Kinetics of Amino Acid Transport Across Bone Marrow Cell Membranes

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ABSTRACT Dog bone marrow nucleated cells were incubated in media containing labeled L-amino acids, and the cellular accumulation of radioactivity as a function of time was measured and analyzed according to a three-compartment model.

(a) The turnover half-time of intracellular histidine arising from extracellular sources was 6.0 ± 0.7 (SEM) min. Similar turnover half-time for serine was 10 ± 2 (SEM) min; for tryptophan, 6.5 ± 1.2 (SEM) min; and for methionine, 4.4 ± 0.6 (SEM) min. Loss of the intracellular amino acids to the extracellular space accounted for the major portion of their turnover.

(b) Each of the four amino acids noted above appeared to be actively transported into the cell.

(c) At physiologic extracellular histidine concentrations, histidine entered the cell predominantly by a facilitated process with an apparent Michaelis constant of 0.28 mmole/liter and a limiting flux of 14×10^{-3} mmole/min per cell. Loss of histidine from the cell appeared to be substantially facilitated with an apparent Michaelis constant greater than that for histidine entry.

(d) Insulin and glucagon had no measurable effect on histidine transport across the bone marrow cell membrane.

(e) Methionine depressed the influx and the fractional turnover rate of the intracellular pool of both histidine and serine.

(f) The extent of cellular accumulation of α -N-formiminoglutamate and α -N-formylglutamate was about 1/100 that of histidine. α -N-formiminoglutamate added to the culture was about $\frac{1}{4}$ as effective as histidine in providing monocarbon fragments for DNA thymine synthesis.

INTRODUCTION

Incorporation of label into a product from a labeled amino acid administered to intact cells depends upon its

transport across the cell membrane as well as upon its metabolism within the cell. Interpretation of data describing such incorporation requires quantitative information concerning transport and equilibration between extracellular and intracellular amino acid pools.

In the present work, the time course of accumulation of labeled L-amino acids inside dog bone marrow nucleated cells was analyzed according to a three-compartment model. From such analyses, it was possible to evaluate the fractional turnover rate of the intracellular amino acid pool and the rates of its entry into and exit from the cell.

METHODS

All radiochemicals were obtained from either Nuclear-Chicago Corp. (Des Plaines, Ill.) or New England Nuclear Corp. (Boston, Mass.); L-amino acids, from Calbiochem (Los Angeles, Calif.); Gey's Balanced Salt Solution (Gey's BSS) and basal medium of Eagle with L-glutamine (BME), from Grand Island Biological Co. (Grand Island, N. Y.); heparin without preservatives, from Abbott Laboratories (North Chicago, Ill.); dextran (average mol wt 250,000), from Pharmacia (Uppsala, Sweden); and ammonium oxalate, from General Chemical Co. (New York). Stock solutions of insulin (24 U/ml) and glucagon (0.15 mg/ml) were made by dissolving crystalline bovine insulin (Sigma Chemical Co., St. Louis, Mo.) and crystalline glucagon (Calbiochem), respectively, in 0.002 N HCl; the solutions were kept at 4°C for no longer than 1 wk, and diluted with Gey's BSS immediately before the individual experiments. For experiments on effects of methionine, BME without methionine was obtained from Grand Island Biological Co., and serine was added to obtain a final concentration of 0.017 or 0.040 mmole/liter. The resultant medium was also referred to as BME.

