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Effects of ethinyl estradiol on intestinal membrane structure and function in the rabbit

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Structural and functional properties of the small intestinal microvillus membrane were evaluated in the rabbit after administration of ethinyl estradiol, a synthetic estrogen with a demonstrated propensity to alter hepatic membrane lipid fluidity, and promote cholestasis. In the jejunum, no estrogen-induced changes in microvillus membrane total lipid, cholesterol or phospholipid content were observed. However, the ileal microvillus membrane in estradiol-treated animals demonstrates significant reductions vs. controls (per mg protein) in total lipid (0.55 μg vs. 0.89 μg) and phospholipid (206.7 μg vs. 304.91 μg) ($p < 0.001$) content, as well as modifications in specific phospholipid species. The increase in the ileal microvillus membrane cholesterol: phospholipid molar ratio (0.65 vs. 0.51, $p < 0.05$) was associated with a significant decrease in membrane lipid fluidity reflected by an increase in fluorescence anisotropy measurements utilizing diphenyl hexatriene as the fluorophore (r at 25°C = 0.306 vs. 0.282, $p < 0.05$). Thermotropic lipid phase transitions, assessed by Arrhenius plots of both fluorescence data and ileal microvillus membrane *p*-nitrophenyl-phosphatase activity demonstrate that phase changes occur between and 24 and 28°C in both treated and untreated groups. Within the temperature range studied (40–10°C) no differences from control were observed in microvillus membrane alkaline phosphatase activity following estrogen treatment. These data therefore indicate that ethinyl estradiol-induced effects on microvillus membrane lipid composition and physical properties occur predominantly in the ileum and appear to be related, in part, to specific alterations in the availability of phospholipid following estrogen treatment.

Introduction

The relationship between intestinal microvillus membrane lipid fluidity and membrane associated enzyme functions has been characterized utilizing membrane alkaline phosphatase activity and Na^+-dependent bile acid transport in the terminal ileum [1,2], as well as glucose transport in jejunum [1]. Clear differences are recognized between these lipid dependent, 'intrinsic' functions and lipid independent, 'extrinsic' functions [3–6]. In the liver, administration of ethinyl estradiol, a synthetic estrogen has been demonstrated to result in a reduction in bile flow [7]. Although the molecular basis of this abnormality is unknown and possibly complex, several studies have implicated in its

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pathogenesis, an increase in both the rat hepatocyte plasma membrane cholesterol content and in the cholesterol/phospholipid molar ratio; and, these changes in themselves are associated with a decrease in membrane lipid fluidity [7–9]. Bile flow is resumed following administration of Triton WR-1339 to the estrogen-treated rats, with a restoration towards normal of the plasma membrane lipid composition and fluidity [9]. Similarly, oral administration of *S*-adenosyl-L-methionine has also been observed to improve bile flow [10] possibly owing to an increase in methylation of membrane phosphatidylethanolamine to phosphytidylcholine [10,11]. These observations together with the demonstration of ethinyl estradiol-induced inhibition in (Na^+-K^+)-ATPase activity occurring in the absence of a modification in hepatocellular protein synthesis [9], suggest that the decrease in bile flow is directly influenced by associated membrane lipid changes.

In the present study, we have sought to evaluate the effects of estrogen treatment on membrane lipid composition and lipid fluidity of the rabbit small intestinal microvillus membrane, which represents the final common pathway for intestinal solute absorption. We also consider possible influences of these effects on membrane intrinsic (i.e., lipid-associated) enzymatic processes.

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Methods

Microvillus membrane vesicles. For all studies, male New Zealand white rabbits 7–8 weeks of age (Ace Breeders, Boyertown, PA) were used. Animals were permitted at least a 7 day period of equilibration in our animal facility before study and were maintained on Wayne Rabbit Chow (Brookshire Feeds, Lansdale, PA). Estrogen-treated animals were subcutaneously injected with ethinyl estradiol dissolved in propylene glycol at a dose of 5 mg per kg per day for 5 days. Control rabbits were injected with the same volume of the propylene glycol vehicle. Rabbits were housed in wire bottom cages to prevent solid coprophagia, and fasted overnight (except for water ad lib). Although animals were not pair-fed, no weight differences between treated and control groups were noted at the time of the study. All animals were killed by intracardiac pentobarbital injection at 9:00 a.m. The small intestine was then removed from the ligament of Treitz to the ileocecal valve, and divided into three equal length segments, with the proximal two thirds designated as jejunum and the distal third as ileum. Microvillus membranes were isolated as described in Ref. 12 using a modification of the method of Kessler et al. [13], where following homogenization of mucosal scrapings, the homogenates were treated with 10 mM magnesium chloride as described by Hauser et al. [14] and the microvillus membrane fraction was obtained by differential centrifugation.

