The rate of protein degradation in developing brain

Methodological considerations

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Recently we reported that the rate of protein breakdown decreases during development. Breakdown rates were calculated from the rates of protein synthesis and the changes in brain protein content with age. A different study, measuring breakdown by monitoring the loss of label from brain protein after an $\text{H}^{14}\text{CO}_3^-$ pulse, came to the opposite conclusion: that the rate of breakdown is low in immature brain and increases during development. We have now investigated some of the factors (the distribution of label in protein and the potential for recycling) that might introduce errors into these measurements. The specific radioactivities of both protein-bound and free amino acids were determined in the brains of young rats several days after an intraperitoneal pulse of $\text{H}^{14}\text{CO}_3^-$. For a number of amino acids the specific radioactivity of the free amino acid is high compared with that of the protein-bound amino acid, and therefore recycling could result in an underestimate of the degradation rate. Because glutamic acid had a relatively low specific-radioactivity ratio, $[1\text{-}^{14}\text{C}]\text{glutamic acid}$ was used in a pulse-labelling experiment to measure degradation. The rate so obtained, $0.6\% \cdot \text{h}^{-1}$, is twice the rate found with $\text{H}^{14}\text{CO}_3^-$ labelling (based on total protein-bound radioactivity). Insofar as recycling is a possible complication, $0.6\% \cdot \text{h}^{-1}$ may be a minimum value. Although somewhat higher degradation rates are found after labelling with an intracranial pulse, which was considered as a possible route to limit recycling, there are difficulties in interpreting these data.

The brain grows rapidly in rats in the first weeks after birth. It is clear that the ratio of the protein synthesis rate to the protein degradation rate in developing brain must be higher than the ratio in adult brain, which grows only slowly. Both synthesis and degradation rates might be different compared with rates in the mature brain, to achieve growth. Most authors agree that there is a much higher rate of protein synthesis in the brains of young animals compared with adult brain (Johnson, 1976). There are only a few studies on rates of protein breakdown in brain during development. Two different methods have been used in efforts to determine the degradation rate in growing brain, and the results are not in agreement.

The first procedure used to determine the degradation rate (Dunlop *et al.*, 1978) in young rats is based on measuring the protein synthesis rate and the rate at which protein accumulates in the brain, and calculating the degradation rate as the difference between these two rates (synthesis rate—growth rate = degradation rate). For example, between 2

and 4 days of age, the rate of brain protein synthesis in the cerebral hemisphere of the rat is found to be about $1.9\% \cdot \text{h}^{-1}$ provided that the animal's body temperature is 38°C . The rate at which protein accumulates during the same period, however, is only $0.6\% \cdot \text{h}^{-1}$, so that if transport of protein out of the brain is quantitatively unimportant, the rate of degradation must be substantial ($1.3\% \cdot \text{h}^{-1}$) (Dunlop *et al.*, 1978). From similar data the rate of degradation in adult brain is found to be considerably lower ($0.65\% \cdot \text{h}^{-1}$) and, since growth is relatively slow, is practically equal to the synthesis rate. More recent studies by the same approach also indicate higher degradation rates in both mouse and rat brain (Banay-Schwartz *et al.*, 1979; Lajtha *et al.*, 1979) in the first few weeks after birth.

The other method used to measure protein degradation rates in growing brain is based on monitoring the decay of label in the brain protein after pulse labelling (Dahl & Weibel, 1979). Garlick *et al.* (1976) demonstrated that the average degradation rate of mixed proteins can be determined by

monitoring the decline with time in protein radioactivity after a pulse label. One prerequisite for accurate determinations by this method is that the label should exhibit a low degree of recycling. It has been shown that glutamic acid and aspartic acid labelled in the carboxy group via the administration of $\text{NaH}^{14}\text{CO}_3$ meet this requirement in adult rat liver (Garlick *et al.*, 1976) and brain (Chee & Dahl, 1978). With a variation of this procedure, using total protein-bound radioactivity rather than that in aspartate and glutamate, a very low rate of protein degradation was found in brain when the pulse was administered to 5-day-old rats and the decay monitored over the next 12 days. The rate may be estimated from the data in that study (Dahl & Weibel, 1979) to be of the order of $0.3\% \cdot \text{h}^{-1}$, about half the rate seen in adult rat brain and one-quarter of the rate calculated from synthesis- and growth-rate data for 5-day-old rats.

