Biochimica et Biophysica Acta, 1111 (1992) 59–64

BBAMEM 75746

Developmental changes in hepatic basolateral membrane lipid composition and fluidity

Donald Novak [a], William Ray [a], Jane Carver [a] and Steven Grossman [b]

[a] *Department of Pediatrics, University of South Florida College of Medicine, Tampa, FL (USA) and* [b] *Department of Chemistry, University of South Florida College of Medicine, Tampa FL (USA)*

(Received 28 January 1992)
(Revised manuscript received 18 May 1992)

Key words: Developmental change; Hepatocyte; Basolateral membrane; Lipid; Lipid composition; Membrane fluidity

Membrane fluidity and lipid composition influence the activity of a variety of membrane proteins. Decreased rates of hepatic ion clearance are associated with the neonatal period. We postulated that hepatic basolateral membranes derived from suckling animals might be less fluid than those from adult animals. Basolateral membrane vesicles were prepared from the livers of 1-week-old (SBLMV) and adult (ABLMV) rats by a Percoll gradient method. Na^+/K^+-ATPase activities were similar in the two groups. Double bond index, cholesterol and cholesterol/phosphorus ratios were significantly higher in SBLMV compared with ABLMV, while lipid phosphorus and relative percentages of phospholipid subclasses did not differ. Fluorescence anisotropy measured using diphenylhexatriene as well as 2-(9-anthroyloxy)stearate was significantly greater in SBLMV compared with ABLMV, while measurements made with 12-(9-anthroyloxy)stearate were similar in both age groups. Mean excited state lifetimes, lifetime distributions, and rotational correlation times were similar in both groups. These data suggest that hepatic basolateral membranes derived from suckling rats are less fluid than those from adult animals and further suggest that this difference may be due to increased cholesterol in hepatic basolateral membranes derived from suckling animals.

Introduction

Suckling animals undergo an array of physiologic changes as they develop from fetal to adult life, including dramatic alterations in the activities of a variety of membrane associated proteins. These transformations are particularly evident in the hepatocyte, as illustrated by the postnatal development of taurocholate transport across both the basolateral [1,2] and canalicular [3] membranes, as well as by the ontogeny of hepatic Na^+/K^+-ATPase activity [1]. Mechanisms controlling these transformations have not been clearly defined, although preliminary data suggest that rates and timing of protein insertion into the membrane, as well as protein modification either prior or subsequent to insertion may play important roles [1]. in addition, membrane composition is known to influence transmembrane protein activities. Examples include modulation of sodium phosphate co-transport by cholesterol in brush border membranes derived from rat kidney [4], as well as modification of alkaline phosphatase activity by cholesterol [5] and fatty acid composition [6] in intestinal brush border membranes. Other examples include the effect of membrane fluidity upon the activity of Na^+/K^+-ATPase in the liver [7], as well as upon intestinal brush border D-glucose uptake [8]. Therefore, studies of the ontogeny of transmembrane protein mediated substrate transport across the hepatocyte basolateral membrane must take into account developmental changes in membrane lipid composition. Kapitulnik et al. [9] demonstrated that hepatic microsomal membranes derived from fetal rats had higher cholesterol and lower phospholipid contents compared with adult animals, while Brasitus and coworkers [10] reported higher proportions of unsaturated fatty acids in intestinal membranes of 6-week-old compared with adult rats. Changes in membrane lipid composition may also alter membrane physical characteristics. The use of fluorescent probes which intercalate into lipid bilayers has allowed the determination of membrane fluidity, defined as the relative motional freedom of

Correspondence to: D. Novak, Pediatric Gastroenterology Section, Department of Pediatrics, Box J296, JHMHSC, University of Florida, Gainesville, FL 32610-0296, USA.

lipid molecules within a lipid bilayer [11]. In addition, membrane structural heterogeneity can be explored through the study of fluorophore lifetime distributions.

We hypothesized that basolateral membranes derived from suckling animals would differ significantly in lipid composition and fluidity from those derived from adult animals. We have defined compositional and structural changes that occur in the hepatocyte basolateral membrane during development.