In each experiment, bone marrow cells were aspirated from the femurs of two to three lightly anesthetized dogs (sodium pentobarbital), anticoagulated, pooled, and subjected to dextran sedimentation to eliminate approximately 99% of the erythrocytes present. After centrifugation of the supernatant fluid in conical tubes at 450 g for 3 min, approximately the upper three-quarters of the sediment formed were resuspended in appropriate medium containing heparin (60 U/ml). The suspension was passed through a mesh (Macalaster Bicknell Company of Connecticut, Inc., New Haven, catalogue No. 9331) to remove cell aggre-

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gates. Cell counting was done in duplicate with a Coulter counter (Coulter Electronics, Chicago, Ill.). In the final suspension, there was one nucleated cell for every one to three erythrocytes present. By morphological characteristics, about 20–30% of the nucleated cells were deemed capable of further mitosis. 2-ml portions of the cell suspension were delivered with volumetric pipets into a series of incubation tubes (Nalge Co., Rochester, N. Y., catalogue No. 21009-400) containing known quantities of amino acids. After equilibration of the suspension at 37°C for 20–30 min, tracer quantities of labeled amino acids were added in equal amounts to all tubes without appreciably changing amino acid concentrations. Volume of incubation was 5 ml in most experiments. The number of nucleated cells remained essentially constant during the incubation (at 37°C with shaking under air) as previously shown for human marrow cells under similar conditions (1). Gross hemolysis was absent. The extracellular concentration and specific activity of amino acids were kept essentially constant by using low cell concentration (1% v/v or less) and short incubation time (2, 3). In most experiments, the cellular uptake of radioactivity was in the range 0.15–3% of that added to the culture. After addition of 5 ml of iced medium to the culture, the nucleated cells present were harvested by centrifugation in a Sorvall RC2-B centrifuge (Ivan Sorvall Inc., Norwalk, Conn.) at –3°C and 2500 *g* for 4 min, resuspension of the packed cells in 8 ml iced 80 mM ammonium oxalate (in distilled water), recentrifugation of the suspension in a conical glass tube at 1200 *g* for 3 min, and decantation of the supernatant hemolysate. During the single washing with ammonium oxalate solution, most of the contaminating erythrocytes were lysed. The entire procedure from the dilution in iced medium to the recovery of nucleated cells took 10 min. Termination of incubation was taken to be at 1 min after the dilution. The entire cell pellet was digested in NCS Solubilizer (4) and assayed for radioactivity with a Nuclear-Chicago Mark I liquid scintillation counter.

Some pellets of harvested cells were subjected to extraction with iced trichloroacetic acid (TCA, 5% w/v) for 30 min and then with hot (88°C) TCA for 20 min. The radio-

activity in these TCA extracts and TCA precipitate was determined by liquid scintillation counting.

To determine specific activity of DNA thymine, 2-ml portions of the cell suspension were delivered into four incubation tubes containing histidine-imidazole-2-¹⁴C (His-¹⁴C₁) or α -N-formiminoglutamic acid-formimino-¹⁴C (FIG-LU-¹⁴C₁) with or without methotrexate. After incubation in a metabolic shaker at 37°C, the specific activity of DNA thymine was determined by a method previously described (5).

To determine intracellular water space, cell suspensions with nucleated cell/erythrocyte ratios greater than 2 were prepared by following the dextran-sedimentation procedure with two successive "differential centrifugation" steps. After 20-min incubation in the presence of tritiated water (HTO) and inulin-carboxyl-¹⁴C, a known quantity of cells was packed by centrifugation at 1200 *g* for 10 min. The intracellular water space of the nucleated cells present (*W*) was taken to be the difference between the calculated HTO space and inulin-carboxyl-¹⁴C space of the pellet. In four experiments, $W = 14 (\pm 2, \text{SEM}) \times 10^{-11}$ ml/cell. This was felt to be an overestimate because of the contaminating erythrocytes in the pellet.

Student's *t* test was used for testing significance of difference between two means.

