Lipid Dynamics and Lipid–Protein Interactions in Rat Enterocyte Basolateral and Microvillus Membranes†

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ABSTRACT: Rat intestinal basolateral membranes undergo a reversible lipid thermotropic transition previously characterized by differential scanning calorimetry and fluorescence polarization. Arrhenius studies of these membranes provide evidence for two groups of intrinsic enzyme activities. 5′-Nucleotidase and adenylate cyclase (basal, NaF stimulated, and prostaglandin E_1 stimulated) show a discontinuity in the Arrhenius plot at 28–30 °C, corresponding to the peak temperature of the lipid transition; $(Na^+ + K^+)$-dependent adenosine triphosphatase, K^+-dependent *p*-nitrophenylphosphatase, and magnesium-dependent adenosine triphosphatase show a break point at 20–22 °C, approximately 5–7 °C below the lower critical temperature of the thermotropic transition. Benzyl alcohol (50 mM) decreased the lipid transition temperature by approximately 6.0 ± 1.0 °C and concomitantly reduced the break point temperatures of the basal and stimulated adenylate cyclase activities by 4–7 °C. Preparations of basolateral and microvillus membranes were studied with five fluorescent lipid probes (2-anthroylstearate, 12-anthroylstearate, dansylphosphatidylethanolamine, diphenylhexatriene, and retinol) by means of steady-state fluorescence polarization, and excited-state lifetimes were determined by single photon counting. The fluorescence anisotropy of each probe was considerably greater in microvillus as compared to basolateral membranes, whereas the excited-state lifetimes were similar. The results indicate that lipid molecules have greater motional freedom in the basolateral membrane. Composition studies suggest that the difference in lipid dynamics between the membranes results from the higher ratios of protein/lipid (w/w), cholesterol/phospholipid (mol/mol), and sphingomyelin/lecithin (mol/mol) in the microvillus membrane.

The microvillus (luminal) and basolateral (contraluminal) plasma membranes of the enterocyte, the predominant cell type lining the small intestine, are highly differentiated for the processes of digestion, absorption, and secretion. These antipodal membranes, whose function is to regulate the exchange of substances between organism and environment, differ from each other in ultrastructure (Bloom & Fawcett, 1968; Oda, 1976), enzyme activities (Douglas et al., 1972; Lewis et al., 1975; Murer et al., 1974, 1976), transport mechanisms (Murer et al., 1974), electrophysiological properties (Rose & Schultz, 1971; Okada et al., 1977), protein components (Fujita et al., 1973), and lipid composition (Forstner et al., 1968; Douglas et al., 1972; Kawai et al., 1974; Lewis et al., 1975). Although there is considerable information concerning individual proteins which mediate specific functions in each membrane type (Murer et al., 1974, 1976; Kenny & Booth, 1978), the role of the lipids has been more obscure in the face of increasing recognition that lipid–protein interactions can influence the protein activities of biological membranes (Fox, 1975; Lee, 1975; Razin, 1975; Melchior & Steim, 1976; Tada et al., 1978; Brasitus et al., 1979). Recently we described studies of the lipid dynamics and lipid–protein interactions in rat intestinal microvillus membranes (Schachter et al., 1976; Schachter & Shinitzky, 1977; Brasitus et al., 1979, 1980). These luminal membranes have characteristically low lipid fluidity,[1] as assessed by fluorescence polarization of lipid-soluble fluorophors (Schachter & Shinitzky, 1977), and undergo a reversible lipid thermotropic transition in the range 23–39 °C, with a peak temperature (T_p)[2] of 31 °C on differential scanning calorimetry (DSC) (Brasitus et al., 1980). Moreover, a number of microvillus membrane protein activities are influenced by the thermotropic transition and are thereby categorized operationally as "intrinsic" activities (Brasitus et al., 1979). Arrhenius plots of *p*-nitrophenylphosphatase, calcium-dependent adenosine triphosphatase (CaATPase), and magnesium-dependent adenosine triphosphatase (MgATPase) and active transport of D-glucose all show a change in energy of activation in the range 25–30 °C, i.e., between the lower critical[3] and

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[1] The term "lipid fluidity" as applied to model bilayers and natural membranes is used throughout this report to express the relative motional freedom of the lipid molecules or substituents thereof. In doing so, we follow a heuristic usage which has become increasingly frequent in the recent literature. It bears emphasis, however, that the term is broad and includes different types of motions, e.g., rotational or lateral diffusion of a molecule in an array, movements of substituent groups of a molecule, and flow of molecules under a pressure gradient in accord with a fluidity which is 1/viscosity of the molecular array (Blank, 1974; Lakowicz et al., 1979a,b). In this report, "lipid fluidity" of natural membranes is assessed by the steady-state fluorescence polarization of lipid-soluble probes. The anisotropy parameters so obtained are probe dependent and reflect the overall motional freedom of the fluorescent molecule without distinguishing the specific mechanisms affecting its motions. These specific mechanisms include alteration of the rate of rotation of the probe owing to viscous drag of the environment, anisotropic rotations, and hindered motions owing to structural factors (Chen et al., 1977; Dale et al., 1977; Kawato et al., 1977; Kinosita et al., 1977; Lakowicz et al., 1979a,b; Veatch & Stryer, 1977).

[2] Abbreviations used: 2AS, DL-2-(9-anthroyl)stearic acid; 12AS, DL-12-(9-anthroyl)stearic acid; CaATPase, calcium-dependent adenosine triphosphatase; DPE, *N*-[5-(dimethylamino)naphthalene-1-sulfonyl]dipalmitoyl-L-α-phosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DSC, differential scanning calorimetry; ΔE, energy of activation; MgATPase, magnesium-dependent adenosine triphosphatase; $(Na^+ + K^+)$ATPase, (sodium plus potassium)-dependent adenosine triphosphatase; PGE_1, prostaglandin E_1; T_p, peak temperature of the thermotropic transition observed by differential scanning calorimetry; P_i, inorganic phosphate.

[3] The lower critical temperature of the lipid transition was determined by differential scanning calorimetry as the point of deviation from the base line of the heating scan. This temperature corresponded closely to the break point observed on the Arrhenius plot of the DPH anisotropy parameter (Brasitus et al., 1980).

