SUMMARY

1. *Proteus vulgaris* possesses an inducible leucine decarboxylase. The formation of this enzyme was dependent on a source of carbon and energy in addition to an inducer, and was more rapid and extensive in growing cultures than in dense, washed suspensions.

2. A variety of compounds structurally related to leucine were tested for their ability to combine with the enzyme (acting as substrates or inhibitors) and to induce its formation. All the known substrates were inducers. *iso*Valerate and *iso*butylamine, which inhibited the enzyme, could not induce its formation, whereas alanine was an inducer but was inert in the enzyme system.

3. Those amino acids which were both substrates and inducers of the enzyme showed an affinity for the induction system at least ten times as great as their affinity for the enzyme.

4. The induction system closely resembles the enzyme system but is distinct from it. This is in keeping with the theory that inducers combine with a bound form of the enzyme.

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The Rate of Incorporation of [^{35}S]Methionine and [^{35}S]Cystine into Proteolipids and Proteins of Rat Brain

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Gaitonde & Richter (1956) have reported that, at 3 hr. after intracisternal injection of [^{35}S]methionine, about 17% of the radioactivity in the brain was found in the trichloroacetic acid-soluble fraction, 3% in the lipids and 80% in the proteins. The specific radioactivities of the three fractions gave evidence of a rapid turnover of labelled methionine in the proteins. The specific radioactivity of the lipid sulphur was low and no definite conclusions were drawn as to the metabolic activity of this fraction; but it was observed that the labelling of lipid sulphur was mainly due to small amounts of lipoproteins present in the lipid extracts.

By extracting fresh ox-brain tissue with chloroform–methanol (2:1, v/v), Folch & Lees (1951) obtained a group of lipoproteins which they designated as proteolipids because of their solubility in lipid solvents. The proteolipid content of the white matter was three to four times that of the grey matter of the brain. Measurable quantities of proteolipids are present in the 7- or 8-day-old mouse brain. The proteolipid content of the brain increases greatly during the period of myelination, and in the 30-day-old mouse it reaches the level in the adult brain (Folch, 1954). These results suggest a possible significance of proteolipids in relation to the formation of myelin. To obtain further information on this point a study has now been made of the turnover of the proteolipids of rat brain at

various stages of growth. This paper deals with the rate of uptake of [^{35}S]methionine sulphur into the proteolipids, proteins and free amino acids of the young-adult (42-day-old) rat brain. The extraction and fractionation techniques employed by Gaitonde & Richter (1955), combined with those of Folch, Lees & Sloane-Stanley (1957), have yielded results that indicate that the proteolipids turn over at a much slower rate than other brain proteins. It was also found that the apparent turnover rate of methionine in proteins increased with time after the injection of [^{35}S]methionine.

METHODS

Experimental animals. Young adult rats of the Wistar albino strain raised in the Laboratory were used. In each series, 6-week-old litter mates of both sexes, weighing 90–108 g. (mean body wt. 99 g.), were injected intraperitoneally with 0·25–0·33 ml. of 0·9% NaCl solution containing 0·5–0·7 mg. of L-methionine equivalent to a radioactive dose of 5 μc of ^{35}S/100 g. body wt. The animals were denied access to food and water during the experimental period. The exchange of labelled methionine *in vivo* was terminated after different periods (2 min.–5 hr.) by dropping the whole animal, or the head after decapitation, into liquid N_2. The frozen brain tissue was removed and pulverized as described by Clouet, Gaitonde & Richter (1957).

Preparation of proteolipid fraction from rat brain. The general scheme for the preparation of the fractions described below is given in Fig. 1.

The frozen pulverized tissue (1–1·2 g.) was quickly weighed and extracted with $CHCl_3$–methanol (2:1,v/v; 19 ml./g. of fresh tissue) at room temperature (Folch *et al.* 1957) for 20–30 min. The suspension was centrifuged and the clear supernatant solution filtered through Whatman no. 30 paper. The tissue residue was re-extracted with 5–10 ml. of the same solvent and kept for further fractionation. The combined filtrates contained lipids together with non-lipid contaminants.

