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# PRODUCTION OF L-ASPARAGINE BY TUMOR CELLS AND THE EFFECT OF ASPARAGINE ANALOGS*

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**Abstract**—A rapid assay of the ability of intact cells (10–50 mg) to synthesize L-asparagine and L-aspartic acid, and its application to the evaluation of potential asparagine analogs has been devised. Washed cell preparations were incubated at 37° in Eagle's medium (supplemented with L-glutamine, 2 mM, and L-aspartic acid, 0·05 mM), and the amounts of L-asparagine and L-aspartic acid formed were determined with an enzymatic assay. Murine tumor cells resistant to L-asparaginase produced 1·0 to 1·6 nmoles L-asparagine/mg cells/hr, while sensitive cells produced less than 0·1 nmole/mg cells/hr. The newly synthesized L-aspartic acid was largely localized in the cells; the newly synthesized L-asparagine, however, was distributed both in the cells and in the medium. 5-Diazo-4-oxo-L-norvaline (0·5 mM) and 5-chloro-4-oxo-L-norvaline (0·05 mM) inhibited L-asparagine biosynthesis by P815Y ascites cells 88 and 95 per cent respectively; no significant inhibition of L-aspartic acid biosynthesis was observed with either analog. 5-Bromo-4-oxo-L-norvaline (0·1 mM) caused 78 per cent inhibition of L-asparagine biosynthesis, but also reduced the biosynthesis of L-aspartic acid to 52 per cent.

PREVIOUS workers have shown that certain types of neoplastic cells are sensitive to L-asparaginase.[1–3] From these results and the independent finding that some leukemic cells require L-asparagine in tissue culture,[4,5] it has been concluded that the tumor cells that are sensitive to L-asparaginase synthesize very little L-asparagine, while resistant lines can synthesize relatively large amounts of this amino acid.[6,7] Most of these studies measured the conversion of $^{14}$C-labeled L-aspartic acid to $^{14}$C-L-asparagine with cell-free extracts or partially purified preparations of L-asparagine synthetase by separation of L-aspartic acid and L-asparagine with high-voltage electrophoresis,[8,9] by anion-exchange chromatography,[10,11] or by alumina column chromatography.[12] By using trinitrophenylation and paper chromatography,[13] Broome and Schwartz[14] also demonstrated a difference in the production of L-asparagine in asparaginase-sensitive and -resistant lymphoma cells in a culture medium. In the current study, a rapid enzymatic method for the assay of L-aspartic acid and L-asparagine[15] has been used to measure the production of L-asparagine and L-aspartic acid by small quantities of intact cells. This method has been applied to the evaluation of a number of potential antimetabolites of L-asparagine.

## MATERIALS AND METHODS

*Chemicals and enzymes.* The following asparagine analogs were prepared by

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Dr. P. K. Chang in this department: 5-diazo-4-oxo-L-norvaline (DONV),[17] 5-diazo-4-oxo-D-norvaline (D-DONV), 5-chloro-4-oxo-L-norvaline (CONV), 5-bromo-4-oxo-L-norvaline (BONV). Eagle's basal medium with Earle's salts was obtained from Microbiological Associates, Inc. L- And D-aspartic acid, L- and D-asparagine, and reduced nicotinamide adenine dinucleotide (NADH) were obtained from Calbiochem Company. Glutamate-oxaloacetate transaminase and malate dehydrogenase were purchased from Boehinger Mannheim, G.m.b.H., Germany. L-Asparaginase from *Escherichia coli* was supplied by Squibb & Sons, New York.

*Tumors.* Tumors were transplanted by the intraperitoneal injection of 1–2 $\times$ $10^6$ cells in 0·1 ml of Fischer's medium with 10% horse serum. The tumor cells used in these studies were P815Y leukemic mast cells, L5178Y lymphoblastic leukemic cells and Sarcoma 180 cells. L-Asparaginase-sensitive and -resistant sublines of 6C3HED lymphosarcoma cells, designated 6C3HED-S and 6C3HED-R, respectively, were kindly provided by Dr. J. D. Broome; and L-asparaginase-resistant 6C3HED-RG1 cells were kindly provided by Dr. J. G. Kidd. The Novikoff hepatoma of rat was kindly provided by Dr. A. B. Novikoff. The cell line P815Y was used for routine tests because of its rapid and reproducible growth, as well as its relatively high rate of L-asparagine biosynthesis.

