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Epidermal Growth Factor and Postnatal Development of Intestinal Transport and Membrane Structure

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ABSTRACT. The effects of epidermal growth factor (EGF) on postnatal development of intestinal transport and the physical composition of the microvillus membrane were examined. New Zealand White rabbits received EGF (40 μg/kg/d) from d 3 of life to d 17 either intraperitoneally or orogastrically. Intestinal H_2O, Na^+, and glucose absorption expressed per cm of intestine were significantly increased in animals receiving EGF by either route. When EGF was given by the orogastric route, nutrient absorption rates normalized to mucosal DNA were not elevated; thus, increased absorption induced by orogastric EGF appeared to be secondary to mucosal hyperplasia. In contrast, systemic EGF up-regulated cellular nutrient transport. To evaluate at which membrane level these changes occurred, brush border membrane vesicles were isolated from both the jejunum and ileum of control and EGF-treated animals. Rates of Na^+-dependent glucose transport into the vesicles revealed that in the ileum systemic EGF up-regulated maximal rates of glucose transport by 54% without affecting the Km. These observations were associated with alterations in the lipid composition and physical properties of the microvillus membrane. EGF-treated animals had significant reductions in membrane cholesterol content and altered ratios of phospholipid subclasses. The net result of these variations was that the microvillus membrane isolated from EGF-treated animals was significantly more fluid than membrane from controls. Thus, these results suggest that EGF modulates development of transport function during the postnatal period both by stimulating mucosal growth and by inducing specific transport processes. Furthermore, these changes are associated with alterations in the physical composition of the microvillus membrane. (*Pediatr Res* 30: 342–350, 1991)

Abbreviations

EGF, epidermal growth factor
BBMV, brush border membrane vesicles
HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid
DPH, 1,6-diphenyl-1,3,5-hexatriene
SCN, thiocyanate
J_{max}, maximum transport rate

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EGF is a small peptide of 53 amino acids (1), originally isolated from the submandibular gland of mice by Cohen (2). It has since been identified in Brunner's glands (3), saliva (4), urine (5), amniotic fluid (6), Paneth cells (7), and breast milk (8) and has been shown to have a variety of biologic effects. These include promotion of DNA synthesis (9–11) and transcription of RNA and subsequent protein synthesis (12).

Receptors for EGF are present along the length of the entire small intestine (13). Because of the presence of high concentrations of EGF in breast milk (8, 14), there has been speculation that this peptide might play a role in modulating postnatal development of the gastrointestinal tract. Although several studies have examined the effect of EGF on enzymatic function of the small intestine during the postnatal period (15, 16), the effect of EGF on nutrient and electrolyte transport function has only been examined with regards to Ca^+ transport (17). Additionally, the effects of systemic *versus* luminal EGF have not yet been clearly defined (16). Intestinal absorptive function continues to mature during the postnatal period. In general, the intestine of suckling animals is more permeable to H_2O and electrolytes and, therefore, a significant proportion of transport occurs via diffusion (18). The suckling intestine has a limited capacity for active transport of electrolyte (19) and active absorption of bile salts appears only at weaning (20). Sodium-dependent glucose transport appears to be present at birth (21). The present study was undertaken to determine the effect of EGF on postnatal maturation of intestinal function.

MATERIALS AND METHODS

Animal model. Suckling New Zealand White rabbits received 40 μg/kg/d EGF (Sigma Chemical Co., St. Louis, MO) either by daily intraperitoneal injection or via an orogastric feeding tube from d 3 of life to d 17. Littermate controls received 154 mM saline in equivalent volumes by the appropriate route. Animals were weighed daily and studied at d 18–20.

Intestinal in vivo perfusion. On the day of study, animals were weighed and then anesthetized with halothane. *In vivo* absorption was measured by a single-pass perfusion technique as previously described (22). In separate experiments, either a 10- to 15-cm segment of jejunum, starting 10 cm distal to the ligament of Treitz, or ileum, ending at the meso appendix, was isolated and cannulated at each end. The segment was perfused at a constant rate of 0.1 mL/min. The perfusate contained 135 mM Na^+, 5 mM K^+, 25 mM HCO_3^-, 115 mM Cl^-, 5 g/L of polyethylene glycol 4000, and 10 μCi/L of [^{14}C]polyethylene glycol as the nonabsorbable marker, osmolality 310 mosmol/L, and pH 7.4 at 37°C. In addition, the perfusate contained either 30 mM glucose and 10 μCi [^{3}H]glucose; 10 mM taurocholic acid and 10 μCi [^{3}H]taurocholic acid; or an equivalent amount of mannitol. Body temperature was monitored by a rectal temperature probe and maintained by a heating mattress. After a 60-min equilibra-

tion period, studies were initiated with the mannitol-electrolyte solution. Three consecutive 20-min samples were collected from the distal site. The perfusate was then changed to the glucose solution. After 40 min of equilibration, three more 20-min samples were collected. In a separate group of animals, ileal absorption of bile salt was assessed by perfusing with the solution containing taurocholate during the second study period. Intraluminal hydrostatic pressure was constantly monitored and maintained below 3 cm H_2O. After the second study period, the intestinal segment was removed and weighed, and its length measured. Mucosa was then scraped from the perfused segment, homogenized, and analyzed for DNA (23) and protein content (24). An adjacent segment of intestine was removed, scraped, and used for measurement of Na^+K^+ATPase activity (25). The collected samples were analyzed for Na^+ by flame spectrophotometry and for glucose, bile salt, and polyethylene glycol by scintillation spectrometry. Polyethylene glycol recovery ranged from 95–105% and did not differ between groups. Net fluxes of H_2O, Na^+, glucose, and bile salts were calculated using standard formulas (26) and were expressed as μL or nmol/cm or mg DNA/min.

Brush Border Membrane Studies. Vesicle preparation. BBMV were prepared from the proximal and distal one half of the small intestine of control and intraperitoneal EGF-treated animals by a magnesium chloride precipitation method (27). Purification was assessed by measuring sucrase sp act in the original homogenate and the final preparation (28). Basolateral membrane contamination was monitored by assaying Na^+K^+ATPase activity (25). Transport was calculated based upon the protein content of the vesicles as measured by the method of Lowry (24).

