# A regional survey of myelin development: some compositional and metabolic aspects

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**Abstract** A survey of differences in composition and metabolism of myelin from five areas of the central nervous system was made in brain and spinal cord slices of the rat from 20 days to 20 months postnatal age. Purified myelin from the forebrain areas showed a composition characteristic of immaturity longer than did myelin from the hindbrain and spinal cord. The trend of chemical maturity is in agreement with the anatomical observations that myelination begins in the hindbrain and proceeds rostrally. Myelin recovery per 100-mg slice increased continually from 20 days to 20 months of age, while the uptake of [1-$^{14}$C]acetate into myelin lipid and of [1-$^{14}$C]leucine into myelin protein decreased precipitously with age. Taking into account the continuous increase in myelin during maturation, a calculation was made of the total amount of incorporation of labeled material into lipids or proteins per 100-mg slice for each region at each age. The metabolic characteristics of myelin from the cerebral cortex (including the corpus callosum), the thalamic area, and the cerebellum were very similar, while myelin from brainstem and spinal cord was metabolically more active, especially at the early ages. Synthesis of lipid in the myelin sheath represents about 50% of the lipid synthesis of the whole brain and about 75% of that of the spinal cord. The proportion of myelin-related protein synthesis is much less, probably less than 10% of the protein synthesis occurring in whole brain and about 15% of that in the spinal cord except at early ages.

**Supplementary key words** metabolism · lipid composition · myelination

One of the fundamental and necessary events in the normal development of the vertebrate central nervous system is the formation of a myelin sheath for insulation of the nerve axon. Myelination proceeds in a regulated and ordered fashion, appearing first in the spinal cord and hindbrain, then advancing rostrally to the forebrain (1). Therefore, not all central nervous system nerve tracts become ensheathed simultaneously, but myelin formation proceeds at maximal rates at somewhat different times in the various brain regions early in the life of the animal. A detailed anatomical study of the times of appearance of myelin in the different brain structures in the rat has been documented by Jacobson (2).

In some degenerative diseases, certain areas of the brain seem to be especially susceptible to demyelinative lesions; for instance, those resulting from exposure to cyanide occur primarily in the corpus callosum (3). A similar localization of the lesion is described for Marchiafava-Bignami disease (4), while in central pontine myelinolysis demyelination is limited to a focus in the pons (5). Metabolic differences of the myelin of these different areas may result in varying vulnerability to the disease process.

The present study was directed toward in vitro measurement of biochemical and metabolic correlates of myelin formation and maintenance in five areas of the central nervous system of the rat throughout the course of development from 20 days to 20 months of postnatal life. A similar survey was reported previously in which whole brain, spinal cord, and peripheral nerve were compared (6).

Rats younger than 20 days were not included in this study because, although myelination is well advanced in the rat hindbrain and spinal cord at 20 days, the forebrain is just beginning to yield measurable amounts of myelin. Furthermore, a higher risk of contamination of the myelin with other ill-defined fractions exists in very young rats in which myelin morphology and composition are changing rapidly.

## METHODS

Wistar rats maintained on Purina lab chow were decapitated at ages 20, 25, 30, 45, 60, and 90 days, and 6, 12, 15, 18, and 20 months, and their brains and spinal cords were quickly removed. The cerebellum and brainstem were cut off at the midbrain and separated from each other, and the thalamic area was carefully peeled out from the cerebrum leaving the corpus callosum attached to the cerebral cortical area. The four parts of the brain, cerebral cortex plus corpus callosum, thalamus, cerebellum,

TABLE 1. Composition of myelin lipid

| | Cerebral Cortex | Thalamic Area | Cerebellum | Brainstem | Spinal Cord |
|---|---|---|---|---|---|
| | *% of total lipid* | | | | |
| 20 days | | | | | |
| Total phospholipid | 56.45 | 56.0 | 50.2 | 52.2 | 51.1 |
| PS + Sphing + PI[a] | 13.8 | 13.4 | 11.5 | 11.4 | 12.9 |
| Lecithin | 23.6 | 22.0 | 18.6 | 16.8 | 16.6 |
| Ethanolamine phosphatide[b] | 19.2 | 21.2 | 19.8 | 21.8 | 20.1 |
| Galactolipid | 20.2 | 20.1 | 27.8 | 25.5 | 26.3 |
| Cholesterol | 23.2 | 23.8 | 23.6 | 24.7 | 23.7 |
| 25 days | | | | | |
| Total phospholipid | 52.0 | 51.9 | 50.2 | 46.3 | 47.8 |
| PS + Sphing + PI | 13.0 | 12.8 | 12.4 | 11.8 | 13.1 |
| Lecithin | 18.5 | 18.6 | 17.7 | 14.9 | 13.7 |
| Ethanolamine phosphatide | 20.5 | 20.5 | 20.4 | 19.4 | 20.7 |
| Galactolipid | 25.2 | 26.4 | 29.5 | 34.0 | 31.0 |
| Cholesterol | 22.8 | 21.6 | 20.3 | 20.3 | 21.1 |
| 30 days | | | | | |
| Total phospholipid | 46.6 | 47.8 | 46.1 | 44.0 | 45.4 |
| PS + Sphing + PI | 11.5 | 12.2 | 12.5 | 12.1 | 12.6 |
| Lecithin | 15.5 | 15.7 | 13.9 | 12.9 | 13.1 |
| Ethanolamine phosphatide | 19.6 | 20.1 | 19.7 | 19.1 | 19.6 |
| Galactolipid | 32.3 | 31.5 | 34.3 | 36.3 | 35.5 |
| Cholesterol | 21.1 | 20.6 | 19.6 | 20.5 | 20.2 |
| 60 days | | | | | |
| Total phospholipid | 47.1 | 45.8 | 44.7 | 45.6 | 45.5 |
| PS + Sphing + PI | 12.9 | 12.5 | 12.5 | 12.6 | 13.1 |
| Lecithin | 13.7 | 12.8 | 12.8 | 12.2 | 12.3 |
| Ethanolamine phosphatide | 19.9 | 20.2 | 20.1 | 20.4 | 20.2 |
| Galactolipid | 29.6 | 32.5 | 32.9 | 33.3 | 33.1 |
| Cholesterol | 23.2 | 21.8 | 20.9 | 22.9 | 21.6 |
| 90 days | | | | | |
| Total phospholipid | 44.2 | 43.3 | 42.8 | 42.6 | 42.7 |
| PS + Sphing + PI | 11.6 | 12.6 | 13.5 | 13.7 | 13.8 |
| Lecithin | 13.5 | 13.3 | 10.3 | 10.3 | 10.9 |
| Ethanolamine phosphatide | 19.0 | 18.8 | 18.4 | 18.75 | 18.5 |
| Galactolipid | 33.5 | 34.5 | 37.9 | 33.4 | 33.6 |
| Cholesterol | 22.3 | 22.7 | 19.8 | 22.0 | 22.5 |
| 6 months | | | | | |
| Total phospholipid | 42.0 | 43.3 | 42.7 | 41.8 | 42.0 |
| PS + Sphing + PI | 11.5 | 11.0 | 12.1 | 11.8 | 13.1 |
| Lecithin | 12.5 | 11.8 | 11.8 | 11.5 | 9.8 |
| Ethanolamine phosphatide | 18.8 | 19.5 | 18.8 | 18.5 | 19.3 |
| Galactolipid | 34.5 | 34.2 | 36.0 | 36.9 | 35.4 |
| Cholesterol | 22.9 | 22.5 | 21.4 | 24.0 | 22.3 |

Each number represents a single determination.