*Assay of* L-*asparagine and* L-*aspartic acid net production by cell suspensions.* Tumor cells from ascites fluid were collected by centrifugation and washed three times at 25° with Eagle's medium containing 10 units/ml of heparin. Unless otherwise stated, each incubation contained 4 ml of a cell suspension (10 mg/ml) in the same medium supplemented with L-aspartic acid (0·05 mM) and L-glutamine (2 mM). The cell suspension was incubated in precleaned glass scintillation counting vials at 37°, with moderate shaking. One ml of the suspension was withdrawn at zero time and at various intervals after incubation and pipetted into capped, polycarbonate Spinco tubes and immediately heated for 10 min in a boiling-water bath. After centrifugation at 105,000 *g* for 60 min, the supernatant fluid was assayed for L-aspartic acid and L-asparagine content according to the enzymatic method of Cooney *et al.*[15] The net increase of L-aspartic acid and L-asparagine at various times after incubation was calculated by subtracting the zero time values. Reproducibility was within 5 per cent if cell suspensions of 20 mg/ml were used. Up to 25 assays could be performed in 120 min.

*Incorporation of* L-*asparagine and* L-*aspartic acid into acid-insoluble fractions.* [$^{14}$C]L-asparagine (1·8 $\mu$c, 0·012 $\mu$moles) was added to the cell suspension at zero time. After various periods of incubation, portions of the cell suspension were heated in a boiling-water bath for 10 min and centrifuged at 105,000 *g* for 30 min. The supernatant fraction of each portion was assayed for radioactivity and L-asparagine content, and the specific activity of the total soluble pool of L-asparagine was calculated. Since the cell lines used do not contain asparaginase, radioactivity could be attributed to L-asparagine; and this was confirmed by paper chromatography of the extracts. To the other fraction was added an equal volume of 5% trichloroacetic acid (TCA). The acid-insoluble residue was washed three times with 3 ml of 5% TCA containing 10 mM unlabeled L-asparagine on a Millipore filter (0·22 $\mu$, pore diameter). The radioactivity in the insoluble residue was determined in a liquid scintillation counter with appropriate corrections for quenching. The incorporation of [$^{14}$C]L-aspartic acid into acid-insoluble residue was similarly determined.

The assay technique for L-asparagine synthetase was that described by Horowitz *et al.*[9] with the following modifications. The assay mixture contained L-aspartic acid (0·15 mM), [$^{14}$C]L-aspartic acid (uniformly labeled; 1·5 × $10^6$ counts/min, 154 mc/mole), L-glutamine (20 mM), ATP (10 mM), $MgCl_2$ (10 mM), dithiothreitol (5 mM) and Tris-HCl buffer (0·1 M, pH 8·0) in a final volume of 1·0 ml. To this was added a 105,000 *g* supernatant fraction (2–3 mg protein) from a homogenate of the cells prepared with a Potter–Elvehjem homogenizer in 0·1 M Tris, pH 8·0, containing 5 × $10^{-4}$ M dithiothreitol. After incubation at 37°, the reaction was stopped by heating in a boiling-water bath for 10 min. The per cent conversion to $^{14}$C-L-asparagine was measured by high-voltage electrophoresis (30 V/cm) on Whatman 3-MM filter paper with sodium phosphate buffer (0·1 M, pH 7·0).

## RESULTS

The optimal production of L-asparagine and L-aspartic acid by cell lines resistant to L-asparaginase required supplementation of the incubation medium with L-glutamine (2 mM) (Table 1). In some experiments, an apparent loss of free L-aspartic acid or L-asparagine in the total incubation mixture was observed with L-asparaginase-sensitive lines. It was generally found that cell suspensions of 5–30 mg/ml were optimal for L-aspartic acid and L-asparagine production during periods of up to 120 min.