Transport measurements. Glucose uptake studies were carried out by a modification of Hopfer *et al.*'s (29) rapid filtration technique as previously described (30). Under the conditions used in these experiments, preliminary studies demonstrated that rates of glucose uptake were linear up to 10 s, the overshoot peaked at 30 s, and uptake reached equilibrium at 2 min. Therefore, an incubation time of 5 s was used to estimate initial uptake rates of glucose at concentrations of glucose in the incubation medium ranging from 10 μM to 8 mM. To start the reaction, 10 μL of freshly prepared vesicles in a buffer containing 250 mM mannitol, 10 mM HEPES, and 10 mM Tris at pH 7.5 were added to 50 μL of incubation buffer containing varying concentrations of [^{3}H]glucose, 100 mM NaSCN, 50 mM mannitol, and 20 mM HEPES at pH 7.5. The diffusional component of uptake was measured by replacing 100 mM *cis*-NaSCN with an equivalent concentration of KSCN. This component was subtracted from total uptake to give Na^+-dependent D-glucose uptake. The reaction was stopped by the addition of 3 mL of ice-cold stop solution (20 mM HEPES, 100 mM Na^+Cl^-, and 150 mM mannitol). The resultant mixture was filtered on a prewetted and chilled 0.45-μm nitrocellulose filter (Millipore, Mississauga, Ontario, Canada) and washed with 9 mL of stop solution. Filters were then dissolved in scintillation vials by 10 mL of scintillation fluid and counted. Nonspecific binding of [^{3}H]glucose to filters was measured by filtration of incubation medium, stop solution, and vesicles (without allowing mixing of medium and vesicles). All results were subsequently corrected for nonspecific binding. Volume of vesicles was routinely measured during each transport study by incubating vesicles with medium for 2 h. The final volume of vesicles ranged from 0.6 to 1.1 μL/mg protein and did not differ between groups. Experiments were performed on at least four different freshly prepared vesicle preparations on different days. Uptake data are reported as nmol glucose absorbed/min/mg of vesicle protein.

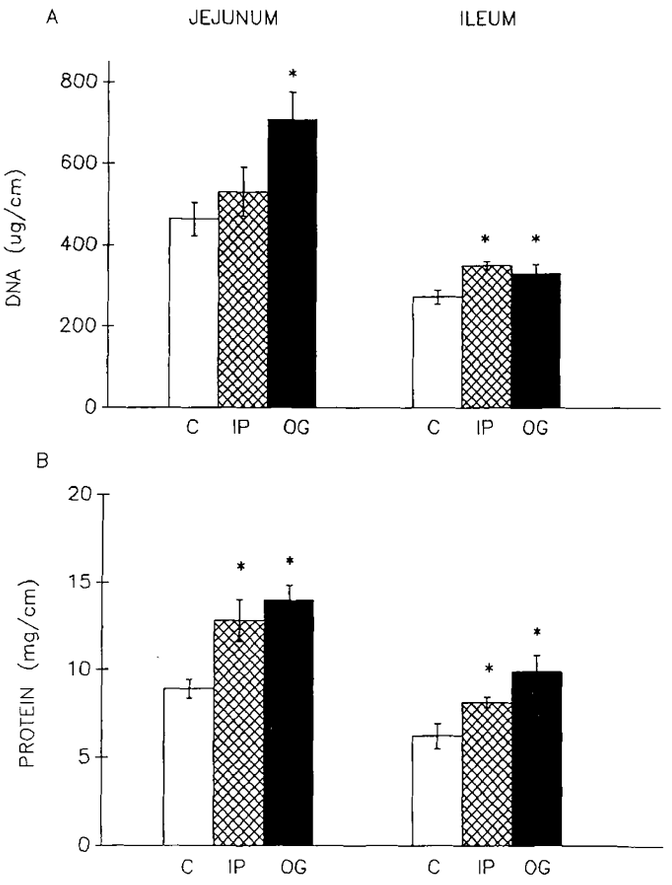

Fig. 1. DNA (*A*) and protein (*B*) content of mucosa from jejunal and ileal intestinal segments. *C*, control (n = 12); *IP*, intraperitoneal EGF (n = 5); *OG*, orogastric EGF (n = 9). Values are means ± SEM. *, $p < 0.05$ compared with controls.

Kinetic analysis. Initial rates of glucose uptake are presented as a function of the medium glucose concentration. To determine if the transport curves were better described by a one or two transporter model, the data were analyzed by the following equations, using weighted nonlinear regression techniques as previously described (31).

$$J = \frac{J_{max}C}{Km + C} \quad (1)$$

Equation 1 describes a single transport system where J represents the rate of transport, J_{max} the maximum transport rate, C the glucose concentration, and Km the concentration at which half-maximal transport rates occur.

$$J = \frac{J^1_{max}C}{Km^1 + C} + \frac{J^2_{max}C}{Km^2 + C} \quad (2)$$

Equation 2 describes a two transporter system containing two separate J_{max} and Km. To determine which model best described the transport system, the sums of squared residuals for each determination were compared using an F test (31).

Membrane physical properties. Steady state fluorescence polarization studies were carried out using an SPF 500-C spectrofluorometer (SLM-Amino, Urbana, IL).The lipid soluble fluorophores DPH and n-(9-anthroyloxy) stearic acid (Molecular Probes, Junction City, OR), where n took the values of 3, 6, 9, and 12, and 16-(9-anthroyloxy) palmitic acid were used to assess membrane physical properties. Vesicles were loaded with probe and measurements were performed as previously described (32). Data are reported as the steady state anisotropy parameter, r_S. Inasmuch as fluorescent lifetime cannot be directly obtained with steady-state techniques, total fluorescence was measured instead. Because total fluorescence is directly proportional to fluorescent lifetime, an absence of change in the former parameter was construed as evidence that the latter also had not changed. Finally, these techniques quantitate motional freedom of the probes utilized. The term membrane fluidity is used in its

