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# Activity of myelin membrane $Na^+/K^+$-ATPase and 5′-nucleotidase in relation to phospholipid acyl profiles, ganglioside composition and phosphoinositides in developing brains of undernourished rats *

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**The relationship between undernutrition-induced alterations in some myelin membrane-bound enzyme activities and phospholipid fatty acid composition of this membrane was ascertained in developing rat brains. Undernutrition was imposed in pregnant dams through gestation and lactation, (last 10 days of gestation, and through lactation) by feed restriction. Experimental groups of animals received 50% of the amount of diet consumed by controls. Pups born to these mother rats were killed at day 7, 14 or 21 of postnatal age. Myelin membrane was isolated from the major regions of the brain, and analysed for phospholipid fatty acid profiles, phosphoinositides and ganglioside species. While there were no diet-related differences in the activities of 5′-nucleotidase (EC 3.1.3.5), myelin phospholipids from cerebella and brain stems of experimental rats exhibited lowered proportions of the long-chain polyunsaturated fatty acids, $C_{20:4}$ $(n-6)$ and $C_{22:6}$ $(n-3)$ concomitant with elevated activities of ouabain-sensitive $Na^+/K^+$-ATPase (EC 3.6.1.4). Levels of diphosphoinositide, triphosphoinositide and trisialogangliosides also decreased in myelin from brains of experimental animals. These results suggest a relationship between myelin phospholipid fatty acid profiles as indicators of membrane unsaturation, and the possibility of allosteric modification of $Na^+/K^+$-ATPase activity.**

## Introduction

The dependence of activity of membrane-bound enzymes on a hydrophobic environment supplied by the membrane lipid bilayer, is well established. The extent of polyunsaturation present in the membrane, appears to be involved in modulation of activity of such membrane-bound enzymes [1]. Manipulation of nutritional status, which induces changes in acyl compositions of biological membranes in various tissues, is known to be accompanied by alterations in the activity of membrane-associated enzymes [2].

$Na^+/K^+$-ATPase activity, which exhibits an overall dependence on membrane phospholipids, is influenced by both the length and degree of unsaturation of acyl moieties of phospholipids [1]. Fatty acyl profiles of myelin membrane phospholipids are specifically altered in response to dietary deprivation, during the brain spurt [3], and earlier workers [4] have documented the impaired capacity of brain microsomal elongation and desaturation systems to synthesise the long chain polyunsaturated fatty acids characteristic of myelin membrane lipids, in such experimental animals. The existence of $Na^+/K^+$-ATPase (EC 3.6.1.4) and 5′-nucleotidase (EC 3.1.3.5) activities in myelin membrane have been reported recently [5,6]. The activity of these two enzymes in the myelin sheath suggests that myelin membrane may play a significant role in biological transport of cations.

Our study thus basically aims at assessment of the influence of altered phospholipid acyl profiles of myelin membrane under nutritional inadequacy, on the activity of myelin $Na^+/K^+$-ATPase and 5′-nucleotidase. Since myelinogenesis proceeds at different rates in various regions of the brain, we were interested in determining whether (i) myelin phospholipid fatty acyl composition would be differentially affected in the cerebrum, cerebellum and brain stem, and (ii) as a corollary, myelin

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membrane bound $Na^+/K^+$-ATPase and 5′-nucleotidase would also exhibit such regional differences in vulnerability to the stress Gangliosides profiles of myelin were simultaneously studied, since these complex lipids are known to influence membrane surface potentials and ionic permeabilities [7,8] In addition to the glycosphingolipids, the polyphosphoinositides are also implicated in transmembrane cation flux in the perinodal loops of myelin [9], necessitating a study of their levels as well to enable an understanding of the various modulatory factors that influence myelin membrane bound $Na^+/K^+$-ATPase activity

## Materials and Methods

*Materials* Substrates and chemicals used for enzyme assays were obtained from Sigma Chemicals, St Louis, MO Solvents used were of analytical grade

*Nutritional studies* Typically, undernutrition was imposed in 10 day pregnant Wistar strain rats by feed restriction through gestation and lactation Rats fed a 22% protein diet (ad lib, adequate with respect to all other essential nutrients, fats carbohydrates, vitamins and minerals) were used as control animals, while those fed 50% of the diet consumed by the controls were treated as experimental (undernourished) animals Pups nursed by these two groups of dams were considered controls and experimentals, respectively Litters were culled to a size of 8 at birth, and brain tissues from control and undernourished groups of pups used for analyses, at 7 14 and 21 days of postnatal age