Protein and enzyme assays. Total protein of intestinal homogenate and microvillus membrane fractions was determined by the method of Lowry et al. [15], with bovine serum albumin as the protein standard. Disaccharidase assays were performed at 37°C in 250 μl volume containing 0.05 M sodium phosphate, 0.002% Triton X-100 buffer at pH 5.8, with 0.0375 M sucrose as substrate. The amount of glucose released was determined by incubation for 20 min at 37°C with 250 μl Glucostat reagent (Worthington Biochemical Corp., Freehold, NJ) prepared in 0.5 M Tris at pH 7.0. Termination of lactase and sucrase activity was effected by the added Tris.

Membrane-bound alkaline phosphatase was assayed utilizing 5 mM *p*-nitrophenyl phosphate (Sigma) as substrate [16]. Incubations were carried out at pH 9.5 in glycine buffer (0.034 M) with added $ZnCl_2$ (0.34 mM) and $MgCl_2$ (0.34 mM); and, the reaction was stopped after 10 min by addition of 1.0 M NaOH.

Lipid analysis. Lipids were extracted from jejunal and ileal microvillus membranes immediately following isolation by the method of Folch et al. [17]. Recovery was determined by co-extraction of tracer quantities of [^{3}H]cholesterol and [^{14}C]phosphatidylethanolamine added to microvillus membrane vesicle suspensions, with results (> 90% recovery in all extractions) expressed as total counts recovered/total counts added. Measurements of total microvillus membrane lipid were made by gravimetric analysis (Cahn Electro-

balance, Cahn Inst., Paramount, CA) of triplicate samples from N_2-dried, Folch-extracted material.

Microvillus membrane cholesterol was quantified by gas-liquid chromatography utilizing a Varian model 1400 Gas-Liquid Chromatograph (Varian Corp., Palo Alto, CA) equipped with a flame ionization detector. Triplicate samples were injected onto a 6 ft. (2 mm internal diameter) Gas-Chrom Q column packed with 3% OV-17 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) with injector, detector and column temperatures at 305, 315, 295°C, respectively. This method employs an internal standard-area technique using stigmasterol as the internal standard. Areas under the curve were computed using a Spectra Physics Computing Integrator (Spectra-Physics, Lanham, MD). Cholesterol ester content was determined according to the method of Brown et al. [18].

Microvillus membrane phospholipid content was determined by the method of Vaskovsky [19], which measures inorganic phosphate. Individual phospholipid species were measured following separation in one dimension by thin-layer chromatography, using a modification of the method of Touchstone et al. [20]. Folch-extracted lipid was applied to Silica gel G plates (Analtech, Inc., Newark, DE) following heat activation at 120°C for 0.5 h, and developed in a solvent containing chloroform/ethanol/triethylamine/water (30 : 34 : 30 : 5, v/v). Following drying at room temperature, plates were developed a second time in the same solvent, dried and sprayed with phosphate reagent as described by Vaskovsky [19]. Individual spots were scraped and eluted in four washes of chloroform/methanol/7 M ammonium hydroxide (13 : 7 : 1, v/v). Recoveries of phospholipid standards (phosphatidylcholine; phosphatidylethanolamine; sphingomyelin; phosphatidylinositol; phosphatidylserine; lysophosphatidylcholine) were $\geqslant 95\%$ when evaluated by migration of both individual species and phospholipid mixtures, and no co-migration of phospholipid species was apparent.

