Thermotropic Transitions in Rat Intestinal Plasma Membranes Studied by Differential Scanning Calorimetry and Fluorescence Polarization†

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ABSTRACT: The microvillus (luminal) and basolateral (contraluminal) regions of the plasma membrane of the rat small intestinal enterocyte were isolated separately and examined by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene and by differential scanning calorimetry. Calorimetry of the microvillus membrane preparations showed a reversible lipid thermotropic transition in the range 23–39 °C with T_p of 31 °C and an enthalpy of ~0.1 cal/g. Corresponding studies of the basolateral membranes revealed a similar lipid transition in the range 27–40 °C with T_p of 28–30 °C and an enthalpy of ~0.15 cal/g. Total lipid extracts, both hydrated and unhydrated, of the enterocyte membranes yielded similar thermotropic transitions, but the enthalpies exceeded those of the whole membranes. Irreversible transitions owing to protein denaturation were observed with both membrane types at temperatures in excess of ~60 °C. Fluorescence polarization experiments detected the lower critical temperature of the lipid transition in each membrane, i.e., at 23 °C in microvillus membranes and at 26 °C in basolateral membranes. Lipid fluidity as assessed by diphenylhexatriene fluorescence polarization was considerably greater in dispersions of the extracted lipids as compared to the intact membranes and in basolateral as compared to microvillus preparations. The results are explicable in terms of protein–lipid interactions and the higher cholesterol/phospholipid molar ratio of microvillus as compared to basolateral membrane lipid. The calorimetric observations indicate that the enterocyte membranes function in vivo at or near the upper critical temperature of the lipid transition.

Prior publications (Schachter et al., 1976; Schachter & Shinitzky, 1977; Brasitus et al., 1979a) describe observations on the lipid fluidity of isolated rat intestinal microvillus membranes as assessed by steady-state fluorescence polarization of lipid-soluble fluorophores. The studies indicate that the lipid fluidity of this specialized plasma membrane organelle is low compared to other mammalian membranes and that a lipid thermotropic transition can be detected in the vicinity of 26 ± 2 °C. The lipid transition influences the function of certain of the microvillus membrane proteins. Arrhenius plots of D-glucose transport, *p*-nitrophenylphosphatase, and calcium-dependent and magnesium-dependent adenosine triphosphatase activities show a discontinuity in slope near the lipid transition temperature (Brasitus et al., 1979a). Since these functions experience the lipid transition, they may appropriately be termed "intrinsic" activities. In contrast, the microvillus membrane digestive enzymes lactase, maltase, sucrase, leucine aminopeptidase, and γ-glutamyl transpeptidase yield one slope on an Arrhenius plot and may be classified as "extrinsic" activities, functionally independent of the lipid transition.

The characterization of lipid thermotropic transitions has provided a useful tool for the study of lipid–protein interactions in a number of membrane types (Tourtellotte, 1972; Linden et al., 1973; Fox, 1975; Lee, 1975, 1977a,b; Razin, 1975; Melchior & Steim, 1976) and in plasma lipoproteins (Tall et al., 1978). Accordingly, it was of interest to isolate separately the microvillus (luminal) and basolateral (contraluminal) plasma membranes of the rat enterocyte and to examine the membrane preparations by differential scanning calorimetry (DSC)[1] and fluorescence polarization. The results described below demonstrate lipid thermotropic transitions which are relatively broad and of low enthalpy in each of these plasma membranes.

Experimental Procedures

Membrane Preparations. Albino male rats of the Sherman strain weighing 250–300 g were fasted 18 h with water ad libitum before removal of the small intestine. Microvillus membranes were prepared as previously described (Schachter & Shinitzky, 1977; Brasitus et al., 1979a) by using procedures reported by Schmitz et al. (1973) and Hopfer et al. (1973). Preparations were maintained at 2–5 °C throughout. Mucosal scrapings of the proximal half of the small intestine of each of 6–12 rats were pooled, homogenized, and treated with 10 mM $CaCl_2$ (Schmitz et al., 1973), and the brush border particulate fraction was obtained by differential centrifugation. After homogenization at high speed (Hopfer et al., 1973), the microvillus membranes were isolated by differential centrifugation and suspended in 13 mM Tris buffer of pH 7.4. When not tested immediately, the membranes were stored frozen at −15 °C. The purity and comparability of these preparations were assessed by estimations of sucrase and *p*-nitrophenylphosphatase specific activities. Preparations used were purified from 10- to 20-fold as compared to the original homogenates.

Basolateral membranes were prepared from enterocytes which were isolated from the proximal half of the small intestine by the procedure of Stern & Jensen (1966) as modified by Murer et al. (1974). Enterocytes prepared from each of 6–12 rats were suspended in an ice-cold buffer (final pH 7.5) consisting of 0.25 M sucrose, 10 mM triethanolamine hydrochloride, and 0.5 mM EDTA (STE buffer). The pooled suspension containing ~30 g wet weight of cells was sedimented at 500*g* for 10 min, and the cells were washed 3 times

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[1] Abbreviations used: ATP, adenosine 5′-triphosphate; ATPase, adenosine triphosphatase; DPH, 1,6-diphenyl-1,3,5-hexatriene; DSC, differential scanning calorimetry; STE buffer, sucrose (0.25 M), triethanolamine hydrochloride (10 mM), and EDTA (0.5 mM); T_p, peak temperature of the thermotropic transition.