Animals were decapitated, brains dissected out into major anatomical regions (except for 7d brains which were used whole), and rapidly transferred to preweighed chilled vials Myelin was isolated and $Na^+/K^+$-ATPase assayed according to the method of Reiss et al [5], while 5′-nucleotidase was assayed as described by Cammer et al [6] Brain tissues from control and experimental animals were always processed simultaneously, and isolated myelin membrane fractions were assessed for purity at all stages studied, using suitable marker enzymes (glucose-6-phosphatase [10] for microsomal contamination, succinate dehydrogenase [10] for mitochondrial, thiamine pyrophosphatase [10] for golgi, 5′-nucleotidase [10] for plasma membrane, and acetylcholine esterase [11] for axolemmal contamination) All enzyme assays were done in duplicate, and the amount of protein used and time of incubation were within linear range of the enzyme reactions Myelin $Na^+/K^+$-ATPase was always assayed in the presence of 5 mM azide to exclude the contribution of mitochondrial ATPase Assays were performed in the presence and absence of (a) 3 mM ouabain, and (b) 0 01% deoxycholate (DOC)

Myelin lipids were extracted by the method of Folch et al [12] Individual phospholipids were separated by two dimensional thin layer chromatography (TLC), with modified solvent system for the second dimension [13,14] Resolved bands were identified with iodine vapours (by comparing with standard lipids run simultaneously) Identified phospholipids were scraped off the plates, eluted with suitable solvents, and the eluates taken to dryness under nitrogen The phospholipid fatty acids were then transesterified with 5% methanolic NaOH for 30 min, neutralised with 0 35 M acetic acid, washed twice with methanol/water (1 1 v/v) and extracted into petroleum ether, finally being taken to dryness under nitrogen and suspended in iso-octane

The phospholipid fatty acid methyl esters were separated in a Varian Model 3700 gas chromatograph equipped with a flame ionisation detector and a steel column (1/8 inch internal diameter, containing Silar 10C as stationary phase, adsorbed on 80–100 chromosorb W, Supelco Inc Bellefonte, PA) Nitrogen was used as the carrier gas at a rate of 20 ml/min Oven temperature was 200°C Peaks were identified using reference standards supplied by Sigma Chemicals, St Louis Peak area computation was done on a Varian 4270 electronic integrator

Myelin membrane cholesterol was estimated according to Zlatkis et al [15] Polyphosphoinositides were extracted from myelin membrane samples by the method of Hauser et al [16] and separation carried out by TLC as described by Pappu and Hauser [17]. Lipid phosphorus was quantified by a micro-method [18] Blanks of 60 mg of silica gel gave readings equivalent to 0 06 μg of phosphorus Appropriate corrections for lipid phosphorus were made by eliminating phosphorus contained in silica gel Gangliosides of myelin membrane were extracted and resolved by TLC according to the procedure adopted by Suzuki et al. [19], detection was carried out using resorcinol spray [19] and ganglioside NANA (*N*-acetylneuraminic acid) estimated by the method of the same authors Membrane protein was quantitated by the procedure of Lowry et al [20] using crystalline bovine serum albumin as a standard

*Statistical analysis* All data were statistically analysed and the significance of differences between the means of control and experimental groups was calculated using Student's '*t*'-test [21]

## Results

*Body weight and brain weight differences in undernourished rat pups*

Pre- and early post-natal undernutrition caused a significant reduction of body weights of experimental pups (to 54 9% of control values, $P < 0.01$) by the third week of life Brain weights of these animals were spared considerably from such an effect Pooled brain-region weights in undernourished progeny were 90–92% of control values in all the regions studied

