Synthesis and Turnover of Cerebrosides and Phosphatidylserine of Myelin and Microsomal Fractions of Adult and Developing Rat Brain

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The synthesis and turnover of cerebrosides and phospholipids was followed in microsomal and myelin fractions of developing and adult rat brains after an intracerebral injection of [U- 14 C]serine. The kinetics of incorporation of radioactivity into microsomal and myelin cerebrosides indicate the possibility of a precursor-product relationship between cerebrosides of these membranes. The specific radioactivity of myelin cerebrosides was corrected for the deposition of newly formed cerebrosides in myelin. Multiphasic curves were obtained for the decline in specific radioactivity of myelin and microsomal cerebrosides, suggesting different cerebroside pools in these membranes. The half-life of the fast turning-over pool of cerebrosides of myelin was 7 and 22 days for the developing and adult rat brain respectively. The half-life of the slowly turning-over pool of myelin cerebrosides was about 145 days for both groups of animals. The half-life of the rapidly turning-over microsomal cerebrosides was calculated to be 20 and 40 h for the developing and adult animals respectively. The half-life of the intermediate and slowly turning-over microsomal cerebrosides was 11 and 60 days respectively, for both groups of animals. The amount of incorporation of radioactivity into microsomal cerebrosides from L-serine was greatly decreased in the adult animals, and greater amounts of the precursor were directed towards the synthesis of phosphatidylserine. In the developing animals, considerable amounts of cerebrosides were synthesized from L-serine, besides phosphatidylserine. The time-course of incorporation indicated that a precursor-product relationship exists between microsomal and myelin phosphatidylserine. The half-life of microsomal phosphatidylserine was calculated to be about 8 h for the fast turning-over pool in both groups of animals.

Previous metabolic studies on myelin have indicated that various components of myelin membranes in adult animals are relatively stable (Davison, 1970). Recent studies, however, have suggested that some of the myelin lipids and proteins have substantial metabolic turnover (Ansell & Spanner, 1967; Smith, 1968; Horrocks, 1969; Wood & King, 1971; Miller *et al.*, 1975). Our studies, after an intracerebral injection of [32 P]P_i, [14 C]glycerol, [14 C]ethanolamine (Jungalwala & Dawson, 1971) and [14 C]choline (Jungalwala, 1974a) have suggested that the phospholipids of myelin appear to be in a fairly rapid equilibrium with phospholipids of other subcellular membranes. For example, the apparent half-life of the rapidly turning-over phosphatidylcholine of myelin and microsomal fraction was 4–6 days, measured after an intracerebral injection of [methyl- 14 C]choline. We have also reported that the apparent half-life of myelin cerebro-

side sulphate (sulphatides) was much longer, of the order of 120 days, after an intracerebral injection of Na₂ 35 SO₄ into developing animals (Jungalwala, 1974b). The microsomal sulphatides in the same animals, however, turned over rapidly, with a half-life of about 1 day for the fast turning-over pool and 50 days for a slowly turning-over pool.

Cerebrosides are one of the major lipid components of myelin membranes. They are also present, in low concentration, in other subcellular membranes of brain (Lewin & Hess, 1965). Only a few studies on the turnover rates of cerebrosides *in vivo* have been reported. Earlier metabolic studies with labelled galactose have indicated that the galactose moiety of cerebrosides in the rat brain undergoes turnover (Moser & Karnovsky, 1959). Burton *et al.* (1964), in a preliminary report, indicated a half-life of about 45 days for the brain cerebrosides, with [1- 14 C]galactose as a precursor, in 13-day-old rats. Radin *et al.* (1957) calculated the half-life of total rat brain cerebrosides to be about 13 days, with [1- 14 C]galactose injected