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with STE buffer, resuspended in 10 mL of ice-cold STE buffer, and homogenized with 25 strokes in a tight-fitting Dounce homogenizer. The homogenate was diluted 1:1 with cold STE buffer, and basolateral membranes were prepared by the method of Murer et al. (1974), except that a tight-fitting Dounce homogenizer was used throughout, as described by Schlatz et al. (1978). Following discontinuous sucrose density gradient centrifugation, the basolateral membrane fraction collected at the 20%/30% interface was removed, diluted with STE buffer, pelleted by centrifugation at 140000*g* for 30 min, and finally suspended in the same buffer. When not tested immediately, the membranes were stored at −15 °C. The purity and comparability of the preparations were assessed by estimations of the marker enzymes $(Na^+ + K^+)$-dependent adenosine triphosphatase [$(Na^+ + K^+)$-ATPase] and 5′-nucleotidase (see below). Basolateral membrane preparations were purified from 10- to 15-fold as compared to the starting homogenates.

The basolateral and microvillus membrane preparations were also tested for contamination with either microsomal or mitochondrial membranes by using the enzyme markers NADPH-cytochrome *c* reductase and succinate dehydrogenase, respectively. For these marker enzymes, respectively, the activity ratios (isolated membranes)/(original homogenates) were on the average 0.09 (range 0–0.12) and 0.09 (range 0–0.13) for the microvillus membranes and 0.09 (range 0.06–0.11) and 0.08 (range 0.05–0.10) for the basolateral preparations.

Lipid Extracts and Liposomes. Total lipids were extracted from microvillus or basolateral membranes by the method of Folch et al. (1957). For preparation of sonicated dispersions of lipid (liposomes), the dried, extracted lipid was suspended in phosphate-buffered saline (Dulbecco & Vogt, 1954) to a final concentration of ~0.3 mg/mL and the mixture sonicated for 10 min, under N_2, at 5 °C. Thereafter, the preparations were centrifuged for 10 min at 10000*g*, and the final supernatant suspensions were used.

Fluorescence Polarization Studies. Membranes and liposome suspensions were treated with the lipid-soluble fluorophore 1,6-diphenyl-1,3,5-hexatriene (DPH), and steady-state fluorescence polarization measurements were made in an SLM polarization spectrofluorometer, as previously described (Schachter & Shinitzky, 1977). The polarization of fluorescence was expressed as the fluorescence anisotropy *r*, and the anisotropy parameter $(r_0/r - 1)^{-1}$ was calculated by using a value of $r_0 = 0.362$ for DPH (Shinitzky & Barenholz, 1974). The anisotropy parameter varies directly with the rotational relaxation time of the probe and hence is related inversely to the lipid fluidity (Shinitzky & Inbar, 1976). For examination of the effects of temperature, preparations were warmed initially to 40 °C and the fluorescence polarization was estimated every 1 to 2 °C as the suspensions cooled slowly to 0 °C. Plots of log $(r_0/r - 1)^{-1}$ vs. 1/K were constructed to detect thermotropic transitions (Shinitzky & Barenholz, 1978).

Differential Scanning Calorimetry. Samples of membranes (equivalent to 4–10 mg of membrane protein) or extracted membrane lipids (0.8–3.0 mg of lipid) were hermetically sealed in 75-μL stainless steel pans and examined in a Perkin-Elmer differential scanning calorimeter, Model DSC-2, as previously described (Tall et al., 1977). The protein/lipid ratios (w/w) of the microvillus and basolateral membrane samples, respectively, were 2.02 ± 0.30 and 0.60 ± 0.21. For examination of lipid extracts, a chloroform–methanol solution of the lipids was added to the DSC pan, dried under a stream of N_2, and then dried under vacuum. In some experiments lipids were hydrated by addition of 0.15 M NaCl (pH adjusted to 8.4 with NH_4OH) followed by incubation at 24 °C for 2 h. Heating and cooling rates of 2.5 or 5 °C/min were used, and most measurements were made at full sensitivity (0.1–0.5 mcal/s). The instrument was calibrated with cyclohexane and indium standards and with aqueous samples of dimyristoylphosphatidylcholine, as previously described (Tall et al., 1977). Enthalpy measurements were obtained from the areas under the thermal transitions as compared to the standards. Each sample was scanned at least 3 times in the temperature range of interest, and from 3 to 5 samples of each membrane preparation and of its extracted lipid were examined.

