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LIPID FLUIDITY AND COMPOSITION OF INTESTINAL MICROVILLUS MEMBRANES ISOLATED FROM RATS OF DIFFERENT AGES

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The lipid composition and fluidity of microvillus (luminal) membranes isolated from the small intestines of Fisher 344 rats aged 6, 17, and 117 weeks were compared. Lipid fluidity, as assessed by the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene, was significantly greater in rats aged 6 weeks as compared to 17 or 117 weeks. A lipid thermotropic transition was observed at 17.5 ± 1.3°C in the membranes of the youngest group, approx. 5–6°C lower than that of the older animals. The differences in lipid composition which account for the higher fluidity of the youngest preparations include a decreased cholesterol/phospholipid molar ratio in both the proximal and distal halves of the small intestine and, in the proximal half alone, increases in the lipid/protein ratio and double bond index. The foregoing reduction in cholesterol/phospholipid ratio derives mainly from a higher content of total phospholipid, and the increment in double bond index results from an increase in arachidonic acid residues. The results demonstrate an age-dependent decrease in fluidity of intestinal microvillus membranes in the early post-weaning period in the rat. This pattern was unlike that of the microvillus membrane *p*-nitrophenylphosphatase, whose specific activity declined progressively in the older age groups.

Introduction

A number of studies indicate that aging influences the composition and function of cell membranes [1–8]. The mechanisms which mediate such alterations, however, are not well understood. For example, the extent to which aging can affect membrane functions via primary changes in lipid composition and fluidity ** remains to be determined. To provide further information on this question we have compared the lipid composition and fluidity of microvillus (luminal) membranes isolated from the small intestines of rats aged 6, 17, and 117 weeks. The intestinal microvillus membrane is highly specialized for transport and digestive functions [9], and there is considerable evidence that many of its protein-mediated functions are influenced by the composition and physical state of the lipids [10–14]. Moreover, a broad thermotropic transition of the microvillus membrane lipids in the range of 23–39°C has been characterized by differential scanning calorimetry [11], and the lower critical temperature, 23–26°C, can be detected by fluorescence polarization studies with the hydrocarbon fluorophore 1,6-diphenyl-1,3,5-hexatriene [11,15].

Several authors have reported evidence of de-

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** A discussion of our use of the term 'lipid fluidity' as applied to anisotropic bilayer membranes is given in Ref. 13. Briefly, the term is used here to express the relative motional freedom of the lipid molecules or substituents thereof, combining in the one term concepts of the rate of movement (correlation time) and the extent of movement (limiting-hindered anisotropy).

creased order in the intestinal microvillus membranes of very young animals. Pang et al. [16] used electron spin resonance to detect decreased order in microvillus membranes prepared from the intestines of newborn as compared to adult rabbits. Schwarz et al. [17] studied the fluorescence polarization of diphenylhexatriene and observed greater fluidity of the microvillus membranes of suckling rats and rabbits as compared to adults. The results of the experiments described below also demonstrate greater fluidity in the youngest rats aged 6 weeks. A trend of decreased fluidity with age, however, was not observed on comparison of rats aged 17 and 117 weeks.

Materials and Methods

Animals and membrane preparations. Male Fisher 344 rats obtained from the National Institute of Aging colony and maintained by the Charles River Breeding Laboratories (Wilmington, MA) were used in all the experiments. Three age groups were obtained: 5 weeks old (or 1 week after weaning) at the time of arrival at our facility; 16 weeks of age; and 116 weeks old. Mean weights were 71, 267, and 381 g, respectively. All animals had been maintained under barrier-reared conditions from birth and fed a chow diet containing 22% casein, which was continued in our facility. Rats were kept for 1 week prior to use in standard non-metabolic cages in rooms of controlled temperature and humidity. Most animals lost weight in the first 48 h after arrival but gained thereafter; animals which did not restore their body weight were not used. Groups of either six (6 weeks old) or three (17 and 117 weeks old) animals were fasted for 18 h with water ad libitum, killed rapidly by cervical dislocation and the entire small intestine was excised. Each gut was divided equally into proximal and distal halves, and mucosal scrapings were obtained and pooled separately for the proximal and distal segments. Microvillus membranes were prepared from the pooled scrapings as described previously [10–14], and the purity and comparability of the isolated membranes were assessed by estimation of the specific activity of the marker enzyme *p*-nitrophenylphosphatase. As indicated in Table I, mean purification ratios (membrane specific activity/homogenate specific activity) ranged from 10 to 12. Marker enzymes for basolateral, microsomal and mitochondrial membranes, i.e., ouabain-sensitive ($Na^+ + K^+$)-dependent adenosine triphosphatase, NADPH-cytochrome *c* reductase and succinate dehydrogenase, respectively, were also assayed. Mean values for these specific activity ratios membranes/homogenates were 0.5–1.0 and individual values did not exceed 1.5. Marker enzymes were assayed as described previously [10–14] under conditions of substrate excess and no more than 5% of a given substrate was consumed in any assay.