TABLE 1. NET PRODUCTION OF L-ASPARTIC ACID AND L-ASPARAGINE BY P815Y CELLS IN EAGLE'S MEDIUM WITH AND WITHOUT SUPPLEMENTATION OF L-GLUTAMINE AND L-ASPARTIC ACID*

| | L-aspartic acid (nmoles/mg of cells) | | L-asparagine (nmoles/mg of cells) | |
|---|---|---|---|---|
| | 30 min | 60 min | 30 min | 60 min |
| Eagle's medium | 0·06 | −0·03 | 0·07 | −0·03 |
| Eagle's medium + 0·05 mM L-aspartic acid | −0·07 | 0·23 | 0·23 | 0·16 |
| Eagle's medium + 2·0 mM L-glutamine | 1·29 | 2·38 | 0·62 | 1·20 |
| Eagle's medium + 2·0 mM L-glutamine + 0·05 mM L-aspartic acid | 1·26 | 2·37 | 0·67 | 1·25 |

*Data presented are the average of two assays in which 10 mg/ml cell suspensions were used.

Figure 1 shows the time course of the production of L-aspartic acid and L-asparagine in various murine tumor cells. L5178Y lymphoblastic leukemic cells, P815Y leukemic mast cells or 6C3HED-S lymphosarcoma cells produced similar amounts of L-aspartic acid (2·3 nmoles/mg/hr). L-Asparagine production in these same cell lines, however, was quite different. L-Asparaginase-sensitive cells such as 6C3HED-S and L5178Y were relatively unable to synthesize L-asparagine (less than 0·1 nmole/mg/hr), but the L-asparaginase-resistant P815Y leukemia synthesized large amounts of L-asparagine (i.e. 1·0–1·6 nmoles/mg/hr). It should be noted, however, that cell-free extracts of this sensitive tumor have very low asparagine synthetase activity compared to most resistant cell lines (Table 2). The production of L-asparagine by different

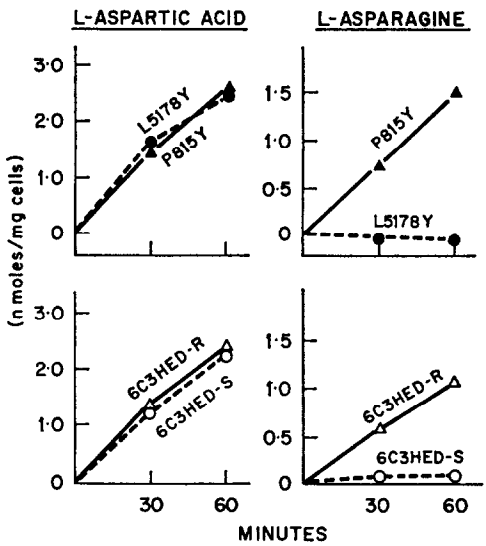


FIG. 1. Time course of L-aspartic acid and L-asparagine production in murine tumor cells. One per cent cell suspensions were used for all experiments as described in Materials and Methods.

sublines of tumor cells from mice also was studied. Prolonged treatment of mice given the L-asparaginase-sensitive 6C3HED-S cells with subcurative doses of L-asparaginase caused the emergence of an L-asparaginase-resistant subline, 6C3HED-R cells.[14] Only this L-asparaginase-resistant subline of the tumor elaborated significant amounts of L-asparagine (Fig. 1). Whole cell suspensions of other resistant tumor

TABLE 2. L-ASPARTIC ACID AND L-ASPARAGINE BIOSYNTHESIS IN VARIOUS TUMOR CELLS AND CELL-FREE EXTRACTS

| Tumor cells | Host strain | Net production of L-aspartic acid (nmoles/mg cells/hr)* | Net production of L-asparagine (nmoles/mg cells/hr) | L-Asparaginase sensitivity | Activity of L-asparagine synthetase in cell-free extracts (nmoles/mg protein/hr) |
|---|---|---|---|---|---|
| Mouse | | | | | |
| P815Y (14)† | $AKD_2F_1$ | 3·51 ± 0·27 | 1·33 ± 0·09 | Resistant | 0·20 ± 0·02 |
| 6C3HED-S (2) | C3H | 1·82 ± 0·01 | 0·02 ± 0·02 | Sensitive | 0·17 ± 0·03 |
| 6C3HED-R (2) | C3H | 2·21 ± 0·72 | 1·15 ± 0·05 | Resistant | 4·84 ± 0·23 |
| 6C3HED-RG1 (2) | C3H/HEJ | 2·11 ± 0·35 | 1·32 ± 0·15 | Resistant | 12·69 ± 0·46 |
| L5178Y (2) | $AKD_2F_1$ | 2·64 ± 0·31 | −0·18 ± 0·02 | Sensitive | 0·01 ± 0·01 |
| Sarcoma 180 (2) | Swiss | 3·01 ± 0·42 | 0·54 ± 0·02 | Resistant | 2·63 ± 0·22 |
| Rat | | | | | |
| Novikoff hepatoma (2) | Holtzman albino | 3·67 ± 0·15 | 1·38 ± 0·05 | Resistant | 2·55 ± 0·09 |

*Figures represent the mean value ± standard error.