Compartmental analysis. The cellular uptake of radioactivity was analyzed according to the compartmental model illustrated in Fig. 1. The assumptions inherent in this model are as follows: (a) Labeled amino acids are introduced into the extracellular fluid at zero time without appreciably disturbing the prevailing steady state. The concentration and the specific activity of the extracellular amino acid are kept essentially constant thereafter. (b) When a labeled amino acid is metabolized or chemically altered in the cell, the label is "translocated" to other substances. The translocation of labels to other acid-soluble materials is assumed to be negligible compared to the translocation to acid-insoluble materials, proteins in particular (6). Further, the estimated order of magnitude for the fractional turnover rate of various mammalian proteins *in vitro* and *in vivo* is 1%/hr (2). Thus, during a short incubation period, most of the translocated label remains in the cell and the re-

FIGURE 1 Compartmental model for analysis of intracellular accumulation of labels from extracellular labeled amino acids. The model assumes: (a) that intracellular metabolism of the labeled amino acid results in "translocation" of labels primarily to substances which do not leave the cell at significant rate, (b) that such intracellular "fixation" of the labeled amino acid follows prior equilibration of the labeled amino acid in the intracellular free amino acid pool with little fixation directly from the extracellular pool, and (c) that recycling of labels back to the intracellular amino acid pool via biosynthesis and reutilization is negligible compared to the entry of labels from the extracellular pool. k_{12} , k_{21} , and k_{23} represent the fractional rates of entry, exit, and metabolism of the labeled amino acid, respectively; A_1 , A_2 , and A_3 represent the amounts of the amino acid label in compartments I (extracellular pool), II (intracellular pool), and III ("translocated"), respectively.

FIGURE 2 Measured radioactivity content in whole cells (open circles), cold TCA extract (triangles), hot TCA extract (closed circles), and TCA precipitate (boxes) upon incubating 1.0×10^8 nucleated dog bone marrow cells in Gey's BSS with L-histidine-imidazole- $2\text{-}^{14}\text{C}$ at 0.077 mmole/liter (SA 3.7 dpm/ μmole). The upper curve represents the least squares best fit form of equation 1 to the radioactivity content in whole cells. The middle curve [$A_2(t)$] and the lower curve [$A_3(t)$] represent the radioactivity content in compartments II and III, respectively, calculated by application of the model to the radioactivity content in whole cells.

cycling of label back to the intracellular amino acid pool via amino acid biosynthesis or protein catabolism is negligible compared to the steady inflow of labels from the extracellular pool. (c) It is felt that amino acids may be incorporated directly into cellular proteins without prior passage through intracellular amino acid pools (7, 8). This model assumes that such direct fixation is negligible compared to indirect fixation.

This model generates an equation describing the cellular accumulation of radioactivity of the form:

$$A_o(t) = K_1(1 - e^{-rt}) + K_2t \quad (1)$$

where

$A_o(t)$ = intracellular radioactivity as a function of time after introduction of (N) quantity of radioactivity to the medium

$$K_1 = (k_{12}N/r) - (k_{12}k_{23}N/r^2) \quad (2)$$

$$K_2 = k_{12}k_{23}N/r \quad (3)$$

r = fractional turnover rate of the intracellular amino acid pool = $k_{21} + k_{23}$ (4)

Solving equations 2, 3, and 4 for the unknown fractional rates, we obtain:

$$k_{12} = (rK_1 + K_2)/N \quad (5)$$

$$k_{21} = r^2K_1/(rK_1 + K_2) \quad (6)$$

$$k_{23} = rK_2/(rK_1 + K_2) \quad (7)$$

K_1 , K_2 , and r were found by fitting the measured cellular uptake of radioactivity with equation 1 using a program (9)

for minimization of squares of differences on a CDC 6600 digital computer. The amino acid influx (I) was calculated as the product of k_{12} and the amount of the amino acid in question initially added to the extracellular fluid. I/r underestimated the amount of the amino acid in compartment II (intracellular pool size) by its endogenous fraction derived from biosynthesis and reutilization; I/rW underestimated the intracellular concentration of the amino acid assuming its uniform distribution throughout the cell water. The quotient, flux ratio (influx/efflux) divided by concentration ratio (extracellular/intracellular), was underestimated by $I/k_{21}C_oW$ where C_o is the extracellular concentration of the amino acid. When the quotient was greater than unity, the amino acid was considered to be actively transported into the cell (10-13).