TABLE I

*Activities of myelin membrane $Na^+/K^+$ ATPase (μmol / mg protein per h)*

Myelin membrane isolated from whole brain/regional homogenates according to Reiss et al [5] and $Na^+/K^+$-ATPase assayed as described in Methods. Enzyme activities are expressed as μmol $P_i$ formed per mg protein per h. The values are means ± S.E. for six independent experiments in each case. Differences between control and undernourished groups assessed for statistical significance by Student's *t*-test. * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$. (Eight whole brains at day 7, five cerebra/eight cerebella/eight brain stems at days 14 and 21, were pooled to yield myelin membrane samples in every experiment)

| Age Group | Whole brain (1) | | |
|---|---|---|---|
| 7d Control | 14.1 ± 2.04 | | |
| Experimental | 14.5 ± 2.55 | | |
| | Cerebrum (1) | Cerebellum (1) | Brain stem (1) |
| 14d Control | 18.6 ± 1.47 | 12.3 ± 1.49 | 16.1 ± 0.54 |
| Experimental | 21.3 ± 1.94 | 19.4 ± 0.76 ** | 18.5 ± 0.70 ** |
| 21d Control | 14.5 ± 0.63 | 13.5 ± 0.76 | 16.5 ± 2.00 |
| Experimental | 21.0 ± 1.39 ** | 19.6 ± 1.14 ** | 24.5 ± 0.48 ** |

*Myelin $Na^+/K^+$-ATPase and 5′-nucleotidase*

Activity of $Na^+/K^+$-ATPase in myelin membrane was significantly elevated in both the cerebella and brain stems of undernourished rat pups at 14 and 21 days of age (activity in 14d experimental cerebella and brain stems being 156% and 115% of corresponding control values, $P < 0.01$, Table I). By the third week of life, the cerebral myelin $Na^+/K^+$-ATPase activity had also increased in experimental brains, to 144% of that in control cerebral myelin membrane. Activity of this enzyme in myelin from the brain stems increased further by day 21 in undernourished rat pups (from 115% of control values at 14d, to 148% of control values at 21d, $P < 0.01$). Presence of deoxycholate yielded an approximately 3.7–3.9-fold stimulation of myelin $Na^+/K^+$-ATPase activity during the second and third weeks of life (Table II), in both control and experimental animals. Myelin membrane protein, expressed as mg protein/g brain, did not exhibit significant differences between these two groups. 5′-nucleotidase activities were not altered in the brain regions studied in experimental animals, at any stage. That the isolated myelin membrane fractions were reasonably free from contamination with other subcellular membranes, was evident from the data obtained on marker enzyme relative specific activities in myelin (data not presented).

*Myelin phospholipid acyl chain composition and cholesterol*

A study of the myelin phospholipid fatty acyl profiles (Table III) indicates that there are significant decreases in the degree of membrane unsaturation, as shown by the unsaturation index of myelin phospholi-

TABLE II

*Effect of deoxycholate on myelin membrane $Na^+/K^+$ ATPase*

All values represent means ± S.E. of six independent experiments. Enzyme assays in the presence of deoxycholate contained 0.01% (DOC as final concentration) of the detergent; all assays were performed in duplicate. C3 cerebrum, CL cerebellum, BS brain stem.

| | | $Na^+/K^+$ ATPase (μmol P / mg protein per h) | | Ratio stimulated activity / basal activity |
|---|---|---|---|---|
| | | basal (− DOC) | stimulated (+ DOC) | |
| 7d | Control | 14.1 ± 2.04 | 39.6 ± 4.48 | 2.80 |
| | Exptal | 14.6 ± 2.55 | 41.4 ± 5.16 | 2.84 |
| 14d CB | Control | 18.6 ± 1.47 | 69.1 ± 5.84 | 3.71 |
| | Exptal | 21.3 ± 1.94 | 79.7 ± 6.18 | 3.74 |
| 14d CL | Control | 12.4 ± 1.49 | 45.8 ± 4.86 | 3.70 |
| | Exptal | 19.4 ± 0.76 | 72.8 ± 5.08 | 3.75 |
| 14d BS | Control | 16.1 ± 0.54 | 61.6 ± 6.96 | 3.82 |
| | Exptal | 18.5 ± 0.70 | 71.0 ± 7.16 | 3.83 |
| 21d CB | Control | 14.5 ± 0.64 | 55.2 ± 3.66 | 3.80 |
| | Exptal | 21.0 ± 1.39 | 80.8 ± 5.67 | 3.83 |
| 21d CL | Control | 13.5 ± 0.76 | 51.3 ± 5.03 | 3.86 |
| | Exptal | 19.6 ± 1.15 | 75.0 ± 7.11 | 3.86 |
| 21d BS | Control | 16.5 ± 2.08 | 63.8 ± 6.48 | 3.86 |
| | Exptal | 24.5 ± 0.48 | 94.7 ± 6.86 | 3.86 |

TABLE III

*Myelin membrane unsaturation index and contents of phospholipid long chain polyunsaturated fatty acids*