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peak temperatures of the lipid transition. In contrast, the microvillus membrane digestive enzymes lactase, maltase, sucrase, leucine aminopeptidase, and γ-glutamyl transpeptidase yield one slope on an Arrhenius plot and are classified as "extrinsic" activities, functionally independent of the lipid transition.

This report focuses on the lipid dynamics and lipid–protein interactions in rat intestinal basolateral membranes as compared to microvillus membranes. Basolateral membranes examined by DSC also undergo a reversible lipid transition in the range 27–40 °C with a T_p of 28–30 °C (Brasitus et al., 1980), and it was of interest to construct Arrhenius plots of a number of basolateral membrane enzyme activities. As described below, 5′-nucleotidase and adenylate cyclase activities show a discontinuity at approximately 28–30 °C whereas ($Na^+ + K^+$)-dependent adenosine triphosphatase [($Na^+ + K^+$)ATPase], K^+-dependent *p*-nitrophenylphosphatase, and MgATPase all yield a break point at approximately 20–22 °C. In addition, steady-state fluorescence polarization studies with five lipid-soluble probes indicate that the motional freedom of lipid molecules is considerably greater in the basolateral membrane than in the antipodal microvillus membrane.

Experimental Procedures

Membrane Preparations. Albino male rats of the Sherman strain weighing 250–300 g were fasted 18 h with water ad libitum prior to removal of the small intestine. Microvillus membranes were isolated from mucosal scrapings of the proximal half of the small intestine and basolateral membranes were isolated from enterocytes prepared from the proximal half of the small intestine, as described previously (Brasitus et al., 1979, 1980). Purity of the microvillus preparations was assessed by estimating the specific activity of the marker enzymes sucrase and *p*-nitrophenylphosphatase. Final ratios of the specific activities in the isolated membranes to the initial homogenates varied from 10 to 20. Basolateral membrane preparations were evaluated via the marker enzymes ($Na^+ + K^+$)ATPase and 5′-nucleotidase, and the final specific activity ratios of membranes/homogenates varied from 10 to 15. In addition, the enzymes NADPH–cytochrome *c* reductase and succinate dehydrogenase, marker enzymes for microsomal and mitochondrial membranes, respectively, were also assayed, and the specific activity ratios of membranes/homogenates averaged 0.08–0.09 and did not exceed 0.12 (Brasitus et al., 1980).

Fluorescence Polarization Studies. Five lipid-soluble fluorophors were used: 1,6-diphenyl-1,3,5-hexatriene (DPH; purchased from Aldrich Chemical Co.), *all-trans*-retinol (Sigma Chemical Co.), DL-2-(9-anthroyl)stearic acid (2AS; Molecular Probes Inc.), DL-12-(9-anthroyl)stearic acid (12AS; Sigma Chemical Co.), and *N*-[5-(dimethylamino)-naphthalene-1-sulfonyl]dipalmitoyl-L-α-phosphatidylethanolamine, i.e., dansylphosphatidylethanolamine (DPE; Molecular Probes Inc.). The methods used to load the membranes with either DPH, retinol, or 12AS and the quantification of the polarization of fluorescence in an SLM polarization spectrofluorometer have been described (Schachter & Shinitzky, 1977). The procedures for 2AS were identical with those for 12AS. The DPE probe was dissolved in absolute ethanol (final concentration 25 mM), and 3.0 μL of this solution was added to 4 mL of a suspension containing membranes equivalent to 150–200 μg of protein in an isotonic buffer (145 mM NaCl, 4 mM KCl, 0.5 mM $CaCl_2$, and 5 mM sodium phosphate, pH 7.4). After shaking for 20 min at room temperature, the membranes were pelleted by centrifugation at 31000*g* for 20 min at 4 °C, washed once with 5 mL, and finally suspended in 2.0 mL of the same buffer. Fluorescence polarization measurements of the loaded membranes were made as previously described for DPH (Schachter & Shinitzky, 1977). The polarization of fluorescence was expressed as the fluorescence anisotropy, *r*, and as the anisotropy parameter, $[(r_0/r) - 1]^{-1}$, which varies inversely with the rotational relaxation time of the probe, as indicated by the Perrin equation $r_0/r = 1 + 3\tau/\rho$, where τ is the mean lifetime of the excited state of the fluorophor and ρ is the rotational relaxation time. The values of r_0, the maximal limiting anisotropy, used in the calculations were as follows: DPH, 0.362 (Shinitzky & Barenholz, 1974); retinol, 0.367 (Schachter et al., 1976); 2AS and 12AS, 0.285 (Schachter & Shinitzky, 1977); DPE, 0.321 (Shinitzky, personal communication). The mean lifetime of the excited state, τ, of each probe in both microvillus and basolateral membranes was determined by time-resolved single photon counting (Photochemical Research Associates, London, Ontario; Model 1000 single photon counter). From the observed values of τ and *r*, an apparent rotational relaxation time, ρ, could be calculated from the Perrin equation on the assumption that the depolarizing rotations were isotropic and unhindered.

