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CHANGES IN FLUIDITY AND 22:6($n-3$) CONTENT IN PHOSPHOLIPIDS OF TROUT INTESTINAL BRUSH-BORDER MEMBRANE AS RELATED TO ENVIRONMENTAL SALINITY

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Phospholipids and fatty acids analyses were carried out on brush-border membranes isolated from trout intestine. Phosphatidylcholine (PC) is the principal phospholipid of these membranes. When animals are transferred from fresh water to sea water, the content of the 22:6($n-3$) fatty acid strongly increases at the level of phospholipids, mainly PC. Concomitantly, an important increase in the fluidity of the lipid core of the membrane was detected by steady-state fluorescence anisotropy. It is suggested that the molecular species of PC (especially rich in $n-3$ fatty acids) may have an important part to play in marine organisms according to the osmoregulation problems met in these animals.

Introduction

Although much work has been done on the lipid composition and metabolism of fish [1,2] we have only a few reports concerning adaptive changes in sea water and fresh water such as those of phospholipid metabolism in eel or trout [3–6]. If some slight variations were observed in the relative phospholipid composition of salt transporting epithelia [3] the main changes were recorded at the level of phosphate or polar headgroup renewal. Numerous studies on the regulation of membrane lipid composition during temperature adaptation have focused the interest on the importance of the fatty acyl chain [7,8]. Thus, it appears that alterations of membrane lipids to changes in environmental temperature are the main responses used by poikilothermic organisms to regulate membrane function through its fluidity and lipid-protein interactions. Similar biochemical adaptations occurring during osmoregulatory process were investigated only in the eel gills [6,9,10] and in various organs of the guppy [11]. Although some discrepancies appear between gills studies [9,10], these works suggest that the fatty acid moieties of membrane phospholipids might be concerned with homeoviscous adaptation during changes in environmental salinity. Since it is accepted that the permeability properties of membrane are in part determined by the phospholipid headgroup and the nature of the fatty acyl group [12], these lipid components were investigated at the level of the brush-border membrane of trout intestine. Previous results [13] have shown that modifications of the lipid composition of these membranes, including essential fatty acid, cholesterol and phosphatidylcholine contents, induced significant changes of their salt permeability. The observation of a modification in transport function observed at the brush-border level soon after the sea water transfer of trout [14] prompted us to investigate the lipid composition of intestinal

apical membrane along this high salinity adaptation.

Materials and Methods

Experiments were conducted on rainbow trout (*Salmo gairdneri* R.) weighing 200–250 g, obtained from a local hatchery and maintained in outdoor tanks supplied with air saturated running fresh water (12–13°C). Fish were fed a 8% cod liver oil diet prepared according to a standardized formula [15] for one month (1% live weight per day) before their rapid transfer in sea water ($S = 32‰$). Brush-border membranes were prepared according to a method already described [16] from the anterior intestine of trout sampled in fresh water and after one day, two days and seven days in sea water. These samples being a pool from six animals were investigated at each adaptation time for their phospholipid pattern and fatty acid composition and fluidity by fluorescence anisotropy determination. The phospholipid content of membrane preparations was studied after extraction and two-dimensional TLC according to methods already described [17,18]. Phospholipid spots were located with Dittmer's reagent [19] and the determination of lipid phosphorus was carried out with the Fiske-SubbaRow reagent slightly modified [20]. The purification of three phospholipid components (phosphatidyl-choline, -ethanolamine and a mixture of phosphatidyl-inositol and -serine) and their fatty acid analysis were run as described in a previous paper [16]. Cholesterol content was measured by a colorimetric method [21] on the neutral lipid fractions. Protein was estimated by the Lowry procedure [22] on aliquots of membrane suspensions. Steady-state fluorescence anisotropy of 1,6-diphenylhexatriene (DPH) in brush-border membrane suspensions was determined on a SLM-8000 SC spectrophotopolarimeter. The basic procedure used for labelling has been previously described [23]. Excitation and emission wavelengths were 340 and 425 nm, respectively. A low turbidity of the sample, less than 0.1 absorbance unit at 340 nm, corresponding to approximately 20 μg/ml protein, precludes any correction for light scattering [24]. In all experiments, the molar ratio phospholipids/diphenylhexatriene was about 300.