Values are means ± S.E. of six independent observations in each case. Unsaturation index [21] = sum of (percentage of each fatty acid × number of double bonds). C, control; E, experimental; CB, cerebrum; CL, cerebellum; BS, brain stem. Differences between control and experimental groups are assessed for statistical significance by Student's *t*-test. ** $P < 0.01$ *** $P < 0.001$

| | | $(n-6)+(n-3)$ | Unsat index | $C_{20:1}/C_{20:4}$ |
|---|---|---|---|---|
| 7d | C | 8.4 ± 0.3 | 59.1 | 0.15 ± 0.015 |
| | E | 9.0 ± 0.5 | 58.7 | 0.15 ± 0.016 |
| 14d CB | C | 22.0 ± 0.3 | 127.6 | 0.29 ± 0.018 |
| | E | 16.2 ± 0.2 ** | 98.2 | 0.42 ± 0.036 ** |
| 14d CL | C | 23.8 ± 0.3 | 136.8 | 0.36 ± 0.034 |
| | E | 14.8 ± 0.2 *** | 97.3 | 0.84 ± 0.081 *** |
| 14d BS | C | 25.1 ± 0.3 | 142.6 | 0.31 ± 0.034 |
| | E | 16.6 ± 0.4 *** | 107.6 | 0.74 ± 0.078 *** |
| 21d CB | C | 27.1 ± 0.6 | 158.1 | 0.40 ± 0.046 |
| | E | 16.9 ± 0.3 *** | 121.0 | 0.89 ± 0.096 *** |
| 21d CL | C | 29.7 ± 0.3 | 166.5 | 0.31 ± 0.034 |
| | E | 17.4 ± 0.5 *** | 113.4 | 0.72 ± 0.077 *** |
| 21d BS | C | 34.3 ± 0.7 | 193.3 | 0.33 ± 0.039 |
| | E | 19.9 ± 0.5 *** | 118.2 | 0.71 ± 0.081 *** |

pids from the cerebellum and brain stem (at both, the 14d and 21d stages) in experimental animals (Table III) The differences in unsaturation index in these regions, between control and undernourished groups, exceeded those observed in the cerebral myelin from these groups Surprisingly, the 20 3/20 4 ratios in membrane phospholipids from all brain regions of nutritionally deprived animals were significantly increased, akin to the condition of essential fatty acid deficiency (EFA), where an increased triene/tetraene ratio is utilised as an indicator of EFA insufficiency (Galli et al [22]) While phosphatidyl-choline, serine and -inositol in experimental myelin accounted for about 10% each, of the overall increase in phospholipid $C_{20\,3}$, phosphatidylethanolamine accounted for the maximal increase of $C_{20\,3}$ (78%) in these food-restricted pups

Myelin maturation was accompanied by an increase in relative proportions of the polyunsaturated fatty acid (PUFA) content of phospholipids the total $(n-6)+(n-3)$ fatty acids increased from 8 4 to 34 3% between 7d and 21d after birth, as against 9 0 to 19 9 during the same period in control and experimental animals respectively (Table III) While myelin membrane phospholipids from undernourished pups tended to exhibit higher percentages of saturated ($C_{16\,0}+C_{18\,0}$) and monounsaturated ($C_{16\,1}+C_{18\,1}+C_{10\,1}$) fatty acids than corresponding control pups, such differences were not statistically significant The ratio $(n-6)/(n-3)$ remained constant in both, control and food-restricted groups, however, despite significant diminutions, at both, 14 and 21d in the $(n-6)$ and $(n-3)$ series fatty acids, in experimental animals By the third week of life, the $(n-6)+(n-3)$ contents of myelin obtained from cerebra, cerebella, and brain stems of undernourished animals, constituted 62%, 59% and 58% of corresponding control values, respectively ($P<0\,001$, Table III)

No changes were observed in the headgroup distribution of myelin membrane phospholipids between the two groups of animals (data not shown) Analyses of fatty acyl profiles of individual phospholipids revealed that diacylglycerophosphoryl-ethanolamine(dGPE) and -inositol (dGPI) were maximally affected by the stress of nutritional inadequacy, while no significant alterations were recorded in the diacylglycerophosphorylcholine, or serine classes All the three regions examined indicated drastic reductions in dGPE arachidonate ($C_{20\,4}$) and docosahexaenoate ($C_{22\,6}$) in myelin from the experimental group (Figs 1–3)