[a] Phosphatidylserine (PS), sphingomyelin (Sphing), and the inositide phosphoglycerides (PI) were pooled as one determination.

[b] Ethanolamine phosphatide contained both the plasmalogen and the diacyl compound.

and brainstem, represented about 52, 20, 13, and 12%, respectively, of the wet weight of the brain; the spinal cord served as the fifth area for study. Tissues from each of these areas from two to six rats (equal numbers of males and females) were pooled, and 0.5-mm-thick slices were prepared with a Stadie-Riggs tissue slicer. About 600 mg of slices (wet wt) was used for each incubation.

The tissue slices were incubated for 2 hr in 25-ml Erlenmeyer flasks containing 6 ml of 5 mM glucose in Krebs-Ringer bicarbonate buffer, pH 7.4, and 10 $\mu$Ci each of DL-[1-$^{14}$C]leucine and [1-$^{14}$C]sodium acetate. The radioactive compounds were purchased from New England Nuclear Corp., Boston, Mass. The specific activities were 6.0 mCi/mmole for leucine and 2.0 mCi/mmole for sodium acetate. The flasks were gassed with 95% $O_2$–5% $CO_2$. At the end of the 2-hr incubation at 37°C the flasks were chilled. The slices were centrifuged free from the medium, washed with ice-cold distilled water, and then centrifuged again. Purified myelin and nonmyelin residual fractions were prepared according to methods previously described (7) involving an initial separation on a discontinuous sucrose gradient (10.5% sucrose layered over 30% sucrose), two water washes of the separated fractions, and a second discontinuous gradient. These fractions were prepared as quantitatively as possible, and the nonmyelin fraction was also subjected to the second gradient centrifugation in order to recover as much myelin as possible.

The myelin and nonmyelin pellets were extracted with chloroform–methanol 2:1 (v/v), and the lipids and proteins were isolated. Specific activity measurements were made according to previously published methods (7). Myelin lipids were separated by one-dimensional thin-layer chromatography using chloroform—methanol—7 N $NH_4OH$ 120:70:9 (v/v/v). The spots were visualized by iodine vapor, and appropriate areas of silica gel were scraped from the plates. Phospholipid, galactolipid, and cholesterol were measured by methods previously described (7).

Lipid weights were used as a measure of recovery of all fractions. For purposes of quantitation of the recovered fractions, myelin was considered to consist of 80% lipid and 20% protein, while the residual membranes were assumed to be 50% lipid and 50% protein (8). Two series of analyses were completed, and experiments at some ages were carried out a third time.

There was some crossover of the two $^{14}$C-labeled compounds, i.e., some radioactive acetate was incorporated into protein and some radioactive leucine into lipid. Acetate labeling of protein was less than 10% of the labeling with leucine. All proteins were homogenized in acidified chloroform–methanol, but some of the label may have resulted from residual lipid bound to the protein. Leucine labeling of lipid was less than 5% of the labeling with acetate.

## RESULTS

### Regional changes in myelin composition with age

The lipid composition of the purified myelin from the younger rats was not constant from area to area. At 20 days of age, the myelin phospholipid represented a slightly higher proportion of total lipids in the cerebral cortex and

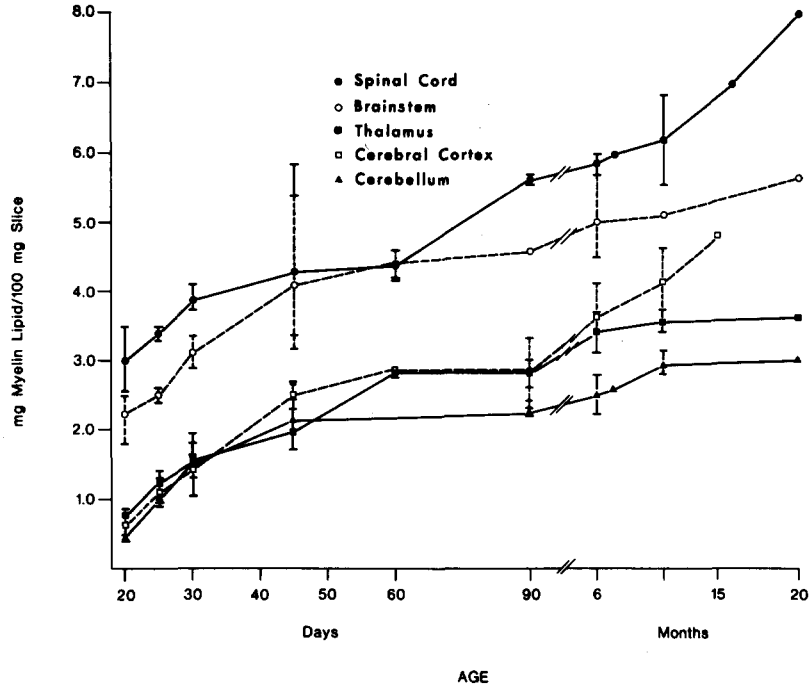


**Fig. 1.** Recovery of myelin lipid/100-mg slice from five areas of the central nervous system of rats aged 20 days to 20 months. Each point represents two or three different experiments; vertical lines indicate ranges.

thalamus than in the cerebellum, brainstem, and spinal cord (Table 1). Furthermore, at early ages the proportion of lecithin was considerably higher and that of galactolipid considerably lower in myelin from the forebrain areas than in myelin from the hindbrain and spinal cord. As maturation progressed, a constant composition typical of normal adult myelin (9) was gradually attained. The values shown in Table 1 are the results of a single determination, and much variability in stage of maturation was evident among individual animals. Nevertheless, the lipid compositions showed a trend toward earlier maturation in cerebellum, brainstem, and spinal cord, with myelin lipid from cerebral cortex and the thalamic area reaching the same adult composition somewhat later.

