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^{35}S-Sulphate incorporation into myelin sulphated mucopolysaccharides during rat brain development

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Summary. Rat brain myelin acid mucopolysaccharides (AMPS) incorporate 15%, 8%, 5.5% and 4% of tojtal associated ^{35}S-sulphate, 14, 21, 30 and 75 days after birth, respectively. The course of ^{35}S-sulphate incorporation into total rat brain mucopolysaccharides, as well in those from myelin, had a similar feature with peak on the 2nd week and a significant decrease on the 3rd and 4th week postnatally.

The presence of acid mucopolysaccharides (AMPS) in central and peripheral nervous tissue has been demonstrated chemically and histochemically many times[1-3] and it was indicated that these compounds are necessary to maintain normal brain function[4]. Changes in AMPS content in the course of rat brain development and maturation have been reported in a number of papers[5-7]. These macromolecules have been closely studied as mambrane constituents, especially within synaptic plasma membrane, and their possible role in the cell surface regulation of metabolic function has been suggested.

In our previous experiments we identified and quantified hyaluronic acid (HA), heparitin sulphate (HS) and chondroitin sulphates (AéÁC) in myelin isolated from the whole rat brain as well as from different rat brain regions[8-10]. The results obtained induced us to attempt a more precise identification and localization of these compounds in myelin, by studying the incorporation of ^{35}S-sulphate into AMPS extracted from purified rat brain myelin as a function of postnatal development.

White-Hood male rats, 14-, 21-, 30- and 75-day-old, were injected i.p. with 60 µC/100 g b.wt of $Na_2{}^{35}SO_4$ (640 µC/mM) dissolved distilled destilled water and sacrificed by decapitation 16 h later. After decapitation the brains were rapidly removed and weighed; 5% homogenate in 0.32 M sucrose was prepared from whole brain and myelin was isolated and purified according to the method described by Norton and Poduslo[11]. Aliquots from brain homogenate and myelin suspension were used for protein[12] and radioactivity determination (10% Biosolv in a scintillation liquid based on toluol). Water-washed myelin was freeze-dried and weighed.

Purified and lyophilized myelin was delipidated by extraction with chloroform-methanol (2:1) and this was repeated twice. After digestion with N/10 NaOH and neutralization with N/10 HCl, myelin was subjected to proteolysis with activated papain solution[13] at 65 °C for 48 h. Repeated extraction with chloroform-methanol (2:1) and (1:2) were then carried out. After the addition of Lloyd's reagent, the suspension was centrifuged at 2000× g for 15 min. In the supernatant, the remaining proteins were precipitated by 10% trichloracetic acid following centrifugation. Acid mucopolysaccharides contained in supernatant were separated and purified by Ecteola Cellulose microchromatography[13]. After washing the column with 0.025 M NaCl and subsequent elution with 3 M NaCl, the eluate was dialyzed for 48 h. at 4 °C in distilled water by constant stirring. The dialyzed aliquot was used for uronic acid[13] determination, as well as for radioactivity determination[14]. Following lyophilization, individual AMPS were separated by microzone electrophoresis on Cellulose-polyacetate strips in buffered $CuSO_4$ solution (pH 3.4). Individual AMPS wer identified after staining with Alcian blue and subsequent densitometry by comparing with standards running simultaneously (figure 1). Fugure 2 shows that 35% of ^{35}S-sulphate is incorporated into sulphated mucopolysaccharides extracted from the whole rat brain on the 14th day

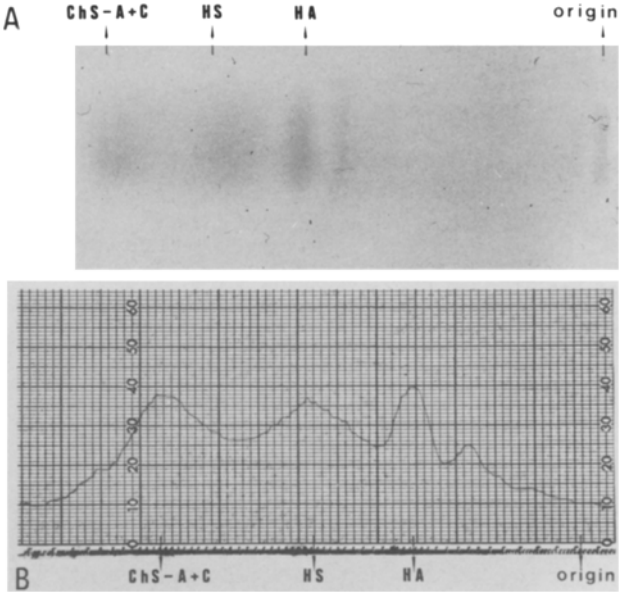

Fig. 1. *A* Electrophoretic profile of individual acid mucopolysaccharides (AMPS) extracted from 14-day-old rats myelin and resolved on cellulose polyacetate strips after staining with Alcian blue. *B* Densitometer scans of individual AMPS resolved electrophoretically and identified by comparing electrophoretic mobility, after staining by Alcian blue, of clearly marked fractions from the analyzed samples, with migration of individual AMPS from standard solutions consisting of highly pure AMPS submitted to electrophoresis simultaneously. Electrophoresis was performed by the method described by Stefanović[17].

of postnatal development. This percentage markedly decreases on the 21st and 30th day (13% and 12% of the total homogenate radioactivity respectively) and then increases, reaching 21% on the 75th day. The highest incorporation of ^{35}S-sulphate into myelin occurs on the 21st day of postnatal development (17% of total homogenate radio activity) and then decreases. On the 30th and 75th day it is 11%. Of the total ^{35}S-sulphate associated with myelin, sulphated mucopolysaccharides extracted from myelin incorporate 15% of ^{35}S-sulphate on the 14th day, 8% on the 21st day, 5.5% on the 30th and 4% on the 75th day postnatally (figure 2).