Materials and Methods

Animals

7-day-old Sprague-Dawley rats (8–12 pups/litter) with their dams, as well as adult male Sprague-Dawley rats were obtained from Harlan Laboratories, Indianapolis, IN. Animals were housed in a temperature-controlled room with 12-h light-dark cycles. Adult rats were fed standard rat chow and water ad libitum, while rat pups were suckled. All animals were killed by decapitation in the fed state. These studies were approved by the University of South Florida Laboratory Animal Medical Ethics Committee.

Chemicals

All chemicals were of reagent grade or of the highest grade commercially available. Solvents were glass distilled.

Preparation of rat liver basolateral membrane vesicles

Rat liver basolateral membranes were prepared by the method of Blitzer and Donovan [12], with modifications as described by Suchy et al. [2]. The membrane aliquots were rapidly frozen in liquid nitrogen and stored in liquid nitrogen until used.

Determination of marker enzyme activities and protein concentration

Na^+/K^+-ATPase and Mg^{2+} ATPase activities were assayed by the method of Scharschmidt [13]. Protein concentrations were determined by the method of Lowry et al. [14], using bovine serum albumin as standard.

Lipid composition studies

Total lipids were extracted from the membrane vesicles by the method of Folch et al. [15]. Cholesterol was measured utilizing the method of Zlatkis et al. [16], while total phosphorus concentrations were measured by the method of Bartlett [17]. Phosphoglyceride classes were separated on washed and dried Silica gel G plates (Analtech, Newark, DE) using the solvent system of Zail and Pickering [18]. Plates were dried under nitrogen, sprayed with 50% sulfuric acid and charred. Separated phospholipid bands were then scraped and relative phosphorus concentrations determined [19]. Fatty acids were methylated by the method of Morrison and Smith [20]. Fatty acid methyl esters were separated using a Perkin-Elmer model Sigma 2000 with a 30 meter DB 225 Megabore column. Gas liquid chromatography columns were obtained from J&W Scientific (Folsom, CA). Column temperature was maintained at 170°C for 8 min, then increased by 2°C/min to 210°C. Carrier gas flow rate was 30 ml/min. Identification of individual fatty acid methyl esters was performed via comparison of retention times to those of authentic standards (NuChek Prep, Elysian, MN or Supelco, Bellefonte, PA).

Florescence polarization studies

Three fluorophores were utilized: 1,6-diphenyl-1,3,5-hexatriene(DPH) (Aldrich, Milwaukee, WI), DL-2-(9-anthroyl)stearic acid (2-AS) and DL-12-(9-anthroyl)stearic acid (12-AS) (Molecular Probes, Junction City, OR). Steady-state and dynamic polarization measurements were made on an SLM 48000 multifrequency phase modulation fluorometer, in conjunction with an IBM PC-XT computer, utilizing previously described methods [21]. All measurements were performed at 25°C. Loading of membranes with fluorophores was done as described by Brasitus et al. [22]. Measurements of steady-state fluorescent polarization were made in the T format, utilizing an excitation wavelength of 365 nm. Emitted light was passed through a 408 nm cutoff filter. Steady state anisotropies were determined according to the relationship $(I_{vv} - GI_{vh})/(I_{vv} + 2GI_{vh})$, where I_{vv} is the intensity of light emitted through a vertically aligned polarizer after sample excitation with vertically aligned light, and I_{vh} represents said light after emission through a horizontally aligned filter. G is a grating correction factor, defined as the ratio of vertically to horizontally polarized emitted light after horizontal excitation. Readings were corrected as necessary for fluorescence noted both in the absence of fluorophore (membrane + buffer) and in the absence of membrane (buffer and fluorophore). In all cases, combined corrections were less than 3% of fluorescent intensities noted in the intact preparation. Membrane preparations [2] were also examined for the effect of light scattering as described by Lisinger and Flores [23]. No effects of scatter depolarization were noted upon steady-state anisotropy as measured utilizing DPH within the concentration ranges used in this study. Lifetime measurements (DPH) were obtained utilizing a reference solution containing dimethyl POPOP in absolute ethanol. Measurements were obtained at a minimum of seven frequencies between 0 and 100 MHz, with excitation light polarized at 0 degrees and emitted light polarized at 55 degrees. Phase and modulation data were then examined utilizing SLM software, version 1.4 (SLM instruments, Champaign-Urbana, IL). Specifically, one,