Results

The phospholipid and cholesterol contents of the membrane preparations are reported in Table I. Since no significant variations were detected during sea water adaptation (from 1 to 7 days), only the one day sea water data are compared to the fresh water ones. It appears that the phospholipid and cholesterol contents are slightly but not significantly decreased one day after the transfer and their relative concentrations are not modified. The phospholipid composition, expressed in per cent of total phospholipid, is reported in Table II and as for the Table I, data are restricted to fresh water and one day sea water groups. As previously described [16], the main phospholipids are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). It appears that sea water adaptation does not modify the relative proportion of phospholipids in the brush border membrane. Table III details the fatty acid composition of phosphatidylcholine, phosphatidylethanolamine and the mixed fraction phosphatidylserine (PS) + phosphatidylinositol (PI). As the important variations in the fatty acid composition of phospholipids were observed bteween membranes prepared from fresh water and one day sea water animals, only these data are presented. Moreover, the gross composition and the main lipid indexes are given for all the experimental groups in Table IV. The most notable finding in Table III is that one of the major saturated fatty acid (stearic acid, 18 : 0) is less abundant in PC and PE after one day in sea

TABLE I

INFLUENCE OF ACCLIMATION TO SEA WATER ON PHOSPHOLIPID AND CHOLESTEROL CONTENT OF TROUT INTESTINAL BRUSH-BORDER MEMBRANE

Chol, cholesterol; PL, total phospholipids. Since no variations were detected during sea water (SW) adaptation only the one day data are given. Mean value ± S.D. of three samples, each sample containing six membrane preparations. FW, fresh water.

Parameters	Acclimation medium	
	FW	SW (1 day)
μg PL/mg protein	291 ± 35	210 ± 15
μg Chol/mg protein	167 ± 40	121 ± 6
μmol Chol/μmol PL	1.22 ± 0.41	1.17 ± 0.11

TABLE II

INFLUENCE OF ACCLIMATION TO SEA WATER ON PHOSPHOLIPID DISTRIBUTION OF TROUT INTESTINAL BRUSH-BORDER MEMBRANE

The results (including diacyl, alkyl and alkenyl form of the phospholipid classes) are given in weight per cent of phospholipid and are expressed as mean values ± S.D. of three samples, each sample containing six membrane preparations. Since no variations were detected during sea water adaptation, only the one day data are given. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SP, sphingomyelin; DPG: cardiolipin; LPC: lysophosphatidylcholine. Only traces of phosphatidic acid were detected. FW, fresh water; SW, sea water.

Phospholipid	Acclimation medium	
	FW	SW (1 day)
PC	46.6 ± 3.6	44.2 ± 1.4
PE	22.5 ± 1.3	23.7 ± 0.4
PS	9.1 ± 1.6	8.2 ± 1.1
PI	6.3 ± 0.4	7.4 ± 0.5
SP	4.5 ± 0.5	5.7 ± 0.7
DPG	8.5 ± 0.9	8.2 ± 0.9
LPC	3.0 ± 1.0	2.4 ± 0.3

water whereas the relative amount of docosahexaenoic acid (22 : 6, $n-3$) in both phospholipids is higher in the same experimental conditions; these changes being maintained during 1, 2 or 6 days of sea water adaptation. Consequently, this balance between the two fatty acids 18 : 0 and 22 : 6 ($n-3$) is reflected in the values of the unsat./sat. ratio and the unsaturation index (double bond number/sat.) which are lower in PC and PE of membranes prepared from fresh water trout as compared to membranes from sea water trout (Table IV). It can be noticed that the greatest changes in fatty acid composition are observed in the PC fraction and that the mixed PI + PS fraction is only slightly modified during sea water adaptation.

Fluorescence anisotropies r were measured and then averaged for three samples obtained from both fresh water and one day sea water animals. These measurements were done at seven temperatures between 5 and 35°C. The results were expressed as the anisotropy parameter $((r_0/r)-1)^{-1}$,

TABLE III

INFLUENCE OF ACCLIMATION TO SEA WATER ON THE FATTY ACID COMPOSITION OF THE BRUSH-BORDER MEMBRANE OF TROUT INTESTINE

Since no variatoins were detected during sea water adaptation, only the one day data are given. Mean value ± S.D. of three samples, each sample containing six membrane preparations. The significance of the difference between fresh water (FW) and sea water (SW) is indicated by ** ($P < 0.01$, Student's t-test).