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intraperitoneally into young rats. Smith (1968) reported a half-life of greater than 1 year for myelin cerebroside, with [$1\text{-}^{14}\text{C}$]acetate as the precursor injected intraperitoneally in developing and adult rats. In view of these conflicting results, we have re-investigated the problem of the turnover of cerebroside in purified myelin in the adult and developing rat brain. Cerebrosides are mainly synthesized in the endoplasmic reticulum (microsomal fraction) (Morell *et al.*, 1970). The turnover of cerebroside in microsomal fractions was also determined simultaneously for comparison. [$U\text{-}^{14}\text{C}$]Serine was selected as the precursor of cerebroside because relatively more L-serine than galactose or acetate is incorporated (L. W. Hayes & F. B. Jungalwala, unpublished results). [$U\text{-}^{14}\text{C}$]Serine was injected intracerebrally, since after an intracerebral injection the brain is subjected to a sudden pulse of radioactivity instead of slow diffusion of a small percentage of the dose, as after an intravenous or intraperitoneal injection, owing to the blood/brain barrier. Also an intracerebral route of injection leads to a higher incorporation of radioactivity into brain lipids (Jungalwala & Dawson, 1971; Jungalwala, 1974a,b).

Significant amounts of radioactivity from L-serine were incorporated into phospholipids of brain, particularly into phosphatidylserine and phosphatidylethanolamine. The turnover of myelin phosphatidylserine, derived from the direct precursor L-serine, has not been studied. We have therefore also examined the metabolism of phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine of myelin and microsomal fractions of the rat brain.

Experimental

Intracerebral injections

Female adult Sprague-Dawley rats weighing 200 ± 10 g were purchased from Charles River Laboratories, Wilmington, MA, U.S.A. The rats were anaesthetized with ether and each was injected intraventricularly with $20 \mu\text{Ci}$ of L-[$U\text{-}^{14}\text{C}$]serine ($0.167 \mu\text{mol}$) in a $10 \mu\text{l}$ volume, by using a Stoelting stereotaxic instrument essentially as previously described (Jungalwala & Dawson, 1971). The young animals (19 days old), of either sex from the same litter and of similar weight (33–37 g), were injected intracerebrally with $20 \mu\text{Ci}$ of L-[$U\text{-}^{14}\text{C}$]serine ($0.167 \mu\text{mol}$) in a $10 \mu\text{l}$ volume (Jungalwala & Dawson, 1971).

Preparation of subcellular fractions

Purified myelin, microsomal and the acid-soluble fractions from the brain homogenate were prepared as described previously (Jungalwala & Dawson, 1971; Jungalwala, 1974a,b). The purity of the myelin was checked for microsomal and mitochondrial contamination by marker-enzyme assays (Jungalwala &

Dawson, 1971). NADPH-cytochrome *c* reductase was used as a microsomal marker, and cytochrome *c* oxidase was used as a mitochondrial marker. Protein was measured by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as standard.

Extraction and fractionation of lipids

The subcellular fractions obtained from two to three brains for each time-point were suspended in 2 ml of 0.32 M-sucrose. A portion was used for marker-enzyme assay and protein determinations, and the remainder was extracted with 20 vol. of chloroform/methanol (2:1, v/v) (Jungalwala & Dawson, 1971). The lipid extract was washed with 0.2 vol. of 0.9% NaCl. The lower phase was then washed three times with 0.2 vol. of chloroform/methanol/water (1:15:15, by vol.) containing 0.1% DL-serine, and three times with the same mixture but without serine. The lipid extract was evaporated to dryness under N_2 , redissolved in chloroform, and then chromatographed on a column (5 mm $\times$ 7 cm) of silicic acid as described by Vance & Sweeley (1967). The less-polar lipids were eluted with chloroform, the galactolipids with acetone and the phospholipids with methanol.

Analysis of galactolipids

The galactolipid fraction was concentrated to about $200 \mu\text{l}$ volume and chromatographed on a silica-gel G thin-layer plate [previously washed with chloroform/methanol/0.5 M-HCl (10:10:3, by vol.) and reactivated at 120°C], with chloroform/methanol/water (24:7:1, by vol.) as solvent. Lipids were detected by brief exposure to I_2 vapours. Bands corresponding to cerebroside and sulphatide standards were individually scraped from the plate, slurried with developing solvent, transferred to a small silicic acid column (5 mm $\times$ 1 cm) and the galactolipids were eluted with the developing solvent used for t.l.c. About 0.3–1.0 mg of cerebroside was isolated from myelin and 0.1–0.3 mg from the microsomal fraction. Radioactivity was determined on a portion of the eluted samples. The rest of the material was used for the quantitative determination of cerebroside, by a micro-adaptation of the orcinol/ H_2SO_4 method (Balazs *et al.*, 1971) and sulphatides (Kean, 1968) in triplicate.

