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Ontogeny of proximal colon basolateral membrane lipid composition and fluidity in the rabbit

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Basolateral membranes from rabbit proximal colon were prepared from isolated colonocytes throughout postnatal maturation, using a modification of published techniques. In suckling (14–20 day) and post-weaning/mature (35–49 day) animals, membranes were purified approx. 10-fold, based upon the enrichment of ouabain-sensitive, sodium-potassium dependent adenosine triphosphatase activity. Membrane lipid analyses demonstrated age-dependent increases in total cholesterol and the cholesterol/phospholipid molar ratio, as well as decreases in phosphatidylethanolamine content and the fatty acid unsaturation index. Fluidity of basolateral membranes and membrane liposomes, determined from fluorescence anisotropy measurements using the lipid probes 1,6-diphenyl-1,3,5-hexatriene and DL-12-(9-anthroyl)stearic acid, demonstrated significant, ontogenic decreases in fluidity; and, additional studies showed that fluidity changes occurred early in the weaning period (by day 24 postnatally). Arrhenius plots of liposome anisotropies suggested a bilayer lipid thermotropic transition temperature of 22°C in sucklings 26°C in mature rabbits. These findings demonstrate that ontogeny of colonic basolateral membranes is associated with significant modulations in lipid composition and fluidity.

Introduction

The epithelium of the mammalian colon, similar to the small intestinal mucosa, exhibits marked structural and functional heterogeneity [1–4]. For example, colonic luminal (apical) cells demonstrate fluid and electrolyte absorption, while crypt cells are involved in active fluid secretion [3,4]. These transport processes take place across the polarized surface of the colonocyte, which consists of biochemically and functionally distinct luminal (brush-border) and abluminal (basolateral) plasma membranes [5]. Until recently, little information has been available concerning the biophysical properties of colonocyte membranes. Isolation of these membranes had been hampered by cellular heterogeneity [6] and a thick, adherent colonic mucus layer [7]. Recently, several investigators have overcome these difficulties and described methods of membrane purification from both scraped mucosa [8] and isolated epithelial cells [9]. Thus, both luminal and basolateral membranes (BLM) from adult rat [5,9,10] and rabbit [8] colon have been isolated in high purity, based upon enzyme marker analysis.

During postnatal development of all mammalian species studied, brush-border and basolateral membranes of the small intestinal epithelial cell undergo significant changes in composition, biophysical properties and membrane function [11–14]. Membrane structure–function relationships have been described during ontogenesis and in mature animals [15–18]. At present, however, no studies elucidating developmental characteristics of colonic plasma membranes have been reported. The work presented herein describes modified techniques, based on previously published methods [9], for purification of BLM from isolated proximal colonocytes throughout postnatal maturation of the rabbit. Further analyses characterize ontogenic changes in lipid composition and fluidity of these membranes.

Materials and Methods

Materials. Unless otherwise specified, all materials were obtained from Sigma Chemical Co., St. Louis, MO.

Cell isolation. For studies of mature animals (age 35–49 days), male New Zealand white rabbits (Charles River Laboratories, Kingston, NY) ranging in age from 35 to 42 days were transported to the animal facilities at New York Medical College at least one week prior to study (35-day-old animals were obtained from litters