†Figures in parentheses indicate the number of experiments. In each experiment, tumor cells from two to five animals were pooled.

lines such as 6C3HED-RG1, Sarcoma 180 or Novikoff hepatoma produced significant amounts of L-asparagine (Table 2), a result consistent with the data from cell-free extracts reported in earlier studies.[8,9,11,16]

*Distribution of newly synthesized* L-*aspartic acid and* L-*asparagine in cell suspensions.* When the P815Y cells were incubated in asparagine-free medium as described above and then separated into supernatant medium and cells by centrifugation at 1700 rev/min for 5 min at 25°, the total amount of newly synthesized L-aspartic acid was largely localized in the cells compared to the medium, since the relative volume of cells to medium is 1:125; the total amount of newly synthesized L-asparagine, however, was more evenly distributed in the cells and in the medium (Fig. 2). A similar

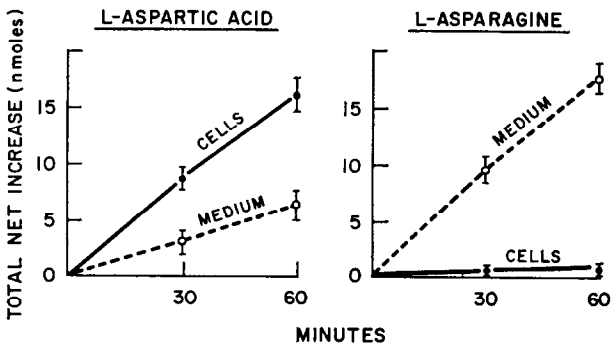


FIG. 2. Distribution of newly synthesized L-aspartic acid and L-asparagine in the cells and in the medium. Each point on the chart represents the amount of amino acid in a 2·0-ml sample. At zero time, the total L-aspartic acid in the medium (2·0 ml) was 100 nmoles, and in the cells (16 mg) was 18·6 ηmoles; total L-asparagine in the same medium was 10·2 nmoles and in the cells was 2·4 nmoles. All values were obtained from the average of four experiments ± standard error.

pattern of distribution was observed with the 6C3 HED-R, 6C3HED-RG1 and Novikoff hepatoma cells. When tracer amounts of uniformly labeled [$^{14}$C]L-asparagine (3 nmoles/ml) were added to P815Y or 6C3HED-RG1 cells, the total radioactivity in the medium decreased during incubation at 37°, while the total amount of radioactivity in the cells increased. Thus, L-asparagine is in a state of efflux concurrent with the active inward transport, presenting a dynamic bidirectional exchange of the amino acid across the cell membrane. The specific activity of the L-asparagine in the medium decreased rapidly as production of this amino acid by the cell progressed. Using average values for the specific activity of L-asparagine in the cell, it can be calculated that approximately 0·45 nmoles L-asparagine was incorporated into the acid-insoluble fractions of 6C3HED-RG1 and P815Y cells per mg per hr respectively (Fig. 3). When uniformly labeled [$^{14}$C]L-aspartic acid was added to similar cell suspensions, at least 98 per cent of the radioactivity remained in the medium; no appreciable increase in the radioactivity in the cells occurred during a 60-min incubation. Furthermore, radioactivity from [$^{14}$C]L-aspartic acid was not incorporated into the acid-insoluble fractions of either the P815Y or the 6C3HED-RG1 cells (Fig. 3). Addition of L-asparaginase (0·7 I.U./ml) to suspensions of P815Y cells at the beginning or at the end of a 60-min incubation made no difference in the total amount of L-aspartic acid produced by the system (cells plus medium). These results suggested

