Biochimica et Biophysica Acta, 774 (1984) 138–146
Elsevier

BBA 72147

LIPID COMPOSITION AND FLUIDITY OF RAT ENTEROCYTE BASOLATERAL MEMBRANES

REGIONAL DIFFERENCES

THOMAS A. BRASITUS * and DAVID SCHACHTER

Departments of Physiology and Medicine, Columbia University College of Physicians and Surgeons, New York, NY 10032 (U.S.A.)

(Received November 3rd, 1983)
(Revised manuscript received March 12th, 1984)

Key words: Basolateral membrane; Lipid composition; Membrane fluidity; ($Na^+ + K^+$)-ATPase; (Rat enterocyte)

The lipid composition and fluidity of basolateral membranes prepared from the mucosa of the proximal, middle and distal thirds of the rat small intestine were determined. Fluidity, as assessed by the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene and a series of anthroyloxy fatty acid derivatives, is decreased in the distal third as compared to the proximal segments. This pattern is similar to that described previously for microvillus membranes. The decrease in fluidity of the distal as compared to the proximal membranes results from an increase in cholesterol content, cholesterol/phospholipid molar ratio and degree of saturation of the fatty acid residues. In the middle and distal thirds of the gut, the degree of saturation of the fatty acid residues is higher in microvillus as compared to basolateral membranes, accounting in part for the characteristically lower fluidity of the luminal membranes. The specific activity of the basolateral membrane ($Na^+ + K^+$)-dependent adenosine triphosphatase is significantly lower in the distal as compared to the proximal and middle thirds of the intestinal mucosa. Studies of the binding of [^{3}H]ouabain indicate that this pattern results from fewer enzyme sites in the distal membranes.

Introduction

The enterocyte, the major absorptive cell of the intestinal mucosa, is differentiated along the length of the small intestine for specialized functions. Corresponding to the functional specialization, regional differences in the lipid fluidity ** and composition of the rat enterocyte microvillus (luminal) membrane have been demonstrated [1–3]. Microvillus membranes of the distal, ileal mucosa contain more cholesterol [3] and are less fluid than the proximal membranes. Another aspect of enterocyte differentiation is that the luminal microvillus membrane is considerably less fluid than the contraluminal basolateral membrane [4,5]. The present studies were undertaken to determine whether basolateral membranes also vary in lipid composition and fluidity along the gut and to characterize more completely the lipid composition of both basolateral and microvillus membranes in various intestinal segments. The results below demonstrate a pattern of decreased fluidity of the basolateral membrane in the distal intestinal mucosa. Moreover, the regional variations in fluidity of both the microvillus and basolateral membranes, as well as

* Present address: Division of Gastroenterology, Department of Medicine, Michael Reese Hospital and Medical Center, Chicago, IL 60616, U.S.A.

** A discussion of our use of the term 'lipid fluidity' as applied to anisotropic bilayer membranes is given in Refs. 4 and 15. Briefly, it is used here to express the relative motional freedom of the lipid molecules or substitutents thereof, combining in the one term concepts of the rate of movement and the extent of movement.

the fluidity difference between these antipodal membranes, result from differences in cholesterol/phospholipid molar ratio and the degree of saturation of the fatty acid substituents.

Methods

Membrane preparations. Albino male rats of the Sherman-Wistar strain were maintained on a pelleted diet (Camm Maintenance Rodent Diet) with water and food ad libitum. Rats weighing 250–300 g were fasted for 18 h with water ad libitum prior to removal of the small intestine. The intestine was divided into proximal, middle and distal thirds. Basolateral membranes were isolated from enterocyte suspensions and microvillus membranes were isolated from mucosal scrapings derived from each third of the intestine, using methods described previously [4–6]. Purity of the basolateral membranes was assessed via the marker enzyme ($Na^+ + K^+$)-dependent adenosine triphosphatase (($Na^+ + K^+$)-ATPase), and the final specific activity ratios of (purified membranes)/(crude homogenates) varied from 10 to 20. The microvillus membrane marker enzymes were *p*-nitrophenylphosphatase and sucrase, and the final specific activity ratios of isolated membranes to crude homogenates also varied from 10 to 20. All preparations were also assayed for NADPH-cytochrome *c* reductase and succinate dehydrogenase, marker enzymes for microsomal and mitochondrial membranes, respectively; specific activity ratios of membranes to homogenates averaged 0.10 and did not exceed 0.20.

