Effect of Medium-Chain Glycerides on Physiological Properties of Rabbit Intestinal Epithelium *in Vitro*

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Medium chain glycerides (MCGs) have been reported to enhance intestinal absorption of hydrophilic drugs. However, the mechanisms involved in absorption enhancement are not well understood. The effects of MCGs (CapMul MCM) on physiological properties of rabbit ileum and distal colon, including active ion transport, transepithelial resistance (R_t) and passive permeability, have been investigated *in vitro*. CapMul MCM inhibited active ion transport (measured as a decrease in short-circuit current, I_{sc}) in both intestinal segments in a concentration-dependent manner. The inhibition of I_{sc} was rapidly reversible (within 100 min) upon removal of CapMul MCM. The data indicate that CapMul MCM preferentially affected ion transport by villus cells in the ileum and surface cells in the distal colon. Ion transport in crypt cells in both segments was not significantly altered. R_t of the ileum was not significantly affected by 5% CapMul MCM, while mannitol transport was 6 fold enhanced. Treatment of distal colon with 1% CapMul MCM reduced R_t by 95%, while mannitol transport was 100 fold enhanced. In a parallel experiment, mucosal(m)-to-serosal(s) transport of cephalexin, a β-lactam antibiotic, in the ileum was about 40% reduced in the presence of 5% CapMul MCM, whereas transport in the s-to-m direction was 2.5 fold enhanced. Treatment of the distal colon with 1% CapMul MCM resulted in 25 fold enhancement of cephalexin transport in either direction. These results suggest that absorption enhancement by MCGs results from an increased permeability of the intestine confined to the villus or surface epithelium.

KEY WORDS: active ion transport; CapMul MCM; enhancer; intestinal transport; medium-chain glycerides; transepithelial resistance; Ussing chamber; passive permeability.

INTRODUCTION

A wide variety of enhancers have been described to increase the gastrointestinal absorption of otherwise poorly absorbed hydrophilic drugs (1–3). Most of these enhancers are micelle-forming soluble amphiphiles, i.e. detergents. Detergent monomers can partition into the plasma membrane, where they form defects in the lipid bilayer. At high enough detergent concentrations the membrane can be dissolved in detergent-membrane mixed micelles. Detergents can also extract proteins from the plasma membrane of the cell. Treatment of cells with a detergent will generally have a cytotoxic effect.

Some promising results have been obtained *in vivo* with medium-chain glycerides (MCGs, mixtures of mono-, di-, and triglycerides with medium-chain length (C_8-C_{12}) fatty acids) (4–9). Beskid et al. reported that a formulation incorporating CapMul 8210 (a mixture of glyceryl mono- and dicaprylate) enhanced enteral, rectal, and oral (from enteric-coated capsules) absorption of sodium ceftriaxone in rats, rabbits and squirrel monkeys (7). Sekine and coworkers used MGK®, a mixture of glyceryl mono- and dicaprylate, to enhance absorption of sodium cefmetazole from rabbit rectum and from different intestinal segments in the dog (8,9). In addition, Van Hoogdalem and coworkers demonstrated enhanced rectal delivery of sodium cefazolin in rats by MGK® (5).

It has been suggested that the absorption enhancing effect of MCGs is due to their effects on phospholipid bilayer structure (10). Higaki et al. have demonstrated that incorporation of MCGs in liposomal phospholipid bilayers results in an enhanced permeability of phenol red. MCGs added to preformed liposomes also promoted the release of phenol red (11–13). In addition, MCGs have been shown to cause denudation of rectal epithelium (14).

The objectives of the present study were (i) to investigate whether MCGs alter physiological function of the intestinal epithelium, such as the ability to support ion and solute transport, and to determine the effect of MCGs on passive permeability and carrier-mediated transport of selected marker molecules (ii) to investigate the reversibility of alterations in physiological functions, (iii) to determine whether MCGs exert different effects in different intestinal regions, e.g. ileum vs colon and whether these effects occur in all cell types e.g. villus or surface and crypt cells.

An *in vitro* model utilizing rabbit ileal and distal colonic tissues mounted in Ussing chambers was used in this study. The rationale for selecting this model is that (i) the ion/solute absorptive and secretory functions in rabbit ileum and distal colon have been well characterized and correlated to human intestinal transport (15,16) and (ii) this *in vitro* model can provide a rapid method for evaluating effects of absorption enhancers on transport of drugs (17,18). The advantages of the Ussing chamber technique are: (i) tissue viability and integrity can be continuously monitored by measuring short-circuit current (I_{sc}) and transepithelial resistance (R_t), (ii) local effects of agents can be determined from changes in I_{sc} and R_t, (iii) mechanisms of drug transport and metabolic stability can be identified and (iv) correlation between transport changes and tissue morphology can be investigated (17,19–21).