Fatty acid	PC		PE		PS + PI	
	FW	SW	FW	SW	FW	SW
16 : 0	29.1 ± 1.5	31.5 ± 0.4	25.3 ± 0.2	23.6 ± 1.3	23.0 ± 2.0	26.2 ± 4.0
18 : 0	22.4 ± 0.3	11.9 ± 1.3 **	24.5 ± 5.1	16.3 ± 1.5	34.6 ± 5.1	28.9 ± 1.3
20 : 0	2.5 ± 0.1	1.0 ± 0.2 **	2.2 ± 0.7	1.5 ± 0.2	3.0 ± 1.2	2.9 ± 0.6
22 : 0	3.0 ± 0.8	1.3 ± 0.4	2.7 ± 0.7	1.6 ± 0.3	3.7 ± 0.5	2.7 ± 0.2
24 : 0	1.3 ± 0.2	0.9 ± 0.3	1.2 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	1.8 ± 0.2
16 : 1($n-9$)	1.8 ± 0.2	1.8 ± 0.1	2.3 ± 0.1	1.3 ± 0.1	3.0 ± 0.1	1.9 ± 0.5
18 : 1($n-9$)	9.0 ± 0.8	7.0 ± 0.1	7.4 ± 2.8	6.0 ± 0.4	7.8 ± 2.2	5.9 ± 1.6
20 : 1($n-9$)	1.3 ± 0.1	1.3 ± 0.1	2.4 ± 0.5	2.9 ± 0.2	–	0.9 ± 0.5
18 : 2($n-6$)	0.8 ± 0.3	0.7 ± 0.1	1.6 ± 0.8	1.0 ± 0.3	1.0 ± 0.1	0.7 ± 0.4
20 : 3($n-6$)	–	0.6 ± 0.1	–	–	2.0 ± 0.6	4.6 ± 0.6
20 : 4($n-6$)	5.5 ± 1.8	4.4 ± 2.2	2.9 ± 1.3	4.2 ± 1.6	3.0 ± 0.2	4.4 ± 1.7
22 : 5($n-6$)	1.3 ± 0.2	–	2.1 ± 0.8	2.0 ± 1.2	0.9 ± 0.1	1.5 ± 0.4
18 : 3($n-3$)	2.1 ± 0.1	–	0.8 ± 0.1	0.8 ± 0.4	2.1 ± 0.8	2.1 ± 0.4
20 : 3($n-3$)	1.6 ± 0.1	–	0.5 ± 0.1	0.5 ± 0.3	1.1 ± 0.2	2.0 ± 0.6
20 : 4($n-3$)	2.1 ± 0.2	3.3 ± 0.1	2.1 ± 1.0	1.3 ± 0.2	–	–
20 : 5($n-3$)	–	0.7 ± 0.4	1.2 ± 0.2	1.2 ± 0.3	2.3 ± 0.2	–
22 : 5($n-3$)	–	0.7 ± 0.1	1.1 ± 0.3	–	–	–
22 : 6($n-3$)	10.6 ± 0.9	28.0 ± 3.6 **	12.1 ± 1.3	24.4 ± 3.1 **	5.8 ± 0.2	8.9 ± 2.4

TABLE IV

INFLUENCE OF ACCLIMATION TO SEA WATER ON THE PARAMETERS OF THE FATTY ACYL CHAIN OF BRUSH-BORDER PHOSPHOLIPID FROM TROUT INTESTINE

These parameters are calculated from Table III. d.b./sat. = double bonds per 100 moles of fatty acid/per cent saturated fatty acid. Since no variations were detected during sea water adaptation, only the one day data are given. Mean value ± S.D. of three samples, each sample containing six membrane preparations. The significance of the difference between the adaptation media is indicated by * or ** when $P < 0.05$ or $P < 0.01$, respectively (Student's t-test). FW, fresh water; SW, sea water.