Lipid extracts and analysis. Total lipids were extracted from the purified membranes by the method of Folch et al. [7]. Where appropriate, sonicated dispersions (liposomes) of the dried lipid extracts were prepared as described previously [5]. The lipid composition of the extracts was examined by thin-layer chromatography according to the procedure of Katz et al. [8], as previously described [3,4], except that the glycolipid moiety was not quantified. Hence, the percentage of each lipid component described below (Table III) represents the quantity of that component divided by total lipids minus glycolipids, and the resulting values are approx. 23% greater than comparable data reported previously [3,4]. Total cholesterol and phospholipids were also estimated by the methods of Zlatkis et al. [9] and Ames and Dubin [10], respectively. The fatty acid composition of the total lipid extract was determined by methylating the acyl chains as described by Gartner and Vahouny [11]. The fatty acid methyl esters were quantified in a JEOL JGC-20K gas chromatograph equipped with a flame-ionization detector and interfaced with a Hewlett-Packard 3390A integrator, using authentic fatty acid methyl ester standards to identify the sample peaks by retention times.

Fluorescence polarization studies. The fluidity of the membrane or liposome suspensions was assessed by the steady-state fluorescence polarization of the following lipid-soluble fluorophores: 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co.); DL-2-(9-anthroyloxy)stearic acid; DL-7-(9-anthroyloxy)stearic acid; DL-9-(9-anthroyloxy)stearic acid; DL-12-(9-anthroyloxy)stearic acid and DL-16-(9-anthroyloxy)palmitic acid. All the anthroyloxy derivatives were purchased from Molecular Probes, Junction City, OR. The methods used to load the membranes and liposomes and the estimation of the fluorescence polarization in an SLM polarization spectrofluorometer have been described previously [4]. The polarization of fluorescence was expressed as the fluorescence anisotropy, r, and as the anisotropy parameter, $[(r_0/r) - 1]^{-1}$, where r_0 is the maximal limiting anisotropy, taken as 0.362 and 0.285 for diphenylhexatriene and the anthroyloxy probes, respectively [4]. The fluorescence anisotropy values of diphenylhexatriene reflect mainly the static component of membrane fluidity, i.e., the maximal hindered anisotropy, r_∞ [12–14]. The anthroyloxy probes, on the other hand, have relatively low r_∞ values [15,16] and the fluorescence anisotropy reflects mainly the dynamic component of fluidity, i.e., the correlation times or rates of rotation. Possible changes in excited-state lifetime of the various probes were monitored by calculation of the total intensity of fluorescence, $F = I_{\parallel} + 2I_{\perp}$, where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities oriented, respectively, parallel and perpendicular to the plane of polarization of the exciting light [17]. Changes in r described below were not the result of changes in the excited-state lifetime as assessed by F.

($Na^+ + K^+$)-dependent adenosine triphospha-

tase. Assays of $(Na^+ + K^+)$-ATPase activity were performed as described previously [5]. Basolateral membranes, prepared as described above, were stored at 5 °C and assayed within 18 h. To quantify the number of $(Na^+ + K^+)$-ATPase sites, [^{3}H]ouabain binding, estimated as described by Liberman et al. [18], was assayed within 2 days of preparing the membranes. The reaction mixture consisted of 1.0 ml membrane suspension (25–50 μg membrane protein) in a medium of the following final composition: NaCl, 100 mM; $MgCl_2$, 5 mM; Tris-ATP, 5 mM; Tris-HCl, 50 mM (pH 7.4) and [^{3}H]ouabain (12.7 Ci/mmol; New England Nuclear) plus carrier ouabain to yield concentrations from 0.8 to 80 μM. All components minus Tris-ATP were preincubated for 3–5 min at 37 °C, and the reaction was started by addition of Tris-ATP and the samples were shaken for 5 min at 37 °C. Thereafter, aliquots of 0.2 ml were filtered with suction through 0.45 μm Millipore filters (Millipore Corp., Bedford, MA) in quadruplicate. The filters were premoistened with ice-cold 2.5 mM Tris-EDTA (pH 7.4) and after the sample filtration, the filter was washed rapidly and successively with three 1.0-ml aliquots of the ice-cold Tris-EDTA. Under these conditions, control filters retained less than 0.2% of the applied [^{3}H]ouabain. Washed filters were dissolved in 10 ml aquasol II scintillation solution (New England Nuclear) and the radioactivity was estimated in a liquid-scintillation spectrometer to quantify the bound [^{3}H]ouabain. Unbound [^{3}H]ouabain in the reaction mixture was calculated as the difference between the total [^{3}H]ouabain and the bound moiety. To correct for nonspecific binding, membranes were incubated with a 1000-fold excess of ouabain in media lacking NaCl, $MgCl_2$ and ATP. The values used for correction were approx. 15–25% of the total radioactivity bound.