In an attempt to clarify this situation we have pulse-labelled the brain protein of 5-day-old rats with bicarbonate and estimated the possible degree of recycling at some later periods. A degradation rate has also been measured by using $[1-^{14}\text{C}]$ glutamic acid as label. Finally we thought if possible that an intracranial pulse might concentrate the label to such an extent in the brain as to decrease substantially recycling from tissues outside the brain, and we have obtained some decay rates after labelling by this route.

Methods

Intraperitoneal $\text{H}^{14}\text{CO}_3^-$ labelling

Wistar rats (5 days old) were given intraperitoneal injections of 2.6 ml of $\text{NaH}^{14}\text{CO}_3/100\text{ g}$ body wt. (all labelled compounds were obtained from New England Nuclear, Boston, MA, U.S.A.). The $\text{H}^{14}\text{CO}_3^-$ solution was prepared by dissolving 3.98 mCi of $\text{NaH}^{14}\text{CO}_3$ (sp. radioactivity 6.3 Ci/mol) in 2 ml of 0.9% NaCl, pH 7.4. The rats were returned to the mother's cage, which was enclosed in plastic. Air was passed slowly through the enclosure and into a basic solution of BaCl_2 to trap CO_2 for disposal. At 2 days after the injection, the rats were killed by decapitation. Each brain was homogenized in 2 ml of 3% sulphosalicylic acid. The homogenate was centrifuged at 7000g for 10 min and the supernatant removed and frozen for amino acid analysis. The pellet was resuspended in 3% sulphosalicylic acid and frozen. Later those samples were thawed and poured on to Whatman GF/B glass-fibre filters on a Millipore manifold, where the residue was rinsed with 3% sulphosalicylic acid. The vacuum was turned off and 5 ml of 5% trichloroacetic acid was added to the sample well. The mixture was left for 5 min, and then the vacuum was applied and the solvent drawn through the filter. This

procedure was repeated with 8 ml each of methanol, chloroform/methanol (1:1, v/v) and diethyl ether. At this point the approx. 20 mg of dry, flaky residue, which is largely protein, was weighed and placed in a tube with 3 ml of constant-boiling 6 M-HCl. The tube was sealed under vacuum and heated at 106°C for 24 h. After hydrolysis, the HCl was removed by evaporation in an air stream. The residue was dissolved in water and subjected to amino acid analysis. The effluent of the ion-exchange column was split so that part was used to determine the quantity of amino acid in the sample by fluorimetry with *o*-phthaldialdehyde and part used for liquid-scintillation counting as previously described (Patterson & Greene, 1965). The counting efficiency was 95%. In this way the specific radioactivity of each amino acid is determined. Samples of liver protein were treated in exactly the same manner. The extracts of free amino acids were desalted before amino acid analysis. This was done with a small (2 cm) column of Bio-Rad AG 50W (X8) ion-exchange resin. Samples of blood were collected from the neck wound at the time of decapitation, but the amounts of radioactivity in these plasma samples were below detectable limits, so that specific radioactivities could not be determined.

Intracranial injections

For these injections a 50 μl syringe was used with a Hamilton mechanical plunger advance. The needle of the syringe was connected by a piece of polyethylene tubing to a length of 30-gauge needle, which was used for puncturing the brains of young rats. In each case the volume injected was 3 μl at a depth of 3.1 mm from the surface. The animals were then returned to the cage with the rest of the litter and the mother for an appropriate period. The rat was then decapitated and the brain removed and divided into right and left cerebral hemispheres and cerebellum. A sample of liver was also taken. Since the total radioactivity is required, it was necessary to process the entire cerebral hemispheres, which in older rats had to be divided into two or three separate pieces. The tissue was either chopped to a thickness of 0.25 mm and shaken with 5% trichloroacetic acid or homogenized in 5% trichloroacetic acid, and the resulting suspension was centrifuged. In either event the solid material was extracted three times with trichloroacetic acid to remove acid-soluble radioactivity. The solids were then separated from the trichloroacetic acid by passing the mixture through a Whatman GF/B glass-fibre filter. The filter and the solid material were transferred to a scintillation vial with 2 ml of 1 M-NaOH. After heating to digest the trichloroacetic acid-insoluble material, 0.5 ml of acetic acid was added to acidify the sample, and finally 5 ml of

water and 8 ml of scintillation fluid (Patterson & Greene, 1965). The trichloroacetic acid-soluble extracts were collected, evaporated to dryness and similarly subjected to scintillation spectrophotometry. When the acid-soluble radioactivity became an insignificant fraction of the protein-bound radioactivity, the extraction was omitted and the tissue was digested and counted for radioactivity directly.

