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The Turnover of Protein in Discrete Areas of Rat Brain

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1. Rats were injected serially with [^{14}C]glucose to obtain a constant specific radioactivity of brain amino acids. Measurements with this system for periods of up to 8h gave an apparent mean half-life for protein in whole brain of 85h (indicating the presence of a protein fraction with much more rapid turnover than this). 2. The half-lives of proteins in the granule-cell, molecular and white-matter layers of cerebellum were also determined. These had values of 33, 59 and 136h respectively. In addition, the incorporation into protein in six layers of the cerebral cortex, subjacent white matter and five layers of Ammon's horn was studied. All cell-body layers incorporated amino acids at about the same rate irrespective of location, and these rates were considerably higher than those for incorporation into proteins in areas rich in dendrites or fibre tracts. 3. A new method for measuring small amounts of glutamate with a cyclic enzyme system is presented.

Proteins turn over in brain as in other tissues and the average half-life for these proteins has been estimated as about 14 days (Lajtha, 1964; Piha *et al.*, 1966; Richter, 1959). A protein specific to the nervous system, S100, has a half-life of 16 days (Cicero & Moore, 1970).

Hungen *et al.* (1968) determined the half-lives of proteins of subcellular fractions from brain and found that these lie between 10 and 20 days. In general these determinations have been made on heterogeneous pieces of tissue, by measuring the rate of decay of labelled protein after either continuous feeding of labelled amino acid or pulse labelling into the circulation or into the brain. Protein that is rapidly turning over cannot be assessed accurately by these methods, nor is it possible to examine different cell types in small samples of the brain because of the relatively low specific radioactivity of the protein after it has been turning over for some time.

Protein turnover studies can be done in a system in which the labelled precursors are in a state of equilibrium. This situation is difficult to achieve with labelled amino acids because of problems of transport from the blood to the brain and the possible subsequent gradient of radioisotope throughout the brain. However, glucose is rapidly transported into brain cells and the intracellular glucose is converted to a large degree into amino acids, particularly glutamate, aspartate and alanine, and these amino acids are incorporated into brain proteins (Winzler *et al.*, 1952; Geiger *et al.*, 1960; Vrba, 1962; Vrba *et al.*, 1962; Shimada *et al.*, 1970; Yoshino & Elliott, 1970*a*,*b*).

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In the experiments reported here, labelled amino acids in the brain were kept under equilibrium conditions by repeated injections of labelled glucose. The distribution and turnover rate of proteins were determined within small areas of the tissue comprised of relatively homogeneous cell types.

Materials and Methods

Animals

Weanling Sprague–Dawley rats (50–70g) were obtained from A. R. Schmidt, Madison, Wis., U.S.A., and used shortly after purchase.

Reagents

Radioactive glucose. D-[U-^{14}C]Glucose, of specific radioactivity 190–210mCi/mmol, was purchased from International Chemical and Nuclear Corp., Irvine, Calif., U.S.A. Each batch was checked by paper chromatography and found to have only one radioactive component.

Chemicals and solvents. These were all of reagent-grade quality unless otherwise stated.

Methods

Protein-turnover experiments. Each animal was anaesthetized by intraperitoneal injection of a mixture of phenobarbital sodium and pentobarbital such that the respective doses were 100mg/kg and 25mg/kg.

Repetitive subcutaneous injections of [^{14}C]glucose were given; each injection contained either 120 or 5μCi (0.48μmol) of [^{14}C]glucose. Rats were injected

at times 0, 10, 20, 60, 90, 120, 150, 180, 210, 270 and 330min. The final injection was given 30min before the rat was killed.

Sampling. Blood samples were collected and the serum glucose concentrations determined as described by Nelson *et al.* (1968), by using 1 μl of serum. Samples (2 μl) of serum were added to scintillation fluid for counting. Rats were killed by decapitation and the heads were dropped immediately into Freon 12 (CCl_2F_2, DuPont) at −150°C.

Labelled intermediates in brain. Samples of brain (about 25mg of frontal cortex) were placed on 300 μl of a frozen solution of 0.3M-$HClO_4$ at −20°C. The temperature of the mixture was adjusted to −12°C in an ice–salt bath and the brain was ground with the viscous $HClO_4$ solution. After grinding, the contents of the tube were homogenized in an all-glass homogenizer.