Other methods and materials. Protein was estimated by the method of Lowry et al. [19] using bovine serum albumin as the reference standard. Radioactive materials were purchased from New England Nuclear. Fatty acid methyl esters, columns for gas-liquid chromatography and various pure lipid standards were obtained from Applied Science (State College, PA) and Supelco (Bellefonte, PA).

Results

Fluidity studies

Basolateral membranes and liposomes of the lipid extracts were prepared from each third of the small intestine and the fluorescence anisotropy of diphenylhexatriene at 25 °C was examined. The results in Table I indicate that the mean $[(r_0/r) - 1]^{-1}$ value of the membranes was approx. 56% greater in the distal third as compared to the proximal two-thirds of the intestine ($P < 0.01$), indicative of an increase in lipid order [12–14]. Correspondingly, the mean diphenylhexatriene $[(r_0/r) - 1]^{-1}$ value of the liposomes was approx. 26% greater in the distal preparations ($P < 0.05$). Further noteworthy in Table I is that the $[(r_0/r)$

TABLE I

FLUORESCENCE ANISOTROPY OF DIPHENYLHEXATRIENE IN BASOLATERAL MEMBRANES PREPARED FROM DIFFERENT SEGMENTS OF THE SMALL INTESTINE

Estimations were at 25 °C and values are means (±S.E.) for four different preparations. *P* values for differences between the distal third and either the proximal or middle third were calculated by Student's *t*-test of paired comparisons.

Preparation	Intestinal segment (third)	Fluorescence anisotropy (r)	Anisotropy parameter $[(r_0/r)-1]^{-1}$	P
Basolateral membranes	proximal	0.239	1.93 ± 0.10	< 0.01
	middle	0.235	1.85 ± 0.10	< 0.01
	distal	0.271	2.95 ± 0.12	
Liposomes	proximal	0.201	1.25 ± 0.09	< 0.05
	middle	0.201	1.25 ± 0.09	< 0.05
	distal	0.222	1.58 ± 0.11	

$-1]^{-1}$ values of the intact membranes consistently exceed those of the corresponding liposomes, a pattern observed previously in microvillus membranes as well [2–5] and ascribed to the effects of the membrane proteins on the lipid fluidity.

To determine whether the foregoing regional differences in fluidity are temperature-dependent, Arrhenius plots of the diphenylhexatriene $[(r_0/r) - 1]^{-1}$ values against 1/K were examined and the results for both intact membranes and liposomes are illustrated in Fig. 1. The anisotropy parameter of the distal membranes or liposomes exceeds that of the corresponding proximal preparations throughout the temperature range of 0–40 °C. Break points were observed at approx. 24 and 20 °C, respectively, for the distal basolateral membranes and liposomes. As described previously [5], these break-point temperatures correspond to the lower critical temperatures of broad lipid thermotropic transitions.

Basolateral membranes prepared from each third of the small intestine were treated with a number of anthroyloxy derivatives and the values of the anisotropy parameters are shown in Table II. Although the regional differences were not as marked as for diphenylhexatriene, the $[(r_0/r) - 1]^{-1}$ values of the 2-, 7- and 16-anthroyloxy derivatives were significantly greater ($P < 0.05$) in the distal third as compared to either the proximal and middle segments (2- and 7-derivatives) or the most proximal segment (16-derivative). The $[(r_0/r) - 1]^{-1}$ value of 12-anthroyloxystearate was significantly greater in the distal segment ($P < 0.05$; paired t-test) as compared to the grouped proximal plus middle segment values. Only the 9-anthroyloxystearate value was not significantly greater in the distal third. The results indicate that in addition to an increase in lipid order, the dynamic component of membrane fluidity is decreased in the basolateral membranes of the distal segment.