RESULTS

Figs. 2, 3, and 6 present individual measurements of the cellular uptake of radioactivity and the least squares best fit form of equation 1 (weighted according to the reciprocal of the radioactivity uptake). The fit was generally satisfactory.

Fig. 2 shows the fit in an experiment in which cells were incubated with His- ^{14}C in Gey's BSS in duplicate series. One set of pellets of harvested cells was digested for assay of cellular radioactivity; the other set was subjected to extraction with TCA as described in Methods. From the results of fitting equation 1 to the whole cell radioactivity, the radioactivity in compart-

ments II and III was calculated. The measured radioactivity in the cold TCA extract and that in the TCA precipitate corresponded roughly to those calculated to be in compartments II and III of the model, respectively.

Table I summarizes results of compartmental analysis of cellular uptake of labeled amino acids at near physiologic concentrations. It shows the following: (a) The calculated intracellular concentrations of the four amino acids were higher than their corresponding external concentrations. (b) They appeared to be all actively transported into these cells. (c) When BME was the suspending medium, as compared to when Gey's BSS was used at similar histidine concentrations, histidine had slower entry ($P < 0.0005$) and in spite of its smaller "intracellular pool size" (I/r , $P < 0.0005$) had smaller fractional efflux ($P < 0.025$). This finding was felt to be evidence of competition for transport between histidine and other amino acids present in BME. (d) Even at external concentrations lower than those used with histidine and methionine, serine had faster entry and greater "intracellular pool size" (I/r) than histidine ($P < 0.005$) and methionine ($P < 0.025$). Thus serine was concentrated more readily and to greater extents than histidine and methionine. (e) The rate of exit of the amino acid from the cell much exceeded its rate of metabolism within the cell as might be seen from the ratio, k_{21}/k_{32} . (f) With BME as the medium, the turnover halftime of the intracellular pool ($0.693/r$) of histidine was 6.0 ± 0.7 (SEM) min. Similar turnover half-time for serine was 10 ± 2 (SEM) min; for tryptophan, 6.5 ± 1.2 (SEM) min; and for methionine, 4.4 ± 0.6 (SEM) min.

The time course of ^{14}C accumulation within cells incubated in the presence of His- ^{14}C in Gey's BSS at various histidine concentrations was analyzed. As shown in Table II, the fractional influx of histidine decreased with its increasing extracellular concentration, but its fractional efflux stayed essentially unchanged with its rising intracellular level.

By use of an appropriate minimization program (9), the histidine influx at various extracellular histidine concentrations was fitted with equation 8:

$$I = k_a S + V_{\max} S(S + K_m)^{-1} \quad (8)$$

where S = extracellular concentration, k_a = apparent diffusion constant, K_m = apparent Michaelis constant, and $V_{\max}$ = limiting flux of the saturable component. Weighting the squares of deviation of the influx from equation 8 according to the reciprocal of the influx, the minimization gave the following estimates for histidine entry: $k_a = 4.2 \times 10^{-8}$ $\mu\text{mole}/\text{min}$ per cell per $\text{mmole}/\text{liter}$, $K_m = 0.28$ $\text{mmole}/\text{liter}$, and $V_{\max} = 14 \times 10^{-8}$ $\mu\text{mole}/\text{min}$ per cell. The fit of the influx to equation 8