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transported at day 21–28 postnatally). Animals were housed in wire bottom cages to prevent solid coprophagia and were allowed free access to water and standard rabbit chow. Following an overnight fast, except for water ad libitum, animals were killed by intracardiac pentobarbital injection following sedation with intramuscular ketamine, and colonocytes were obtained as described by Brasitus and Keresztes [9]. For investigation of suckling rabbits (age 14–20 days, either sex), litters with their does were transported to the animal facility at 7 days postnatally and were allowed to suckle normally until studied. Pups were not fasted; however, gastrointestinal tracts were grossly devoid of milk at time of cell isolation, in order to limit the contribution of intraluminal milk lipid to subsequent analyses. After suckling rabbits were killed as described above, colonocytes were obtained by a modification of published procedures [9]. The abdomen was opened longitudinally and the proximal half of the colon (excluding the cecum) was excised and flushed with 20 ml ice-cold 145.0 mM NaCl, 1.0 mM dithiothreitol (DTT), to remove intestinal contents. Proximal colonic segments were then manually perfused at a rate of 0.5 ml per s with 20 ml 10% fetal calf serum in 1.5% Joklik's modified minimal essential medium (GIBCO, Grand Island, NY) at 37°C. Loops were made by tying one end of the segment, and these were filled with sodium citrate buffer (27.0 mM sodium citrate, 1.5 mM KCl, 96.0 mM NaCl, 5.6 mM KH_2PO_4, pH 7.3). The open ends were ligated, the sacs were placed in a 250 ml Erlenmeyer flask containing 100 ml sodium citrate buffer and incubated in a shaking water bath for 15 min at 37°C. Sacs were then emptied and the intraluminal buffer (containing apical cells) was saved on ice. Sacs of proximal colon were refilled with a solution containing 145.0 mM NaCl, 4.0 mM KCl, 5.0 mM NaH_2PO_4, 5.0 mM Na_2HPO_4, 1.54 mM EDTA and 0.5 mM DTT, pH 7.2 (EDTA buffer). Following a 30 min incubation at 37°C in a 250 ml Erlenmeyer flask containing 100 ml EDTA buffer, the intraluminal solution containing suspended colonocytes was combined with the reserved citrate incubation buffer and centrifuged at $650 \times g$ for 10 min to pellet the isolated cells.

Membrane preparation. The pelleted colonocytes obtained from the citrate and EDTA-buffer incubations were suspended in 40 ml ice-cold 5.0 mM histidine, 5.0 mM imidazole, 0.5 mM EDTA, pH 7.0 (HIE buffer), containing 250 mM sucrose, and homogenized with 20 strokes in a tight-fitting, glass-glass Dounce homogenizer. Cell homogenates were filtered through a single layer of Nitex™ nylon mesh, 100 μm pore size (Tetko, Inc., Elmsford, NY) and washed through with 10 ml of HIE buffer. An aliquot of each cell homogenate was saved for subsequent analysis. Following a series of differential centrifugations [9], the resultant 'crude' membrane pellet was suspended in 4 ml 60% sucrose/HIE buffer (w/v) and dounced with five strokes. This material was layered on the bottom of a cellulose nitrate tube and a discontinuous sucrose gradient was formed by successive 3 ml additions of 40 and 30% sucrose/HIE buffer, followed by 20% sucrose/HIE buffer sufficient to fill the tube. The discontinuous gradients were placed in a Beckman SW 41Ti swinging bucket rotor (Beckman Instruments, Fullerton, CA) and centrifuged at $140000 \times g$ for 120 min in a Beckman L5-50 ultracentrifuge. BLM were collected from the 30/40% sucrose interface, suspended in HIE buffer (minus sucrose) and pelleted at $105000 \times g$ for 30 min. Final pellets were suspended in 1 ml HIE buffer and stored at −20°C for further analysis.

Protein and enzyme assays. The total protein of the cell homogenate and BLM were determined by the method of Lowry et al. [19], using bovine serum albumin as the standard. Sodium-potassium dependent adenosine triphosphatase (Na^+/K^+-ATPase, EC 3.6.1.3), an enzyme marker for BLM, was measured according to the method of Kinsolving [20], after freeze-thawing samples three times and treating with 0.6 mM sodium deoxycholate (12 μM final concentration). Enzyme activity was calculated as the difference between the total and ouabain-insensitive ATPase activities. To determine contamination of the final BLM preparation by co-isolated cellular elements, specific activities of cysteine-sensitive alkaline phosphatase (EC 3.1.3.1), a luminal membrane marker, and mitochondria associated succinate dehydrogenase (EC 1.3.99.1) were assayed by published methods [9,21].

Lipid analysis. Lipids from proximal colon BLM were extracted by a modification [22] of the method of Folch et al. [23], and recovery (> 90% in all preparations) was determined by coextraction of tracer lipids. Total cholesterol was determined by gas-liquid chromatography (GLC) utilizing a Shimadzu mini-3 gas chromatograph (Shimadzu, Inc., Columbia, MD) equipped with a flame ionization detector. Separation was accomplished using a 1.7 m glass column (2 mm i.d.) packed with 3% OV-17 on 100/200 Supelcoport (Supelco, Inc., Bellefonte, PA), and quantitation was achieved by using stigmasterol as an internal standard. Cholestryl ester content was measured according to the method of Brown et al. [24].