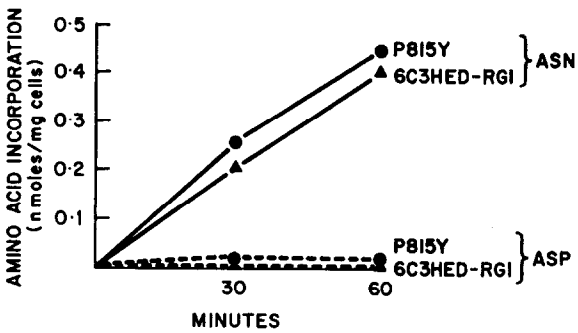


FIG. 3. Incorporation of L-asparagine and L-aspartic acid into the acid-insoluble fraction by P815Y and 6C3HED-RG1 cells. Uniformly labeled [$^{14}$C]L-asparagine or [$^{14}$C]L-aspartic acid was added at zero time to 1% cell suspensions. Incorporation of amino acids was calculated by measuring the specific activities of the L-asparagine and L-aspartic acid pools and determining radioactivities in the acid-insoluble residues as described in Materials and Methods.

that the destruction of newly released L-asparagine in the medium did not increase the production of L-asparagine by the cells during these short time periods.

*Effects of* L-*asparagine analogs and structurally related compounds on* L-*asparagine and* L-*aspartic acid production in P815Y cells.* Since L-asparaginase is believed to exert its therapeutic effect by eliminating L-asparagine from the vascular space, compounds which may inhibit L-asparagine biosynthesis within cancer cells could augment the lethal effect by eliminating endogenous supplies of this amino acid. DONV had no effect on L-aspartic acid biosynthesis but was a good inhibitor of L-asparagine biosynthesis at concentrations ranging from 0·1 to 0·5 mM (Fig. 4).

Table 3 indicates the inhibition of L-asparagine and L-aspartic acid production in P815Y cells by a group of asparagine analogs. L-DONV inhibited L-asparagine biosynthesis quite selectively; however, the D-isomer of DONV does not affect the biosynthesis of either L-asparagine or L-aspartic acid. CONV was an even more potent inhibitor. BONV was an only slightly more potent inhibitor of L-asparagine biosynthesis than the L-DONV, but it also caused some inhibition of L-aspartic acid biosynthesis. The hydrolysis product of these analogs, 5-hydroxy-4-oxo-L-norvaline, did not affect the biosynthesis of either amino acid. L-Asparagine was shown to be a potent inhibitor of its own biosynthesis. When the medium was supplemented with this amino acid (Fig. 5) at $10^{-4}$ M, no net increase of L-asparagine could be observed. The concentration for 50 per cent inhibition was $2{\cdot}5 \times 10^{-5}$ M, which was equivalent to or somewhat lower than the concentration found in the plasma or ascites fluid of the animal. These results confirm the metabolic significance in whole cells of the feedback inhibition by L-asparagine previously demonstrated with preparations of asparagine synthetase. In contrast, D-asparagine, L-aspartic acid or D-aspartic acid at the level of $5 \times 10^{-4}$ M had no effect on either L-asparagine or L-aspartic acid production. The glutamine analog, 6-diazo-5-oxo-L-norleucine ($1 \times 10^{-4}$ M), which does not inhibit L-asparaginase,[17] was a potent inhibitor of L-asparagine biosynthesis (98 per cent inhibition), a result consistent with the postulated role of glutamine as the amide donor in this reaction. The following compounds, at $5 \times 10^{-4}$ M level, had no significant effects on either L-asparagine or L-aspartic acid

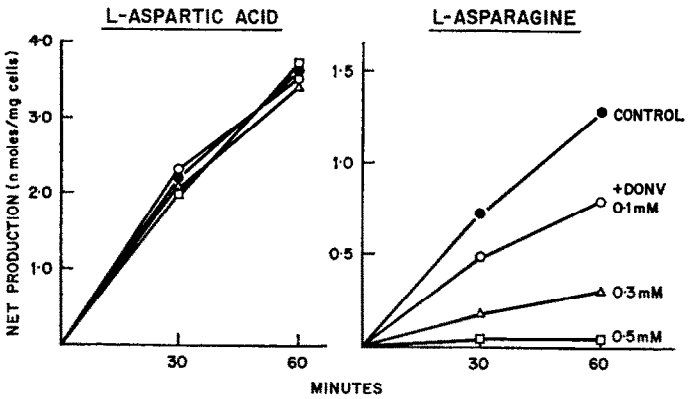


FIG. 4. Inhibition of L-aspartic acid and L-asparagine biosynthesis in P815Y cells by DONV. All figures represent net synthesis in the incubation mixture.