The lipid extract (30 ml.) contained ^{35}S-labelled compounds, which had to be removed before measurements of the specific radioactivity of proteolipids could be made. The lipid extract was first washed with a one-fifth volume of distilled water as described by Folch *et al.* (1957), and the resulting two phases were separated by centrifuging. The upper phase (washing) containing strandin, non-lipid contaminants and traces of lipids was siphoned off. The lower phase (proteolipids) was washed six times in a similar manner with 5–10 ml. portions of the pure upper phase of $CHCl_3$–methanol–water (8:4:3, by vol.; Folch *et al.* 1957). The washings were collected separately and oxidized with $HClO_4$–HNO_3 for measurement of the specific radioactivity. The first washing contained considerable amounts of S; to the remaining washings carrier sulphate (0·12 mg. of S) was added to obtain a satisfactory precipitation of ^{35}S as benzidine sulphate. About 95% of the radioactivity removed by all the washings was present in the first three washings of the lower phase; a small but detectable amount of radioactivity (approximately 1%) was extracted in each of the remaining washings. Paper chromatography of hydrolysates of the washings showed that most of the amino acids were removed in the first two washings; only traces of serine, glutamic acid and alanine were present in subsequent washings. All lipid extracts were, therefore, washed three times with the pure upper phase before oxidation to inorganic sulphate.

Since the upper phase (combined washings of the lipid extract) contained free amino acids, salts, strandin and traces of lipids, it was dialysed for 24–48 hr. with frequent changes of water. The strandin fraction remaining in the dialysis bag was hydrolysed and paper-chromatographed for its amino acid composition. The dialysate (diffusible fraction) was subjected to ion exchange and paper chromatography for the determination of free amino acids.

Treatment of the lipid-free tissue residue. The tissue residue remaining after treatment with $CHCl_3$–methanol was immediately suspended in 10 ml. of cold 10% trichloroacetic acid solution, centrifuged and filtered at room temperature. The precipitate was washed again three times with the same solution. The combined trichloroacetic acid filtrates were washed four times with ether, dried at room temperature, and used for paper chromatography or oxidized for the determination of specific radioactivity of S. The trichloroacetic acid-insoluble precipitate was designated as protein residue. In series 2, the protein residue

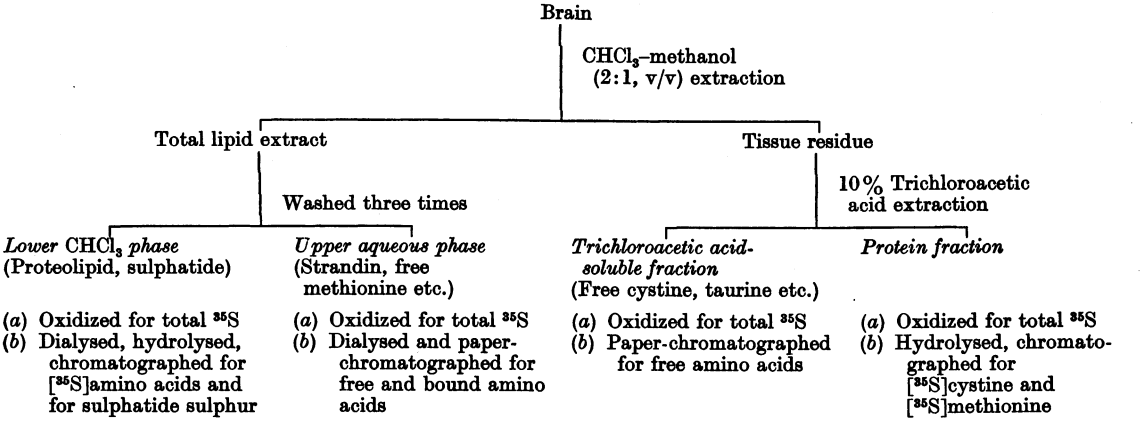

Fig. 1. Scheme of tissue fractionation.

was washed further with 20 ml. of $CHCl_3$–methanol–HCl (200:100:1, Folch & LeBaron, 1953).

Hydrolysis of samples. Samples containing protein were hydrolysed with 6N-HCl by refluxing for 18–24 hr. on a hot sand bath or by heating in a sealed tube at 105°. The hydrolysate was filtered and dried under reduced pressure. The residue was dissolved in water and subjected to ion-exchange chromatography as described below.