Intraperitoneal [1^{14}C]glutamic acid labelling

Wistar rats (5 days old) were given an intraperitoneal injection of [1^{14}C]glutamic acid (sp. radioactivity 50 Ci/mol) dissolved in 0.9% NaCl. The dose was $0.2 \mu\text{Ci/g}$ in a volume equal to 2% of the body weight ($2.8 \mu\text{Ci}$ for a 14 g rat). As discussed elsewhere, this dose results in rather low radioactivity in the brain 12 days later and might profitably be substantially increased in any similar experiments. Four rats were decapitated at 4 h, 6 days and 12 days after the pulse. Each brain was removed, cut into left and right cerebral hemispheres, chopped to a thickness of 0.47 mm on a Mickle tissue chopper and placed in a weighed scintillation vial containing 4 ml of 3% sulphosalicylic acid. The slices were shaken for 15 min and the sulphosalicylic acid was removed. Similarly the slices were then extracted twice with 5% trichloroacetic acid, once with methanol, twice with chloroform/methanol (1:1, v/v) and finally with 8 ml of diethyl ether. After drying in air, the protein from the right cerebral hemisphere was dissolved in 2 ml of 1 M-NaOH by heating in boiling water. When the sample was almost dry, 0.5 ml of acetic acid was added followed by 5 ml of water and 8 ml of scintillator. Liver protein was similarly treated. The protein from the left cerebral hemispheres was weighed and transferred to Pyrex tubes for hydrolysis in constant-boiling 6 M-HCl for 24 h under vacuum at 110°C . The HCl was removed by evaporation and the hydrolysates were separated into individual amino acids as described above. The radioactivity was determined for each peak.

Results

To obtain some indication of the possible degree of recycling of label in the brain protein of young rats after an intraperitoneal pulse of $\text{NaH}^{14}\text{CO}_3$, we measured the concentration and radioactivity content of various amino acids in the free pool and in protein at an arbitrary time of 2 days after the injection of 5-day-old rats. When the distribution of radioactivity in protein is compared with similar data (1 day after injection) obtained by others (Swick & Ip, 1974; Chee & Dahl, 1978), it appears that there is considerably less label in glutamate and aspartate in the brain proteins of the young rats and relatively more in serine, glycine and alanine (Table

1). There are also differences in the pattern seen in young and adult liver but these are smaller than those in brain.

From specific-radioactivity data (Table 2) we see that the specific-radioactivity ratios (the specific radioactivity of the free amino acid divided by the specific radioactivity of the protein-bound amino acid) for brain and liver in young rats are generally similar and that, whereas the ratios for aspartate, glutamate, alanine and arginine are of the order of 10–30%, the ratios of the specific radioactivity of the free amino acid to that of the protein-bound are considerably higher for glycine (liver and brain) and serine (in brain). These data suggest that the potential for recycling of all the highly labelled amino acids in brain and liver of young rats is substantial, and particularly so for serine and glycine, which account for a considerably larger portion of the protein-bound label in young compared with adult brain.

That the specific-radioactivity ratios in brain and liver (Table 2) are, with the exception of serine, fairly similar suggests that the equilibrium effect of exchange of tissue free amino acid with that in plasma is not overwhelming. For example, the specific radioactivity of aspartate in liver protein is considerably higher than that in brain, so one might expect that the rapid exchange of amino acid

Table 1. *Distribution of label among amino acids in hydrolysates of brain protein 2 days after $\text{NaH}^{14}\text{CO}_3$ labelling of young rats compared with values reported for adults*

For this experiment 5-day-old rats were given an intraperitoneal pulse of $\text{NaH}^{14}\text{CO}_3$ and killed 2 days later. Hydrolysates of protein from brain and liver were separated into individual amino acids by ion-exchange chromatography. Results are means $\pm$ S.D. or ranges. Data cited for adult rats were obtained at 1 day rather than 2 days after labelling.