two and three component decay models were compared with unimodal or bimodal Lorentzian distributions [24]. Estimated experimental errors were set at 0.2 degrees for phase and 0.002 for modulation analyses. Fluorescent intensities (2-AS and 12-AS) were calculated according to the formula $I_{vv} + 2I_{vh}$ [25]. Rotational correlation times were estimated via differential polarized phase fluorometry utilizing the method of Lakowicz [21].

Statistical methods

All results represent mean ± standard deviation. Differences between groups were determined using Student's two-tailed *t*-test.

Results

Marker enzyme activities

To be used in developmental studies, membrane preparations must be: (1) highly enriched in the desired membrane fraction relative to homogenate and (2) comparable between age groups studied. The marker enzyme profile of the hepatocyte basolateral membrane vesicle preparation utilized in this study reveals it to be both highly purified and similar in adult and neonatal animals [1]. Contamination by endoplasmic reticulum, lysosomes, and mitochondria has previously been shown to be minimal and similar in neonatal and adult animals [1]. Because of the wealth of previous information and characterization of the preparation utilized, we chose to examine only Na^+/K^+-ATPase and Mg^{2+}-ATPase activities, markers for the basolateral and canalicular membranes. Relative enrichments (Table I) did not differ significantly between the two age groups studied and were in the range obtained by previously by us [26] as well as other prior investigators utilizing this preparation [1,2,12]. The preparations were significantly (21–26-fold) enriched in membranes derived from the basolateral surface of the hepatocyte, while only modestly enriched (5–6-fold) in membranes derived from the canalicular surface.

TABLE I

Marker enzyme activities of plasma membrane

Values are means ± S.D. in μmol P_i/mg protein per h. Numbers in parentheses are number of membrane preparations.

	Na^+/K^+-ATPase	Mg^{2+}-ATPase
Homogenate		
1 w/o	0.48 ± 0.24	2.12 ± 0.70
Adult	0.60 ± 0.46	2.73 ± 0.70
Basolateral fraction		
1 w/o	10.36 ± 4.50	9.95 ± 3.29
Adult	10.22 ± 4.44	16.00 ± 4.65
Relative specific activity		
1 w/o	26.24 ± 13.69 (8)	5.12 ± 2.27 (8)
Adult	21.16 ± 8.83 (13)	6.05 ± 2.13 (13)

TABLE II

Fatty acid composition of hepatic basolateral membrane vesicles derived from SBLMV and ABLMV rats (expressed as weight % of total fatty acid)

All values represent means ± S.D. least eight preparations. Significance: * $P < 0.05$, ** $P < 0.005$, compared with values in vesicles derived from ABLMV. Double bond index = Σ[(% each fatty acid) (number of double bonds per fatty acid)].

Fatty acid	ABLMV	SBLMV
16:0	27.3 ± 5.2	31.1 ± 7.8
16:1	0.6 ± 0.8	0.0 ± 0.0
18:0	23.0 ± 2.7	24.2 ± 3.4
18:1	9.7 ± 1.8	5.3 ± 2.3 **
18:2(*n* − 6)	11.7 ± 1.7	5.7 ± 1.3 **
20:0	0.2 ± 0.3	0.1 ± 0.2
20:1	0.8 ± 1.0	0.9 ± 0.9
20:2(*n* − 6)	0.1 ± 0.1	0.1 ± 0.1
20:3(*n* − 3)	0.2 ± 0.4	0.1 ± 0.2
18:3(*n* − 3)	0.3 ± 0.3	0.1 ± 0.2
20:4(*n* − 6)	15.4 ± 2.2	17.7 ± 1.8 *
22:0	0.9 ± 1.0	0.4 ± 0.3
20:5(*n* − 3)	0.7 ± 0.4	0.2 ± 0.5
24:0	1.3 ± 0.8	1.3 ± 1.0
24:1	0.6 ± 0.7	0.5 ± 0.4
22:5(*n* − 3)	1.4 ± 0.5	2.1 ± 1.0
22:6(*n* − 3)	5.8 ± 1.1	10.0 ± 2.3 **
Double bond index	1.44 ± 0.17	1.62 ± 0.24 **
F.A. > 18C	0.25 ± 0.03	0.32 ± 0.04 **
Saturated	0.53 ± 0.05	0.57 ± 0.06
Monoenoic	0.12 ± 0.02	0.07 ± 0.02 **
(*n* − 3)	0.08 ± 0.01	0.13 ± 0.03 **
(*n* − 6)	0.27 ± 0.04	0.24 ± 0.02 *
(*n* − 6)/(*n* − 3)	3.40 ± 0.39	2.10 ± 0.88 **