Ion-exchange chromatography. To obtain good resolution for the identification and quantitative determination of amino acids on paper chromatograms, it was desirable to remove salts and breakdown products of lipids by using ion-exchange resins before two-dimensional chromatography of the upper phase, trichloroacetic acid extracts and hydrolysates of the samples. A sample representing 1 g. of fresh tissue was transferred to a column (0·9 cm. × 15 cm.) of Permutit Q (H^+ form) and washed with 25–30 ml. of water. Under these conditions, taurine present in the upper phase and in the trichloroacetic acid-soluble fraction was obtained in the washings (effluent) of the resin. The amino acids were eluted from the column with 50 ml. of N-NH_3 solution followed by 20 ml. of distilled water (eluate). The combined eluate was filtered and evaporated to dryness at 40–50° under reduced pressure. The residue was dissolved in 10 ml. of water and traces of NH_3 were removed by repeated distillation under reduced pressure. Finally the residue was dissolved in 100 μl. of 0·05N-HCl and 50 μl. was used for paper chromatography.

Paper chromatography. Two-dimensional paper chromatography (Dent, 1948) was used for the analysis of amino acids in tissue fractions. The chromatograms were developed with acetic acid–butan-1-ol–water (1:4:5, by vol.; upper phase used) and aqueous 80% (w/w) phenol in the presence of traces of NH_3. The papers were dried, heated and sprayed with 0·5% ninhydrin solution in methanol at pH 5·0. The spots were cut, eluted in 5 ml. (or more) of 60% (v/v) ethanol and the extinction was measured at 570 mμ.

Separation of proteolipid sulphur from sulphatide sulphur. The total washed lower phase containing proteolipids and sulphatide was hydrolysed. The hydrolysate was transferred to a column of Permutit Q to absorb all amino acids including cystine and methionine. The washings (effluent) of the resin containing sulphate were designated as sulphatide sulphur and the ammonia eluate of the resin as proteolipid sulphur. Both fractions were oxidized for the determination of specific radioactivity.

Separation of cyst(e)ine sulphur from methionine sulphur. The observation that under suitable conditions permolybdic or performic acid oxidizes methionine quantitatively to its sulphone and cystine to cysteic acid (Toennies & Kolb, 1941; Toennies & Homiller, 1942) has been made the basis of a simple and reliable method for the determination of the specific radioactivities of the two amino acids in tissue proteins. The hydrolysates of proteins (30–80 mg.) were dried to a syrupy consistency and treated with 0·5 ml. of 30% H_2O_2 for 24 hr. at 5°. The contents of the flask were warmed on a water bath (50°) for 10 min., diluted with 10–15 ml. of water and concentrated at the same temperature with a Rinco (rotating vacuum-type evaporator). Under these conditions, methionine was oxidized to methionine sulphone and cyst(e)ine to cysteic acid. The residue in the flask was quantitatively transferred with 2–3 ml. of water to a column of Permutit Q prepared as described above. The washings (effluent, 30 ml. of water) of the resin contained cysteic acid. Methionine sulphone was eluted from the resin with NH_3. The separation of methionine sulphone from cysteic acid was quantitative. The respective fractions were concentrated and oxidized for the determination of specific radioactivity.

Determination of sulphur and radioactivity. Total S and its radioactivity were determined as described previously (Gaitonde & Richter, 1955). The precipitate of benzidine sulphate was collected on a filter paper within a fixed area of 2·8 cm.^{2} and radioactivity measured with a modification of Robinson's (1950) windowless gas-flow counter.

RESULTS

Isolation of proteolipid and strandin fractions

The presence of bound amino acids in the strandin fraction (upper aqueous phase) of ox brain was reported by Folch, Arsove & Meath (1951) and Folch & Lees (1959) and in ox-brain mucolipid by Rosenberg & Chargaff (1958). This was confirmed in rat brain in the present experiments. The strandin fraction obtained after dialysis gave no ninhydrin-positive spots on paper chromatograms, but after hydrolysis showed the presence of large amounts of hexosamine and of the following amino acids: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, valine, leucine and cyst(e)ine.