	Acclimation medium	Total sat.	Total $n-6$	Total $n-3$	Unsat./ Sat.	d.b./ Sat.
PC	FW	58.3 ± 0.8	8.3 ± 2.3	17.7 ± 1.9	0.71 ± 0.02	2.36 ± 0.07
	SW	46.3 ± 1.8 *	6.0 ± 2.3	33.4 ± 3.7 *	1.16 ± 0.09 **	4.94 ± 0.53 **
PE	FW	55.8 ± 6.6	7.7 ± 3.0	18.6 ± 1.7	0.81 ± 0.21	2.67 ± 0.58
	SW	44.3 ± 0.9	9.0 ± 2.6	28.6 ± 2.8 *	1.26 ± 0.05	4.90 ± 0.26 *
PS + PI	FW	65.8 ± 2.5	8.1 ± 1.2	12.4 ± 0.1	0.52 ± 0.05	1.58 ± 0.15
	SW	62.5 ± 3.9	13.2 ± 0.5 *	13.5 ± 2.6	0.61 ± 0.10	2.04 ± 0.36

where r_0, the maximal limiting anisotropy of the probe, was taken as 0.362 [23]. This anisotropy parameter varies directly with the rotational relaxation time of the fluorophore, and hence is related inversely to the 'lipid fluidity' (the term lipid fluidity being used to express the relative motional freedom of the lipid molecule) [25]. The accuracy on $\bar{r}$ is $\Delta\bar{r} = \pm 0.001$, resulting in $\Delta(\ln[(r_0/\bar{r}) - 1]^{-1}) = \pm 0.015$. By considering the temperature dependence shown on Fig. 1, the one day sea water samples exhibit from 15 up to 35°C a significant increase of lipid fluidity compared to fresh water samples; for example, at 15°C and 35°C the mean value for the anisotropy parameter decreases by 6% and 14%, respectively.

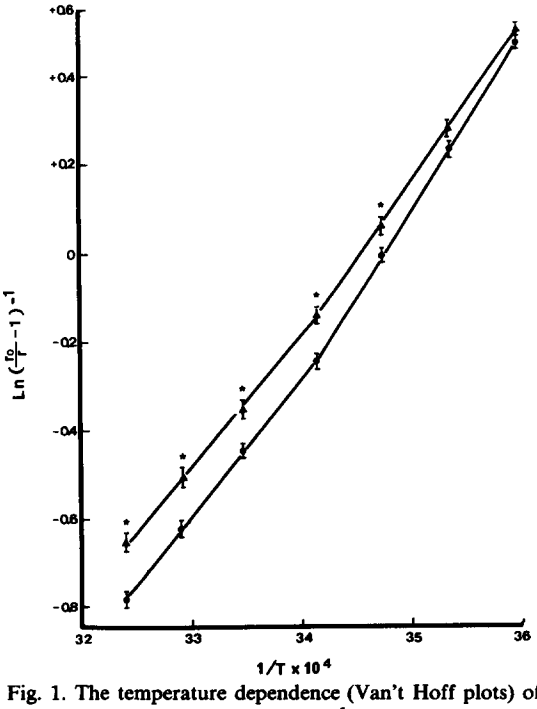

Fig. 1. The temperature dependence (Van't Hoff plots) of the anisotropy parameter $((r_0/r)-1)^{-1}$ of DPH embedded in brush border membranes: upper curve (▲), fresh water sample; lower curve (●), one day sea water sample. Mean value ± S.D. of three samples, each sample containing six membrane preparations. Asterisks denote significant differences between fresh water and sea water samples ($P < 0.05$).

Discussion

It is well known that sea water fish possess abundant amounts of highly unsaturated long chain fatty acids [26]. Recently, it was established that $(n-3)$ series of polyenic acids are essential dietary factors in trout [1] but the molecular functions of these constituents at the membrane level are unclear. It may be speculated that the high fluidity conferred by the $(n-3)$ fatty acids as related to the one of $(n-6)$ fatty acids contributes to the homeoviscous regulation of cellular membrane [27]. These mechanisms might be reasonably considered as playing an important role in the control of their permeability to electrolytes and/or non electrolytes.

