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Correlative Biochemical and Morphological Studies of Myelination in Human Ontogenesis*

I. Myelination of the Spinal Cord

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Summary. Biochemical, light and electron microscopic observations in six human fetuses between the 16th and 34th weeks of gestation and five infants, 1 day to 3 years old, are presented. The results indicate that myelination of the human spinal cord started before the 16th week of gestation, as a considerable amount of myelin is isolated at this time biochemically, and occasionally axons with loose myelin coils are observed in the electron microscope. It is also stressed that morphological studies are insufficient to evaluate the completion time of the myelination process, as it can be shown biochemically that qualitative myelin maturation takes a long time.

Key words: Human ontogenesis – Spinal cord myelination

In some nervous system diseases of the childhood it is suggested that there is a connection of the morbid process with changed intrauterine development. Very often, the problem cannot be solved unequivocally because only incomplete data concerning the development of human nervous system are available. The process of myelination is one of the indicators of maturation. Information for the central and peripheral nervous system of man comes chiefly from histological investigations (Langworthy, 1932; Tridon, 1959; Larroche, 1965). Due to unavailability of human fetal material, relatively little biochemical work has been done (Brante, 1949; Cumings et al., 1958, 1965; Svennerholm and Vanier, 1972). Thus, a large part of the biochemical and morphological work relies on the animal material (Kishimoto and Randin, 1958; Hausner, 1968; Cuzner and Davison, 1968; Norton and Poduslo, 1973; Benjamins et al., 1973; Agrawal et al., 1974; Luse, 1956; Peters, 1964; Samorajski and Friede, 1968; Matheson, 1970).

In the present work, it was our purpose to assess, in biochemical and morphological terms, the myelination of the human spinal cord at the selected stages of fetal development.

Material and Methods

The material consisted of six human fetuses between the 16th and 34th weeks of gestation and five children aged 1 day to 3 years. The spinal cord was taken from fetuses 1.5–3 h after termination of pregnancy, and in other cases 9–28 h after death (Table 1).

For histological studies, the material was fixed in 10% calcium-formalin solution for 7–10 days. The section C_8-Th_1 and part of the lumbosacral section were cut and embedded in paraffin. Microtome sections were stained by the Klüver-Barrera method.

For electron microscope examination, the lumbosacral section of the spinal cord was taken and fixed first in 5% glutaraldehyde and then in 1% osmium tetroxide, both in cacodylate buffer, for 2 h at 4° C. Dehydrated in increasing concentrations of alcohol and acetone, the material was embedded in Epon 812, sectioned in LKB III ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a JEM 7 electron microscope. The myelin fractions isolated for biochemical studies were similarly processed to evaluate their purity.

For biochemical investigations, the thoracic section of the formalin-fixed spinal cord was used. The material was washed several times in distilled water at 4° C to remove formalin, dried on Whatman paper grade 1, and weighed. Myelin was separated by the method of Agrawal et al. (1974) in VAC601 (Janetzki) ultracentrifuge in a discontinuous sucrose gradient (Fig. 1). The separated fractions were washed in distilled water and weighed after centrifugation at $100{,}000 \times g$. Light, heavy, and membrane fractions were obtained at sucrose concentrations of 0.32 M, 0.55–0.75 M, and 0.85 M, respectively. Their separation is shown in Fig. 2. Since we worked with formalin-fixed material, the purity of the fractions was determined only by electron microscope examination. Lipids from the fractions were extracted by the method of Folch et al. (1957). Total lipids were determined by the method of Skipski and Barclay (1969). Neutral lipids were analyzed by the use of thin-layer chromatography, according to the micromethod of Müller and Vahar-Matiar (1974).