Total membrane phospholipids were determined by measuring extracted lipid inorganic phosphate [25]. Individual phospholipids were assayed by thin-layer chromatography in one dimension, according to a modification [12] of the method of Touchstone et al. [26]. Fatty acids were separated and identified by GLC, employing a Shimadzu GC-9A gas chromatograph, following derivitization to fatty acid methyl esters using 14% boron trifluoride/methanol. Samples were injected

onto a 1.7 m glass column (3 mm i.d.) packed with 10% SP-2330 on 100/200 Supelcoport, and fatty acids were separated using a temperature program mode as previously described [12]. Individual peaks were identified by comparison to retention times of authentic fatty acid methyl ester standards.

Fluorescence polarization studies. To determine the fluidity of proximal colon BLM during ontogenesis, the lipid soluble fluorescence probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and DL-12-(9-anthroyl)stearic acid (12-AS) were employed. These fluorophores were prepared and stored as previously described [14]. All studies were carried out within 2 weeks of membrane isolation. Membrane fluidity was calculated following fluorescence polarization measurements using a Shimadzu RF-540 spectrofluorophotometer fitted with a thermoregulated sample chamber (Perkin-Elmer, Inc., Norwalk, CT) and automatic polarizers (C.N. Wood, Newtown, PA), as previously described [14,16]. Values for the steady-state fluorescence anisotropy r (reciprocal of fluidity) were determined using the equation:

$$r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$$

where $I_{\parallel}$ and $I_{\perp}$ equal fluorescence intensities parallel and perpendicular, respectively, to the excitation plane. Scattered light plus ambient medium fluorescence contributed $< 5\%$ to the total fluorescence intensity throughout the temperaure range utilized in all studies. Limiting hindered anisotropy r_∞, which represents the static component of fluidity related to bilayer molecular packing and hindrance to probe rotation [27], was calculated from DPH fluoresecence polarization measurements according to the equation of van der Meer et al. [28], which may be written:

$$r_\infty = (r_0 r^2)/(r_0 r + (r_0 - r)^2/m)$$

where r_0 equals the maximal limiting anisotropy in the frozen state (0.365 for DPH [29]) and $m = 1.71$ for DPH [28]. Although specific determination of r_∞ from time-resolved studies (dynamic depolarization using DPH [30]) was not carried out in these studies, the above calculation provides a reasonable approximation for r_∞ under the conditions utilized herein [28]. Constancy of fluorescence probe lifetimes was assessed from measurements of total fluorescence intensity, F, where $F = I_{\parallel} + 2I_{\perp}$ [31]. This value did not vary with temperature, or among membrane/liposome preparations (comparisons by analysis of variance). To evaluate thermotropic transitions (break points) of membrane lipids, Arrhenius plots of log r vs. $(1/K) \times 10^3$ were constructed. Accuracy of transitions was tested by measuring the phase transition of multilamellar liposomes of dimyristoylphosphatidylcholine, using DPH fluorescence polarization as previously described [11].

Liposomes. Liposomes were prepared from extracted, N_2-dried membrane lipids after suspension in phosphate-buffered saline containing DPH (lipid concentration approx. 0.3 mg per ml). Lipid suspensions were sonicated under N_2 for 3 min at 4°C, and then centrifuged at $10000 \times g$ for 10 min. The supernatant contained liposomes with the incorporated fluorescence probe (probe/phospholipid molar ratio approx. $2 \cdot 10^{-3}$).

Statistical analysis. Student's t-test for unpaired samples was utilized for comparisons between BLM preparations at 14–20 and 35–49 days old.

