Biochimica et Biophysica Acta 860 (1986) 325–335
Elsevier

BBA 73221

Homeoviscous adaptation under pressure. III. The fatty acid composition of liver mitochondrial phospholipids of deep-sea fish

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(Received November 25th, 1985)
(Revised manuscript received March 17th, 1986)

Key words: Lipid composition; Homeoviscous adaptation; High pressure; (Deep-sea fish)

Homeoviscous adaptation of biological membranes to high hydrostatic pressure has been investigated by determining the differences in lipid composition of membranes from fish obtained from depths between 200 and 4000 m. The fatty acid composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine/inositol and cardiolipin from a liver mitochondrial fraction was analysed by capillary gas-liquid chromatography. The ratio of saturated to unsaturated fatty acids was significantly and negatively related to depth in PC and PE as predicted by homeoviscous adaptation to pressure. Thus, deep sea species possess greater proportions of unsaturated fatty acids than shallow species. Cardiolipin showed the opposite trend. An unsaturation index was not significantly related to depth in any phospholipid fraction.

Introduction

The two principal thermodynamic variables, temperature and pressure, have significant effects upon the physical properties of biological membranes [1]. Thus, low temperature and high pressure exert similar ordering effects upon the hydrophobic core of artificial bilayers [2] and biological membranes [3]. Many living organisms compensate for the direct effects of temperature variations by compensatory adjustments of membrane structural order, a phenomenon termed 'homeoviscous adaptation'. The evidence for this is of two types, namely, biochemical and biophysical. Cold-acclimation invariably results in reduced percentage of saturated fatty acids in membrane phospholipids, a response which on the basis of other experiments is expected to offset the ordering effects of cold [4]. Experiments with probes of membrane structure demonstrate reduced bilayer order in membranes of cold-acclimated animals relative to those of warm-acclimated animals [5,6].

Homeoviscous theory predicts that membranes are also adapted to offset other bilayer-ordering factors, notably pressure. In comparison with temperature the study of the adaptation of membranes to hydrostatic pressure has scarcely begun. This is partly due to the recently acquired understanding of how pressure affects membranes and partly to the difficulty of obtaining material from organisms which live in the deep sea. Nevertheless, there are a few observations which are consistent with homeoviscous adaptation to pressure. Lewis [7] found that the proportion of 18:1 fatty acids increased with depth at the expense of saturated and longer chain unsaturated fatty acids. Patton [8] found that deep water and antarctic fish species possessed more polyunsaturated fatty acids compared to tropical fish species. However, he was unable to observe any significant difference between the deep sea and antarctic species, which might be related specifically to a pressure adapta-

tion. Recently, a more detailed examination of fish brain gangliosides has been reported for shallow and 2000 m depth species [9]. The lipids from the latter contained a relatively low proportion of saturated fatty acids, which is consistent with our prediction.

Recently, we have shown the bilayer order of membranes isolated from fish obtained from depths of 4000 m (or 400 atm, 40 MPa) is consistent with homeoviscous adaptation to pressure [10]. Of the membrane fractions isolated at sea, only one was available in sufficient quantity to enable its lipids to be characterised, namely, the liver mitochondrial fraction. We present here the fatty acid composition of phosphoglycerides from this membrane fraction. We demonstrate a statistically significant relationship between lipid saturation and depth (pressure) of capture, as required by homeoviscous theory [11].

Materials and Methods

Animals. Fish were trawled in the vicinity 50°N, 13°W (North Atlantic) from known sea floor depths, down to 4000 m during two research cruises; for details see Ref. 10. The depth of capture is a good indicator of the animals' normal ambient pressure [11]. Pressure increases linearly 10 atm (approx. 1 MPa) for each 100 m of water.

Membrane fractionation and lipid extraction. A mitochondrial fraction was prepared from freshly excised liver as described previously [10]. An aliquot of this fraction (about 0.1–0.2 ml) was immediately extracted by addition of 2 ml chloroform/methanol (2:1, v/v) containing 0.005% (w/v) butylated hydroxytoluene. This single phase solution was sealed in a glass ampoule and stored under nitrogen at −20°C at sea. After the cruise a further 2 ml of chloroform/methanol solution

TABLE I

THE FATTY ACID COMPOSITION OF PHOSPHATIDYLCHOLINE FROM A MITOCHONDRIA-RICH FRACTION ISOLATED FROM FISH LIVERS

The fish were collected from the depths indicated, in the North Atlantic on two separate cruises. The values are the mean % weight of n replicate animals, ±S.D. in brackets. Fatty acids which were tentatively identified but not shown include 10:0, 12:0, 14:1($n-9$),