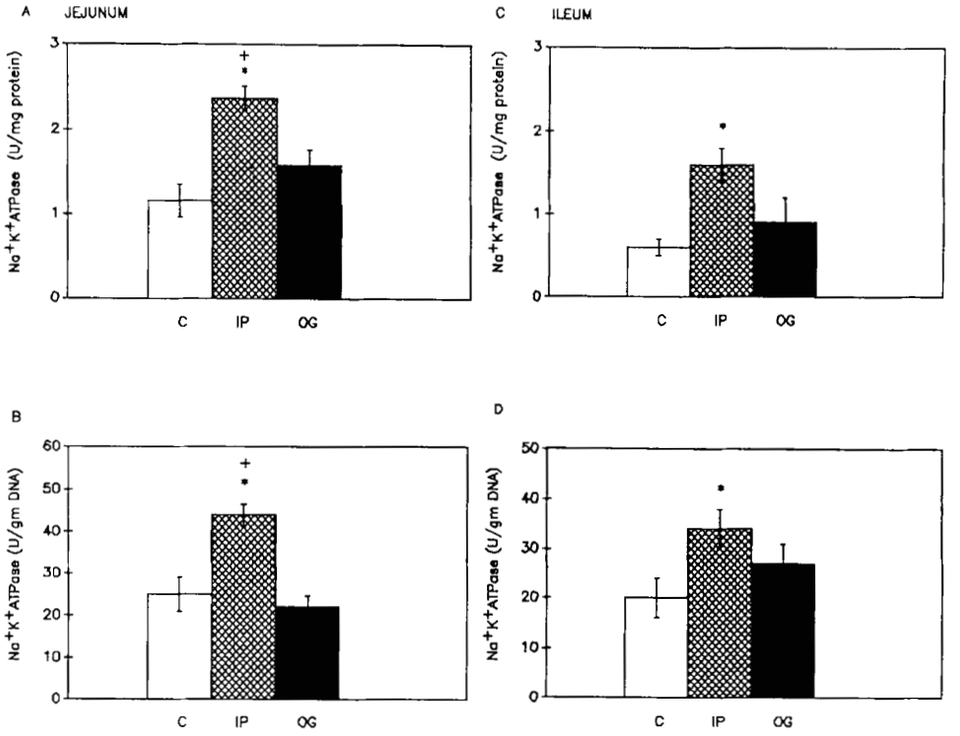

Fig. 2. Activity of Na^+K^+ATPase in the jejunum and ileum expressed as units/mg protein (*A* and *C*) or units per mg DNA (*B* and *D*). *C*, control ($n = 12$); *IP*, intraperitoneal EGF ($n = 5$); *OG*, orogastric EGF ($n = 9$). Values are means ± SEM. *, $p < 0.05$ compared with controls; +, $p < 0.05$ IP compared with OG.

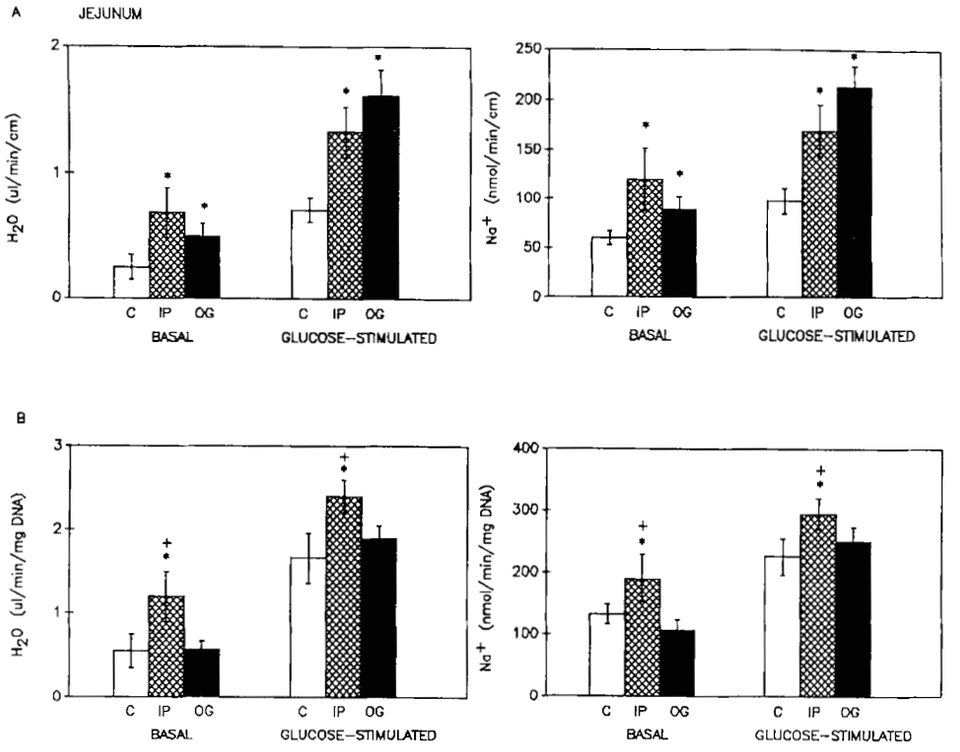

Fig. 3. *In vivo* net fluxes of H_2O and Na^+ in jejunum under basal conditions (mannitol) and in the presence of glucose. Fluxes were calculated as either nmol/min/cm (*A*) or as nmol/min/mg DNA (*B*). *C*, control ($n = 12$); *IP*, intraperitoneal EGF ($n = 5$); *OG*, orogastric EGF ($n = 9$). Each *bar* represents the mean ± SEM. *, $p < 0.05$ compared with controls, +, $p < 0.05$ IP compared with OG.

broadest sense here. Increased motional freedom of the probe is referred to as increased membrane fluiditiy.

Membrane lipid composition. Membrane lipids were extracted by the method of Folch *et al.* (33). Total phospholipid content of the membrane extract was determined by the method of Ames and Dubin (34) and cholesterol content, by a cholesterol oxidase enzymatic method (Boehringer Mannheim, Dorval, Quebec, Canada). Aliquots of extracted lipid were plated on Whatman LK-5D TLC plates and developed as previously described (30). The resultant phospholipid species were identified by simulta-