MATERIALS AND METHODS

Materials

CapMul MCM (C8/C10 (64/36) mono-/di-glycerides (45/45)), Lot# J770, was obtained from Karlshamns Lipid Specialties (Columbus, OH, USA). [^{3}H]-cephalexin (SK&F 35488) with a specific activity of 3.64 Ci/mmol was synthesized by the Radiochemistry Department at SmithKline Beecham Pharmaceuticals, (King of Prussia, PA, USA). The [^{3}H]-cephalexin was stored at −80°C in ethanol/H_2O (1:1) at a radioconcentration of 5.7 μCi/10μl. [^{14}C]-mannitol was

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purchased from New England Nuclear (Boston, MA, USA). Except where otherwise indicated, all compounds used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Tissue Preparation

New Zealand White rabbits (2–3 kg) were maintained on standard chow and water *ad libitum*. Animals were sacrificed by cervical dislocation and 15–20 cm sections of distal ileum and distal colon were removed, opened along the mesenteric border and rinsed of luminal contents with iced bicarbonate Ringer solution (112 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.4 mM NaH_2PO_4, 1.6 mM Na_2HPO_4 and 25 mM $NaHCO_3$). The epithelium was stripped of the underlying musculature as previously described (22,23). Sheets of the stripped tissue (2–3 cm) were mounted in an Ussing chamber apparatus (exposing 1.13 cm^2 surface area). The tissues were bathed on both sides with 10 ml bicarbonate Ringer solution maintained at 37°C. Bathing solutions were gassed continuously with 95% O_2/5% CO_2 and circulated by gas lift. Under these conditions, the pH of the bicarbonate Ringer was 7.4. Mannitol (10 mM) was added to the mucosal bathing solution and glucose (10 mM) to the serosal bathing solution except when indicated otherwise.

Electrical Measurements

Tissues were equilibrated (30–60 min for ileum or 60–120 min for distal colon) and continuously short-circuited by automatic voltage clamps (Physiologic Instrument VCC600, Precision Instrument Design, Tahoe City, CA, USA) which compensated for the fluid resistance between the bridge tips. Transepithelial electrical potential difference (PD, mV), referenced to the mucosal bathing solution, and short-circuit current (I_{sc}, $\mu Amp/cm^2$) were measured at the desired time intervals. Transepithelial resistance (R_t, ohm cm^2) was calculated from the open-circuit PD and I_{sc} ($R_t = PD/I_{sc}$) or from the current required to clamp the PD briefly (<5 sec) to 5 mV when the basal PD was less than 0.5 mV.

Enhancer Studies

After the equilibration period, different concentrations of CapMul MCM were added to the mucosal bathing solution. At all concentrations used, CapMul MCM forms an emulsion in the mucosal bathing solution. Ileum was exposed to CapMul MCM for 20 minutes, and colon for 10 min. At the end of this incubation period, the mucosal bathing solution was rapidly removed and the chamber rinsed with 100 ml fresh mucosal bathing solution (without CapMul MCM) preincubated at 37°C. PD and I_{sc} were measured continuously during the experiment.

At the end of the experiment, the ability of the tissues to support active ion and solute transport was determined from the basal I_{sc} and the change in I_{sc} resulting from mucosal addition of L-leucine (5 mM) and D-glucose (10 mM) to ileal tissues or amiloride (0.01 mM) to colonic tissues (15,24,25), and serosal addition of prostaglandin E_1 (PGE_1, 0.01 mM) to both tissues (26). Na^+-coupled leucine or glucose transport was demonstrated by an increase in I_{sc}. Inhibition of Na^+ influx through the apical channel by the mucosal addition of amiloride was demonstrated by a decrease in I_{sc}. Stimulation of Cl^- secretion by the serosal addition of PGE_1 was measured by an increase in I_{sc}. In all cases the maximum increase or decrease in I_{sc} occurring within 5 min is reported.

Transepithelial Unidirectional Flux Measurements

Transepithelial unidirectional flux measurements were carried out in Ussing chambers, using [^{14}C]-mannitol and [^{3}H]-cephalexin as marker molecules. Sections of ileum and distal colon from the same animal were studied simultaneously. Ten mM mannitol was added to the mucosal bathing solution, and 2.5 mM mannitol and 7.5 mM glucose to the serosal bathing solution. Cephalexin was added to both bathing solutions to a final concentration of 0.1 mM. After a 60-120 min equilibration, 5 μCi of [^{3}H]-cephalexin and 3 μCi of [^{14}C]-mannitol were added to mucosal or serosal bathing solution for measurement of mucosal-to-serosal (m-to-s) or serosal-to-mucosal (s-to-m) fluxes, respectively. The rationale for including unlabeled mannitol and cephalexin in both the donor and receiver bathing solutions is to avoid nonspecific binding. Inclusion of unlabeled mannitol and cephalexin in both bathing solutions will not alter interpretation of the transport results since radiolabeled tracer is being measured. One ml samples were obtained from the receiver (cold) chamber at 30 and 60 min after addition of radiolabeled markers (control period). CapMul MCM was added immediately after collection of the 60 min sample and the receiver chamber was sampled again at 75 and 105 min. After each sample was taken, 1 ml of cold bathing solution was added back to maintain constant volume. One hundred μl samples were taken from the donor (hot) chamber at 30, 60, 75 and 105 min. after addition of radiolabeled marker molecules. These volumes were not replaced. The samples were mixed with 10 ml scintillation liquid (Ready Safe, Beckman Instruments, Inc., Fullerton, CA) and radioactivity counted in a Packard Tri-Carb 4640 scintillation counter. Counts per minute (cpm) were converted to disintegration per minute (dpm) using the external standard channels ratio. Permeability (P, cm/h) was calculated by dividing flux with the initial concentration of the transported molecule in the donor chamber. During the transport experiments, tissues were continuously short-circuited except for brief intervals (<10 sec) during which time R_t was measured.