The course of incorporation of ^{35}S-sulphate into sulphated mucopolysaccharides of the total brain homogenate and myelin has a similar trend (figure 3). The highest specific activity is reached on the 14th day postnatally and on the 21st day decreases 71% in myelin. On the 30th day specific activity in the total brain homogenate continues to decrease, while in myelin there is a marked decrease (55%). On the 75th day of the postnatal development, the specific activity shows an increase both in homogenate (54%) and in myelin (63%) compared with the previously studied age (figure 3).

Percentage distribution of individual acid mucopolysaccharides from total acid mucopolysaccharides

	Myelin			Total brain		
Age (days)	HA	HS	ChS(A+C)	HA	HS	ChS(A+C)
14	12	51	37	13	28	59
21	19	28	53	6	25	69
30	27	30	44	10	26	64
75	22	32	44	15	26	59

HA, hyaluronic acid; HS, heparitin sulphate; ChS(A+C), chondroitin sulphate.

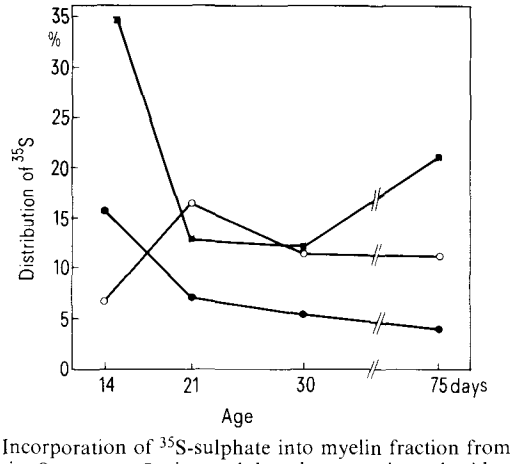

Fig. 2 Incorporation of ^{35}S-sulphate into myelin fraction from total rat brain ○——○; into sulphated mucopolysaccharides from myelin ●——●; into sulphated mucopolysaccharides from total rat brain ▮——▮. Each point represents the mean of 5 experiments. In each experiment 30–35 g wet wt of rat brain tissue was used.

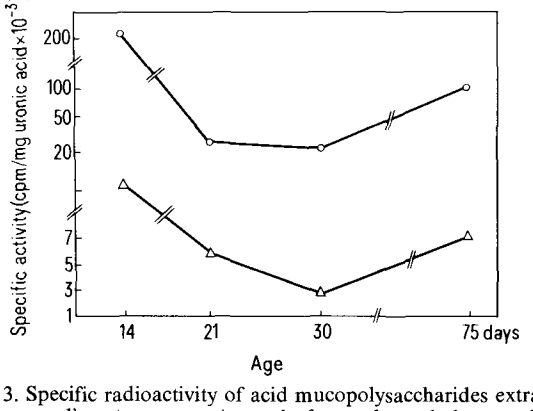

Fig. 3. Specific radioactivity of acid mucopolysaccharides extracted from myelin △——△ and from the whole rat brain ○——○ at various postnatal ages. Each point represents the mean of 5 experiments.

Comparing individual AMPS isolated and identified from the total brain homogenate and myelin (table 1), one can notice that the proportion of sulphated mucopolysaccharides is highest not only in the total brain homogenate, but also in myelin during all periods of postnatal development in our study, although myelin also containes a higher level of hyaluronic acid, especially in adult stage.

These results, which demonstrate in vivo incorporation of ^{35}S-sulphate into acid mucopolysaccharides (AMPS) associated with rat brain myelin, confirm histochemical[15,16] and biochemical findings already reported[8–10] indicating that AMPS are a myelin constituents in the central and peripheral nervous system. With the method of myelin isolation which we used in our work, we were able to establish that AMPS are specifically associated with myelin and are not the result of myelin contamination, as has been suggested by some authors[17,18].

The profile of AMPS distribution within total brain homogenate during postnatal brain development, high level in premyelination stage and rapid fall during myelination[5,6,8,9,19] accompanied by similar AMPS-sulphotransferase fluctuations[6–20,21], contribute to the hypothesis that there is a close relationship between brain maturation and AMPS synthesis. However, the fact that during premyelination period highest incorporation of ^{35}S-sulphate occurs not only into sulphated AMPS of total brain, as reported by Saxena[6], but also into AMPS associated myelin (figure 2), may suggest that the synthesis of these heteromacromolecules may be a prerequisite for myelination.

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