Enzyme Studies. Specific activities of a number of basolateral membrane enzymes were determined over the temperature range 10–40 °C, and Arrhenius plots were constructed. Assay conditions were chosen to ensure linear kinetics and excess of substrate, i.e., maximal velocity, throughout the test period, and no more than 5% of a given substrate was consumed in any assay. ($Na^+ + K^+$)ATPase was quantified by a modification of the method of Siegel & Goodwin (1972), as previously described (Brasitus et al., 1980). Enzyme activity was calculated from the increment in release of [^{32}P]P_i from [γ-^{32}P]ATP in the presence as compared to the absence of NaCl plus KCl. Potassium-dependent *p*-nitrophenylphosphatase was estimated by a modification of a method described previously (Kowarski & Schachter, 1973). The reaction mixtures (0.7 mL final volume) consisted of membranes equivalent to 100 μg of protein, 62.5 mM glycine buffer, pH 9.5, 6.5 mM $MgCl_2$, 6.0 mM bis(Tris) *p*-nitrophenyl phosphate (Sigma Chemical Co.), and either 150 mM KCl or an equivalent volume of water. Following the assay period, 1 mL of ice-cold 25 mM Tris, pH 7.4, was added, and the optical density at 410 nm was determined immediately to quantify the *p*-nitrophenol released. Under these conditions, the absorbance values remained stable for 10–15 min. The activity of 5′-nucleotidase was estimated according to the procedure of Dipple & Houslay (1978). Adenylate cyclase activity was determined by the method of Salomon et al. (1974) as modified by Schlatz et al. (1978). Assay mixtures contained membranes equivalent to 25–50 μg of protein in a final volume of 50 μL consisting of 1.2 mM [α-^{32}P]ATP (0.4 μCi; New England Nuclear), 250 mg/mL of pyruvate kinase (Sigma Chemical Co., Type III), 10 mM phosphoenolpyruvate (Sigma Chemical Co.), 10 mM caffeine, 5 mM $MgCl_2$, and 50 mM Tris-HCl, pH 7.5. Samples were incubated with shaking for 10 min, and enzyme activity was assessed by isolation of cyclic adenosine monophosphate by column chromatography on Dowex 50 cation-exchange resin (Bio-Rad Laboratories; AG WX4) followed by neutral alumina (Sigma Chemical Co.) [method C of Salomon et al. (1974)]. Sucrase, *p*-nitrophenylphosphatase, NADPH–cytochrome *c* reductase, and succinate dehydrogenase were assayed as previously described (Brasitus et al., 1980).

Other Methods. Total lipids were extracted from membrane preparations by the method of Folch et al. (1957). The lipid composition of the extracts was examined by thin-layer

Table I: Fluorescence Polarization and Excited-State Lifetime Studies

probe	membrane type	anisotropy parameter,[a] $[(r_0/r) - 1]^{-1}$	P	mean fluorescence anisotropy, r	excited-state lifetime,[b] τ (ns)	app rotational relaxation time,[c] ρ (ns)
diphenylhexatriene	microvillus	3.69 ± 0.14 (16)	<0.001	0.285	11 (3)	133
	basolateral	1.53 ± 0.10 (11)		0.219	11 (3)	51
retinol	microvillus	3.00 ± 0.35 (5)	<0.01	0.275	8 (1)	72
	basolateral	1.52 ± 0.10 (5)		0.221	8 (1)	37
12-anthroylstearate	microvillus	0.62 ± 0.04 (9)	<0.01	0.109	14 (2)	26
	basolateral	0.40 ± 0.04 (10)		0.081	16 (2)	19
2-anthroylstearate	microvillus	0.89 ± 0.04 (5)	<0.02	0.134	12 (2)	32
	basolateral	0.61 ± 0.02 (5)		0.108	12 (2)	22
dansylphosphatidyl-ethanolamine	microvillus	1.02 ± 0.09 (5)	<0.025	0.162	14 (2)	43
	basolateral	0.80 ± 0.01 (9)		0.142	14 (2)	34

[a] Values are means ± SE; values in parentheses are the numbers of preparations examined. P values for the differences between microvillus and basolateral membranes are indicated. [b] Values are means; values in parentheses are the numbers of preparations examined. [c] Calculation discussed under Experimental Procedures and text footnote 3.

chromatography according to the procedure of Katz et al. (1976). Protein was estimated by the method of Lowry et al. (1951) with bovine serum albumin as standard.

Results

Fluorescence Studies. Preparation of basolateral and microvillus membranes were treated with each of five lipid-soluble fluorescent probes, and Table I summarizes the results of steady-state fluorescence polarization studies and of determinations of τ, the mean lifetime of the excited state. Whereas τ for each probe was similar in the microvillus as compared to the basolateral membrane, the anisotropy parameter, $[(r_0/r) - 1]^{-1}$, was uniformly higher in the microvillus preparation. Ratios of $[(r_0/r) - 1]^{-1}$ in microvillus/basolateral membranes were 2.4 (DPH), 2.0 (retinol), 1.5 (12AS), 1.5 (2AS), and 1.3 (DPE).[4] It is further noteworthy that the anisotropy parameter of 2AS exceeded that of 12AS in both membrane types. In accord with prior observations in multilamellar vesicles of dipalmitoyllecithin (Cadenhead et al., 1977), the anthroyl fluorophor of 12AS experiences greater freedom of motion than that of 2AS, presumably because the latter is localized in the bilayer closer to the aqueous interfaces (Bashford et al., 1976; Cadenhead et al., 1977).

Membrane Composition. Differences in membrane composition are expected to underly the differences in lipid dynamics noted above, and the composition of four preparations of microvillus membranes and three of basolateral membranes was examined. The ratios of protein/lipid (w/w) in the microvillus and basolateral membranes, respectively, were 1.8 ± 0.1 and 0.6 ± 0.2, in agreement with prior reports of relatively more protein in the microvillus membrane (Forstner & Wherrett, 1973; Schachter & Shinitzky, 1977) and relatively more lipid in the basolateral membrane (Douglas et al., 1972). The lipid extracts of each membrane were further examined by thin-layer chromatography, and the results are summarized in Table II. Expressed as the percent by weight of total lipids, the cholesterol content of the membranes did not differ significantly whereas the total phospholipid content of the basolateral lipid was approximately 51% greater than that of the microvillus lipid ($P < 0.025$). Accordingly, the cholesterol/phospholipid molar ratio of microvillus membranes, 0.87 ± 0.06, considerably exceeded that of basolateral membranes, 0.62 ± 0.04 ($P < 0.025$). These values agree with prior studies of rat microvillus membrane lipid (Forstner et al., 1968) and basolateral membrane lipid (Douglas et al., 1972). Moreover, comparable differences between microvillus and basolateral membranes, i.e., higher ratio (w/w) of protein/lipid and higher molar ratio of cholesterol/phospholipid in the microvillus membrane and considerably greater content of total phospholipids in the basolateral membrane, were reported for mouse preparations (Kawai et al., 1974). The values in Table II suggest that the greater phospholipid content of rat basolateral as compared to microvillus membranes is attributable mainly to lecithin and to a lesser extent to sphingomyelin. The molar ratios of sphingomyelin/lecithin in microvillus and basolateral membranes, respectively, were 0.67 ± 0.04 and 0.45 ± 0.06 ($P < 0.05$). An additional noteworthy feature is that the lysolecithin content of microvillus lipid was approximately 6 times that of basolateral lipid ($P < 0.001$). This result suggests that phospholipase A activity, reported to be relatively high in rat intestinal brush borders (Subbaiah & Ganguly, 1970), may account in part for the difference in lecithin content of the two membranes.