Statistics

Statistical comparisons between experimental tissues and their corresponding control were performed with a paired Student's t-test. A level of $P < 0.05$ was considered significant. Results are reported as means ± S. E.

RESULTS AND DISCUSSION

The Ussing chamber technique allows for continuous monitoring of the electrical properties, and hence viability, of the tissue and simultaneous measurement of transport across the tissue. The presence of a short-circuit current signifies that the mucosal cell is actively transporting ions

across the mucosa. Changes in short-circuit current indicate a change in active ion transport. The resistance reflects the "leakiness" of the tissue to ions. A change in R_t may result from an alteration in tissue integrity, or, from a change in a specific ion conductance of the cell membrane.

Effect of CapMul MCM on I_{sc}, R_t and Active Ion Transport in Rabbit Ileum

Sections of rabbit ileum (45 tissues from 8 animals) were found to have a basal I_{sc} and R_t in the range of 1.6–3.4 μEq/h cm^2 and 34–43 ohms cm^2. Addition of CapMul MCM to the mucosal bathing solution produced an initial increase in I_{sc} over the first 5 min, followed by a decrease. The secondary decrease in I_{sc} is concentration dependent (Fig. 1). Following a 20 min incubation with 0.05, 0.5 and 5% CapMul MCM, I_{sc} was reduced to 65, 45 and 30% of the initial control value. R_t was not affected by incubation with CapMul MCM (data not shown).

The effect of CapMul MCM on active ion transport was determined by measuring the maximal change in I_{sc} (within a 5-min time period) in response to consecutive additions of (i) 5 mM L-leucine and (ii) 10 mM D-glucose to the mucosal bathing solution and (iii) 0.01 mM PGE_1 to the serosal bathing solution at the end of the CapMul MCM incubation period. The results are shown in Fig. 2. Changes in I_{sc} produced by L-leucine, D-glucose and PGE_1 in the presence of 0.05% and 0.5% CapMul MCM were not significantly different from control values (0% CapMul MCM). However, approximately 70–80% of the Na^+-coupled active transport of leucine and glucose was found to be inhibited in the presence of 5% CapMul MCM. The PGE_1 response, on the other hand, was not inhibited in the presence of 5% CapMul MCM (Fig. 2). The Na^+-glucose and Na^+-leucine cotransporters are located in the apical membrane of the villus cells, while the PGE_1-sensitive Cl^--channel is located in the apical membrane of the crypt cells. The selective inhibition of the Na^+-glucose and Na^+-leucine cotransporters suggests that the effect of Capmul MCM is largely restricted to villus cells.

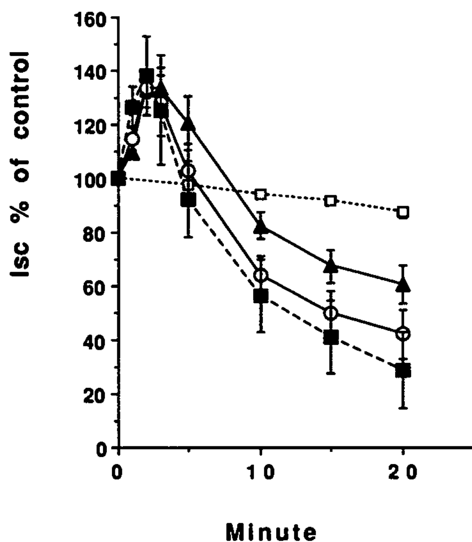

Figure 1. Change in I_{sc} in rabbit ileum in the presence of different concentrations of CapMul MCM as a function of time. CapMul MCM was added to the mucosal chamber at time zero (30–60 min after mounting tissues). Results are means ± 1 S. E. for 5–10 tissues from 5 animals. (□), 0% CapMul MCM; (▲), 0.05% CapMul MCM; (○), 0.5% CapMul MCM; (■), 5% CapMul MCM.