Two-dimensional chromatography of the dialysate (diffusible fraction) showed the presence of several free amino acids. Determination of the approximate amounts of amino acids in this fraction and also in the trichloroacetic acid-soluble fraction indicated that mainly neutral amino acids were present in the original unwashed chloroform–methanol extract. The results of a typical experiment are given in Table 1. Methionine which was detectable only in the dialysate of the upper phase could not be measured quantitatively. The total amino acid content given in Table 1 is in good agreement with the data reported by other workers (Ansell & Richter, 1954; Tallan, Moore & Stein, 1954, 1956; Porcellati & Thompson, 1957; Berl & Waelsch, 1958). The lower values for glutamine obtained in the present work may be due to partial destruction during ion-exchange chromatography. β-Alanine (tentatively identified) was detected in the upper phase (55%) and in the trichloroacetic acid-soluble fraction (45%). The values given for histidine, lysine and arginine are given with some reserve since these amino acids did not separate on the paper chromatograms and were measured together.

Uptake of ^{35}S into lipid and non-lipid fractions

Upper aqueous phase (strandin fraction). The initial high specific radioactivity of the upper phase (Fig. 2, curve *A*) indicated that a fair amount

Table 1. *Amino acid content of the dialysate of the upper phase and the trichloroacetic acid extract of rat brain*

For details see Methods. Methionine and β-alanine were present in quantities too small to be measured.

	μg./g. of fresh brain		
	Upper phase	Trichloroacetic acid extract	Total
Serine	49·4	89·4	138·8
Glycine	57·0	55·5	112·5
Threonine	36·2	19·3	55·5
α-Alanine	39·6	13·1	52·7
β-Alanine	+ +	+	+ + +
Valine	6·9	2·2	9·1
Methionine	+	Not detectable	+
Phenylalanine	4·5	Not detectable	4·5
Leucine	9·9	3·2	13·1
γ-Aminobutyric acid	206·0	54·7	260·7
Tyrosine	5·9	1·9	7·8
Aspartic acid	38·6	342·0	380·6
Glutamic acid	329·0	1070·0	1399·0
Glutamine	262	66·2	328·2
Ethanolamine	Trace	42·0	42·0
Taurine	63·4	181·5	244·9
Histidine + lysine + arginine	22·6	100·0	122·6
N-Acetylaspartic acid*	Lost	432·0	—
Glutathione + cystine†	9·8	74·5	84·3

* Determined from the aspartic acid formed after hydrolysis of the washings of the cation exchanger.
† Expressed as total cystine.

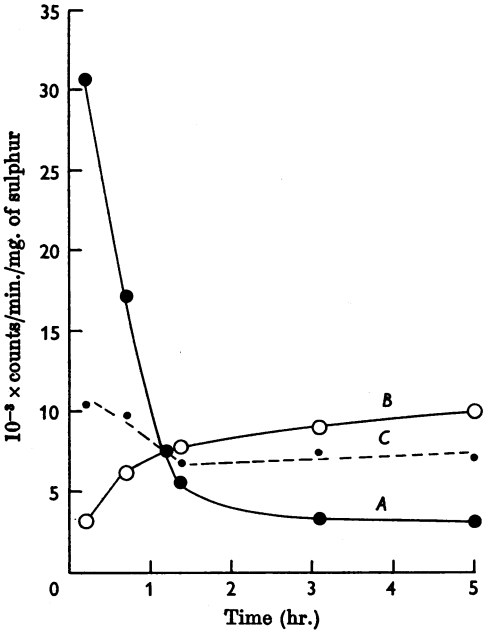

Fig. 2. Rate of disappearance of free [^{35}S]methionine in rat brain after intraperitoneal injection of L-[^{35}S]methionine. *A*, Upper aqueous phase (washings of the lipid extract contain [^{35}S]methionine); *B*, trichloroacetic acid-soluble fraction, contains [^{35}S]cystine. *C* represents the specific radioactivity of the total sulphur of the free amino acid pool. The values were calculated from those of the other two curves as follows: (counts/min. in upper phase + counts/min. in trichloroacetic acid extract)/(mg. of S in upper phase + mg. of S in trichloroacetic acid extract).