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Table 1. Material

Number of case	Age	Methods of examination applied			
		biochemical	histological	electron microscopic	
1	16–17 weeks	+	+	+	Fetal cases; interruption of pregnancy for medical or social reasons
2	18 weeks	+	+	–	
3	20 weeks	+	+	+	
4	23–24 weeks	–	–	+	
5	25 weeks	+	+	–	
6	27 weeks	+	+	+	
7	34 weeks	+	+	–	
8	1 day	+	+	–	Infantile cases; death due to diseases not pertaining to the nervous system.
9	10 days	+	+	–	
10	3 months	+	+	+	
11	8 months	+	+	–	
12	3 years	+	+	–	

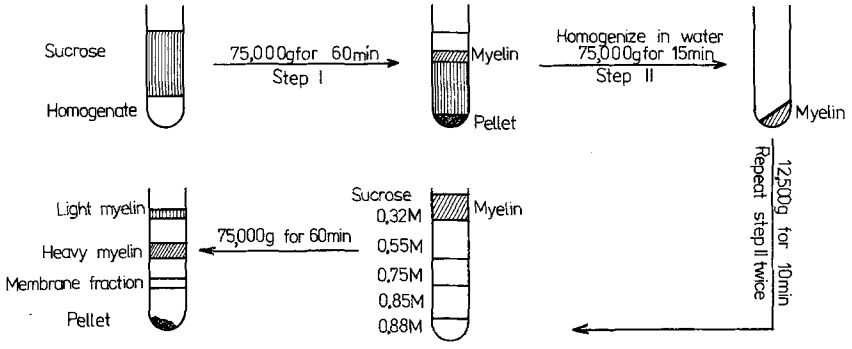

Fig. 1. Isolation of myelin subfractions according to Agrawal et al. (1974)

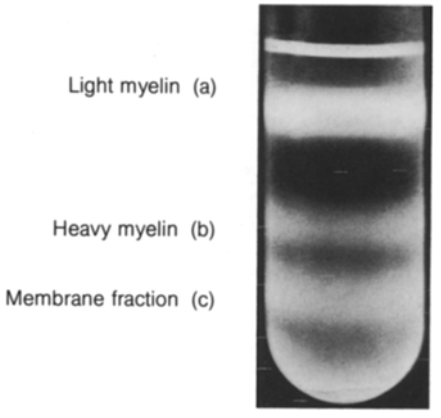

Fig. 2. Myelin subfractions isolated from spinal cord

The lipids were applied in amounts of 16 – 27 µg on microscopic glass plates coated with Silica Gel H (Merck). The chromatograms were sprayed with 10% sulphuric acid and the various fractions were visualized by exposure to 120° C. For identification, R_f values were compared with those of standards (Sigma). The percentage composition of individual myelin fractions was determined by planimetry of the absorption curves at 560 mµ obtained with a Zeiss (Jena) ERJ-63 densitometer. The purity of myelin fractions in electron microscope is shown in Fig. 3. To examine the influence of formalin fixation on lipid studies, results for both the fresh and the formalin-fixed material obtained from one of the fetuses were analyzed. No significant differences were found.

Results

Morphological Studies

Myelination gliosis takes place in the spinal cord in the 18th to 27th weeks of fetal life (Table 2). The fibers that undergo myelination earliest are those of the anterior horns of the cervical and lumbosacral part of the spinal cord in the 18th and 20th weeks of fetal life, respectively. In the long tracts of the cervical spinal cord, myelination of fibers was seen to begin earliest, in the 20th week of fetal life, in the cuneate fasciculus (Burdach's column), whereas in the phylogenetically oldest ascending and descending tracts it was not seen until the 27th week (Table 3). In the lumbosacral section, myelination of the long tracts begins later (Table 3).

Examination of the anterior horn and ventral funiculus of the lumbosacral section with electron

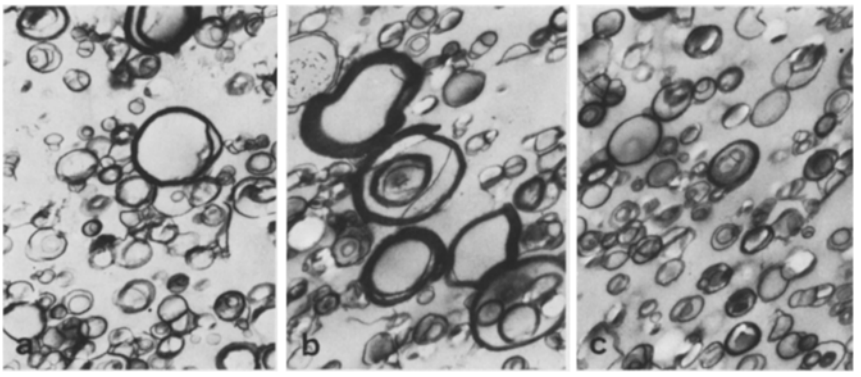

Fig. 3a—c. Electron micrographs of myelin subfractions prepared from the spinal cord. **a** Light myelin; **b** heavy myelin; **c** membrane fraction. ×9,000