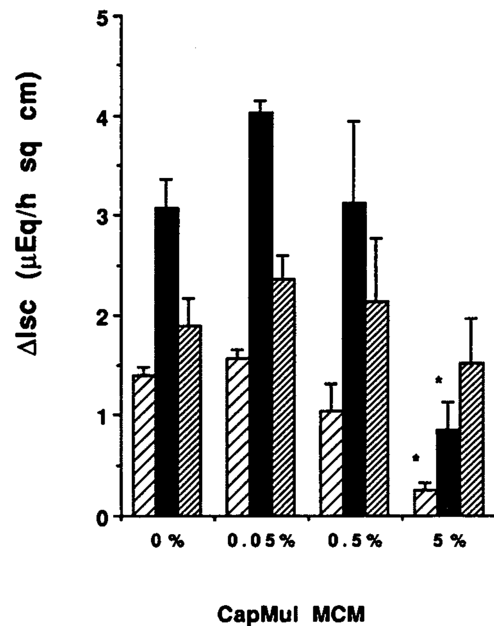

Figure 2. Change in I_{sc} in rabbit ileum upon addition of mucosal leucine (5 mM) and glucose (10 mM), and serosal PGE_1 (0.01 mM) in the presence of different concentrations of CapMul MCM in the mucosal chamber, as described in Materials and Methods. In other experiments, the effects on I_{sc} were found to be independent of the order of addition of leucine, glucose and PGE_1. Data shown are means ± 1 S. E. for 5–10 tissues from 5 animals. (▨), response to L-Leucine; (■), response to glucose; (▩), response to PGE1. * indicates significant difference from control ($p < 0.05$).

To investigate the reversibility of the CapMul MCM-induced change in I_{sc}, CapMul MCM (5%) was removed and the mucosal chamber rinsed with 100 ml mucosal bathing solution preincubated in a water bath at 37°C. Fig. 3 shows I_{sc} returned to 85% of the control value within 25 min after the rinse and completely returned to the control value within 75 minutes. The Na^+-coupled glucose transport and PGE_1 response of the tissues had also returned to the control level at the end of the tissue recovery period (data not shown).

Effect of CapMul MCM on Short-circuit Current, Resistance and Active Ion Transport in Rabbit Distal Colon

Rabbit distal colon exhibited a basal I_{sc} in the range of 1.0–3.0 μEq/h cm^2 and R_t of 200–300 ohms cm^2 (30 tissues from 5 animals). Both I_{sc} and R_t were reduced upon addition of CapMul MCM in a time and concentration-dependent manner. Figure 4 shows that following a 10 minute incubation with CapMul MCM (0.05, 0.1 and 0.3%) I_{sc} was reduced while at a higher concentration of CapMul MCM (1%), the I_{sc} changed polarity. After CapMul MCM was removed, I_{sc} returned toward control values within 95 min. Unlike ileal tissues, R_t of colonic tissues decreased in the presence of CapMul MCM (Fig. 5). Addition of 1% CapMul MCM reduced R_t to 10% of the control value and R_t did not recover

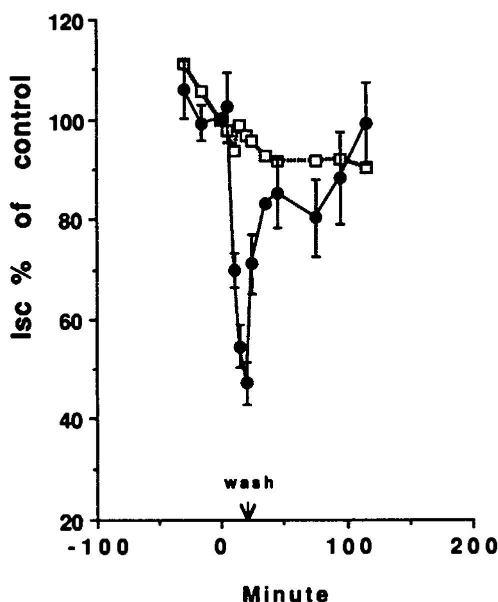

Figure 3. Reversibility of CapMul MCM-induced change in I_{sc} in rabbit ileum. CapMul MCM was added to the mucosal chamber at time zero (30–60 min after mounting of the tissues). After 20 min CapMul MCM was removed by washing the mucosal chamber with 100 ml mucosal buffer solution without CapMul MCM. Data at 0% CapMul MCM are means for 6 tissues from 2 animals, and data at 5% CapMul MCM are means ± 1 S. E. for 9 tissues from 3 animals. (□), 0% CapMul MCM; (●), 5% CapMul MCM.

after CapMul MCM was removed. At lower CapMul MCM concentrations, R_t was reduced to 95, 80, and 45% of control values by 0.05, 0.1, and 0.3% CapMul MCM, respectively, and slowly recovered to >80% within 100 min after CapMul MCM removal.

Frizzell et al. (24) have demonstrated that active Na^+ transport is the major determinant of the I_{sc} in rabbit distal colon. Na^+ movement into the surface cell occurs through