Lipid composition studies

To characterize the composition changes which underly the fluidity pattern described above, lipid extracts of basolateral membranes prepared from each third of the intestine were examined by thin-layer chromatography and the results are sum-

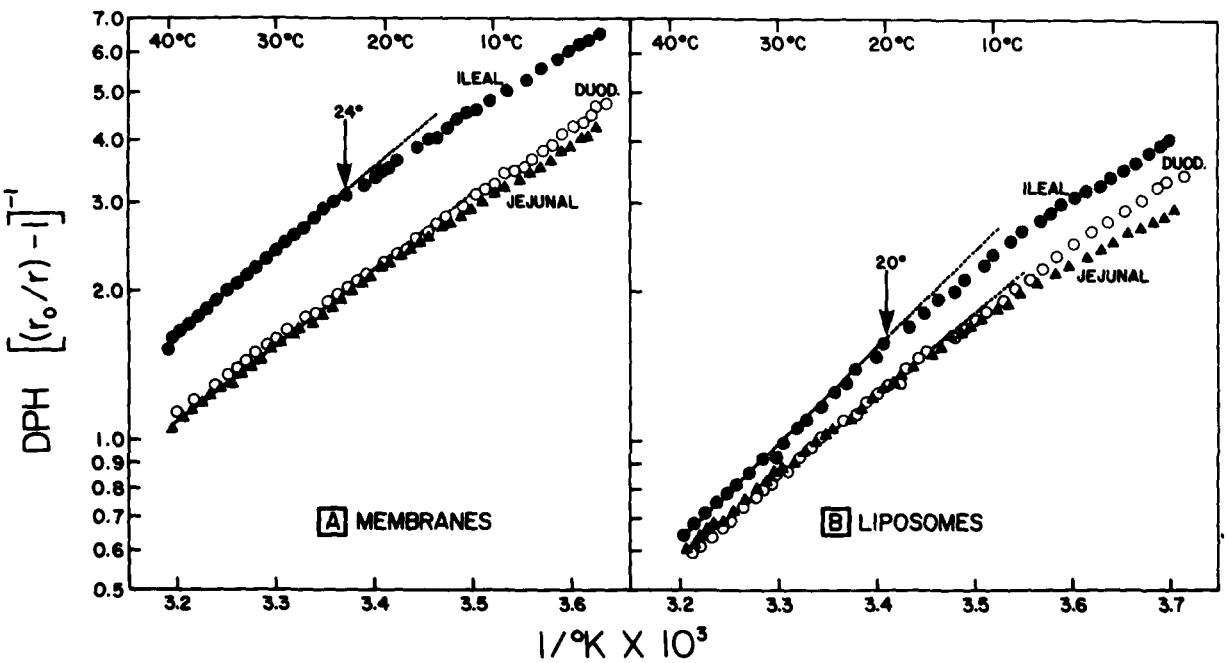

Fig. 1. Arrhenius plots of the fluorescence anisotropy parameter, $[(r_0/r)-1]^{-1}$, of diphenylhexatriene in (A) basolateral membranes and (B) liposomes prepared from the basolateral membrane lipid. The membranes were prepared from the proximal (○), middle (▲) and distal (●) thirds of the rat small intestine. DPH, diphenylhexatriene.

TABLE II

FLUORESCENCE ANISOTROPY OF ANTHROYLOXY FATTY ACIDS IN BASOLATERAL MEMBRANES PREPARED FROM DIFFERENT REGIONS OF THE SMALL INTESTINE

Estimations were at 25 °C and values are means (±S.E.) for four different preparations. *P* values for differences of the distal third from either the proximal or middle third were calculated by Student's *t*-test of paired comparisons.

Probe	Segment (third)	Fluorescence anisotropy (r)	Anisotropy parameter $[(r_0/r)-1]^{-1}$	*P*
2-(9-Anthroyloxy)stearate	proximal	0.097	0.52 ± 0.03	< 0.05
	middle	0.096	0.52 ± 0.03	< 0.05
	distal	0.103	0.58 ± 0.03	
7-(9-Anthroyloxy)stearate	proximal	0.098	0.54 ± 0.03	< 0.05
	middle	0.097	0.53 ± 0.03	< 0.05
	distal	0.103	0.59 ± 0.02	
9-(9-Anthroyloxy)stearate	proximal	0.080	0.40 ± 0.03	
	middle	0.079	0.39 ± 0.02	
	distal	0.082	0.42 ± 0.03	
12-(9-Anthroyloxy)stearate	proximal	0.068	0.31 ± 0.01	
	middle	0.065	0.30 ± 0.02	
	distal	0.069	0.33 ± 0.02 [a]	
16-(9-Anthroyloxy)palmitate	proximal	0.049	0.21 ± 0.01	< 0.05
	middle	0.058	0.26 ± 0.02	
	distal	0.057	0.26 ± 0.03	

[a] Differs significantly ($P < 0.05$) by paired *t*-test against all proximal segments.