	Lepidorhombus whiffiagonis 200 m ($n = 3$)	*Lophius budegassa* 200 m ($n = 3$)	*Phycis blennoides* 900 m ($n = 4$)	*Lepidion eques* 800–900 m ($n = 6$)	*Alepocephalus bairdii* 1170 m ($n = 4$)
16:0	15.9 (0.8)	22.2 (1.9)	23.9 (0.6)	22.0 (3.8)	18.4 (2.1)
16:1($n-7$)	3.1 (0.5)	4.7 (1.8)	2.7 (0.6)	2.5 (0.4)	2.0 (1.0)
18:0	2.3 (0.7)	4.7 (1.0)	2.8 (0.5)	3.4 (0.9)	3.3 (0.9)
18:1($n-7$)	2.0 (0.2)	2.6 (1.0)	2.1 (0.5)	2.4 (0.2)	2.5 (1.1)
18:1($n-9$)	7.9 (1.4)	10.3 (1.2)	9.4 (2.2)	8.7 (0.7)	10.7 (0.9)
18:2($n-6$)	0.6 (0.1)	1.1 (0.2)	0.3 (0.1)	0.4 (0.2)	0.6 (0.1)
18:3($n-6$)	0.4 (0.3)	0.8 (0.4)	1.1 (1.3)	2.3 (1.7)	0.8 (0.9)
20:1($n-9$)	1.6 (0.8)	1.3 (0.6)	0.5 (0.6)	1.0 (0.2)	2.3 (0.9)
20:4($n-6$)	6.4 (0.6)	4.9 (0.4)	3.4 (0.5)	4.7 (0.9)	3.9 (0.8)
20:5($n-3$)	10.1 (0.5)	7.2 (2.4)	8.4 (1.2)	8.6 (1.2)	10.7 (1.0)
22:5($n-3$)	4.4 (0.4)	1.4 (0.4)	1.5 (0.4)	1.1 (0.6)	2.9 (0.5)
22:6($n-3$)	32.1 (0.9)	28.7 (3.1)	28.6 (2.9)	23.9 (4.2)	22.5 (2.6)
Unknowns	7.2 (1.6)	5.8 (1.7)	9.4 (2.3)	12.5 (3.1)	12.8 (1.6)
Σ saturated	21.8 (0.6)	29.5 (1.63)	29.8 (1.2)	28.9 (4.9)	25.7 (2.5)
Σ monounsaturated	16.3 (1.4)	19.5 (4.4)	17.4 (2.4)	16.6 (1.4)	19.5 (0.4)
Σ polyunsaturated	54.8 (1.1)	45.2 (4.4)	43.4 (1.5)	42.0 (5.8)	42.0 (3.7)
Unsaturation index	312.0 (5.4)	262.3 (23.0)	256.5 (12.1)	236.0 (35.1)	244.0 (18.8)
Saturation ratio	0.306 (0.014)	0.457 (0.025)	0.493 (0.043)	0.457 (0.129)	0.421 0.066)

was added together with 0.5 vol. (v/v) 0.8% KCl. The emulsion was separated by centrifugation at $200 \times g$ for 10 min. The chloroform layer was removed and reduced in volume under nitrogen and stored under nitrogen in sealed glass ampoules at $-20°C$.

Phospholipid separation, and separation of fatty acid methyl esters. Phospholipids were separated by two-dimensional thin-layer chromatography, as described previously [12]. The plates were sprayed with an aqueous solution of 0.005% (w/v) Rhodamine 6G and the phospholipids were visualised under ultraviolet illumination. The choline, ethanolamine and serine/inositol phosphoglycerides and cardiolipin fractions were eluted with 2 ml chloroform/methanol solution and methylated with BF_3-methanol as described by Morrison and Smith [13]. The methyl esters were stored under nitrogen in sealed glass ampoules at $-20°C$.

Gas-liquid chromatography. The fatty acid methyl esters were identified by capillary gas-liquid chromatography using a Carlo Erba chromatograph (Model Fractovap 4160) and an SP-1000 capillary. Hydrogen was used as the carrier gas and a good resolution of peaks was obtained with a continuous thermal ramp (2.5 Cdeg/min) following an initial equilibration at 100°C. The injector temperature was 225°C and the flame ionization detector was 240°C. Peaks were quantified by a Hewlett-Packard recording integrator whose calibration was checked by manual triangulation. Methyl esters were identified by comparison of retention times with those of authentic standards obtained from Supelco Inc., (Bellefonte, PA) and Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.), supplemented by standards from the Institute of Marine Biochemistry, Aberdeen.

15:0, 16:1($n-9$), 16:1($n-9$), 16:2, 16:3($n-3$), 16:4($n-?$), 17:0, 18:1($n-7$), 18:3($n-3$), 18:4($n-3$), 20:0, 20:1($n-11$), 20:1($n-7$), 20:2($n-6$), 20:3($n-6$), 20:3($n-3$), 20:4($n-3$), 21:1($n-5$), 22:1($n-11$), 22:1($n-9$), 24:0, 24:1($n-9$).