Table 2. Correlative biochemical and morphological investigations of the spinal cord

Number of case	Age	Biochemical studies		Morphological studies		
		Myelin content[a]	Myelin fractions[b]			Electron microscopy[c]
				C_8-Th_1	L–S	L–S
1	16–17 weeks	159.5	1:0.2:0.4	–	–	8
2	18 weeks	230.6	1:0.4:0.8	○ ±	–	
3	20 weeks	167.1	1:0.7:0.5	○ +	○ ±	
4	23–24 weeks					21
5	25 weeks	217.5	1:0.8:0.9	○ +	○ ±	
6	27 weeks	583.3	1:0.7:0.8	○ + +	○ +	25
7	34 weeks	650.0	1:0.7:1.4	+ + +	+	
8	1 day	585.9	1:1.1:1.3	+ + +	+ +	
9	10 days	678.0	1:1.0:1.2	+ + +	+ +	
10	3 months	629.2	1:1.1:1.0	+ + +	+ +	42
11	8 months	568.1	1:1.1:1.5	+ + +	+ + +	
12	3 years	610.0	1:3.3:6.9	+ + +	+ + +	

[a] Myelin content in mg per g of wet weight
[b] Myelin fractions as a ratio of light myelin: heavy myelin: membrane fraction
[c] No. of myelin lamellae; – myelinated fibers not visible; ± few fibers with myelin; + more numerous myelin fibers; + + numerous myelin fibers; + + + a lot of myelin fibers; ○ myelination gliosis

Table 3. The appearance of first signs of myelination in the spinal cord in histological examination

	C_8-Th_1	L-S	
Anterior horn	18 weeks	20 weeks	Foetal life
Cuneate fasciculus	20 weeks	27–34 weeks?	
Other phylogenetically oldest tracts	27 weeks	34 weeks	
Anterior pyramidal tract	34 weeks	–	
Lateral pyramidal tract	1 month	3 months	Postnatal life

microscope revealed in the entire material occasional axons with loose myelin coils in the 16th week of fetal life (Table 2, Fig. 4). Somewhat more numerous axons with tight myelin sheaths were seen in the 20th and also 23rd to 24th week (Table 2). In the 27th week of fetal life and 3rd month of postnatal life, the number of axons undergoing myelination was considerably higher (Figs. 5 and 6). The anterior horns contained in the 20th week of fetal life fibers undergoing myelination (Table 3).