The dGPE fractions from experimental cerebral, cerebellar and brain stem myelin were more affected, in terms of reductions in these long chain PUFAs ($P<0\,001$, Figs 1–3) The (dPGE)$C_{20\,4}$ in myelin from these regions, constituted 39 8% 27 9%, 31 3%, (dGPE)$C_{22\,6}$ was 32 5%, 24 8%, 22 9%, respectively, of control figures Percent values of $C_{20\,4}$ in the dGPI fractions of

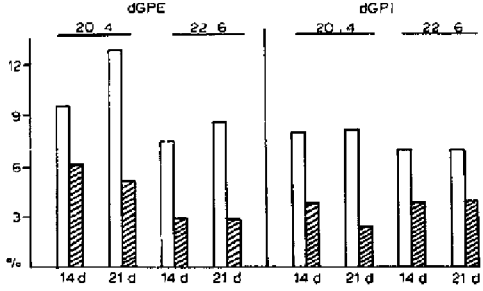


Fig 1 The proportion of 20 4 $(n-6)$ and 22 6 $(n-3)$ in ethanolamine(dGPE) and inositol-(dGPI) phosphoacylglycerols from cerebral myelin membrane fractions during dietary inadequacy The undernutrition was initiated prenatally upto 21d of age (see text for details) Results (expressed as percentage values of total fatty acid methyl esters of respective phospholipid) are the mean percentages of acyl group composition from three individual samples (d = age, in days) □, Control, ▨, undernourished

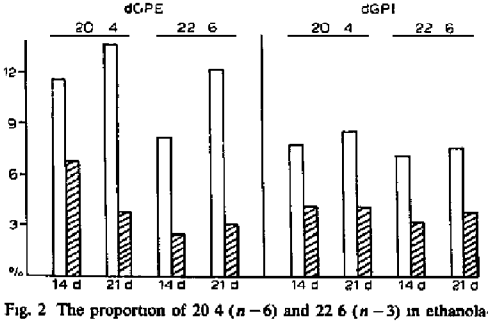


Fig. 2 The proportion of 20 4 $(n-6)$ and 22 6 $(n-3)$ in ethanolamine-(dPGE) and inositol-(dGPI) phosphoglycerides from cerebellar myelin membrane fractions Results are the mean percentages of acyl group composition from three individual samples (d = age in days) □, Control ▨, undernourished

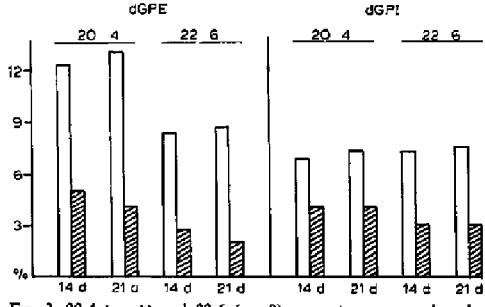


Fig 3 20 4 $(n-6)$ and 22 6 $(n-3)$ proportions in myelin phospholipids from the brain stem Results are the mean percentages of acyl group composition from three individual samples (d = age, in days) □, Control, ▨, undernourished

TABLE IV

*Profiles of myelin membrane gangliosides isolated from the developing rat brain*

Values represent means ± S E of six observations each and are expressed as mole percent (mole fraction × 100) Mole percent of any given gangliosides =

$$\frac{\text{No of moles of the ganglioside}}{\text{Sum of No of moles of all gangliosides } (G_{M_1}+G_{D_{1a}}+G_{D_{1b}}+G_{T_1})}$$

gangliosides derived from pooled aqueous upper-phase [12] extracts of myelin samples determined by the method of Suzuki et al [19] after resolution by thin-layer-chromatography $G_1$(trisialoganglioside) $G_2$ and $G_3$(disialogangliosides $G_{D1a}$ and $G_{D1b}$, respectively) $G_4$ (monosialoganglioside) Gangliosides are termed according to Korey and Gonatas [32] with the corresponding Svennerholm nomenclature in parentheses [33] Statistical significance of the difference between control and experimental groups ** $P < 0.01$, *** $P < 0.001$