Mora moro 1170 m ($n = 2$)	*Nezumia aequalis* (1982) 1170 m ($n = 3$)	*Nezumia aequalis* (1981) 1200 m ($n = 4$)	*Coryphenoides pupestris* 1300 m ($n = 3$)	*Antimora rostrata* 2000 m ($n = 5$)	*Conocara murrayi* 2000 m ($n = 2$)	*Coryphenoides armatus* 4000 m ($n = 3$)
22.7	19.7 (2.4)	19.3 (3.0)	13.9 (2.9)	21.5 (3.1)	13.2	15.6 (0.2)
2.7	2.1 (1.8)	1.2 (1.4)	1.7 (0.8)	3.1 (0.2)	5.0	2.2 (0.1)
3.1	3.5 (1.1)	7.1 (5.9)	3.1 (0.3)	2.0 (0.6)	3.0	3.4 (0.5)
1.6	15.9 (2.7)	2.9 (2.3)	3.7 (0.8)	2.7 (0.4)	3.5	3.9 (0.2)
9.2		15.9 (2.7)	16.0 (5.2)	10.4 (1.3)	14.2	12.2 (2.5)
0.6	1.2 (0.2)	0.6 (0.8)	1.4 (0.5)	–	0.2	0.2 (0.3)
4.8	0.1 (0.2)	–	–	–	–	–
0.7	2.4 (0.4)	2.2 (2.4)	6.6 (2.1)	1.3 (0.3)	2.2	3.2 (1.0)
3.2	4.8 (0.8)	7.0 (3.3)	3.0 (1.5)	4.3 (1.3)	7.6	3.4 (0.6)
8.5	11.1 (2.2)	10.9 (4.7)	12.6 (4.3)	9.9 (1.3)	7.7	6.2 (0.6)
1.6	1.2 (0.1)	3.1 (1.7)	2.6 (0.2)	1.7 (0.4)	2.7	1.6 (0.6)
21.4	18.1 (1.1)	23.5 (7.1)	27.4 (4.0)	36.5 (3.6)	25.8	35.1 (3.4)
10.1	12.4 (1.8)	4.5 (3.4)	2.8 (2.0)	4.3 (1.5)	6.7	8.1 (1.6)
30.6	26.2 (3.4)	27.7 (5.4)	18.4 (3.3)	25.4 (2.6)	20.5	20.4 (0.5)
16.4	22.8 (4.0)	22.7 (4.2)	31.4 (7.2)	17.5 (1.8)	27.2	24.8 (3.9)
42.9	38.5 (3.6)	45.1 (9.5)	47.4 (8.7)	52.8 (4.5)	45.6	46.7 (2.6)
245.2	223.5 (17.6)	264.4 (54.8)	287.9 (39.6)	312.9 (23.6)	256.9	288.0 (13.1)
0.515	0.433 (0.093)	0.423 (0.141)	0.235 (0.048)	0.362 (0.053)	0.282	0.286 (0.016)

Results

The percent weight composition of the principal identified fatty acids of choline (PC), ethanolamine (PE), serine (PS)/inositol (PI), phosphoglycerides and cardiolipin are presented in Tables I–IV, respectively. The legend to Table I includes a complete list of peaks which were tentatively identified as fatty acids. Some samples possessed peaks that were not identifiable by our normal procedures. Because significant quantities of these unidentified components invalidated any quantitative comparisons between species, those samples which contained in excess of 15% unknown peaks have been arbitrarily excluded from the data in Tables I–IV. This was a greater problem for the minor phosphoglycerides, PS and cardiolipin, than for the major phosphoglycerides, PC and PE. For example, for PS, 18 out of 40 samples exceeded this arbitrary limit, whilst for cardiolipin there were 19 out of 40 samples. The corresponding values for PC and PE were 11/53 and 8/53, respectively. The variability between individuals of most species was generally similar to that observed in previous studies of laboratory-acclimated freshwater species [14] though some species showed appreciable variation. Data for *Nezumia aequalis* based on fish trawled in 1981 was confirmed by fish trawled in the following 1982 cruise (Table I). The corresponding results for PE did not show the same reproducibility, but the data for *N. aequalis* in 1982 was based on only two specimens (Table II).

In PC the principal fatty acids were 16:0, 18:1, 20:5($n-3$) and 22:6($n-3$) with a number of smaller components (18:0, 16:1($n-7$),

TABLE II

THE FATTY ACID COMPOSITION OF PHOSPHATIDYLETHANOLAMINE FROM A MITOCHONDRIA-RICH FRACTION ISOLATED FROM FISH LIVERS

Other details as noted in legend to Table I.