Fluorescence polarization studies. The lipid soluble fluorophore 1,6-diphenyl-1,3,5-hexatriene (Aldrich Chemical Co.) was used throughout. The methods used to load the membranes and to quantify the fluorescence anisotropy in an SLM polarization spectrophotometer have been described [15]. The polarization of fluorescence was expressed in terms of the fluorescence anisotropy, *r*. The fluorescence anisotropy of this probe in bilayer membranes is determined mainly by the maximal hindered anisotropy and provides an estimate of the static component of 'fluidity', i.e., of lipid order [19–21]. The temperature dependence of the fluorescence anisotropy was determined over the range of 0–40°C and Arrhenius plots were made to detect thermotropic transitions [11–15]. Total fluorescence intensity was monitored to detect changes in fluorescence lifetime; no such changes were observed to account for the fluorescence anisotropy alterations described below.

Membrane composition studies. Total lipids were extracted from the membranes by the method of Folch et al. [22]. The lipid composition of the extracts was examined by quantitative thin-layer chromatography according to the procedure of Katz et al. [23]. Glycolipids were not estimated in these studies, and the relative composition of the extracts is given below as a percentage of the neutral lipids plus the phospholipids. To determine the acyl chain composition, fatty acids of the total lipid extracts were derivatized as described by Gartner and Vahouney [24]. The fatty acid methyl esters were quantified on a JEOL JGX-20K gas chromatograph equipped with a flame ionization detector and interfaced with a Hewlett-Packard 3390A integrator. Authentic fatty acid methyl esters were used to identify retention

times as previously described [24]. Membrane protein was estimated by the method of Lowry et al. [25], using bovine serum albumin as the standard.

Materials. Fatty acids, methyl esters, columns for gas-liquid chromatography and lipid standards were purchased from Applied Science Corp. or Supelco. Unless otherwise indicated, all other materials were obtained from Sigma Chemical Co. or Fisher Chemical.

Results

Comparability of membrane preparations

For meaningful comparisons it is essential that the isolation procedure for microvillus membranes yield similarly purified suspensions from rats of various ages. As indicated in Table I the purification ratios of the marker enzyme *p*-nitrophenylphosphatase were very similar in preparations from different age groups and different segments. Values of the specific activity of the enzyme, moreover, were greatest in the preparations from the youngest rats and decreased progressively with age in crude homogenates, in isolated microvillus membranes, and in both proximal and distal samples. The data of Table I also demonstrate much higher specific activity values in the proximal as compared to the distal mucosa of each age group ($P < 0.01$).

Fluorescence polarization studies

The effects of temperature on the diphenylhexatriene fluorescence anisotropy, r, in microvillus membranes prepared from the proximal intestine of each age group are illustrated by composite Arrhenius plots in Fig. 1. At each temperature tested the mean value of the youngest age group was significantly lower ($P < 0.01$) than that of the others. A change in slope of the Arrhenius plot, indicative of the lower critical temperature of the lipid thermotropic transition [11,15], was observed in all three groups. The mean break point temperature of 17.5 ± 1.3°C in the youngest preparations, however, is approximately 5–6°C lower than that of the older groups ($P < 0.01$), as indicated in Table II. Values of the diphenylhexatriene fluorescence anisotropy, r, at 25°C for each age group and segment are also summarized in Table II. In both the proximal and distal segments the preparations from the youngest age group exhibit greater fluidity, i.e., lower r values ($P < 0.01$), as compared to the other age groups. In addition, proximal segment membranes from the 117-week-old rats yielded somewhat lower r values than those from the 17-week-old animals ($P < 0.05$). It is also noteworthy that the fluorescence anisotropy was consistently higher in the distal as compared to the proximal segment ($P < 0.01$), a pattern which has been reported previously [15,26].