TABLE III

COMPOSITION OF LIPID EXTRACTS OF BASOLATERAL MEMBRANES FROM DIFFERENT SEGMENTS OF THE SMALL INTESTINE

Values are means (±S.E.) for lipid extracts of five different membrane preparations. Values are given in percent by weight of total lipids minus glycolipid. *P* values were calculated by Student's *t*-test of paired comparisons for differences between the proximal and distal thirds.

Component	% (by wt.)			*P*
	Proximal third	Middle third	Distal third	
Cholesterol	14.4 ± 1.7	17.4 ± 1.4	22.3 ± 2.1	< 0.01
Cholesterol esters	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	
Triacylglycerols	3.3 ± 0.3	4.0 ± 0.4	3.3 ± 0.3	
Fatty acids	9.6 ± 1.1	9.7 ± 1.2	7.9 ± 1.8	
Total phospholipids	71.7 ± 5.2	67.8 ± 6.2	65.5 ± 6.4	
Phosphatidylcholine	44.6 ± 3.7	42.4 ± 4.1	47.7 ± 4.7	
Lysophosphatidylcholine	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	
Phosphatidylethanolamine	9.0 ± 1.5	7.8 ± 1.5	4.2 ± 1.2	< 0.01
Sphingomyelin	17.5 ± 0.5	17.0 ± 0.5	13.0 ± 0.4	< 0.01
Cholesterol/phospholipid molar ratio	0.40 ± 0.06	0.51 ± 0.04	0.68 ± 0.06	< 0.01
Sphingomyelin/lecithin molar ratio	0.39 ± 0.04	0.40 ± 0.04	0.27 ± 0.06	< 0.05

marized in Table III. Both the cholesterol content and the cholesterol/phospholipid molar ratio increased in the distal as compared to the proximal segment. The cholesterol content was 55% greater ($P < 0.01$) and the cholesterol/phospholipid ratio was 70% greater ($P < 0.01$) in the distal as compared to the proximal third. In addition, the distal lipid extracts contained 53% less phosphatidylethanolamine ($P < 0.01$) and 26% less sphingomyelin ($P < 0.01$) than the corresponding proximal extracts.

The fatty acid composition of the basolateral and microvillus membrane lipid extracts prepared from each third of the intestine was determined and the values for each fatty acid are shown in Table IV. The major differences between the segments are illustrated by the mean values plotted in Fig. 2. In both membrane types, the most striking changes from the proximal to the distal segments were a marked reduction in linoleic acid (18 : 2) ($P < 0.0025$) and a less marked increase in palmitic acid (16 : 0) ($P < 0.02$). The stearic acid (18 : 0) content of the microvillus membranes was also significantly greater in the distal and middle thirds as compared to the proximal third ($P < 0.005$). The net effect of these changes is an increase in the saturation of the acyl chains of the more distal membranes, as illustrated further in Fig. 3, where values of the saturation index (see Fig. 3 legend) and of the ratios of saturated/total fatty acids and saturated/unsaturated fatty acids are plotted. All