of ^{35}S had passed through the blood–brain barrier. The evidence obtained by paper chromatography indicated that this fraction contained free methionine, cyst(e)ine, taurine and other amino acids. Most of the radioactivity in this fraction was present as free [^{35}S]methionine together with a small amount of [^{35}S]cyst(e)ine which was mostly present in the form of glutathione. Taurine, which accounted for most of the sulphur of the upper phase, contained very little radioactivity. The total sulphur content of the upper phase was approximately 100 μg. of sulphur/g. of fresh tissue, and, if all the tissue methionine (12·2 μg./g. of fresh tissue; Schurr, Thompson, Henderson, Williams & Elvehjem, 1950) were present in the upper phase, the true specific radioactivity of methionine would be at least 35–40 times higher than that recorded in Fig. 2 (curve *A*). For practical purposes, the observed specific radioactivity of the upper phase may be regarded as approximately proportional to the true specific radioactivity of free methionine in the pool. The curve, therefore, indicates a rapid disappearance of free [^{35}S]methionine in the brain. At the end of 90 min., the specific radioactivity of methionine had declined to 16% of the observed maximum value and, during the next 90 min., it reached a plateau at 11% of the original value.

Lower phase (*proteolipid fraction*). The rate of uptake of ^{35}S into the washed lipid extract is shown in Fig. 3 (curve *A*). At 12 min. after an intraperitoneal injection of [^{35}S]methionine, a considerable amount of radioactivity (1422 counts/min./mg. of S) was present in the lipid extract containing proteo-

lipids. There was a gradual increase in the amount of ^{35}S in this proteolipid fraction over a period of 5 hr. The slope of the curve suggested that a progressive labelling of proteolipids was taking place over a long period. These findings are in agreement with the view that the proteolipid contains at least two components of different turnover rates.

The washed lower phase contains both proteolipids and sulphatides (Folch *et al.* 1957). In order to measure the relative rates of incorporation of ^{35}S into these two fractions, the washed lower phase was exhaustively dialysed against water to remove any traces of free [^{35}S]methionine and hydrolysed, and the specific radioactivity was determined for the combined cystine and methionine present in the hydrolysate. Any inorganic sulphur formed during hydrolysis and derived from sulphatides was excluded from the proteolipid protein sulphur. Thus it was found that proteolipids were labelled as early as 2–5 min. after injection of [^{35}S]methionine (Fig. 3, curve *B*), but little or no radioactivity was detected in the sulphatide fraction during the 2–60 min. period.

Uptake of ^{35}S into the lipid-free tissue residue

The residue remaining after the extraction of lipids contained most of the tissue proteins. In order to complete the removal of acid-soluble sulphur-containing compounds, this protein residue was extracted with trichloroacetic acid.

Trichloroacetic acid-soluble fraction. This fraction contained 65–70 % of the total acid-soluble sulphur together with other free amino acids: paper-chromatographic evidence (Table 1) suggested that cystine (90 %) and taurine (74 %) accounted for most of the sulphur in this fraction. The radioactivity in this fraction was mainly due to [^{35}S]-cyst(e)ine. Although the presence of taurine sulphur of low specific radioactivity would greatly decrease the true specific radioactivity of cystine sulphur, the specific-radioactivity–time relation of this fraction (Fig. 2, curve *B*) may be considered as an index of the rate of formation of cystine from methionine. A rapid fall in the specific radioactivity of lipid washings (methionine, curve *A*), concomitant with a gradual increase in the specific radioactivity of trichloroacetic acid-soluble fraction (cystine, curve *B*), is in agreement with the previous observation of a rapid methionine metabolism (Gaitonde & Richter, 1957).

Protein residue. The sharp increase in the specific radioactivity of the protein sulphur during the first 30 min. (Fig. 4, curve *A*) was in agreement with the previous observations that the injected [^{35}S]methionine was rapidly incorporated into the brain proteins. After the initial 30 min., the rate of incorporation of ^{35}S into proteins decreased considerably, but it seemed to proceed linearly with time. Thus the shape of the curve *A* suggested that ^{35}S of two different specific radioactivities was being