FIGURE 3 Accumulation of labeled L-amino acids inside nucleated dog bone marrow cells incubated in BME as a function of time. Cellular accumulation of labeled histidine served as an internal "control" in each of the experiments. The measured activity of ^3H is shown as circles and that of ^{14}C as triangles. The least squares best fit form of equation 1 to each set of data is represented by the corresponding curves. Ordinate values are not directly comparable between any two curves since: (a) the components of the figure represent separate experiments performed on different bone marrow cell samples at different times, (b) amino acid concentration and specific activity varied among different amino acids and among individual experiments. Experiment 1: 7.2×10^7 cells, 0.052 mM histidine-imidazole-2,5- ^3H (SA 18 dpm/ μmole), and 0.043 mM serine-3- ^{14}C (SA 4.2 dpm/ μmole). Experiment 2: 3.6×10^7 cells, 0.054 mM histidine-imidazole-2,5- ^3H (SA 18 dpm/ μmole), and 0.024 mM tryptophan-methylene- ^{14}C (SA 21 dpm/ μmole). Experiment 3: 4.4×10^7 cells, 0.056 mM histidine-imidazole-2- ^{14}C (SA 9.7 dpm/ μmole), and 0.050 mM methionine-methyl- ^3H (SA 26 dpm/ μmole).

TABLE I
*Analysis of the Uptake of Labeled Amino Acids by Dog Bone Marrow Nucleated Cells**

Medium.....	Gey's balanced salt solution	Basal medium of Eagle			
	Histidine-imidazole-2- ¹⁴ C 3	Histidine-imidazole-2- ¹⁴ C or -2, 5- ³ H 8	Serine-3- ¹⁴ C 3	Tryptophan-methylene- ¹⁴ C 2	Methionine-methyl- ³ H 3
Labeled L-amino acid.....					
No. of experiments.....					
Extracellular concentration (C _e), mmole/liter.....	0.052	0.052-0.056	0.040-0.043	0.024-0.025	0.050
Fractional turnover rate of intracellular pool (r), min ⁻¹	0.161 ± 0.004	0.12 ± 0.01	0.08 ± 0.02	0.11 ± 0.02	0.16 ± 0.03
Influx (I) × 10 ⁸ , μmole/min per cell	2.59 ± 0.06	0.26 ± 0.02	0.55 ± 0.08	0.24 ± 0.02	0.23 ± 0.02
Fractional efflux (k ₂₁), min ⁻¹	0.152 ± 0.004	0.10 ± 0.01	0.05 ± 0.01	0.11 ± 0.02	0.13 ± 0.02
Fractional metabolic flux (k ₂₂), min ⁻¹	0.009 ± 0.000	0.024 ± 0.003	0.028 ± 0.006	0.006 ± 0.005	0.033 ± 0.004
Intracellular pool size‡ (I/r) × 10 ⁸ , μmole/cell	16.1 ± 0.4	2.3 ± 0.3	8 ± 2	2.3 ± 0.6	1.5 ± 0.2
Intracellular concentration‡ (I/rW), mmoles/liter	1.15	0.16	0.57	0.16	0.11
Flux ratio/concentration ratio‡§ (I/k ₂₁ C _e W)	23	3	19	7	3

* Results, shown below the thin line, are means and standard errors of the means.

‡ These results were calculated as the quantities in parentheses and were underestimates (see text).

§ The quotient of (influx/efflux) divided by (extracellular concentration/intracellular concentration).

with the above optimized parameters is shown in Fig. 4.

At physiologic extracellular histidine concentrations, histidine entered the cell predominantly by the saturable process when Gey's BSS not containing other amino

acids was the suspending medium (Fig. 4). When BME containing other competing amino acids was used, histidine influx was inhibited to about 1/10 of that obtained with Gey's BSS (Table I). Fig. 5 shows effect of methionine on histidine uptake from Gey's BSS and

TABLE II
*Results of Compartmental Analysis of Intracellular Accumulation of Radioactivity in Dog Bone Marrow Nucleated Cells Incubated in 5 ml Gey's BSS with L-Histidine-Imidazole-2-¹⁴C at Various L-Histidine Concentrations**