The preparation was centrifuged, washed with about 0.1vol. of 0.3M-$HClO_4$ and a portion of the supernatant was neutralized with a calculated volume of 5M-$KHCO_3$. The precipitated $KClO_4$ was removed by centrifugation. Concentrations of the following intermediates in this acid-soluble fraction were measured enzymically: glucose (Nelson *et al.*, 1968), glutamate and alanine (Young & Lowry, 1966) and aspartate (Fleming & Lowry, 1966). Samples (5 μl) were chromatographed on Whatman no. 1 paper in a phenol–water (22:3, w/v) solvent system overnight. R_F values of intermediates identified were as follows: aspartate, 0.025; glutamate, 0.082; lactate, 0.179; glucose, 0.276; alanine, 0.484; glutamine, 0.480; γ-aminobutyrate, 0.745.

A Vanguard Autoscanner 880 strip scanner was used to locate the radioactive areas on the chromatographic strips. The R_F values of each were compared with those of spots obtained by running standard solutions of the above substances under identical conditions. Each radioactive spot was cut out from the paper and eluted with the buffer appropriate to the enzyme assay procedure. The concentration of glucose or amino acid was determined in each extract, together with the amount of radioactivity.

The location and homogeneity of intermediates on the chromatograms were checked by two-dimensional paper chromatography with butan-1-ol–acetic acid–water (12:3:5, by vol.) as the second developing solvent system. Alanine and glutamine were separated by this system with R_F values of 0.30 and 0.17 respectively. Radioautography was performed on X-ray film after exposure times of 3 days.

In some experiments the specific radioactivity of glutamate was determined after separation on an ion-exchange column. For this purpose miniature columns (2.5mm diam.) were used. They were packed with Dowex 1 (X12; 200–400 mesh; Cl^- form) to a bed volume of 40–50 μl and the resin was converted into the acetate form by washing successively with 5ml of 0.2M-NaOH, 5ml of water, 10ml of 0.2M-sodium acetate and 5ml of water. Each washing was repeated once. Then 10 μl of brain extract was added to the column of resin and allowed to drain into it. The column was washed with 800 μl of water to elute neutral and basic substances. Further elution was done with 0.5M-acetic acid and the first 50 μl was discarded. Glutamate was eluted quantitatively in the next 50 μl. A subsequent 200–300 μl sample contained the aspartate. The concentration and radioactivity of the glutamate was determined as described above.

Brain protein. The precipitated material remaining after $HClO_4$ extraction was prepared as described by Morgan & Austin (1968). The proteins were dissolved in 0.5ml of formamide with heating to 120°C for 2h. Samples were used for radioactivity counting and protein determination (Lowry *et al.*, 1951).

Glutamate content of rat brain protein. Two rat brains, each weighing about 1g, were homogenized in 10vol. of cold 0.3M-$HClO_4$ and the precipitated proteins treated as described above. These proteins were finally dissolved in 10ml of 1M-NaOH and the solutions were clarified by centrifugation. Each solution was diluted 1:10 with water and 150 μl of each was hydrolysed in 1.0ml of 6M-HCl at 110°C for 24h in sealed tubes. The hydrolysates were neutralized and the glutamate and aspartate concentrations were measured.

Radioactive brain protein was also hydrolysed and the glutamate and aspartate separated by ion-exchange chromatography on columns (0.5cm × 4.0cm) of Dowex 1 essentially as described above. The dicarboxylic acid eluates were counted for radioactivity by scintillation counting.

Micro-sample preparation. Samples of cell layers from cerebral cortex, Ammon's horn and cerebellum were dissected from freeze-dried tissue sections (Lowry, 1953).

Each sample was weighed on a quartz 'fish-pole' balance (0.5–5 μg) and then placed in a tube (50mm × 5mm). Acid-soluble components were extracted with 10 μl of 0.3M-$HClO_4$ at 0°C, and this was retained for chromatography. The samples were then washed with 25 μl of 0.3M-$HClO_4$ at 0°C, followed by 10 μl of 0.3M-trichloroacetic acid at 60°C for 1h to solubilize and remove glycogen and nucleic acids. Lipids were extracted by washing at least three times with approx. 250 μl of ethanol followed by hexane. The hexane was removed with a stream of air.