## Recovery

Although recovery was somewhat variable at some ages, it is clear that the amount of myelin as measured by my-

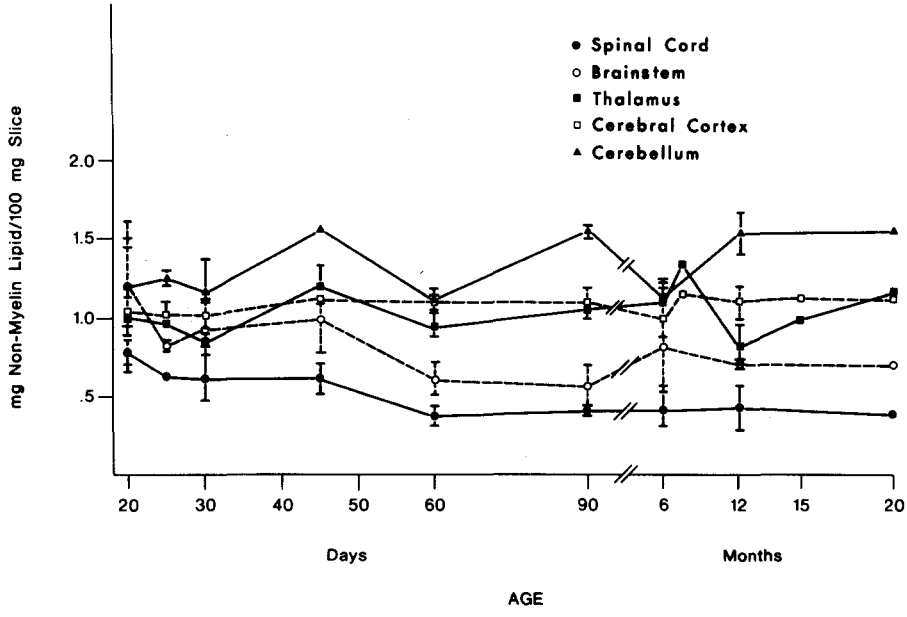


**Fig. 2.** Recovery of nonmyelin lipid/100-mg slice from five areas of the central nervous system as in Fig. 1.

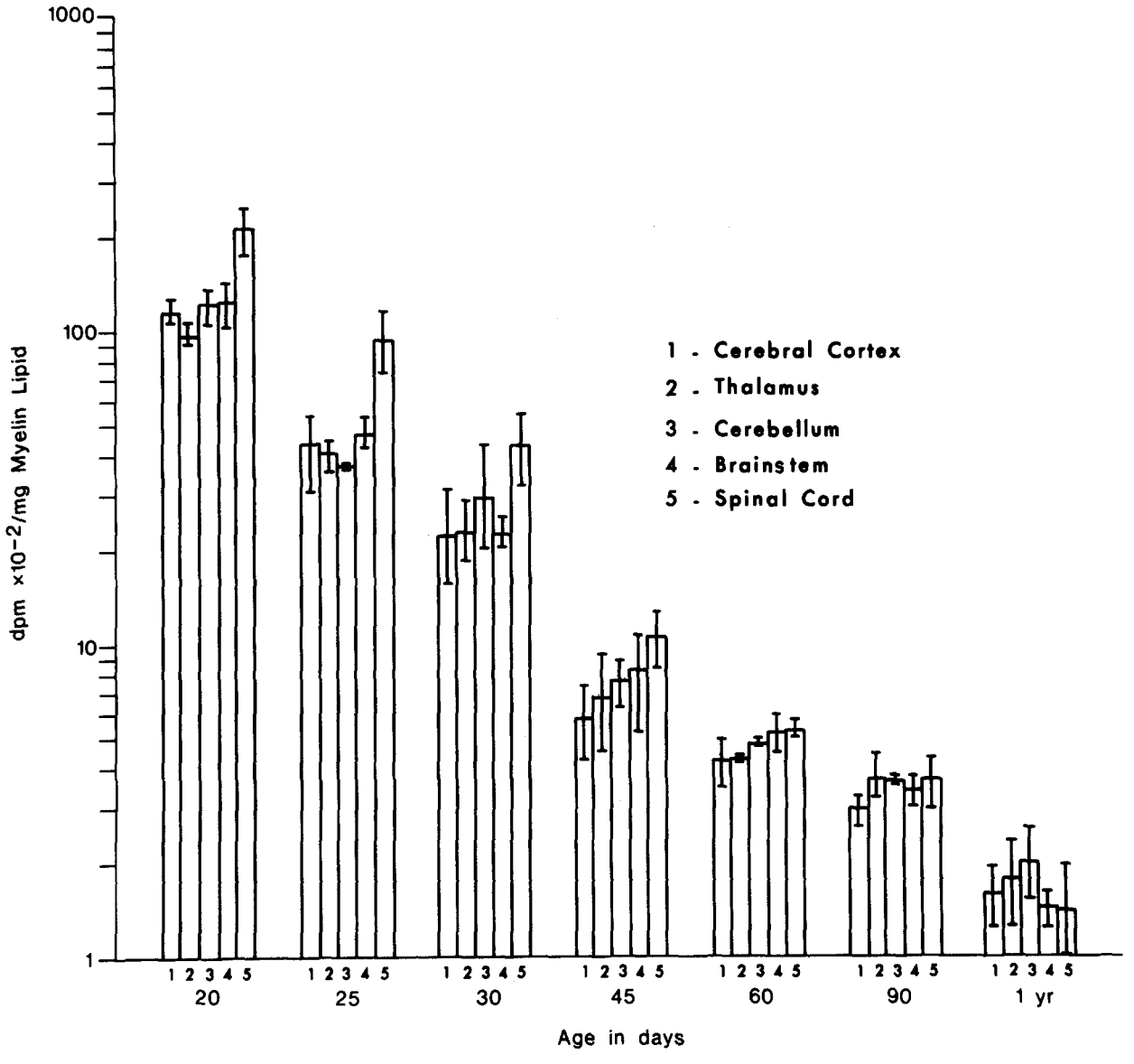


**Fig. 3.** Specific activities of lipids of myelin purified from 600 mg of slices from five areas of the central nervous system of rats of different ages after 2-hr incubation at 37°C in 6 ml of Krebs-Ringer bicarbonate buffer, containing 5 mM glucose, 10 $\mu$Ci of [1-$^{14}$C]sodium acetate, and 10 $\mu$Ci of DL-[1-$^{14}$C]leucine. Each point represents the average of two or three different experiments; vertical lines indicate ranges.

elin recovery per 100-mg slice increased throughout the life of the rat in all areas studied (Fig. 1). The myelin content of the slices increased at the greatest rate between 20 and 45 days, but after this time there was a slow, steady increase even after 1 yr. It has been noted previously that the myelin in the whole brain increases throughout the life of the rat (6, 10), and our measurements show that the amount of myelin per fixed weight of tissue also increases. The recovery of myelin lipid per 100-mg slice was approximately the same for cerebral cortex, thalamic area, and cerebellum up to 30 days, after which divergence in the total content occurred. The recovery of lipid in the residual nonmyelin fraction of the cerebral cortex, thalamic area, and cerebellum did not change appreciably during development of the rat, although this fraction in the brainstem and spinal cord appeared to decrease until 60 days of age, and then it remained relatively constant (Fig. 2). The increase in myelin, therefore, does not appear to occur at the expense of the nonmyelin fraction.