Analysis of phospholipids

The phospholipid fraction was concentrated and analysed in two different ways. For young animals, the phospholipids were subjected to alkaline ethanolysis, and the water-soluble glycerophosphatides were analysed by paper chromatography and high-voltage electrophoresis as described by Dawson *et al.* (1962). The spots on the paper were detected and counted for radioactivity by the method of Jungalwala & Dawson (1971). For adult animals, the phospholipids were analysed by two-dimensional t.l.c. by the method of

Getz *et al.* (1970) as modified by Eichberg *et al.* (1973). By this method, phosphatidylserine is very well separated from phosphatidylinositol and other phospholipids. The phospholipid spots were made visible with I_2 vapour and marked. The I_2 was allowed to evaporate. The spots were scraped from the t.l.c. plate and the phospholipids were extracted from the silica gel three times with 10 vol. of chloroform/methanol/acetic acid/water (50:39:1:10, by vol.). A measured portion of the extract was evaporated to dryness and used for determining radioactivity. The rest of the extract was used for determination of phosphorus by the procedure of Bartlett (1959) as modified by Dittmer & Wells (1969).

Determination of radioactivity

Samples in organic solvents were evaporated to dryness, and those containing galactolipids were counted for radioactivity in a toluene-based scintillation solution containing 5% ethanol (Jungalwala & Dawson, 1971) in a Tri-Carb liquid-scintillation counter. All other samples were counted in the toluene-based scintillation solution without ethanol. Samples in water were counted in Bray's (1960) solution.

Distribution of ^{14}C label in cerebroside

The labelled cerebroside with about 500 μ g of added carrier cerebroside were hydrolysed with methanolic HCl and the radioactivity was determined in the fatty acid, sphingosine base and hexose components (Hoshi *et al.*, 1973).

Materials

L-[U- ^{14}C]Serine (sp. radioactivity 120 mCi/mmol) was purchased from ICN Pharmaceuticals, Cleveland, OH, U.S.A. Galactocerebroside, sulphatides and phospholipid standards were purchased from Supelco, Bellefonte, PA, U.S.A. Unisil (activated silicic acid) was from Clarkson Chemical Co., Williamsport, PA, U.S.A. Uniplates coated with silica gel G and silica-gel HR plates were from Analtech, Newark, DE, U.S.A. Solvents and reagents were of analytical grade.

Results

Purity of myelin fraction

The assessment of the purity of an isolated membrane fraction, such as myelin, is of utmost importance, since contamination by other membrane fractions could lead to serious error in the interpretation of radioisotopic-incorporation experiments. Adams & Fox (1969) have pointed out that the labelled microsomal material is very difficult to remove from a myelin preparation. In our experiments the myelin was purified by washing the crude myelin fraction five times with 0.32M-sucrose and isolating myelin

after centrifugation at very low centrifugal force (755g_{av.}). The myelin was then given an osmotic shock and further purified on a continuous density gradient. The purified myelin had about 5–7% contamination by the microsomal proteins as measured by the NADPH-cytochrome *c* reductase assay. There was negligible mitochondrial contamination (cytochrome *c* oxidase activity) in the myelin and microsomal fractions. Each myelin preparation was checked for its purity by the marker-enzymes assay.