Extracellular concentration of histidine, (S)	Fractional rate of entry, (k ₁₂) × 10 ¹²	Fractional rate of exit, (k ₂₁)	Fractional turnover rate of intracellular pool, (r)	"Intracellular pool size," (I/r) × 10 ⁸	Intracellular concentration of histidine‡ (I/rW)
mmoles/liter	min ⁻¹ /cell	min ⁻¹	min ⁻¹	μmole/cell	mmoles/liter
0.052	100	0.15	0.16	16	1.1
0.102	78	0.15	0.16	24	1.7
0.503	41	0.16	0.17	61	4.4
1.00	30	0.17	0.18	84	6.0
1.50	25	0.17	0.17	110	7.9
2.00	21	0.15	0.16	136	9.7
3.00	15	0.13	0.13	173	12
4.00	15	0.16	0.17	181	13

* For each histidine concentration, the values given for the results are means from one to three experiments.

‡ Calculated assuming that intracellular water was 14 × 10⁻¹¹ ml/cell (see text for results of measurements of intracellular water space).

FIGURE 4 Rate of L-histidine entry into cells as a function of extracellular L-histidine concentration (S) in dog bone marrow nucleated cells incubated in Gey's BSS in the presence of L-histidine-imidazole-2-¹⁴C. Circles represent the calculated rate of entry based on fitting equation 1 to data of ¹⁴C accumulation inside cells. The upper curve represents the least squares best fit form of equation 8 to the calculated rate of entry. The lower two curves represent the two components of the upper curve showing the predominance of the saturable component $[V_{max}S/(S + K_m)]$ over the nonsaturable component ($k_a S$) at physiologic concentrations.

from BME. In terms of fractional (relative to control) inhibition of histidine uptake, the apparent effectiveness of methionine was less with BME than with Gey's BSS as the suspending medium. In further experiments with BME shown in Fig. 6 and Table III, methionine substantially depressed the influx and fractional turnover rate of the intracellular pool of both histidine and serine when methionine was administered at a concentration comparable to the aggregate concentration (3.1

mmoles/liter) of other neutral amino acids present in BME.

Insulin added in vitro has been reported to diminish the K_m for α -aminoisobutyric acid entry into isolated rat diaphragms (14). Recent work from this laboratory on ¹⁴CO₂ production from His-¹⁴C in rats suggested the possibility that glucagon may alter histidine transport (15). To evaluate possible role of these pancreatic hormones in histidine transport, the time course of cellular

TABLE III
Effects of 3.2 mM L-Methionine on the Kinetics of the Intracellular Pools of L-Histidine and L-Serine in the Nucleated Cells of Dog Bone Marrow Incubated in BME

Concentration, mmole/liter	L-histidine		L-serine	
	Exp. 1 0.054	Exp. 2 0.052	Exp. 1 0.024	Exp. 2 0.040
Fractional turnover rate of intracellular pool (r)	66	55	75	56
Influx (I)	57	69	59	59
Fractional efflux (k_{21})	65	55	80	55
Fractional metabolic flux (k_{22})	83	55	46	56
"Intracellular pool size" (I/r)	86	127	79	105

The kinetic quantities listed are presented as per cent of the corresponding control values.

FIGURE 5 Effect of L-methionine on accumulation of L-histidine-imidazole-2- ^{14}C inside nucleated dog bone marrow cells in the absence (Gey's BSS, experiment 1) and presence (BME, experiment 2) of other "essential" amino acids in the medium. Most "essential" amino acids present in the BME were neutral amino acids which had an aggregate concentration of 3.1 mmoles/liter. Cell number and histidine concentration and specific activity differed between the two separate experiments, and ordinate values are not comparable between the two components of the figure.

accumulation of His- $^{14}\text{C}_1$ (0.052 or 0.102 mmole/liter in Gey's BSS) in the absence and presence of these hormones was analyzed according to the compartmental model presented in Fig. 1. Neither insulin (0.1 U/ml) in three experiments nor glucagon (1 $\mu\text{g}/\text{ml}$) in two experiments had substantial effect on the calculated model parameters and corresponding fluxes of histidine.