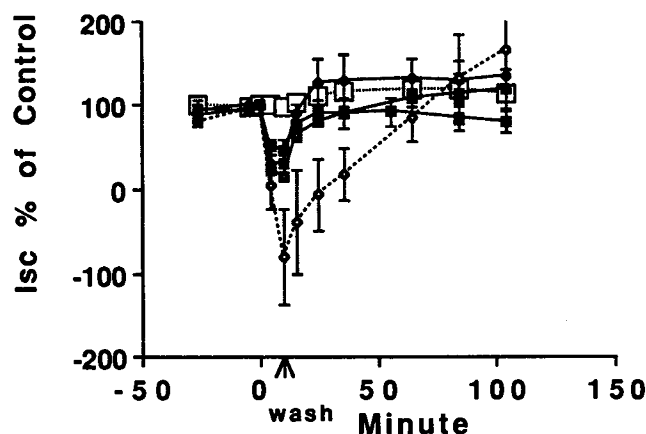

Figure 4. Reversibility of CapMul MCM-induced change in I_{sc} in rabbit distal colon. CapMul MCM was added to the mucosal chamber at time zero (60–120 min after mounting tissues). After 10 minutes CapMul MCM was removed by rinsing the mucosal chamber with 100 ml of mucosal buffer solution without CapMul MCM. Data are means ± 1 S. E. for 5–10 tissues from 5 animals. (□), 0% CapMul MCM; (♦), 0.05% CapMul MCM; (□), 0.1% CapMul MCM; (■), 0.3% CapMul MCM; (◇), 1% CapMul MCM.

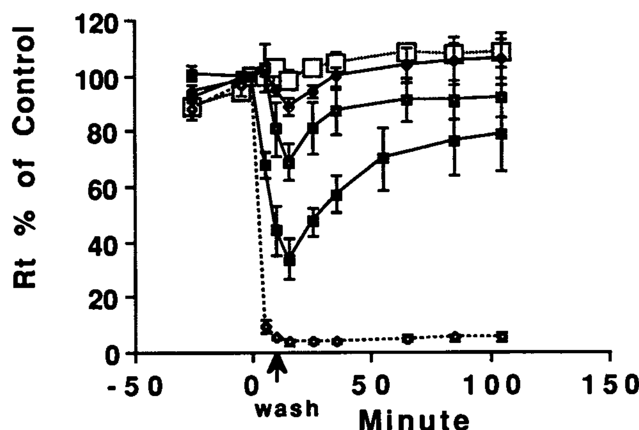

Figure 5. Reversibility of CapMul MCM-induced change in Rt in rabbit distal colon. CapMul MCM was added to the mucosal chamber at time zero (60 – 120 min after mounting tissues). After 10 minutes, CapMul MCM was removed by rinsing the mucosal chamber with 100 ml of mucosal buffer solution without CapMul MCM. Data are means ± 1 S. E. for 5–10 tissues from 5 animals. (□), 0% CapMul MCM; (♦), 0.05% CapMul MCM; (□), 0.1% CapMul MCM; (■), 0.3% CapMul MCM; (◇), 1% CapMul MCM.

conductive channels that can be specifically blocked by the mucosal addition of amiloride (16,28,29). After removal of CapMul MCM (1%) from the distal colonic tissue I_{sc} returned to control values within 90 minutes. However, at this time, I_{sc} could only partially be blocked by amiloride (Fig. 6). Hence, the observed reversal of I_{sc} is due to transport of ions other than Na^+ or to Na^+ transport by an amiloride-insensitive pathway. Following treatment with 0.05, 0.1, and 0.3% CapMul MCM and recovery from this treatment, the response to amiloride was indistinguishable from untreated control tissues, indicating complete reversal of effects. On the other hand, the PGE_1 response was not significantly different between the CapMul MCM-treated tissues (at all concentrations tested) and the control tissues (Fig. 6). These results suggest that the effect of CapMul MCM on colonic tissue was primarily confined to surface cells.

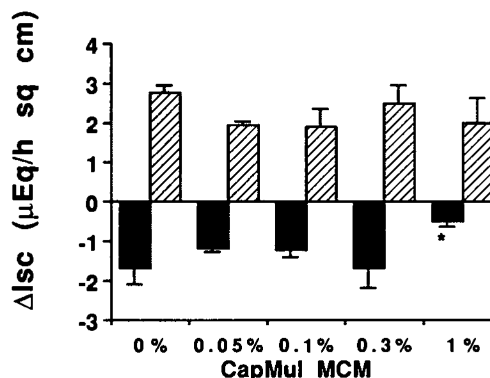

Figure 6. Change in I_{sc} of CapMul MCM-treated rabbit distal colon upon addition of mucosal amiloride (10 μM) and serosal PGE_1 (10 μM), 100 min *after* removing CapMul MCM from the mucosal chamber, as described in Materials and Methods. Data are means ± 1 S.E. for 5–10 tissues from 5 animals. *indicates significant difference from control ($p < 0.05$). (■), response to ameloride; (□), response to PGE1.