	Radioactivity (%)			
	Brain protein	Liver protein	Adult brain protein [results of Chee & Dahl (1978)]	Adult liver protein [results of Swick & Ip (1974)]
Asp	9.5 ± 1.9	$5 \ 15.3 \pm 1.0$	75	25–30
Glu	19.7 ± 2.9	$5 \ 17.8 \pm 1.4$		
Ser	9.5 ± 2.0	$5 \ 4.5 \pm 0.5$	*	
Gly	12.6 ± 3.1	$5 \ 5.8 \pm 0.4$	4	
Ala	14.9 ± 3.6	$5 \ 7.0 \pm 0.9$	15	
Arg	24.3 ± 2.4	$3 \ 21.9 \pm 0.1$	2	25
Total	90.5	72.3	100*	75–85

* With 10% reported in Ser + Pro + Arg.

Table 2. *Specific radioactivities of free and protein-bound amino acids in brain and liver 2 days after a pulse of $\text{NaH}^{14}\text{CO}_3$ was administered to 5-day-old rats*

The ratio of total radioactivity per mg of protein in brain to liver was 0.69. Glutamine and asparagine are converted in the hydrolysates into glutamate and aspartate respectively. In each case four or more determinations were made with the following exceptions. Two measurements were made for all arginine values. With the extracts of brain free amino acids, a peak of radioactivity emerges from the ion-exchange column immediately before aspartate. In only one sample was this contaminant sufficiently separated from aspartate so that the specific radioactivity could be measured. The means ($\pm$ S.D.) of the specific radioactivities are indicated. Numbers in parentheses indicate the value for free/protein-bound amino acid specific radioactivity $\times 100$.

	Specific radioactivity (d.p.m./ μmol)					
	Brain		Liver		Brain/liver	
	Free	Protein-bound	Free	Protein-bound	Free	Protein-bound
Asp	164	748 $\pm$ 130 (22)	456 $\pm$ 79	2097 $\pm$ 303 (22)	0.36	0.36
Ser	791 $\pm$ 116	1070 $\pm$ 137 (73)	301 $\pm$ 40	864 $\pm$ 141 (36)	2.6	1.2
Ala	365 $\pm$ 21	1701 $\pm$ 89 (21)	386 $\pm$ 63	1256 $\pm$ 73 (31)	0.94	1.35
Glu	195 $\pm$ 33	1270 $\pm$ 121 (16)	227 $\pm$ 22	2015 $\pm$ 268 (11)	0.86	0.63
Gly	1077 $\pm$ 282	1761 $\pm$ 270 (61)	584 $\pm$ 156	897 $\pm$ 76 (65)	1.8	2.0
Arg	—	4570 $\pm$ 905	2045 $\pm$ 375	8486 $\pm$ 735 (24)	—	0.53

between plasma and the tissues would tend to depress the specific radioactivity of free aspartate in liver, whereas that in brain would be elevated. Yet the specific radioactivity of free aspartate in liver is, like protein-bound aspartate, also well above the value in brain. The difference in specific-radioactivity ratios in brain versus liver for glutamate, alanine and glycine could be interpreted as a slight levelling effect. In general, however, it appears that the specific radioactivity of the free amino acid is largely determined by the specific radioactivity of that amino acid in the local proteins, at least in this early period after labelling.

The relative amounts of label found in the different amino acids of brain protein change with time (Table 3) in directions that would be appropriate if the free/protein-bound amino acid specific-radioactivity ratio (Table 2) is an indication of the degree of recycling. That is, aspartate and glutamate have relatively low specific-radioactivity ratios compared with those for glycine and serine and should be subject to a lower degree of recycling. Thus, with the passage of time, the percentage label in aspartate and glutamate should decline, whereas the percentage label in protein-bound serine and glycine should increase.

Decay after intracranial injection of label

We wanted to determine whether intracranial labelling would, by concentrating the label in the brain, lessen recycling and yield a higher degradation rate. Fig. 1 shows the decay of radioactivity with time after intracerebral injection of young rats with $\text{NaH}^{14}\text{CO}_3$ or with $[1-^{14}\text{C}]$ glutamic acid. The data have been normalized by setting the total

Table 3. *Distribution of radioactivity in brain protein 4 h, 2 days and 5 days after labelling 5-day-old rats with $\text{NaH}^{14}\text{CO}_3$*

Rats received an intraperitoneal injection of $\text{H}^{14}\text{CO}_3^-$. At various times thereafter samples of brain protein were prepared and hydrolysed. The radioactivity in each sample was separated into component amino acids by ion-exchange chromatography. Two samples were analysed at each point. Results are means $\pm$ S.D.