His- $^{14}\text{C}_1$ has been in use for labeling monocarbon (C_1) pool as a tool in differential diagnosis of megaloblastic anemia (16). However, only a small fraction of administered His- $^{14}\text{C}_1$ is metabolized to FIGLU- $^{14}\text{C}_1$ and thus useful for labeling the C_1 pool. To explore the possibility of substituting FIGLU- $^{14}\text{C}_1$ for His- $^{14}\text{C}_1$ as a $^{14}\text{C}_1$ source, specific activity of DNA thymine isolated from cells incubated in Gey's BSS with FIGLU- $^{14}\text{C}_1$ was determined. Similar experiments were performed with His- $^{14}\text{C}_1$, which enters the cell as histidine but is metabolized intracellularly to FIGLU- $^{14}\text{C}_1$. Table IV shows that the DNA thymine specific activity in experiments with His- $^{14}\text{C}_1$ was approximately four times as great as that in experiments with FIGLU- $^{14}\text{C}_1$. Data in Table V suggest that the differences in DNA thymine specific activity were related to marked differences in cell membrane transport of these two substances. After 30 min incubation, the intracellular concentration of FIGLU arising from the extracellular FIGLU (0.075 mmole/liter) was calculated to be only 0.010 mmole/liter. Similar experiments with α -N-formylglutamic acid (FOGLU) produced similar results. Cellular uptake of His- $^{14}\text{C}_1$ was over 100 times as great as that of FIGLU- $^{14}\text{C}_1$.

DISCUSSION

The satisfactory fit of equation 1 to the cellular radioactivity data supports the validity of the compartmental model used. If a significant amount of radioactivity is

TABLE IV
Incorporation of ^{14}C from L-Histidine-Imidazole-2- ^{14}C (His- $^{14}\text{C}_1$) and α -N-Formimino-L-Glutamate-Formimino- $^{14}\text{C}_1$ (FIGLU- $^{14}\text{C}_1$) into DNA Thymine in Dog Bone Marrow Cells in the Presence and Absence of Methotrexate

	Exp. 1		Exp. 2	
Incubation time, hr.	1		2	
Concentration of methotrexate when present, moles/liter. . .	10^{-4}		10^{-6}	
	His- $^{14}\text{C}_1$	FIGLU- $^{14}\text{C}_1$	His- $^{14}\text{C}_1$	FIGLU- $^{14}\text{C}_1$
Concentration, mmoles/liter.	1	1	0.075	1
Specific activity (SA), $\mu\text{Ci}/\mu\text{mole}$	1.67	1.67	1.67	1.67
SA of DNA thymine, $\text{dpm}/\mu\text{mole}$				
Without methotrexate	444	119	81	16
With methotrexate	bgd*	bgd*	49	9

* Sample counting rate not significantly different from the background counting rate.

translocated in the cell from the labeled amino acid to those substances that leave the cell at significant rates, the model would be a poor approximation to reality. The present study has not excluded such a possibility. Further, different kinds of marrow nucleated cells might have different fractional rates for a given amino acid flux; thus, the present results represent average values for the mixed nucleated cell population of dog bone marrow. Furthermore, if a significant quantity of amino acids becomes "fixed" intracellularly without first equilibrating in the intracellular free amino acid pools (7, 8), much of the calculations performed in this study would be invalid.

The results of fractionation of cellular radioactivity by TCA extraction (Fig. 2) indicate the following: (a) The intracellular histidine of compartment II is acid soluble. (b) During the period of incubation, the rate of possible intracellular recycling of labeled histidine from proteins to compartment II is negligible compared to its influx from the extracellular pool.