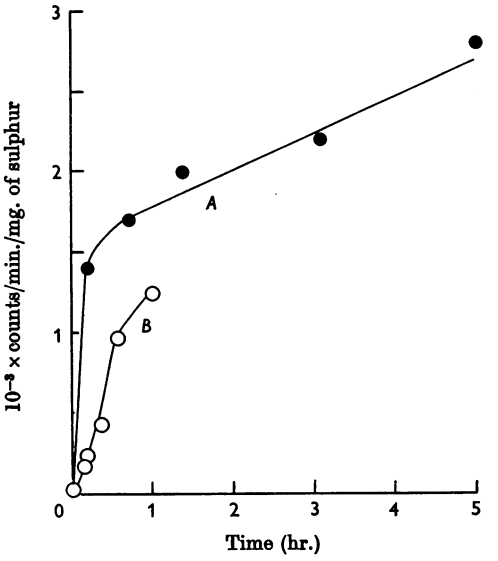

Fig. 3. Rate of uptake of [^{35}S]methionine and [^{35}S]cystine into proteolipids of rat brain after injection of L-[^{35}S]-methionine. *A*, Washed lipid extract (proteolipid); *B*, [^{35}S]cystine + [^{35}S]methionine of washed proteolipid after dialysis to remove free [^{35}S]methionine.

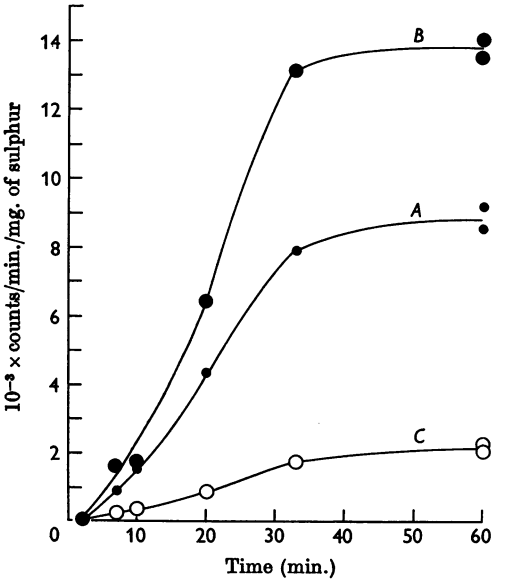

Fig. 4. Rate of uptake of [^{35}S]methionine and [^{35}S]cystine into the protein residue of rat brain. *A*, Total sulphur of protein residue; *B*, methionine of protein; *C*, cystine of protein.

incorporated into proteins. The biphasic nature of incorporation of ^{35}S into proteins is consistent with the observed changes of specific radioactivities of the free methionine and free cyst(e)ine fractions (Fig. 2, curves *A* and *B*). Thus the rapid uptake of ^{35}S during the first half hour was mainly due to incorporation of [^{35}S]methionine. The subsequent reduction in the rate of uptake indicated that labelled methionine in proteins was being diluted by incorporation of methionine of lower specific radioactivity. However, any dilution of ^{35}S in proteins was gradually being counterbalanced by incorporation of [^{35}S]cystine with a specific radioactivity considerably lower than that of methionine.

Rate of incorporation of methionine and cystine into proteins. The specific radioactivity of methionine and cystine combined into brain proteins reached a maximum at 30 min. (Fig. 4).

Since brain proteins contain 4·37 mg. of methionine sulphur and 4·26 mg. of cystine sulphur/g. of protein (M. K. Gaitonde, unpublished observation) the data on the specific radioactivities of protein-bound cystine and methionine (Table 2) indicated that during the first 60 min. after injection of [^{35}S]methionine about 16–20 % of the total radioactivity in proteins was present as [^{35}S]cystine.

The apparent specific radioactivities of free methionine and free cyst(e)ine at different times after injection of [^{35}S]methionine were obtained from the specific radioactivities of the upper phase (free methionine fraction) and the trichloroacetic acid-soluble fraction [free cyst(e)ine fraction] by assuming that the bulk of the tissue sulphur due to taurine, which is metabolically less active than that of methionine and cyst(e)ine, was equally distributed between the two fractions. Thus the apparent turnover rate of the two amino acids in brain proteins may be calculated from: $S_p K / S_f t \times 60 \times 1000$ μmoles of amino acid/g. of protein/hr., where S_p and S_f are the specific radioactivities of methionine or cyst(e)ine in the protein and in the free amino acid pool, K is mg. of sulphur of the amino acid/g. of protein, and t, time of exchange in minutes. The apparent turnover rates given in Table 2 would imply that methionine was incorporated into the brain proteins at an increasing rate. In contrast, the apparent turnover rate of [^{35}S]cyst(e)ine, which was as high as that of methionine at 7 min. in the first series and higher at 5 min. in the second series, decreased with the time of exchange.