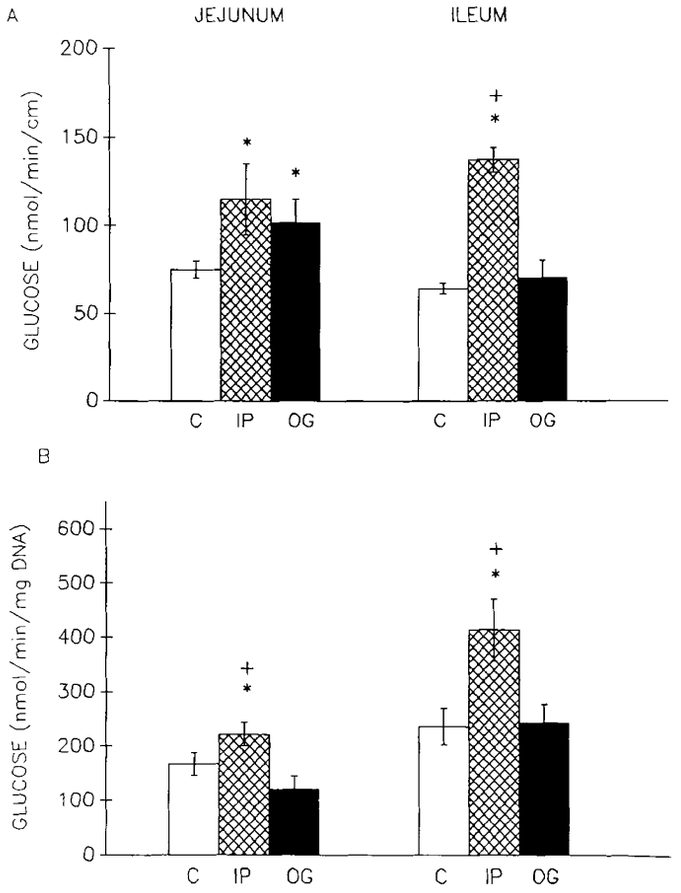

Fig. 4. *In vivo* net absorption of glucose in jejunal and ileal intestinal segments. Absorption was calculated as either nmol/min/cm (*A*) or as nmol/min/mg DNA (*B*). *C*, control ($n = 12$); *IP*, intraperitoneal EGF ($n = 5$); *OG*, orogastric EGF ($n = 9$). Each *bar* represents the mean ± SEM. *, $p < 0.05$ compared with controls; +, $p < 0.05$ IP compared with OG.

neously running authentic standards (Supelco, Bellafonte, CA). Identified phospholipids were aspirated into reaction tubes after drying under nitrogen. Pentadecanoic acid was added as an internal standard and fatty acid methyl esters prepared by standard techniques (32). These were separated and quantitated using gas-lipid chromatography, also as previously described (30).

Statistical Analysis. Results are expressed as means ± SEM. Analysis of variance was used to compare results between groups.

RESULTS

Final body weight was not affected by the administration of EGF. At 18–20 d of age, mean body weight of intraperitoneally (291 ± 13 g) and orogastrically (288 ± 15 g) treated animals did not differ from controls (298 ± 7 g).

Mucosal DNA and Protein Content and Na^+K^+ATPase Activity. As we have previously shown (16), EGF had a trophic effect on the jejunal mucosa. Orogastric EGF caused a significant increase in jejunal mucosal DNA (Fig. 1*A*), whereas both intraperitoneal and orogastric EGF caused an increase in jejunal mucosal protein content (Fig. 1*B*). In the ileum, intraperitoneal and orogastric EGF caused significant increases in both mucosal DNA (Fig. 1*A*) and protein (Fig. 1*B*). Animals receiving systemic EGF demonstrated a significant increase in jejunal and ileal Na^+K^+ATPase compared with controls when calculated either per g protein (Fig. 2*A* and *C*) or mg DNA (Fig. 2*B* and *D*).

In Vivo Perfusion. Jejunum. In vivo perfusion of the jejunum demonstrated a significant increase in H_2O and Na^+ absorption during the basal period in animals receiving orogastric ($n = 9$) or intraperitoneal ($n = 5$) EGF compared with controls ($n = 12$) (Fig. 3*A*). The presence of 30 mM glucose in the perfusate significantly ($p < 0.01$) stimulated H_2O and Na^+ absorption in all groups (Fig. 3*A*). However, H_2O and Na^+ absorption remained significantly greater in the EGF-treated animals. Net absorption of glucose was also increased in the EGF-treated animals compared with controls (Fig. 4*A*). Because increased nutrient absorption per cm length of intestine might be secondary to either an increase in mucosal mass or induction of cellular transport, absorption was also calculated based on mucosal DNA. The increase in H_2O and Na^+ absorption induced by orogastric EGF disappeared when the data were normalized to mucosal DNA (Fig. 3*B*). A similar pattern was seen with glucose absorption (Fig. 4*B*). Increased absorption rates induced by orogastric EGF per cm of intestine are no longer apparent when expressed per cell (DNA). These findings suggest that the jejunal response to orogastric EGF is due to the observed mucosal hyperplasia and not to an increase in the rate of absorption per cell. In contrast, in animals receiving systemic EGF, absorption remained significantly elevated when normalized to mucosal DNA, indicating an induction of cellular transport (Figs. 3*B* and 4*B*).

Ileum. Similar results were observed when an ileal loop was perfused. In the basal period, H_2O absorption and Na^+ absorption were significantly increased with systemic EGF, and Na^+ absorption was significantly increased with orogastric EGF when expressed per cm length of intestine (Fig. 5*A*). The addition of either glucose (intraperitoneally, $n = 7$; orogastrically, $n = 9$) or taurocholate (intraperitoneally , $n = 6$; orogastrically, $n = 5$) to the perfusate caused a significant ($p < 0.05$) increase in H_2O and Na^+ absorption in both EGF groups, whereas only taurocholate ($n = 14$) increased ($p < 0.05$) absorption in control animals. Glucose ($n = 14$) had no effect. In the presence of glucose and taurocholate, absorption remained elevated in the EGF groups compared with control values. When the data were expressed per mg mucosal DNA (Fig. 5*B*), H_2O and Na^+ absorption remained significantly elevated in the systemically treated animals. In the orogastric group, absorption was only increased in the presence of glucose. Net absorption was only increased in the presence of glucose. Net absorption of glucose (Fig. 4) and taurocholate (Fig. 6) was also enhanced by systemic EGF, and this effect was apparent regardless of whether the data were normalized per cm length or per mucosal DNA content.

Brush Border Membrane Studies. Purification and validation. To determine if intraperitoneal EGF produced its effect at the level of the microvillus membrane, this subcellular fraction was isolated from both control and systemically treated animals. The purification of membrane vesicles based upon enrichment of sucrase activity was similar in controls and EGF-treated animals (Table 1). There was no detectable Na^+K^+ATPase activity in microvillus membrane in either group, indicating negligible contamination by basolateral membranes.