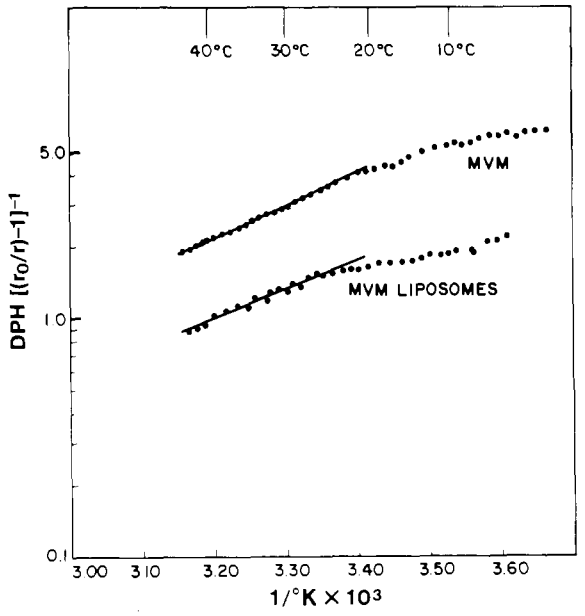

FIGURE 1: Arrhenius plots of the anisotropy parameter of diphenylhexatriene in a sample of isolated microvillus membranes (MVM, upper curve) and in liposomes prepared from a lipid extract of these membranes (lower curve).

Other Methods. Sucrase and *p*-nitrophenylphosphatase were assayed as described previously (Brasitus et al., 1979a). $(Na^+ + K^+)$-ATPase was estimated by a modification of the procedure of Siegel & Goodwin (1972). A sample of membrane containing 1 to 2 μg of protein was added to 40 μL of a reaction mixture containing 10 mM $MgCl_2$, 10 mM imidazole hydrochloride buffer of pH 7.1, 5 mM adenosine 5′-triphosphate (ATP), 0.1 μCi of [γ-^{32}P]ATP (New England Nuclear, 10–20 Ci/mmol), 120 mM NaCl, and 20 mM KCl. After incubation for 15 min at 37 °C, the [$^{32}P_i$]phosphate released was quantified (Siegel & Goodwin, 1972) and compared to the corresponding release in the absence of NaCl plus KCl. The activity of 5′-nucleotidase was estimated as described by Murer et al. (1974). The release of inorganic phosphate from adenosine 5′-monophosphate minus the corresponding release from adenosine 3′-monophosphate was determined as the "specific" 5′-nucleotidase activity. NADPH-cytochrome *c* reductase was assayed by the method of Masters et al. (1967) and succinate dehydrogenase by the procedure of King (1967). The composition of total lipid extracts of microvillus and basolateral membrane preparations was examined by thin-layer chromatography according to the procedure of Katz et al. (1976). Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Results

Fluorescence Polarization Studies. The effects of temperature on the anisotropy parameter, $(r_0/r - 1)^{-1}$, of DPH

Table I: Thermotropic Transition Temperatures and Diphenylhexatriene Anisotropy Parameters in Membranes and Liposomes[a]

membrane	preparation	no. of prep-ara-tions	tran-sition temp (°C)	DPH $(r_0/r - 1)^{-1}$ 25 °C	DPH $(r_0/r - 1)^{-1}$ 37 °C
microvillus	intact membrane	20	23 ± 1.1	3.51 ± 0.21	2.30 ± 0.32
	liposomes	3	23 ± 2.2	1.47 ± 0.10	1.08 ± 0.01
basolateral	intact membrane	12	26 ± 1.5	1.41 ± 0.04	1.01 ± 0.01
	liposomes	3	25 ± 1.7	0.73 ± 0.05	0.47 ± 0.01

[a] Values are means ± SE.

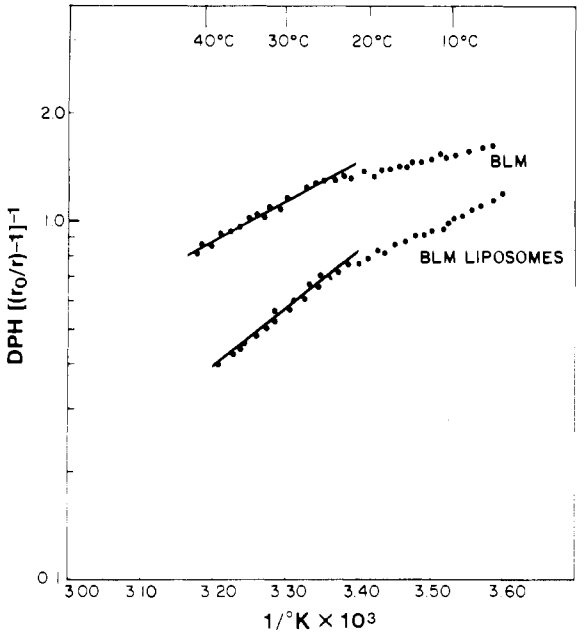

FIGURE 2: Arrhenius plots of the anisotropy parameter of diphenylhexatriene in a sample of basolateral membranes (BLM) and in liposomes prepared from a lipid extract of these membranes (lower curve).

in microvillus membranes and in liposomes prepared from microvillus membrane lipid are illustrated by representative Arrhenius plots in Figure 1. As noted previously (Schachter & Shinitzky, 1977), the plots appear reasonably linear as the temperature falls from approximately 40 to 23 °C, but a distinct change in slope occurs with further cooling and the plots become somewhat curvilinear. The lipid thermotropic transition denoted by the change in slope was observed in 20 preparations of microvillus membranes and in 3 preparations of the corresponding liposomes. The results summarized in Table I indicate that the transition temperatures are similar for the membranes and liposomes, 23 ± 1.1 and 23 ± 2.2 °C, respectively. On the other hand, the anisotropy parameter for DPH at 25 or 37 °C is over twice as great in the membranes as in the liposomes, indicating that the fluidity of the lipids is considerably greater in the liposomes.