Incorporation of radioactivity from [^{14}C]serine in vivo into young rat brains

Cerebroside. The specific radioactivity of the cerebroside in myelin and microsomal fraction after an intracerebral injection of [^{14}C]serine into 19-day-old rats is shown in Fig. 1. The specific radioactivity in the acid-soluble pool, mainly representing the free precursor, declined rapidly with time. After 2–3 days the remaining radioactivity was only 10% of that measured at 0.5 h after the injection. The total amount of radioactivity present in the acid-soluble pool per brain was determined. The rats were injected with 44×10^6 d.p.m. per brain. The total amount of radioactivity in the acid-soluble pool left per brain at 0.5, 1, 2, 4, 8 and 12 h was 3.0×10^6 , 1.7×10^6 , 1.5×10^6 , 0.8×10^6 , 0.5×10^6 and 0.4×10^6 d.p.m. respectively, and at 1, 2, 3, 4, 7, 10, 60 and 100 days it was 0.5×10^6 , 0.4×10^6 , 0.35×10^6 , 0.27×10^6 , 0.16×10^6 , 0.08×10^6 , 0.04×10^6 and 0.006×10^6 d.p.m. respectively. The specific radioactivity of the microsomal cerebroside reached a maximum 4 h after the injection and then rapidly declined, whereas the specific radioactivity of myelin cerebroside reached a maximum 2–3 days after the injection and then declined with time. It has been shown (Burton *et al.*, 1963; Norton & Poduslo, 1973), that in the developing rat brain the maximum rate of synthesis of cerebroside and deposition of myelin cerebroside takes place when the rats are 15–30 days old. Thereafter the deposition of cerebroside continues throughout maturity but at a lower rate. In the present study, rats were injected with L-serine when they were 19 days old. Thus the apparent initial decline in the specific radioactivity, especially of myelin cerebroside, in these animals could, at least in part, be due to rapid deposition of newly formed non-radioactive cerebroside in brain. It has been reported that more than 70% of the cerebroside of the brain are localized in myelin (Norton & Poduslo, 1973). The rate of deposition of cerebroside in brain was estimated by determining the amount of cerebroside in a sample of the rat brain homogenates at different time-intervals. The values obtained were similar to those reported in the literature (Hauser, 1968; Norton & Poduslo, 1973). The ratio of the amount of cerebroside at different time-intervals to the amount of cerebroside in the brain homogenate at 19 days was calculated.

Fig. 1. Specific radioactivity of cerebroside of myelin and microsomal fractions of brain after an intracerebral injection of [U - ^{14}C]serine into 19-day-old rats

The specific radioactivities of cerebroside of microsomal fraction (●), myelin (▲) and of myelin corrected for dilution by newly formed non-radioactive cerebroside (■) are shown. The specific radioactivity of the acid-soluble pool (Δ) is expressed as d.p.m./ng of phosphorus. Brains from two to three animals were pooled for each time-point. The inset shows the specific radioactivity of myelin cerebroside after correction, plotted on a semi-logarithmic plot for the determination of the half-life.

This ratio for each time-interval was multiplied by the apparent specific radioactivity to obtain the corrected specific radioactivities of the myelin fractions owing to the deposition of newly synthesized cerebroside. The corrected values were replotted (Fig. 1); after correction, the position of the peak was not significantly altered, indicating that the newly formed cerebroside did not contribute effectively towards the apparent steady state reached. Although there is a considerable scatter of values for the corrected specific radioactivity of myelin cerebroside, it is evident that the curve is biphasic. After a rise at the early time-points, there is a rapid decline in the specific radioactivity of myelin cerebroside, and the later time-points indicate a slow decline in the specific radioactivity.

Distribution of ^{14}C in myelin and microsomal cerebroside. The distribution of ^{14}C label in the cerebroside isolated from total homogenate at all time-points, as well as from myelin and microsomal fractions at early (1 and 3 days) and at late (100 and 130 days) time-points, was checked. It was determined in the sphingosine, galactose and fatty acid moieties of the cerebroside after methanolysis and phase partitioning (Hoshi *et al.*, 1973). There was no significant difference between the distribution of label in myelin and microsomal cerebroside at the time-points examined. Some 68–72% of the total radioactivity was found in the sphingosine moiety of cerebroside isolated at 1 and 3 days after the injection. About

28–30% of the radioactivity was in the fatty acid moiety, but only 1–2% was in the carbohydrate moiety. At 100 and 130 days after the injection, the distribution of the label in the sphingosine moiety was 77–80%, in the fatty acid 16–18% and in the carbohydrate moiety 2–5%. The distribution of the label in the cerebroside in the whole homogenate at all the time-points checked was similar to that described for microsomal and myelin fractions. Carter & Kanfer (1974) reported a similar distribution of label from [^{14}C]serine into cerebroside of 9-day-old rat brain, 5–10 h after intracerebral injection.