In this study, the principal phospholipid of trout intestinal brush-border membrane is repre-

sented by PC. Similar results showing an important content of PC (about 30 to 40% of total phospholipids) have been performed in tortoise and rat intestinal membranes [28,29]. It must also be pointed out that the most important change of the 22 : 6($n-3$) content during sea water adaptation occurs at the level of PC. A similar situation where PC metabolism is mainly checked by the degree of the environmental salinity has been related in various tissues of fishes [3,4]. Consequently, the PC molecule appears to be especially implicated in marine animals during the osmoregulation problems. Besides, the judicious observation that the presence of ($n-3$) fatty acids in the diet reduced mortality in trout grown in sea water suggests a membrane function [30] of these components. The abundance of ($n-3$) fatty acids in the phosphatides of salt-secreting epithelia from marine fish species [31] and crab gills [32] also suggests that these fatty acids may have a specific role in membrane structure of these tissues. As a conclusion, these findings, together with the present results, demonstrate that the content of ($n-3$) fatty acids (especially 20 : 5, $n-3$ and 22 : 6, $n-3$) appears to be implicated in the osmoregulation mechanism of marine animals. Nevertheless, the functions and essentiality of the ($n-3$) acyl groups remain to be learned in these aquatic organisms.

Since it is shown in the present work that sea water transfer caused an important increase in 22 : 6 ($n-3$) in the brush border membrane with a concomitant decrease in saturated fatty acids, the influence of the dietary source on the membrane composition cannot be solely considered. Indeed, in our experiments, trout were fed the same pelleted food before and during sea water adaptation. Similar results concerning the increased proportion of 22 : 6 ($n-3$) in phospholipid of the digestive tract of the guppy in sea water adaptation have been recently reported [11]. In this species, it was observed a balance between ($n-3$) and ($n-6$) fatty acids of intestinal PC and PE, but this can originate from the complex composition of the live food used in that study. It cannot be stated from our results that there is a firmly change in essential fatty acids requirement of trout according to the salinity of their environment. Metabolic studies of the bioconversion of precursors (18 : 2, $n-6$, and 18 : 3, $n-3$) in various tissues would be necessary to solve the important problem of the complex relationship between membrane lipid composition and osmotic regulation.

Nevertheless, our results clearly show that the increase in 22 : 6 ($n-3$) content associated with the decrease in the proportion of saturated fatty acids is concomitant with the increase of membrane fluidity. It must be pointed out that these fluidity changes are observed in the absence of any variations at the level of cholesterol content and phospholipid polar headgroups. Then, the increase of membrane fluidity can be correlated only with the increase of the lipid unsaturation which is known to be a critical parameter monitoring the dynamic properties of the hydrocarbon chains of membranal lipid core [33] as well as its permeability properties [34].

The decrease in NaCl permeability previously observed at the enterocyte apical border soon after the transfer of trout from fresh water to sea water [14] may reflect either a direct effect of the high content of docosahexaenoic acid on ion permeability or an indirect effect on ion pumping efficiency at the basal membrane level. This precise point deserved further investigations on the apical permeability of the enterocyte using brush border membrane vesicles. If the observed increase in the brush border membrane fluidity can be assigned to the high 22 : 6 ($n-3$) level, other lipid metabolites may be also involved and further work would be needed to assess a precise role for prostaglandins and hydroxy fatty acid derivatives of the ($n-6$) and ($n-3$) series which may be synthesized in intestinal epithelium [35]. The rapidity of the observed alterations of the lipid phase is likely to be correlated with a change in the substrate selectivity or affinity of key enzymes such as choline or ethanolamine phosphotransferase which may be the main control of the distribution of 22 : 6 ($n-3$) in animal cells [30]. It would be valuable then to find out what controls these enzymes themselves. A modification in the activity of phospholipase and transacylase reactions under the influence of a change in the luminal salt content cannot be excluded. During sea water adaptation, Mg^{2+} and Ca^{2+} reach high concentrations [36] due to the limited permeability of the intestinal epithelium towards these divalent cations absorbed with drunk water. Thus, it can be pos-

tulated that a rapid increase in Mg^{2+} and Ca^{2+} near the external lipid layer of the brush-border membrane would be able to alter several key steps of the lipid metabolism. Studies of trout intestine under controlled luminal perfusion would be of value to examine the mechanism of the membrane lipid modification which we have shown to be present soon after the freshwater-sea water transfer. The studies on the time course changes of fatty acid uptake during the early adaptation process would help to clear the important problem of the membrane homeoviscous adaptation related with alteration of ion transport properties.