| Group/region of brain | $G_3$ ($G_{T_1}$) | $G_2$ ($G_{D_{1a}}$) |
|---|---|---|
| 14d Control cerebrum | 11 77 ± 0 099 | 13 68 ± 0 143 |
| 14d Experimental cerebrum | 11 65 ± 0 119 | 13 75 ± 0 171 |
| 14d Control cerebellum | 11 75 ± 0 066 | 13 72 ± 0 284 |
| 14d Experimental cerebellum | 11 10 ± 0 130 ** | 15 07 ± 0 112 *** |
| 14d Control brain stem | 11 81 ± 0 092 | 13 83 ± 0 079 |
| 14d Experimental brain stem | 8 73 ± 0 010 *** | 17 14 ± 0 170 *** |
| 21d Control cerebrum | 11 80 ± 0 660 | 13 71 ± 1 114 |
| 21d Experimental cerebrum | 11 80 ± 0 052 | 13 72 ± 0 031 |
| 21d control cerebellum | 11 50 ± 0 086 | 13 91 ± 1 160 |
| 21d Experimental cerebellum | 8 78 ± 0 106 *** | 16 03 ± 0 057 *** |
| 21d Control brain stem | 11 78 ± 0 078 | 13 61 ± 0 166 |
| 21d Experimental brain stem | 8 46 ± 0 057 *** | 17 03 ± 0 057 *** |

experimental myelin membrane were 29 6%, 47 6%, 57.9% while $C_{22\,6}$ represented 57%, 50% and 42 1% of corresponding control figures for the cerebra, cerebella and brain stems, respectively (Figs 1–3) Membrane cholesterol contents did not vary between the two groups studied

*Gangliosides and polyphosphoinositides of myelin membrane*

Food restriction-induced changes in gangliosides species composition are illustrated in Table IV The total amounts of *N*-acetylneuraminic acid/mg myelin (ganglioside sialic acid per mg of lyophilised myelin membrane) remained unaltered between control and undernourished groups However, distribution of lipid-bound sialic acid among various ganglioside species did exhibit differences Characteristically, the mole fractions of $G_2$($GD_{1a}$) in the myelin gangliosides from experimental cerebella and brain stems at day 14 increased at the expense of lowered amounts of the higher ganglioside, $G_1$($GT_1$) ($P < 0.05$ and 0.001, respectively) These patterns were evident at the 21d stage too, albeit with a further drop of $G_1$ ($GT_1$) levels in the cerebellum gangliosides, as compared to the 14d stage, in undernourished pups ($P < 0.001$, Table IV)

Phosphatidylinositol phosphate levels in experimental cerebellar myelin showed a lowering at the 14d stage, while by day 21 significant differences were obvious between control and experimental myelin phosphatidylinositol phosphate (PIP) and phosphatidylinositol diphosphate ($PIP_2$) from all brain regions studied, the deficits being most pronounced in the experimental cerebellum ($P < 0.001$, Table V)

## Discussion

Our data demonstrate that nutritional inadequacy mediated alterations in myelin membrane phospholipid

TABLE V

*Levels of phosphatidyl inositol phosphate (PIP) and phosphatidyl inositol diphosphate ($PIP_2$) in myelin membrane isolated from various regions of the developing brain in postnatally undernourished rat pups*

Myelin isolated from brains of control and experimental (see text for details) rat pups aged 7 14 and 21d was processed for extraction of polyphosphoinositides [15] The polyPI lipid fraction was then resolved by TLC [16] into phosphatidylinositol phosphate (PIP) and phosphatidylinositol diphosphate ($PIP_2$), and phosphorus in isolated bands determined [17] (a) denotes percent (percent of total myelin lipid weight) of phosphatidyl inositolphosphate (PIP) while (b) denotes percent (percent of total myelin lipid weight) of phosphatidyl inositoldiphosphate ($PIP_2$) Differences between control and experimental groups assessed for statistical significance by the Student's *t*-test * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ All values are means ± S E of six independent observations.

| Group | Whole brain | | | | | |
|---|---|---|---|---|---|---|
| | (a) | (b) | | | | |
| 7d Control | 1 61 ± 0 052 | 0 93 ± 0 030 | | | | |
| 7d Experimental | 1 54 ± 0 055 | 0 91 ± 0 017 | | | | |
| | Cerebrum | | Cerebellum | | Brain stem | |
| | (a) | (b) | (a) | (b) | (a) | (b) |
| 14d Control | 1 65 ± 0 092 | 0 87 ± 0 028 | 1 76 ± 0 013 | 0 95 ± 0 008 | 1 76 ± 0 067 | 0 98 ± 0 078 |
| 14d Experimental | 1 59 ± 0 012 | 0 96 ± 0 013 | 1 49 ± 0 023 *** | 0 94 ± 0 047 | 1 64 ± 0 024 | 1 01 ± 0 031 |
| 21d Control | 1 81 ± 0 027 | 1 06 ± 0 036 | 1 67 ± 0 028 | 1 08 ± 0 018 | 1 74 ± 0 088 | 1 05 ± 0 038 |
| 21d Experimental | 1 56 ± 0 028 *** | 0 93 ± 0 017 ** | 1 35 ± 0 033 *** | 0 78 ± 0 021 *** | 1 52 ± 0.044 * | 0 95 ± 0 006 * |