	Lepidorhombus whiffiagonis 200 m ($n=3$)	*Lophius budegassa* 200 m ($n=3$)	*Helicolenus dactylopterus* 200 m ($n=3$)	*Phycis blennoides* 900 m ($n=4$)	*Lepidion eques* 830 m ($n=4$)	*Mora moro* 1170 m ($n=2$)
16:0	13.6 (1.2)	18.2 (1.1)	15.3 (4.1)	13.7 (0.6)	10.9 (1.8)	12.8
16:1($n-7$)	2.2 (0.1)	1.3 (0.1)	4.2 (0.8)	0.5 (0.5)	1.1 (1.2)	1.4
18:0	5.8 (0.6)	8.3 (1.1)	12.9 (1.9)	9.0 (1.9)	8.4 (0.5)	8.9
18:1($n-7$)	6.4 (0.6)	4.5 (0.8)	6.4 (0.9)	4.3 (2.1)	5.5 (0.3)	3.9
18:1($n-9$)	7.5 (0.7)	5.0 (0.6)	11.4 (0.5)	8.5 (1.6)	5.8 (1.1)	9.2
18:2($n-6$)	0.8 (0.1)	0.8 (0.3)	1.3 (0.4)	–	0.6 (0.4)	0.9
18:3($n-6$)	0.4 (0.1)	1.2 (0.3)	4.0 (1.8)	2.1 (2.4)	3.6 (1.8)	2.9
20:1($n-9$)	6.0 (1.3)	0.9 (0.9)	2.1 (0.4)	2.6 (1.4)	2.6 (0.6)	1.9
20:4($n-6$)	2.5 (0.6)	4.1 (0.3)	3.7 (0.6)	2.5 (0.4)	4.4 (0.7)	2.7
20:5($n-3$)	4.1 (0.6)	2.4 (0.6)	5.8 (0.1)	6.4 (1.3)	6.7 (0.8)	8.0
22:5($n-3$)	1.9 (0.2)	0.9 (0.2)	1.6 (0.3)	1.3 (0.7)	1.0 (0.2)	1.3
22:6($n-3$)	37.6 (1.3)	38.1 (1.1)	27.3 (0.9)	34.3 (3.7)	29.4 (3.8)	34.6
Unknowns	8.3 (0.7)	11.6 (1.7)	7.2 (0.6)	13.4 (0.9)	11.3 (1.5)	10.6
Σ saturated	20.8 (1.2)	29.0 (0.5)	28.7 (2.4)	23.4 (1.4)	22.1 (3.2)	22.3
Σ monounsaturated	23.0 (2.2)	11.9 (1.5)	20.8 (1.2)	16.6 (1.4)	18.6 (1.7)	16.4
Σ polyunsaturated	47.9 (1.0)	47.5 (1.0)	43.3 (2.1)	46.5 (2.4)	48.0 (4.6)	50.8
Unsaturation index	293.5 (3.3)	266.6 (14.6)	250.0 (8.4)	276.6 (17.2)	266.5 (25.5)	297
Saturation ratio	0.293 (0.022)	0.486 (0.013)	0.449 (0.05)	0.372 (0.031)	0.334 (0.062)	0.333

20 : 4($n-6$) and 22 : 5($n-3$)). 18 : 1 was present as two isomers with the ratio between them remaining at approximately 4 : 1 ($n-9/n-7$) for all species. There was considerable variability between species though it was not consistently related to depth of capture. For example, the proportion of 16 : 0 in the fish trawled at 4000 m was somewhat lower than for most species caught at shallower depths, but *Lepidorhombus whiffiagonis* (200 m) was similar to the 4000 m fish. The proportion of the principal polyunsaturated fatty acid, 22 : 6($n-3$), varied in an apparently random manner with depth. The values for the two species caught at 2000 m lay almost at the extremes of the range observed for all species!

In comparison with the fatty acids in PC, PE showed an increased proportion of 18 : 0 with a slightly lower proportion of 16 : 0. The principal unsaturated fatty acids were again 20 : 5($n-3$) and 22 : 6($n-3$). The trend of composition as a function of depth was more clearcut than PC. Thus, 16 : 0 and 18 : 0 became progressively reduced as the animals' depth increased, whilst the monounsaturated fatty acids became progressively greater. The polyunsaturated fatty acids, however, remained at similar levels over the entire depth range (47–50%). Fish trawled at depths greater than 1200 m possessed somewhat higher proportions of 20 : 1($n-9$) (5–7%) than those trawled in shallower depths (1–4%). Again, *L. whiffiagonis* provides an exception to this general trend.

PS contained comparatively large quantities of stearic (18 : 0) and arachidonic acid (20 : 4($n-6$)), with a corresponding reduction in 16 : 0 and 20 : 5($n-3$). There were no obvious trends in fatty acid compositions of PS or cardiolipin with depth.