TABLE 3. INHIBITION OF L-ASPARTIC ACID AND L-ASPARAGINE BIOSYNTHESIS IN P815Y CELLS BY ASPARAGINE ANALOGS RELATED TO 4-OXO-NORVALINE

| Compound | Concentration (M) | Per cent inhibition* | |
|---|---|---|---|
| | | L-Aspartic acid | L-Asparagine |
| 5-Diazo-4-oxo-L-norvaline | $1 \times 40^{-4}$ | 0 | 51 |
| | $5 \times 10^{-4}$ | 5 | 88 |
| 5-Diazo-4-oxo-D-norvaline | $5 \times 10^{-4}$ | 0 | 0 |
| 5-Chloro-4-oxo-L-norvaline | $1 \times 10^{-6}$ | 2 | 9 |
| | $1 \times 10^{-5}$ | 0 | 62 |
| | $5 \times 10^{-5}$ | 0 | 95 |
| 5-Bromo-4-oxo-L-norvaline | $1 \times 10^{-5}$ | 0 | 23 |
| | $1 \times 10^{-4}$ | 52 | 78 |
| 5-Hydroxy-4-oxo-L-norvaline | $5 \times 10^{-4}$ | 0 | 0 |

*Per cent inhibition of the net increase of L-aspartic acid and L-asparagine was measured at the end of a 60-min incubation as described in Materials and Methods.

production by P815Y cell suspensions under these same conditions: 4-oxo-S-methyl sulfoxide-L-norvaline, DL-β-methyl aspartic acid, S-methyl-L-cysteine sulfoxide, S-methyl-L-cysteine sulfoximine, L-cysteine sulfinic acid, β-cyanoalanine, aspartic acid β-chlorethylamide and L-2,4-diamino butyric acid. S-carbamyl-L-cysteine had been shown by Adamson and Fabro[18] to have minimal antitumor activity, but this analog did not inhibit L-asparagine or L-aspartic acid biosynthesis as tested.

## DISCUSSION

Since the net increases of both L-aspartic and L-asparagine after incubation of cell suspensions were measured by subtracting zero time values, the result represents the algebraic sum of their biosynthesis, incorporation and metabolism. By measuring the incorporation of trace amounts of added [$^{14}$C]L-asparagine into the acid-insoluble

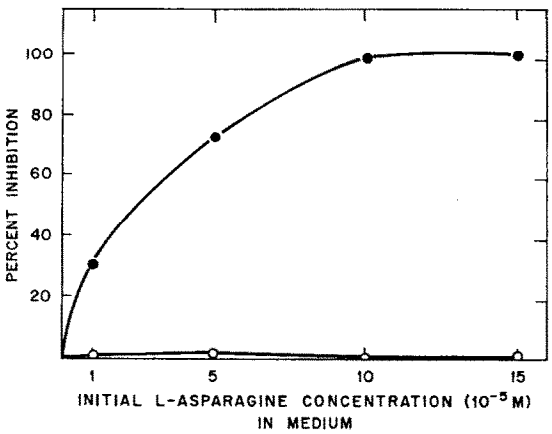


FIG. 5. Effect of L-asparagine on L-asparagine and L-aspartic acid production. Effect on L-asparagine production, ●——●; effect on L-aspartic acid production, ○——○. P815Y cell suspensions (1 mg/ml) were used with the assay procedure described in Materials and Methods.

fraction of the cells and coupling this with the average specific radioactivity of L-asparagine through the incubation period, an estimate of the fraction of asparagine that was diverted to protein synthesis was obtained. On this basis, P815Y cells incorporated 0·45 nmole L-asparagine into the acid-insoluble fraction in 60 min (Fig. 3), in addition to the net increase of 1·40 nmoles L-asparagine in the soluble portion of the cells and medium during this same period. No detectable amount of added [$^{14}$C]L-aspartic acid entered P815Y cells or was incorporated into acid-insoluble fractions (Fig. 3), indicating the very strong permeability barrier to this compound.