Acid-soluble extracts. Portions (8.7 μl) of the above acid extracts were spotted on Whatman no. 1 paper, together with 2 μl of a mixture containing 5mg of each of the following/ml; glucose, glutamate, aspartate, alanine and γ-aminobutyrate to act as carrier. These compounds were chosen as they were the most significantly labelled intermediates in the extracts.

To the spot on the filter paper was added 2 μl of 1.3M-$KHCO_3$ to neutralize the $HClO_4$. If this was not

done, bad streaking resulted. The chromatograms were developed with the phenol–water system overnight. After drying, each strip was lightly sprayed with ninhydrin and the relevant spots eluted, added to a 2.54cm (1in) stainless-steel planchet, dried and counted for radioactivity.

Protein radioactivity. The sections remaining after extraction were re-weighed to determine the protein content and placed on a 2.54cm (1in) stainless-steel planchet. Formic acid (2–5μl) was pipetted on to the section to dissolve and spread the protein. After drying each sample was counted for radioactivity.

Radioactivity measurements. All scintillation counting was done in an 'aqueous' scintillation fluid (Bruno & Christian, 1961) by using a Packard Tri-Carb scintillation spectrometer. Each sample was counted to an S.E.M. of ±2%. Samples were checked for quenching by addition of [^{14}C]benzoic acid standard (Packard). Efficiencies were all within the range of 53–58%.

Planchet samples were all counted for radioactivity at infinite thinness by using a Nuclear–Chicago gas-flow counter with a background of 1.5c.p.m. Each sample was counted to an S.E.M. of ±5% or better.

Glutamate estimations in micro-samples. Glutamate was measured in freeze-dried samples dissected from the several layers of cerebellum, cerebrum and Ammon's horn.

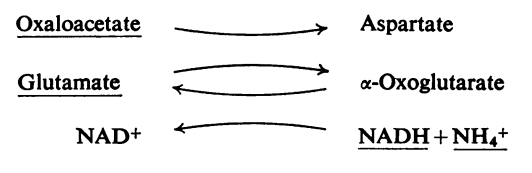
Because of the small sample size a new method was developed, which increases the sensitivity 25-fold in the present instance, but which is capable of much greater 'amplification'. The cyclic reaction, catalysed by glutamate–oxaloacetate transaminase and glutamate dehydrogenase, is shown in Scheme 1. The NAD^+ formed is measured fluorimetrically.

The reagent for the cyclic reaction has the following composition: 50mM-imidazole acetate buffer, pH6.7 (33mM-imidazole acetate, 17mM-imidazole base), 10mM-ammonium acetate, 0.02% bovine serum albumin, 0.2mM-ADP, 0.15mM-NADH, 0.2mM-oxaloacetate, glutamate–oxaloacetate transaminase (5μg/ml), glutamate dehydrogenase (5μg/ml). The oxaloacetate is added directly from a 200mM stock solution in 1M-HCl stored frozen (it does not keep except in strong acid). The transaminase as currently supplied contains added α-oxoglutarate, which must be removed. This is accomplished by centrifuging the $(NH_4)_2SO_4$ suspension with subsequent washing three times by resuspension and centrifugation, by using equal volumes of fresh 3M-$(NH_4)_2SO_4$. The enzyme is finally dissolved and stored in 50% glycerol containing 0.01M-imidazole buffer, pH7.

Each sample (average weight 0.5μg) is added to 10μl of 0.05M-NaOH in a tube (6mm×50mm) and heated for 2min at 100°C to inactivate the enzymes. (It is important to destroy the last traces of malate dehydrogenase and lactate dehydrogenase, otherwise NAD^+ will be formed from oxaloacetate or from the pyruvate formed by some oxaloacetate breakdown.) After heating, the entire 10μl sample is transferred to a fluorimeter tube (10mm×75mm) containing 50μl of the reagent for the cyclic reaction, which is kept cold in an ice bath. (The technique of total transfer is to draw the entire sample, not too rapidly, into a constriction pipette somewhat smaller than the volume concerned. At least 99% of the sample can be readily transferred with good precision. The original vessel should have stood quietly for a few minutes before transfer, to be sure all the fluid has drained off the upper walls.)