TABLE I

RELATIVE PURITY OF RAT INTESTINAL MICROVILLUS MEMBRANE PREPARATIONS AS ASSESSED BY *p*-NITROPHENYLPHOSPHATASE ACTIVITY

Values are means ± S.E. for three groups aged 6 and 17 weeks and four groups aged 117 weeks.

Intestinal half	Age group (weeks)	*p*-Nitrophenylphosphatase specific activity (μmol·min^{-1}·(mg protein)$^{-1}$)		
		Crude homogenate (*a*)	Microvillus membranes (*b*)	Purification ratio (*b*/*a*)
Proximal	6	0.76 ± 0.06 [a]	8.49 ± 0.52 [a]	11.2
	17	0.39 ± 0.01	4.61 ± 0.24	11.8
	117	0.30 ± 0.01	3.43 ± 0.13	11.4
Distal	6	0.09 ± 0.01 [a]	0.94 ± 0.07 [b]	10.4
	17	0.04 ± 0.01	0.52 ± 0.12	13.0
	117	0.03 ± 0.01	0.32 ± 0.04	10.7

[a] All age groups differ from each other significantly ($P < 0.01$) within the same intestinal half.
[b] All age groups differ from each other significantly ($P < 0.05$) in the distal segment.

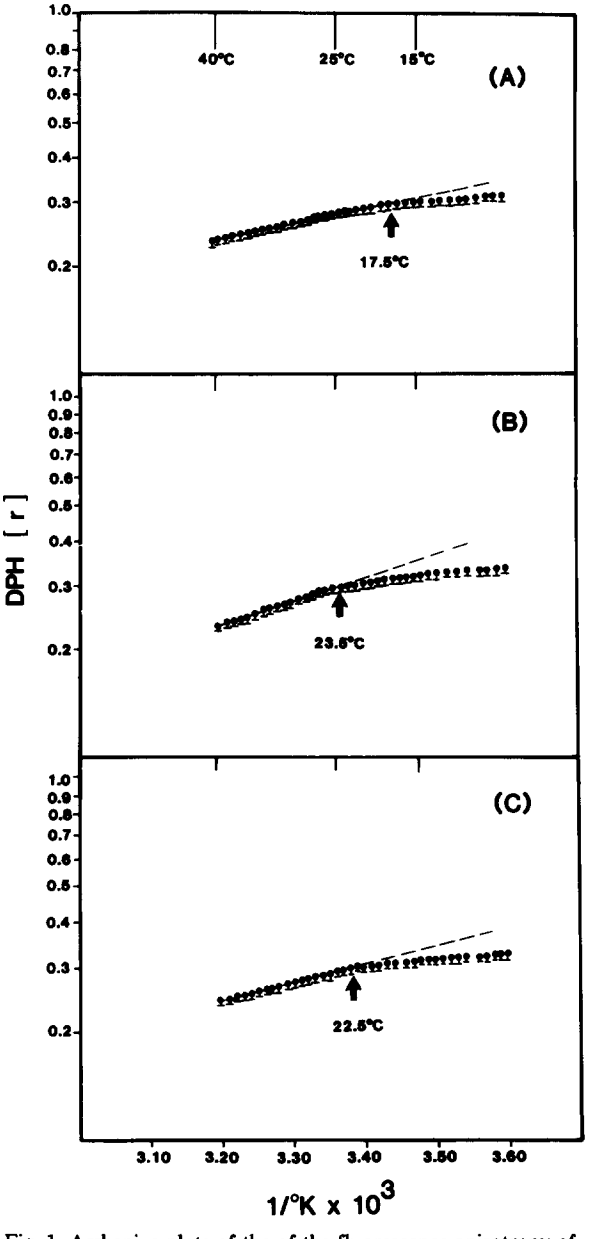

Fig. 1. Arrhenius plots of the of the fluorescence anisotropy of diphenylhexatriene in proximal microvillus membranes of rats aged 6 weeks (A), 17 weeks (B) and 117 weeks (C). The numbers of groups and determinations are listed in Table I.