Time after injection	Proportion of total protein bound radioactivity (%)		
	... 4 h	2 days	5 days
Asp	8.2 $\pm$ 0.9	8.6 $\pm$ 2.2	6.0 $\pm$ 1.1
Glu	22.3 $\pm$ 0.3	17.3 $\pm$ 0.8	14.5 $\pm$ 0.1
Glu + Asp	30.5	25.9	20.5
Ser	7.4 $\pm$ 0.8	7.2 $\pm$ 0.4	8.6 $\pm$ 0.7
Gly	10.2 $\pm$ 1.2	11.7 $\pm$ 0.8	14.8 $\pm$ 1.3
Ser + Gly	17.6	18.9	23.4
Ala	18.4 $\pm$ 1.2	16.8 $\pm$ 0.1	14.9 $\pm$ 0.7
Total (%)	66.5	61.6	58.8

radioactivity present at the first time point to an arbitrary value. For reference, the decay in brain protein radioactivity in 5-day-old (Dahl & Weibel, 1979) and adult (Chee & Dahl, 1978) rats receiving an intraperitoneal pulse of $\text{NaH}^{14}\text{CO}_3$ is indicated. The decay of radioactivity in brain protein proceeds at much higher rates after intracranial injection of labelled leucine (results not shown), glutamate or $\text{H}^{14}\text{CO}_3^-$ than after intraperitoneal $\text{H}^{14}\text{CO}_3^-$ injection. The decay rates can be calculated from such

Fig. 1. Decay of radioactivity in brain after pulse labelling

For details see Table 5. Symbols: ●, intraperitoneal injection of $[1-^{14}\text{C}]$ glutamate, 5-day-old rats, radioactivity in total protein, first point at 4 h after injection; ○, same as ●, except radioactivity in glutamic acid only; ▲, intracranial injection of $[1-^{14}\text{C}]$ glutamate, 5-day-old rats, radioactivity in total protein, first point at 4 h; ■, intracranial injection of $\text{NaH}^{14}\text{CO}_3$, 5-day-old rats, radioactivity in total protein, first point at 2 h; ----, intraperitoneal injection of bicarbonate, 5-day-old rats, radioactivity in total protein, first point at 4 h (Dahl & Weibel, 1979); - · - · -, intraperitoneal injection of bicarbonate, adult rats, first point at 4 h (Chee & Dahl, 1978). For comparison, the curves have been normalized so that the protein-bound radioactivity in the brain at the first point is 1260 d.p.m.

curves, even though they are not linear in a semi-logarithmic plot, by determining the area under the curve and the maximum extent of labelling (Garlick *et al.*, 1976). The degradation rate so obtained, by weighing cutouts of photocopies of the curves, is shown in Table 4.

Intraperitoneal $[1-^{14}\text{C}]$ glutamate labelling

The data presented in Table 2 related to intraperitoneal $\text{H}^{14}\text{CO}_3^-$ pulse-labelling suggest that a more accurate rate measurement might be obtained if the radioactivity in glutamic acid were considered rather than total protein-bound radioactivity. However, since a number of other amino acids are highly labelled by $\text{H}^{14}\text{CO}_3^-$ and these might contribute

modest amounts of radioactivity to glutamate, and since it was conceivable that the use of glutamate as label might result in much of the radioactivity being concentrated in protein-bound glutamate, obviating the requirement for isolation of that amino acid, we used $[1-^{14}\text{C}]$ glutamic acid to label the protein in 5-day-old rats.

When the decay of label in brain protein is determined at 4 h, 6 and 12 days after intraperitoneal administration of $[1-^{14}\text{C}]$ glutamate a non-linear plot is observed on semi-logarithmic paper (Fig. 1). As a rough estimate we calculated the degradation rate as if the protein behaved as a single species and obtained rates of roughly $0.47\% \cdot \text{h}^{-1}$ between 4 h and 6 days and $0.31\% \cdot \text{h}^{-1}$ between 6 and 12 days. The overall rate may be estimated as $0.38\% \cdot \text{h}^{-1}$ from 4 until 12 days. This value is slightly higher than that seen after an $\text{H}^{14}\text{CO}_3^-$ pulse ($0.30\% \cdot \text{h}^{-1}$).

However, when the radioactivity in glutamic acid is measured we find that the percentage of the total declines with time. Even at 4 h only 60% of the protein-bound radioactivity is in glutamic acid (Table 5). When these data are plotted (also in Fig. 1) a much higher degradation rate is evident. Again the plot is non-linear, but a rough approximation of the rate is $0.61\% \cdot \text{h}^{-1}$ between 4 h and 12 days after injection.