### Uptake of [1-$^{14}$C]acetate into membrane lipids

The specific activities of the total lipids extracted from purified myelin from the different areas of the brain did not show appreciable differences from each other at any of the ages studied, although these values decreased in all areas with increasing age (Fig. 3). The specific activity of myelin from spinal cord, however, was almost double that of the brain areas up to 30 days of age, then gradually decreased to the same levels as the brain. Although synthesis of myelin lipids did not appear to differ much among the

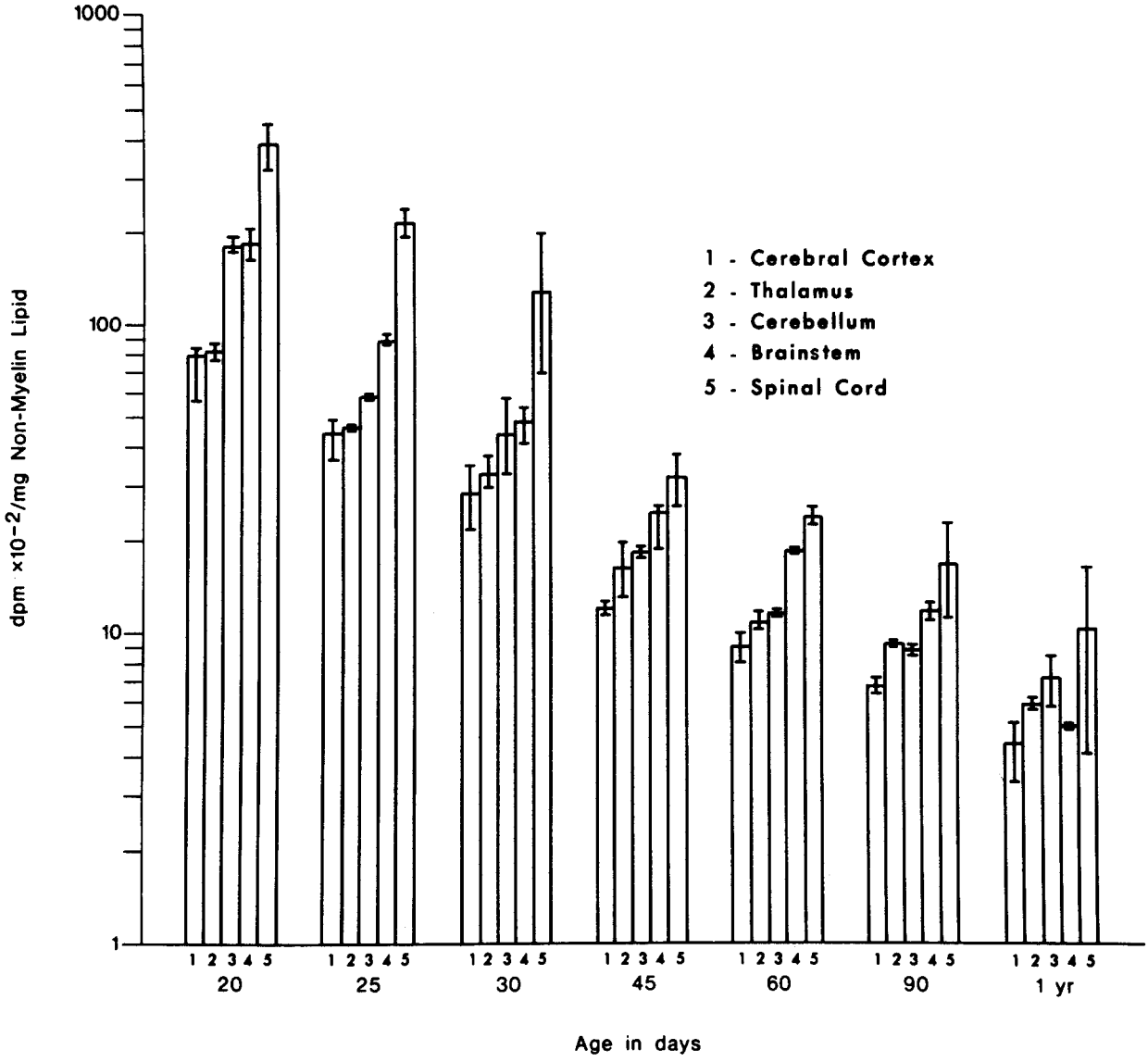


**Fig. 4.** Specific activity of lipids of the residual nonmyelin fraction after myelin is removed from slices from five areas of the central nervous system incubated as in Fig. 3.

different brain structures at any one age, lipid synthesis in the nonmyelin membrane fractions showed much more variability (Fig. 4). The lipids in the residual membranes from the cerebral cortex and thalamus were the least radioactive at all ages, and those of the spinal cord the most radioactive, especially at the early ages.

### Uptake of [1-$^{14}$C]leucine into membrane protein

Incorporation of [1-$^{14}$C]leucine into myelin protein was relatively uniform for the five areas of the central nervous system (Fig. 5). Although there were some differences at early ages, the specific activity of the spinal cord protein was in the same range as the protein of myelin from the four brain areas. Although the rate of synthesis decreased precipitously at early ages, a fairly stable rate was reached by 60 days. Whereas the rate of uptake of radioactive precursors into the membrane components of myelin lipid and protein and nonmyelin lipid declined rapidly to a small fraction of the rate at 20 days, the uptake of [1-$^{14}$C]leucine into nonmyelin residual protein declined much less with age and still showed comparatively high activity after 1 yr (Fig. 6). After 20 days of age, the cerebral cortex and cerebellum generally showed the lowest rate of protein synthesis in the nonmyelin fraction, the spinal cord the highest.

