Phase Transitions in Myelin from Lamellar into Water in Oil and Oil in Water Patterns* (34132)

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Previous studies from this laboratory adduced evidence indicating that phase transitions from the lamellar pattern into o/w and w/o systems may occur in the myelin sheath and in cellular membranes (1–5). It has been shown that after treatment with 1 *M* NaCl, but not after treatment with 0.5 *M* $CaCl_2$, about 50% of the myelin constituents could be extracted with water. About one half of the remaining constituents could be extracted with water after treatment with KCNS or KI (4). Histochemical studies of membrane-bound enzymes indicated that in the w/o state (induced by Ca^{2+} or CNS ion treatments) most enzymes were inactivated (5). Recent work (6) has shown that in the presence of atmospheric oxygen phase transitions in membranes often become irreversible. The phase transitions are assumed to be due to changes in the degree of hydrophilia of the fixed ionic charges on the membranes in accordance with data known for over 50 years (7).

Three fractions can be obtained from crude homogenates of cerebral white matter and peripheral nerve: the "Na" fraction (extractable with water after NaCl treatment), the "CNS" fraction (extractable after KCNS treatment), and the "R" (residue) fraction. Available evidence suggests that the "Na" fraction is of the o/w type whereas the "CNS" fraction corresponds to the w/o type of emulsion. The "R" fraction is not emulsifiable in water under the presently discussed conditions. Assuming that the extracted myelin micelles can undergo phase transitions so that in the o/w pattern the more hydrophilic polar groups would be on the outside of the particles, and in the w/o pattern less hydrophilic groups will face the suspending medium, such suspensions should differ in their electrophoretic mobility. The question whether the active, more and less hydrophilic groups, belonging to polar lipids, to proteins, or to both, is not relevant to the present investigation. The present study was made in order to test this notion, care being taken not to expose the extracts to different concentrations of ions during the electrophoretic runs.

Material and Methods. White matter of freshly killed cattle was used. The techniques of homogenization and extraction have been previously described (3, 4). The extracts were lyophilized and treated as follows.

In the first experiment two 100-mg samples of lyophilized "Na" fraction were used. To one sample 0.1 ml of 0.5 *M* $CaCl_2$ was added and mixed for 30 min in a magnetic stirrer at room temperature, and then 1 ml of a "standard" solution, consisting of a phenol acetic acid/water (2:1:1, w/v/v) mixture containing 2 *M* urea, was added. The "control" sample was mixed with 1 ml of the same "standard" solution to which 0.1 ml of 0.5 *M* $CaCl_2$ solution had been added. From each of these mixtures 0.02 ml, containing 610 μg of protein according to the procedure of Lowry *et al.* (8) were run in disc electrophoresis as described by Takayama (9) for 2 hr at 5 mA/column at 4°.

In the other two experiments 100-mg samples of the "CNS" fraction were used. In one experiment one sample was first treated for 30 min with 0.1 ml of 1 *M* NaCl and then solubilized as above, whereas the control sample was mixed with 1.1 ml of the stand-

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ard solution containing 0.1 ml of 1 *M* NaCl.

In the third experiment one 100-mg sample of the "CNS" fraction was first treated with 0.1 ml of 0.5 *M* $CaCl_2$ and then solubilized in 1 ml of the standard solution, and the control sample was mixed directly in 1.1 ml of standard solution containing 0.1 ml of 0.5 *M* NaCl. In the last two experiments 0.1 ml of mixtures each containing 955 μg of protein were used for the electrophoretic runs, which were performed as above.

The samples were run simultaneously and were stained for proteins for 2 hr with 0.5% amido black in 7% acetic acid. Staining for lipids was done with oil red O.

Results. The electrophoreic patterns obtained are presented in Figs. 1, 2, and 3. It can be seen in Fig. 1 that treatment with $CaCl_2$ completely changed the distribution pattern of the "Na" fraction proteins with appearance of fast-moving (presumably basic) proteins. Furthermore, this treatment caused the appearance of a lipophilic band.