acyl chain composition are associated with elevated activities of myelin $Na^+/K^+$-ATPase in experimentally food-restricted, growing rat pups While such changes in myelin phospholipid fatty acid profiles have been reported in brains of undernourished animals before [3], there have been no attempts to simultaneously examine functional parameters of the membrane under these conditions Thus, these observations are of relevance, in terms of a functional role for myelin membrane, since the existence of an intrinsic $Na^+/K^+$-ATPase in this membrane implicates that the former is involved in regulation of cation fluxes

Definitive evidence for the association of an enzyme with myelin membrane isolated from young, myelinating brains, is rendered difficult owing to the fact that the distribution of membrane material in a sucrose gradient changes dramatically during growth of the rat pup Marker enzyme profile characterisation, which was carried out to enable an assessment of the purity of isolated myelin fractions, firmly ruled out significant contamination with other subcellular organelles/membranes Characteristics other than activity under a single standard set of conditions should provide a more reliable index of the intrinsic nature of a membrane bound enzyme [6] We, therefore, chose to determine the response of myelin membrane $Na^+/K^+$-ATPase to DOC treatment The 2 8–3 9-fold stimulation of $Na^+/K^+$-ATPase activity is in conformity with the observations of Reiss et al [6], and proves that the enzyme being assayed under these experimental conditions is myelin-associated $Na^+/K^+$-ATPase Besides, the fold stimulation factor, representing the ratio stimulated/basal $Na^+/K^+$-ATPase activity was similar between control and experimental myelin membrane clearly confirming that the proportion of vesicularised myelin membrane was similar these samples Lack of differences in amounts of myelin membrane protein between control and undernourished groups indicated that the documented increases in specific activities of myelin $Na^+/K^+$-ATPase of experimental animals did not arise from overall differences in membrane protein contents, though it did not rule out the possibility of an alteration in the amount of this enzyme protein in such animals

Ouabain-sensitive $Na^+/K^+$-ATPase in cerebellar and brain stem myelin membrane, was significantly elevated in experimental animals at both the 14d and 21d stages, concomitant with decreases in the double bond index and proportions of long chain polyunsaturated fatty acids This occurrence is probably indicative of an adaptative change in enzyme activity, in response to an altered phospholipid acyl profile of myelin phospholipids That cerebral myelin at 14d does not exhibit changes in $Na^+/K^+$ ATPase activity provides convincing enough evidence that the cerebral hemispheres are not myelinating at the same rate as the other two regions (cerebellum and brain stem), and are therefore not affected to a similar extent at this stage

The $(n-6)+(n-3)$ values from myelin phospholipids of undernourished animals remained below those of control counterparts at all the ages examined, and are definitively indicators of impairments in the capacity of the growing brain under nutritional stress, to elaborate long chain PUFAs characteristic of these membrane phospholipids Vallet-Strouve and Tordet-Candroit [23] have shown a similar decrease of the enzymatic desaturation of linoleic acid ($C_{18\,2}$ $(n-6)$ which is the precursor of the $(n-6)$ serie fatty acids [24] in intrauterine-growth-retarded rat brains Secondly, it has been demonstrated that both, $(n-6)$ and $(n-3)$ series are metabolised competitively [25], however, in this case, as the total $(n-6)+(n-3)$ fatty acids from experimental myelin phospholipids was modified, as compared to controls (as opposed to constant $(n-6)/(n-3)$ ratios), we presume this modification is not a reflection of enzymatic competition between the two polyunsaturated series