Membrane composition studies

Membrane composition was examined in order to determine the factors responsible for the foregoing differences in membrane fluidity. The values in Table III demonstrate that the cholesterol/phospholipid molar ratio is significantly lower in the

TABLE II

FLUORESCENCE ANISOTROPY OF DIPHENYLHEXATRIENE IN MICROVILLUS MEMBRANES PREPARED FROM RATS OF VARIOUS AGES

Membranes were isolated from the proximal and distal halves of the small intestine. Values shown are means ± S.E. for three groups aged 6 and 17 weeks and four groups aged 117 weeks. Values in parenthesis are total number of determinations. Thermotropic transition temperatures were determined from Arrhenius plots illustrated in Fig. 1.

Intestinal half	Age group (weeks)	Diphenylhexatriene fluorescence anisotropy (25°C) (*r*)	Thermotropic transition temperature (°C)
Proximal	6	0.279 ± 0.003 [a] (6)	17.5 ± 1.3 [a] (4)
	17	0.292 ± 0.001 [b] (6)	23.5 ± 0.6 (4)
	117	0.289 ± 0.001 (8)	22.5 ± 0.6 (4)
Distal	6	0.285 ± 0.002 [a] (6)	
	17	0.295 ± 0.002 (6)	
	117	0.293 ± 0.002 (8)	

[a] $P < 0.01$ as compared to samples of the same half of the intestine from rats aged 17 or 117 weeks.

[b] $P < 0.05$ as compared to samples of the proximal intestine from rats aged 117 weeks.

proximal and distal membrane preparations of the youngest age group as compared to the older rats. In addition, the lipid/protein ratio and the double bond index are higher in the proximal segment membranes of the youngest age group. All three changes are expected to increase the lipid fluidity of the membranes of the 6-week-old group. Further results of the analyses of total neutral lipids and phospholipids are listed in Table IV. A significant increase in the total phospholipid content of the preparations from the youngest animals, both in the proximal and distal intestinal segments, is the major determinant of the relatively low cholesterol/phospholipid ratio. In distal segment preparations the cholesterol content is also reduced in the 6-week-old as compared to the older groups. Additional significant changes include higher phosphatidylethanolamine and lower triacylglycerol and fatty acid contents in the lipid extracts of the youngest age group.

The proximal segment lipid extracts of the 17-week and 117-week animals also differ in composition, and the differences are concordant with the fluidity change monitored by diphenylhexatriene

TABLE III

COMPOSITIONAL PARAMETERS OF MICROVILLUS MEMBRANES ISOLATED FROM RATS OF VARIOUS AGES

Values are means ± S.E. for three groups aged 6 or 17 weeks and four groups aged 117 weeks. Values in parenthesis are numbers of determinations. Double bond index was calculated from the date in Table V as the sum of the fraction of each fatty acid times the number of double bonds in that acid. PC, phosphatidylcholine.

Parameter	Proximal half of intestine			Distal half of intestine		
	6 weeks	17 weeks	117 weeks	6 weeks	17 weeks	117 weeks
Cholesterol/ phospholipid (mol/mol)	0.3 ± 0.06 [a] (7)	1.07 ± 0.08 (4)	1.00 ± 0.09 (10)	1.00 ± 0.18 [b] (5)	1.40 ± 0.16 (5)	1.32 ± 0.05 (9)
Sphingomyelin/ PC (mol/mol)	0.30 ± 0.05 (8)	0.32 ± 0.01 (4)	0.18 ± 0.01 [c] (10)	0.25 ± 0.04 (5)	0.26 ± 0.05 (5)	0.15 ± 0.02 (9)
Double-bond index	1.26 ± 0.03 [d] (4)	0.84 ± 0.04 (4)	1.03 ± 0.05 [e] (4)	1.14 ± 0.07 (4)	1.04 ± 0.05 (4)	1.13 ± 0.04 (4)
Lipid/protein (w/w)	0.77 ± 0.02 [f] (3)	0.61 ± 0.02 (3)	0.53 ± 0.01 (4)			