Blanks and standards are provided, the latter consisting of blanks to which have been added 1 and 2μl samples of 25μM-glutamate. The rack of tubes is incubated for 60min at 38°C, then chilled in ice and acidified with 10μl of 1M-HCl to destroy excess of NADH. Fluorescence is developed from the NAD^+ by adding 1ml of 6M-NaOH (with rapid mixing) and heating for 10min at 60°C (Lowry *et al.*, 1957). Readings were made in a Farrand (filter) fluorimeter.

Greater sensitivity, when needed, can be achieved by increasing the concentrations of the two enzymes and by incubating for longer periods.



Scheme 1. *Cyclic reaction catalysed by glutamate–oxaloacetate transaminase and glutamate dehydrogenase*

The initial substrates are underlined. After incubation the NAD^+ formed is measured fluorimetrically.

Results

Separation of labelled metabolites

Although many intermediates in the brain would be expected to have high specific radioactivities, only a few occur in concentrations high enough to contribute significantly to the total acid-soluble radioactivity. These are glucose, lactate, glutamate, glutamine, aspartate, alanine and γ-aminobutyrate.

To check whether the relevant spots were free from contaminating radioactive components, extracts were chromatographed in two dimensions by using the phenol–water solvent system first, followed by the butan-1-ol–acetic acid–water system in the second dimension. Radioautographs of these chromatograms showed only two components other than those listed above. One of these had an R_F value greater than that of γ-aminobutyrate in the phenol–water

system and therefore did not interfere with the components of interest. The other had the same R_F value as glutamate in that solvent system. It was, however, a very minor contaminant, which would have contributed less than 5% of the radioactivity in the glutamate spot.

Specific radioactivity of blood glucose

The injection schedule, based on preliminary experiments, was designed to maintain a plateau concentration of [^{14}C]glucose in the blood and hence a constant concentration of ^{14}C-labelled amino acids, particularly glutamate, in the brain. This was monitored by regular measurements of blood glucose specific radioactivity during the course of each experiment. As shown in Fig. 1, this procedure successfully maintained a relatively constant specific radioactivity of blood glucose for periods of up to 8h. From the observed values a mean value for blood glucose specific radioactivity was calculated in each experiment.

Specific radioactivity of blood glutamate

With the results obtained for the blood glucose specific radioactivity, it was possible to estimate the variation of brain glutamate radioactivity during the period of injection on the assumption that this would fluctuate with fluctuations in blood glucose radioactivity. This assumption is supported by the close correlation between blood glucose and brain glutamate specific radioactivities at the time of death (Table 1). Only in one early sample was there evidence of a substantial time lag. The results reported by Vrba (1962), Yoshino & Elliott (1970*a*) and many others also indicate rapid equilibration between blood glucose and brain glutamate. Mean brain glutamate specific radioactivities during each experiment were calculated, by using the values for the mean blood glucose specific radioactivities, the specific radioactivity of blood glucose at death (final) and the specific radioactivity of brain glutamate at death (final). The equation used was:

Mean sp. radioactivity of brain glutamate = Final sp. radioactivity of brain glutamate × (mean sp. radioactivity of blood glucose)/(final sp. radioactivity of blood glucose).

Glutamate content of rat brain protein

Brain protein hydrolysates contained 0.93mmol of glutamate and 0.49mmol of aspartate per g of protein. The glutamate value agrees with that reported by Yoshino & Elliott (1970*b*), of 0.90mmol/g; the aspartate concentration is somewhat lower than their value of 0.69mmol/g. Glutamate and aspartate were of equal specific radioactivity in the protein 2h after the initial injection. Approx. 80% of the radioactivity in the protein hydrolysate was found to be from glutamate and aspartate. Therefore 52% of brain protein radioactivity was contributed by the glutamate residues.