Alepocephalus bairdii 1170 m ($n=3$)	*Nezumia aequalis* (1981) 1200 m ($n=4$)	*Nezumia aequalis* (1982) 1170 m ($n=2$)	*Coryphenoides rupestris* 1300 m ($n=3$)	*Antimora rostrata* 2000 m ($n=5$)	*Conocara murrayi* 2000 m ($n=3$)	*Coryphenoides armatus* 4000 m ($n=6$)
13.1 (1.1)	9.1 (0.8)	10.0	9.3 (0.7)	10.8 (1.5)	10.0 (3.8)	6.1 (2.3)
0.8 (0.1)	0.8 (0.1)	0.4	0.4 (0.4)	0.5 (0.4)	1.0 (0.1)	0.4 (0.2)
9.6 (1.7)	8.8 (0.8)	10.9	5.6 (1.4)	4.4 (0.9)	6.9 (2.1)	4.4 (0.7)
3.4 (0.3)	13.1 (0.)	3.9	6.3 (1.0)	7.9 (0.9)	6.2 (1.4)	10.7 (1.7)
9.3 (0.4)	13.1 (0.6)	13.2	14.8 (2.8)	11.3 (1.3)	12.4 (4.0)	14.5 (1.9)
0.5 (0.4)	1.1 (0.2)	0.4	1.3 (0.4)	–	6.2 (1.4)	0.5 (0.1)
0.7 (0.8)	–	0.3	–	–	0.8 (1.1)	–
4.6 (0.3)	1.7 (1.0)	2.3	7.1 (2.0)	7.7 (0.8)	5.4 (0.7)	7.4 (1.2)
4.5 (0.7)	2.9 (0.3)	2.6	1.9 (0.2)	2.8 (0.3)	8.6 (2.7)	2.0 (0.4)
9.7 (1.6)	6.2 (0.8)	5.5	13.3 (3.2)	8.3 (1.9)	11.9 (4.3)	4.9 (0.6)
1.1 (0.2)	1.7 (0.5)	0.6	1.6 (0.5)	1.9 (0.7)	1.5 (0.4)	0.8 (0.2)
23.3 (3.1)	37.4 (4.4)	32.0	28.6 (3.3)	40.3 (5.1)	24.2 (2.5)	34.7 (5.6)
12.4 (2.1)	9.5 (2.7)	11.7	6.2 (3.3)	3.2 (2.0)	7.6 (2.8)	8.1 (4.7)
25.7 (0.8)	18.3 (1.3)	22.0	15.4 (2.0)	15.7 (2.7)	17.5 (2.6)	11.1 (3.5)
20.9 (0.7)	22.7 (1.2)	22.7	31.7 (2.4)	27.6 (2.1)	27.3 (1.0)	36.4 (2.6)
41.0 (2.8)	49.5 (4.2)	43.4	46.8 (5.7)	53.4 (5.3)	47.6 (1.4)	44.4 (6.3)
240.0 (19.0)	301.5	263.2	288.0 (28.6)	328.4 (29.1)	277.6 (15.0)	287.0 (38.2)
0.416 (0.026)	0.255 (0.033)	0.335	0.198 (0.029)	0.196 (0.047)	0.233 (0.027)	0.143 (0.062)

TABLE III

THE FATTY ACID COMPOSITION OF PHOSPHATIDYLSERINE/PHOSPHATIDYLINOSITOL FROM A MITOCHONDRIA-RICH FRACTION ISOLATED FROM FISH LIVERS

Other details as noted in the legends to Table I.

	Lepidorhombus whiffiagonis 200 m ($n = 2$)	*Phycis blennoides* 900 m ($n = 2$)	*Lepidion eques* 850 m ($n = 2$)	*Alepocephalus bairdii* 1170 m ($n = 2$)	*Nezumia aequalis* 1200 m ($n = 5$)	*Coryphenoides rupestris* 1300 m ($n = 1$)	*Conocara murrayi* 2000 m ($n = 2$)	*Antimora rostrata* 2000 m ($n = 5$)	*Coryphenoides armatus* 4000 m ($n = 1$)
16:0	11.9	8.5	6.4	7.7	10.1 (2.3)	9.8	9.0	12.6 (2.3)	13.9
16:1($n-7$)	2.7	–	–	0.9	0.5 (0.6)	–	1.3	0.8 (0.8)	–
18:0	10.9	28.7	20.9	37.9	21.6 (2.5)	25.3	22.1	14.3 (7.1)	23.4
18:1($n-7$)	2.5	2.6	2.6	0.5	3.7 (0.8)	4.8	3.0	3.4 (0.6)	6.6
18:1($n-9$)	12.9	8.0	7.2	5.2	11.6 (3.7)	9.5	11.2	9.1 (3.4)	9.3
18:2($n-6$)	0.5	–	–	0.9	–	–	–	–	–
18:3($n-6$)	2.5	4.5	8.7	0.6	0.3 (0.6)	–	–	–	–
20:1($n-9$)	3.8	–	0.9	0.9	2.9 (2.0)	1.2	5.8	4.3 (1.8)	2.9
20:4($n-6$)	16.2	21.0	19.52	14.9	21.3 (4.2)	28.8	17.2	14.4 (4.8)	13.4
20:5($n-3$)	8.2	5.5	4.0	4.8	5.8 (2.0)	6.8	11.6	8.8 (3.4)	9.2
22:5($n-3$)	1.9	–	0.5	1.6	2.2 (1.9)	–	2.1	1.0 (1.0)	–
22:6($n-3$)	15.7	7.1	12.3	6.8	6.5 (0.7)	8.3	9.8	25.7 (8.8)	16.7
Unknowns	7.6	8.3	11.9	9.7	10.6 (1.7)	5.7	6.9	3.8 (4.1)	4.6
Σ saturated	25.9	39.0	31.2	49.0	33.4 (5.3)	35.1	31.1	25.5 (7.3)	37.3
Σ monoun-saturated	21.0	14.5	11.6	11.8	20.1 (3.8)	15.4	21.3	20.5 (4.7)	18.8
Σ polyun-saturated	45.4	38.2	45.3	29.6	36.0 (5.9)	43.8	40.7	50.1 (6.4)	39.3
Unsaturation index	239.5	182.7	212.6	147.4	184.5 (24.4)	213.9	217.0	263.4 (80.7)	218.6
Saturation ratio	0.393	0.737	0.548	1.289	0.605 (0.146)	0.592	0.502	0.375 (0.147)	0.642

TABLE IV

THE FATTY ACID COMPOSITION OF CARDIOLIPIN FROM A MITOCHONDRIA-RICH FRACTION ISOLATED FROM FISH LIVERS

Other details as noted in the legend to Table I.

