THE NATURE OF THE AMINO ACID POOL USED FOR PROTEIN SYNTHESIS IN RAT BRAIN SLICES

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Abstract—The incorporation into brain slice protein of externally provided [$1\text{-}^{14}\text{C}$]valine was measured at varying levels of valine in the medium, under conditions of constant protein synthesis and equilibration of intracellular valine specific activity. The results indicate that the valine pool used for protein synthesis is not identical to the pool of total free valine. Neither does the incorporation solely occur from an extracellular pool which is in equilibrium with the incubation medium. The data are compatible with a two-site activation model in which aminoacylation of tRNA occurs at both an internal site utilizing amino acid from the intracellular pool and an external (possibly membranous) site converting extracellular valine directly to valyl-tRNA. A good fit to the experimental observations is also provided by a compartmented intracellular valine pool model.

IN STUDIES on protein synthesis or breakdown in brain slices, it is of obvious importance to identify the amino acid pool that serves as the precursor for proteins. Only little information is available on the nature of this precursor pool, and its location within the tissue is still an unsettled question (JONES & McILWAIN, 1971; DUNLOP *et al.*, 1974). In contrast, many studies have been carried out using a variety of peripheral mammalian tissues. In fact, several lines of evidence suggest that the precursor pool for protein synthesis might be distinct from the total intracellular pool of free amino acids in rat skeletal muscle preparations (KIPNIS *et al.*, 1961; HIDER *et al.*, 1971;), pancreas fragments (VAN VENROOY *et al.*, 1972), liver slices (GUIDOTTI *et al.*, 1964), perfused liver (MORTIMORE *et al.*, 1972), cultured hepatoma cells (HOD & HERSHKO, 1976) and liver *in vivo* (VIDRICH *et al.*, 1977). Yet, other data suggest that the internal amino acid pool serves as the source of amino acids used for protein synthesis in rat skeletal muscle preparations (LI *et al.*, 1973), perfused heart (MORGAN *et al.*, 1971; MOWBRAY & LAST, 1974) and liver as well as other tissues *in vivo* (LOFTFIELD & HARRIS, 1956; FERN & GARLICK, 1974; SETA *et al.*, 1973). These apparently conflicting data may be explained by recently proposed models in which both intracellular and extracellular amino acid pools have been implicated (HOD & HERSKO, 1976; VIDRICH *et al.*, 1977).

In the present study we measured the incorporation into brain slice protein of externally provided [$1\text{-}^{14}\text{C}$]valine at varying levels of valine in the incubation medium, under conditions of constant protein synthesis. Steady-state equilibration of the specific activity of intracellular valine was established, the plateau level of which varied with the concentration of valine in the medium. The results indicate that the valine pool used for protein synthesis is not identical to the pool of total free valine.

MATERIALS AND METHODS

^{14}C -Labelled amino acids. The amounts of L-[$1\text{-}^{14}\text{C}$]valine (NEN, Boston, MA, 57.8 mCi/mmol) used per incubation were 0.2, 0.4, 0.6 and 2.4 μCi at 0.01, 0.04, 0.1 and 1.0 mM concentration of L-valine in the medium respectively.

Lysine incorporation was measured by addition of 0.4 μCi L-[$U\text{-}^{14}\text{C}$]lysine (Radiochemical Centre, Amersham, England, 336 mCi/mmol) per incubation at 0.04 mM concentration of L-lysine in the medium.