Comparable evidence for a lipid thermotropic transition was obtained in 12 preparations of basolateral membranes and in 3 preparations of the corresponding liposomes. Figure 2 shows the Arrhenius plots for one preparation, and Table I summarizes all the experiments. Again, the transition temperatures are similar for the intact membranes and the liposomes, 26 ± 1.5 and 25 ± 1.7 °C, respectively. The transition temperatures do not differ significantly from those of the microvillus membranes. At 25 and 37 °C the DPH $(r_0/r - 1)^{-1}$ values are approximately twice as high in the membranes as compared to the liposomes (Table I), indicating greater lipid fluidity in the liposomes. Finally, it is noteworthy that the $(r_0/r - 1)^{-1}$ values are considerably higher in the microvillus as compared to the corresponding basolateral preparations.

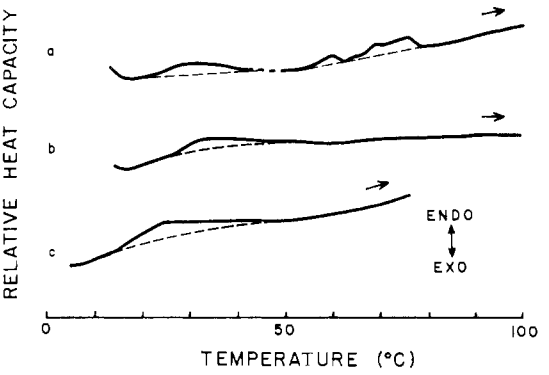

FIGURE 3: Differential scanning calorimetry heating curves of (a) intact microvillus membranes, (b) the same sample following heating to 100 °C, and (c) hydrated lipids of microvillus membranes. The sample contained 4.5 mg of lipid in (a) and (b) and 3 mg of lipid in (c). Samples were heated at 5 °C/min. Sensitivity settings for (a) were 0.1 mcal/s at temperatures below the dashed curve (i.e., in the region of the lipid transition) and 1.0 mcal/s at the higher temperatures (i.e., in the region of the denaturation endotherms). Sensitivity settings for (b) and (c) respectively were 0.1 and 0.2 mcal/s. Endo and exo represent the directions of endothermic and exothermic transitions.

Differential Scanning Calorimetry. Each of five preparations of microvillus membranes demonstrated a broad, reversible thermotropic transition between 23 and 39 °C (Figure 3a), attributable to lipid, since a similar transition was seen in the lipid extracts (Figure 3c). The peak temperature (T_p) for all the microvillus membrane samples was 31 ± 2 °C. Although this transition was observed in all the samples, its enthalpy was very small, ~0.1 cal/g of lipid. When the membranes were heated to higher temperatures, a complex endotherm appeared (Figure 3a). This probably represents denaturation of membrane proteins, since it was irreversible (Figure 3b) and absent from scans of the lipid extracts (Figure 3c). The total enthalpy of the denaturation endotherm, ~1.5 cal/g of protein, is similar to the value reported for erythrocyte spectrin, 2.0 cal/g (Brandts et al., 1977). In all the samples a major peak of the denaturation endotherm was observed at 74–78 °C and a smaller peak at 60–63 °C. Heating scans of three preparations of rehydrated microvillus membrane lipid showed the lipid transition in the range 14–38 °C (Figure 3c), with T_p of 27 ± 2 °C. The enthalpy, ~0.4 cal/g of lipid, was greater than the corresponding value for the intact membranes. On cooling to −60 °C and reheating, there was no significant change in the transition, indicating no thermotropic crystallization of lipids.

Three preparations of basolateral membranes and two preparations of rehydrated basolateral membrane lipid were examined by DSC (Figure 4). The intact membranes showed a broad, reversible transition between 27 and 40 °C and a complex, irreversible, denaturation endotherm (parts a and b of Figure 4). The T_p of the lipid transition was at 28–30 °C, and the enthalpy was ~0.15 cal/g of lipid. The denaturation endotherm included a major peak of T_p = 63–66 °C and smaller, more variable peaks at 60, 70, and 77 °C. The total enthalpy of the denaturation endotherm was 2.4 cal/g