No obvious changes occurred in the distribution pattern of the "CNS" fraction treated with $CaCl_2$, as can be seen in Fig. 2. Marked changes were caused, however, in this fraction by treatment with NaCl, as can be seen in Fig. 3, also here associated with appearance of lipophilic bands.

Discussion. The findings show that myelin lipoprotein or protein emulsions change their electrophoretic characteristics after treatment with concentrated salt solutions. As the effects of salts were obvious in comparison to controls (which had equal final concentrations of the electrolytes) the results indicate that the phase transitions are either not readily or not reversible. The observations which indicate that the "Na" fraction is affected by $CaCl_2$ treatment and the "CNS" fraction is affected by NaCl but not by KCNS is in accordance with previous findings: the "Na" fraction is an o/w type of emulsion which can be transformed into a w/o pattern by an excess of Ca ions; the "CNS" fraction is a w/o type of emulsion which is not affected by Ca ions, but is transformed into an o/w pattern by Na ions. It might be useful to point out that the concept of o/w and w/o patterns does not necessarily indicate that completely hydrophilic oil-like groups face the exterior or interior surface of the micelles. The hydrophobic groups are simply less hydrophilic than the hydrophilic groups.

Summary. Cerebral white matter was extracted with water after treatment with 1 *M* NaCl and the resuidue was again extracted

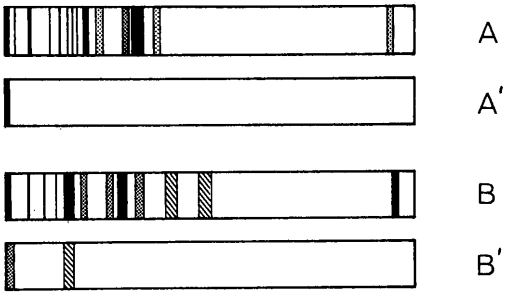

FIG. 1. Electropherograms of samples of the "Na" fraction of white matter. *A* and *A'* are controls. *B* and *B'* experiments treated with 0.1 ml of $CaCl_2$ before solubilization. *A* and *B* were stained for proteins, *A'* and *B'*, stained for lipids.

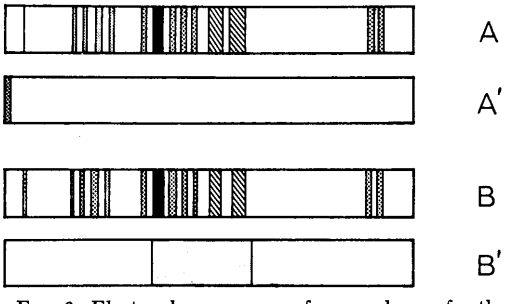

FIG. 2. Electropherograms of samples of the "CNS" fraction. *A* and *A'* are controls. *B* and *B'* were first treated with 0.1 ml of $CaCl_2$. *A* and *B* were stained for proteins; *A'* and *B'*, for lipids.

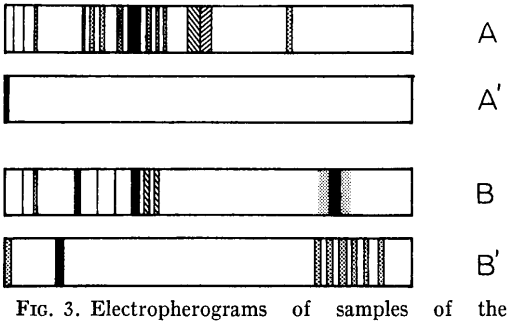

FIG. 3. Electropherograms of samples of the "CNS" fraction. *A* and *A'* are controls. *B* and *B'* were first treated with 0.1 ml of NaCl. *A'* and *B'*, stained for lipids.

after treatment with 1 *M* KCNS. Acrylamide gel electrophoresis of the extracted lipoproteic complexes treated with ions which were previously shown to preserve or revert phase patterns have shown that phase transitions (w/o and o/w) do occur in myelin.

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