Lipid composition

Fatty acid composition. There was no significant difference in percentage of saturated fatty acids between ABLMV and SBLMV (Table II). The double bond index was significantly higher in SBLMV due predominantly to increases in percentages of decosahexaenoic (22:6(*n* − 3)) and arachidonic (20:4(*n* − 6)) acids. Percentages of linoleic acid (18:2(*n* − 6)), total

TABLE III

Analysis of cholesterol and total lipid phosphorus from SBLMV and ABLMV

Cholesterol and total lipid phosphorus values are expressed as μmol/mg protein and represent mean ± S.D. of at least eight preparations.

	Cholesterol (*C*)	Lipid phosphorus (*P*)	*C*/*P*
ABLMV	0.17 ± 0.04	0.31 ± 0.08	0.57 ± 0.14
SBLMV	0.26 ± 0.03	0.37 ± 0.08	0.72 ± 0.15
P value	< 0.001	N.S.	< 0.05

TABLE IV

Analysis of phospholipid subclasses from SBLMV and ABLMV

Individual phospholipids are expressed as molar percent of total phospholipid content and represent means ± S.D. of at least five preparations. PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; SP, sphingomyelin; PI, phosphatidylinositol.

	PC	PE	SP	PS + PI
ABLMV	51.0 ± 6.0	26.1 ± 4.3	8.4 ± 3.1	15.2 ± 3.7
SBLMV	47.4 ± 7.8	26.5 ± 7.8	7.9 ± 3.8	19.3 ± 13.8
P value	N.S.	N.S.	N.S.	N.S.

monounsaturated fatty acids and ($n - 6$) fatty acids were higher in ABLMV, while fatty acids greater than 18 carbons in length were lower.

Cholesterol / phospholipid (C / P) ratios and phospholipid subclasses (Tables III and IV). Total cholesterol content was significantly higher in SBLMV compared with ABLMV (0.26 μmol/mg protein vs. 0.17 μmol/mg protein) whereas total lipid phosphorus contents were not significantly different. As a result, the *C/P* ratio was significantly elevated in SBLMV (0.72) as compared with ABLMV (0.57). There were no significant differences in relative concentrations of phospholipid subclasses between the two age groups.

Fluidity studies

Steady-state anisotropy. Steady-state anisotropy values as measured by DPH were significantly decreased in ABLMV versus SBLMV (Table V). Anisotropy measurements made utilizing 2-AS were significantly increased in SBLMV compared with ABLMV, whereas no significant differences were noted with the probe 12-AS.

Dynamic lifetime measurements. Measurements of lifetime of the excited states were performed utilizing DPH. Best fit was obtained utilizing a discrete bi-exponential decay model (Table VI), in accordance with

TABLE V

Steady-state anisotropy studies of hepatic basolateral membrane vesicles

All measurements performed at 25°C. Values represent mean + S.D. of at least seven determinations. Significance: * $P < 0.02$ compared to values obtained from ABLMV; † $P < 0.01$ compared to values obtained from ABLMV.