In radiorespirometric studies, the appearance of $^{14}\text{CO}_2$ in the breath after the administration of ^{14}C -labeled amino acids has been used as a measure of in vivo metabolic processes (16). It is clear that such measurements are influenced by a variety of "nonmetabolic" processes, such as transport of the labeled amino acid

TABLE V
Radioactivity Uptake by 4.4×10^7 Dog Bone Marrow Nucleated Cells Incubated in Gey's BSS with L-Histidine-Imidazole-2- ^{14}C ($\text{His-}^{14}\text{C}_1$) or α -N-Formimino-L-Glutamate-Formimino- ^{14}C ($\text{FIGLU-}^{14}\text{C}_1$) or α -N-Formyl-L-Glutamate-Formyl- ^{14}C ($\text{FOGLU-}^{14}\text{C}_1$)

	$\text{His-}^{14}\text{C}_1$	$\text{FIGLU-}^{14}\text{C}_1$	$\text{FOGLU-}^{14}\text{C}_1$
Concentration, mmole/liter	0.075	0.075	0.075
Specific activity, dpm/ μmole	5.0	5.4	5.4
Radioactivity uptake (dpm) *			
At 1 min	4,420	40	20
5 min	16,400	30	50
15 min	35,500	260	280
30 min	48,600	320	330

across cell membranes and turnover of the bicarbonate pool containing the ^{14}C label on carbon dioxide (17). The magnitude of turnover time of the intracellular amino acid pool measured in the present in vitro study suggests that the rate of equilibration between the extracellular and intracellular pools of an amino acid- ^{14}C constitutes a significant "delay" in the production of labeled metabolites, such as $^{14}\text{CO}_2$.

The fit shown in Fig. 4 supports the notion that histidine entry into nucleated dog bone marrow cells

FIGURE 6 Accumulation of radioactivity inside 3.0×10^7 nucleated dog bone marrow cells incubated in BME with both L-histidine-imidazole-2,5- ^3H at 0.054 mM (SA 42 dpm/ μmole) and L-serine-3- ^{14}C at 0.024 mM (SA 20 dpm/ μmole) in the presence and absence of 3.20 mM L-methionine. The measured cellular activity of ^3H is shown as open (control) and closed (3.20 mM L-methionine) circles and that of ^{14}C as triangles (control) and boxes (3.20 mM L-methionine). The best fit form of equation 1 to each set of data is represented by the corresponding curves.

can be described as a combination of an apparent first order process and a facilitated process (18) describable by Michaelis-Menten kinetics. Similar behavior has been observed in human erythrocytes (19), rat diaphragms (20), guinea pig lymph node cells (21), and Ehrlich ascites tumor cells (22) for certain other neutral amino acids. The apparent diffusion constant for histidine entry expressed as k_a/W relative to the same frame of reference as that for k_m is 0.30 min^{-1} . Calculated k_m of histidine shown in Tables I and II are upper "limits" of its simple diffusion constant and are all smaller than 0.30 min^{-1} . This suggests that the apparent first-order component of histidine influx shown in Fig. 4 does not represent a single simple diffusion process but rather a summation of simple diffusion and one or more facilitated processes with high K_m values.

In this study, histidine exodus appeared to have a substantial susceptibility to analogue inhibition (Tables I and III) but appeared to exhibit an apparent first-order kinetics (Table II). Such findings suggest that a substantial component of histidine exodus in dog bone marrow cells involves facilitation associated with high K_m values (23).

The calculated concentration ratios (intracellular/extracellular) of FIGLU and FOGLU after 30 min of incubation are both much smaller than unity indicating either one or both of the following possibilities: (a) At the concentrations used, their net entry is so slow that their intracellular concentrations can not closely approach steady-state concentrations during the experiment. (b) Their steady-state intracellular concentrations are far below their extracellular concentrations.

At neutral pH, glutamine and glutamate- γ -methyl ester (both virtually devoid of net charges) exhibit evidences of active absorptive transport across intestinal walls whereas glutamate (with net negative charges) fails to do so (24). Judged by such findings of others (24), the poor uptake of FIGLU and FOGLU in the present experiments may be related to their net charges at neutral pH (roughly a single net negative charge on FIGLU in accordance with its pK_a values [25] and two net negative charges on FOGLU).

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