Slice preparation and incubation procedure. Male albino rats of an inbred Wistar strain (TNO, Zeist, The Netherlands), weighing 120–140 g, were decapitated and the brain was rapidly removed. The brain cortex was sliced on a McIlwain tissue chopper to 0.3 mm thickness and was placed in incubation medium (30 ml per cortex of one brain), which was gassed with O_2 at 37°C. The medium concentrations after adjustment to pH 7.4 at 20°C (DUNLOP *et al.*, 1975a) were: Na^+ , 118 mM; K^+ , 7.4 mM; Mg^{2+} , 1.3 mM; Ca^{2+} , 2.6 mM; Cl^- , 127.6 mM; SO_4^{2-} , 1.3 mM; PO_4^{3-} , 1.5 mM; glucose, 12 mM; and HEPES (*N*-2-hydroxyethyl piperazine *N'*-2-ethane sulphonic acid), 25 mM. The incubation medium contained various levels of unlabelled L-valine (0.01, 0.04, 0.1 and 1.0 mM) or, in one experiment, L-lysine, as indicated in the Figures and Tables. After separation of the tissue into individual slices, the medium con-

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Abbreviations used: SSA, sulphasalicylic acid; HEPES *N*-2-hydroxyethyl piperazine *N'*-2-ethane sulphonic acid.

taining the slices was divided over the incubation flasks (10 flasks per cortex of one brain) resulting in approx 50 mg cortex tissue per incubation flask, as checked later on by protein determinations. Subsequently, each flask was supplemented with 2 ml fresh medium (composition, see above) after withdrawal of the preincubation medium, and the incubation was continued under continuous gassing with O_2 at $37^\circ C$ in a Gyrotory water bath shaker (New Brunswick). The 15 min involved in this procedure was equivalent to a preincubation. Unless otherwise specified the procedure for each flask was as follows. The radioactive amino acid was added at time zero. After 20, 40 or 60 min 1 ml medium was withdrawn for amino acid analysis and the incorporation was stopped at the same moment by addition of 5 ml ice-cold standard medium (incubation medium without added L-valine or L-lysine).

Separation of the extracellular from the intracellular precursor pool. After the incubation had been stopped, slices were subjected to a short cold-wash procedure according to LIPTON & HEIMBACH (1977), in order to remove extracellular amino acid. The stop-medium was withdrawn, the slices were washed briefly with 5 ml ice-cold standard medium and subsequently shaken with a fresh 5 ml portion of ice-cold standard medium for 8 min in a bath with ice-water. Next, the slices were washed briefly with another 5 ml and transferred to tubes with 2 ml 3% (w/v) sulphosalicylic acid (SSA) and stored at $-80^\circ C$ for later analysis of intracellular radioactivity.

This procedure was checked in an experiment, where slices were incubated with $0.2 \mu Ci$ L[4,5- 3H]leucine (The

Radiochemical Centre, Amersham, England; 50 Ci/mm) in a final concentration of 0.04 mM and with [^{14}C -carboxy]-inulin (The Radiochemical Centre, Amersham, England; 7.7 mCi/mmol) in a final concentration of 0.02% (w/v) simultaneously at 37° for 30 min. Figure 1 shows the amount of radioactivity remaining in the tissue upon the subsequent cold-washes expressed as percentage of the initial radioactivity. As reported for guinea-pig hippocampal slices (LIPTON & HEIMBACH, 1977), a short washout period appeared to be suitable to remove the extracellular portion of exogenously added labelled amino acid while preserving the intracellular amino acid. From the data in Fig. 1 it may be calculated that an 8 min wash removes 89% of the total [^{14}C]inulin extractable at $0^\circ C$ (first inulin space, COHEN *et al.*, 1968), suggesting that the slices are efficiently freed of extracellular contamination, without producing an appreciable loss of intracellular [3H]leucine (ca 6% from the non-inulin space). Similar results were obtained using [$1-^{14}C$]valine at a final concentration of 0.04 mM. Therefore, cold-wash intervals of 8 min were routinely used.