Fluorescence polarization studies. Fluorescence polarization measurements were performed on a Perkin-Elmer 650-10S Fluorescence Spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) fitted with an automatic polarizer (C.N. Wood Mfg., Newtown, PA). In all experiments, the lipid-soluble fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was used as previously described [12]. A 2.0 mM stock solution of 1,6-diphenyl 1,3,5-hexatriene in tetrahydrofuran was prepared and stored protected from light at −20°C. Immediately prior to use, an aqueous suspension of the probe was prepared by diluting the 1,6-diphenyl-1,3,5-hexatriene stock solution in 2000 vols. of 0.02 M phosphate-buffered saline, pH 7.4, and stirred vigorously for 2–3 h at 25°C until no odor of tetrahydrofuran could be detected. The resultant dispersion of 1.0 μM 1,6-diphenyl-1,3,5-hexatriene was clear and devoid of fluorescence. Microvillus membranes from jejunal or ileal epithelium (either freshly prepared or stored at −20°C for up to 2 weeks post-isolation) equivalent to 100–200 μg of protein were incubated in 3 ml of 1,6-diphenyl-1,3,5-hexatriene suspension at 37°C for 1 h. Fluorescence emission intensities (excitation wavelength 360 nm, emission wavelength 430 nm) were sequentially recorded parallel and perpendicular to the excitation plane, during which time the temperature in the thermoregulated sample chamber was reduced gradually from 40 to 10°C at a rate of 0.5 Cdeg/min. Fluorescence polarization was expressed as the fluorescence anisotropy r, determined from 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 exciting plane. The anisotropy parameter $[(r_0/r) - 1]^{-1}$ was calculated by employing a value of $r_0 = 0.362$ for 1,6-diphenyl-1,3,5-hexatriene [21]. According to the Perrin equation, a form of which can be written: $r_0/r = 1 + 3\tau/p$, where τ = excited state lifetime and p = rotational relaxation time of the probe, these values vary directly with p under conditions of constant τ, and are therefore considered to be inversely related to lipid fluidity [22]. In these experiments, τ was assessed by measurement of fluorescence intensity $F = I_{\parallel} + 2I_{\perp}$. This value did not differ significantly among membrane preparations. The contribution of light scattering (fluorescence intensity of membrane suspension minus probe) plus fluorescence of the incubating buffer were routinely examined and corrections were $<$ 5% of the total fluorescence intensity F. In order to detect thermotropic transitions (breakpoints),

Arrhenius plots of r vs. $(1/K) \times 10^3$ were constructed. Accuracy of transitions were tested by measuring the thermotropic phase transition of multilamellar liposomes of dimyristoylphosphatidylcholine using 1,6-diphenyl-1,3,5-hexatriene fluorescence anisotropy, with transition temperatures (upper limit of major transition at 25°C) closely approximating values previously reported [23].

The term 'lipid fluidity' is used to described the motional freedom of lipid molecules or molecular probes (e.g. 1,6-diphenyl-1,3,5-hexatriene) within a membrane bilayer. Since determination of absolute fluidity is limited in an anisotropic medium (as opposed to a homogeneous isotropic system) because of the inability to accurately reproduce the three-dimensional structure of the hydrophobic bilayer, the steady-state fluorescence polarization measurement r, is employed to estimate relative degrees of fluidity (the anisotropic behavior of the membrane probe) following probe incorporation into the bilayer. As discussed by Van Blitterswijk et al. r in cell membranes or liposomes can be resolved into a static and dynamic component [24]. The static component, or limiting hindered anisotropy r_∞, is related to the structural order of the membrane and reflects the degree of hindrance to probe rotation by molecular packing of bilayer lipids. The dynamic, or kinetic factor r_f, is related to the rotational relaxation time of the probe. In relatively ordered phases (i.e. $r > 0.2$), such as exists in most biological membranes, the contribution of r_f is small, and r_∞ approximates r. In these studies values for r_∞ were estimated from the fluorescence anisotropy as previously described [24].

Liposomes. Liposomes were prepared from extracted, dried membrane lipid following suspension of lipid in phosphate-buffered saline containing the fluorescence probe as described above, to a final concentration of 0.3 mg lipid/ml. The lipid suspension was then sonicated for 10 min at 4°C under N_2; and, liposomes were then used for fluorescence polarization studies.

Statistics. For comparison of all variables studied between treated and control groups, Student's t-test for unpaired samples was employed.