Specific radioactivity of brain protein

This was measured in proteins extracted from rat brains labelled in two experiments, one in which the dose of radioisotope was 120μCi per injection and

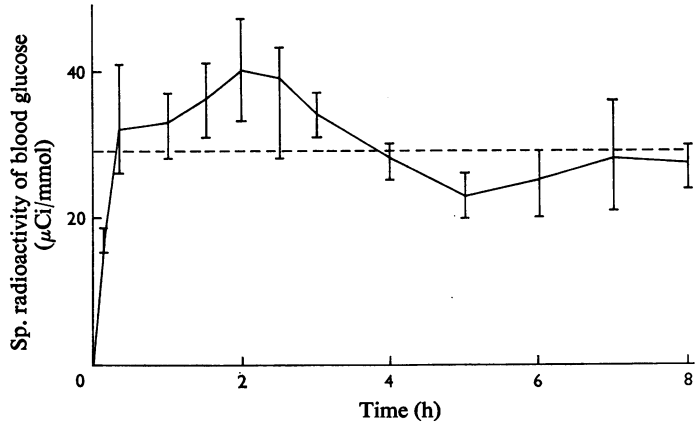


Fig. 1. *Specific radioactivity of blood glucose over an 8h period*

[U-^{14}C]Glucose (5μCi) was injected subcutaneously into three anaesthetized rats at each of the times corresponding to the points shown on the curve. Blood was withdrawn from the tail at the same time and the specific radioactivity of the blood glucose determined. Each value is the mean for the three rats, together with the range at each time-interval. The dashed line indicates the mean blood specific radioactivity for the 8h period.

Table 1. *Specific radioactivity of blood glucose and brain (frontal cortex) glutamate at death*

Rats were given multiple injections of [U-^{14}C]glucose subcutaneously as described in the Materials and Methods section. Each row of values represents the results with a separate animal.

Time (h)	Sp. radioactivity at death (mCi/mmol)		Brain glutamate/ blood glucose sp.-radioactivity ratio
	Blood glucose	Brain glutamate	
1.25	0.50	0.05	0.10
	0.67	0.23	0.34
2	1.53	0.51	0.33
	1.25	0.43	0.34
4	1.10	0.42	0.38
	1.41	0.36	0.25
6.5	0.39	0.125	0.32

the other 5μCi per injection (Table 2). The specific radioactivity of the protein glutamate was calculated from the total radioactivity of the protein on the basis of the results from the preceding paragraph. The ratio between specific radioactivities of protein glutamate and of free glutamate indicated the glutamate turnover and hence the protein turnover. There was close agreement between the two experiments, indicating a mean turnover half-time of about 85h. There was no significant difference in the rate of labelling from 1.25 to 8h.

Incorporation rates of ^{14}C-labelled amino acids into layers of cerebellar cortex

The incorporation of ^{14}C into glutamate and protein was measured in three layers of cerebellum, after labelling periods of 1.25, 2 and 4h. The free glutamate concentrations and the free glutamate specific radioactivities were very nearly the same in both granule-cell and molecular layers (Table 3). The free glutamate content of white matter, on the other hand, was considerably less, but the specific radioactivity tended to be higher than in the other layers.

The ratios of incorporation into protein were not uniform in that, as might be anticipated, the protein of the cell-body layer had a higher specific radioactivity than either the molecular layer, which is rich in dendritic processes, or the white matter, which is rich in fibres.

Yoshino & Elliott (1970*b*) found little variation in the glutamate content of protein from various brain regions, including white matter. In view of this, and the fact that each cell contains a mixture of thousands of proteins, we have assumed that the glutamate content of protein from all three layers of cerebellum is constant and equal to that of protein glutamate as determined for cerebral-cortex protein, i.e. 0.93mmol/g of protein. By using this value the specific radioactivity of protein glutamate was calculated in the same manner as for the large samples of Table 2. The relative specific radioactivity of protein glutamate and free glutamate gives a measure of the turnover during the labelling period.

Table 3 shows a turnover rate at the 4h labelling period, which decreases in the order granule-cell layer, molecular layer, white matter, with half-times ranging from 33 to 136h.