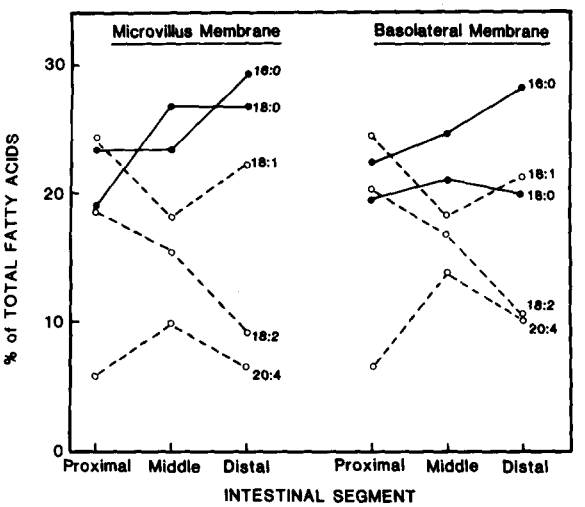

Fig. 2. Content of individual fatty acid residues in the lipid extracts of microvillus and basolateral membranes prepared from the proximal, middle and distal thirds of the rat small intestine. Values plotted are expressed as the percent by weight of the total fatty acid residues. Values are means of from 3–5 separate membrane preparations. Total fatty acid content and S.E. values for each estimation are listed in Table IV. Acids shown are palmitic (16 : 0), stearic (18 : 0), oleic (18 : 1), linoleic (18 : 2) and arachidonic (20 : 4).

TABLE IV

FATTY ACID COMPOSITION OF LIPID EXTRACTS OF MICROVILLUS AND BASOLATERAL MEMBRANES ISOLATED FROM THE PROXIMAL, MIDDLE AND DISTAL THIRDS OF THE RAT SMALL INTESTINE

Values are means ± S.E. for four preparations (except five and three preparations, respectively, for basolateral membranes of the proximal and middle thirds). n.d., not determined.

Fatty acid	Relative content (%)					
	Microvillus membrane			Basolateral membranes		
	Proximal	Middle	Distal	Proximal	Middle	Distal
14:0	1.50 ± 0.16	0.87 ± 0.09	1.27 ± 0.11	0.98 ± 0.10	1.17 ± 0.44	2.76 ± 0.66
14:1	0.43 ± 0.03	0.42 ± 0.02	0.63 ± 0.03	0.46 ± 0.07	0.47 ± 0.01	0.71 ± 0.11
16:0	23.30 ± 1.45	23.36 ± 0.68	29.10 ± 0.94	22.33 ± 1.35	24.77 ± 0.97	28.34 ± 0.88
16:1	2.71 ± 0.54	2.00 ± 0.53	1.59 ± 0.04	1.37 ± 0.08	0.80 ± 0.05	1.85 ± 0.19
18:0	19.05 ± 1.39	26.81 ± 1.25	25.58 ± 1.53	19.49 ± 0.19	21.15 ± 0.76	19.95 ± 0.55
18:1	24.11 ± 1.23	18.18 ± 1.26	22.25 ± 0.68	24.62 ± 2.54	18.33 ± 0.85	21.22 ± 0.41
18:2	18.67 ± 0.74	15.47 ± 1.30	9.10 ± 0.53	20.33 ± 1.58	16.80 ± 2.83	10.60 ± 0.67
20:0	0.55 ± 0.08	0.61 ± 0.21	0.71 ± 0.12	0.38 ± 0.09	0.84 ± 0.37	0.72 ± 0.28
20:1	1.46 ± 0.08	1.05 ± 0.36	1.96 ± 0.15	0.98 ± 0.14	0.77 ± 0.58	0.60 ± 0.34
20:2	0.27 ± 0.09	0.28 ± 0.09	0.43 ± 0.03	n.d.	n.d.	n.d.
20:3	1.03 ± 0.10	1.18 ± 0.07	1.23 ± 0.13	n.d.	n.d.	n.d.
20:4	5.94 ± 0.77	9.80 ± 1.26	6.51 ± 0.89	6.51 ± 1.30	13.76 ± 1.78	10.57 ± 1.20

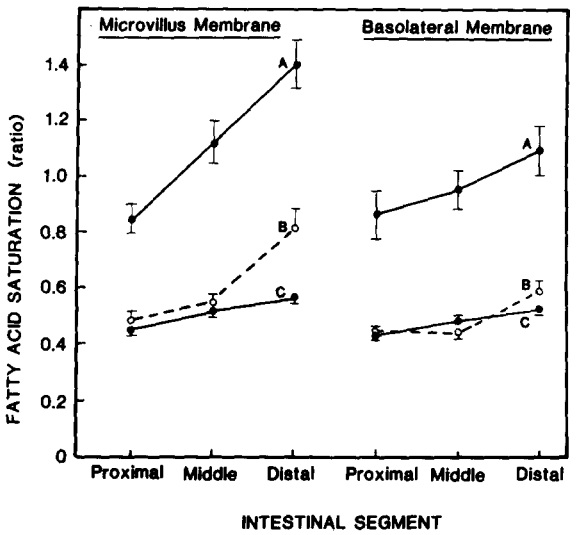

Fig. 3. Saturation of the fatty acid residues of lipid extracts prepared from basolateral and microvillus membranes. Values are mean ± S.E. calculated from the data listed in Table IV and illustrated partially in Fig. 2. The values are expressed as the ratio of saturated/unsaturated residues (A), the saturation index (B) and the ratio of saturated/total fatty acid residues (C). The saturation index was calculated as (the total number of saturated residues)/(the sum of the number of each type of unsaturated residue multiplied by the number of double bonds in that residue).

three indices of the degree of acyl chain saturation are significantly greater in the distal as compared to the proximal segments ($P < 0.01$).