Results

Membrane isolation

Table I shows the enrichment (-fold) and recovery (yield) relative to starting colonocyte homogenates, of proximal colon BLM, as measured by specific activities of appropriate marker enzymes. During both the suckling (14–20 days) and post-weaning (35–49 days) peri-

TABLE I

Enzyme marker specific activities of cell homogenates and purified basolateral membrane (BLM) [a]

Age (days)	Enzyme marker	Specific activity		Enrichment (-fold)	Yield [b] (%)
		homogenate	BLM		
14–20	Na^+/K^+-ATPase (nmol/mg per min)	6.1 ± 0.8	58.0 ± 12.6	9.6 ± 1.4	10.3 ± 1.1
35–49	Na^+/K^+-ATPase	5.6 ± 2.0	38.1 ± 9.4	8.8 ± 2.0	12.8 ± 2.5
14–20	alkaline phosphatase (μmol/mg per min)	6.6 ± 0.9	7.1 ± 2.3	1.2 ± 0.3	0.4 ± 0.2
35–49	alkaline phosphatase	15.7 ± 7.5 [c]	14.6 ± 4.7 [c]	0.9 ± 0.4	0.3 ± 0.2
14–20	succinate dehydrogenase (μmol/mg per min)	0.7 ± 0.3	0.4 ± 0.3	0.6 ± 0.2	0.8 ± 0.3
35–49	succinate dehydrogenase	0.9 ± 0.4	0.5 ± 0.2	0.5 ± 0.3	0.6 ± 0.4

[a] Values are means ± S.E. of duplicate determinations from at least four membrane preparations in each age group.
[b] Yield (%) = (total BLM enzyme/total homogenate enzyme units) × 100.
[c] $P < 0.05$, compared to 14–20 days.

TABLE II

Composition of basolateral membrane lipid extracts [a]

Component (μmol/mg protein)	14–20 days	35–49 days
Cholesterol	0.20 ± 0.03	0.27 ± 0.03 [b]
Cholestryl esters	< 0.01	< 0.01
Phospholipid	0.47 ± 0.04	0.43 ± 0.05
Cholesterol/phospholipid (mol/mol)	0.43 ± 0.02	0.67 ± 0.05 [b]

[a] Values are means ± S.E. of triplicate determinations on at least four membrane preparations.
[b] $P < 0.05$, compared to 14–20 days.

ods, the specific activity of BLM-associated Na^+/K^+-ATPase was enriched 9–10-fold. By contrast, activities of cysteine-sensitive alkaline phosphatase (a luminal membrane marker) and succinate dehydrogenase (a mitochondrial membrane marker) were purified a mean of 1.0–1.2- and 0.5–0.6-fold, respectively, indicating relative absence of these membranes in the final BLM preparation. Recoveries of total Na^+/K^+-ATPase units (total BLM enzyme units per total colonocyte homogenate units) were similar for suckling and mature BLM preparations.

Lipid composition

Ontogenic changes in BLM lipid composition are indicated in Tables II–IV. Analysis of membrane cholesterol and phospholipid content (Table II) demonstrated an age-dependent increase in total cholesterol (suckling vs. mature = 0.20 ± 0.03 vs. 0.27 ± 0.02 μmol/mg protein, $P < 0.05$) without a change in total phospholipid content, resulting in a significant increase in the cholesterol/phospholipid molar ratio in older animals (0.43 ± 0.02 vs. 0.67 ± 0.05, $P < 0.05$). In both age groups, membrane cholestryl ester content was negligible. Although no age-related differences in total BLM phospholipids were measured, analysis of individual phospholipids (Table III) showed a significant decrease in phosphatidylethanolamine content in 35–49-day animals. A trend towards age-dependent increases in phosphatidylcholine and decreases in phosphatidylserine content was also noted; however, these differences were not statistically significant.

TABLE III

Distribution of phospholipids [a]

Phospholipid	% (w/w) of total lipid-associated phosphate of basolateral membranes	
	14–20 days	35–49 days
Phosphatidylcholine	21.5 ± 6.6	28.8 ± 7.5
Lysophosphatidylcholine	< 1	< 1
Sphingomyelin	16.2 ± 3.1	18.4 ± 2.3
Phosphatidylserine	21.2 ± 5.2	16.1 ± 2.1
Phosphatidylinositol	20.5 ± 4.9	18.7 ± 5.3
Phosphatidylethanolamine	23.6 ± 1.0	16.0 ± 2.3 [b]

[a] Values are means ± S.E. of duplicate determinations on three membrane preparations.
[b] $P < 0.05$, compared to 14–20 days.