Tissue fractionation. Tissue samples were processed as described before (REITH *et al.*, 1978). Briefly, the slices from one incubation were homogenized in 2 ml 3% (w/v) SSA and centrifuged. In the incorporation experiments with valine, the supernatant was assayed for the content and specific activity of valine by amino acid analysis. The pellet was washed three times with 5% (w/v) trichloroacetic acid (TCA) and assayed for radioactivity. Radioactivity measurements were carried out using xylene/triton scintillation mixtures; the results were expressed as disintegrations per min after correction for quenching by external standardization (SCHOTMAN *et al.*, 1977; REITH *et al.*, 1978). The protein content of the homogenate was quantified by the method of LOWRY *et al.* (1951). In a separate experiment, the TCA-insoluble material was treated subsequently with hot TCA, chloroform; methanol (1:1, v/v) and ether (REITH *et al.*, 1978). In samples from incubations at 0.01, 0.04, 0.1 and 1.0 mM concentration of valine, the hot TCA step and lipid extraction released only 8–12% of the cold TCA-insoluble radioactivity suggesting that the bulk of the acid-insoluble radioactivity resided in protein.

Amino acid analysis. The SSA extracts and incubation media were analysed for valine with an automated amino acid analyser system (TSM; Technicon) with split stream, using fluorescence detection with *o*-phthalaldehyde (SERVA, Heidelberg) and 2-mercaptoethanol (ROTH & HAMPAL, 1973). The appropriate fractions from the analyzer were collected and assayed for radioactivity. [$1-^{14}C$]Norleucine (NEN) was diluted with [^{12}C]norleucine and added to all fractions as an internal standard (SCHOTMAN, in prep).

FIG. 1. Wash-out of [^{14}C]insulin and [3H]leucine from brain slices. Slices were incubated with [^{14}C]inulin, 0.02% (w/v) and [3H]leucine, 0.04 mM at $37^\circ C$ for 30 min. They were then either removed and gently blotted for measurement of initial radioactivity, or cold-washed during various time intervals. The tissue was homogenized in ice-cold incubation medium and analyzed for radioactivity and protein. The data are plotted as the % of the original radioactivity remaining in the tissue at each time interval, $\circ$ — $\circ$ 3H leucine, $\bullet$ — $\bullet$ ^{14}C inulin. The shaded areas represent respectively the calculated loss of intracellular [3H]leucine from the non-inulin space, and [^{14}C]inulin trapped in the second inulin space, inextractable at $0^\circ C$ (COHEN *et al.*, 1968). Each point is the mean of 2 or 3 incubations.

RESULTS

Steady state conditions of protein synthesis in brain slices incubated at different concentrations of valine

The experiments described in this paper are aimed at measuring the incorporation of valine into protein at various specific activities of intracellular [^{14}C] valine. These conditions were obtained by varying the concentration of valine in the incubation medium; slices were incubated in medium containing 0.01, 0.04, 0.1 and 1.0 mM-L-valine. It was important to establish

TABLE 1. INFLUENCE OF THE CONCENTRATION OF VALINE IN THE MEDIUM ON THE INCORPORATION RATE OF L-[U- 14 C]-LYSINE INTO PROTEIN OF BRAIN SLICES

Valine concentration in medium (mM)	Incorporation (d.p.m./min/mg protein)
0.01	$8.3 \pm 0.5^*$
0.04	7.6 ± 0.5
0.1	8.4 ± 0.4
1	7.4 ± 0.4

* Mean $\pm$ S.E.M. for 5 incubations.

† 0.04 mM concentration of L-lysine in the medium.

No significant difference between the groups were found using Student's *t*-test with a 5% limit of confidence.

that under the various experimental conditions used, the rate of protein synthesis remained equal. For that purpose, the incorporation of L-[U- 14 C]lysine was measured over a period of 40 min at various concentrations of L-valine in the medium ranging from 0.01 to 1.0 mM. Lysine was chosen as precursor since lysine and valine are likely to utilize different transport sites at their primary site of entry into the cells (BLASBERG & LAJTHA, 1965). As shown in Table 1, there were no significant differences in incorporation between the various groups, suggesting that protein synthesis rates are essentially constant within the range of external valine concentrations used.