Incorporation rates of ^{14}C-labelled amino acids into cerebral-cortex proteins

There was very little difference between the molecular layer (layer I) and the various cellular layers (layers II–VI) with regard to free glutamate content (Table 4). However, as in the cerebellum, the value in white matter was lower than in the other layers. The specific radioactivity of the free glutamate was fairly uniform throughout all layers, including white matter. Protein glutamate specific radioactivity showed a wider range than that of free glutamate, being higher in the more superficial cell-body layers than in either the molecular layer or the white matter. Deeper cell-body layers also showed a gradation of protein glutamate specific radioactivities. The turnover rates reflect these differences in specific radioactivity. The protein in the most superficial cell layer (II) had a half-time of 25h, compared with 83h for white-matter protein.

Incorporation rates of ^{14}C-labelled amino acids into proteins of layers of Ammon's horn

The fibre-enriched layer, the alveus, was found to have a lower free glutamate content than other layers, as for white matter in both cerebral cortex and cerebellum (Table 5). Low concentrations of glutamate were also found in rabbit alveus layer (Young & Lowry, 1966). The glutamate contents in the other layers of Ammon's horn were consistently higher

Table 2. *Incorporation of label into proteins of forebrain*

Rats were injected subcutaneously, as described in the text, with serial injections of [^{14}C]glucose. The dose per injection was 120μCi (Expt. 1) or 5μCi (Expt. 2). Results were obtained from 25mg samples. Each entry represents a different animal. Protein glutamate specific radioactivity is calculated by attributing 52% of the total specific radioactivity to glutamate and dividing by 0.93 (the mmol of glutamate/g of protein). The calculation of mean specific radioactivity of free glutamate is described in the text.

	Specific radioactivity			Turnover of protein glutamate		
				(%)		
Time (h)	Protein (μCi/g)	Protein glutamate (μCi/mmol)	Mean free glutamate (μCi/mmol)	Total	(per h)	$t_{\frac{1}{2}}$ (h)
Expt. 1						
1.25	0.91	0.51	52	0.98	0.78	
1.25	3.71	2.1	230	0.90	0.72	
2	13.2	7.4	510	1.46	0.73	
2	12.2	6.8	430	1.59	0.80	
4	23.0	12.8	420	3.05	0.76	
4	22.6	12.7	360	3.53	0.88	
				Mean	0.78	89
Expt. 2						
2	0.28	0.156	8.5	1.84	0.92	
2	0.43	0.24	10.5	2.29	1.14	
2	0.31	0.174	11.4	1.53	0.77	
4	0.66	0.37	14.4	2.56	0.64	
4	1.06	0.59	23.1	2.55	0.63	
4	0.76	0.42	11.8	3.59	0.90	
6	1.40	0.78	14.9	5.25	0.87	
6	1.19	0.66	15.2	4.36	0.73	
6	1.12	0.63	13.4	4.69	0.78	
8	1.37	0.77	7.5	10.2	1.27	
8	1.32	0.74	12.4	6.0	0.75	
8	1.25	0.70	12.9	5.4	0.67	
				Mean	0.84	83

than in layers of cerebellum or cerebrum. The specific radioactivity of the glutamate showed little variation among the layers but protein glutamate specific radioactivities ranged from a high value (19μCi/mmol) in the pyramidal-cell layer to a low value (10μCi/mmol) in the molecular layer. Consequently there is a range of values for half-times of protein from 29h for the cell-body layer to 60h for the molecular layer.

Discussion

As the results show, it is a relatively simple procedure to maintain a constant concentration of radioactive glucose in the blood by repeated subcutaneous injections of radioactive glucose. Calculations of mean protein turnover rates in tissues can then readily be made if the precursor amino acid pool formed from glucose is of a constant specific radioactivity. Glucose is rapidly transported from the blood into brain tissue and converted into a range of products, particularly acidic amino acids (Vrba *et al.*, 1962; Lindsay & Bachelard, 1966; O'Neal *et al.*, 1966; Shimada *et al.*, 1970; Yoshino & Elliott, 1970*a*). More than half the ^{14}C of labelled glucose is retained in acidic amino acids of the brain within 15min (Vrba *et al.*, 1962), and equilibrium is reached between glucose and amino acids, particularly glutamate and aspartate, in the perfused cat brain within 50min (Barkulis *et al.*, 1960). In the experiments reported here, the brain glutamate specific radioactivity, relative to the blood glucose specific radioactivity, remained reasonably constant over the period 1.25–6.5h (Table 1) although there was evidence of a lag during the first 1.25h. Calculations based on a constant glutamate specific radioactivity at the 4h point would therefore appear to be valid. In all the experiments the rats were anaesthetized. Yoshino & Elliott (1970*b*) have shown that this does not affect the labelling rate.