Discussion

The rate of protein degradation in young brain has been measured by two different procedures, which give markedly different results. The methods involved do in fact measure two somewhat different parameters, so the results need not be equivalent. When the degradation rate is calculated from the synthesis and growth rates measured within 1–2 days of 5 days of age, the rate should apply at that age. On the other hand, when the pulse-labelling procedure is used it is necessary to monitor the decay curve for many days under conditions that do not meet the criteria that the proteins be in a steady state (Garlick *et al.*, 1976), since the brain is growing rapidly during this interval. Since the rapidly degraded proteins are lost, the more slowly metabolized species would be subject to the changing conditions, and it would thus be difficult to predict what value might be expected from this approach, but we suggest it should be intermediate between the values observed by the mass-action method at the initial and final ages involved in the measurement. The value observed by Dahl & Weibel (1979), however, is well below that reported in adult brain and the data obtained by these two methods are thus not compatible.

As stated previously, the weakest point in the synthesis/growth measurements of the degradation

Table 4. Average protein degradation rate measured after intracranial or intraperitoneal pulse labelling of young rats compared with that obtained by other procedures

Unless indicated otherwise, results are from the present work.

Method	Age (days)	Rate (% · h ⁻¹)	Reference
Intraperitoneal			
[1- ¹⁴ C]Glutamate (total-protein d.p.m.)	5-17	0.4*	
[1- ¹⁴ C]Glutamate (d.p.m. in Glu)	5-17	0.6*	
Intracranial pulse (using total protein d.p.m.)			
[1- ¹⁴ C]Glutamate	5-26	0.74†	
H ¹⁴ CO ₃ ⁻	5-17	0.8†	
[U- ¹⁴ C]Leucine	3-18	0.5-0.6†	
[1- ¹⁴ C]Leucine	3-21	0.5-0.6†	
Intraperitoneal H ¹⁴ CO ₃ ⁻ (total protein d.p.m.)	5-17	0.3*	Dahl & Weibel (1979)
Synthesis rate minus growth rate	5	1.3	Dunlop <i>et al.</i> (1978)
	1-2‡	0.74	Lajtha <i>et al.</i> (1979)
	1-2‡	0.84‡	Lajtha <i>et al.</i> (1979)
Synthesis = degradation	Adult	0.65	Dunlop <i>et al.</i> (1977)
Intraperitoneal	Adult	0.55†	Chee & Dahl (1978)
H ¹⁴ CO ₃ ⁻ (d.p.m. in acidic amino acids)			

* These rates are approximated from the equation $\ln P_0/P = k_d t$, where P_0 is the total protein-bound radioactivity in the brain at the initial point (4 h after injection) and P is the radioactivity at the last point measured (12 days after injection). Since none of these curves is linear in a semi-logarithmic plot, these values are approximations.

† The rate is calculated from the initial radioactivity (4 h after injection) divided by the integral of the decay curve (plotted on regular graph paper, not semi-logarithmic paper). The integral of the curve was determined by cutting and weighing photocopies of the curve. Strictly speaking the curve should be extrapolated to zero radioactivity, or an estimate made at a time when the tissue radioactivity reaches 10% of the initial value (Garlick *et al.*, 1976). Only the curves for intracranial [1-¹⁴C]glutamic acid and for H¹⁴CO₃⁻ in adult rats (Chee & Dahl, 1978) can be extended to zero radioactivity with confidence.

‡ Newborn mice in this case.

Table 5. Distribution of radioactivity in brain protein after [1-¹⁴C]glutamate labelling by intraperitoneal injection of 5-day-old rats

The total radioactivity in whole brain at 4 h, 6 days and 12 days was 2289 ± 308, 1232 ± 76 and 843 ± 44 d.p.m. The counting efficiency was 95%. The numbers of samples counted are shown in parentheses.