Steady state conditions of isotope equilibration in brain slices incubated at different valine concentrations

In order to compare the incorporation into protein of [14 C]valine with the respective intracellular specific activity, it is essential that the specific activity

of intracellular valine is at a steady state condition. As shown in Fig. 2, at an externally provided L-valine concentration of 0.01 mM, the intracellular to medium specific activity (IC/EC ratio) was equilibrated at least 20 min after addition of radioactive valine to the medium, and remained constant from 20 to 60 min. Since similar values of the specific activity of valine in the medium were found at 20, 40 and 60 min, equilibration of the IC/EC ratio within 20 min implicates that the intracellular specific activity was equilibrated within 20 min. The incorporation into protein was linear in the 20–60 min interval. Also at 0.4, 0.1 and 1.0 mM concentrations of L-valine in the medium, linear incorporation curves were found in this time interval.

Fig. 3 shows the time courses of the equilibration of intracellular [14 C]valine specific activities at externally provided L-valine concentrations of 0.01, 0.04, 0.1 and 1.0 mM in the medium. In all cases, the specific activities of valine in the medium remained constant from 20 to 40 min. A plateau level of intracellular valine specific activity was reached at all valine concentrations; within 20 min the lowest equilibration level was found at 0.01 mM-valine. This phenomenon, also described for other systems, has been suggested

FIG. 2. Time-course of the equilibration of the specific activity of intracellular [14 C]valine and its incorporation into protein at an externally applied concentration of L-valine of 0.01 mM. ●—● ratio of specific activity of intracellular valine (IC) to that of valine in the medium (EC). ○—○ incorporation of [14 C]valine into protein. The specific activity of valine in the medium remained constant during the incubation periods. Values are mean $\pm$ S.E.M. of 6 incubations. The actual levels of valine in the medium measured were 0.015, 0.014 and 0.016 mM at 20, 40 and 60 min respectively.

FIG. 3. Time-course of the equilibration of intracellular specific activity of [14 C]valine at various concentrations of valine in the medium. See Fig. 2 for details. In all cases, the specific activities of valine in the medium remained constant up to 40 min after the start of the incubation.

FIG. 4. Changes in intracellular free valine (nmol/mg protein) with increasing concentrations of valine (mM) in the medium. Following incubation for 40 min, the pool size of intracellular free-valine was determined as described under Experimental Procedures. Values are mean $\pm$ S.E.M. of 6 incubations.

to be due to the constant production of unlabelled amino acid by protein breakdown (MORTIMORE *et al.*, 1972; LI *et al.*, 1973; HOD & HERSHKO, 1976). The IC/EC ratio at equilibrium was ca 0.5 and 0.7 at 0.01 and 0.04 mM-valine respectively. The intracellular specific activity approached the medium specific activity at 0.1 and 1.0 mM-valine, the small difference in steady state IC/EC ratio between 0.1 and 1.0 mM-valine not being statistically significant. The influence of varying external valine concentrations on the IC/EC ratio is related to the effects on inward transport and the pool size of intracellular valine. Figure 4 shows that there is indeed a large increase in intracellular free valine at high concentrations of valine in the medium.

Comparison of incorporation of [14 C]valine into protein with its intracellular specific activity

Figure 5 (upper panel) shows the incorporation of

FIG. 5. Relationship between the incorporation of [14 C]valine into protein and its intracellular specific activity. L[1- 14 C]valine was added in amounts of 0.2, 0.4, 0.6 and 2.4 μ Ci per incubation at 0.01, 0.04, 0.1 and 1.0 mM-valine concentration respectively. All data were normalized to a specific activity of 19 d.p.m./pmol valine in the medium corresponding to the specific activity at 0.01 mM external valine. This was done by multiplying the results by the ratio of the specific activity of medium valine at 0.01 mM to that observed at the actual concentration. Upper panel, $\bullet$ — $\bullet$ the incorporation of [14 C]valine into protein, as determined from the slope of incorporation between 20 and 40 min. $\circ$ — $\circ$ Specific activity of intracellular valine, being the mean of the specific activity at 20 and 40 min (see also Figs. 2 and 3). Lower panel, incorporated radioactivity divided by intracellular specific activity (IC s.a.). Values are means $\pm$ S.E.M. calculated from 6 incubations at each time point and valine concentration.