To determine if the uptake of D-glucose was into an osmotically sensitive intravesicular space rather than binding to membrane surfaces, BBMV from both controls and EGF-treated animals were incubated in medium of increasing osmolarity to decrease intravesicular space. The amount of glucose taken up by vesicles prepared from both groups was linearly related to the reciprocal of medium osmolarity and had a y-intercept not significantly different from zero (data not shown). These data indicate that glucose uptake was into an osmotically sensitive space and make it unlikely that adsorption contributed significantly.

Kinetic results. Figure 7 illustrates Na^+-dependent rates of glucose uptake into BBMV from proximal (*panel A*) and distal (*panel B*) intestine. These data have been corrected for rates of transport observed in the absence of an Na^+ gradient. Systemic EGF had little effect on the kinetics of Na^+-dependent glucose transport in BBMV from the proximal intestine. However, in BBMV from distal intestine, systemic EGF produced a significant increase in maximal rates of glucose transport.

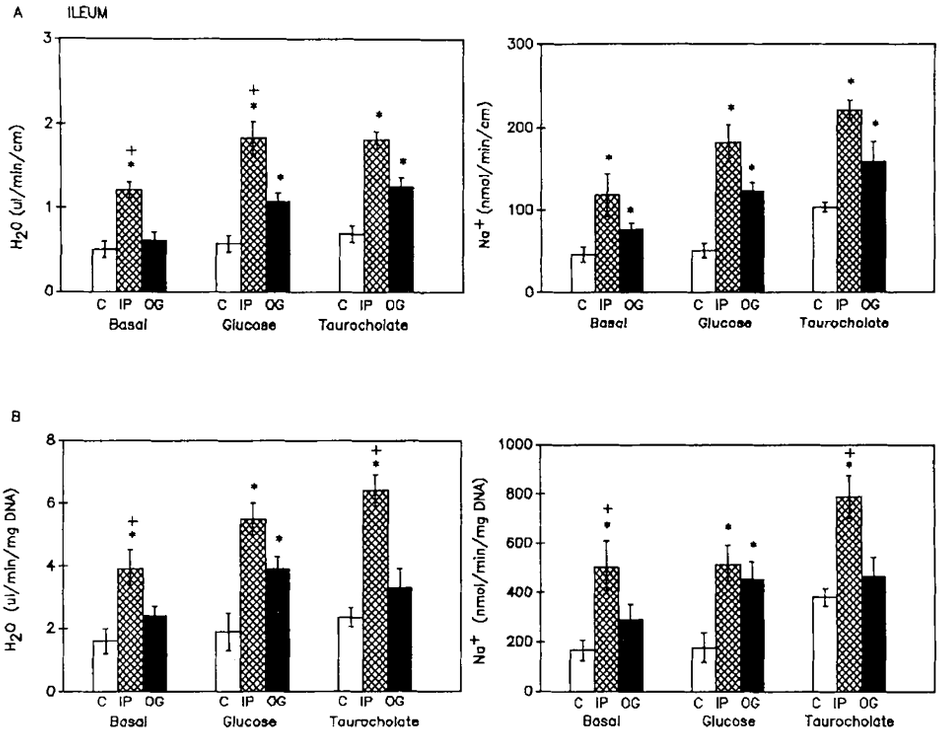

Fig. 5. *In vivo* net fluxes of H_2O and Na^+ in ileum under basal conditions (mannitol) and in the presence of either glucose or bile salts. Fluxes were calculated as either nmol/min/cm (*A*) or as nmol/min/mg DNA (*B*). *C*, control (n = 14); *IP*, intraperitoneal EGF (n = 7); *OG*, orogastric EGF (n = 9). Each *bar* represents the mean ± SEM. *, $p < 0.05$ compared with controls; +, $p < 0.05$ IP compared with OG.

The kinetic parameters that describe these curves were defined by nonlinear regression techniques as outlined in Materials and Methods. Initially, the data were fit to a model that incorporated either one or two transporters. No statistically significant difference was observed in the "goodness of fit" parameter between models (F test). Thus, the data were fit to an equation involving a single transporting site. The kinetic parameters observed in BBMV from proximal intestine in EGF-treated animals (J_{max}, 2.2 ± 0.5 nmol/min/mg protein; Km, 489 ± 120 μM) were not different from those observed in control animals (J_{max}, 3.1 ± 0.4 nmol/min/mg protein; Km, 279 ± 26 μM). However, in BBMV isolated from the distal intestine of EGF-treated animals, a significant ($p < 0.05$) increase in maximal glucose transport rates, J_{max}, was observed (4.2 ± 0.2 *versus* 2.7 ± 0.2 nmol/min/mg protein). Values for the Km did not differ (216 ± 21 *versus* 334 ± 55 μM). The passive component of transport measured in the absence of an Na^+ gradient was not different between control and EGF-treated animals (at 4 mM glucose: 1.7 ± 0.8 *versus* 2.7 ± 0.8 nmol/min/mg protein).

Membrane physical properties. Because membrane physical properties may affect the kinetics of Na^+-dependent glucose transport (35), physical properties and lipid composition of BBMV from controls and animals receiving systemic EGF were quantitated. Membrane physical properties were estimated by determining both the static and dynamic components of membrane fluidity. The former was assessed using the probe DPH, and the latter was evaluated at several depths within the bilayer using a series of n-(9-anthroyloxy) fatty acids, where n took the value of 3, 6, 9, 12, and 16. Thus, 3-(9 anthroyloxy) stearic acid probes events taking place near the surface of the bilayer, whereas 16-(9-anthroyloxy) palmitic acid localizes close to the core of the membrane.

Table 2 summarizes the data regarding the static component of membrane fluidity. The observed steady-state anisotropy parameters for DPH were significantly lower in BBMV obtained from EGF-treated animals. Although fluorescent lifetime cannot be directly measured with steady-state techniques, it can be indirectly assessed by measuring the total fluorescence of the sample. Because total fluorescence for a constant amount of membrane and probe did not differ between groups, we infer that the difference in anisotropy was unlikely to be secondary to changes in fluorescent lifetime. The observed differences in anisotropy parameter translate into a significant reduction in membrane order associated with the administration of EGF.

These differences in membrane physical properties induced by EGF were not limited to the static component of membrane fluidity. As illustrated in Figure 8, the dynamic component of membrane fluidity was also altered and involved almost all depths of the bilayer examined by these probes. In all cases, EGF treatment was associated with a reduction in the anisotropy parameter of the probe, a change that suggests increased motional freedom for the fluorescent group. Once again, no significant differences were observed in total fluorescence (data not shown), implying that these differences were unlikely to be secondary to differences in fluorescent lifetime.