Time after pulse	Radioactivity (%)		
	4 h	5 days	12 days
Asp	6.2 (2) ±0.4	—	—
Glu	60.2 (4) ±7.2	36.0 (1)	29.2 (2) ±4.0
Gly	9.2 (2) ±0.8	—	16.5 (1)
Ala	6.8 (2) ±0.8	—	—
Arg	15.0 (3) ±5.0	29.3 (4) ±2.3	30.9 (2) ±2.9

rate is probably the assumption that the female is able to maintain the pups' body temperature, and hence the protein synthesis rate, at a constant value throughout the day. We have no additional data on

this question, but note that we have found the protein degradation rate in slices of young brain to be similar to that calculated by the mass-balance approach *in vivo* and to show similar variations with age and brain region (Dunlop *et al.*, 1981). This correspondence could, of course, be a fortuitous artefact.

In a study in which the synthesis rate was measured over a period of 30 h the degradation rate measured was 0.74% · h⁻¹ in newborn rats and 0.84% · h⁻¹ in newborn mice. In this case the pups were maintained with the female in a cage at 31°C. However, the protein synthesis rate observed in this experiment was significantly lower than that seen in pups whose brain temperature is stabilized at 37-38°C. It is therefore possible that the rates observed (both synthesis and degradation) may be low. In any event these data would presumably give a minimum degradation rate.

Some of the assumptions involved in the pulse-labelling procedure are more easily examined. The H¹⁴CO₃⁻ pulse labelling procedure applied previously to young brain is a variation of the usual approach in that one ordinarily isolates the label in glutamic acid and aspartic acid. The reason for using the label in total brain protein in the latter study rather than that in the acidic amino acids was that in

adult brain fully 75% of the label was recovered in these two amino acids (Chee & Dahl, 1978) and the degradation rate in adult brain was similar calculated on the basis of either total label or label in glutamate and aspartate. It is assumed that the distribution of label is similar and that the degree of recycling of this label is also low in young brain, as it must be in adult brain, since the apparent degradation rate therein is similar to that calculated by other methods (i.e. it is nearly equal to the synthesis rate; growth is negligible).

However, as shown in Table 1, we find a markedly different distribution of label among the protein-bound amino acids in young brain 2 days after labelling. There is a smaller portion of the label in glutamate and aspartate and relatively more in the other amino acids.

To obtain some estimate of the possible effect of recycling on this rate measurement, we determined the specific radioactivities of free and protein-bound amino acids in brain 2 days after administering $\text{NaH}^{14}\text{CO}_3$. Although there is much discussion about the precise source of amino acids for protein synthesis, and whether extracellular or intracellular free amino acids may be preferentially used for protein synthesis in different tissues, the data available on brain would suggest that the specific radioactivity of the intracellular pool is likely to be close to that of the precursor for protein. Certainly if the specific radioactivities of the free amino acids are very low, compared with those for the protein, recycling is unlikely to be a major problem. However, as seen in Table 2, the specific radioactivities of most of the highly labelled free amino acids are of the order of magnitude as the specific radioactivities of the same amino acids in brain protein. Recycling may therefore significantly lower the decay rate measured with an $\text{H}^{14}\text{CO}_3^-$ pulse in young brain.

The problem of high specific-radioactivity ratios for free and protein-bound amino acids is compounded by the different distribution of label in the protein of young brain (Table 1). Chee & Dahl (1978) found 75% of the label in adult brain protein at 1 day after injection in glutamate plus aspartate, 15% in alanine and another 10% scattered between proline, serine and arginine. We found in young rats only 30% of the label in glutamate plus aspartate; 60% of the label was found in serine, glycine, alanine and arginine, and of these, serine and glycine appear likely to be subject to very heavy re-incorporation. That this may be the case is shown in Table 3, where the distribution of label in protein is shown 4 h, 2 days and 5 days after an $\text{H}^{14}\text{CO}_3^-$ pulse. The fraction of label in aspartate and glutamate is declining with time, whereas that in glycine and serine is increasing.

We note that, whatever the degree of recycling, its

contribution in a growing tissue is greatly amplified. The rat brain at 5 days of age contains approx. 31 mg of protein, whereas 12 days later the value is over 100 mg, so that a minimum of 69% of the final protein was synthesized during the study.

We considered using intracranial administration of a labelled protein precursor as an alternative to intraperitoneal administration on the grounds that this route would concentrate the label in brain protein and hence the 'external' recycling would be lessened.

Dunn (1975) found lower incorporation of labelled amino acid administered by intraperitoneal pulse into adult mouse brain protein after an intracranial puncture. We have confirmed that observation and show that this effect is indeed due to an inhibition of brain protein synthesis. More pertinent to the experiments described here is the finding that intracranial puncture does not inhibit protein synthesis in young rat brain.