Results

Microvillus membrane vesicle isolation

Jejunal and ileal microvillus membrane vesicles were isolated at 15-fold purification based on sucrase specific activity. No significant differences in recovery or in the relative purification were noted between groups. Criteria for analysis in subsequent studies was a purification of at least 12-fold. Representative preparations were examined and were found to be free of contamination, judged by the absence of both $(Na^+ + K^+)$-ATPase, a basolateral membrane enzyme, and succinate dehydrogenase activities [25,26]. Electron microscopic views of microvillus membrane vesicles confirmed the absence of co-isolated cellular elements.

Effects of ethinyl estradiol on intestinal microvillus membrane lipid composition

The principal changes in intestinal microvillus membrane lipid composition resulting from ethinyl estradiol treatment are presented in Table I. Total

TABLE I

EFFECTS OF ETHINYL ESTRADIOL (EE) ON SMALL INTESTINAL MVM LIPID COMPOSITION

Results are presented as means ± S.E. of three determinations on each of four preparations.

	Jejunum		Ileum	
	Control	EE-treated	Control	EE-treated
Total lipid (μg/mg protein)	0.57 ± 0.05	0.63 ± 0.03	0.89 ± 0.03	0.55 ± 0.02 [a]
Cholesterol (μg/mg protein)	87.3 ± 5.6	90.0 ± 8.1	65.3 ± 3.2	74.9 ± 4.9
Phospholipid (μg/mg protein)	227.3 ± 13.2	213.5 ± 6.5	304.9 ± 21.4	206.7 ± 7.07 [a]
Cholesterol : phospholipid (mol : mol)	0.73 ± 0.05	0.61 ± 0.07	0.51 ± 0.01	0.65 ± 0.02 [b]

[a] $p < 0.001$ compared to control.
[b] $p < 0.05$ compared to control.

TABLE II

EFFECTS OF ETHINYL ESTRADIOL (EE) ON MVM PHOSPHOLIPID COMPOSITION (% TOTAL PHOSPHOLIPID)

Results are presented as means ± S.E. of three determinations on each of four preparations.

	Jejunum		Ileum	
	Control	EE-treated	Control	EE-treated
Lysophosphatidylcholine	2.36 ± 1.04	2.95 ± 0.39	1.60 ± 1.10	4.79 ± 1.49
Sphingomyelin	12.65 ± 0.64	13.67 ± 1.30	15.22 ± 1.48	15.71 ± 2.14
Phosphatidylcholine	23.85 ± 2.95	21.82 ± 0.74	22.66 ± 1.69	21.04 ± 1.27
Phosphatidylserine	16.10 ± 3.67	23.56 ± 5.38	16.05 ± 0.48	26.18 ± 0.84 [a]
Phosphatidylinositol	7.46 ± 1.22	8.94 ± 0.49	6.73 ± 0.16	9.22 ± 0.29 [b]
Phosphatidylethanolamine	34.12 ± 4.93	24.79 ± 7.65	33.68 ± 1.00	22.73 ± 7.33 [b]

[a] $p < 0.001$ compared to control.
[b] $p < 0.002$ compared to control.

lipid content, cholesterol and phospholipid concentrations and the relative ratios are similar in the jejunum of estrogen-treated animals and in the controls. In all membranes studied, cholesterol ester content was negligible. In the ileum, however, a highly significant decrease in total lipid content occurs, largely due to a decrease in phospholipid concentration. This is translated as a significant change or increase in the cholesterol: phospholipid ratio. In order to determine if this is the result of a reduction in a specific phospholipid, analyses to provide quantification of individual phospholipid species were performed for both the jejunum and ileum. These data, Table II, demonstrate that the overall reduction of phospholipid in estrogen-treated rats is due principally to a decrease in ileal phosphophatidyl ethanolamine content with a proportionate increase in phosphatidylserine. These estrogen mediated changes in ileal phospholipid content result in a significant difference in the phosphatidylcholine: phosphatidylethanolamine ratio between control (0.67 ± 0.06) and estradiol-treated (1.02 ± 0.22) animals ($p < 0.05$).

Effects on microvillus membrane lipid fluidity

Estimates of lipid fluidity in membrane preparations isolated from jejunum and ileum were assessed by steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5 hexatriene as the lipid soluble fluorophore.