When measurements of protein turnover rates are

Table 3. *Incorporation of label into protein of cerebellar layers*

Freeze-dried samples were prepared and extracted as described in the Materials and Methods section and radioactivities were measured in the free glutamate and protein fractions. Free glutamate concentrations, measured in separate samples from the 4h rat, were 89±1.8, 86±1.5 and 49±1.9 mmol/kg of protein (i.e. lipid-free dry weight) in granular, molecular and white-matter layers respectively. Each value is the mean of at least six samples ±S.E.M. For calculations see Table 2 and the text. Turnover indicates the percentage of protein glutamate that has become labelled.

		Specific radioactivity			Turnover	
Time (h)	Layer	Protein (μCi/g)	Protein glutamate (μCi/mmol)	Mean free glutamate (μCi/mmol)	(%/h)	$t_{\frac{1}{2}}$ (h)
1.25	Granular cell	3.7±0.3	2.1	135± 7	1.24	
	Molecular	2.3±0.1	1.3	163±36	0.64	
	White matter	2.8±0.4	1.6	248±16	0.52	
2	Granular cell	11.2±0.1	6.3	115± 9	2.75	
	Molecular	7.8±0.1	4.4	118± 4	1.86	
	White matter	8.0±0.2	4.5	117±20	1.93	
4	Granular cell	38.8±5.3	21.8	261± 6	2.08	33
	Molecular	23.2±2.3	13.0	279±14	1.17	59
	White matter	18.4±1.3	10.3	505±28	0.51	136

Table 4. *Incorporation of label into proteins of layers of cerebral cortex*

The procedure and details are as described for Table 3. Each value is the mean of at least six samples, except that in one case (*) there were only two samples. The duration of the experiment was 4h. Layer VII is the subjacent white matter.

		Specific radioactivity			Turnover	
Layer	Free glutamate (mmol/kg of protein)	Protein (μCi/g)	Protein glutamate (μCi/mmol)	Mean free glutamate (μCi/mmol)	(%/h)	$t_{\frac{1}{2}}$ (h)
I	102±3	23.5±3.7	13	260±20	1.25	55
II	101±2	38.5±1.4	22	202±30	2.72	25
III	107±2	41.0±5.7	23	225±40	2.55	27
IV	104±2	33.9±1.4	19	268±20	1.77	39
V	107±1	31.1±2.4	17	316±10	1.35	51
VI	97±4	24.0±4.9	13	282±10	1.15	60
VII	90±3	17.4*	10	302±20	0.83	83

made by using this system with experiments of relatively short duration, only those proteins with short half-lives will incorporate much of the radioactivity. The mean value obtained for the half-life of average proteins of cerebral cortex was 86h. This value is probably derived from a family of proteins, with rates differing substantially from the mean. For example, an apparent half-life of 86h would be obtained in an 8h experiment if the protein were present as two equal groups, one with a half-life of 50h, the other with a half-life of 300h. The results could also be explained by a mixture of 20% protein having a 20h half-life with 80% protein having a 300h half-life.

In contrast with this type of experiment, the decay of radioactivity after pulse-labelling provides reliable turnover values for proteins with long half-lives, but is less suitable for protein fractions with short half-lives. From decay rates, half-lives of 10–20 days have been obtained for one group of brain proteins (Lajtha & Toth, 1966; Hungen *et al.*, 1968) and a much longer half-time for a stable proteolipid fraction (Furst *et al.*, 1958). As discussed, the present results demonstrate the existence of a third fraction with half-times of probably not more than 2 days. The existence of a rapid component can be observed even in decay experiments, as shown by Hungen *et al.*

Table 5. *Incorporation of label into proteins of cell layers of Ammon's horn*

Samples were prepared and analysed as described in Table 3. The results are presented in the same way. The labelling period was 4h.