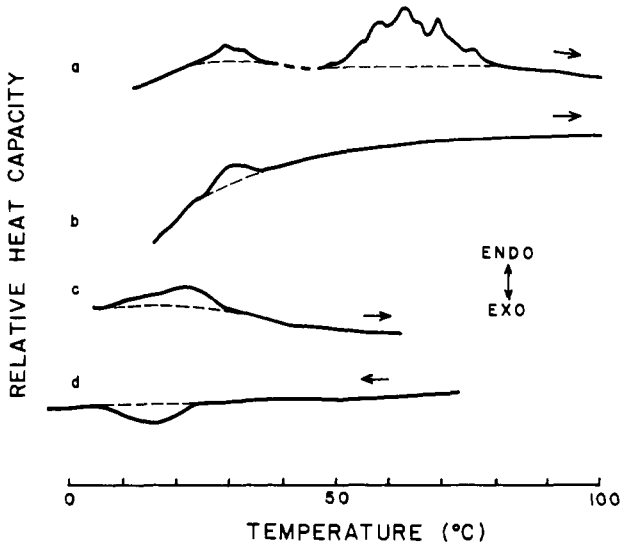

FIGURE 4: Differential scanning calorimetry heating curves of (a) intact basolateral membranes, (b) the same sample following heating to 100 °C, and (c) hydrated lipids of basolateral membranes. In (d) is shown the cooling curve of the hydrated lipids. The sample in (a) and (b) contained 1.3 mg of lipid and that in (c) and (d) contained 2.3 mg of lipid. Samples were heated or cooled at 5 °C/min. Sensitivity settings for (a) were 0.1 mcal/s below the dashed curve (i.e., in the region of the lipid transition) and 0.2 mcal/s above the dashed curve (i.e., for the denaturation endotherm). The sensitivity setting for (b) was 0.1 mcal/s and for (c) and (d) it was 0.2 mcal/s. Endo and exo represent the directions of endothermic and exothermic transitions.

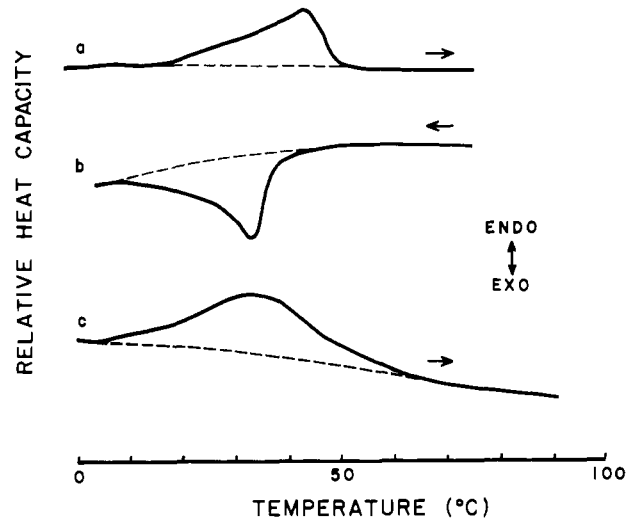

FIGURE 5: Differential scanning calorimetry heating curve (a) and cooling curve (b) of dried microvillus membrane lipids and heating curve (c) of dried basolateral membrane lipids. The samples contained 0.84 mg of lipid in (a) and (b) and 0.70 mg of lipid in (c). All samples were heated or cooled at 5 °C/min at a sensitivity of 0.2 mcal/s. Endo and exo represent the directions of endothermic and exothermic transitions.

of protein. After the membranes were heated to 100 °C, a repeat scan showed only the lipid transition (Figure 4b) with an enthalpy of 0.23 cal/g of lipid. Rehydrated basolateral membrane lipid showed a transition in the range 13–33 °C (Figure 4c), with T_p of 22–24 °C and an enthalpy of 0.55 cal/g of lipid. This enthalpy value exceeds that observed for the lipid transition of the intact membranes.

Two samples of extracted microvillus membrane lipid and one sample of basolateral membrane lipid were examined without prior rehydration, and DSC scans are illustrated in Figure 5. On the initial scan the microvillus lipid showed a broad transition (heating 15–50 °C; cooling 5–40 °C) with T_p on heating of 42 °C (Figure 5a) and on cooling of 34 °C (Figure 5b). The enthalpy of the transition, 2 to 3 cal/g of lipid, greatly exceeded the values for hydrated lipids or intact membranes. The sample of basolateral membrane lipid yielded a very broad transition (5–60 °C) with T_p of 34 °C (Figure 5c) and an enthalpy value of 4.5 cal/g of lipid.

Discussion

The luminal (microvillus) and contraluminal (basolateral) plasma membranes of the enterocyte which lines the small intestine are highly differentiated to regulate the digestion, absorption, and secretion of essential nutrients and other substances. With the experimental results described above, the occurrence of a lipid thermotropic transition in each of these antipodal rat enterocyte membranes is supported by three types of evidence: differential scanning calorimetry, fluorescence polarization observations, and Arrhenius plots of membrane protein activities. The transition temperature detected by fluorescence polarization of DPH (Table I) corresponds to the lower limit of the transition temperature range determined by DSC of each membrane type. For the microvillus membranes the DPH studies yielded a mean transition temperature of 23 °C[2] and DSC indicated a temperature range of 23–39 °C; corresponding values for the basolateral membranes were 26 and 27–40 °C, respectively. As noted previously (Brasitus et al., 1979a), we have thus far been unable to detect the upper critical temperature of the transition by fluorescence polarization. Arrhenius plots of four microvillus membrane activities, *p*-nitrophenylphosphatase, calcium-dependent ATPase, magnesium-dependent ATPase, and active transport of D-glucose, showed a discontinuity in slope (change in energy of activation) at 25–30 °C (Brasitus et al., 1979a). Hence, these protein-dependent activities experience the lipid transition a few degrees above the lower critical temperature indicated by DSC. Similar findings of a broad lipid transition on DSC and a relatively sharp break in the Arrhenius plot of 2-deoxyglucose transport were reported for *Mycoplasma* membranes (Tourtellotte, 1972).[3]