Table I. Permeability (P) of Unidirectional Transport (m-to-s and s-to-m) of [^{14}C]-Mannitol and [^{3}H]-Cephalexin Across Isolated Rabbit Ileum and Distal Colon Segments in the Absence and Presence of CapMul

	P (Mannitol), cm/h				P (Cephalexin), cm/h			
	Control		CapMul[1]		Control		CapMul[1]	
Tissue	m-to-s	s-to-m	m-to-s	s-to-m	m-to-s	s-to-m	m-to-s	s-to-m
Ileum	0.0038	0.0038	0.0273	0.0213	0.0657	0.0070	0.0402	0.0173
	(0.0006)	(0.0005)	(0.0059)	(0.0036)	(0.0055)	(0.0003)	(0.0021)	(0.0024)
Distal Colon	0.0023	0.0024	0.1948	0.2751	0.0061	0.0073	0.1355	0.2124
	(0.0002)	(0.0005)	(0.0171)	(0.0388)	(0.0006)	(0.0007)	(0.0213)	(0.0307)

[1]: 5 and 1% CapMul were used in rabbit ileum and distal colon, respectively.
Numbers in parentheses are S.E.
In all cases, mannitol and cephalexin permeability were significantly different in control and CapMul MCM treated tissues.
Only in the CapMul MCM treated ileum was there a significant difference ($p < 0.05$) between s-to-m and m-to-s permeability of cephalexin. In all other cases m-to-s and s-to-m permeabilities were not significantly different ($p > 0.05$).
In control tissues, mannitol permeability was significantly different from cephalexin permeability ($p < 0.05$), while in CapMul MCM treated tissues there was no significant difference ($p > 0.05$) between mannitol and cephalexin permeability, with the exception of m-to-s transport of cephalexin in the ileum.

Effects of CapMul MCM on Transport of [^{14}C]-Mannitol and [^{3}H]-Cephalexin across Rabbit Ileum and Distal Colon

Unidirectional permeabilities of mannitol and cephalexin across rabbit ileum and distal colon are presented in Table I. I_{sc} and R_t were also determined concurrently with the fluxes (see Table II). As expected for a paracellular marker, mannitol permeability in the m-to-s direction is equal to that in the s-to-m direction in both ileum and distal colon (Table I, control). Hidalgo et al. (32) have demonstrated that cephalexin is transported across rabbit small intestinal mucosa by a carrier-mediated transport mechanism. This transporter is expressed only on the apical surface of jejunum and ileum and is not present in distal colon. In the ileum, the permeability of cephalexin in the m-to-s direction is 9 times greater than that in the s-to-m direction, while the permeabilities in the distal colon are equal in either direction.

Addition of 5% CapMul MCM to the mucosal bathing solution of ileal tissue resulted in a 15% decrease in R_t and an approximately 6 fold enhancement of mannitol transport in both m-to-s and s-to-m directions. Addition of 1% CapMul MCM to distal colonic tissue resulted in a decrease in resistance of 96% and an approximately 100 fold increase in mannitol transport in both directions. In addition, 5% CapMul MCM inhibited the m-to-s transport of cephalexin in the ileum by 40%, while the s-to-m transport was increased 2.5 fold. The inhibition in the m-to-s direction is probably a combination of a perturbation of the carrier-mediated transport mechanism of cephalexin and an enhanced passive permeability of cephalexin. In distal colon, 1% CapMul MCM enhanced both m-to-s and s-to-m transport of cephalexin by approximately 25 fold.

It is interesting to note that, in the CapMul MCM treated tissues, there is no significant difference between mannitol and cephalexin fluxes, except m-to-s transport of cephalexin in the ileum. In the CapMul MCM treated ileum, m-to-s transport of cephalexin is still significantly higher than s-to-m transport. This is most likely due to incomplete inhibition of carrier-mediated transport of cephalexin. Note that these incubation conditions also result in only partial inhibition of I_{sc}.

The above results demonstrate that the distal colon is more sensitive to CapMul MCM than the ileum, with respect to active ion transport, R_t and tissue permeability. Similar findings have been reported *in vivo* in rats, e.g. the colon showed higher sensitivity than the duodenum or ileum to the action of sodium salicylate in mineral oil (33), taurocholate-monoolein mixed micelles (34) and mixed micelles of taurocholate with sodium caprate, laurate and caprylate (35).

Our observations are similar to those made by Moore et al (27) who demonstrated that Triton X100 treatment of guinea pig ileum, mounted in Ussing chambers, resulted in a 47% decrease in I_{sc} and a 32% decrease in R_t and a 3–5 fold increase in solute flux. Removal of the detergent resulted in a rapid recovery of ion transport and R_t and ablation of increased solute flux (in about 1 hour). Morphologic evidence demonstrated that Triton X100 caused a denudation of villus tips, while leaving the lower part of the villus and the crypts

Table II. Short-Circuited Current (I_{sc}) and Resistance (R_t) Measured Across Isolated Rabbit Ileum and Distal Colon Segments in the Absence and Presence of CapMul[1].

	I_{sc} (μEq/h cm^2)		R_t (ohms cm^2)	
Tissue	Control	CapMul[2]	Control	CapMul[2]
Ileum	2.21	1.10*	33.1	27.2*
	(0.33)	(0.21)	(0.76)	(2.04)
Distal Colon	2.89	0.10*	249.0	9.3*
	(0.51)	(0.67)	(12.7)	(2.27)

Numbers in parentheses are S.E.
[1]: I_{sc} and R_t during control period were measured 60 min after addition of radiolabeled molecules. I_{sc} and R_t of CapMul MCM treated tissue were measured 105 min after addition of the radiolabeled molecules, i.e. at the end of the CapMul MCM incubation period. In untreated tissues I_{sc} and R_t remain constant between 60 and 105 min (see Fig. 3, 4 and 5).
[2]: 5 and 1% CapMul were used in rabbit ileum and distal colon, respectively.
*: significantly different from control ($p < 0.05$).

intact. It was argued that the recovery process was too rapid to be accounted for by enhanced cell proliferation and was due to migration of neighboring cells into the denuded area.