### Total radioactivity in myelin

Although the specific activities of the myelin fractions decreased with age, the total amount of myelin that was labeled increased with age, and therefore the newly labeled myelin was diluted by a factor that was constantly increasing. In order to arrive at a more accurate assessment of the real metabolic activity with increasing age, a calculation was performed whereby the average specific

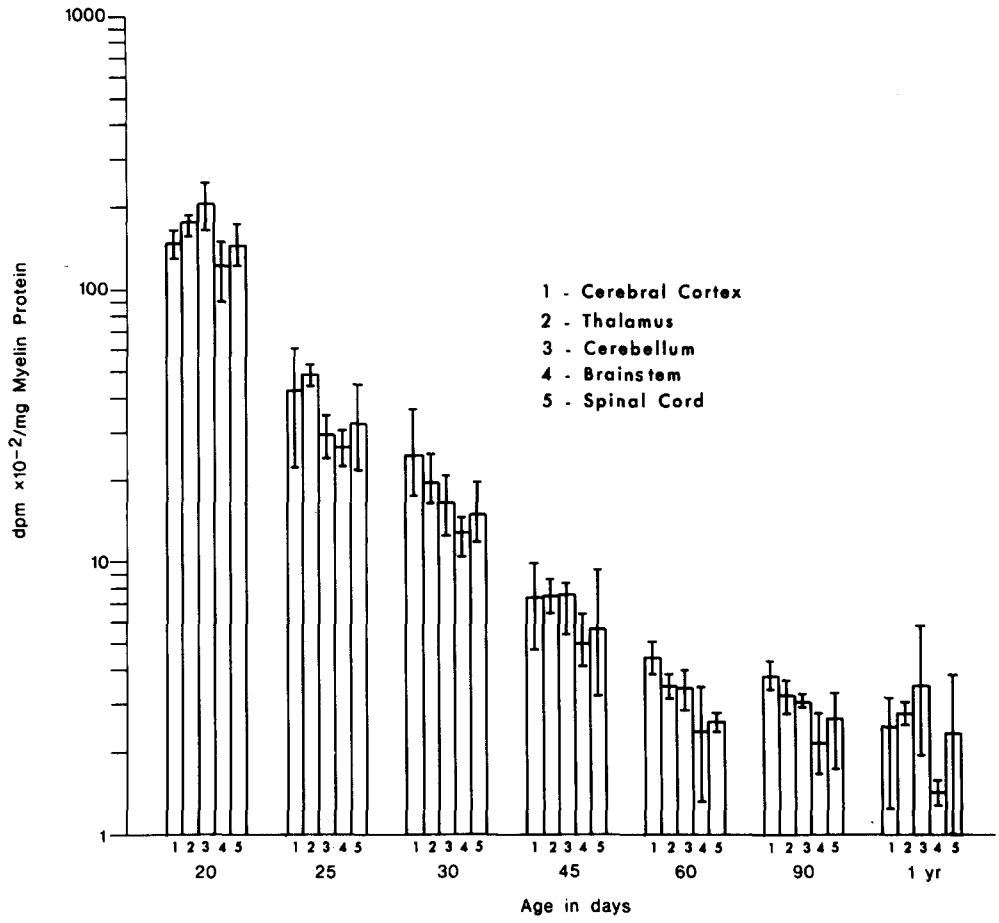


**Fig. 5.** Specific activity of protein of myelin purified from slices from five areas of the central nervous system incubated as in Fig. 3.

activity of the myelin lipid or protein at a given age was multiplied by the amount of myelin recovered per 100-mg slice. This value should be in proportion to the total amount of lipid or protein synthesis in myelin per 100-mg slice. The amount of the total radioactivity in myelin lipid in the cerebral cortex, thalamic area, and cerebellum was comparable at all ages, whereas the brainstem and spinal cord showed much larger values early in the development of the rat brain (Fig. 7). From 6 months of age, the total uptake of radioactivity was comparable in all parts of the brain. A similar pattern was seen for the total uptake into myelin protein except that metabolic activity of the brainstem approached that of the other three parts of the brain by 30 days of age, and by 60 days the levels in all five parts of the central nervous system were similar (Fig. 8). The total uptake of radioactivity in both protein and lipid in myelin decreased with age, but not quite as precipitously as the specific activities.

Using the values obtained for total radioactive uptake, an estimation may be obtained of the proportion of active lipid or protein synthesis attributable to myelin synthesis and/or maintenance under our in vitro resting conditions. The value representing the total uptake of [1-$^{14}$C]acetate into myelin lipids was divided by the sum of the total uptake into myelin lipids and the total uptake in nonmyelin lipids. It has been determined that this sum represents at least 90% of total lipid-bound radioactivity in our slices, with supernates and washings containing less than 10% (7). In each area of the brain, the proportion of lipid synthesis involved in myelin was a fairly constant proportion of that in the whole tissue (Fig. 9). Thus, about 50% of the acetate uptake into the cerebral cortex and thalamic area is found in the myelin sheath at all ages, about 40% in the myelin of cerebellum, except at 20 days where the nonmyelin bulk is relatively high and comparatively active, more than 60% in the brainstem, and approximately 70% in the spinal cord (Fig. 9). Thus, a considerable proportion of acetate uptake into lipids is attributable to myelin metabolism under our in vitro resting conditions.

Similar calculations for leucine uptake into protein showed that except at early ages the synthesis of myelin-bound protein represents a much smaller proportion of total protein synthesized, less than 15% in all areas (Fig. 10).