The fatty acid composition of proximal colonocyte BLM is shown in Table IV. Among the major fatty acids identified, the % total of palmitic acid (16:0) was

TABLE IV

Distribution of fatty acids in basolateral membrane lipid extracts [a]

Fatty acid	% Total fatty acids	
	14–20 days	35–49 days
14:0	1.5 ± 0.7	1.1 ± 1.0
16:0	17.5 ± 1.1	25.4 ± 1.7 [b]
18:0	16.3 ± 1.0	16.6 ± 0.8
18:1	21.0 ± 2.1	24.6 ± 0.7
18:2	22.8 ± 3.1	18.6 ± 1.5
18:3	7.5 ± 0.7	1.8 ± 0.5 [b]
20:0	3.1 ± 0.3	1.9 ± 0.4
20:4	5.2 ± 0.5	4.7 ± 0.5
Unsaturation index [c]	2.6 ± 0.2	1.9 ± 0.1 [b]

[a] Values are means + S.E. of duplicate determinations on four membrane preparations.
[b] $P < 0.05$, compared to 14–20 days.
[c] Calculated as the sum of the % total of each unsaturated acyl chain multiplied by its double bond number, divided by the % total of the saturated acyl chains.

TABLE V

Fluorescence polarization studies of basolateral membranes (BLM) and membrane liposomes, using 1,6-diphenyl-1,3,5-hexatriene (DPH) and DL*-12-(9-anthroyl)stearic acid (12-AS)* [a]

Age (days)	Probe	Preparation	(r) Anisotropy at 25°C	Limiting hindered anisotropy (r_∞)
14–20	DPH	BLM	0.188 ± 0.008	0.151 ± 0.010
	12-AS	BLM	0.068 ± 0.004	
	DPH	Liposomes	0.122 ± 0.005	
35–49	DPH	BLM	0.238 ± 0.014 [b]	0.218 ± 0.019 [b]
	12-AS	BLM	0.099 ± 0.006 [c]	
	DPH	Liposomes	0.181 ± 0.039 [b]	

[a] Values are means ± S.E. of duplicate determinations from at least four membrane/liposome preparations in each age group.
[b] $P < 0.01$, compared to 14–20 day-old rabbits.
[c] $P < 0.05$, compared to 14–20 day-old rabbits.

increased and linolenic acid (18:3) content was decreased in post-weaning/mature rabbits, compared to sucklings. BLM of 14–20-day-old rabbits also demonstrated a significantly higher fatty acyl unsaturation index (2.6 ± 0.2 vs. 1.9 ± 0.1, $P < 0.05$) than did membranes from 35–49-day-old animals.

Fluorescence polarization studies

To determine the influence of ontogenic changes in BLM lipids on bilayer physical characteristics, membrane fluidity was determined from fluorescence polarization measurements, utilizing the fluorophores DPH and 12-AS. These lipid probes were chosen because they differ in structure and steric configuration, and, therefore, localize within unique bilayer domains [32]. For DPH, probe molecular rotation is limited by membrane lipid structure and intrinsic protein constituents (i.e. hindered motion). The r_∞ values (the 'static' component of anisotropy) measured for DPH are, therefore, high and represent the major component of fluorescence anisotropy. For 12-AS, anisotropy measurements more closely reflect the speed of molecular rotation (a 'dynamic' component), as opposed to hindrance to probe motion. Thus, r_∞ values for 12-AS are low, and fluidity of the bilayer region which incorporates this molecule is approximated by the steady-state anisotropy, r. As shown in Table V, significant, age-related increases in both r (DPH and 12-AS) and r_∞ (DPH) at 25°C indicate ontogenic decreases in fluidity of the various lipid domains probed by these fluorophores. To evaluate the contribution of bilayer lipids alone vs. protein-lipid interactions to these fluidity measurements, DPH anisotropies in protein-free BLM liposomes were determined. At all temperatures studied, DPH anisotropy values were lower in liposomes (by ~ 50% in sucklings and ~ 30% in mature rabbits) than in the corresponding intact BLM preparations. These data also demonstrated significant, age-related increases in liposome r values (0.122 ± 0.005 vs. 0.181 ± 0.009, $P < 0.01$) at 25°C.