Selective destruction of surface epithelium, without significantly affecting crypt epithelium, has been described following *in vivo* treatment of porcine colon with deoxycholate (30,31). The extent of denudation was dependent on deoxycholate concentration. Denudation resulted in a loss of ion transport, decrease in R_t and increase in solute flux. Removal of deoxycholate resulted in a rapid restitution of the barrier function. Solute flux returned to normal in 40 min, while ion transport returned to normal in 2 hrs. From these studies it was proposed that active migratory events play an important role in restitution of the barrier.

In summary, CapMul MCM affects the electrical properties and permeability of rabbit intestinal epithelium *in vitro*. The distal colon is more sensitive to the effects of CapMul MCM than the ileum. The CapMul MCM effect appears to be localized to villus cells in the ileum and surface cells in the distal colon. The effect of CapMul MCM on electrical properties in the ileum was rapidly reversible at all concentrations measured, whereas the effect in the colon was reversible only at the lower concentrations. The similarities between our studies and those cited above (14,27,30,31) suggest that the CapMul MCM-induced decrease in ion transport and resistance and increase in solute transport may be caused by enhanced sloughing of cells from the surface epithelium. This hypothesis will required further investigation.

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REFERENCES

1. S. Muranishi. Absorption enhancers. *Critical Reviews in Therapeutic Drug Carrier Systems* 7:1–33 (1990).
2. E. S. Swenson and W. J. Curatolo. Means to enhance penetration, (2) Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. *Adv. Drug Del. Rev.* 8:39–92 (1992).
3. E. J. Van Hoogdalem, A. G. De Boer, and D. D. Breimer. Intestinal drug absorption enhancement: an overview. *Pharmac. Ther.* 44:407–443 (1989).
4. S. Muranishi. Modification of intestinal absorption of drugs by lipoidal adjuvants. *Pharm. Res.* 3:108–118 (1985).
5. E. J. Van Hoogdalem, A. M. Stijnen, A. G. De Boer, and D. D. Breimer. Rate-controlled absorption enhancement of rectally administered cefazolin in rats by a glyceride mixture (MGK). *J. Pharm. Pharmacol.* 40:329–332 (1988).
6. K. Higaki, I. Kishimoto, H. Komatsu, M. Hashida, and M. Hitoshi. Effect of medium-chain glycerides on the intestinally and the hepatobiliary transport of phenol red. *Int. J. Pharm.* 36:131–139 (1987).
7. G. Beskid, J. Unowsky, G. R. Behl, J. Siebelist, J. L. Tossounian, C. M. McGarry, N. H. Shah, and R. Cleeland. Enteral, oral, and rectal absorption of ceftriaxone using glyceride enhancers. *Pharmacology* 34:77–84 (1988).
8. M. Sekine, K. Sasahara, T. Kojima, K. Hasegawa, and R. Okada. Improvement of bioavailability of poorly intestinally absorbed drugs from medium-chain glyceride base. Enhancement of the rectal absorption of cefmetazole sodium in rabbits. *Chem. Pharm. Bull.* 32:4189–4192 (1984).
9. M. Sekine, H. Terashima, K. Sasahara, K. Nishimura, R. Okada, and S. Awazu. Improvement of bioavailability of poorly absorbed drugs. II. Effect of medium chain glyceride base on the intestinal absorption of cefmetazole sodium in rats and dogs. *J. Pharmacobio-Dyn.* 8:286–295 (1985).
10. N. Muranushi, N. Takagi, S. Muranishi, and H. Sezaki. Effect of fatty acids and monoglycerides on permeability of lipid bilayer. *Chem. Phys. Lipids.* 28:269–279 (1981).
11. K. Higaki, N. Takechi, M. Hashida, and H. Sezaki. Enhancement of membrane permeability to a poorly absorbed drug by medium-chain glycerides: Effect of medium-chain glycerides on the release of phenol red from egg phosphatidylcholine liposomes. *Chem. Pharm. Bull.* 36:1214–1217 (1988).
12. K. Higaki, M. Kato, M. Hashida, and H. Sezaki. Enhanced membrane permeability to phenol red by medium-chain glycerides: studies on the membrane permeability and microviscosity. *Pharm. Res.* 5:309–312 (1988).
13. K. Higaki, N. Takechi, M. Kato, M. Hashida, and H. Sezaki. Effect of medium-chain glycerides on the intestinal absorption of phenol: studies on mechanisms of the promoting effect. *J. Pharm. Sci.* 79:334–338 (1990).