In a prior study (Schachter & Shinitzky, 1977), we concluded that the thermotropic transition temperature observed in microvillus membranes by fluorescence polarization is sufficiently below 37 °C to preclude a physiological role for the change in state of the lipids. The present DSC studies, however, indicate that the microvillus and basolateral membranes function in vivo near or at the upper extremity of the thermotropic transition. The physiological implications of this arrangement are unknown, although Linden et al. (1973) and Lee (1975) have pointed out that lateral compressibility of the membrane lipids is markedly enhanced when the temperature

[2] This temperature agrees well with prior results (Schachter & Shinitzky, 1977) if one takes into account the region of the small intestine from which the microvillus membranes were prepared. In the prior studies the transition temperatures determined for duodenal, jejunal, and ileal preparations, respectively, were 21–22, 26, and 28–34 °C; i.e., they increased with distance from the proximal to the distal end of the small intestine. The mean ± SE value for all segments was 26 ± 2 °C. Preparations in the present studies used the proximal half of the intestine, a mixture of duodenal and jejunal microvillus membranes.

[3] Evidence that both the upper and lower critical temperatures of a lipid transition can be detected by Arrhenius plots of membrane protein activities has been described (Linden et al., 1973; Fox, 1975). However, we are thus far unable to detect the upper critical temperature of the transition in enterocyte membranes by comparable experiments (Brasitus et al., 1979a).

is lowered through the upper extremity of the lipid transition and two phases coexist. Presumably, the increased compressibility could facilitate the insertion of substances into the membrane and thereby enhance processes of transport or membrane assembly (Linden et al., 1973). Whether this or other factors are involved, it is worthy of emphasis that the function of enterocyte membranes may be modulated by changes in state of the lipids in the range of physiological body temperatures.

The reversible lipid transitions of microvillus and basolateral membranes seen by DSC are broad and of low enthalpy. Broad transitions, often attributed to low cooperativity (Oldfield & Chapman, 1972; Hinz & Sturtevant, 1972a; McElhaney et al., 1973; Lee, 1975), are frequently observed for complex mixtures of lipids with different transition temperatures and have been reported for membranes of *Mycoplasma* (Steim et al., 1969; Tourtellotte, 1972; Razin, 1975), *Escherichia coli* (Hoest et al., 1974; Melchior & Steim, 1976), and other bacterial species (Hoest et all., 1974). Factors which probably account for the relatively low enthalpy of the enterocyte transitions include cholesterol and the membrane proteins. In aqueous dispersions of a defined phospholipid, cholesterol decreases the enthalpic change of the major transition (Ladbrooke & Chapman, 1969; Oldfield & Chapman, 1972; Hinz & Sturtevant, 1974a; Estep et al., 1978). In biological membranes, the transition enthalpy observed for *Azotobacter laidlawii* was lowered on increasing the membrane cholesterol to 12% by weight of the total membrane lipid (de Kruyff et al., 1972), and comparison of two strains of *Mycoplasma mycoides* showed a lipid transition in an adapted strain, whose cholesterol comprised 3% of the membrane lipids, but no transition in the native strain with 22–25% cholesterol (Rottem et al., 1973). The cholesterol content (percent by weight of total lipid) of our microvillus and basolateral membranes, respectively, was 14.3 ± 2.5 and 16.0 ± 2.7%, values in reasonable agreement with published ranges of 13.7–15.8% for microvillus membranes (Forstner et al., 1968b; Forstner & Wherrett, 1973) and 12.3–16.3% for basolateral preparations (Douglas et al., 1972). The cholesterol content of the enterocyte membranes is thus sufficient to decrease the transition enthalpy but evidently not to obliterate the transition. Human erythrocyte membranes, which contain 24% cholesterol (Farquhar, 1962), were reported to show no transition on DSC unless cholesterol was removed (Ladbrooke et al., 1968).

Ladbrooke & Chapman (1969) reported that the interactions of cholesterol and lecithin molecules which underlie the changes in transition enthalpy are observed only in the presence of water, and on removal of water these components "crystallize separately". Separation of cholesterol-poor lipid domains in the unhydrated lipid extracts of enterocyte membranes may account for the higher enthalpy values observed, e.g., 4.5 cal/g for the basolateral membrane lipids. Although less than the 8–15 cal/g reported for a number of pure phospholipids (Ladbrooke & Chapman, 1969; Hinz & Sturtevant, 1972b), this value is comparable to that of *Mycoplasma* membrane lipids, 3 to 4 cal/g (Reinert & Steim, 1970; Tourtellotte, 1972). Hydration of pure lecithins has been reported to narrow the temperature range and to lower the midpoint temperature of the thermotropic transition (Ladbrooke & Chapman, 1969). Similar effects are observed in the scans of dried vs. hydrated enterocyte membrane lipids (Figures 3–5). For example, hydration lowered the peak transition temperature of the basolateral lipid from 34 °C (Figure 5c) to 23 °C (Figure 4c) and decreased the breadth of the transition temperature range from 55 to 20 °C.