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References

1 Watanabe, T. (1982) Comp. Biochem. Physiol. 73B, 3–15
2 Leger, C. and Fremont, L. (1981) in Nutrition des Poissons (Fontaine, M., ed.), pp. 215–246, CNRS, Paris
3 Zwingelstein, G. (1981) in Nutrition des Poissons (Fontaine, M., ed.) pp. 249–260, CNRS, Paris
4 Zwingelstein, G. (1979) Oceanis 5, 117–30
5 Girard, J.P., Thomson, A.J. and Sargent, J.R. (1977) FEBS Lett. 73, 267–270
6 Hansen, H.J.M. and Abraham, S. (1979) Comp. Biochem. Physiol. 63B, 483–490
7 Hazel, J.R. and Sellner, P.A. (1980) in Animals and Environmental Fitness (Gilles, R., ed.), Vol. 1, pp. 541–560, Pergamon Press, Oxford
8 Smith, M.W. and Miller, N.G.A. (1980) in Animals and Environmental Fitness (Gilles, R., ed.), Vol. 1, pp. 521–540, Pergamon Press, Oxford
9 Hansen, H.J.M. and Abraham, S. (1983) Comp. Biochem. Physiol. 75B, 581–587
10 Thomson, A.J., Sargent, J.R. and Owen, J.M. (1977) Comp. Biochem. Physiol. 56B, 223–228
11 Daikoku, T., Yano, I. and Masui, M. (1982) Comp. Biochem. Physiol. 73A, 167–174
12 Jain, M.K. and Wagner, R.C. (1980) Introduction in Biological Membranes, pp. 117–142, J. Wiley and Sons, New York
13 Di Costanzo, G., Duportail, G., Florentz, A. and Leray, C. (1983) Mol. Physiol. 4, 279–290
14 Leray, C. and Florentz, A. (1983) in Intestinal Transport (Gilles-Baillien, M. and Gilles, R., eds.), pp. 354–368, Springer-Verlag, Berlin
15 Castell, J.D., Sinnhuber, R.O., Wales, J.H. and Lee, D.J. (1972) J. Nutr. 102, 77–85
16 Di Costanzo, G., Florentz, A., Leray, C. and Nonnotte, L. (1983) Mol. Physiol. 4, 111–123
17 Chapelle, S. (1977) Biochem. Syst. Ecol. 5, 241–248
18 Chapelle, S., Dandrifosse, G. and Zwingelstein, G. (1976) Int. J. Biochem. 7, 343–351
19 Dittmer, J.C. and Lester, R.L. (1964) J. Lipid Res. 5, 126–127
20 Portoukalian, J., Meister, R. and Zwingelstein, G. (1978) J. Chromatogr. 152, 569–574
21 Kates, M. (1972) Techniques of Lipidology, North-Holland Publishing Company, Amsterdam
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
23 Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394
24 Dickens, B.F. and Thompson, G.A. (1981) Biochim. Biophys. Acta 664, 211–218
25 Brasitus, T.A., Tall, R.A. and Schachter, D. (1980) Biochemistry 19, 1256–1261
26 Ackman, R.G. (1980) in Advances in Fish Science and Technology (Connel, J.J., ed.), pp. 86–103, Fishing News Books, Ltd, Farnham, Surrey, U.K.
27 Holman, R.T. (1970) Prog. Chem. Fats Lipids 9, 611–682
28 Chapelle, S. and Gilles-Baillien, M. (1981) Biochem. System. Ecol. 9, 233–240
29 Chapelle, S. and Gilles-Baillien, M. (1983) Biochim. Biophys. Acta 753, 269–271
30 Tinocco, J. (1982) Prog. Lipid Res. 21, 1–45
31 Bell, M.V., Simpson, M.F. and Sargent, J.R. (1983) Lipids, 18, 720–726
32 Chapelle, S. and Pequeux, A. (1982) Biochem. System. Ecol. 10, 71–78
33 Stubbs, C.D., Kouyama, T., Kinosita, K. and Ikegami, A. (1981) Biochemistry 20, 4257–4262
34 Deuticke, B. (1978) Rev. Physiol. Biochem. Pharmacol. 1–97
35 Nomura, T. and Ogata, H. (1976) Biochim. Biophys. Acta 431, 127–131
36 Shehadeh, Z.H. and Gordon, M.S. (1969) Comp. Biochem. Physiol. 30, 397–418