	Lepidorhombus whiffiagonis 200 m ($n = 3$)	*Lophius budegassa* 200 m ($n = 2$)	*Lepidion eques* 800 m ($n = 2$)	*Phycis blennoides* 900 m ($n = 1$)	*Alepocephalus bairdii* 1170 m ($n = 5$)	*Nezumia aequalis* 1200 m ($n = 1$)	*Antimora rostrata* 2000 m ($n = 1$)	*Conocara murrayi* 2000 m ($n = 2$)	*Coryphenoides armatus* 4000 m ($n = 4$)
16:0	2.3 (0.6)	3.9	5.7	9.4	7.9 (3.1)	14.9	11.6	9.2	16.0 (5.5)
16:1($n-7$)	17.6 (3.1)	16.2	1.7	1.4	1.2 (1.0)	3.9	3.5	2.7	1.5 (1.1)
18:0	0.7 (0.2)	1.4	1.8	2.5	2.9 (1.4)	5.7	–	3.1	5.8 (2.4)
18:1($n-7$)	4.5 (0.2)	5.1	4.0	6.6	4.3 (1.2)	6.4	8.0	9.6	8.6 (3.8)
18:1($n-9$)	11.9 (3.1)	11.4	6.3	10.1	10.9 (2.8)	12.0	7.4	20.5	8.0 (4.2)
18:2($n-6$)	2.9 (2.3)	4.3	1.8	2.7	1.7 (0.4)	2.7	2.1	1.8	–
18:3($n-6$)	1.2 (0.2)	5.1	2.5	2.8	1.5 (1.7)	–	–	–	–
20:1($n-9$)	2.0 (0.5)	1.7	0.7	–	3.8 (2.3)	5.0	5.2	–	2.9 (2.0)
20:4($n-6$)	0.6 (0.1)	0.3	0.8	–	0.2 (0.2)	1.1	–	0.6	–
20:5($n-3$)	1.2 (0.2)	1.3	1.2	1.6	0.5 (0.5)	–	–	0.6	0.3 (0.5)
22:5($n-3$)	3.5 (0.2)	1.5	0.8	1.7	0.5 (0.7)	3.0	–	4.9	2.2 (1.4)
22:6($n-3$)	34.2 (0.9)	26.9	36.1	38.7	43.6 (7.0)	24.4	45.3	27.5	25.5 (18.6)
Unknowns	9.9 (3.2)	9.3	10.8	13.9	12.1 (2.8)	14.1	10.7	9.2	8.4 (4.2)
Σ saturated	6.2 (1.2)	15.0	29.9	13.3	12.9 (4.5)	22.5	11.6	13.6	26.0 (10.6)
Σ monoun-saturated	37.3 (2.8)	35.2	14.0	22.9	21.4 (3.3)	30.6	24.1	37.8	30.0 (6.2)
Σ polyun-saturated	46.7 (2.5)	41.0	45.3	50.0	53.5 (6.1)	32.8	53.6	39.4	35.7 (17.9)
Unsaturation index	288.0 (8.4)	238.7	261.2	292.4	313.4 (39.7)	208.2	312.5	247.7	240.4 (98.0)
Saturation ratio	0.075 (0.017)	0.198	0.521	0.182	0.178 (0.074)	0.355	0.149	0.179	0.427 (0.231)

Because of the great complexity of the fatty acid profiles and because variations in the proportion of one component affects the proportions of other components, it was necessary to calculate indices of composition. Two indices have been calculated and included in Tables I–IV. The saturation ratio is simply the ratio of the weight percent saturated to unsaturated fatty acids. The unsaturation index is the sum of the weight percent multiplied by the number of double bonds for all identified components in the mixture. Table V provides a summary of the regression analysis of these indices against depth of capture. Unsaturation index was not significantly related (at the 5% level) to depth in any phosphoglyceride class. By contrast, saturation ratio was significantly related to depth in PC ($P < 0.05$), PE ($P < 0.001$) and in cardiolipin ($P < 0.05$). Interestingly, the regression of cardiolipin against depth produced a positive slope, in contrast to PC and PE. The regressions obtained for the saturation ratio of PC and PE against depth were also significant when all samples, including those with greater than 15% unknown fatty acids, were included ($P = 0.013$ and $P < 0.001$, respectively, $n = 53$), so that the exclusion of samples containing excessive unknown components in the regression analyses presented in Table V does not affect the conclusions drawn. Also included in Table V is the regression equation for a mixture of PC and PE (70 : 30) which more closely reflects the bulk composition of the membrane. This regression was also highly significant ($P < 0.003$).