Inasmuch as the fluidity of the basolateral membrane exceeds that of the microvillus membrane [4,5], a comparison of the acyl chain compositions is of interest. The results in Table IV (illustrated in Figs. 2 and 3) indicate that in the proximal third of the intestine the antipodal plasma membranes did not differ significantly in fatty acid composition and saturation indices. By contrast, in the middle and distal thirds, the stearic acid content was significantly greater ($P < 0.02$ and $P < 0.01$, respectively) in the microvillus membranes and the arachidonic acid content was greater ($P < 0.05$ and $P < 0.01$, respectively) in the basolateral membranes. As a result, the saturation index of the microvillus membranes exceeded that of the basolateral membranes in the middle ($P < 0.001$) and distal ($P < 0.01$) thirds (Fig. 3, B).

Basolateral membrane ($Na^+ + K^+$)-ATPase

In view of the differences in lipid fluidity of the basolateral membranes along the length of the small intestine (Tables I and II), we estimated both the specific enzyme activity of the ($Na^+ + K^+$)-ATPase and the number of ouabain-binding sites in preparations from each third of the gut. Prior studies indicate that the activity of this integral membrane enzyme is sensitive to the lipid fluidity of its microenvironment [4,20–23]. As indicated by the values in Table V, the enzyme activity assayed under maximal velocity conditions did decrease by 28% in the distal third as compared to the proximal third ($P < 0.025$). The number of ouabain-binding sites in the distal portion was also decreased by 44% ($P < 0.001$) and as a consequence the calculated turnover number of the enzyme activity was 31% greater in the distal as compared to the proximal preparations ($P < 0.05$).

TABLE V

($Na^+ + K^+$)-DEPENDENT ADENOSINETRIPHOSPHATASE ACTIVITY AND [^{3}H]OUABAIN-BINDING SITES IN BASOLATERAL MEMBRANES OF DIFFERENT SEGMENTS OF THE SMALL INTESTINE

Values are means (± S.E.) for six preparations of membranes. *P* values for difference of the distal and proximal segments were calculated by Student's *t*-test of paired comparisons.

Segment (third)	($Na^+ + K^+$)-ATPase activity (nmol/min per mg protein)	[^{3}H]Ouabain binding		Turnover (min^{-1})
		Total sites (pmol/mg protein)	K_d (M) ($\times 10^5$)	
Proximal	368 ± 38	274 ± 12	3.1 ± 1.4	1351 ± 107
Middle	326 ± 49	208 ± 14	4.3 ± 1.4	1563 ± 203
Distal	266 ± 30	154 ± 13	3.9 ± 1.5	1765 ± 194
P	< 0.025	< 0.01		< 0.05

Discussion

The foregoing results indicate that the lipid fluidity of enterocyte basolateral membranes, like that of the antipodal microvillus membranes [1–3], decreases in the distal third of the small intestine. This difference in fluidity is evident throughout the temperature range 0–40 °C, i.e., below and above the thermotropic transition previously characterized by differential scanning calorimetry and fluorescence polarization studies [5]. At least two differences in lipid composition account for the decreased fluidity of the distal basolateral membranes. The cholesterol content and cholesterol/phospholipid molar ratio of the lipid increase distally (Table III), and the saturation index of the fatty acid residues increases, owing largely to a reduction in linoleic acid and an increase in palmitic acid.

