PROTEINS OF HUMAN GLIAL CELL MEMBRANE

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1. Introduction

Although it is a well-known fact that one special type of glial cells, the oligodendrocytes, are concerned with myelination [1] little is known about the role of glial cell membrane in myelinogenesis. A special "second myelin-like fraction", probably representing a previously undescribed membrane fraction present mainly in the developing central nervous system, was recently discovered in rat brain [2,3]. It was proposed that it may be derived from glial cell membrane and thus be regarded as a transitional form of membrane leading to the synthesis of myelin. Histological observations were cited to support this hypothesis. However, separation of glial cells and myelin was not performed, and it was suggested that pure glial cell membranes should be isolated from developing brain before conclusions as to its true identity.

Separation of highly purified glial cells, myelin and subcellular fractions is now possible with the aid of new ultracentrifugation techniques [4,5] which were utilized in the present study aimed at the investigation of human glial cell membrane proteins. The results were compared to the previously established myelin protein patterns of both mature [6] and developing [7] human myelin. After analysis of the glial cell membrane protein patterns of human pre- and postnatal brains it seems unlikely that the glial cell membrane is the only precursor of myelin.

2. Material and Methods

Two foetal brains were obtained from therapeutic abortions carried out at the Department of Obstetrics and Gynaecology of the University of Helsinki. Three postnatal brains were from patients who died and were autopsied at the Children's Hospital of the University of Helsinki. The crown-rump (CR) lengths of the foetuses and the ages of the children are given in table 1.

The foetal brains were removed as quickly as possible and at least within 2 hr, and the children were autopsied within 24 hr. Meanwhile they were kept at + 4°. The dissected brains were stored at −25°. Myelin, neurons and glial cells were extracted from foetal haemispheres and from postnatal brain cortices [5]. Cell membranes were then separated from neurons and glial cells [4]. "The second myelin-like fraction", as described in the introduction, was also separated [2] and subjected to further analysis.

The purity of the samples was checked after fixation [8] by electron microscopy. The purity of

Table 1
The crown-rump (CR) lengths and approximate ages of the foetuses and children.

Number of specimen	CR length (cm)	Approximate age
Foetuses		
1	16.0	18 weeks
2	17.0	19 weeks
Children		
3	–	1 month
4	–	6 years
5	–	13 years

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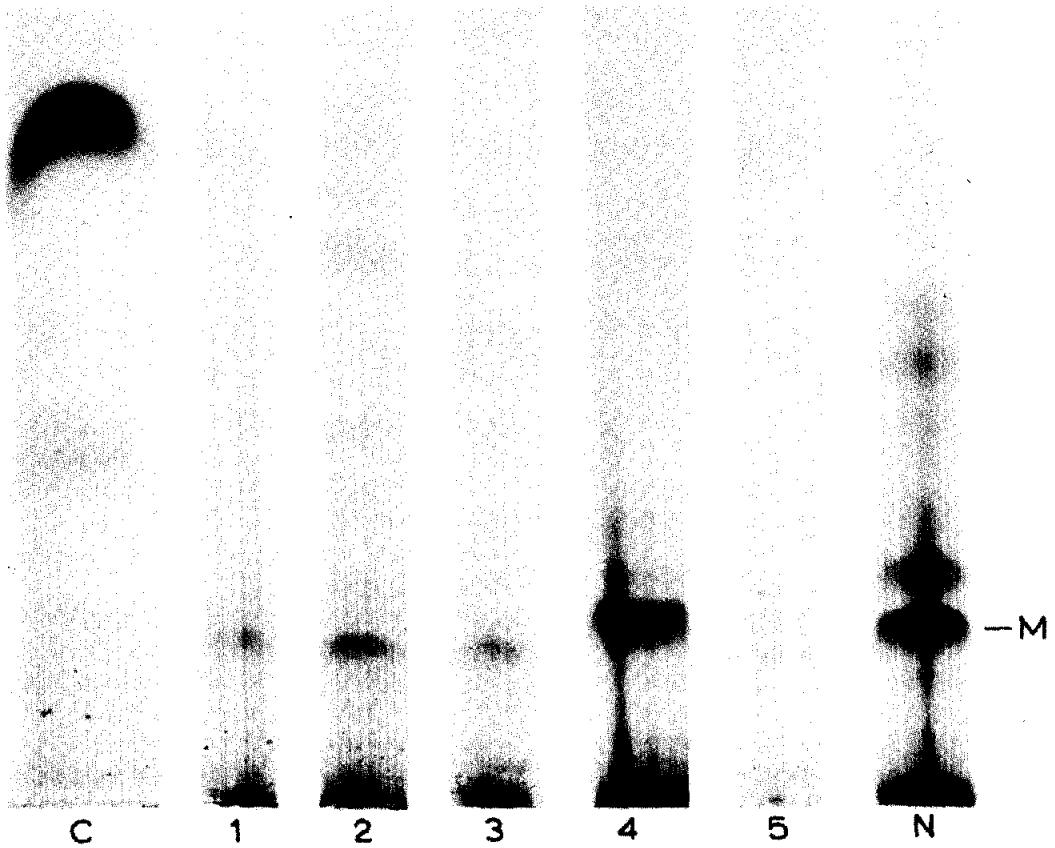

Fig. 1. The protein patterns of glial cell membranes of 2 foetal and 3 postnatal brains. The protein pattern of 1 postnatal neuronal membrane fraction, heavily loaded with protein, is shown on the right. M = membrane protein, N = neuronal membranes, C = cytochrome *c*. The numbers refer to table 1.

the myelin fractions was at least 95% and, on the other hand, no myelin was seen in the corresponding membrane fractions. Synaptosomal contamination of the membrane fractions was evaluated by measuring the activity of acetylcholine esterase (AChE) [9]. No contamination of the membranes was observed by either electron microscopy or enzyme assays.