That the changes documented in long chain PUFAs of myelin membrane from experimental pups do not arise from alterations in phospholipid headgroup variations between groups was clearly testified by the lack of differences in headgroup compositions This part of the study was deemed important because (while bilayer fluidity is basically a property of the motional state of the lipid acyl chains) the lipid headgroups also have shown to have a significant effect on acyl chain motion, and more importantly, membrane unsaturation [1] Large increases in dGPE $C_{20\,3}$, which resulted ultimately in the raised $C_{20\,3}/C_{20\,4}$ ratios of myelin in undernourished animals, most likely owes its origin to the alkenylacylglycerophosphorylethanolamine (aGPE) fraction, since we did not estimate $C_{20\,3}$ levels separately in these two types (dGPE and a GPE) of ethanolamine phosphoacylglycerols The fact that myelin is rich in alkenylacyl GPE makes this a reasonable speculation [26] Furthermore, the absence of significant alterations in percentages of $C_{20\,3}$, $C_{20\,4}$ and $C_{22\,6}$ in dGPC and dGPS are not unexpected, because these two phospholipids normally contain only small amounts of PUFA The dramatic lowering of arachidonate and docosahexaenoate in dPGE and dGPI during the 14 and 21d stages support an involvement of the cytochrome reductase dependent microsomal chain elongation-desaturation systems of the brain in the experimental animals [4] In this context, it is interesting to note that fatty acid deficient liver plasma membrane $Na^+/K^+$-ATPase was shown [27] to have higher $V_{max}$ and $K_m$ compared with controls Decreases in membrane unsaturation of the kind reported here, could be expected to influence physical properties, like 'bulk fluidity' [1] of the membrane bilayer and thereby, mem-

brane-bound enzyme activity, though such changes (of membrane) fluidity have not been monitored in this instance Cholesterol is also known to influence $Na^+/K^+$-ATPase activity [1], by virtue of its ability to inhibit molecular motion/conformational freedom within the phospholipid bilayer No differences were found, however, in myelin cholesterol contents between the adequately fed and restricted group of animals, thus excluding the involvement of cholesterol in the observed changes in $Na^+/K^+$-ATPase activity in experimental animals The greater vulnerability of the cerebellum and brain stem myelin from experimental animals, to undernutrition induced alterations in phospholipid composition are attributable to the earlier onset of myelinogenesis in these regions, since the progress of myelination in the central nervous system is known to be caudo-rostral Lack of an effect of the altered phospholipid acyl composition, on 5'-nucleotidase in experimental myelin corroborates earlier observations [28] indicating that the activity of this enzyme is independent of membrane lipid composition

Myelin membrane possesses a characteristic composition of glycosphingolipids, or gangliosides The presence of gangliosides is known to increase conductance of phosphatidylcholine bilayers, and cause changes of surface potential and molecular packing of lipids [8] Thus, it is conceivable that nutritional inadequacy-mediated lowering of the levels of the higher polysialoganglioside $G_1$ ($G_{T_1}$) in myelin membrane from experimental cerebella and brain stems (Table VI) could be influencing membrane lipid organisation as well This speculation seems to be in general agreement with the altered activities of myelin membrane bound $Na^+/K^+$-ATPase Recent reports have also suggested that gangliosides may alter the activity of membrane-bound enzymes Brain $Na^+/K^+$-ATPase showed an enhancement of 26–43% by exogenously added gangliosides [29] this process was concentration dependent and required stable, irreversible insertion of gangliosides into the membrane bilayer

Previous workers have reported the presence of phosphatidylinositol phosphate (PIP) and phosphatidylinositol diphosphate ($PIP_2$) in packed myelin lamellae, and speculated upon their involvement in regulation of cation fluxes in this membrane [9] Besides, Kahn and Morell [30] have more recently obtained evidence for the turnover of phosphoinositide and phosphatidic acid in myelin, and its stimulation by acetylcholine These findings are suggestive of an influence of phosphoinositides (viz PIP and $PIP_2$) on the activity of cation flux controlling enzymes such as the myelin $Na^+/K^+$-ATPase. The lowered levels of PIP and $PIP_2$ in myelin from undernourished pups thus seem to effectively eliminate their involvement in observed elevations of $Na^+/K^+$-ATPase activity under the conditions of our experiment

In conclusion the data summarised in this account support the role of an adaptative increase in myelin of experimental animals, in an attempt, possibly to counteract the overall effects of decreased unsaturation of membrane phospholipids The consequences of such a phenomenon are presently not understood and deserve further study, in view of the fact that myelin $Na^+/K^+$-ATPase could control both transmyelin flux of ions and fluid [31]

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