Sulphatides. The specific radioactivity of the sulphatides isolated from myelin and microsomal fractions at various time-points is shown in Fig. 2. The specific radioactivity of the microsomal sulphatides reached a maximum about 1–3 days after the injection and then gradually declined. The specific radioactivity of the myelin sulphatides reached a maximum about 3–4 days after the injection and then declined. Hauser (1968) showed that there is a rapid deposition of sulphatides in myelin when the rats are between 17 and 25 days old. A correction for this deposition was applied by determining the rate of deposition of sulphatides in the brain of these rats (Jungalwala, 1974b). The corrected specific radioactivity of the sulphatides of the myelin is given in Fig. 2.

Phospholipids. The specific radioactivity of phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine in myelin and microsomal fractions

Fig. 2. Specific radioactivity of sulphatides of myelin and microsomal fractions of brain after an intracerebral injection of $[U-^{14}C]$ serine into 19-day-old rats

The specific radioactivities of sulphatides of microsomal fraction (●), myelin (▲) and myelin after correction for dilution by newly formed non-radioactive sulphatides (■) are shown. Brains from two to three animals were pooled for each time-point. The inset shows the specific radioactivity of myelin sulphatides after correction, plotted on a semi-logarithmic plot for the determination of the half-life.

Fig. 3. Specific radioactivity of phosphatidylserine (a) and of phosphatidylethanolamine and phosphatidylcholine (b) of myelin and microsomal fractions of brain after an intracerebral injection of $[U-^{14}C]$ serine into 19-day-old rats

Specific radioactivities of myelin and microsomal phosphatidylserine (□, ■), phosphatidylethanolamine (○, ●) and phosphatidylcholine (△, ▲), are shown. □, ○, △, Myelin; ■, ●, ▲, microsomal fraction. Brains from two to three animals were pooled for each time-point.

of the 19-day-old rats after an injection of $[^{14}C]$ serine is shown in Fig. 3. The specific radioactivity of the microsomal phosphatidylserine was maximum 4h after the injection and then appeared to decline rapidly. The specific radioactivity of the myelin phosphatidylserine reached a maximum about 12h

after the injection and then gradually declined (Fig. 3a). The specific radioactivity of the microsomal phosphatidylserine was higher than that found for microsomal phosphatidylethanolamine, which in turn was higher than that of microsomal phosphatidylcholine (Figs. 3a and 3b). A similar relationship

was found for the myelin phospholipids. Again, the specific radioactivity of microsomal phosphatidylserine reached a maximum before that for microsomal phosphatidylethanolamine and phosphatidylcholine. These results indicate that serine was first incorporated into phosphatidylserine, which was then decarboxylated to phosphatidylethanolamine, which in turn could be methylated to phosphatidylcholine.

Incorporation of [^{14}C]serine in vivo into adult rat brains

Cerebrosides. The incorporation of radioactivity, after an intraventricular injection of [^{14}C]serine, into microsomal and myelin cerebrosides, as well as the radioactivity remaining in the acid-soluble pool, is shown in Fig. 4. Only 10% of the radioactivity of the initial maximum measured after 1 h remained in the acid-soluble pool of the brain after 14 h. The total radioactivity in the acid-soluble pool per brain at 1, 2, 5 and 14 h was 8×10^6 , 2.9×10^6 , 1.6×10^6 and 0.7×10^6 d.p.m. respectively. At 1, 2.75, 6, 10, 20, 43, 72, 95 and 121 days it was 0.6×10^6 , 0.36×10^6 , 0.15×10^6 , 0.3×10^6 , 0.19×10^6 , 0.035×10^6 , 0.012×10^6 , 0.01×10^6 and 0.007×10^6 d.p.m. respectively. The specific radioactivity of the microsomal cerebrosides had a maximum about 2–5 h after the injection and then gradually declined with time up to about 10 days after the injection. After this period, the rate of decline in the specific radioactivity of microsomal cerebrosides was very low. The specific radioactivity of the myelin cerebrosides reached a maximum 24 h after the injection and then declined gradually till about 20 days. After this period the rate of

decline appeared to be minimal. Norton & Poduslo (1973) have concluded that myelination is a continual process in rat even during adult life. We have therefore corrected the observed specific radioactivity of myelin as described for developing animals. The corrected specific radioactivity of myelin is also shown in Fig. 4.

The distribution of the label in the cerebrosides isolated from homogenate as well as from the myelin and microsomal fractions was determined. The results were very similar to those found for the developing animals. Some 70–75% of the total radioactivity of the cerebrosides was in the sphingosine moiety, the fatty acid contained 24–30% and the carbohydrate moiety contained 1–4% of the radioactivity at various time-points examined.