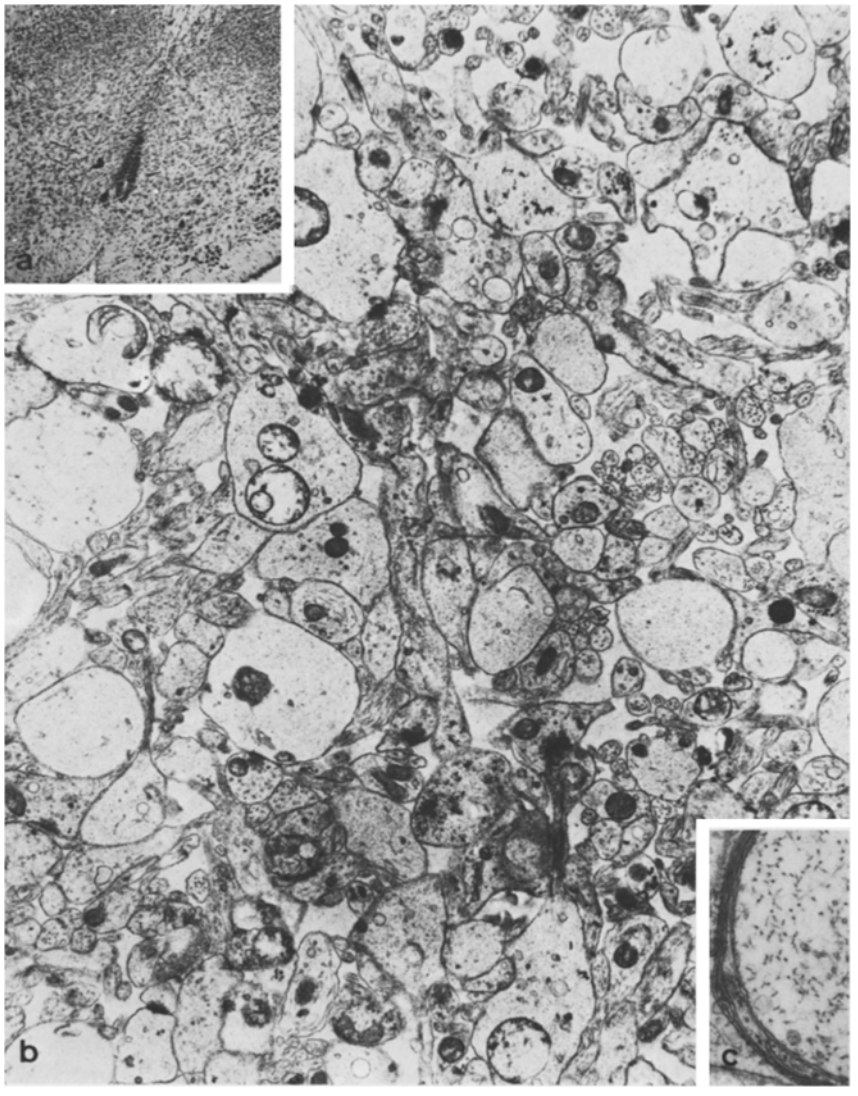

Fig. 4a—c. Spinal cord of human fetus, 16th week. **a** Cross section of the segment L_5. Myelin fibers (myelinated axons) absent. Klüver-Barrera, ×45. **b** Anterior funiculus of segment S_1. Numerous non-myelinated axons of different diameters. ×6,300. **c** Loose turns of myelin lamellae are visible in the single axons in the whole investigated material. ×22,500

Biochemical Studies

The total amount of myelin that could be isolated from the fetal spinal cord increased from the 16th week onward to reach in the 27th week approximately the level found in a 3-year-old child (Table 2). The wet weight of different myelin fractions changed considerably in the process, the amounts of heavy myelin and membrane fraction increasing until the third year of postnatal life. For comparison, it may be noted that in a 24-year-old human the membrane fraction accounts for as little as some 10% of the light myelin. The lipid composition of the myelin fractions was seen to change, too. The cholesterol content increased considerably around the 20th week of fetal life. Cerebrosides accumulated as well, but at a somewhat slower rate, with a more sharp increase in the first months of postnatal life. In the course of further development there was essentially little change in the level of either. On the other hand, the percentage of free fatty acids and triglycerides content decreased, especially in the perinatal period (Fig. 7). Cholesterol esters were not found in the material.

Discussion

The morphological data on myelination obtained from our material are in agreement with those reported by other authors (Langworthy, 1932; Tridon, 1959; Meier, 1976). However, little has been published so far on the biochemistry of the developing human spinal cord. Investigations of the myelination process in our material demonstrated a gradual increase of the total myelin content, a shift in the ratio of heavy myelin and

