_{1/2}$ values of 200 and 400 min (Table 1) obtained in the face of the decreased metabolic processes and general cell deterioration that occurs in the presence of cycloheximide and puromycin emphasize the ability of asparagine to maintain ODC activity under adverse metabolic conditions. It is probable that in this minimal medium this slow loss of ODC activity in the presence of cycloheximide or puromycin closely follows the rate of functional deterioration of the cell. These results suggest that in the presence of optimal concentrations of asparagine in this minimal medium, ODC activity is as perishable as the cell.

Table 2. Effect of removing asparagine (Asn) or adding cycloheximide (CHX) or puromycin (Puro), on the $t_{1/2}$ of ODC activity during various times of the ODC induction curve of Fig. 2

Time after induction curve (hr)	Asn (10 mM)	CHX (50 μg/ml)	Puro (50 μM)	$t_{1/2}$, min
4	−	−	−	12.5*
4	−	+	−	25
4	−	−	+	13
4	+	+	−	420
4	+	−	+	400
8	−	−	−	15*
8	−	+	−	25
8	−	−	+	15
8	+	+	−	240
8	+	−	+	220

ODC activity was induced by 10 mM asparagine under the conditions shown in Fig. 2. At 4 hr and 8 hr the cells were washed twice and reincubated in a salts/glucose medium with (+) or without (−) additives. In the presence of cycloheximide or puromycin, protein synthesis was inhibited more than 95%.
* With a lag period of about 30–50 min.

DISCUSSION

Our understanding of the dynamics of metabolism in the whole animal (14) paved the way for the development of cell-free systems that now permit the dissection of the individual steps in protein synthesis. In a similar fashion, our present-day understanding of the dynamics of hormone action and enzyme induction has been largely dependent upon whole animal studies or upon studies of cells cultured in complex media that permit growth and replication (1, 2, 15, 16).

Such complex integrated cellular expressions as the induction of an enzymatic activity appear to require the integrity of the membrane-bounded cell. Consequently, one available recourse for simplification of such systems (in order to create the "intact cell" counterpart of "cell-free" systems) is the development of a minimal medium that permits the retention of the integrity of the cell and the basal associated metabolic reactions. Given this elementary level of cellular sustenance, we can now start imposing nominal changes to the medium in order to relate these to the chain of metabolic reactions that ensues.

Numerous studies have indicated that ornithine decarboxylase activity can be increased by hormones that affect growth (3, 9, 17). Fausto tested the effects of several amino acids on ODC activity in rat liver (3). Hogan and Murden (15) showed that glutamine added to SWIM77 medium increased ODC activity in HTC cells 8-fold. Prouty (18) obtained similar results for HeLa cells in Joklik-modified Eagle's medium. Our results also reproduce this glutamine effect with neuroblastoma cells in Dulbecco's medium, but we found that asparagine consistently yields better stimulation. However, most significantly, we found that maximal ODC activity in confluent N18 cells can be induced by asparagine alone in a simple salts/glucose medium. The only other active amino acid is glutamine, although glyine, serine, and proline showed low activity.

Cyclic nucleotides have been suggested to be directly involved in the regulation of ODC activity (9) in cells. The results shown in Fig. 3 clearly indicate that 10 mM asparagine alone is necessary and sufficient to bring about maximal ODC activity in neuroblastoma cells in a salts/glucose medium. Cyclic AMP analogs and compounds that can increase the intracellular cAMP level do not induce ODC activity at all in confluent neuroblastoma cells in salts/glucose medium. It appears to us that this simple induction medium offers researchers interested in the mechanism of action of cAMP a good means of evaluating to what extent other reactions, inducible by cAMP, are in effect mediated by asparagine or other amino acids.

Table 1 summarizes the actinomycin D effect on the induction of ODC activity. It emphasizes that the expression of the actinomycin D effect can be modified by the composition of the medium. Consequently it becomes very difficult to interpret the actinomycin D effects in terms of transcriptional or post-transcriptional control. Furthermore, our results raise questions on "paradoxical effects" obtained with other enzyme systems (10). The underlying explanation of the modification of the actinomycin D effect by the composition of the medium remains to be elucidated.