Protein–lipid interactions also contribute to lowering the lipid transition enthalpies of enterocyte membranes. Although the low enthalpy values of the intact membranes are only approximations (Results), they are clearly less than the corresponding values of the extracted, hydrated lipids. The ratios of the transition enthalpies (intact membranes)/(extracted, hydrated lipids) are in the range 0.2–0.5, indicating that the membrane proteins prevent a significant fraction of the lipids from participating in the cooperative transition. Comparison with the comparable ratio reported for *Mycoplasma* membranes, 0.9 ± 0.1 (Reinert & Steim, 1970), suggests that protein–lipid interactions leading to a decrease in transition enthalpy are relatively more effective in the enterocyte membranes. Similar decreases in transition enthalpies of defined phosphatidylcholine suspensions have been noted on inclusion of polypeptides such as mellitin (Mollay, 1976) and chlorothricin (Packe & Chapman, 1972) or of the hydrophobic protein lipophilin, extracted from brain myelin (Papahadjopoulos et al., 1975a,b). As discussed by Lee (1977b), one interpretation of these effects is that a moiety of the lipid is relatively immobilized by interaction with the protein and cannot take part in the thermotropic transition.

Additional evidence of protein–lipid interactions in enterocyte membranes is provided by the fluorescence polarization studies with DPH. The DPH anisotropy parameter varies directly with the rotational relaxation time of the probe (Shinitzky & Inbar, 1976) and hence is related inversely to the lipid fluidity. From the data in Table I, the ratio of the $(r_0/r - 1)^{-1}$ values of (intact membranes)/(liposomes) exceeds 2.0 for the microvillus preparations and is ~2.0 for the basolateral preparations. Lipid fluidity, therefore, is significantly reduced in the presence of the membrane proteins (Schachter & Shinitzky, 1977). The values in Table I also indicate that the lipid fluidity at 25 and 37 °C of the basolateral membrane and of its extracted lipid considerably exceeds that of the corresponding microvillus preparations. This conclusion is supported further by fluorescence polarization measurements with other lipid-soluble fluorophores, retinol and anthroyl stearate (Brasitus et al., 1979b), and by determinations of the excited-state lifetimes of the probes in each membrane type (T. A. Brasitus and D. Schachter, unpublished experiments). The difference in fluidity may result in part from differences in the cholesterol/phospholipid molar ratio. In our preparations the molar ratios observed for microvillus and basolateral membranes, respectively, were 1.17 ± 0.11 and 0.62 ± 0.04, in agreement with published values of 1.10–1.35 for microvillus membranes (Forstner et al., 1968a,b; Forstner & Wherrett, 1973) and 0.49–0.57 for basolateral membranes (Douglas et al., 1972). There is considerable evidence that increasing the cholesterol/phospholipid ratio decreases the fluidity of aqueous phospholipid dispersions above their transition temperatures (Chapman & Penkett, 1966; Hubbell & McConnell, 1971; Oldfield & Chapman, 1971; Cogan et al., 1973; Shinitzky & Inbar, 1976).

The molecular mechanisms which underlie the lipid transitions of enterocyte membranes described here are unknown and could include gel to liquid-crystalline transitions, lateral phase separations (Linden et al., 1973), lipid cluster formation (Lee et al., 1974), or other mechanisms (Lee, 1975, 1977a,b). However, the enterocyte transitions are particularly well-defined owing to the concordant results of DSC, fluorescence polarization, and Arrhenius studies of membrane protein activities. In this respect they are similar to the transitions of *Mycoplasma* membranes (Steim et al., 1969; Tourtellotte, 1972; Rottem et al., 1973; Razin, 1975; Melchior & Steim, 1976) but unlike those of a number of other mammalian membranes. DSC revealed transitions only at low temperatures, 0–5 °C, in sarcoplasmic reticulum (Martonosi, 1974; Davis et al., 1976; Mitsui, 1978) and in rat liver mitochondria

and microsomes (Blazyk & Steim, 1972). Spin-label studies and Arrhenius plots of membrane activities, on the other hand, demonstrated breaks at higher temperatures ranging from 18 to 26 °C in these organellar membranes (Eletr & Inesi, 1972; Tinberg et al., 1972; Williams et al., 1972; Eletr et al., 1973; Lee et al., 1974). Possible molecular mechanisms to account for the transitions which are not detected by calorimetry are discussed by Lee (1975, 1977a,b). At present it appears that the enterocyte transitions described in this report are particularly useful for further exploration of lipid dynamics in mammalian membranes.

References

Blazyk, J. F., & Steim, J. M. (1972) *Biochim. Biophys. Acta 266*, 737–741.

Brandts, J. F., Erickson, L., Lysko, K., Schwartz, A. T., & Taverna, R. D. (1977) *Biochemistry 16*, 3450–3454.

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

Brasitus, T. A., Tall, A. R., Schachter, D., & Mamouneas, T. G. (1979b) *Gastroenterology 76*, 1107 (Abstract).

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

Cogan, U., Shinitzky, M., Weber, G., & Nishida, T. (1973) *Biochemistry 12*, 521–528.

Davis, D. G., Inesi, G., & Gulik-Krzywicki, T. (1976) *Biochemistry 15*, 1271–1276.

de Kruyff, B., Demel, R. A., & van Deenen, L. L. M. (1972) *Biochim. Biophys. Acta 255*, 331–347.

Douglas, A. P., Kerley, R., & Isselbacher, K. J. (1972) *Biochem. J. 128*, 1329–1338.

Dulbecco, R., & Vogt, M. (1954) *J. Exp. Med. 99*, 167–182.

Eletr, S., & Inesi, G. (1972) *Biochim. Biophys. Acta 290*, 178–185.

