Journal of Chromatography, 145 (1978) 307—310
Biomedical Applications
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 105

Note

The diminution of the myelin ethanolamine plasmalogen in brain of the *Jimpy* mouse and brain and spinal cord of the *Quaking* mouse as visualized by thin-layer chromatography

M.H. HACK* and F.M. HELMY

Section of Histochemistry, Department of Medicine, Tulane University, New Orleans, La., 70112 (U.S.A.)

(Received July 5th, 1977)

Within the nervous system of vertebrates it has been long established that the glycolipids are a characterizing component of myelin and are quantitatively related to the degree of myelination as demonstrated by morphological criteria. On the other hand the ethanolamine plasmalogens are both more ubiquitous in nature and uniquely abundant in the myelin sheath; they are histochemically demonstrated by the Feulgen plasmalogen reaction which is also used as the thin-layer chromatographic (TLC) spot test. We have recently shown [1] that the ethanolamine plasmalogens of brain, spinal cord and optic and sciatic nerves can be resolved by TLC into two components, only one of which is peculiar to myelin.

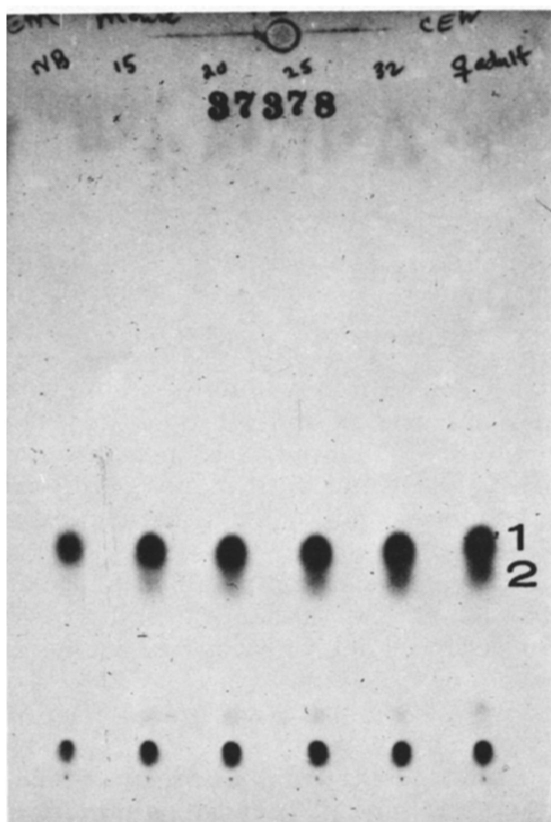
The present report describes our observations on brain and spinal cord of the myelin-deficient mouse mutants *Jimpy* and *Quaking* and may serve to explain the observations of others with regard to the diminution of plasmalogens in the brains of these mutants [2, 3, 4]. Similar plasmalogen diminution has been reported for the demyelination of multiple sclerosis [5] and in Wallerian degeneration of rabbit sciatic nerve [6].

MATERIAL AND METHODS

Normal Swiss mice were used to serve as a base-line for interpreting the observations on the *Jimpy* and *Quaking* mutants. This normal series consisted

*Present address: Faculty of Medicine, King Faisal University, P.O.Box 2114, Dammam, Saudi Arabia.

of adult, new-born, 15-, 20-, 25- and 32-day post-natal specimens. The myelin-deficient mouse mutants, all males, consisted of 6 each 17-day *Jimpy* and litter-mate controls and 2 each 40- and 50-day, respectively, *Quaking* and litter-mate controls. The *Jimpy* and *Quaking* series were obtained directly from the Jackson Laboratory (Bar Harbor, Maine, U.S.A.). The mice were killed by exsanguination, the spinal cord cut at the level of the foramen magnum and the brains removed, frozen with dry ice and lyophilized; the spinal cords of the *Quaking* series were also removed, frozen and lyophilized. Chloroform—methanol (2:1, v/v) extracts were made of each sample with a ratio of 5 ml solvent per 100 mg dry weight of tissue. The chromatographic analysis of the phosphatides and glycolipids was done as explained elsewhere [1], mainly using Schleicher & Schuell F-1500 silica gel thin-layer plastic sheets and the solvent system CEW (chloroform—ethanol—water, 65:25:3).