Table II: Composition of Lipid Extracts of Rat Microvillus and Basolateral Membranes[a]

component	% by wt of total lipid	
	microvillus	basolateral
cholesterol	14.3 ± 1.2	16.0 ± 1.8
cholesterol esters	4.8 ± 2.8	1.1 ± 0.3
triglycerides	1.6 ± 1.0	3.7 ± 0.3
fatty acids	14.2 ± 3.0	8.9 ± 1.2
total phospholipids	34.1 ± 5.2	51.4 ± 0.5
lecithin	11.3 ± 0.3	29.9 ± 2.1
lysolecithin	3.0 ± 0.2	0.5 ± 0.3
sphingomyelin	7.6 ± 0.4	13.3 ± 0.8
glycolipids	29.6 ± 1.4	19.3 ± 0.4

[a] Values are means ± SE for lipid extracts of four preparations of microvillus membranes and three preparations of basolateral membranes.

Temperature Dependence of Enzyme Activities. A number of basolateral membrane enzyme activities were examined to detect break points in the Arrhenius plot and to explore the relationship of such break points to the critical temperatures of the lipid thermotropic transition. Four membrane preparations were examined for 5′-nucleotidase activity and six preparations for adenylate cyclase, either unstimulated (basal) or stimulated with prostaglandin E_1 (PGE_1) or NaF. Each preparation and each activity tested showed a discontinuity in the Arrhenius plot, as illustrated by plots of the mean values

[4] Table I also lists the values of an apparent rotational relaxation time, ρ, calculated from the Perrin equation (Experimental Procedures) on the assumption that the depolarizing rotations are isotropic and unhindered by structural features of the environment. The ratios for (ρ microvillus)/(ρ basolateral) are essentially the same as the anisotropy parameter ratios since the τ values did not vary with the membrane type.

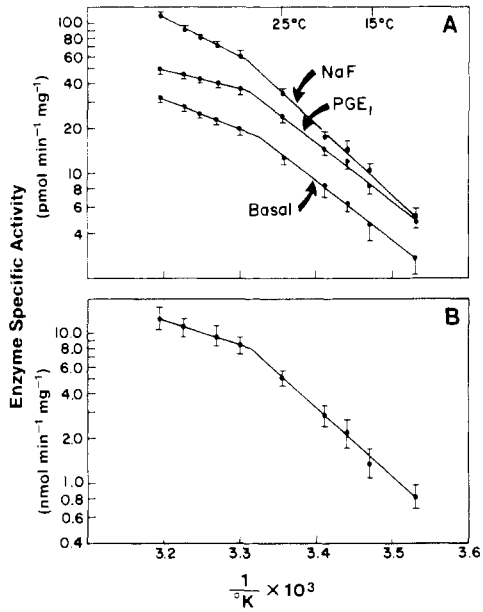

FIGURE 1: Arrhenius plots of rat basolateral membrane enzyme activities. Mean ± SE values of adenylate cyclase (basal, NaF stimulated, and PGE_1 stimulated) are plotted in (A) and values of 5′-nucleotidase in (B). Numbers of preparations tested, break point temperatures, and apparent energies of activation for the individual experiments are listed in Table III. Linear plots shown were determined by the method of least squares.

Table III: Temperature Dependence of Basolateral Membrane Enzyme Activities[a]

enzyme activity	no. of prepn	app break point (°C)	app energy of activation, ΔE (kcal/mol) below break point	app energy of activation, ΔE (kcal/mol) above break point
5′-nucleotidase	4	28.4 ± 2.1	36.1 ± 3.7	16.9 ± 2.1
adenylate cyclase[b]				
basal	6	30.0 ± 1.6	24.7 ± 2.8	8.7 ± 1.9
basal + benzyl alcohol	3	26.0 ± 0.7	23.3 ± 2.4	6.9 ± 1.6
NaF	6	30.2 ± 1.5	30.4 ± 5.3	13.8 ± 1.2
NaF + benzyl alcohol	3	23.1 ± 2.0	35.7 ± 4.7	14.4 ± 3.2
PGE_1	5	28.6 ± 0.6	27.7 ± 0.7	8.7 ± 0.3
PGE_1 + benzyl alcohol	3	22.3 ± 1.1	30.5 ± 0.9	8.4 ± 2.4
$(Na^+ + K^+)$ATPase	7	21.8 ± 1.5	29.8 ± 3.0	12.7 ± 1.5
K^+-dependent *p*-nitrophenylphosphatase	5	20.1 ± 1.0	44.4 ± 6.7	7.5 ± 2.8
MgATPase	11	20.3 ± 1.3	11.3 ± 0.6	5.4 ± 0.9

[a] Values are means ± SE. [b] Activity tested in the absence or presence of benzyl alcohol (50 mM), NaF (10 mM), or PGE_1 (0.05 mM).

in Figure 1. The apparent break point temperatures and energies of activation, ΔE, for all the experiments are summarized in Table III. The break point temperatures observed for 5′-nucleotidase (Figure 1B) and basal and stimulated adenylate cyclase (Figure 1A) are similar, approximately 28–30 °C (Table III), corresponding thereby to the T_p of the lipid thermotropic transition (Brasitus et al., 1980). Stimulation of adenylate cyclase activity was greater with NaF (10 mM) as compared to PGE_1 (0.05 mM), and their effects on ΔE also differed. As indicated in Table III, below the break point, the ΔE values for the basal and stimulated adenylate cyclase activities were similar whereas above the break point the mean ΔE for the NaF-stimulated activity, 13.8 kcal/mol, exceeded the 8.7 kcal/mol observed for the basal ($P < 0.05$) and PGE_1-stimulated ($P < 0.001$) activities.