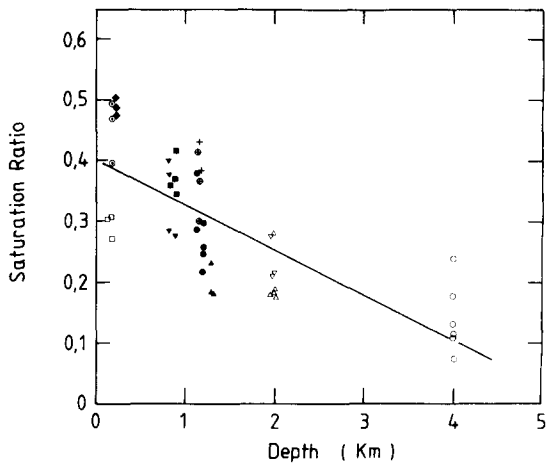

Fig. 1. The regression of saturation ratio for the ethanolamine phosphoglyceride fraction of fish upon the depth of capture. The line was calculated from the regression equation shown in Table V. Each symbol represents a separate species: ○, *Coryphenoides armatus*; ●, *Nezumia aequalis*; △, *Antimora rostrata*; ▲, *Coryphenoides rupestris*; □, *Lepidorhombus whiffiagonis*; ▽, *Conocara murrayi*; ■, *Phycis blennoides*; ▼, *Lepidion eques*; ◆, *Lophius budegassa*; +, *Alepocephalus bairdii*; ⊙, *Helicolenus dactylopterus*; ⊕, *Mora moro*.

TABLE V

SUMMARY OF REGRESSION ANALYSIS FOR SATURATION RATIO AND UNSATURATION INDEX AGAINST DEPTH OF CAPTURE (km)

SR, saturation ratio; UI, unsaturation index; n, number of samples; b, slope; Y-int, intercept at zero depth; P, probability that the slope was significantly different from zero as calculated for ($n - 2$) degrees of freedom (see Ref. 23).

		n	b	Y-int	P
PC	SR	42	−0.039 ± 0.018	0.442	0.036 *
	UI	42	9.22 ± 6.36	253.8	0.151
PE	SR	45	−0.0735 ± 0.0096	0.401	< 0.0001 **
	UI	45	6.655 ± 3.958	273.1	0.096
PS/PI	SR	22	−0.056 ± 0.0825	0.684	0.509
	UI	22	16.07 ± 14.10	189.5	0.267
Cardiolipin	SR	21	0.062 ± 0.027	0.158	0.033 *
	UI	21	−9.497 ± 9.408	286.3	0.327
PC/PE (70 : 30 ratio)	SR	36	−0.040 ± 0.013	0.398	0.003 **

* Significant.
** Highly significant.

Fig. 1 displays the distribution of individual data points around the calculated regression line for PE. This graph illustrates the extent to which the interspecific variation was accounted for by depth of occurrence. Thus, *Lophius* and *Lepidorhombus* occur at 200 m and yet display very different saturation ratios. Nevertheless, the ratios for both species were greater than species which occur at 2000 m or deeper.

Discussion

It is generally held that each type of membrane has a characteristic fatty acid profile and the profiles of phosphoglycerides often differ from those of neutral lipids [15]. This means that the gross analysis of whole tissue samples or complex mixtures of membranes yield only averaged information, which may well obscure relationships with environmental variables [7,8]. Clearly, it is important to analyse the fatty acid composition of specific phosphoglyceride fractions from pure membrane preparations. This study has partially satisfied this requirement in that a single membrane preparation was used for analysis rather than whole tissues or gross extracts, and that specific phosphoglyceride classes were prepared rather than total lipid extracts. Nevertheless, the degree of purity of our mitochondrial-rich fractions remains unknown and, due to the limitations of ship-board centrifugation, it may not be as reproducible as those obtained with more sophisticated and extended preparative procedures. Even experiments in the laboratory with trout liver have shown some contamination with microsomal membranes (J.A.C. Lee, unpublished observations; see also Ref. 14). The isolation at sea of more purified membrane fractions is a major goal for future work.

In our previous comparative study of the membrane order of deep-sea fish there was no significant correlation between diphenylhexatriene polarisation of the mitochondrial-rich fraction used here and the depth (hence pressure) at which the animals live. Similarly, there was no significant correlation between diphenylhexatriene polarisation and either saturation ratio or unsaturation index (data not shown). The liver was found to vary in size and colour even between individuals of the same species, and this was often evident in the degree of pigmentation of the isolated membrane fraction. The presence of pigments and other absorptive material in this membrane fraction severely limits the usefulness of the polarisation technique because of the intrinsic fluorescence of the membranes and the absorption of diphenylhexatriene fluorescence by membrane chromophores. Thus, diphenylhexatriene polarisation is probably an unreliable indicator of membrane properties in this instance.