[a] Differs significantly ($P < 0.025$) from proximal samples of 17- and 117-week-old groups.
[b] Differs significantly ($P < 0.05$) from distal samples of the 117-week group.
[c] Differs significantly from proximal 6-week ($P < 0.05$) and 17-week ($P < 0.001$) samples.
[d] Differs significantly from 17-week ($P < 0.001$) and 117-week ($P < 0.05$) proximal samples.
[e] Differs significantly from 17-week proximal sample ($P = 0.025$).
[f] Differs significantly from 17-week ($P < 0.02$) and 117-week ($P < 0.001$) samples.

TABLE IV

LIPID COMPOSITION OF INTESTINAL MICROVILLUS MEMBRANES ISOLATED FROM RATS OF VARIOUS AGES

Values are means ± S.E. for the three groups aged 6 or 17 weeks and four groups aged 117 weeks described in Table I. Lipid composition is given in percent by weight of total neutral plus phospholipids; glycolipids are not included.

Component	% by wt. of neutral lipids plus phospholipids					
	Proximal half of intestine			Distal half of intestine		
	6 weeks (8) [a]	17 weeks (4)	117 weeks (10)	6 weeks (5)	17 weeks (5)	117 weeks (9)
Cholesterol	24.9 ± 1.9	28.1 ± 1.5	24.5 ± 1.5	27.4 ± 1.5 [b]	31.6 ± 1.6	29.8 ± 0.6
Cholesterol esters	1.3 ± 0.5	2.5 ± 0.5	2.0 ± 0.4	2.0 ± 0.6	2.0 ± 0.5	4.1 ± 0.9
Fatty acids	9.6 ± 1.0 [d]	12.7 ± 2.0	15.7 ± 1.2	12.7 ± 2.0 [e]	16.2 ± 1.7	18.3 ± 1.2
Total phospholipids	63.7 ± 3.4 [c]	52.9 ± 1.5	50.3 ± 2.2	58.6 ± 6.5 [e]	46.5 ± 3.1	45.6 ± 1.3
Phosphatidylcholine	39.0 ± 3.1	33.9 ± 0.3 [f]	40.4 ± 2.0	38.9 ± 3.8	33.3 ± 2.7	33.0 ± 1.3
Lysophosphatidylcholine	1.7 ± 0.8	2.3 ± 0.7	0.3 ± 0.2	1.4 ± 0.7	1.1 ± 0.9	0.9 ± 0.3
Phosphatidylethanolamine	12.5 ± 1.5 [g]	5.9 ± 0.4	4.0 ± 0.4	9.4 ± 1.5 [g]	3.9 ± 1.2	3.5 ± 0.5
Sphingomyelin	10.1 ± 1.5	10.9 ± 0.2	5.6 ± 0.8 [h]	8.9 ± 1.6	8.2 ± 1.3	8.1 ± 0.7

[a] Number of determinations shown in parentheses.
[b] $P < 0.01$ as compared to all proximal preparations from groups aged 17 and 117 weeks.
[c] Significantly different as compared to proximal 17-week ($P < 0.05$) and 117-week ($P < 0.005$) samples.
[d] $P < 0.005$ as compared to proximal 117-week preparations.
[e] $P < 0.025$ as compared to distal 117-week preparations.
[f] $P < 0.05$ as compared to proximal 117-week preparations.
[g] Significantly different from 17-week ($P < 0.02$) and 117-week ($P < 0.001$) preparations of the same segment.
[h] $P < 0.02$ as compared to proximal 6-week or 17-week preparations.