DISCUSSION

The method of Folch *et al.* (1957) was used for the preparation and purification of proteolipids labelled *in vivo*. This resulted in the separation of the free sulphur-compounds into two fractions. The upper-phase (washings) of the chloroform–methanol extract contained approximately 30 % of the total free sulphur compounds and most of the free methionine of the tissue was found in this fraction. The rest of the soluble sulphur compounds which were extracted from the tissue residue with trichloroacetic acid contained most of the labelled cyst(e)ine. The specific radioactivities of the free methionine and free cyst(e)ine in the two fractions were masked (see curve *C*, Fig. 2) by the presence of considerable amounts of other sulphur compounds and especially taurine of low specific radioactivity.

The specific radioactivity of the free methionine (upper phase) of the brain reached a maximum value within 12 min. after the intraperitoneal

Table 2. *Apparent rates of turnover of methionine and cystine in brain proteins*

Rats were injected intraperitoneally with 5 μc of L-[^{35}S]methionine/100 g. body wt. In series 2, proteins were washed further with acidified chloroform–methanol mixture.

Time after injection (min.)	Specific radioactivity of protein-bound (counts/min./mg. of S)		Apparent turnover rate (μmoles/g. of protein/hr.)	
	Methionine	Cystine	Methionine	Cystine
Series 1				
7	1 600	260	107	97
10	1 740	280	86	55
20	6 390	870	195	53
33	13 100	1 760	319	49
60	13 500	2 270	307	31
Series 2				
5	380	90	35	72
10	1 390	270	69	54
21	3 390	730	101	41
30	6 080	1 040	152	32
60	10 200	2 270	232	31

injection and thereafter it decreased rapidly with time: it showed a 50% decrease between 12 and 32 min. after injection and in 3 hr. reached a value of 19% of the maximal value. Similar rates of disappearance of ^{35}S from the non-protein supernatant fraction of brain homogenate have been recently reported by Clouet & Richter (1959) in rats that had received [^{35}S]methionine by intracisternal injection. These findings suggest that although an initially higher rate of incorporation of ^{35}S is obtained after intracisternal injection, the rate of disappearance of free methionine is independent of the route of administration.

The specific radioactivity of cystine present in the trichloroacetic acid-soluble fraction depends on the rate of demethylation of [^{35}S]methionine in the brain and on the permeability of the blood–brain barrier to [^{35}S]cyst(e)ine and [^{35}S]glutathione formed in other tissues by the same process. The precursor–product relationship is well illustrated (Fig. 2 and Table 2) by a decrease in the specific radioactivity of the free methionine fraction and an increase in the specific radioactivity of the cystine fraction. In 90 min. the [^{35}S]methionine had attained a steady state. Under these conditions, the specific radioactivity of cystine increases at a rate directly proportional to the rate of turnover of [^{35}S]methionine in the proteins.

In a previous investigation the rate of incorporation of ^{35}S into proteolipids was measured in lipid extracts obtained by extracting the trichloroacetic acid-insoluble tissue residue with acetone, chloroform–ethanol and ether (Gaitonde & Richter, 1955). In the present investigation, proteolipids were prepared by dispersing the fresh tissue in chloroform–methanol (2:1, v/v) and washing with a one-fifth volume of water (Folch *et al.* 1957). The washed proteolipid extract contained considerable radioactivity, which could be removed by dialysis. The nature of this dialysable ^{35}S is not known, but there is evidence that the specific radioactivity of the dialysable ^{35}S is closely related to that of the free methionine. Thus in experiments of short duration, when the specific radioactivity of free methionine (the upper phase) was very high, it was necessary for the washed proteolipids to be further dialysed before measuring their specific radioactivity. In both investigations it was found that the ^{35}S in the lipid fraction was due mainly to [^{35}S]-methionine and [^{35}S]cystine combined in the proteolipids. The specific radioactivity of lipid extracts obtained by the previous method was approximately 28% of that of the protein residue, whereas in the present work it was only 13%. Unlike the washed lipid extracts used in this investigation, the previous lipid extracts contained strandin and probably phosphatido-peptides (Folch & LeBaron, 1953) which might be released in organic solvents through the action of trichloroacetic acid. Preliminary experiments have indicated marked quantitative differences in aspartic acid, glutamic acid, methionine and basic amino acids of the 'proteolipids' extracted by the two procedures. Since no methionine has been detected in phosphatido-peptides (Folch, 1952) or in strandin, it is likely that the higher uptake of ^{35}S into the lipids prepared by the earlier method was due to their containing additional proteins of higher specific radioactivity.