The mechanisms responsible for the differences in lipid composition and fluidity along the length of the small intestine are unknown. Inasmuch as endogenous cholesterol biosynthesis is greater in enterocytes of the distal as compared to the proximal intestinal mucosa [24], we have suggested previously that enhanced biosynthesis may account for the higher cholesterol content of the distal microvillus membranes [3]. A similar explanation could apply to the basolateral membranes, although it is noteworthy that experimental variation of cholesterol biosynthesis affected the cholesterol content and fluidity of microvillus but not of basolateral membranes of ileal enterocytes [3]. The higher content of linoleic acid in proximal preparations may result from the ingestion of this fatty acid in the diet. Recent studies in our laboratory demonstrate that a diet highly enriched in linoleic acid tends to elevate the content of this acyl residue in all segments of the gut and to eliminate the progressive fall in concentration along the intestine (Fig. 2).

Although the physiological significance of the decrease in lipid fluidity in the distal intestine is unknown, one reasonable hypothesis is that it is related to the well-recognized functional specialization of the ileum for net absorption of sodium and water. An important driving force for this process is active transport of sodium out of the enterocyte across the basolateral membrane. Decreased lipid fluidity of the basolateral membranes is expected to diminish passive back-flux of sodium and, thereby, to enhance the net unidirectional flux owing to the sodium pump. This hypothesis would account for the decrease in titer of [^{3}H]ouabain-binding sites in the distal mucosa (Table V), inasmuch as fewer pump sites would be required in cells with less leaky membranes. The observation that the calculated turnover number for the $(Na^+ + K^+)$-ATPase is increased in the distal basolateral membranes (Table V) indicates that the overall reduction in lipid fluidity does not impede the activity of the enzyme. Presumably, this is because the enzyme molecules are in selected microdomains of higher fluidity, as indicated previously by Arrhenius studies of the enzyme activity [4]. It bears emphasis, however, that the enzyme is sensitive to bulk lipid fluidity, inasmuch as recent experiments in our laboratory show that increases in basolateral membrane fluidity, owing to maintenance of rats on a diet rich in unsaturated triacylglycerols, markedly enhance the $(Na^+ + K^+)$-ATPase activity.

Acknowledgment

The support of National Institutes of Health grants AM21238 and AM01483 is gratefully acknowledged.

References

1 Schachter, D., Cogan, U. and Shinitzky, M. (1976) Biochim. Biophys. Acta 448, 620–624
2 Schachter, D. and Shinitzky, M. (1977) J. Clin. Invest. 59, 536–548
3 Brasitus, T.A. and Schachter, D. (1982) Biochemistry 21, 2241–2246
4 Brasitus, T.A. and Schachter, D. (1980) Biochemistry 19, 2763–2769
5 Brasitus, T.A., Tall, A.R. and Schachter, D. (1980) Biochemistry, 19, 1256–1261
6 Brasitus, T.A., Schachter, D. and Mamouneas, T.G. (1979) Biochemistry 18, 4136–4144
7 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509
8 Katz, S.S., Shipley, G.C. and Small, D.M. (1976) J. Clin. Invest. 58, 200–211
9 Zlatkis, A., Zak, B. and Boyle, A.J. (1953) J. Lab. Clin. Med. 41, 486–492
10 Ames, B.N. and Dubin, D.T. (1960) J. Biol. Chem. 235, 769–775

11 Gartner, S.L. and Vahouny, G. (1972) Am. J. Physiol. 222, 1121–1124
12 Heyn, M.P. (1979) FEBS Lett. 108, 359–364
13 Jähnig, F. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6361–6365
14 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332
15 Schachter, D., Cogan, U. and Abbott, R.E. (1982) Biochemistry 21, 2146–2150
16 Vincent, M., De Foresta, B., Gallay, J. and Alfsen, A. (1982) Biochem. Biophys. Res. Commun. 107, 914–921
17 Shinitzky, M. and Inbar, M. (1974) J. Mol. Biol. 85, 603–615
18 Liberman, U.A., Asano, Y., Lo, C.-S. and Edelman, I.S. (1979) Biophys. J. 27, 127–144
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
20 Grisham, C.M. and Barnett, R.E. (1972) Biochim. Biophys. Acta 266, 613–624
21 Kimelberg, H.K. and Papahadjopoulos, D. (1974) J. Biol. Chem. 249, 1071–1080
22 Giraud, F., Claret, M., Bruchdorfer, K.R. and Chailley, B. (1981) Biochim. Biophys. Acta 647, 249–258
23 Brasitus, T.A. (1983) Biochim. Biophys. Acta 728, 20–30
24 Dietschy, J.M. and Siperstein, M.D. (1965) J. Clin. Invest. 44, 1311–1327