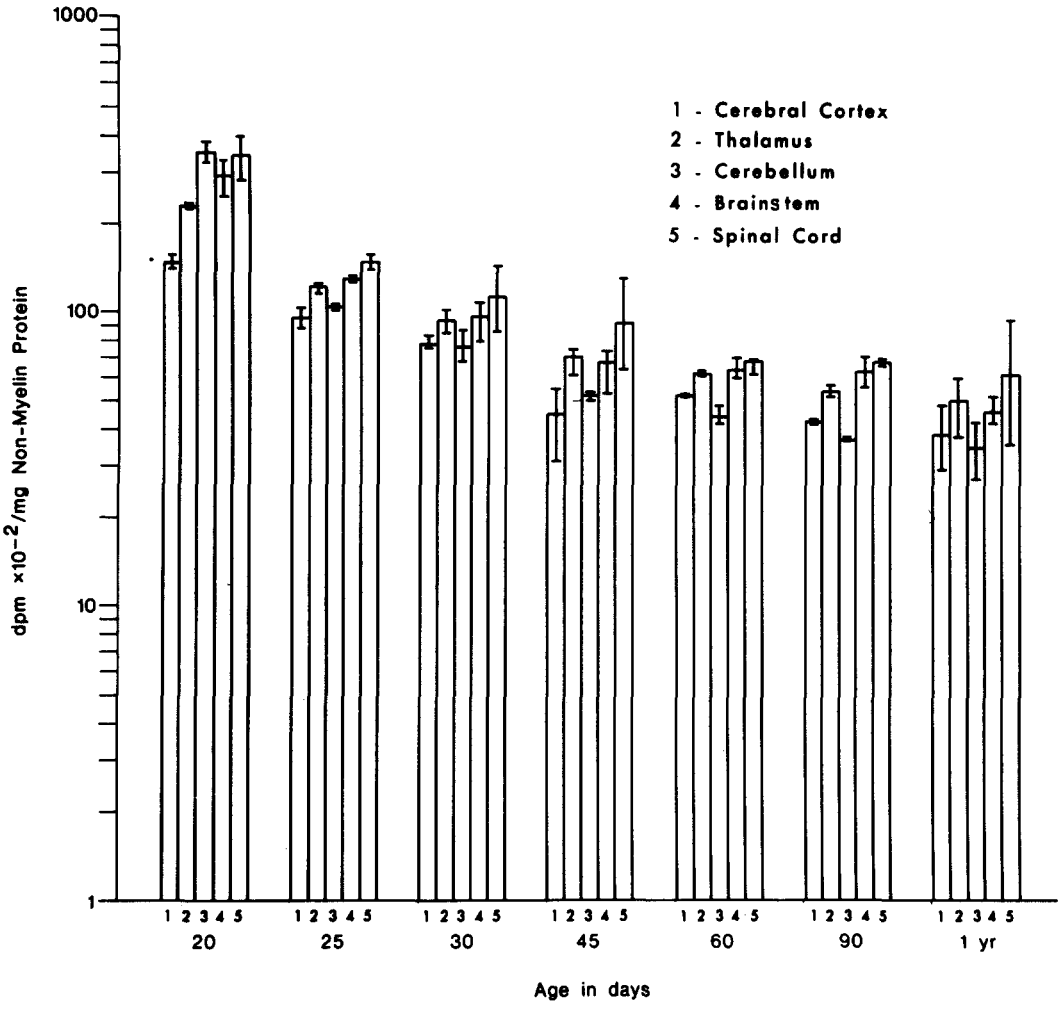


**Fig. 6.** Specific activity of protein of the residual nonmyelin protein from slices from five areas of the central nervous system incubated as in Fig. 3.

## DISCUSSION

Changes in the lipid composition of myelin with age have been noted in whole brain by various investigators (10–13). In general, these changes have shown that immature myelin contains a higher proportion of phospholipid, especially lecithin, and lower amounts of galactolipid than is present in mature myelin. The distribution of lipid in our myelin preparations showed a similar trend. At 20 days the myelin of the cerebral cortex, which is the most immature portion of the brain, had a considerably higher content of lecithin and a lower content of galactolipid than myelin from the more mature brainstem and spinal cord. This relationship held up to 90 days, at which time cerebellum, brainstem, and spinal cord reached the mature composition. At this age lecithin was still higher in cerebral cortex and thalamus. Although our results are based on a single determination, it is evident that the hindbrain attains the lipid composition characteristic of mature myelin before the forebrain. Finally, at 6 months of age, myelin lipid from all areas had a constant composition. It is possible that the various compositions are a reflection of different degrees of contamination in the different preparations by nonmyelin elements, although cerebellar myelin, which is most likely to be contaminated, had a composition that was more mature than that of the myelin preparations from the cerebral cortex. The composition typical of immature myelin has been attributed to a "myelinlike" fraction isolated with the myelin (14). No efforts were made in these experiments to exclude this fraction from the preparations, which were made by a procedure modified from Laatsch et al. (15).

It has been noted previously in our laboratory and elsewhere (6, 10) that myelin is continuously laid down, and therefore increases in total quantity throughout the life of the rat. Whether this also occurs in other vertebrates is not known. Since the weight of the rat brain does not increase appreciably after 60 days, it must be presumed that myelin represents an increasing proportion of the wet weight of the brain. Our experiments have shown that the

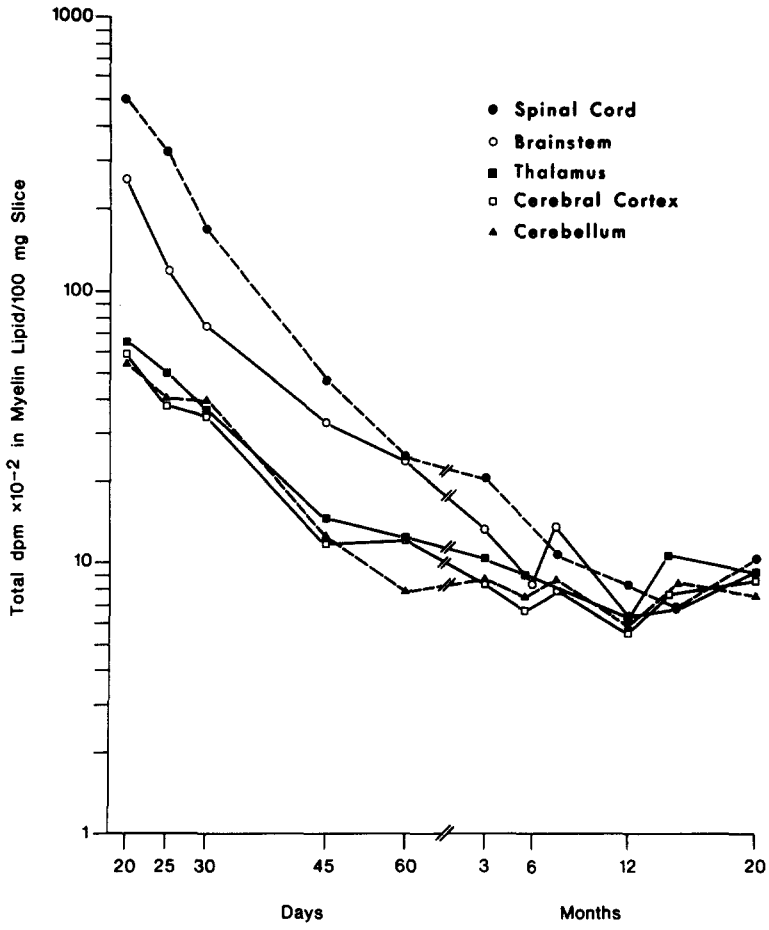


**Fig. 7.** Total radioactivity in myelin lipid/100-mg slice in different areas of the central nervous system from 20-day-old to 20-month-old rats. The values were obtained by multiplying the average recoveries of myelin lipids shown in Fig. 1 by the corresponding average specific activities of myelin lipids in Fig. 3.

myelin recovery per 100-mg slice continually increases in all areas of the brain and spinal cord. The nonmyelin membrane fraction as measured by lipid weight was relatively constant after 60 days. One may speculate as to the nature of the substance replaced as myelin increases without an increase in the weight of tissue. In studies of the composition of the developing brain, Agrawal, Davis, and Himwich (16, 17) found a decrease in water content with increasing age in mouse and dog brains. Thus, it is possible that myelin may replace water.