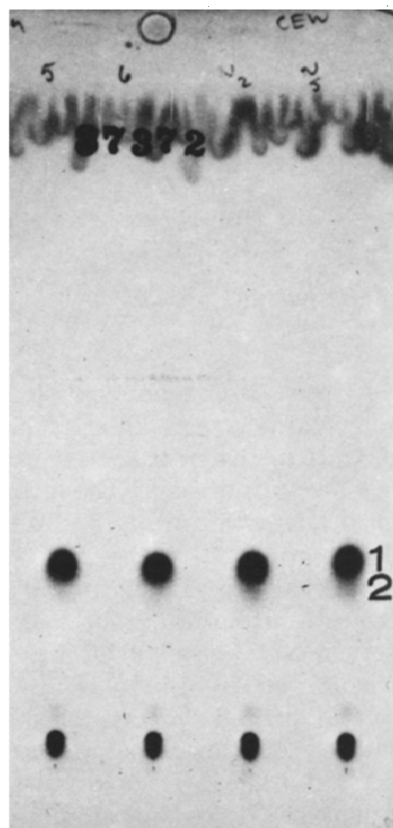


Fig. 1. Brain of normal mice; from left to right: new-born, 15-, 20-, 25- and 32-day post-natal, adult. E. Merck silica gel plastic back sheet with chromatographic development in CEW (see text) and stained by immersion in the Feulgen plasmalogen reagents. The major phosphatide in all specimens is PE-1 (1), mainly as plasmalogen, with PE-2 (2) clearly increasing with age.

Fig. 2. Same chromatographic and staining conditions as Fig. 1; brain of 17-day *Jimpy* (left pair) and litter-mate controls (right pair) showing the increased PE-2 (2) in the controls. The massive solvent front is largely derived from material within the silica gel plate itself.

Validation of the myelin phosphatide was accomplished by co-chromatography with both commercial PE* samples of known purity and the silicic acid column PE-1, PE-2 isolates described earlier [1]. Recognition of the amine component was by use of the fluorescent amine reagent fluorescamine (Roche, Nutley, N.J., U.S.A.) and of the alk-1-enyl (plasmalogen) component by the Schiff leuco-fuchsin reagent. Recognition of the accompanying lipids was readily accomplished by their Rhodamine 6G stainability.

RESULTS

Fig. 1 shows the progressive appearance, in mouse brain, of the characteristic myelin ethanolamine plasmalogen species, PE-2, with increasing post-natal age. Fig. 2 compares two brain specimens each of *Jimpy* and their litter-mate controls revealing the near absence of PE-2 in the myelin-deficient animals and diminished PE-1. Figs. 3a and 3b show the more marked PE-2 differences in the *Quaking* mouse brain, 40 and 50 days post-natal, as compared to 17 days