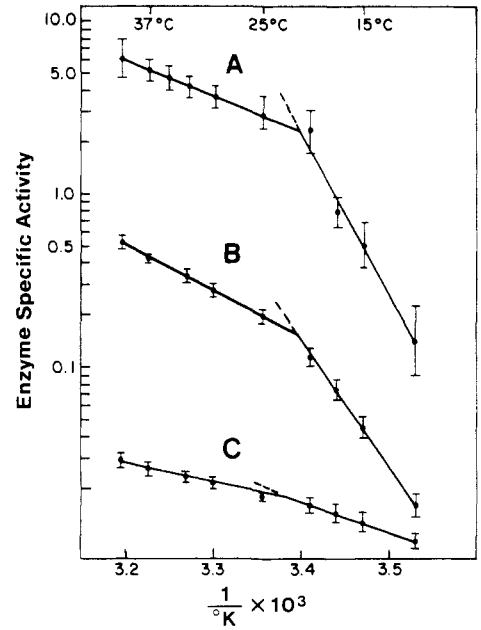

FIGURE 2: Arrhenius plots of rat basolateral membrane K^+-dependent *p*-nitrophenylphosphatase (A; specific activity in nmol min^{-1} mg^{-1}), $(Na^+ + K^+)$-dependent adenosine triphosphatase (B; specific activity in μmol min^{-1} mg^{-1}), and magnesium-dependent adenosine triphosphatase (C; specific activity in μmol min^{-1} per 0.1 mg of membrane protein). Values plotted are means ± SE. Numbers of preparations tested, break point temperatures, and apparent energies of activation for the individual experiments are listed in Table III. Linear plots shown were determined by the method of least squares.

Figure 2 illustrates the results of Arrhenius studies of basolateral membrane $(Na^+ + K^+)$ATPase, K^+-dependent *p*-nitrophenylphosphatase, and MgATPase. It is well established that the first two activities are mediated by the same enzyme complex (Glynn & Karlish, 1975), which comprises the ouabain-sensitive cation pump responsible for active extrusion of intracellular Na^+ in exchange for extracellular K^+, and addition of 1 mM ouabain to our basolateral preparations completely inhibited both of the enzyme activities. A discontinuity in the Arrhenius plot was observed for all three activities in each preparation tested, and the break point temperatures and ΔE values are listed in Table III. The break point temperatures were similar for all three activities, approximately 20–22 °C. The values are 5–7 °C below the lower critical temperature of the lipid transition determined by DSC and fluorescence polarization (Brasitus et al., 1980), and they are approximately 6–10 °C below the corresponding break points observed for 5′-nucleotidase and adenylate cyclase.

Effects of Benzyl Alcohol. A number of reports indicate that benzyl alcohol can increase the fluidity and lower the thermotropic transition temperature of lipid bilayers and biological membranes (Colley & Metcalfe, 1972; Seeman, 1972; Dipple & Houslay, 1978) and can thereby modulate membrane protein activities (Hesketh et al., 1976; Dipple & Houslay, 1978). Seven preparations were loaded with DPH to explore the effects on rat basolateral membranes, and the $[(r_0/r) - 1]^{-1}$ parameter was estimated as a function of temperature, either in the absence or in the presence of 50 mM benzyl alcohol. In each experiment, the alcohol treatment decreased DPH $[(r_0/r) - 1]^{-1}$, as illustrated in Figure 3, and values for the control and treated preparations at 37 °C were 1.58 ± 0.14 and 1.10 ± 0.08 ($P < 0.001$), respectively. (Since addition of benzyl alcohol might cause a shift of membrane DPH to the ambient medium, the suspensions were centrifuged at 20000*g* for 30 min to pellet the membranes, and the DPH

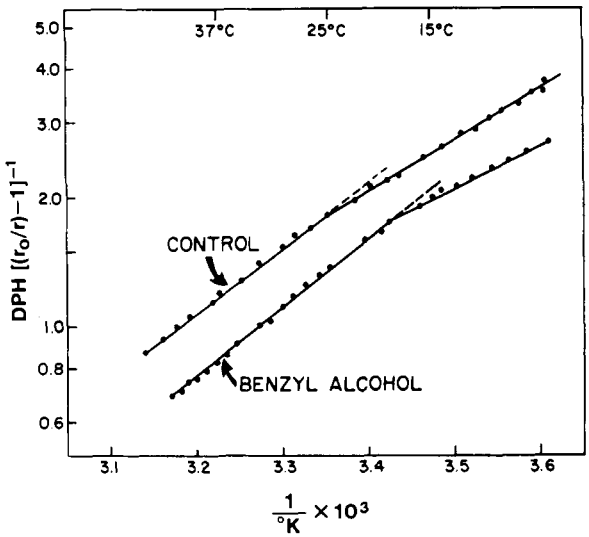

FIGURE 3: Arrhenius plots of the temperature dependence of the diphenylhexatriene anisotropy parameter in rat basolateral membranes. Values for one preparation tested in the absence (control) or presence of 50 mM benzyl alcohol are shown.

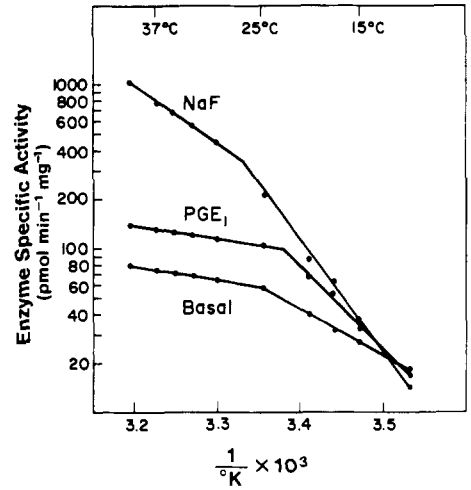

FIGURE 4: Arrhenius plots of rat basolateral membrane adenylate cyclase (basal, NaF stimulated, and PGE_1 stimulated) tested in the presence of 50 mM benzyl alcohol. Values plotted are means. Numbers of preparations tested, break point temperatures, and apparent energies of activation for the individual experiments are listed in Table III.

fluorescence of the supernatant solutions was determined. The supernatant fluorescence in the presence of the alcohol was only 8.0 ± 1.8% of the total fluorescence and did not influence significantly the $[(r_0/r) - 1]^{-1}$ values.) In addition, treatment with benzyl alcohol altered the discontinuity of the Arrhenius plot of DPH $[(r_0/r) - 1]^{-1}$. In five of seven preparations, the break point temperature was lowered from the control value of 26.1 ± 1.2 °C to 20.1 ± 1.4 °C ($P < 0.01$), a decrease of 6.0 ± 1.0 °C (Figure 3). In two preparations, the discontinuity in the Arrhenius plot was not evident following the treatment.