Eletr, S., Zakim, D., & Vessey, D. A. (1973) *J. Mol. Biol. 78*, 351–362.

Estep, T. N., Mountcastle, D. V., Biltonen, R. L., & Thompson, T. E. (1978) *Biochemistry 17*, 1984–1989.

Farquhar, J. W. (1962) *Biochim. Biophys. Acta 60*, 80–89.

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., Sabesin, S. M., & Isselbacher, K. J. (1968a) *Biochem. J. 106*, 381–390.

Forstner, G. G., Tanaka, K., & Isselbacher, K. J. (1968b) *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.

Hinz, H. J., & Sturtevant, J. M. (1972a) *J. Biol. Chem. 247*, 3697–3700.

Hinz, H. J., & Sturtevant, J. M. (1972b) *J. Biol. Chem. 247*, 6071–6075.

Hoest, C. W. M., Verkley, A. J., de Gier, J., Scheek, R., Ververgaert, P. H. R., & van Deenen, L. L. M. (1974) *Biochim. Biophys. Acta 356*, 17–26.

Hopfer, U., Nelson, K., Perrotto, J., & Isselbacher, K. J. (1973) *J. Biol. Chem. 248*, 25–32.

Hubbell, W. L., & McConnell, H. M. (1971) *J. Am. Chem. Soc. 93*, 314–326.

Katz, S. S., Shipley, G. G., & Small, D. M. (1976) *J. Clin. Invest. 58*, 200–211.

King, T. E. (1967) *Methods Enzymol. 10*, 322–331.

Ladbrooke, B. D., & Chapman, D. (1969) *Chem. Phys. Lipids 3*, 304–367.

Ladbrooke, B. D., Williams, R. M., & Chapman, D. (1968) *Biochim. Biophys. Acta 150*, 333–340.

Lee, A. G. (1975) *Prog. Biophys. Mol. Biol. 29*, 3–56.

Lee, A. G. (1977a) *Biochim. Biophys. Acta 472*, 237–281.

Lee, A. G. (1977b) *Biochim. Biophys. Acta 472*, 285–344.

Linden, C. D., Wright, K. L., McConnell, H. M., & Fox, C. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 2271–2275.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Martonosi, M. A. (1974) *FEBS Lett. 47*, 327–329.

Masters, B. S. S., Williams, C. H., Jr., & Kamin, H. (1967) *Methods Enzymol. 10*, 565–573.

McElhaney, R. N., de Gier, J., & van der Neut-Kok, E. C. M. (1973) *Biochim. Biophys. Acta 298*, 500–512.

Melchior, D. L., & Steim, J. M. (1976) *Annu. Rev. Biophys. Bioeng. 5*, 205–238.

Mitsui, T. (1978) *Adv. Biophys. 10*, 97–135.

Mollay, C. (1976) *FEBS Lett. 64*, 65–68.

Murer, H., Hopfer, U., Kinne-Saffran, E., & Kinne, R. (1974) *Biochim. Biophys. Acta 345*, 170–179.

Oldfield, E., & Chapman, D. (1971) *Biochem. Biophys. Res. Commun. 43*, 610–616.

Oldfield, E., & Chapman, D. (1972) *FEBS Lett. 23*, 285–297.

Packe, W., & Chapman, D. (1972) *Biochim. Biophys. Acta 255*, 348–357.

Papahadjopoulos, D., Vail, W. J., & Moscarello, M. (1975a) *J. Membr. Biol. 22*, 143–164.

Papahadjopoulos, D., Moscarello, M., Eylar, E. H., & Isac, T. (1975b) *Biochim. Biophys. Acta 401*, 317–335.

Razin, S. (1975) *Prog. Surf. Membr. Sci. 9*, 257–342.

Reinert, J. C., & Steim, J. M. (1970) *Science 168*, 1580–1582.

Rottem, S., Cirillo, V. P., de Kruyff, B., Shinitzky, M., & Razin, S. (1973) *Biochim. Biophys. Acta 323*, 509–519.

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.

Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J., & Crane, R. K. (1973) *Biochim. Biophys. Acta 323*, 98–112.

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.

Steim, J. M., Tourtellotte, M. E., Reinert, J. C., McElhaney, R. N., & Rader, R. L. (1969) *Proc. Natl. Acad. Sci. U.S.A. 63*, 104–109.

Stern, B. R., & Jensen, W. E. (1966) *Nature (London) 209*, 789–790.

Tall, A. R., Small, D. M., Deckelbaum, R. J., & Shipley, G. G. (1977) *J. Biol. Chem. 252*, 4701–4711.

Tall, A. R., Small, D. M., Atkinson, D., & Rudd, L. (1978) *J. Clin. Invest. 62*, 1354–1363.

Tinberg, H. M., Packer, L., & Keith, A. D. (1972) *Biochim. Biophys. Acta 283*, 193–205.

Tourtellotte, M. E. (1972) in *Membrane Molecular Biology* (Fox, C. F., & Keith, A. D., Eds.) pp 439–470, Sinauer Associates, Stamford, CT.

Williams, M. A., Stancliff, R. C., Packer, L., & Keith, A. D. (1972) *Biochim. Biophys. Acta 267*, 444–456.