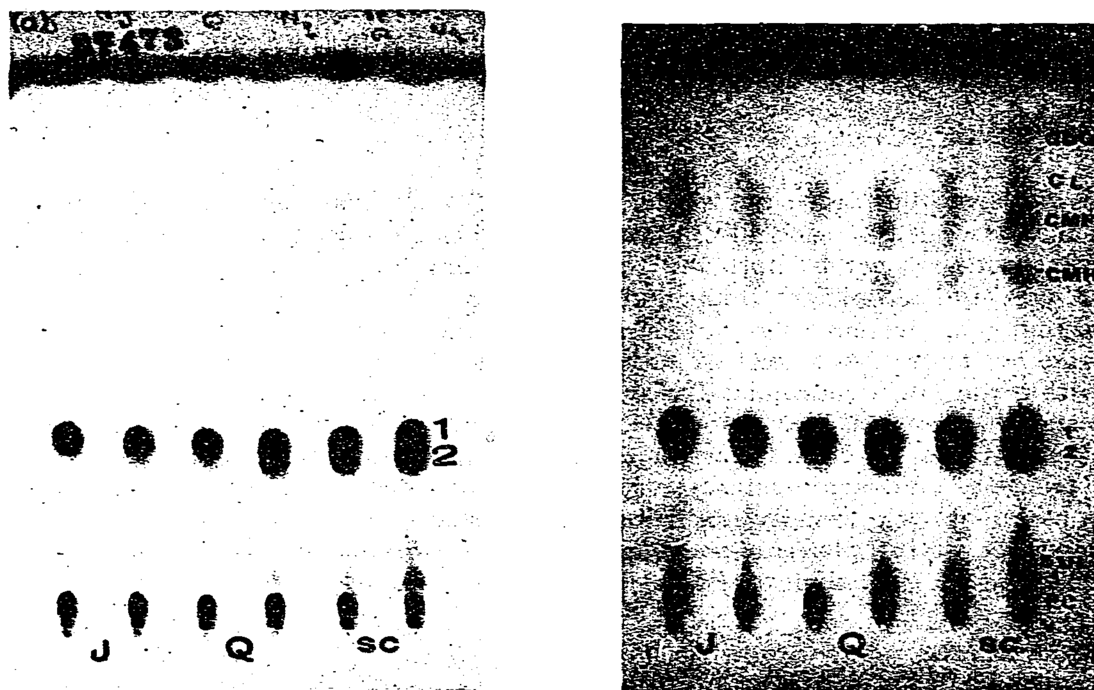


Fig. 3. (a) Schleicher & Schuell F-1500 chromatogram with CEW development of (left to right) *Jimpy* brain and control (J), *Quaking* 40-day brain and control (Q), and *Quaking* 40-day spinal cord and control (sc). The plamalogen reaction stains the PE-1 (1) and PE-2 (2) and the Rhodamine 6G counterstaining reveals the glycolipids and various other phosphatides. (b) The Rhodamine spots are much more visible while the chromatogram is still wet after staining.

*Abbrevations: PE-1 and PE-2 refer to the two phosphatidyl ethanolamine plasmalogens chromatographically resolved from lipids of myelinated nerve tissue. *n*-CMH and *h*-CMH are the major nerve tissue ceramide monohexosides. GDG is galactose diglyceride and CL is the mitochondrial phosphatide cardiolipin.

for *Jimpy*, and in the spinal cord samples of the *Quaking* series. The 50-day *Quaking* is approximately at the myelination level of the 17-day *Jimpy* littermate control. It is clear from fig. 3 that spinal cord samples are to be preferred for this demonstration because of their greater proportion of myelin as compared to the whole brain. Fig. 3b (wet chromatogram) shows more clearly the diminished sulfatide, *n*-CMH, *h*-CMH and GDG of the myelin-deficient specimens as well as what may be ester-CMH (cf. ref. 7). In the CEW chromatographic system CL and *n*-CMH coincide thereby distorting the interpretation of the relative amounts of *n*- and *h*-CMH. Replacement of some of the water with NH_4OH corrects this overlapping by selectively retarding the migration of CL allowing the two major CMH species to be more clearly expressed. The clear identity of the ester-CMH needs to be yet established so that a correct interpretation can be made as to its relationship with the myelination process; similarly the place GDG has in this over-all scheme needs to be more clearly determined.

As described earlier [1], and confirmed here, PE-1 more readily reduced OsO_4 than did PE-2 and therefore represented the more unsaturated molecular species. The specific cleavage by $HgCl_2$ is characteristic of the alk-l-enyl linkage and forms the basis for the plasmalogen reaction with the Schiff leuco-fuchsin reagent.

DISCUSSION

We have interpreted our earlier studies [1] to indicate that the major difference between PE-1 and PE-2 plasmalogen is that the PE-2 seems to be largely 18:1 with respect to both the 1-alk-1′-enyl and 2-acyl groups. Its diminution in the myelin-deficient brain and spinal cord, then, would be generally consistent with current notions [8, 9, 10] that the metabolic defect of *Jimpy* and *Quaking* mice affects one or more enzymes of fatty acid synthetic pathways within the oligodendroglia. The demyelination of multiple sclerosis and Wallerian degeneration and associated loss of plasmalogen (presumably mainly PE-2) would of course have a different explanation relating rather to the damage of the oligodendrocyte itself (or only of its plasma membrane) with subsequent lytic activity occurring. The products of this lysis could show the sequence of the plasmalogen degradation and whether or not the response is different for PE-1 than for PE-2.

REFERENCES

1 M.H. Hack and F.M. Helmy, J. Chromatogr., 135 (1977) 229.
2 N.A. Baumann, C.M. Jacque, S.A. Pollet and M.L. Harpin, Eur. J. Biochem., 4 (1968) 340.
3 J.L. Nussbaum, N. Neskovic and P. Mandel, J. Neurochem., 16 (1969) 927.
4 E. Hogan and K.C. Joseph, J. Neurochem., 17 (1970) 1209.
5 A.N. Davison and M. Wajda, J. Neurochem., 9 (1962) 427.
6 J. Domonkos and L. Heiner, J. Neurochem., 15 (1968) 87.
7 E. Klenk and M. Doss, Z. Physiol. Chem., 346 (1966) 296.
8 N.A. Baumann, M.L. Harpin and J.M. Bourre, Nature (London), 227 (1970) 960.
9 A.A. Kandutsch and S.E. Saucier, Biochim. Biophys. Acta, 260 (1971) 26.
10 I. Goldberg, I. Schechter and K. Bloch, Science, 182 (1973) 497.