In view of the foregoing effects of benzyl alcohol on lipid fluidity, its influence on basal and stimulated adenylate cyclase was examined with three preparations of basolateral membranes. Figure 4 shows the Arrhenius plots of the resulting basal, PGE_1-stimulated, and NaF-stimulated activities, which may be compared to those obtained in the absence of benzyl alcohol (Figure 1A and Table III). A discontinuity in the Arrhenius plot of each activity persisted in the presence of the alcohol but was shifted to a lower temperature, approximately 4–7 °C below the original value (Table III). This downward shift corresponds to the decrease of 6.0 ± 1.0 °C in the break point temperature of DPH $[(r_0/r) - 1]^{-1}$ noted above. Benzyl alcohol, in addition, markedly increased the specific activities of basal and stimulated adenylate cyclase throughout the range of temperatures tested (Figure 4 vs. Figure 1A). However, the relative effect, expressed as the ratio of enzyme specific activity (+benzyl alcohol)/(−benzyl alcohol), varied considerably with temperature and the particular activity assayed. For example, the ratio for basal adenylate cyclase activity was 6.9 at 10 °C and decreased with rising temperature to 2.5 at 40 °C. In contrast, the ratio for NaF-stimulated activity was 2.8 at 10 °C and increased progressively to 9.4 at 40 °C. The ratio for PGE_1-stimulated activity followed an intermediate pattern, increasing from 3.7 at 10 °C to 4.8 at 20 °C and then decreasing in parallel with the basal activity ratio to 2.9 at 40 °C. Lastly, it is noteworthy that in the temperature range studied, benzyl alcohol increased the specific activity of adenylate cyclase from 100 to 800% while it decreased the DPH $[(r_0/r) - 1]^{-1}$ only 30%. Accordingly, at any constant value of $[(r_0/r) - 1]^{-1}$ in the range 1–2, where the treated membranes were at lower temperatures than the untreated, the adenylate cyclase activity was considerably higher in the alcohol-treated membranes.

Discussion

The lipid thermotropic transition of rat basolateral membranes, previously characterized by DSC and fluorescence polarization studies (Brasitus et al., 1980), clearly influences the membrane enzyme activities of 5′-nucleotidase and basal, NaF-stimulated, and PGE_1-stimulated adenylate cyclase. A break point in the Arrhenius plot of each enzyme activity was observed at 28–30 °C, the T_p of the lipid transition on DSC. The enzyme activities of $(Na^+ + K^+)$ATPase, K^+-dependent *p*-nitrophenylphosphatase, and MgATPase, on the other hand, showed a discontinuity at 20–22 °C, some 5–7 °C below the lower critical temperature of the membrane lipid transition. Kimelberg & Papahadjopoulos (1974) reported a similar result from studies of a rabbit kidney $(Na^+ + K^+)$ATPase which was delipidated and subsequently relipidated with various phospholipids. Following reconstitution with dipalmitoylphosphatidylglycerol or distearoylphosphatidylglycerol, a break point in the Arrhenius plot of the enzyme was observed some 6–8 °C below the lower critical temperature of the lipid transition observed on DSC. The results suggest that the rat basolateral membrane $(Na^+ + K^+)$ATPase and K^+-dependent *p*-nitrophenylphosphatase, which represent one enzyme complex, and the MgATPase, presumably a separate enzyme, function in a lipid microenvironment which is more fluid than the bulk lipid and requires a lower temperature for the transition. In accord with this hypothesis (Kimelberg & Papahadjopoulos, 1974), Grisham & Barnett (1972) noted that the lipid associated with purified lamb kidney $(Na^+ + K^+)$ATPase was more fluid than that of the crude microsomal starting material. The ability of an intrinsic enzyme to determine its lipid microenvironment probably derives from strong and relatively specific protein–lipid interactions. Such interactions could also influence the conformation or aggregation of the protein, and the resulting changes in protein structure with temperature might also determine the break point of the Arrhenius plot, as certain authors have emphasized (Thorneley et al., 1975; Anzai et al., 1978; Gómez-Fernández et al., 1979). In summary, our results demonstrate two types of intrinsic membrane enzymes in rat intestinal basolateral membranes. In the first group, 5′-nucleotidase and adenylate cyclase, the physical state of the bulk lipid affects a rate-limiting step of the reaction, and the discontinuity in the Arrhenius plot of the enzyme coincides with the T_p of the bulk lipid transition. The

second group, $(Na^+ + K^+)$ATPase, K^+-dependent *p*-nitrophenylphosphatase, and MgATPase, is influenced additionally by strong protein–lipid interactions which lower the break point temperature of the Arrhenius plot in relation to the bulk lipid T_p either by providing a microenvironment of increased lipid fluidity or by triggering temperature-induced changes in protein structure.