The interpretation of the turnover rates obtained for the proteolipids and residual tissue proteins is difficult in view of the heterogeneity of the tissue proteins and also because of the possibility that their precursor pools *in vivo* might vary widely in their specific radioactivity. The specific radioactivity of proteolipids from 3 to 72 hr. after injection was about one-fifth of that of the residual tissue proteins. These results are consistent with the view that the proteolipids as a group turn over at a slower rate than the other tissue proteins and agree with previous conclusions (Clouet & Richter, 1959; Davison & Dobbing, 1959; Furst, Lajtha & Waelsch, 1958).

The change in the apparent turnover rate of methionine with time of exchange observed in the present investigation may be attributed to the fact that the brain contains proteins that differ in their metabolic activity. Since proteins with rapid turnover will be labelled faster than those with low turnover, the observed turnover rates of methionine will gradually increase with time. The results might also be explained on the basis that labelled intermediates are formed before [^{35}S]methionine is incorporated into the proteins. With [^{35}S]cyst(e)ine that is formed *in vivo* by demethylation of methionine, the labelling of proteins will depend also on (*a*) the rate at which [^{35}S]cystine is formed from the injected [^{35}S]methionine, (*b*) the rate at which [^{35}S]cyst(e)ine is utilized for the synthesis of glutathione, and (*c*) the rate of oxidation of [^{35}S]cyst(e)ine to [^{35}S]cysteic acid and [^{35}S]taurine. In view of the high turnover rates of [^{35}S]cyst(e)ine observed during the first 5–7 min., it appeared unlikely that the rate of demethylation was limiting the rate of incorporation of [^{35}S]cyst(e)ine into proteins. The interpretation of the observed decrease with time of the turnover rates of [^{35}S]-cyst(e)ine in proteins is complicated by the fact that the trichloroacetic acid-soluble fraction obtained in the present work contained 90% of free cyst(e)ine and glutathione together with 75% of the total [^{35}S]taurine in the free pool. Hence the calculated turnover rates of cyst(e)ine are valid only in the early periods when the radioactivity due to [^{35}S]glutathione, [^{35}S]cysteic acid and [^{35}S]-taurine in the trichloroacetic acid-soluble fraction

was relatively small. With increase in time of exchange, the specific radioactivity of the trichloroacetic acid-soluble fraction will no longer remain proportional to the true specific radioactivity of free cyst(e)ine, and therefore, it cannot be used for calculation of turnover rates.

SUMMARY

1. Measurements were made of the labelling of fractions containing (*a*) proteolipid, (*b*) other protein and (*c*) free amino acids of the rat brain after administering L-[^{35}S]methionine *in vivo*.

2. The rate of incorporation of ^{35}S into the proteolipids was much lower than the rate of incorporation into the other proteins. The radioactivity of the lipid fraction was present mainly in the amino acids of the protein component of the proteolipids; the radioactivity in the sulphatide fraction was relatively small.

3. A comparison of the apparent turnover rates of [^{35}S]methionine and [^{35}S]cystine into brain proteins at short intervals after injection of [^{35}S]-methionine suggested that demethylation of [^{35}S]-methionine was probably not limiting the rate of incorporation of [^{35}S]cystine.

4. The apparent turnover rate of [^{35}S]methionine into brain proteins increased with time of exchange of the injected [^{35}S]methionine. The significance of this observation has been discussed.

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The Chromatography of Normal Serum Proteins

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Substituted celluloses are now in widespread use for the chromatography of protein, the most common being diethylaminoethylcellulose and carboxymethylcellulose (Sober & Peterson, 1956), and numerous applications to serum proteins have been described (for a review see Sober & Peterson, 1958). However, chromatography of serum proteins on diethylaminoethylcellulose so far described has failed to resolve them into components homogeneous even as judged by the relatively simple criterion of paper electrophoresis (Sober & Peterson, 1956; Fahey, McCoy & Goulian, 1958).