The thermotropic behavior of BLM fluorescence anisotropies was evaluated by constructing Arrhenius plots of log r(DPH) vs. temperature (Fig. 1). Throughout the temperature range studied (40–5°C), values for r remained higher in weanling-mature rabbit BLM (49 day) than in suckling BLM (18 day). Representative plots of BLM anisotropies from 24-day-old animals were superimposable on those from mature rabbit BLM, indicating that these fluidity changes occurred early in the weaning period. Arrhenius data of r (DPH) in intact membranes suggested a thermotropic transition occurring around 20°C in proximal colon BLM throughout postnatal development. When Arrhenius plots were constructed from anisotropy measurements of membrane liposomes (Fig. 2), similar suckling-mature anisotropy differences throughout this temperature range were found. However, liposome anisotropy results suggested a membrane lipid thermotropic transition occurring at ~ 22°C in suckling animals, and at ~ 26°C in mature rabbits.

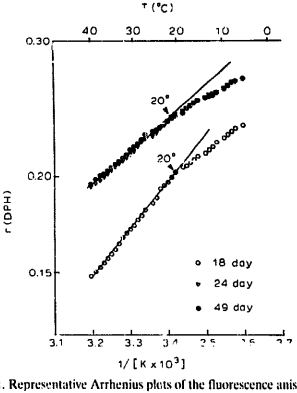

Fig. 1. Representative Arrhenius plots of the fluorescence anisotropy, r(DPH), from 40 to 5°C, in purified BLM from 18-, 24- and 49-day-old rabbits.

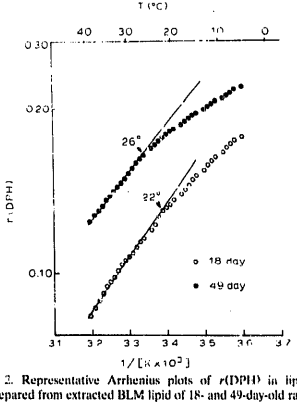

Fig. 2. Representative Arrhenius plots of r(DPH) in liposomes prepared from extracted BLM lipid of 18- and 49-day-old rabbits.

Discussion

The methods described in this report, modified from those previously published [9], comprise effective and reproducible techniques for purification of BLM from rabbit proximal colon epithelial cells. These methods result in membranes from both suckling (14–20 day) and post-weaning/mature (35–49 day) animals being purified ~ 10-fold compared to colonocyte homogenates, based upon enrichment of BLM-associated Na^+/K^+-ATPase, with a 10–13% yield of total enzyme units and little contamination by luminal or mitochondrial membranes. The enrichment data are in close agreement with the results of Brasitus and Kereszties [9] for rat proximal colon membranes, which also were purified from homogenates of isolated colonocytes. One recent study demonstrated purifications of greater than 30-fold (based on enrichment of marker enzyme activities), for adult rabbit distal colon BLM prepared from homogenized mucosal scrapings [8]. This enhanced purification, compared to the present study, is not surprising, since differences in enzyme activities between the initial homogenate and the final BLM fraction would be greater (thus resulting in an apparently higher enrichment-fold) if scraped mucosa, as opposed to colonocytes, were the starting material.

In artifical membranes, higher cholesterol levels, associated with elevated cholesterol/phospholipid molar ratios, result in decreased fluidity of aqueous lipid dispersions above their thermotropic lipid phase transition temperature [33]. Studies of various biological membranes also indicate that cholesterol content, relative to phospholipid, varies inversely with fluidity [12,27,34]. The ontogenic changes in lipid composition and fluidity of proximal colon BLM reported here support this concept, and our results are in close agreement with lipid levels found during postnatal development of rabbit small intestinal BLM [12,14]. Age-related reductions in the fatty acyl unsaturation index should, presumably, also result in decreased membrane fluidity. However, the relationship between fatty acid saturation and fluidity of lipid bilayers is a complex one and involves numerous factors, including length and degree of unsaturation of acyl chains, and interactions with other membrane components, such as proteins, cholesterol and phospholipids [35]. The extent to which developmental differences in BLM palmitic and linolenic acid content, in addition to other lipid changes reported herein, are direct consequences of dietary lipid composition (the transition from a milk to show diet), as opposed to endogenous influences on membrane lipid structure (e.g. cholesterol biosynthesis, enterocyte membrane lipid incorporation/degradation) is likewise unclear and is the subject of ongoing study.