These studies, including calculations of r, r_∞, and $[(r_0/r) - 1]^{-1}$, are summarized in Table III, and the data indicate that the changes in lipid content and phospholipid distribution are reflected by a decrease in membrane lipid fluidity (i.e. increased anisotropy) and that this is confined principally to the ileum. Jejunal values for estradiol-treated and control were unchanged. Arrhenius plots of temperature dependent fluidity measurements between 40 and 10°C were then constructed in order to examine the membrane lipid thermotropic transitions (i.e. liquid-crystal to gel) for both jejunum and ileum. In both jejunal (not shown) and ileal (Fig. 1) membranes clear thermotropic transitions or break points are observed to occur in the range of 25–26°C in both the control and estrogen-treated groups.

TABLE III

FLUORESCENCE PARAMETERS (DIPHENYLHEXATRIENE) AT 25°C IN JEJUNAL AND ILEAL MICROVILLUS MEMBRANES OF CONTROL AND ETHINYL ESTRADIOL-TREATED (ESTROGEN) RABBITS

Values are means ± S.E. for five pairs of membrane preparations.

Membrane	r	r_∞	$((r_0/r)-1)^{-1}$
Jejunum:			
control	0.279 ± 0.010	0.266 ± 0.010	3.57 ± 0.50
estrogen	0.284 ± 0.010	0.274 ± 0.010	3.85 ± 0.80
Ileum:			
control	0.282 ± 0.005	0.275 ± 0.006	3.61 ± 0.34
estrogen	0.306 ± 0.006 [a]	0.299 ± 0.007 [a]	6.09 ± 1.03 [a]

[a] $p < 0.05$ compared to control.

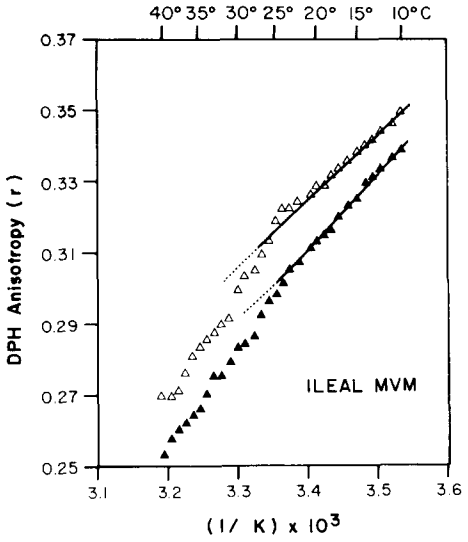

Fig. 1. Representative Arrhenius plot of liposomes prepared from ileal microvillus membrane lipid of ethinyl estradiol-treated rabbits.

TABLE IV

FLUORESCENCE PARAMETERS (DIPHENYLHEXATRIENE) AT 25°C IN ILEAL MICROVILLUS MEMBRANE LIPOSOMES OF CONTROL AND ETHINYL ESTRADIOL-TREATED RABBITS

Values are means ± S.E. for two determinations on each of three liposome preparations.

Treatment	r	r_∞	$((r_0/r)-1)^{-1}$
Control	0.232 ± 0.011	0.209 ± 0.014	1.80 ± 0.24
Estrogen	0.254 ± 0.003 [a]	0.241 ± 0.005 [a]	2.34 ± 0.10 [a]

[a] $p < 0.05$ compared to control.

Effects of ethinyl estradiol on the fluorescence anisotropy of small intestinal microvillus membrane liposomes

Studies of ileal membrane liposome fluidity are summarized in Table IV. These experiments indicate a significant increase in the steady-state (r) and limiting hindered (r_∞) anisotropies as well as in the anisotropy parameter $[(r_0/r)-1]^{-1}$ in ethinyl estradiol-treated animals. Jejunal liposome fluidity did not change following estrogen administration (data not shown). Arrhenius analysis of ileal liposome anisotropy (r) suggests a thermo-

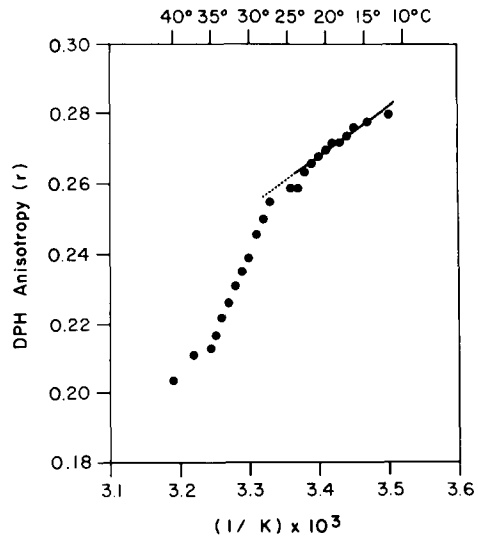

Fig. 2. Representative Arrhenius plots of DPH fluorescence anisotropy, r, of liposomes prepared from microvillus membrane lipid of ethinyl estradiol-treated rabbits.