The incorporation into sulphatides of microsomal and myelin fractions from L-serine in adult rats was too low for reliable measurement of specific radioactivity.

Phospholipids. The specific radioactivity of phosphatidylserine and phosphatidylethanolamine of microsomal and myelin fractions of the adult rat brain after an injection of [^{14}C]serine is given in Fig. 5. The specific radioactivity of the microsomal phosphatidylserine appeared to be maximum 2 h after the injection. The specific radioactivity of myelin phosphatidylserine reached a maximum about 24 h after the injection. These results are very similar to those described for the young animals. Again, the specific radioactivity of the microsomal phosphatidylethanolamine was lower than that of micro-

Fig. 4. Specific radioactivity of cerebrosides of myelin and microsomal fractions of brain after an intraventricular injection of [^{14}C]serine into adult rats

The specific radioactivities of cerebrosides of microsomal fraction (●), myelin (▲) and myelin after correction for dilution by newly formed non-radioactive cerebrosides (■) are shown. The specific radioactivity of the acid-soluble pool (△) is expressed as d.p.m./μg of P. Each time-point represents the average of two separate determinations. Brains from two animals were pooled for each time-point. The inset shows the specific radioactivity of myelin cerebrosides after correction, plotted on a semi-logarithmic plot for the determination of the half-life.

Fig. 5. Specific radioactivity of myelin and microsomal phosphatidylserine and phosphatidylethanolamine of brain after an intraventricular injection of $[U-^{14}C]$ serine into adult rats

Specific radioactivities of myelin and microsomal phosphatidylserine ($\square$, $\blacksquare$) and phosphatidylethanolamine ($\circ$, $\bullet$) are shown. $\circ$, $\square$, Myelin; $\bullet$, $\blacksquare$, microsomal fraction. Other details are the same as in Fig. 4.

somal phosphatidylserine and the former reached a maximum about 14h, after the injection, indicating that microsomal phosphatidylethanolamine was probably derived from microsomal phosphatidylserine by the decarboxylation reaction. The specific radioactivity of the microsomal phosphatidylcholine at the maximum was lower (207 c.p.m./ μ g of P) than that of phosphatidylethanolamine and appeared to reach a maximum about 14h–1 day after the injection (not shown in Fig. 5). These results are similar to those obtained with young animals, even though slightly different methods were used for the analysis of phospholipids.

Discussion

In our experiments with young animals, the precursor L- $[U-^{14}C]$ serine was cleared from the brain at a rapid rate, as indicated by the radioactivity present in the acid-soluble pool. Less than 10% of the radioactivity measured 0.5h after the injection was found 2–3 days after the injection (Fig. 1). Thereafter, the rate of decline of the acid-soluble radioactivity was slow, probably owing to a small amount of recycling of the precursor. The incorporation of $[^{14}C]$ serine into microsomal cerebrosides appeared to reach a maximum 4h after the injection, and at longer time-periods the specific radioactivity began to decline at a

rapid rate. However, the incorporation of $[^{14}C]$ serine into myelin cerebrosides continued to increase until 2–3 days after the injection, when negligible amounts of radioactivity in the acid-soluble pool were present in the brain. This would suggest that the myelin was receiving its complement of cerebrosides from other membrane-bound cerebrosides. It is generally accepted that the enzymes that catalyse the synthesis of complex lipids are not present in myelin. Various reports have indicated that in the system *in vitro*, cerebrosides are mainly synthesized by the microsomal fraction of the brain (Morell *et al.*, 1970; Neskovic *et al.*, 1973; Shah, 1971). However, reports have appeared that indicate that there is some synthesis *in vitro* by the myelin (Neskovic *et al.*, 1973; Constantino-Ceccarini & Suzuki, 1975; Chou & Jungalwala, 1976). The pathway for the biosynthesis of cerebrosides in the system *in vivo* still remains unclear (Carter & Kanfer, 1974). Nevertheless, it is generally accepted that the major site of synthesis of complex lipids, including cerebrosides, is in the endoplasmic reticulum (microsomal fraction). The rate of appearance of radioactivity in myelin cerebrosides suggests that there could be a precursor-product relationship between cerebrosides of the microsomal fraction and myelin. A similar precursor-product relationship between the microsomal fraction and myelin has also been observed for sulphatides

(Herschkowitz *et al.*, 1968; Jungalwala, 1974b), phospholipids (Jungalwala, 1974a), and galactosylglycerides (Inoue *et al.*, 1971).