The specific activities of lipids and protein from the nonmyelin fraction must be proportional to the total radioactive uptake of acetate or leucine into lipid or protein since this fraction did not change per fixed weight of tissue. The amount of myelin, however, increased by a factor of four in the forebrain between 20 and 90 days, while the specific activity of the myelin lipids after a 2-hr incubation with [1-$^{14}$C]acetate decreased by a factor of 40 (Fig. 3). The total uptake of radioactive acetate into myelin lipid per 100-mg slice still decreased markedly, but only by a factor of 7 (Fig. 7). These relationships held throughout the different areas of the nervous system and must be taken into consideration when assessing turnover or metabolic activity. This point has been discussed elsewhere in regard to whole brain, spinal cord, and peripheral nerve (6).

The amounts of total uptake of radioactive acetate into myelin per 100-mg slice from the forebrain areas, the thalamic area, and the cerebellum are very similar, and the curves representing these in Fig. 7 are almost superimposable. This is surprising in view of the late appearance of myelin in the corpus callosum and cerebral cortex (about 18 days of age) relative to the cerebellum (about 12–13 days) (2) and its more immature composition up to 90 days of age. Although at 20, 25, and 30 days the myelin recovery per 100-mg slice was essentially the same for cerebral cortex, thalamic area, and cerebellum, the cerebellum had already achieved 50% of the myelin content at 20 months while the cerebral cortex and thalamic area contained 30 and 40%, respectively, of the myelin present at 20 months. Since myelination begins earlier in the cerebellum, it progresses to a more mature stage by 20 days,

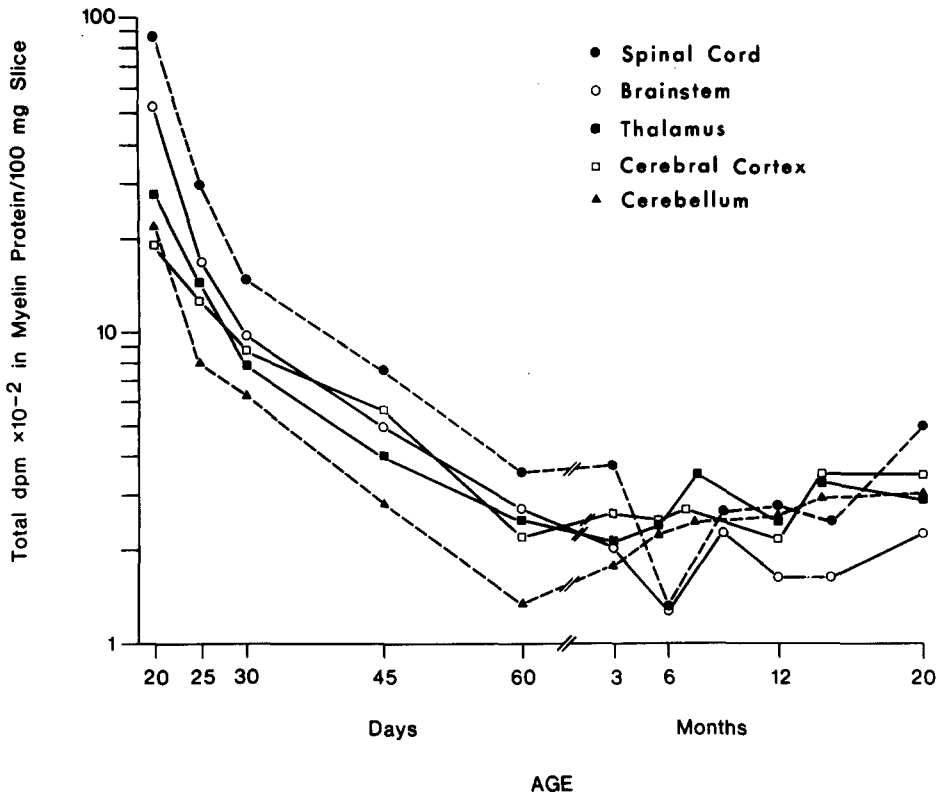


**Fig. 8.** Total radioactivity in myelin protein/100-mg slice in different areas of the central nervous system from rats 20 days old to 20 months old. The values were obtained by multiplying one-fourth of the average recovery of myelin lipid shown in Fig. 1 by the corresponding average specific activity of myelin protein shown in Fig. 5.

the earliest age studied here. If the composition typical of immature myelin is due to contamination of a "myelin-like" fraction (14), the metabolic activity of this fraction could not be appreciably higher than that of myelin of this and other areas.

It is likely that the decreasing rate of uptake of [1-$^{14}$C]acetate into myelin lipids is a result of factors leading to a general "shutdown" of lipid synthesis as a whole, because the proportion of myelin-related lipid synthesis to that in the total brain slice was relatively constant at all ages. One possible mechanism is a change in permeability to certain precursors concomitant with the gradual shift to a glucose-oriented metabolism. This is only a partial explanation, however, because the incorporation of [U-$^{14}$C]glucose into central nervous system myelin lipid also decreases markedly with age (7). Protein synthesis, on the other hand, decreases more in myelin than in the bulk of tissue residue with increasing age. Another factor that may lead to a decrease in the rate of lipid and protein synthesis in myelin is feedback inhibition due to the large amount of myelin. The "shutdown" mechanisms are evidently not irreversible, however, because in certain pathological demyelinating states uptake of [1-$^{14}$C]leucine into myelin protein rises to rates 200–1000% of the normal adult rate (18).

The lipid metabolism of myelin from brainstem and spinal cord differs from that of myelin from other parts of the brain in that the rate of lipid synthesis is far higher at the early ages, and only at 6 months do all areas show the same stable rate of incorporation of radioactive acetate into lipid, after which no further changes occur (Fig. 7). The rate of labeling of myelin protein by [1-$^{14}$C]leucine was stabilized in all areas as early as 60 days, and very little further rate change took place thereafter (Fig. 8). The nonmyelin protein synthesis remained high in all areas throughout, decreasing only by a factor of 3–6 from 20 days to maturity (Fig. 6). Of all areas, the cerebellar protein had the least activity, but since our conditions were for the resting state, it is possible that functional activity may change these relationships.