The adenylate cyclase of enterocyte basolateral membranes is implicated in the regulation of secretion across the small intestinal mucosa and in the pathogenesis of secretory diarrheas induced by cholera and *E. coli* enterotoxins (Field, 1974; Kimberg, 1974). The enzyme complex studied in a number of tissues (Rodbell et al., 1971; Kimberg, 1974; Dipple & Houslay, 1978) is believed to consist of regulatory and receptor components available at the extracellular surface of the plasma membrane and of a catalytic component which mediates cyclic AMP synthesis at the cytosol surface. NaF stimulates directly the inner surface catalytic component, providing thereby a test of the uncoupled enzyme, whereas PGE_1, like hormones generally, stimulates via the receptor and regulatory components and tests the coupled enzyme (Kimberg, 1974). Our results demonstrate that both the uncoupled (NaF-stimulated) and coupled (PGE_1-stimulated) basolateral membrane activities have a similar break point in the Arrhenius plot at the T_p of the bulk lipid. In studies of the adenylate cyclase of rat hepatocyte plasma membranes, on the other hand, the uncoupled and coupled activities responded differently to changes in temperature (Dipple & Houslay, 1978). While the precise effects of membrane lipids on adenylate cyclase may vary with the tissue studied and with other parameters not yet characterized, it is reasonable to conclude that modulation by the lipids can occur. The effects of benzyl alcohol in our experiments and in the hepatocyte membrane studies of Dipple & Houslay (1978) further support this conclusion. When added to basolateral membranes, benzyl alcohol (50 mM) increased lipid fluidity as assessed by DPH $[(r_0/r) - 1]^{-1}$ and decreased the lower critical temperature of the lipid transition by approximately 6 °C. Concomitantly, the Arrhenius plot break points for basal and stimulated adenylate cyclase activities decreased by 4–7 °C (Table III). The benzyl alcohol increased the adenylate cyclase activities 2–9-fold with no significant decrease in ΔE (Table III) and with only a modest increase in lipid fluidity, i.e., 30% reduction in DPH $[(r_0/r) - 1]^{-1}$. Moreover, a comparison of alcohol-treated vs. untreated membranes, assayed at different temperatures so that the DPH $[(r_0/r) - 1]^{-1}$ value was the same for both, showed that the treated membranes, at lower temperature, had a higher specific activity of adenylate cyclase. We conclude that in basolateral membranes benzyl alcohol influences adenylate cyclase only in part via effects on the fluidity and transition temperatures of the bulk lipid and in part via other effects on the protein complex.

Steady-state fluorescence polarization has provided a sensitive technique for examining the lipid fluidity[1] of a variety of artificial and natural membranes (Cogan et al., 1973; Vanderkooi et al., 1974; Shinitzky & Barenholz, 1974, 1978; Jacobson & Papahadjopoulos, 1975), but the information obtained is limited because the observations are probe dependent (Lakowicz et al., 1979b) and do not distinguish different mechanisms affecting the depolarization of the fluorophor. In comparing microvillus and basolateral membranes, therefore, we sought to overcome some of the limitations of the technique by using five fluorescent molecules which differ in structure and shape and which localize in various domains of the bilayer. Accordingly, it is significant that the $[(r_0/r) - 1]^{-1}$ value of each probe is uniformly greater in microvillus as compared to basolateral membranes and that the differences are independent of changes in the excited-state lifetimes. Further, as reported previously (Brasitus et al., 1980), DPH $[(r_0/r) - 1]^{-1}$ is considerably greater in liposomes prepared from microvillus as compared to basolateral membrane lipid, and the difference is maintained throughout the temperature range 0–40 °C, i.e., above and below the thermotropic transition. Although it is not clear from these results to what extent the membrane lipid differences result from viscous forces restraining the rates of rotation of the probes, i.e., "microviscosity" (Weber, 1953; Cogan et al., 1973; Shinitzky & Barenholz, 1978), or from structural parameters which hinder or limit the depolarizing rotations (Chen et al., 1977; Dale et al., 1977; Kawato et al., 1977; Kinosita et al., 1977; Lakowicz et al., 1979a,b; Veatch & Stryer, 1977), the uniform pattern observed with all the probes indicates that lipid molecules in the basolateral membrane generally experience less restraint to motional freedom, and in this sense, the basolateral membrane has greater "lipid fluidity".[1]

The difference in lipid dynamics of the antipodal plasma membranes can be related to differences in their composition. The higher molar ratios of cholesterol/phospholipid and sphingomyelin/lecithin characteristic of the microvillus membrane lipid have been correlated with decreased lipid fluidity in model bilayers and natural membranes (Chapman & Penkett, 1966; Hubbell & McConnell, 1971; Oldfied & Chapman, 1971; Cogan et al., 1973; Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978), and the greater ratio of protein/lipid of the microvillus membrane is also expected to decrease lipid fluidity (Schachter & Shinitzky, 1977; Brasitus et al., 1980). It seems reasonable to suggest that the differences in lipid composition and fluidity of basolateral as compared to microvillus membranes have functional significance and that further studies of these membranes should clarify the questions of function and the mechanisms for maintaining the differences in lipid composition.

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References

Anzai, K., Kirino, Y., & Shimizu, H. (1978) *J. Biochem. (Tokyo) 84*, 815–821.

Bashford, C. L., Morgan, C. G., & Radda, G. K. (1976) *Biochim. Biophys. Acta 426*, 157–172.

Blank, M. (1976) in *Membranes and Diseases* (Bolis, L., Hoffman, J. F., & Leaf, A., Eds.) pp 81–88, Raven Press, New York.

Bloom, W., & Fawcett, D. W. (1968) *A Textbook of Histology*, pp 560–569, W. B. Saunders, Philadelphia, PA.

Brasitus, T. A., Schachter, D., & Mamouneas, T. G. (1979) *Biochemistry 18*, 4136–4144.

Brasitus, T. A., Tall, A. R., & Schachter, D. (1980) *Biochemistry 19*, 1256–1261.

Cadenhead, D. A., Kellner, B. M. J., Jacobson, K., & Papahadjopoulos, D. (1977) *Biochemistry 16*, 5386–5392.

Chapman, D., & Penkett, S. A. (1966) *Nature (London) 211*, 1304–1305.

Chen, L. A., Dale, R. E., Roth, S., & Brand, L. (1977) *J. Biol. Chem. 252*, 2163–2169.