Preparation	Probe	Anistropy (*r*)
ABLMV	DPH	0.201 ± 0.008
	2-AS	0.119 ± 0.002
	12-AS	0.101 ± 0.006
SBLMV	DPH	0.215 ± 0.007 †
	2-AS	0.126 ± 0.005 *
	12-AS	0.100 ± 0.003

TABLE VI

Discrete biexponential analysis of the excited state lifetime of DPH in SBLMV and ABLMV at 25°C

t_1 and t_2: lifetimes in nanoseconds. F_1 and F_2: Fractional intensity.

	t_1	F_1	t_2	F_2	χ^2
SBLMV	3.85 ± 0.94	0.14 ± 0.07	11.10 ± 0.50	0.86 ± 0.07	2.20 ± 1.27
ABLMV	3.74 ± 0.19	0.12 ± 0.01	10.58 ± 0.29	0.88 ± 0.01	2.11 ± 1.47

previous work utilizing DPH [27]. Both long (approx. 11 ns) and short (approx. 4 ns) lifetimes were similar in ABLMV and SBLMV, as were fractional lifetime contributions. Bimodal Lorentzian distribution analysis yielded signficantly lower chi-square values than did unimodal. There were no significant differences between ABLMV and SBLMV in lifetime component centers, distributional widths (full width at half maximum), lifetime fractions or chi-square values. Measurements of total fluorescent intensity were performed utilizing 2-AS and 12-AS. Results did not differ between SBLMV and ABLMV with either fluorophore (in arbitrary units, 2-AS: SBLMV 4.13 ± 0.07 ($n = 4$), ABLMV 4.13 ± 0.02 ($n = 4$); 12-AS: SBLMV 2.41 ± 0.01 ($n = 7$), ABLMV 2.41 ± 0.02 ($n = 7$)).

Rotational correlation times. Rotational correlation times, estimated via multifrequency analysis utilizing the probe DPH, did not differ significantly between ABLMV (0.98 ± 0.13 ns, $n = 3$) and SBLMV (1.18 ± 0.18 ns, $n = 4$).

Discussion

Previous work in vesicles derived from rat renal [28] and rabbit intestinal microvillus [29,30] membranes has documented a decrease in membrane fluidity with postnatal development. These changes have been associated, at least temporally, with increases in the *C/P* ratio with age. Conversely, Kapitulnik et al. [31], demonstrated an increase in membrane fluidity in adult as compared to fetal hepatic microsomal membranes. In contrast to the data presented here, however, changes in fluidity were largely complete by birth and were associated with significant changes in membrane phospholipid content. The present data suggest a different scenario, in which basolateral membrane fluidity continued to increase postnatally and was associated with decreasing contents of membrane cholesterol, while membrane lipid phosphorus content remained stable. The significantly higher levels of cholesterol in SBLMV are consistent with data obtained in intestinal microvillus membrane preparations derived from newborn rabbits [30], and may reflect high levels of fetal hepatic sterol synthesis [32], as well as significant intake of dietary cholesterol [33]. Percentages of membrane fatty acids also differed significantly between

SBLMV and ABLMV. Increased fatty acid chain length and double bond index in SBLMV may be attributable to transplacental accretion of long chain polyunsaturated fatty acids (PUFA) during the fetal period [34]. In addition, breast milk is known to contain significant amounts of PUFA [35]. The capacity for PUFA synthesis in 1-week-old animals may be limited due to diminished hepatic activities of Δ^6- and Δ^5-desaturases during fetal and early neonatal life [34].

Measurements of membrane fluidity utilizing the probe DPH were concordant with the above described data. Cholesterol is known to decrease membrane fluidity, presumably by reducing the motional freedom of membrane phospholipids [36]. Studies of steady-state anisotropy utilizing anthroyloxy probes of varying chain lengths have found that the effect of cholesterol occurs primarily within the 15 angstroms immediately below the membrane surface [37], consistent with the changes observed in our studies utilizing 2-AS, in which the fluorophore component is positioned close to the membrane surface. In contrast, utilization of the fluorophore 12-AS, which is positioned deeper within the bilayer, revealed no changes. Membrane fluidity is also affected by degree of fatty acid desaturation [11]. Introduction of double bonds into fatty acid chains produces conformational changes resulting in increased motional freedom and fluidity. The double bond index of SBLMV was significantly higher than that of ABLMV, yet the fluidity of SBLMV was significantly less than that of ABLMV. This apparent discrepancy may be due to the observation that conformational changes induced with phospholipid acyl chain desaturation are maximal with the initial double bond insertion [37]. Subsequent desaturations induce smaller conformational changes. ABLMV have a significantly higher percentage of monounsaturated fatty acids than do SBLMV, in concordance with observed fluidity differences. Other factors known to influence fluidity, such as phospholipid content, did not differ significantly between the two age groups studied. It is unlikely that the observed changes in anisotropy were due to differences in preparation purity. Previous authors have demonstrated the canalicular hepatocyte membrane to be less fluid than the basolateral surface [38], thus the minimal enhancement in Na^+/K^+-ATPase enrichment noted in SBLMV vs. ABLMV may have caused us to underestimate the difference in anisotropy between the two age groups studied.