TABLE V

FATTY ACID COMPOSITION OF LIPID EXTRACTS OF RAT MICROVILLUS MEMBRANES

Values are means ±S.E. Four membrane preparations of each group (6, 17 and 117 weeks) were tested. Lipid extracts for the analyses consisted of total neutral lipids plus phospholipids.

Fatty acid (carbon atoms: double bonds)	Per cent of total fatty acids					
	Proximal half of intestine			Distal half of intestine		
	6 weeks	17 weeks	117 weeks	6 weeks	17 weeks	117 weeks
14:0	1.7±0.0	2.2±0.3	1.4±0.2	3.3±0.4	2.1±0.2	1.5±0.4
14:1	0.9±0.1	1.3±0.2	0.5±0.2	1.7±0.1	0.4±0.0	0.7±0.2
16:0	18.8±0.9 [b]	22.6±0.3	23.6±1.0	12.7±0.8 [a]	21.0±0.3	20.7±1.3
16:1	3.5±0.4	4.0±0.4	3.4±0.4	8.7±0.3 [b]	4.5±0.3	4.5±0.9
18:0	17.3±0.3	17.3±0.4	16.5±0.4	18.6±0.3 [b]	16.1±0.3	14.7±1.0
18:1	16.3±0.3 [b]	30.3±2.5	24.6±1.3	19.5±0.2 [b]	22.4±0.2	24.0±1.2
18:2	14.5±0.3	14.2±0.7	15.2±1.2	12.4±0.4 [a]	14.4±0.1	15.7±0.9
20:0	3.9±1.0	1.1±0.1	0.8±0.3	3.2±0.1	1.7±0.0	1.5±0.6
20:1	3.6±0.7	1.5±0.6	2.8±0.5	4.2±0.5	5.5±0.3	3.2±1.0
20:2	1.4±0.5	0.5±0.2	0.6±0.2	2.2±0.1	1.1±0.3	1.3±0.5
20:3	2.3±0.9	1.3±0.5	1.6±0.6	2.5±0.4	2.5±0.1	2.0±0.6
20:4	15.8±0.6 [b]	3.6±0.9	8.9±1.3 [c]	10.8±1.7	8.2±1.3	10.2±1.0

[a] $P < 0.02$ as compared to either 17-week or 117-week samples of same segment.
[b] $P < 0.01$ as compared to either 17-week or 117-week samples of same segment.
[c] $P < 0.02$ as compared to 17-week sample of same segment.

(Table II). As indicated in Table III, proximal preparations of the 117-week-old rats have a higher double bond index and lower sphingomyelin/phosphatidylcholine ratio as compared to the 17-week-old animals. The change in ratio results from both a decrease in sphingomyelin and an increase in phosphatidylcholine content (Table IV). These compositional changes are concordant with enhanced fluidity of the membranes of the oldest group.

Values of the double bond index were calculated from the fatty acid analyses summarized in Table V. The results indicate that an increase in arachidonate is the major determinant of the higher double bond index in the proximal preparations of the 6-week-old group. The extracts of the youngest animals also differ significantly from those of the older groups with respect to the content of palmitate and stearate in the proximal preparations and of palmitate, palmitoleate, stearate, oleate and linoleate in the distal preparations. Further, the higher double bond index of the samples prepared from rats aged 117 as compared to 17 weeks (Table III) results from an increase in arachidonate (Table V).

Lastly, the composition studies help account for the pattern of decreased lipid fluidity in the distal as compared to the proximal intestine. This fluidity pattern was most consistent in the group aged 117 weeks, where the distal extracts contained significantly more cholesterol ($P < 0.01$) and less total phospholipid ($P < 0.05$) as compared to the proximal samples (Table IV). These distal extracts exhibited a higher cholesterol/phospholipid ratio ($P < 0.01$) and a higher sphingomyelin/phosphatidylcholine ratio ($P < 0.05$), as shown in Table III.