Some representative values of the degradation rate measured after intracranial injection are shown in Table 4. The degradation rate in the uninjected cerebral hemisphere is equivalent to that in the injected hemisphere for animals receiving $[1-^{14}\text{C}]$ -glutamate or $\text{NaH}^{14}\text{CO}_3$ intracranially, suggesting that for these precursors any damage done to the cells in the immediate vicinity of the injection site is not a factor in the rate measurement. It is noteworthy that the decay rate for the glutamate curve between 14 and 21 days after injection is $0.29\% \cdot \text{h}^{-1}$, which is essentially the rate found by Lajtha *et al.* (1976) for the 'slow component' of adult brain protein.

In a single experiment (results not shown) in which adult (25-day-old) rats received an intracranial pulse of $[\text{U}-^{14}\text{C}]$ leucine the aggregate decay rate was roughly $0.5\% \cdot \text{h}^{-1}$. However, between 7 and 15 days after labelling the rate was considerably less than $0.3\% \cdot \text{h}^{-1}$, indicating that either recycling of this precursor becomes a complication at later times or that the route of administration is labelling a different population of proteins.

The question of whether a different population of proteins may be labelled by an intracranial pulse cannot be answered here. Jakoubek & Tykva (1978) reported a different distribution of label, studied by autoradiography, after intraventricular injections of leucine compared with the intraperitoneal route. Cosgrove & Brown (1981) found the distribution of label among brain proteins separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis to be similar when synthesis in a cell-free system was compared with protein labelled by intracranial injection of precursor. We also note that, even though intraperitoneal or intravenous administration introduces the label to the brain through the physiological distribution channel of blood vessels, this does not necessarily ensure an even

distribution of label for all proteins. For example, since neurons and glial cells have different average protein synthesis rates, the intracellular specific radioactivity of free amino acid, compared with plasma values, should differ in inverse proportion to the rate of synthesis.

In any event the degradation rate may be calculated for the protein exposed to the intracranial pulse and is found to be $0.6\% \cdot h^{-1}$ for leucine and $0.74\% \cdot h^{-1}$ for $[1-^{14}C]$ glutamate. The value of $0.74\% \cdot h^{-1}$ is presumably the lower limit, since, even if external recycling were eliminated, internal re-incorporation would still occur. And as mentioned above, since the proteins in growing brain are not in equilibrium, it may not be possible to measure the average degradation rate by pulse labelling at all in the sense that that value is changing with age and, even with an ideal non-recycling precursor, the value measured over 10 or 15 days should represent some sort of average of the rates during the interval.

Though many of the problems associated with intracranial labelling apply to intraperitoneal pulse-labelling, we sought to obtain a minimum rate measurement by using this more accepted route of administration. Because of the low specific-radioactivity ratio seen in Table 2 for glutamate, and in view of the rapid decay seen after an intracranial pulse of glutamate, we tried $[1-^{14}C]$ glutamic acid as precursor.

From the intraperitoneal $[1-^{14}C]$ glutamate labelling experiment we cannot calculate an accurate degradation rate, since only three points were determined and the radioactivity in glutamate at 12 days after injection is greater than 10% of the initial value (Garlick *et al.*, 1976). However, from the 4 h and 12-day values we obtain a rough estimate of $0.6\% \cdot h^{-1}$. Similarly, if the data are plotted on graph paper and the line between 6 and 12 days is arbitrarily extended to zero radioactivity, the K_d estimated from the initial value and the area under the curve (Garlick *et al.*, 1976) is again about $0.6\% \cdot h^{-1}$, twice the value found previously with an $H^{14}CO_3^-$ pulse. This value will be low if there is substantial recycling of glutamic acid, and we suggest the value is, in any event, an average of the values between 5 and 17 days of age.

Waterlow *et al.* (1978) have summarized much of the data on protein degradation rates in growing tissues. In muscle, rates are higher during development than in the adult, but this does not appear to be the case in liver.

We conclude that degradation of brain protein in young rats is proceeding at a rate at least as high as that for adult brain and, since recycling should contribute substantially more radioactivity to the tissue during rapid growth, rates may well be higher for young than for adult brain. In view of the theoretical difficulties in interpreting degradation rates measured by pulse labelling in a tissue that is not in equilibrium, we suggest that the method of calculating the rate of degradation from synthesis and growth rates, for all its shortcomings, may be the only practical procedure available for studies *in vivo* during development.

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