14. E. J. Van Hoogdalem, C. Vermeij-Kerrs, A. G. De Boer, and D. D. Breimer. Topical effects of absorption enhancing agents on the rectal mucosa of rats in vivo. *J. Pharm. Sci.* 79:866–870 (1990).
15. S. G. Schultz. Salt and water absorption by mammalian small intestine. In L. R. Johnson (eds), *Physiology of The Gastrointestinal Tract,* Raven Press, New York, 1981, pp. 983–989.
16. H. J. Binder and G. I. Sandle. Electrolyte absorption and secretion in the mammalial colon. In L. R. Johnson (eds), *Physiology of The Gastrointestinal Tract,* Raven Press, New York, 1987, pp. 1389–1418.
17. P. M. Reardon, C. H. Gochoco, K. L. Audus, G. Wilson, and P. L. Smith. In vitro nasal transport across ovine mucosa: Effects of ammonium glycyrrhizinate on electrical properties and permeability of growth hormone releasing peptide, mannitol, and lucifer yellow. *Pharm. Res.* 10:553–561 (1993).
18. M. A. Weatley, J. Dent, E. B. Wheeldon, and P. L. Smith. Nasal drug delivery: An in vitro characterization of transepithelial electrical properties and fluxes in the presence and absence of enhancers. *J. Control. Release* 8:167–177 (1988).
19. B. Matuszewska, G. G. Liversidge, F. Ryan, J. Dent, and P. L. Smith. In vitro study of intestinal absorption and metabolism of 8-L-arginine vasopressin and its analogue. *Int. J. Pharm.* 46:1110–1120 (1988).
20. P. L. Smith, C. Mirabelli, J. Fondacaro, F. Ryan, and J. Dent. Intestinal 5-fluorouracil absorption: Use of Ussing chambers to assess transport and metabolism. *Pharm. Res.* 5:598–603 (1988).
21. P. M. Reardon, D. A. Wall, T. K. Hart, P. L. Smith, and C. H. Gochoco. Lack of effect of ammonium glycyrrhizinate on the morphology of ovine nasal mucosa in vitro. *Pharm. Res.* 10:1301–1307 (1993).
22. R. B. Finley and P. L. Smith. Stimulation of chloride secretion by N-formyl-methionylleucylphenylalanine (FMLP) in rabbit ileal mucosa. *J. Physiol.* 417:403–419 (1989).
23. M. Jett, P. Marshall, J. D. Fondacaro, and P. L. Smith. Action of peptidoleukotrienes on ion transport in rabbit distal colon in vitro. *J. Pharmaco. Exp. Ther.* 257:698–705 (1991).
24. R. A. Frizzell, M. J. Koch, and S. G. Schultz. Ion transport by rabbit colon: I. active and passive components. *J. Membrane Biol.* 27:297–316 (1976).
25. R. A. Frizzell and K. Turnheim. Ion transport by rabbit colon: II. unidirectional sodium influx and the effects of amphotericin B and amiloride. *J. Membrane Biol.* 40:193–211 (1978).
26. K. Heintze, C. P. Stewart, and R. A. Frizzell. Sodium-dependent chloride secretion across rabbit descending colon. *Am. J. Physiol.* 244:G357–G365 (1983).
27. R. Moore, S. Carlson, and J. L. Madara. Rapid barrier restitution in an in vitro model of intestinal epithelial injury. *Laboratory Investigation* 60:237–244 (1989).
28. S. G. Schultz. A cellular model for active sodium absorption by mammalian colon. *Annu. Rev. Physiol.* 46:435–451 (1984).
29. N. K. Wills, S. A. Lewis, and D. C. Eaton. Active and passive properties of rabbit descending colon: A microelectrode and nystatin study. *J. Membrane Biol.* 45:81–108 (1979).

30. R. A. Argenzio, C. K. Henrikson, and J. A. Liacos. Restitution of barrier and transport function of porcine colon after acute mucosal injury. *Am. J. Physiol.* 255:G62–G71 (1988).
31. C. K. Henrikson, R. A. Argenzio, J. A. Liacos, and J. Khosla. Morphologic and functional effects of bile salt on the porcine colon during injury and repair. *Laboratory Investigation* 60:72–87 (1989).
32. I. J. Hidalgo, F. M. Ryan, G. Marks, and P. L. Smith. pH-dependent transepithelial transport of cephalexin in rabbit intestinal mucosa. *Int. J. Pharm.* 98:83–92 (1993).
33. J. A. Moore, S. A. Pletcher, and M. J. Ross. Absorption enhancement of growth hormone from the gastrointestinal tract of rats. *Int. J. Pharm.* 34:35–43 (1986).
34. K. Taniguchi, S. Muranishi, and H. Sezaki. Enhanced intestinal permeability to macromolecules. II. Improvement of the large intestinal absorption of heparin by lipid-surfactant mixed micelles in rat. *Int. J. Pharm.* 4:219–228 (1980).
35. T. Ishizawa, M. Hayashi, and S. Awazu. Enhancement of jejunal and colonic absorption of fosfomycin by promoters in the rat. *J. Pharm. Pharmacol.* 39:892–895 (1987).