Although L-asparagine biosynthesis has been studied extensively in both neoplastic tissue extracts and partially purified enzyme preparations, the present studies indicate that the relative L-asparagine synthetase activity in tissue extracts may not necessarily reflect the true L-asparagine-producing capability of the intact cells. For example, a cell-free extract (105,000 *g* supernatant) from P815Y cells (Table 2) showed low L-asparagine synthetase activity, comparable to extracts from the sensitive 6C3HED-S line; however, intact P815Y cells, an asparaginase-resistant form of leukemia in mice, produced as much L-asparagine as other L-asparaginase-resistant tumor cells. A possible explanation is that the homogenization procedure destroyed cell compartmentation and this disturbed a regulatory mechanism; alternatively, the L-asparagine synthetase in different types of cells might have different properties or catalyze L-asparagine biosynthesis by an unknown pathway; or L-asparaginase synthetase in P815Y cells might be bound to cell membranes or organelles and thus not be extracted into the supernatant fraction. The possibility that P815Y cells might contain L-asparaginase was excluded by a direct assay. The P815Y cells also did not incorporate more L-asparagine into protein than did other sensitive or resistant cells as measured by the isotopic studies. Experiments in which equal amounts of extracts from P815Y and 6C3HED-RG1 cells were mixed did not indicate the presence of inhibitory factors in the extracts of the P815Y cells. These unusual relationships are under further study.

Direct assay of L-asparagine and L-aspartic acid showed that practically all newly

synthesized L-aspartic acid was localized in cells, while the newly synthesized L-asparagine was distributed in both the cells and the medium, with an approximately 8-fold higher concentration in the cells. These findings were consistent with the results obtained from experiments using tracer amounts of [$^{14}$C]L-aspartic acid or [$^{14}$C]L-asparagine, in which [$^{14}$C]L-aspartic acid was excluded from the P815Y cells, while [$^{14}$C]L-asparagine was concentrated by the cells from the medium. The fact that a certain fraction of L-asparagine is released into the medium from the cell may be responsible for the finding that some cultured mammalian cells have a population-dependent requirement for some amino acids, including L-asparagine.[19]

Tissue slices from normal rat liver, kidney, spleen or brain liberated virtually no L-asparagine into the incubation medium under the conditions of these assays, possibly due to their higher metabolism of this amino acid. More likely is a more rigorous metabolic control of asparagine biosynthesis in the intact cell exercised by feedback inhibition by endogenous asparagine. The excess production and accumulation of L-asparagine and L-aspartic acid in the neoplastic cells might be related to a lack of proper regulation of this process in the malignant cell. This is of interest, since analysis of cell-free extracts has shown that several normal tissues have measurable amounts of asparagine synthetase.[9,20] No detectable amount of L-asparaginase has been found in the tumor cells used for these studies, but L-asparaginase activity was detected in the rat and guinea pig liver, as reported by others.[20,21] The rate of L-asparagine biosynthesis in many human leukemic cells tested appeared to be low, but this may be a consequence of a rapid decline in the general metabolic capability of cells taken from patients for these assays. Further modification of conditions for application to human leukemic cells is needed.

In the procedures described in this paper to evaluate potential inhibitors of asparagine biosynthesis, it must be shown that the compounds do not interfere with the coupled enzyme assay of L-aspartic acid and L-asparagine which employs glutamate-oxaloacetate aminotransferase, malic dehydrogenase and L-asparaginase. Although L-DONV is a specific inhibitor for L-asparaginase,[22] the total L-asparagine could still be determined by allowing enough time for the complete conversion of L-asparagine to L-aspartic acid in the assay mixture. Of all L-asparagine analogs studied, none was a significant inhibitor or a substrate for glutamate-oxaloacetate aminotransferase or malate dehydrogenase, and they did not interfere with the assay of L-aspartic acid and L-asparagine. Both the incubation medium for the whole cells and the incubation mixture for the cell-free extract contained relatively high concentrations of L-glutamine (2 and 20 mM respectively). These could reduce the effect of DONV and L-asparagine, because studies with a purified L-asparagine synthetase indicate that DONV and L-asparagine are competitive inhibitors of glutamine as a substrate.[23]

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