Discussion

Prior investigators have reported that the fluidity of intestinal microvillus membranes isolated from suckling rats or rabbits is greater than that of adult preparations [16,17]. To determine whether the fluidity difference results from a developmental trend with age, rather than from the dietary and other changes associated with weaning, we chose animals 6 weeks old for our youngest group. Since these were weaned approximately 2 weeks before experimental use, the observation that their

microvillus membranes are more fluid as compared to the older groups provides evidence for an age-dependent change at a time of rapid developmental growth of the intestine. Greater lipid fluidity was demonstrated by the decrease in fluorescence anisotropy of diphenylhexatriene (Table II) and by the reduction in the thermotropic transition temperature (Fig. 1).

Several authors have suggested that the lipid fluidity of membranes in general may decrease progressively with age and account for certain of the concomitants of senescence [3,6]. Experimental evidence concerning this general assertion, however, is sparse and somewhat contradictory. Changes in the hepatic mixed function oxidase activity with age have been ascribed to an increase in microsomal membrane fluidity in CFN rats aged 3, 12, and 26 months [27]. On the other hand, the fluidity of liposomes prepared from the total lipids of human erythrocyte membranes was greater in younger (less than 30 years old) than in older (over 70 years old) subjects [28]. Aging of bovine erythrocytes in vivo is also reported to decrease the membrane fluidity [29]. Comparison of the proximal microvillus membranes of 17- and 117-week-old rats in our studies demonstrated a small but significant increase in fluidity in the older group (Table II). Thus the pattern of fluidity with age in our preparations differs from that of the enzyme *p*-nitrophenylphosphatase, whose specific activity decreases progressively throughout the age range of 6 to 117 weeks (Table I). The specific activity of sucrase and maltase, two other microvillus enzymes, also decreases with age in Fisher rats [30]. Since fluidity is the net result of a number of regulatory mechanisms which determine the biochemical composition of the membrane, it is likely that age-dependent patterns vary in different membranes and in different biological species. In this respect, it is noteworthy that the intestinal microvillus membrane is derived from a regenerating epithelium, and that intestinal mucosal cells with immature features were reported to extend further up the villus in Fisher rats aged 27 months as compared to 4–5 months [30] without a significant change in the mucosal cell turnover rate [31]. Features of microvillus membrane fluidity in relation to age, therefore, are not necessarily representative of other membrane types.

The differences in lipid fluidity in our studies can be accounted for, in a qualitative sense at least, by changes in membrane composition. Studies with model bilayers and biological membranes have shown that above the thermotropic transition temperature the fluidity varies inversely with either the cholesterol/phospholipid molar ratio [21,32] or the sphingomyelin/phosphatidylcholine ratio [21,33]. Further, the fluidity varies directly with the double bond index, i.e., the degree of unsaturation, of the fatty acyl chains [34,35] and with the lipid/protein ratio [15,36]. Microvillus membranes prepared from the 6-week-old animals have a lower cholesterol/phospholipid ratio owing mainly to an increase in total phospholipid (Table IV); and a small but significant decrease in cholesterol as compared to the older groups is also observed in the distal segment preparations. Proximal segment preparations from the 6-week-old rats also have a higher lipid/protein ratio and double-bond index, and the latter results from an increase in arachidonate (Table V). Certain of these compositional changes with rat age have been reported for other tissues: decreases in total lipid or phospholipid were observed in rat liver [37,38], kidney [38] and pancreas [39]; increases in cholesterol/phospholipid ratio were noted in rat erythrocytes [40], liver and skeletal muscle [37] and in microsomal and mitochondrial fractions of rat liver [41]; and a tendency for the fatty acids of some membranes to become more saturated with age has been noted [3]. The values in Table IV also point to a decrease in phosphatidylethanolamine with age, particularly in the proximal intestinal segment. A similar decrease with age has been observed in rat liver plasma membranes [1].

Two changes in composition underlie the small increase in microvillus membrane fluidity of the proximal segments of 117-week-old as compared to 17-week-old rats. The sphingomyelin/phosphatidylcholine ratio is lower and the double-bond index is higher in the oldest rats (Table III). The increase in double-bond index results mainly from an increment in arachidonate content (Table V). The concordance of the fluidity with the compositional changes in these two age groups is notable. Both kinds of change were observed only in the proximal and not in the distal intestinal segment.

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