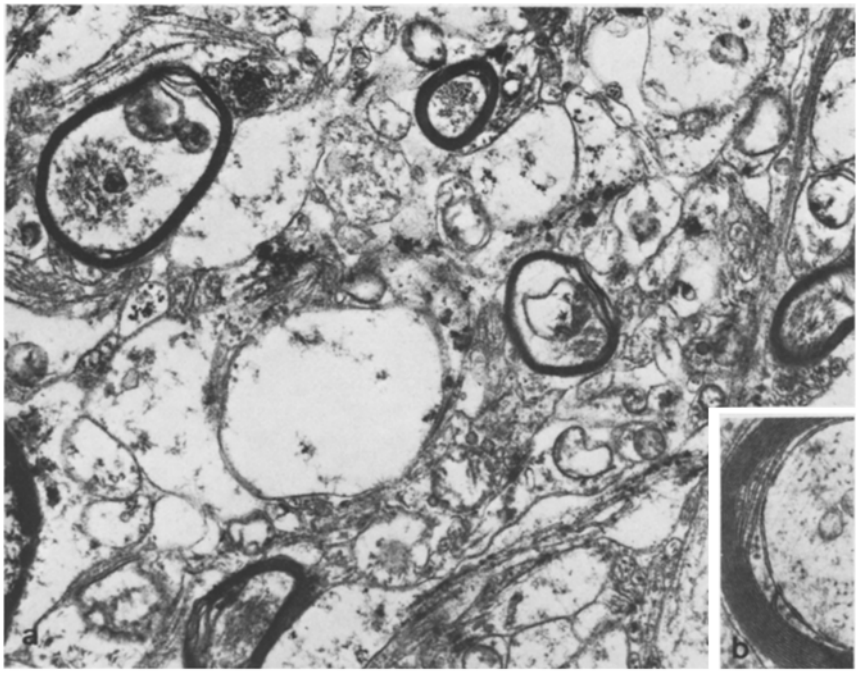

Fig. 5a and **b**. Spinal cord of human fetus, 27th week. Anterior funiculus of segment S_1. **a** Numerous fibers with different thickness of myelin sheath. ×3,800. **b** Numerous closely adhering myelin lamellae. ×21,900

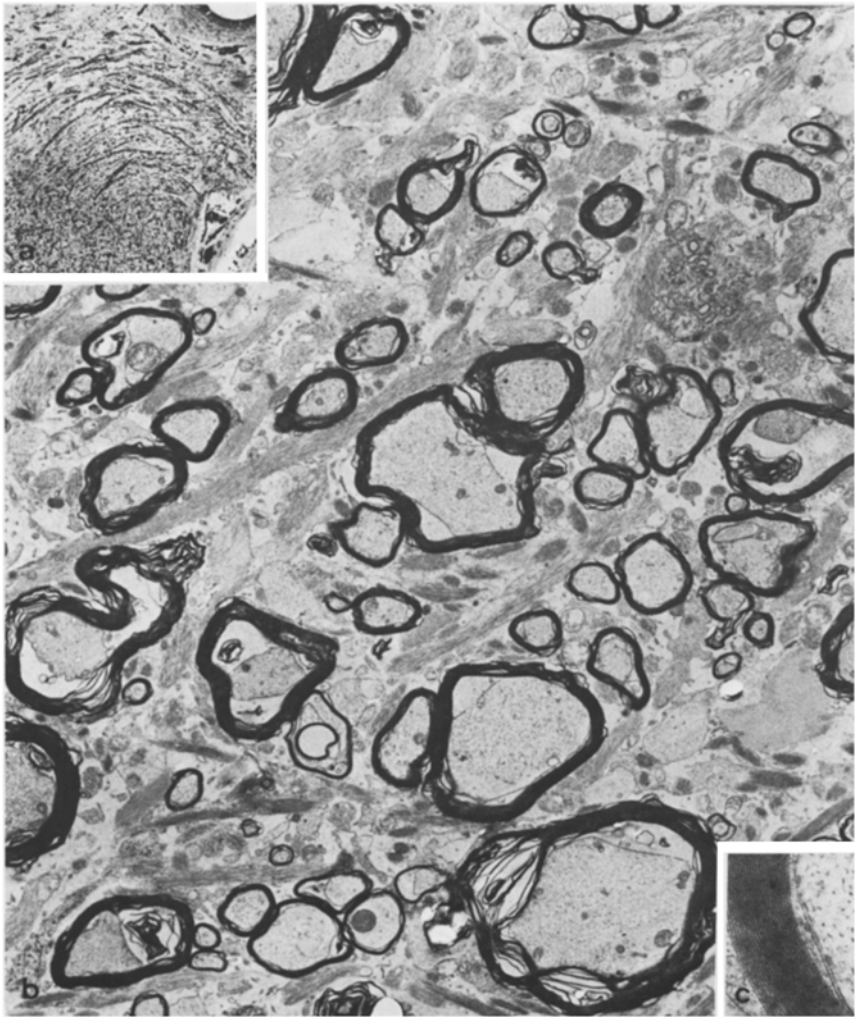

Fig. 6a—c. Infant's spinal cord, 3 months. **a** Advanced myelination of anterior funiculi and anterior commisure in the segment L_4. Klüver-Barrera, ×70. **b** Anterior funiculus of segment L_5. Numerous fibers of different diameters and thickness of myelin sheath. ×2,600. **b** Axon with 24 lamellae of myelin. ×21,000.