Table 3 summarizes the lipid composition of the BBMV. Microvillus membrane isolated from EGF-treated animal was not only more fluid, but also had a significantly lower cholesterol content than membrane isolated from control animals. This alteration was primarily responsible for a significant reduction in the cholesterol:phospholipid ratio observed in these membranes. Inasmuch as this parameter is one of the major determinants of membrane physical properties, these alterations may explain many of the observed alterations in membrane fluidity. However, it was not the only factor involved. In BBMV from the distal intestine, EGF administration also led to alterations in the relative abundance of phospholipid subclasses. A significant decrease in the phosphatidylethanolamine to phosphatidylcholine and sphingomyelin to phosphatidylcholine ratios were observed. These changes would also contribute to the observed increase in membrane fluidity.

DISCUSSION

Our results suggest that during the postnatal period, EGF may modulate the transport capacity of the small intestine and alter

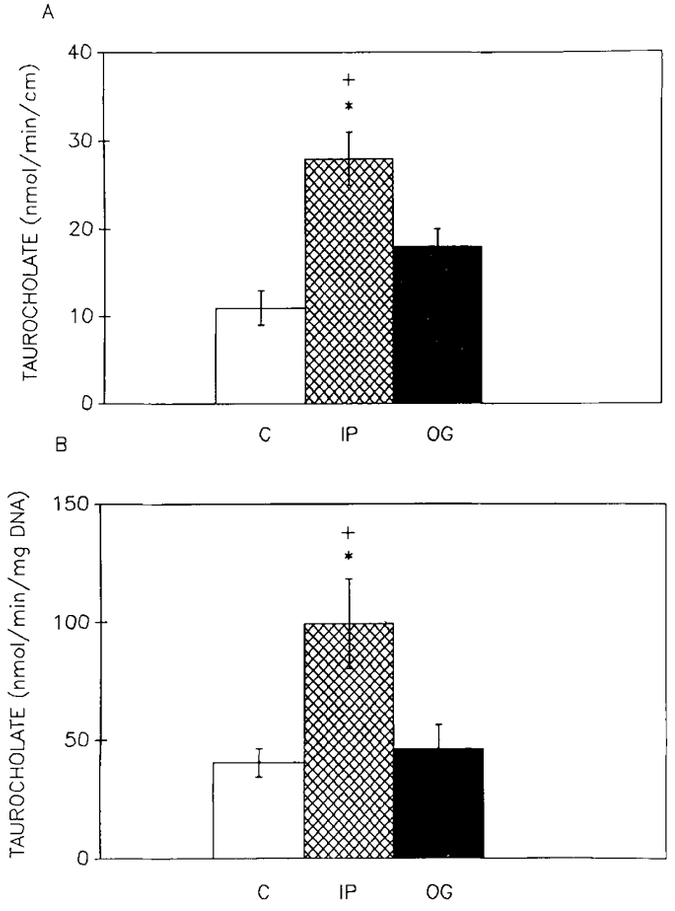

Fig. 6. *In vivo* net absorption of taurocholate in the ileal intestinal segment. Absorption was calculated as either nmol/min/cm (*A*) or as nmol/min/mg DNA (*B*). *C*, control ($n = 14$); IP, intraperitoneal EGF ($n = 6$); *OG*, orogastric EGF ($n = 5$). Each *bar* represents the mean ± SEM. *, $p < 0.05$ compared with controls, +, $p < 0.05$ IP compared with OG.

brush border membrane composition and function in suckling rabbits.

The effects of EGF on cellular proliferation and maturation appear to be dependent upon both the time of exposure (17) and the route of administration (36, 16, 37). In this study, the administration of EGF via an oral route had a trophic effect that was greatest in the jejunum and decreased toward the distal ileum. Systemically administered EGF had a trophic effect in both jejunum and ileum.

The presence of a normal proximal-distal gradient in EGF absorption in suckling animals (38) would result in exposure of the jejunum to much higher levels of EGF compared with the ileum in experimental animals receiving oral supplementation of EGF. In contrast, in animals receiving systemic EGF, both proximal and distal regions of the intestine would be exposed to similar EGF levels at the basolateral membrane surface. Inasmuch as intestinal epithelial cells are extremely sensitive to EGF concentration (15), the differing effects of oral *versus* systemic administration may be partially related to the actual concentration reaching the cells. In addition, the presence of receptors for EGF on the brush border of nondividing villus tip cells (39), together with the observation that receptors are also located on the basolateral membrane (40), leads to the speculation of a mechanism for EGF absorption and action similar to that proposed for polyamines (41). In this model, villus cells would take up EGF and release the peptide into the interstitium where it would be carried via the local circulation to basolateral membranes of crypt cells. Evidence to support this idea comes from the studies by Ulshen *et al.* (42) in which the intraluminal infusion of EGF into the ileum resulted in an increase in DNA synthesis in the jejunum.

The small intestine undergoes several developmental changes that affect transport capacity and function during the postnatal period. The immature intestine is more permeable to water and electrolytes (19), possibly due to a more disordered structure of the microvillus membrane (32, 43). Maturation of the small intestine involves decreases in both brush border (43) and basolateral membrane (44) fluidity associated with increases in the cholesterol/phospholipid molar ratio (32). In the present study, EGF administration resulted in an increase in the fluidity of microvillus membrane. Membrane fluiditiy is controlled by a variety of regulatory mechanisms involving membrane lipid composition. Multiple factors seemed to be responsible for this alteration. First and foremost, EGF led to a reduction in the cholesterol:phospholipid ratio, a prime determinant of membrane physical properties. Secondly, in the ileum EGF increased the relative concentration of phosphatidylcholine. This resulted in reduction of both the phosphatidylethanolamine:phosphatidylcholine and sphingomyelin:phosphatidylcholine ratios. Both would be expected to increase the observed fluidity of the microvillus membrane. The underlying mechanisms of these alterations can only be speculated upon given our data. However, stimulation of transmethylation reactions converting phosphatidylethanolamine to phosphatidylcholine (45) is possible. Furthermore, luminal EGF has been shown to decrease the microclimate pH along the apical membrane surface in rat jejunum (46). Similar alterations have been demonstrated to result in increased fluidity due to a displacement of Ca^+ ions (47).