Fluorescent decay kinetics may be used to elucidate membrane physical properties. DPH, because of its distribution in the hydrophobic acyl regions of a variety of membrane domains [39], as well as its responsiveness to changes in membrane dielectric properties, is a useful probe for such studies. Lifetimes of the excited state (DPH) can be described in terms of mono or multi-exponential decay. Bi-exponential decay models have generally proven to be most suitable in complex biologic membranes and typically reveal a predominant long (approx. 10 ns) lifetime component as well as a minor short (approx. 4 ns) component. In the current study, lifetimes obtained in ABLMV and SBLMV did not differ when examined by a biexponential model, nor did they differ when examined by means of a continuous lifetime distribution, in which lifetime values are weighted both by proportion and width [24]. The lack of difference between lifetime widths noted in the present study may be explained by the high C/P ratios (> 0.5) observed in both ABLMV and SBLMV. Hui et al. [40], demonstrated that onset of phase separation occurred below 20°C in erythrocyte membranes with C/P ratios greater than 0.5. Therefore, differences in heterogeneity secondary to membrane cholesterol content may have been minimal in the present study which utilized a temperature of 25°C. The lack of difference in fluorescent lifetimes noted with DPH is supported by the similarity in fluorescence intensities (suggesting similar fluoresence lifetimes) noted between ABLMV and SBLMV in studies using 2-AS and 12-AS. Finally, the lack of significant difference in rotational correlation times between ABLMV and SBLMV is not surprising in light of previous work in which membrane cholesterol content has had minimal effect upon this parameter [37].

The above described ontologic changes in the hepatocyte basolateral membrane differ from those previously defined in other tissues, although they concur with changes noted by Storch and coworkers in the differentiating 3T3F442A cell [41]. Such differences suggest that these alterations are specific and serve distinct teleologic purposes, including, perhaps, modulation of developing membrane transport processes. Potential regulatory mechanisms for membrane lipid/physiochemical development include changes in the hormonal and/or dietary milieu of the developing animal.

References

1 Suchy, F.J., Bucuvalas, J.C., Goodrich, A.L., Moyer, M.S. and Blitzer, B.L. (1986) Am. J. Physiol. 251, G665–G673.
2 Suchy, F.J., Courchene, S.M. and Blitzer, B.L. (1985) Am. J. Physiol. 248, G648–G653.
3 Novak, D.A., Sippel, C.J., Anantharayan, M. and Suchy, F.J. (1991) Am. J. Physiol. 260, G743–G751.
4 Levi, M., Baird, B.M. and Wilson, P.V. (1990) J. Clin. Invest. 85, 231–237.
5 Brasitus, T.A., Dahiya, R., Dudeja, P.K. and Bissonnette, B.M. (1988) J. Biol. Chem. 263, 8592–8597.
6 Stenson, W.F., Seetharam, B., Talkad, V., Pickett, W., Dudeja, P. and Brasitus, T.A. (1989) Biochem. J. 263, 41–45.
7 Keefe, E.B., Scharschmidt, B.F., Blankenship, N.M. and Ockner, R.K. (1979) J. Clin. Invest. 64, 1590–1598.
8 Brasitus, T.A., Dudeja, P.K., Bolt, M.H.G., Sitrin, M.D. and Baum, C. (1989) Biochim. Biophys. Acta 979, 177–186.