[^{14}C]valine into protein and the intracellular valine specific activity under steady state conditions as defined above at varying concentrations of valine in the medium. Both the incorporation and intracellular specific activity increased from 0.01 to 0.04 mM, reaching a plateau at 0.1 mM. However, the two curves do differ; for example, at 0.01 mM medium valine concentration, the intracellular specific activity was about 48% of the maximal equilibration, whereas the incorporation was around 69% of the plateau level. Figure 5 (lower panel) shows the rates of valine incorporation based on total intracellular specific activity. These rates are high at the low external valine concentrations and decline at high valine concentrations to a constant level.

DISCUSSION

Measurement of intracellular and medium amino acid specific activities

A commonly used procedure to distinguish extracellular from intracellular amino acids consists of washing the tissue at low temperature. This has been employed for rat liver slices (GUIDOTTI *et al.*, 1964), diaphragm (KOSTYO, 1968), muscle (HIDER *et al.*, 1971) and pancreas (VAN VENROOY *et al.*, 1972). However, less data are available on cold-washing of brain slices (DUNLOP *et al.*, 1974; LIPTON & HEIMBACH, 1977). The data presented in Fig. 1 confirm earlier observations in our laboratory (REITH, 1975) indicating that the cold-wash procedure can be a useful technique for brain slices provided that the incubation at low temperature is restricted to short intervals. The wash-out curve found for [^3H]leucine from rat brain slices is very similar to the curve reported for [^{14}C]lysine from hippocampal slices of guinea pig (LIPTON & HEIMBACH, 1977). The errors in our data due to residual extracellular amino acid after an 8 min cold-wash and the small loss of intracellular amino acid were estimated to be 3% or less. Furthermore, the cold-wash procedure circumvents the problem inherent to calculations of intracellular specific activities by subtracting amino acid in the extracellular space: the assumption that extracellular specific activity is equivalent to that in the medium. The validity of this assumption has been contested for incubations with low concentrations of labelled amino acids in the medium, during which the specific activity within the extracellular space may be many fold lower than that in the medium by the passage of nonradioactive amino acid from protein breakdown (LI *et al.*, 1973). In that case, the specific activity in the extracellular space would be overestimated resulting in an underestimation of the intracellular specific activity.

Effect of varying valine levels on lysine incorporation

Although valine as a neutral amino acid and lysine as a large basic amino acid most likely utilize different primary transport sites in brain slices, there can be considerable overlap between the various amino acids

and in fact it has been shown that, in mouse brain slices, the accumulation of large basic amino acids at medium concentrations of 2 mM is inhibited to some extent by external concentrations of 10 mM of virtually all neutral amino acids (BLASBERG & LAJTHA, 1965). In the lysine incorporation experiment presented in Table 1 the medium concentrations were lower: 0.04 mM-lysine and 0.01 to 1.0 mM-valine. Under these conditions there were no significant differences between the acid-soluble or totally recovered ^{14}C -radioactivities of brain slices incubated at 0.01, 0.04, 0.1 and 1.0 mM external valine, suggesting that the uptake of labelled lysine is similar at all valine concentrations. From the lysine incorporation data it is therefore concluded that valine levels up to 1 mM do not influence the actual rate of protein synthesis in this brain slice system. Similar results have been reported by DUNLOP *et al.* (1974), and there are indications that also *in vivo* high valine levels do not inhibit protein synthesis rates at least during the first 2 h after gross injection of valine (DUNLOP *et al.*, 1975b; REITH *et al.*, 1978).