The proteins of the cell membranes and myelin were analysed by horizontal polyacrylamide electrophoresis [10], and the gels were scanned by a Canalco model E microdensitometer. Cytochrome *c* served as a reference in the estimation of the relative migratory values (M_c) of the various proteins [11].

3. Results

The protein patterns of the glial cell membranes of 2 foetal and 3 postnatal brains are shown in fig. 1, where the protein pattern of one postnatal neuronal membrane fraction is also given for comparison. The protein patterns of pre- and postnatal myelin are shown in fig. 2. It is evident that the protein patterns of both pre- and postnatal myelin are entirely different from the cell membrane proteins.

The 3 protein groups characteristic to myelin are recorded. The 3 basic proteins, including the encephalitogenic protein, move with M_c values from 0.7–0.9; the 2 proteolipid proteins move with values from 0.3–0.5, and the acidic proteins remain near the origin [6]. The protein pattern of foetal myelin is identical to

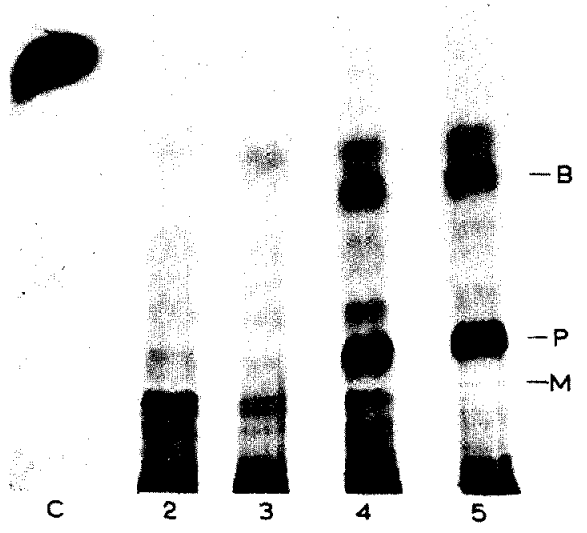

Fig. 2. The protein patterns of 1 foetal and 3 postnatal myelin fractions. M = membrane protein, P = proteolipid protein, B = encephalitogenic basic protein, C = cytochrome *c*. The numbers refer to table 1.

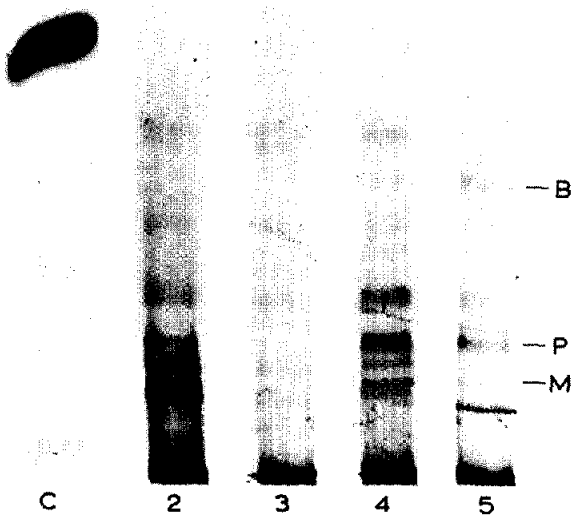

Fig. 3. The protein patterns of "the second myelin-like fractions" of 1 foetal and 3 postnatal brains. See the legend of fig. 2 for explanations.

that reported earlier with no encephalitogen present and with a typical proteolipid protein pattern [7].

Only one major protein band with M_c 0.2 is present in the cell membrane patterns (fig. 1). It is very dominant also in foetal myelin and in "the second myelin-like fraction", as is illustrated in fig. 3, but is relatively faint although clearly visible in adult myelin. Another protein band, obviously rather characteristic to the membranes, is discernible above the major protein. It is especially well visualized when large amounts of neuronal membranes are electrophoresed. This loading visualizes a faint band of the encephalitogen in the neuronal membranes. It should be mentioned here that not even traces of it could be demonstrated after loading with glial cell membranes or foetal myelin [7].

Analysis of the protein pattern of "the second myelin-like fraction" shows, in addition to the main membrane protein, a number of other proteins present also in myelin. Their maturation seems to follow the developmental changes observed in myelin and their pattern is at all stages distinctly different from those of the neuronal and glial cell membranes.

4. Discussion

The results of the present investigation indicate that "the second myelin-like fraction" [2,3] is a mixture of cell membranes and myelin with rather constant proportions. This theory is supported by the maturational changes in its protein pattern, analogous to those observed in myelin [7].

The presence of the main membrane protein in myelin can be explained if one considers the classical myelin model with the winding of myelin forming cell around the axon [12]. It is obvious that the cell membrane must also participate in that structure and give rise to the appearance of the major membrane protein band when myelin proteins are analysed. However, it is now less evident that the glial cell membrane is the precursor of myelin. Not even traces of the encephalitogenic basic protein, the most integral and vulnerable part of myelin [13,14] could be demonstrated in glial cell membranes. Traces of encephalitogen were detected in neuronal membranes but only after a heavy loading. It is possible that this protein is derived from the small amounts of synaptic encephalitogen left on the surface of neuronal perikarya.

Acknowledgements

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