9 Kapitulnik, J., Weil, E., Rabinowitz, R. and Krausz, M.M. (1987) Hepatology 7, 55–60.
10 Brasitus, T.A., Kwo-Yih, Yeh, Holt P.R. and Schachter, D. (1984) Biochim. Biophys. Acta 778, 341–348.
11 Schacter, D. (1984) Hepatology 4, 140–151.
12 Blitzer, B.L. and Donovan, C.B. (1984) J. Biol. Chem. 259, 9295–9301.
13 Scharschmidt, B.F., Keeffe, E.B., Blankenship, N.M. and Ockner, R.K. (1979) J. Lab. Clin. Med. 93, 790–797.
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
15 Folch, J., Lees, M. and Sloane Stanley, G.H. (1956) J. Biol. Chem. 226, 497–509.
16 Zlatkis, A., Zak, B. and Boyle, A.J. (1953) J. Lab. Clin. Med. 41, 486–491.
17 Bartlett, G.R. (1959) Biochemistry 234, 466–468.
18 Zail, S.S. and Pickering, A. (1979) Br. J. Haematol. 43, 399–402.
19 Vaskovsky, V.E., Kostetsky, E.Y. and Vasendin, I.M. (1975) J. Chromatogr. 114, 129–141.
20 Morrison, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608.
21 Lakowicz, J.R., Prendergast, F.G. and Hogen, D. (1979) Biochemistry 18, 508–519.
22 Brasitus, T.A., Schacter, D. and Mamouneas, T.G. (1979) Biochemistry 18, 4136–4144.
23 Storch, J., Schacter, D., Inoue, M. and Wolkoff, A.W. (1983) Biochim. Biophys. Acta 727, 209–212.
24 Williams, B.W. and Stubbs, C.D. (1988) Biochemistry 27, 7994–7999.
25 Schacter, D. and Shinitzky, M. (1977) J. Clin. Invest. 59, 536–548.
26 Novak, D.N., Ryckman, F.C. and Suchy, F.J. (1989) Hepatology 10, 447–453.
27 Fiorini, R., Valentino, M., Wang, S., Glaser, M. and Gratton, E. (1987) Biochemistry 26, 3864–3870.
28 Medow, M.S. and Segal, S. (1987) Biochem. Biophys. Res. Commun. 142, 849–856.
29 Schwarz, S.M., Ling, S., Hostetler, B., Draper, J.P. and Watkins, J.B. (1984) Gastroenterology 86, 1544–1551.
30 Pang, K., Bresson, J.L. and Walker, W.A. (1983) Biochim. Biophys. Acta 727, 201–208.
31 Kapitulnik, J., Tshershedsky, M. and Barenholz, Y. (1979) Science 206, 843–844.
32 Hahn, P. (1982) Annu. Rev. Nutr. 2, 91–111.
33 Roberts, D.C.K., Huff, M.W. and Carroll, K.K. (1979) Nutr. Metab. 23, 476–486.
34 Coleman, R.A. (1989) Semin. Perinatol. 13, 180–191.
35 Uauy, R., Treen, M. and Hoffman, D.R. (1989) Semin. Perinatol. 13, 118–130.
36 Fiorini, R.M., Valentino, M., Glaser, M., Gratton, E. and Curatola, G. (1988) Biochim. Biophys. Acta 939, 485–492.
37 Sawyer, W. (1988) in Advances in Membrane Fluidity (Aloia, R.C., Curtain, C.C. and Gordon, L.M., ed.), Vol. 1, pp. 161–192, Alan R. Liss, New York.
38 Eisenger, J. and Flores, J. (1985) Biophys. J. 48, 77–84.
39 Karnovsky, M.J., Kleinfeld, A.M., Hoover, R.L. and Klausner, R.D. (1982) J. Cell. Biol. 94, 1–6.
40 Hui, S.W., Stewart, C.M., Carpenter, M.P. and Stewart, T.P. (1980) J. Cell Biol. 85, 283–291.
41 Storch, J., Shulman, S.L. and Kleinfeld, A.M. (1989) J. Biol. Chem. 264, 10527–10533.