Layer	Free glutamate (mmol/kg of protein)	Specific radioactivity: Protein (μCi/g)	Specific radioactivity: Protein glutamate (μCi/mmol)	Specific radioactivity: Mean free glutamate (μCi/mmol)	Turnover (%/h)	Turnover $t_{\frac{1}{2}}$ (h)
Alveus	80±5	23.8±2.8	13.4	177±23	1.89	37
Oriens	140±1	26.0±1.1	14.6	206±16	1.77	39
Pyramidalis	119±4	33.4±2.6	18.7	195±11	2.40	29
Radiata	144±3	24.6±2.2	13.8	175±16	1.98	35
Molecular	116±3	16.9±2.2	9.5	207±16	1.15	60

(1968). These authors found the rate of decay of protein label to be first order but biphasic. The major portion of the curve indicates a half-life of 14 days but the initial segment, representing about half of the proteins has a more rapid turnover with $t_{\frac{1}{2}}$ of 2–4 days, which is compatible with our findings. Yoshino & Elliott (1970*b*) present evidence of a small fraction of brain protein with a half-time of only a few hours. Our observation of a nearly linear increment in protein specific radioactivity for 8h, with precursor specific radioactivities kept nearly constant (Table 2), does not support this.

The proteins in cell-body areas turn over more rapidly than those in areas rich in dendritic or axonal processes (Tables 3–5). Although this is not unexpected, the rate of incorporation into the non-cell-body areas is surprisingly high. This reflects either a high rate of protein turnover in supporting cells, a rapid transport of protein into the dendrites and axons or some synthesis of protein in the dendrites and axons themselves. The relatively rapid turnover in dendrites may be due to local synthesis, since ribosomes are commonly found in dendritic processes (Palay & Palade, 1955). Some synthesis may also occur in the axons, despite the inability to demonstrate the presence of ribosomes by electron microscopy (Koenig, 1965; Edström, 1966). Radioautography consistently shows that glia incorporate amino acids into protein, although at a much lower rate than in neurons (Peterson *et al.*, 1968; B. S. McEwan, personal communication) and consequently the labelled protein found in white matter may be due to glial-cell protein. In addition, some local axonal synthesis may contribute to the labelled protein of white matter (Edström, 1966; Giuditta *et al.*, 1968). When various amino acids are injected *in vivo* and whole cell preparations of neurons and glia are subsequently isolated from brain, the incorporation into neurons is 2–3 times higher than that into glia (Blomstrand & Hamberger, 1969; Tiplady & Rose, 1971). This may be compared with the present results for cerebellar cortex, indicating a turnover rate 4 times faster in the granule-cell layer than in white matter.

It is well known that protein is transported along axons at two rates, one of about 40μm/h and the other 10000μm/h (McEwan & Grafstein, 1968; Bray & Austin, 1968) and either of these mechanisms could partially account for the appearance of newly synthesized protein in the white matter layers. Nevertheless the rate of appearance of labelled protein at the shortest time measured, 1.25h, is about the same in all layers and is therefore unlikely to be entirely due to slow protein transport by axoplasmic flow. The proportion of protein that flows at the faster rate is only about 20% of that at the slow rate (Bray & Austin, 1969) and it seems unlikely that white matter could be labelled to the degree shown by this flow of protein.

Areas rich in cell bodies from different parts of the brain incorporate amino acids into protein at about equal rates (Tables 3–5). This applies to granule cells of the cerebellum, pyramidal cells of Ammon's horn and the upper cell layers of cerebral cortex. However, the lower cell layers of cerebral cortex (layers V and VI) incorporate amino acids somewhat more slowly. This could be due to an intrinsic difference in rates of protein synthesis, or to a lesser proportion of cell-body cytoplasm. There is also a general consistency among dendritic layers from different areas and among fibre-tract layers; incorporation into both of these types of layer is consistently lower than in the case of cell bodies. However, in all layers studied there is a considerable amount of protein that turns over rapidly (in less than 4 days) compared with most of the cellular protein (10–20 days). The role of this protein fraction is unknown but it may control some of the unique functions of nerve cells.

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