The effects of altering phospholipid composition on membrane function are not well understood. There is no simple relationship that exists between any specific phospholipid and transport function. Alterations in membrane fluidity have been shown to affect glucose transport (30, 35), Na^+K^+-ATPase activity (48), and sodium uptake and water permeability (49). In the intestine, a proximal distal gradient exists in membrane lipid composition and fluidity, with the jejunum being more fluid than the ileum (50). The jejunum is also more permeable than the ileum, because of both decreased paracellular resistance and less rigid brush border membrane. The apparent fluidization of membranes by EGF administration resulted in an ileal brush border membrane that was similar in degree of fluidity to normal control jejunal membranes. This suggests that the presence of EGF in the gut may be instrumental in the maintenance of the normal proximal distal gradient in fluidity.

Table 1. *Enzymatic characterization of microvillus membrane preparations**

		Sucrase activity (U/g protein)			Na^+K^+ATPase (U/mg protein)	
Group	Treatment	Homogenate	Microvillus membrane	Enrichment factor (fold)	Homogenate	Microvillus membrane
Jejunum	Control	4.0 ± 2.0	66.1 ± 33.0	16.5	1.0 ± 0.2	ND
	EGF i.p.	6.0 ± 2.0	114.0 ± 47.0	19.0	1.9 ± 0.4	ND
Ileum	Control	1.1 ± 0.6	25.1 ± 13.0	23.0	0.5 ± 0.3	ND
	EGF i.p.	2.5 ± 1.0	44.7 ± 19.0	18.0	1.4 ± 0.4	ND

* Values are means ± SEM; ND, not detectable; i.p., intraperitoneally.

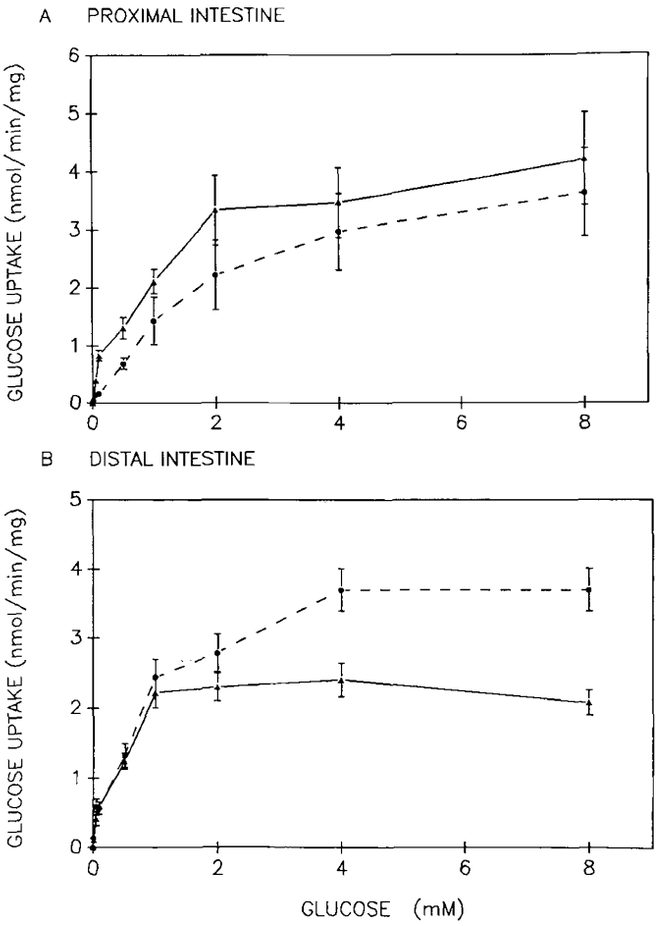

Fig. 7. Initial rates of D-glucose transport as a function of D-glucose concentrations in the medium in the proximal (*A*) and distal (*B*) small intestine of control (———) and intraperitoneal EGF-treated (— — —) animals. The results shown represent the difference between D-glucose uptake in the presence and absence of sodium. Each data point represents the mean ± SEM of eight separate membrane preparations from both groups.

EGF significantly increased absorption rates for H_2O, Na^+, and glucose. However, the increases observed in EGF-treated animals appeared to be due to two different responses that could be separated by examining transport data normalized to either intestinal length or mucosal DNA. In general, increased transport rates per unit length of intestine may be due to an increase in the number of absorptive cells or to increased transport per cell. These two effects can be differentiated by examining transport based on mucosal DNA as an index of mucosal cell number. Oral administration of EGF induced mucosal hyperplasia and increased rates of absorption per cm of intestine. However, when expressed per mg of DNA, rates of absorption, in general, were no different than in control animals. This would imply that the major effect of oral EGF was to increase absorptive cell number. This was not the case for systemic EGF. Not only did systemic EGF result in mucosal hyperplasia and increased rates of nutrient absorption, but these absorption rates were still significantly increased when expressed per mg of mucosal DNA. This suggests that systemic EGF induces mucosal hyperplasia as well as increased rates of nutrient transport per cell. The increased fluidity in both proximal and distal regions of EGF-treated animals may contribute to the increased transport *in vivo* due to an increased cellular permeability and corresponding passive component. Additionally, because the activity of Na,K-ATPase per unit DNA was also increased after EGF administration, a case could be made for increased sodium extrusion across the basolateral membrane and a secondary enhancement of Na^+-dependent absorption rates. This does not appear to be the entire explanation, however, at least in the ileum, inasmuch as studies specifically localized to the apical membrane demonstrated increased rates of glucose transport. The glucose kinetic studies demonstrated a significant increase in maximal Na^+-dependent glucose transport rates, J_{max}, in BBMV isolated from ileum of EGF-treated animals. The passive component was also increased, but did not reach levels of significance.