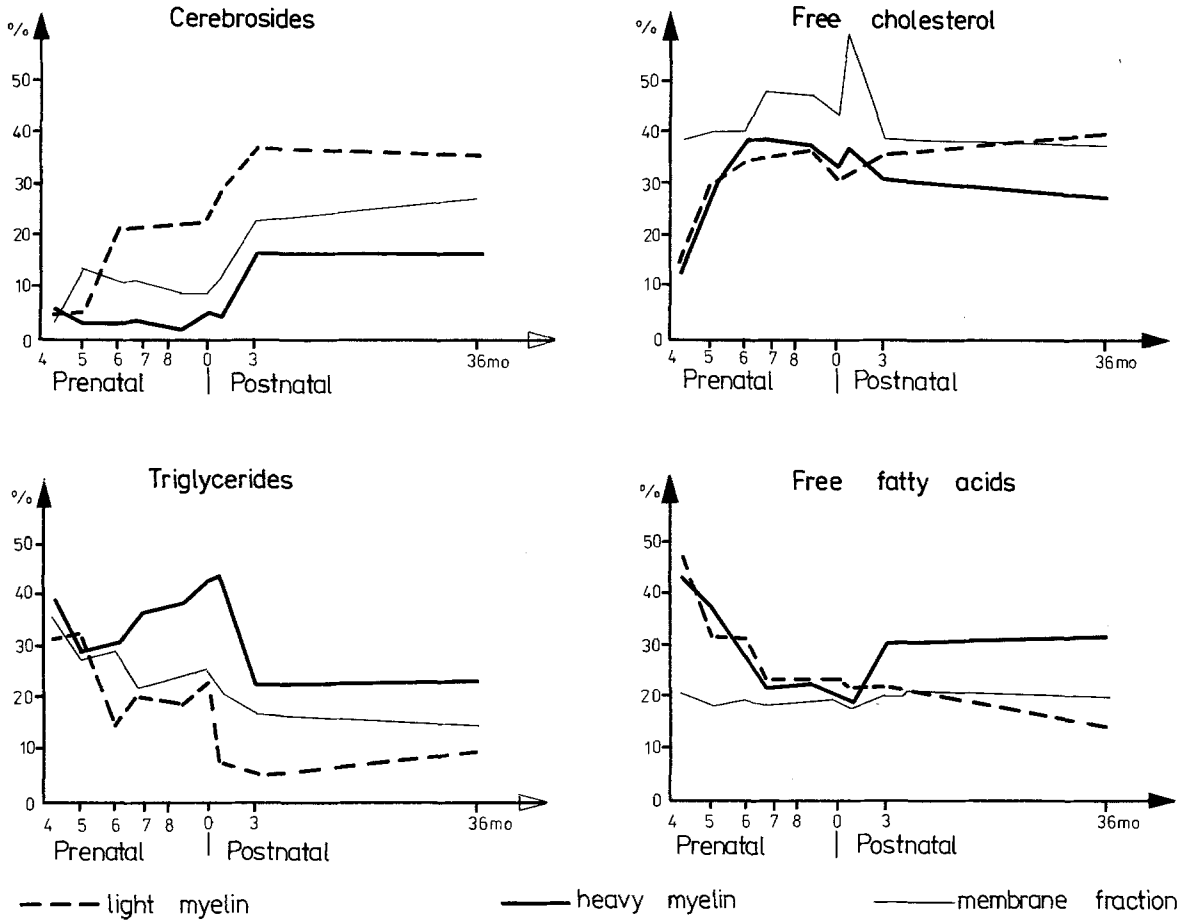

Fig. 7. Qualitative neutral lipids composition of spinal cord myelin fractions

the membrane fraction to light myelin, as well as changes in the neutral lipid composition of the individual myelin fractions.

