proteins is also possible. This enrichment coincides, not only with the late period of differentiation of the granule cells (the most abundant cells in the cerebellum), but also (from day 30) with synapse formation between parallel fibres and Purkinje-cell dendrites in the external region of the molecular layer, and to the coating of the Purkinje dendrites in the same region by Bergman glia (Altman, 1972b).

At birth, AcNeu, Gal, GlcNAc and Man are the predominant protein-bound sugars (we have taken into account neither glucose, which must be largely derived from glyco-
gen, nor ribose, presumably derived from RNA). For the following 13 days, the first three sugars increase in parallel (mol of Gal or of AcNeu/mol of GlcNac both remain constant at around 0.42) at a rate slower than that of proteins. From the day 20, however, their increase becomes faster than that of protein, linear with time until adult values are reached, and parallel (mol of Gal or of AcNeu/mol of GlcNac at around 0.53) for the three sugars.

Fucose increases at a rate equal to that of protein (from birth to day 10 and from day 30 to adulthood) or slightly faster (from day 10 to day 30). Mannose increases at a rate faster than that of protein during the whole cerebellar development. At day 40, almost all the protein-bound AcNeu (96-98% of the adult value) is present, whereas slightly lower values (92%) are attained at this date for the total protein-bound sugars.

Age-dependent changes in the molar ratio between sugars indicate the constant modification of glycan composition during development. The most striking change is the abundance, between days 10-13 and day 24, of glycans rich in mannose and fucose. These changes probably represent changes in glycoprotein population, and not changes in the glycan composition of the same proteins: cerebellar particulate glycoproteins which bind to concanavalin A show age-dependent changes in electrophoretic patterns (J. P. Zanetta, M. S. Ghandour, G. Gombos & G. Vincendon, unpublished work).

Altman, J. (1972b) J. Comp. Neurol. 145, 399–444
Del Cerro, M. P. & Snider, R. S. (1972) J. Comp. Neurol. 144, 131–164

Changes of Ganglioside Pattern during Postnatal Development of Rat Cerebellum

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Modifications of ganglioside patterns occur during the development of the nervous system (for a review, see Tettamanti, 1971). Gangliosides of the nervous system are
mainly localized on the plasma membrane of neural cells, with the possible exception of ganglioside \( G_{M1} \), also present in myelin (Suzuki et al., 1967, 1968). If ganglioside distribution is representative of cell plasma-membrane ganglioside pattern, and not of the accumulation of different intermediates of ganglioside metabolism, changes in distribution during development could either represent modifications of the plasma membrane of each developing neural cell during differentiation, or changes in the proportions of the different cell types, each with a different ganglioside composition.

In the cerebellum, granule cells are by far the most numerous type of cell (Palay & Chan-Palay, 1974). In rat cerebellum, the multiplication of the bulk of progenitor cells takes place during the second week, but the migration and differentiation of granule cells continues up to day 30 (Altman, 1972a,b,c; Del Cerro & Snider, 1972). Thus changes of ganglioside composition in cerebellum, during this period and after, are largely due to one type of cell, the granule cell. Merat & Dickerson (1973) have investigated ganglioside changes in developing rat cerebellum but, for the crucial period of cerebellar development, their investigation was limited to gangliosides \( G_{T1} \), \( G_{D1a} \) and \( G_{D1b} \).

At birth, litters of our inbred Wistar rats were decreased to six animals; animals were killed and, when necessary, cerebella removed from one or more litters were pooled. Gangliosides were extracted as described by Suzuki (1964) and measured by the technique of Miettinen & Takki-Luukainen (1959). They were fractionated by t.l.c. as described by van den Eijnden (1971), made visible with Bial's orcinol-HCl reagent, and their distribution was determined by densitometry (Klenk & Gielen, 1961).

As shown in Fig. 1, the accumulation rate of all eight gangliosides relative to that of protein can be divided into two phases. The first phase from birth to 13 days is similar for all gangliosides, and it coincides with the early maturation of Purkinje and Golgi neurons (Altman, 1972b).

The time-course of proliferation of progenitor cells of cerebellar-cortex interneurons (mostly granule cells) corresponds to the fall in the ganglioside/protein ratio. During the following period, from 13 to 30 days, which roughly corresponds to granule-cell differentiation (Altman, 1972c), the increase of gangliosides follows four different

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![Fig. 1. Age-dependent changes of rat cerebellum gangliosides](image)

\( G_{M1} \); \( G_{Q1} \); \( G_{D1a} \); \( G_{M3} \); \( G_{D3} \); \( G_{M2} \); \( G_{D1b} \); \( G_{T1} \).

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patterns. After a short period of rapid accumulation gangliosides GD1a and GM3 increase at a rate slower than protein, whereas gangliosides GD1b and GT1 increase at the same rate as protein. Gangliosides GM2 and GD1a accumulate at a rate progressively slower than protein, whereas gangliosides GQ1 and GM1 accumulate at a rate progressively faster than protein. The increase of ganglioside GM1 could reflect myelination, but changes of the other gangliosides, especially those that occur after day 30, indicate mostly modifications of granule-cell plasma membranes.

Another important point is that at birth 20% of ganglioside AcNeu is in polysialogangliosides (adult value 41%). Tri- and tetra-sialogangliosides appear to be specific to nerve cells (Wiegandt, 1968). However, whereas adult nervous tissue is rich in these compounds, normal and tumoral neuroblasts or glioblasts cultured in vitro are very poor in polysialogangliosides, even after induction of morphological differentiation (Rebel et al., 1973a, b; Robert et al., 1975). Since at birth most cells in the cerebellum (with the possible exception of neurons of deep cerebellar nuclei, and maybe of a few incoming axons) are neuroblasts, glioblasts and progenitor cells, it appears that immature nerve cells in vitro contain polysialogangliosides. The absence of tri- and tetrasialogangliosides in cultures of normal or tumoral cells cannot be simply related to the immaturity of these cells, but rather to conditions inherent to the culture or to the tumoral origin of some cells.

Altman, J. (1972b) J. Comp. Neurol. 145, 399–464
Del Cerro, M. P. & Snider, R. S. (1972) J. Comp. Neurol. 144, 131–164

Cerebroside Accumulation, Biphasic Myelination and Fatty Acid Synthesis in Pig Spinal Cord during Development
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Myelination is a unique morphological change which takes place in developing nervous tissue and is accompanied by significant changes in lipid composition. Cerebroside, a characteristic myelin lipid, contains galactose as the predominant hexose with the ceramide residue being esterified with either α-hydroxy fatty acids or non-hydroxy long-chain fatty acids. The close correlation between myelination of central and peripheral nerve and the accretion of cerebrosides has been noted by several research groups.

Over the past few years we have been engaged in the systematic chemical character-