Whether the different rates of active metabolism seen in the forebrain and the hindbrain are a reflection of differing rates of turnover is not known. Sammeck, Martenson, and Brady (19) have shown that during active myelination incorporation of [$^{3}$H]tryptophan into myelin basic protein and the subsequent decrease in specific activity is greater in the brainstem than in the forebrain. The extent to which the changing bulk of myelin during the course of the experiment influenced the specific activity of the protein was not determined, however. The slow synthesis and

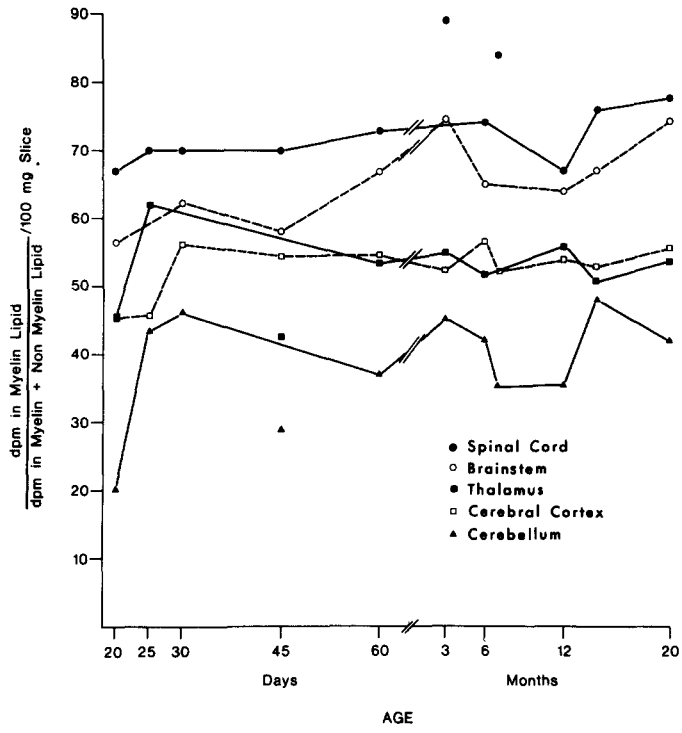


**Fig. 9.** Percentage of total lipid radioactivity in myelin lipid. This number is obtained by the calculation: (total dpm in myelin lipid [Fig. 7])/(total dpm in myelin + nonmyelin lipid).

late maturity of myelin in the forebrain could possibly be related to the susceptibility of the area (i.e., the corpus callosum) to certain metabolic poisons such as cyanide.

Although myelin is commonly believed to be "metabolically inert" with a low rate of turnover, the results of the present study show that the synthesis of myelin lipids may represent a substantial proportion of the lipid synthesis of the brain and spinal cord of both immature and mature rats. In the cerebral cortex and thalamic area, the lipid synthesis in the myelin represented about half the total membrane-bound lipid synthesis in the slice; even in the cerebellum, where myelin is a minor part of its diverse ele-

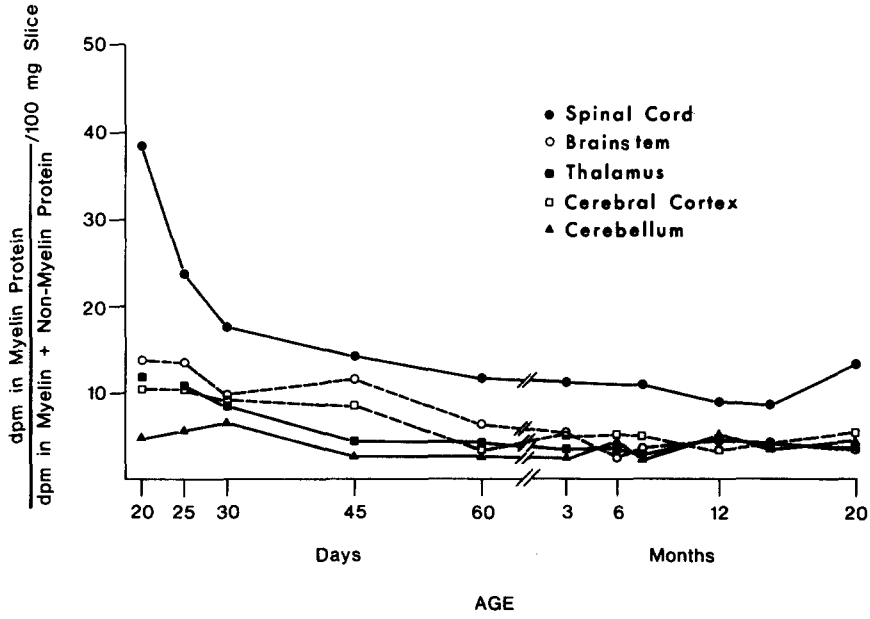


**Fig. 10.** Percentage of total protein radioactivity in myelin protein. This number is obtained by the calculation: (total dpm in myelin protein [Fig. 8]/(total dpm in myelin + nonmyelin protein).

ments, myelin lipid synthesis represented about 40% of the total, while the proportion of myelin-related lipid synthesis in the brainstem and spinal cord was much higher. These estimates reflect the amount of white matter in a given structure and the relatively high proportion of lipid in myelin.

The absence of appreciable turnover of myelin lipid previously found in our in vivo experiments (8, 9) is difficult to explain in view of these in vitro studies. In a recent study by Sun and Horrocks (20), however, the turnover of [1-$^{14}$C]palmitic acid in myelin was found to be much more rapid, with an estimated half-life on the order of 15 days. Extensive reutilization of the $^{14}$C appeared to occur. The recycling phenomenon may be responsible for the long half-lives previously obtained in this laboratory in experiments in which [1-$^{14}$C]acetate (9) and [U-$^{14}$C]glucose (8) were used as lipid precursors where the lipids synthesized are labeled at many sites. Partial breakdown and reutilization of the labeled lipids on a continuing basis may have given the appearance of metabolic stability. It is also possible that palmitic acid, the labeled lipid used by Sun and Horrocks (20), is not representative of the longer-chain fatty acids found especially in the sphingolipids of myelin, and the turnover rate of these may be slower than that of the shorter-chain acids. It would appear, however, that myelin lipid is more active than previously thought, but certain permeability barriers limit its transport away from the myelin structure.

The low proportion of myelin-related protein synthesis reflects the low amount of protein (20–30%) in the myelin membrane. The amount of [1-$^{14}$C]leucine incorporated into myelin did not represent a constant proportion of the incorporation into the membrane-bound protein of the whole slice (Fig. 10), because nonmyelin protein synthesis decreased at a much slower rate than that in myelin. Except in the spinal cord, protein synthesis in the myelin membrane probably represents less than 10% of the total protein synthesis of the central nervous system. This number may be even smaller under nonresting conditions.

This work was supported by the Veterans Administration and by grant no. NS-02785 from the National Institutes of Health. Mrs. Betsy Allyn performed technical assistance.

*Manuscript received 18 July 1972 and in revised form 29 March 1973; accepted 19 April 1973.*

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