It should be clearly noted that this discussion is in terms of "ODC activity" and no correlation with amount of ODC can be made. Such studies will have to await the availability of completely reliable methodology for such a determination.

It is interesting to note here that in the salts/glucose medium the inhibition of protein synthesis by cycloheximide or puromycin is not in itself enough to cause a rapid decay in ODC activity but rather it is dependent on the presence of asparagine. At this stage, although it is early to say that intracellular ODC activity in animals or in cultured cells depends solely on the presence of asparagine (or glutamine), the data obtained from N18 neuroblastoma cells in salts/glucose medium and our unpublished data with mouse leukemic cells and 3T3 fibroblasts strongly suggest that the "stabilizing" effect by asparagine (or glutamine) is a general phenomenon.

The particular role of asparagine remains to be elucidated. The correlation between this amide, the surface glycoproteins, and cellular activity (20), the suggestion that the amide groups of aspartic and glutamic acids may be involved in the process of aging of proteins (21), and the essential role that asparagine appears to play in the metabolism of cancer cells (22) are only some of the questions now available for detailed examination in the minimal medium.

It is probable that we will have to individually tailor the composition of the minimal maintenance media for various cell lines. For instance, we have not been able to maintain murine P-388 or L-1210 cells for any significant period of time in the salts/glucose media described above.

The requirements for the "minimal media" of each cell line will probably prove to be characteristic of the individual metabolic needs of the cell line. In this way we should be able not only to study the commonality of all cells but also to emphasize and understand the uniqueness of the individual cell.

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1. Knox, W. E. (1951) *Br. J. Exp. Pathol.* **32**, 462–469.
2. Rechcigl, M., Jr., ed. (1971) *Enzyme Synthesis and Degradation in Mammalian Systems* (University Park Press, Baltimore, London, Tokyo).
3. Fausto, N. (1971) *Biochim. Biophys. Acta* **238**, 116–128.
4. Amano, T., Richelson, E. & Nirenberg, M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 258–263.
5. Chen, K. Y., Heller, J. S. & Canellakis, E. S. (1976) *Biochem. Biophys. Res. Commun.* **68**, 401–408.
6. Ross, E. & Schatz, G. (1973) *Anal. Biochem.* **54**, 304–306.
7. Chen, K. Y., Tsai, C. M. & Canellakis, E. S. (1975) *Cancer Res.* **35**, 2403–2412.
8. Bonner, W. M. & Lasky, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
9. Bachrach, U. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3087–3091.
10. Russel, D. H. & Snyder, S. H. (1969) *Mol. Pharmacol.* **5**, 253–262.
11. Beck, W. T., Bellantone, R. A. & Canellakis, E. S. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1649–1655.
12. Morris, D. R. & Fillingame, R. H. (1974) *Annu. Rev. Biochem.* **43**, 303–325.
13. Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1858–1862.
14. Schoenheimer, R. (1942) *The Dynamic State of Body Constituents* (Harvard University Press, Cambridge, MA).
15. Hogan, B. L. M. & Murden, S. (1974) *J. Cell. Physiol.* **83**, 345–352.
16. Lee, K. L. & Kenney, F. T. (1971) *J. Biol. Chem.* **246**, 7595–7601.
17. Clark, J. L. (1976) *Biochemistry* **13**, 4668–4674.
18. Prouty, W. F. (1976) *J. Cell. Physiol.* **89**, 65–76.
19. Hershko, A. & Tomkins, G. M. (1971) *J. Biol. Chem.* **246**, 710–714.
20. Fidler, I. J., Montgomery, P. C. & Cesarini, J. P. (1973) *Cancer Res.* **33**, 3176–3180.
21. Robinson, A. B. & Rudd, C. J. (1974) *Curr. Top. Cell. Regul.* **8**, 248–295.
22. Cooney, D. A. & Handschumacher, R. E. (1970) *Annu. Rev. Pharmacol.* **10**, 421–440.