Location of the valine pool used for protein synthesis

A discrepancy was observed between the incorporation of valine into protein and its intracellular specific activity at lower external valine concentrations (Fig. 5, upper panel), under conditions of constant protein synthesis (Table 1) and steady-state levels of intracellular specific activities (Figs. 2 and 3). Calculation of incorporation rates based on total intracellular specific activities results in upward deviations at low external valine concentrations (Fig. 5, lower panel); similar findings have been reported for other systems (MORTIMORE *et al.*, 1972; HOD & HERSHKO, 1976). The data shown in Fig. 5 indicate that the total intracellular free valine pool is not identical to the precursor pool for protein synthesis. If it were, the calculated incorporation rates, accordingly, would have remained constant at all concentrations of medium valine. The data also indicate that the incorporation does not occur from an extracellular pool which is in equilibrium with the medium, since, in that case, the observed incorporation rates would have been similar at all valine concentrations.

For peripheral tissues both internal and external amino acids have been suggested to contribute to the precursor pool for protein synthesis (HOD & HERSHKO, 1976; VIDRICH *et al.*, 1977). An intracellular nonexpandable pool has been postulated in perfused liver (MORTIMORE *et al.*, 1972) and in brain slices (NEIDLE *et al.*, 1975). We applied these models on the data obtained in our brain slice system and compared the predicted values of various parameters with the values actually measured (Tables 2, 3 and 4).

In the model proposed by HOD & HERSHKO (1976) for hepatoma cells, there is only one pool of intracellular free valine, but there are two sites of aminoacylation of tRNA, an internal site utilizing valine from the intracellular pool, and an external (possibly mem-

TABLE 2. OBSERVED VALUES OF a_s/a_i COMPARED WITH VALUES CALCULATED ACCORDING TO THE MODEL PROPOSED BY HOD & HERSHKO (1976)*

Valine concentration in medium	Observed a_i/a_e	Observed a_s/a_i	Calculated a_s/a_i					
			$R_e/(R_e + R_i)$					
			0.1	0.3	0.4	0.5	0.7	0.9
0.01	0.46	1.48	1.12	1.35	1.47	1.59	1.82	2.06
0.4	0.68	1.21	1.05	1.14	1.19	1.24	1.33	1.42

* a_s/a_i was calculated from their equation:

$$\frac{a_s}{a_i} = \frac{R_e \cdot (a_e/a_i) + R_i}{R_e + R_i}$$

See text for definition of the symbols used.

branous) site converting extracellular valine directly to valyl-tRNA. The mixing of valyl-tRNA originating from both sources is very rapid, and this mixed pool is the immediate precursor for protein synthesis. Depending on the relative values of the rates of formation valyl-tRNA from the external site (R_e) and internal site (R_i) the model predicts a relationship between the ratio of intracellular to extracellular valine specific activity (a_i/a_e) and the ratio of tRNA-bound to free, intracellular valine specific activity (a_s/a_i). Table 2 presents the values of a_s/a_i calculated for varying ratios of $R_e/(R_e + R_i)$ using the equations of HOD & HERSHKO (1976) for our data. The observed values of a_s/a_i , which can be computed by dividing calculated incorporation on basis of intracellular specific activity by the calculated incorporation at high (0.1 and 1.0 mM) valine concentrations, are in close agreement with the values calculated according to the model at $R_e/(R_e + R_i) = 0.4$, i.e. the rates of formation of valyl-tRNA from the external and internal site being approximately equal. Such a model appears feasible based on the present knowledge of aminoacyl-tRNA synthetases from eukaryotic cells. Synthetases have been found to be present both in a soluble form (TSCHERNE *et al.*, 1973; TANAKA *et al.*, 1976) and particulate form, as part of ribosomal (ROBERTS & COLEMAN, 1972; TSCHERNE *et al.*, 1973;

TANAKA *et al.*, 1976) and non-ribosomal (BANDYOPADHYAY & DEUTSCHER, 1971) complexes.

Internal and external valine pools are also implicated in the model proposed by VIDRICH *et al.*, (1977) for liver. However, according to their theory, the transmembrane concentration gradient determines the specific activity of tRNA-bound valine. Table 3 presents the values of a_s calculated using the equation of VIDRICH *et al.*, (1977) for our data, and the observed values of a_i at the various external concentrations of valine. The calculated values of a_s/a_i approached unity at all external valine concentrations. This is sharp contrast with the observed values of a_s/a_i which indicate that at low external concentrations of valine the specific activity of the precursor pool is higher than the measured intracellular specific activity. It thus seems that our data do not support the predictions of this concentration gradient model.