The total amount of incorporation of radioactivity from L-serine into adult myelin and microsomal cerebroside was relatively low compared with the developing animals (Figs. 1 and 4). The rate of elimination of the injected precursor (as represented by the radioactivity in the acid-soluble pool) as well as the rate of incorporation into myelin and microsomal cerebroside in adult animals appeared to be similar to that in the young animals. The presence of negligible amounts of radioactivity in the acid-soluble pool just before the maximum specific radioactivity of myelin cerebroside was attained, and the kinetics of incorporation of radioactivity into microsomal and myelin cerebroside, indicate the possibility of a precursor-product relationship between cerebroside of these membranes of the adult animals.

In both developing and adult animals, biphasic curves for the decline in specific radioactivity of myelin cerebroside were obtained (Figs. 1 and 4). The persistence of radioactivity in the cerebroside at longer time-periods might result from recycling of the precursor. However, the very low amount of radioactivity found in the acid-soluble pool, especially at longer time-periods, as well as similar distribution of label, in the cerebroside moiety at various time-intervals cannot completely account for the recycling of the precursor effectively contributing towards the synthesis of cerebroside. The biphasic curves then would suggest the existence of two different pools with different turnover rates. Such biphasic decay curves were also observed in our study on the turnover of phospholipids in myelin and other brain subcellular membranes (Jungalwala & Dawson, 1971; Jungalwala, 1974a) and also by other investigators (Pasquini *et al.*, 1973). The half-lives of the fast turning-over pool of cerebroside of myelin in developing and adult animals were 7 and 22 days respectively (insets of Figs. 1 and 4). The half-life of the slowly turning-over pool of myelin cerebroside was around 145 days, both for developing and adult animals. The difference in the half-life of the fast turning-over pool of the developing and adult animals indicates a different rate of metabolism of cerebroside in myelin of developing and adult animals. The turnover rates of more stable myelin cerebroside in both groups of animals are about the same. It should be pointed out that the turnover times reported here for myelin are probably overestimated, since the labelling in the myelin cerebroside is presumably acquired from the microsomal fraction and thus is not a pulse-labelling.

The specific radioactivity of microsomal cerebroside from both developing and adult rats appeared to decline in a triphasic manner (Figs. 1 and 4). The half-life of the rapidly turning-over microsomal cerebro-

side was calculated to be 20h for the developing animals and 40h for the adult animals. The microsomal cerebroside, which turned over at an intermediate rate, had half-lives of about 11 days for both groups of animals, whereas the slowly turning-over microsomal cerebroside had a half-life of about 60 days for both groups.

The presence of pools with different turnover rates could be explained in many different ways, such as recycling of the precursor, different cell types having different metabolism, various molecular species, such as various fatty acid chains or possibly glucocerebroside turning over at different rates, or topographical location of certain molecules in a membrane being different from the others. It would be of interest to determine which of these factors contribute towards the various metabolic pools.

The results obtained with the incorporation of [^{14}C]serine into sulphatides of microsomal and myelin fractions of developing brains were similar to those reported with $^{35}\text{SO}_4$ as the precursor (Jungalwala, 1974b). With [^{14}C]serine, the incorporation into sulphatides of myelin and microsomal fractions is delayed compared with $^{35}\text{SO}_4$ since [^{14}C]serine presumably is first incorporated into its obligatory intermediate cerebroside which are then sulphated. $^{35}\text{SO}_4$, however, is converted more readily into adenosine phosphate sulphatophosphate, which then donates the sulphate moiety to cerebroside. The half-life of myelin sulphatides in developing animals, based on corrected specific radioactivity, was 160 days (see inset of Fig. 2). The half-life is longer than the half-life (about 120 days) calculated with $^{35}\text{SO}_4$ as the precursor in the developing animals. The longer half-life obtained with [^{14}C]serine could be due to the metabolic nature of the precursor. Cerebroside that are formed from [^{14}C]serine have a relatively low turnover rate and thus do not act as a precursor-pulse for the sulphatides.