Amount of Myelin

In the 27th week of fetal life, the total myelin content in the spinal cord is 2.5 times higher than that in the 16th week of fetal life (Table 2). Parallel histological examinations revealed distinct myelination in the ascending and descending tracts of the cervical part (Table 2, Fig. 5), and its beginning in the lumbosacral part. Electron microscope examination demonstrated a rise in the number of fibers undergoing myelination. The largest number of myelin lamellae was 25 (Table 2). A considerable amount of myelin was isolated as early as the 16th week. Signs of myelination were observed at this time only in the electron microscope.

Myelin Fractions

Based on the density, cerebral myelin can be subdivided into fractions having different chemical compositions (Waehneldt and Mandel, 1972; Agrawal et al., 1974). A myelin-like or membrane fractions and other membrane fractions isolated from young animals by Agrawal et al. probably represent myelin at different stages of maturity (Agrawal et al., 1974). The myelin-like or membrane fraction is marking the first step of myelination. This view is also in agreement with studies that have shown "young" myelin to differ in chemical composition from "mature" myelin (Horrocks, 1968; Cuzner and Davison, 1968; Norton and Poduslo, 1973). The changes we have found in the course of ontogenesis in the proportions of particular myelin fractions in the spinal cord also confirm this view. The early low levels of heavy myelin and of the membrane fraction, and their slight rise by the age of 25th week of fetal life, as well as the concurrently small quantitative changes, coincide with the stage of myelination gliosis and continuing paucity of fibers acquiring a myelin sheath in the anterior horn and dorsal funiculi. The number of such fibers does not increase considerably until the 27th week of fetal life, when the level of total myelin rises 2.5 times.

Qualitative Composition of Myelin Neutral Lipids

Biochemical analyses have shown the human developing brain to change in its content of cholesterol, gangliosides, cerebrosides, sulfatides, and phospholipids (Brante, 1949; Cumings et al., 1958; Svennerholm and Vanier, 1972). No data are available concerning the qualitative neutral lipid changes in course of human spinal cord maturation.

In our material, the cholesterol content is relatively high even at the 16th week of fetal life, especially in the membrane fraction. A sharper increase is, however, observed in the 20th week (Fig. 7). Histological examination showed at that time only a few fibers undergoing myelination in the posterior funiculi and anterior horns of the cervical and lumbosacral sections of the spinal cord (Table 3). In the latter, electron microscope observation showed 6–21 myelin lamellae in fibers undergoing myelination. In the developing CNS cholesterol is rapidly acquired during the period of myelination (Cuzner and Davison, 1968) and even before (Horrocks, 1968). Transient occurrence of estrified cholesterol in the developing CNS was also reported (Alling and Svenerholm, 1969; Svenerholm and Vanier, 1972). In our material, cholesterol esters were never observed.

Accumulation of cerebrosides in the CNS of man (Cumings et al., 1958; Clausen et al., 1965; Svennerholm and Vanier, 1972) is an accepted indicator of myelination. In animal material cerebrosides accumulate actively during myelination, and continue to increase at a slower rate until the later stages of the animal life (Kishimoto and Radin, 1959; Hausner, 1968). In our material, the content of cerebrosides in the 16-week-old fetus is extremely low, an evident increase is observed from the 20th week of fetal life, lasting up to the age of 3 years (Fig. 7). In the 3rd and 8th months of postnatal life, histological examination showed already an apparent myelination of all tracts (Fig. 6), apart from the lateral pyramidal tract. Further accumulation of cerebrosides and cholesterol suggested continuing myelination or biochemical myelin maturation, although the rate is very slow.

Progressing myelination of the spinal cord is accompanied by a decreasing level of free fatty acids and triglycerides (Fig. 7). The function of the latter in the nervous system is not yet clear enough. The hypothesis has been put forward that their presence is a defensive reflex protecting the cell membrane against damage by free fatty acids (Wood and Dawson, 1974). It is possible that during myelination triglycerides store fatty acids but their importance decreased during development.

In conclusion, it is evident from the presented correlative studies that myelination begins earlier than it could be previously demonstrated by simple morphological examinations, and that the onset of myelination is marked by quantitative and qualitative changes in the accumulated myelin. Biochemical studies also indicate that the myelin maturation process is long-lasting in spite of morphologically mature myelin appearance.

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