MORTIMORE *et al.* (1972) proposed for liver an intracellular, nonexpandable pool (S_p) which does not equilibrate significantly with the expandable precursor pool for protein synthesis. S_p would receive amino acids from proteolysis and would be inaccessible to amino acids entering the cell from the medium. Sequestered amino acid pools in brain slices, receiving amino acids from protein breakdown, have been also

TABLE 3. OBSERVED VALUES OF a_s/a_i COMPARED WITH VALUES CALCULATED ACCORDING TO THE MODEL PROPOSED BY VIDRICH *et al.* (1977)*

Valine concentration in medium (mM)	Observed		Calculated	
	a_i	a_s/a_i	a_s	a_s/a_i
0.01	8.8	1.48	9.6	1.09
0.04	13.3	1.21	14.0	1.05
0.1	19.9	0.97	19.7	0.99
1	17.9	1.02	18.2	1.02

* a_s and a_s/a_i were calculated from their equation:

$$a_s = a_e \left(\frac{[e]}{[i] + [e]} \right) + a_i \left(\frac{[i]}{[i] + [e]} \right),$$

in which $[i]$ and $[e]$ denote the concentration of internal and external valine respectively; see text for definition of other symbols.

TABLE 4. CALCULATED INCORPORATION ASSUMING A NON-EXPANDABLE POOL OF INTRACELLULAR VALINE*

Valine concentration in medium (mM)	Calculated incorporation (pmol/min/mg protein)	
	S_p (nmol/mg protein)†	
	0.26	0.30
0.01	1.23 ± 0.20‡	1.15 ± 0.19
0.04	1.20 ± 0.22	1.09 ± 0.20
0.1	0.94 ± 0.11	0.91 ± 0.11
1	1.20 ± 0.20	1.20 ± 0.20

* Incorporation rates were calculated on basis of the specific activity of valine in the expandable pool (total intracellular pool minus nonexpandable pool S_p).

† Nonexpandable pool.

‡ Mean ± S.E.M. calculated from data of Figs. 4 and 5.

suggested by NEIDLE *et al.* (1975). These authors estimated a sequestered valine pool of 32 nmol per g tissue, corresponding to ca 0.3 nmol per mg protein. Table 4 presents incorporation rates of valine into protein calculated from our data accounting for an S_p of 0.26 and 0.30 nmol/mg protein respectively. The errors in the final figures are computed by taking into account the errors in the measured incorporations, intracellular specific activities and pool sizes. The results are compatible with the possibility that the precursor pool for protein synthesis is identical to the expandable intracellular amino acid pool (total intracellular pool minus S_p) since the calculated incorporation rates are essentially similar at all valine concentrations.

As pointed out above, the present data are compatible with both the two-site activation model of HOD & HERSHKO (1976) and the compartmented intracellular pool model of MORTIMORE *et al.* (1972) and KHAIRALLAH & MORTIMORE (1976). However, on kinetic data alone it is difficult to distinguish between the two models as pointed out by MCKEE *et al.* (1978). Both models appear to be feasible: two-site activation of tRNA is supported by the localization of aminoacyl-tRNA synthetases (see above) and recently WARD & MORTIMORE (1978) presented evidence for an intralysosomal location of the nonequilibrating valine pool in liver. However, since brain is a much more complex tissue than is liver, additional factors may have to be taken into account to explain our results with brain slices. Amino acids may be unequally distributed between different cell types, such as neurons and glia or even within the same cell if rapid equilibration between nerve endings and perikarya does not take place. Whatever the mechanism for this compartmentation, experiments on protein synthesis in brain slices could lead to erroneous conclusions if calculations are based on the assumption of a single uniform precursor pool. These errors can be minimized if relatively high levels of amino acids are present in the incubation medium.

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