Fluorescence polarization studies, utilizing the structurally unique fluorophores DPH and 12-AS, demonstrate that developmental reductions in membrane lipid fluidity are found in various bilayer lipid regions, and these changes are initially evident early in the weaning period (by 24 days postnatally). Parallel, age-related increases in fluorescence anisotropies in BLM and in liposomes prepared from membrane lipid, confirm that fluidity modulations are directly related to lipid compositional changes. Lower liposome anisotropy values, compared with intact BLM, reflect protein effects on these measurements [5]. Liposome studies also suggest a thermotropic phase transition at approximately 22°C for suckling rabbit BLM lipid, similar to that previously reported for adult rat colon BLM [14], while a break point in mature rabbit colon BLM liposomes is found at ~ 26°C. This age-related, upward shift in apparent thermotropic lipid phase transition of liposome preparations is expected, based upon the lower lipid fluidity of mature membranes [14]. The reasons underlying differences in apparent break points between liposomes and intact membranes in both age groups likely involve the interfering effects of bilayer proteins on DPH anisotropy measurements.

Previous investigations in adult animal models have demonstrated dependence of several enterocyte membrane functions on lipid fluidity in the small intestine [15–18,36–38]. For example, rates of Na^+-coupled, D-glucose uptake by rat jejunal brush-border membrane vesicles isolated from cells along the crypt-villus axis, can be correlated with maturational (crypt to villus) changes in enterocyte membrane fluidity [18]. Glucose transport across rat jejunal brush-border membranes may be altered, in vitro, following membrane fluidization by specific *n*-aliphatic alcohols [37]. Modulations in activity of the enterocyte membrane glucose carrier, manifested by changes in the transport V_{max}, have also been achieved in vivo by dietary triacylglycerol-induced modifications in bilayer lipid saturation and fluidity [38]. Studies from our laboratory [16] suggest that estrogen-related inhibition of Na^+/K^+-ATPase activity in rabbit ileal BLM is regulated, at least in part, by decreases in membrane fluidity.

Although brush-border and basolateral membrane structure/function associations in mammalian colonic membranes have not been extensively studied, in comparison with the large body of experimental data in the small intestine, available evidence indicates that such interactions are involved in regulating sodium and water fluxes. Recent investigations have, in fact, demonstrated the influence of bilayer lipid dynamics on transport of fluid and electrolyte across isolated colonocyte membranes. Osmotic water permeation in the colon occurs via a lipid-mediated, nonfacilitated pathway, which is characterized by a very low permeability coefficient [8]. Studies in rats [39] have shown that both osmotic water permeability and sodium-hydrogen exchange are enhanced with decreasing molecular order

(increased fluidity) of colonic brush-border membranes. Thus, changes in bilayer fluidity in the range of 5–6% are associated with a 36% increase in the V_{max} of amiloride-sensitive proton efflux across membrane vesicles [38,39]. These studies suggest that alterations in BLM fluidity, of the magnitude reported here, might be expected to influence similar colonic functions in the maturing rabbit. Nevertheless, the physiological significance of these developmental modulations in lipid dynamics with respect to ontogenesis of BLM enzyme and transport function, has not yet been demonstrated. In this regard, enzyme data presented here do not suggest age-related differences in proximal colon BLM Na^+/K^+-ATPase specific activity, despite ontogenic decreases in fluidity. Based upon our earlier studies in rabbit ileum [16], lower enzyme specific activity would be expected in the less fluid mature rabbit colon BLM. Additional studies (measurement of ^{3}H-ouabain binding sites to assess total membrane-associated enzyme units, Arrhenius plots of enzyme specific activity) are required to address this issue and elucidate structure/function relationships in the developing colon.

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