Nevertheless, the acyl group composition of the major phospholipids of the liver mitochondrial-rich fraction did show a variation with depth in a direction and by an amount which was consistent with the predictions of the hypothesis of homeoviscous adaptation, and this constitutes strong evidence of adaptation to pressure. The change of saturation ratio with depth is large. The regression analysis indicates a change in ratio for PC, from 0.442 at the surface to 0.286 at 4000 m (400 atm) whilst the corresponding values for PE are 0.401 and 0.105. Converting these values to percent saturated fatty acids, gives a reduction from 30.6 to 22.4% for PC and from 28.6 to 9.5% for PE. On the other hand, cardiolipin showed the opposite trend with an increasing saturation ratio with increasing depth. The meaning of this result is quite obscure, though the trend is entirely consistent with the change in fatty acid composition in red muscle mitochondria of temperature-acclimated carp [22].

The shallow water fish live at approximately 10°C whilst those at 4000 m depth experience a highly constant temperature of approximately 2°C. In addition, the pressure difference between the two environments, 400 atm, causes an ordering effect in goldfish brain membranes equal to a reduction in temperature of 7 Cdeg [3,16]. Therefore, the normal ambient conditions of the deep water fish membranes we have examined are at the equivalent of a temperature some (7 + 8) Cdeg lower than the 10°C at the surface. The reduction of saturation ratios for PC and PE, 0.442–0.286 and 0.401–0.105 respectively, over the 4000 m depth range, should therefore be judged in relation to the ordering effect of this apparent 15 Cdeg decrease in temperature. Corresponding data for other marine fish mitochondria are not availa-

ble, but the freshwater green sunfish, acclimated at 25 and 5°C shows the following differences in the saturation ratios of its liver mitochondria PE, 0.510–0.390; PC, 0.515–0.402 [14]. It would appear, therefore, that the changes in the saturation ratios for the deep sea fish mitochondria reported here are at least as great as those of the temperature-acclimated fish. Furthermore, as the unknown fatty acids are likely to be unsaturated or branched chain, the ratios given are minimal. The decrease in the proportion of straight chain saturated fatty acids is probably even greater than shown.

Although the regressions of saturation ratio upon depth were statistically significant it is quite clear from Fig. 1 that there were substantial differences between individuals and especially between species that were not explained by the regression. Thus *L. whiffiagonis* had very low saturation ratios compared to *Lophius budegassa* even though both were caught at 200 m. The proportion of variation in saturation ratio that is explained by the regression is provided by the coefficient of determination (r^2, Ref. 23), which for the saturation ratios of PE, PC and cardiolipin were 0.58, 0.10 and 0.22, respectively. The corresponding value calculated for a mixture of PC and PE (70 : 30) was 0.24. This probably reflects the properties of the bulk bilayer more closely than either phosphoglyceride class alone. Bearing in mind the large number of samples involved and the errors that arise from membrane fractionation, phosphoglyceride separation and acyl group analysis, the values place the initial hypothesis in perspective and suggest that other factors are not without importance. Foremost amongst these is uncertainty regarding the thermal and pressure history of the individuals prior to capture and the fact that temperature also varies over the depth range studied. However, neither of these are sufficient to account for the difference between *Lepidorhombus* and *Lophius* and it seems that other biological differences remain such as lifestyle, reproductive status, nutritive condition, etc. Finally, it may well be that the saturation ratio is not a completely representative index of acyl group composition in relation to the physiologically relevant, physical properties of the bilayer of which they form a part. The relationship between lipid composition and membrane order is undoubtedly complex and saturation ratio does not take account of many of these subtleties. There is, for example little quantitative information which takes account of the effect upon membrane order of positional specificity of fatty acids, of variations in molecular species composition or of intermolecular interactions such as between cholesterol and fatty acids [17].

The poor relationship of unsaturation index with depth is probably due to the domination of this index by the proportion of $22:6(n-3)$. This fatty acid varied by up to 14% but the proportion of total polyunsaturated fatty acids remained relatively constant. Unsaturation index has been previously found to be poorly related to adaptive changes in membrane order during temperature acclimation [17]. The physical properties of artificial membranes are most significantly altered by the incorporation of a double bond into a saturated acyl chain [18–20]. The incorporation of additional double bonds has progressively smaller effects upon the thermotropic phase transition and upon diphenylhexatriene polarisation. Thus, changes in the unsaturation index which are brought about by altering the types of polyunsaturated fatty acids may have comparatively little effect upon bulk membrane properties as detected by calorimetry or by diphenylhexatriene polarisation and, in consequence, it is a poor index of membrane fluidity. By contrast, changes resulting from alterations in the proportions of monounsaturated and saturated fatty acids should have more significant effects, but this will not have much influence upon the unsaturation index. Previous studies of membranes from temperature-acclimated fish have shown a good correlation between compensations of diphenylhexatriene polarisation and saturation ratio (or percent saturated fatty acids) [17,21].

Acknowledgements

We thank the Master and crew of R.V. Challenger for invaluable assistance. We also thank the following colleagues for their assistance either at sea or in the laboratory: Dr. A. Rice, N. Merett and colleagues (Institute of Oceanographic Science, Wormley), Professor J. Sargent and Dr. J.

Henderson (Institute Marine Biochemistry, Aberdeen), Mr. J.A.C. Lee, Mr. R. Raynard, Mrs. J. Bridson, Mrs. A. Hall and Miss A. Callaghan for secretarial assistance. This work was supported by a grant to A.G.M. from the Natural Environment Research Council.

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