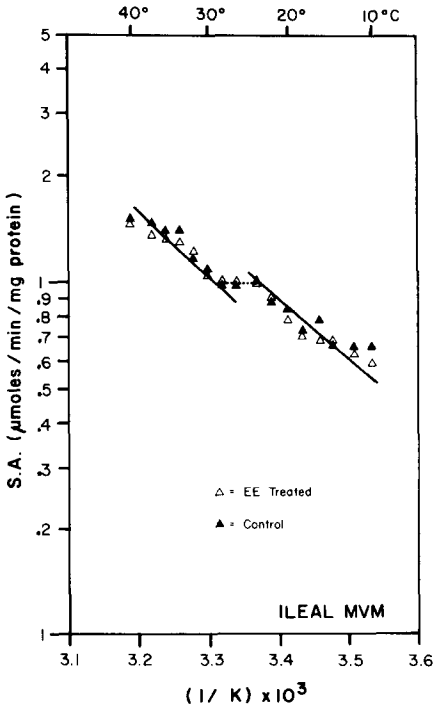

Fig. 3. Arrhenius plot of ileal MVM p-nitrophenylphosphatase activities in control and ethinyl estradiol-treated rabbits ($n = 4$, S.E. not shown where error smaller than symbol).

tropic transition of 25–26°C for both control (not shown) and treated groups (Fig. 2).

Effects on ileal membrane p-nitrophenylphosphatase activity

In order to evaluate possible effects of ethinyl estradiol-mediated lipid composition and fluidity changes on membrane function, *p*-nitrophenylphosphatase activity was measured. Previously, membrane-bound activity sites associated with this enzyme have been shown to manifest characteristics of intrinsic (i.e. membrane lipid dependent) membrane proteins [1]. Over the range of temperature evaluated (40–10°C), ileal alkaline phosphatase specific activity is apparently unaltered by estrogen treatment (Fig. 3). The Arrhenius plots of enzyme activities indicate a thermotropic transition occurring between 24 and 28°C in both groups, confirming the data derived from fluorescence polarization experiments.

Discussion

The data presented here indicate that treatment with ethinyl estradiol results in changes which are not confined solely to the liver but influence the small intestinal microvillus membrane lipid composition and its physical properties as well. These are manifested specifically by a reduction in ileal membrane total lipid content, a reduced phospholipid content, with an altered distribution of phospholipid species, and an increased cholesterol : phospholipid molar ratio. These factors combined are associated with changes in membrane lipid fluidity as determined by 1,6-diphenyl-1,3,5-hexatriene anisotropy.

Studies of ethinyl estradiol administration in rats demonstrate that similar hormone mediated effects to those noted here occur in the liver. Specifically, estrogen treatment results in decreased hepatocyte membrane lipid fluidity, and is accompanied by an increase in the cholesterol : phospholipid molar ratio [7]. Since these changes have been demonstrated to occur in the absence of changes in cellular protein synthesis [9], estrogen effects appear to be exerted primarily through modifications in lipid production(s). It has been suggested that estradiol treatment results in increases in membrane cholesterol esterification; and, subsequent alterations in membrane lipid-dependent ($Na^+ + K^+$)-ATPase activity contribute to inhibition of bile flow [9]. Recent studies have demonstrated amelioration of the estradiol cholestasis, following a starve refeed regimen [27]. This dietary induced phenomenon occurs presumably via changes in phospholipid desaturase activities [28] affecting membrane lipid fluidity and bile flow rates. The data presented here demonstrate no alterations in membrane cholesterol or increases in the negligible cholesterol ester content; but rather, lipid analysis indicates that the estradiol-induced decrease in ileal membrane fluidity is a function primarily of changes in phospholipid content and distribution. In this regard, the significant increase in the phosphatidylcholine : phosphatidylethanolamine ratio in estrogen-treated rabbits suggests an influence on phospholipid methylation rates [29]. Additional investigation will be required to fully assess the nature of altered phospholipid synthesis and/or turnover in this experimental model. Localization of decreased phospholipid content to the ileum, together with the known reduction in bile flow which accompanies ethinyl estradiol administration suggests that the observed microvillus membrane phospholipid changes are a consequence of decreased availability of biliary phospholipid, which under normal circumstances participates in an enterohepatic circulation [30].