The biosynthesis of phosphatidylserine *in vivo* in mammalian tissues still remains unknown. It has been demonstrated in a system *in vitro* that phosphatidylserine is biosynthesized by a base (serine) exchange reaction with pre-existing phospholipids (Porcellati *et al.*, 1971; Saito *et al.*, 1975). The incorporation of [^{14}C]serine into rat brain microsomal phosphatidylserine was as rapid as that found for cerebroside in both the developing and adult animals. The specific radioactivity of the microsomal cerebroside of the developing rat brain was slightly lower than that of phosphatidylserine at the maximum, 45 and 72 d.p.m./nmol respectively. However, in the case of adult animals the specific radioactivity of the microsomal cerebroside at the maximum was only 7.7 d.p.m./nmol, as compared with 55.6 d.p.m./nmol found for phosphatidylserine. If it is assumed that the microsomal fractions of young and adult rat brain have the same proportion of cerebroside to phosphatidyl-

serine, then these results would indicate that in the adult animals, the synthesis of microsomal cerebro-sides from L-serine is greatly decreased and large amounts of the precursor are directed towards the synthesis of phosphatidylserine. In the developing animals, however, considerable amounts of cerebro-sides are being synthesized from L-serine, besides phosphatidylserine.

The time-course of the incorporation into myelin phosphatidylserine indicated that a precursor-product relationship could exist between microsomal and myelin phosphatidylserine. The half-life of myelin phosphatidylserine in developing and adult rat brain could not be calculated accurately for the fast turning-over pool, owing to insufficient information at the critical points of decline. The half-life of the slowly turning-over myelin pool was about 45–50 days for both adult and developing animals. The half-life of microsomal phosphatidylserine was calculated to be about 8 h for the fast turning-over pool, in both developing and adult animals.

It has been suggested that nervous tissue has limited ability to synthesize the choline moiety of phosphoglycerides by a successive methylation of ethanolamine phosphoglycerides (Ansell & Spanner, 1967). We found that radioactivity from L-[¹⁴C]serine was incorporated into phosphatidylethanolamine and phosphatidylcholine of brain microsomal fraction and myelin (Fig. 3b). This incorporation is likely to represent initial decarboxylation of phosphatidylserine to phosphatidylethanolamine (Wilson *et al.*, 1960; Abdel-Latif & Abood, 1966) and possible subsequent methylation to phosphatidylcholine (Bremer *et al.*, 1960). In earlier studies (Jungalwala & Dawson, 1971) we also found some incorporation into phosphatidylcholine after an intracerebral injection of [¹⁴C]ethanolamine. Since in both cases the phospholipids were subjected to alkaline ethanolysis, the presence of radioactivity in the choline phospholipids is not due to label in the fatty acid moiety. However, the exact position of this label in glycerylphosphorylcholine was not determined. Therefore the possibility that the labelling found in the glycerylphosphorylcholine could be due to some incorporation into glycerol moiety from serine cannot be ruled out (Ansell & Spanner, 1962). Henning & Stoffel (1969) have reported that the bases of phosphatidylethanolamine and phosphatidylcholine of liver are predominantly derived from sphingosine, when serine and the long-chain base are simultaneously injected into rats. The possibility thus exists that some of the incorporation observed into these phospholipids in brain could be via the breakdown products of sphingosine derived from serine. It was observed, in developing animals, that the specific radioactivity of phosphatidylethanolamine of myelin was slightly higher than that of the microsomal fraction at most of the time-intervals examined (Fig. 3b). This appears

unusual, but it should be pointed out that we did not examine the critical time-interval between 1 and 2 days, where the specific radioactivity of phosphatidylethanolamine in the microsomal fraction could be still increasing. Alternatively, the significant difference in the pool size of diacylglycerolphosphorylethanolamine of myelin and the microsomal fraction could account for this result. The pool size of the myelin diacylglycerolphosphorylethanolamine is very small (20%), and most of the ethanolamine phosphoglycerides are present in the alkenylacyl and alkylacyl forms (80%), whereas more than 50% of the microsomal ethanolamine phosphoglycerides are of the diacyl type.

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