Cogan, U., Shinitzky, M., Weber, G., & Nishida, T. (1973) *Biochemistry 12*, 521–528.
Colley, C. M., & Metcalfe, J. C. (1972) *FEBS Lett. 24*, 241–246.
Dale, R. E., Chen, L. A., & Brand, L. (1977) *J. Biol. Chem. 252*, 7500–7510.
Dipple, I., & Houslay, M. D. (1978) *Biochem. J. 174*, 179–190.
Douglas, A. P., Kerley, R., & Isselbacher, K. J. (1972) *Biochem. J. 128*, 1329–1338.
Field, M. (1974) *Gastroenterology 66*, 1063–1084.
Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.
Forstner, G. G., & Wherrett, J. R. (1973) *Biochim. Biophys. Acta 306*, 446–459.
Forstner, G. G., Tanaka, K., & Isselbacher, K. J. (1968) *Biochem. J. 109*, 51–59.
Fox, C. F. (1975) in *Biochemistry of Cell Walls and Membranes* (Fox, C. F., Ed.) Vol. 2, pp 279–306, University Park Press, Baltimore, MD.
Fujita, M., Kawai, K., Asano, S., & Nakao, M. (1973) *Biochim. Biophys. Acta 307*, 141–151.
Glynn, I. M., & Karlish, S. J. D. (1975) *Annu. Rev. Physiol. 37*, 13–55.
Gómez-Fernández, J. C., Goñi, F. M., Bach, D., Restall, C., & Chapman, D. (1979) *FEBS Lett. 98*, 224–228.
Grisham, C. M., & Barnett, R. E. (1972) *Biochim. Biophys. Acta 266*, 613–624.
Hesketh, T. R., Smith, G. A., Houslay, M. D., McGill, K. A., Birdsall, N. J. M., Metcalfe, J. C., & Warren, G. B. (1976) *Biochemistry 15*, 4145–4151.
Hubbell, W. L., & McConnell, H. M. (1971) *J. Am. Chem. Soc. 93*, 314–326.
Jacobson, K., & Papahadjopoulos, D. (1975) *Biochemistry 14*, 152–161.
Katz, S. S., Shipley, G. G., & Small, D. M. (1976) *J. Clin. Invest. 58*, 200–211.
Kawai, K., Fujita, M., & Nakao, M. (1974) *Biochim. Biophys. Acta 369*, 222–233.
Kawato, S., Kinosita, K., Jr., & Ikegami, A. (1977) *Biochemistry 16*, 2319–2324.
Kenny, A. J., & Booth, A. G. (1978) *Essays Biochem. 14*, 1–43.
Kimberg, D. V. (1974) *Gastrenterology 67*, 1023–1064.
Kimelberg, H. K., & Papahadjopoulos, D. (1974) *J. Biol. Chem. 249*, 1071–1080.
Kinosita, K., Kawato, S., & Ikegami, A. (1977) *Biophys. J. 20*, 289–305.
Kowarski, S., & Schachter, D. (1973) *J. Clin. Invest. 52*, 2765–2773.
Lakowicz, J. R., Prendergast, F. G., & Hogen, D. (1979a) *Biochemistry 18*, 508–519.
Lakowicz, J. R., Prendergast, F. G., & Hogen, D. (1979b) *Biochemistry 18*, 520–527.
Lee, A. G. (1975) *Prog. Biophys. Mol. Biol. 29*, 3–56.
Lewis, B. A., Gray, G. M., Coleman, R., & Michell, R. H. (1975) *Biochem. Soc. Trans. 3*, 752–753.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Melchior, D. L., & Steim, J. M. (1976) *Annu. Rev. Biophys. Bioeng. 5*, 205–238.
Murer, H., Hopfer, U., Kinne-Saffran, E., & Kinne, R. (1974) *Biochim. Biophys. Acta 345*, 170–179.
Murer, H., Ammann, E., Biber, J., & Hopfer, U. (1976) *Biochim. Biophys. Acta 433*, 509–519.
Oda, T. (1976) in *Recent Progress in Electron Microscopy of Cells and Tissues* (Yamada, E., Mizuhira, V., Kurosumi, K., & Nagano, T., Eds.) pp 13–17, University Park Press, Baltimore, MD.
Okada, Y., Irimajiri, A., & Inouye, A. (1977) *J. Membr. Biol. 31*, 221–232.
Oldfield, E., & Chapman, D. (1971) *Biochem. Biophys. Res. Commun. 43*, 610–616.
Razin, S. (1975) *Prog. Surf. Membr. Sci. 9*, 257–342.
Rodbell, M., Birnbaumer, L., & Pohl, S. L. (1971) in *Colloquium on the Role of Adenyl Cyclase and Cyclic 3',5'-AMP in Biological Systems* (Rall, T. W., Rodbell, M., & Condliffe, P., Eds.) p 59, Fogarty International Center, U.S. Government Printing Office, Washington, DC.
Rose, R. C., & Schultz, S. G. (1971) *J. Gen. Physiol. 57*, 639–663.
Salomon, Y., Londos, C., & Rodbell, M. (1974) *Anal. Biochem. 58*, 541–548.
Schachter, D., & Shinitzky, M. (1977) *J. Clin. Invest. 59*, 536–548.
Schachter, D., Cogan, U., & Shinitzky, M. (1976) *Biochim. Biophys. Acta 448*, 620–624.
Schlatz, L. J., Kimberg, D. V., & Cattieu, K. A. (1978) *Gastroenterology 75*, 838–846.
Seeman, P. (1972) *Pharmacol. Rev. 24*, 583–653.
Shinitzky, M., & Barenholz, Y. (1974) *J. Biol. Chem. 249*, 2652–2657.
Shinitzky, M., & Inbar, M. (1976) *Biochim. Biophys. Acta 433*, 133–149.
Shinitzky, M., & Barenholz, Y. (1978) *Biochim. Biophys. Acta 515*, 367–394.
Siegel, G. J., & Goodwin, B. G. (1972) *J. Biol. Chem. 247*, 3630–3637.
Subbaiah, P. V., & Ganguly, J. (1970) *Biochem. J. 118*, 233–239.
Tada, M., Yamamoto, T., & Tonomura, Y. (1978) *Physiol. Rev. 58*, 1–79.
Thorneley, R. N. F., Eady, R. R., & Yates, M. G. (1975) *Biochim. Biophys. Acta 403*, 269–284.
Vanderkooi, J., Fischkoff, S., Chance, B., & Cooper, R. A. (1974) *Biochemistry 13*, 1589–1595.
Veatch, W. R., & Stryer, L. (1977) *Biophys. J. 17*, 69a.