Of interest in these studies is the apparent increase in jejunal cholesterol : phospholipid ratio when compared to ileal membranes in control animals. We have previously reported a proximal to distal increase in this value in rabbit intestine [12]. Here, differences could be related to effects of estrogen in the mid-intestine, not previously examined in our prior study. Alternatively, actions of the propylene glycol vehicle, or further intraspecies variability, must be considered.

Conforming to the Singer-Nicolson model for biological membranes, the molecular structure of microvillus membrane lipid is comprised of a phospholipid bilayer and free cholesterol [31]. Accordingly, this lipid environment influences the functioning of several enzymatic and transport proteins. These 'intrinsic' proteins possess activity sites embedded within the bilayer which are therefore uniquely sensitive to lipid structural proper-

ties [1,22]. Alterations in the fluid state of membrane lipids may effect changes in protein conformation as a function of the physical state of boundary lipids and restriction of both lipids and protein molecular motion [32,33]. Accordingly, the ethinyl estradiol-induced decrease in hepatocyte surface membrane fluidity is associated with a reduction in $(Na^+ + K^+)$-ATPase activity [7,9]. Furthermore, normal enzyme activity can be restored following reversal of the fluidity changes (associated with a reduction in membrane cholesterol) by administration of the non-ionic detergent Triton WR-1339 [7,34]. Studies of hepatocyte bile secretory failure in estrogen-treated animals, and its reversibility following either Triton-mediated membrane fluidization [9], administration of methyl-donating agents [10] or dietary modulation [27], further suggest that lipid compositional changes and alterations in membrane physical properties influence intrinsic membrane function.

We have previously reported on the relationship between microvillus membrane structure and transport function in a consideration of the postnatal development of sodium-dependent bile acid transport in the rabbit ileum [12]. In the present study, estrogen-induced changes in ileal microvillus membrane lipids appear to be unassociated with significant effects on membrane *p*-nitrophenylphosphatase activity. The apparent membrane lipid phase transition assessed by Arrhenius plots of the fluorescence anisotropy *r* occurs around 25–26°C in both groups, despite the observed fluidity and lipid compositional differences. Further studies (e.g. differential scanning calorimetry [22]) will be required to fully address this phenomenon. Additionally, Arrhenius behavior of microvillus membrane *p*-nitrophenylphosphatase activity indicates a break point (change in slope of specific activity) in the range of 24–28°C in both control and treated groups. Although the nature and significance of such a transition in slope is not clear at present, prior evidence indicates that plots of 'intrinsic' membrane enzyme and transport functions manifest similar break points around the observed thermotropic transition temperature [1,4,22]. The apparent lack of functional change in lipid-associated enzyme activity, despite significant lipid modifications, has been reported to be a consequence of differential effects on membrane bulk vs. annular lipid [4].

These investigations therefore demonstrate that estrogen treatment results in significant changes in ileal but not jejunal membrane lipid composition and fluidity. Furthermore, the significant increases in ileal membrane liposome anisotropy values (decreased fluidity) in estrogen-treated animals demonstrate that the observed effects are mediated, at least in part, by altered membrane lipid composition. Although not specifically studied here, the marked differences between membrane and liposome anisotropies probably affect hindrance to fluorescence probe motion by membrane proteins [35]. Within the magnitude of observed lipid effects, no apparent estrogen-induced alterations in membrane lipid-associated alkaline phosphatase activity is noted. Investigation of other specific ileal functions (e.g. bile acid transport) will be required to fully assess estradiol-induced effects on membrane function. Nevertheless, the profound changes in phospholipid composition in the ileum observed here may represent a model for the study of lipid metabolic changes in cholestatic states.

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