The mechanism by which EGF increased glucose transport rates in ileal BBMV is unclear. Controversy exists as to the existence of a second glucose transporter protein in the small intestine similar to that observed in renal brush border membranes (51). A number of investigators have argued for the presence of two distinct transporters based on kinetic evidence (52–57), whereas others have claimed that differences in Km can be explained by either differences in membrane fluidity (30) or changes in *cis*-Na^+ concentration and resultant conformational changes in the transporter (58). In the present study, no firm conclusions could be drawn concerning this matter. Because our data could be equally well described by a model containing either one or two transporters, we chose the conservative approach and used a single transporter model. This, of course, does not exclude the possibility of a second transporter protein. Furthermore, many investigators describe a low affinity transporter with a Km in the range of 4–12 mM. Inasmuch as the upper concentration of glucose used in this study was 8 mM, we cannot reliably evaluate the presence or absence of such a transporter. Therefore, we only conclude that in the ileum systemic EGF increases the apparent J_{max} for glucose transport across the apical membrane of the enterocyte. Thus, the increase in net glucose absorption observed *in vivo* is at least partially explained by an effect of EGF localized to the microvillus membrane.

In the suckling period, active Na^+-dependent ileal transport of bile salts is deficient (59). The majority of bile salts are returned to the enterohepatic circulation via passive absorption in the jejunum. The appearance of ileal active transport coincides with weaning in the rabbit (21). We have previously demonstrated that chronic administration of EGF increases both bile salt pool size and hepatic excretion of bile salts (60). The present study demonstrates that EGF is capable of increasing taurocholate absorption and Na^+-coupled transport. This increased intestinal absorption might contribute to the increased bile salt pool size previously observed (60).

In summary, EGF is able to enhance nutrient transport capac-

Table 2. *Static component of microvillus membrane fluidity**

Group	Treatment	Steady state anisotropy (r_s)	Limiting hindered anisotropy (r_∞)	Order parameter (s_{DPH})
Proximal	Control	0.266 ± 0.002	0.255 ± 0.003	0.839 ± 0.005
	EGF i.p.	0.259 ± 0.003†	0.245 ± 0.003†	0.823 ± 0.004†
Distal	Control	0.271 ± 0.001	0.261 ± 0.002	0.849 ± 0.003
	EGF i.p.	0.248 ± 0.004†	0.231 ± 0.004†	0.799 ± 0.006†

* The static component of membrane fluidity was measured with DPH. Values are means ± SEM from eight to 12 separate vesicle preparations in each group. i.p., intraperitoneally.

† $p < 0.01$ compared with controls.

Table 3. *Membrane phospholipid and cholesterol content**

Parameter	Jejunum		Ileum	
	Control	EGF i.p.	Control	EGF i.p.
Cholesterol (nmol/mg protein)	262 ± 12	203 ± 13†	273 ± 22	216 ± 16†
Phospholipid (nmol/mg protein)	233 ± 8	267 ± 22	235 ± 11	246 ± 20
Cholesterol:phospholipid (mol:mol)	1.12 ± 0.04	0.78 ± 0.08†	1.17 ± 0.08	0.86 ± 0.04
Phospholipid subclasses (wt%)				
Phosphatidylinositol	11.6 ± 1.9	13.0 ± 1.7	16.2 ± 1.7	15.2 ± 1.7
Phosphatidylserine	8.9 ± 1.7	11.0 ± 1.9	12.1 ± 1.7	10.6 ± 1.7
Sphingomyelin (SPH)	16.8 ± 0.5	20.0 ± 2.4	18.5 ± 2.0	17.2 ± 1.0
Phosphatidylcholine (PC)	37.6 ± 3.0	36.0 ± 2.0	32.3 ± 2.0	45.0 ± 2.5†
Phosphatidylethanolamine (PE)	25.2 ± 2.0	19.9 ± 1.6	20.8 ± 2.6	15.4 ± 2.8
Lysophosphatidylcholine	<1	<1	<1	<1
Ratios (wt:wt)				
PE/PC	0.67 ± 0.11	0.53 ± 0.11	0.64 ± 0.13	0.34 ± 0.0
SPH/PC	0.45 ± 0.05	0.56 ± 0.12	0.57 ± 0.03	0.37 ± 0.0

* Values are means ± SEM of four determinations in each of six membrane isolations.
† $p < 0.01$ compared to control values in each segment.

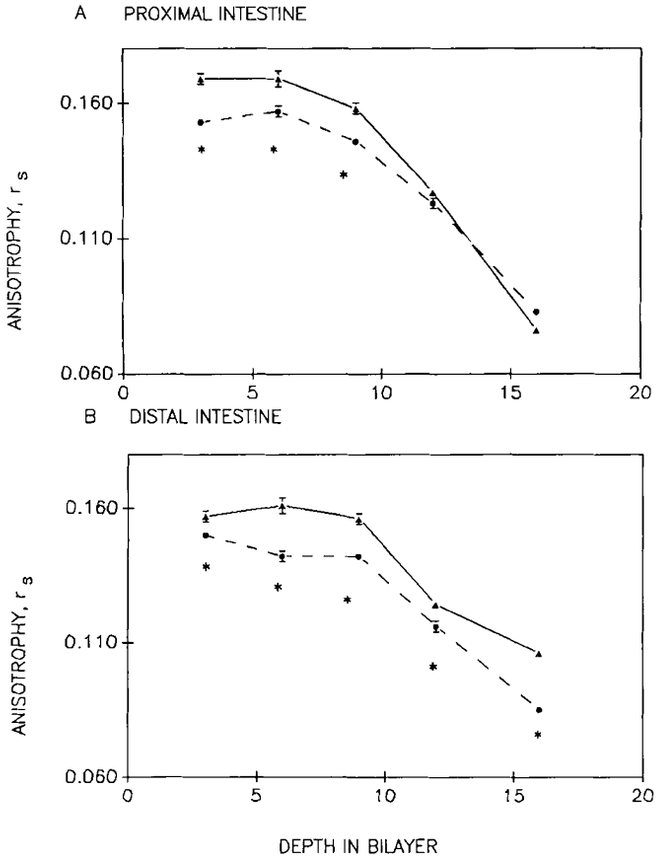

Fig. 8. Dynamic component of membrane fluidity as a function of depth in the bilayer in the proximal (*A*) and distal (*B*) regions. The *horizontal axis* represents the different probes of the *n*-(9-anthroyloxy)-stearic or palmitic acid series. The *vertical axis* shows the fluorescence anisotropy, *r* values. Each data point represents the mean ± SEM of six membrane preparations from both control (———) and intraperitoneal EGF-treated (— — —) animals. *, $p < 0.01$ compared with controls.

ity of the small intestine during the postnatal period both by increasing epithelial cell mass and inducing cellular transport processes. Furthermore, EGF has profound effects on the composition and physical properties of the microvillus membrane of the enterocyte.

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