Interactions of intestinal lymph flow and secretion

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ALTHOUGH THE SMALL INTESTINE is normally an absorbing organ, there are many pathological conditions that result in massive fluid loss into its lumen (4, 9, 13). A net movement of fluid and solute into the intestinal lumen can result from an increased net filtration pressure across the intestinal capillary membrane or stimulation of active solute transport into the lumen.

An increased net capillary filtration pressure can be induced by 1) increasing capillary pressure, 2) decreasing plasma colloid oncotic pressure, or 3) increasing capillary permeability. In most tissues, capillary pressure can increase significantly before interstitial edema occurs. The resistivity to edema formation (safety factor) following an increase in net capillary filtration pressure is due to a readjustment of the capillary and tissue forces, i.e., tissue pressure and lymph flow increase, and tissue colloid osmotic pressure decreases. The changes in tissue forces have been demonstrated after elevation of venous pressure (15, 22) and plasma volume expansion (7, 14). In the small intestine, an increase in venous pressure or a decrease in plasma colloid oncotic pressure of 12-15 mmHg will result in passive fluid movement (filtration secretion) into the intestinal lumen (17). Therefore, the total safety factor against filtration secretion in the small intestine is only 12-15 mmHg.

Stimulation of active transport processes in the mucosal epithelium can also cause net fluid and solute movement into the intestinal lumen. Several substances have been shown experimentally to elicit fluid secretion in the small intestine; these include extracellular products of V. cholerae (18), E. coli (9), and C. perfringens (8), vasopressin (20), and various prostaglandins (18). Recent in vitro studies suggest a role of CAMP in the active chloride secretion induced by the previously mentioned "secretagogues" (16). At present, choleragenic secretion is believed to be totally independent of capillary filtration forces.

Several investigations have analyzed the effect of an increased filtration force on steady-state lymph flows; however, only a limited number of these studies have actually dealt with the transient nature of lymph flow changes following these perturbations (3, 6).

The present study was designed to determine the transient responses of lymph flow and filtration secretion rate after either a rapid elevation of intestinal venous pressure (to 30 mmHg) or a decrease in plasma colloid oncotic pressure caused by intravenous Tyrode infusion. In addition, the intestinal lymph flow and secretion rate were studied after cholera toxin exposure.

METHODS

Animal Preparation

Female cats (2-3 kg) were fasted for a period of 24 h, except for the feeding of a milk and cream mixture (50 ml) approximately 12-16 h prior to surgery. Each cat was initially anesthetized with ketamine (30 mg/kg im) and supplementary doses of sodium pentobarbital were given as required to maintain light anesthesia. A tracheal cannula was used to facilitate breathing and as a means of artificially respiring the cats if they failed to breathe spontaneously. Following a midline abdominal incision, the greater omentum, spleen, stomach, large intestine, and most of the small intestine were surgically removed. A loop of ileum weighing 25-50 g, with intact innervation and lymphatic drainage, was isolated and autoperfused by the intact mesenteric artery. Atropine (1 mg/kg) was administered intravenously for the prevention of excessive intestinal motility. Body temperature was maintained at 37°C with an overhead
infrared lamp and a heating pad placed beneath the animal. All exposed tissue was moistened with saline-soaked gauze and covered with a plastic sheet in order to minimize evaporation and tissue dehydration. Plexiglass plugs containing inflow and outflow cannulas were tied into each end of the isolated ileal segment. A femoral artery was catheterized and connected to a Statham transducer (P23A) for measurement of systemic arterial pressure, and a femoral vein was catheterized for infusion of solutions, anesthetics, etc. Venous outflow pressure of the intestinal loop was monitored through a T connector inserted into the superior mesenteric vein. The T connector and associated pressure transducer (Statham, P-BC) were positioned at heart level. In those experiments that required the maintenance of a constant venous pressure (cholera toxin, increased venous pressure), a large venous outflow cannula was inserted into the superior mesenteric vein, and the venous outflow was passed through a reservoir system via an extracorporeal flow circuit. The intestinal venous pressure could be set at any desired level by adjusting the reservoir height. After passage through the external reservoir, blood was returned to the animal by way of a cannula inserted into the right external jugular vein. Heparinized blood from a freshly sacrificed donor cat was used to fill all tubing of the extracorporeal flow circuit. Venous outflow pressure was obtained through a T connector inserted into the venous outflow circuit immediately distal to the superior mesenteric vein. A polygraph recorder was used to continuously record arterial and venous outflow pressures (Grass Model 7B).

**Determination of Lymph Flows**

The lymphatic vessels of the intestine were clearly marked 12-16 h after the cat had ingested the mixture of milk and cream. With the aid of a dissecting microscope, a cannula was inserted in the large lymphatic vessel emerging from the mesenteric pedicle. Entrance to the vessel was made at a point between the pedicle and before the vessel entered into the superior mesenteric lymph node. Lymph flow was determined by observing the lymph movement in a calibrated pipette (1 ml full scale) that had been connected into the lymphatic catheter. The pipette was positioned horizontally and at the level of the intestinal segment under study in order to eliminate any hydrostatic factors. Smaller lymphatics emerging from the mesenteric pedicle were occluded; therefore, with the greater part of the small intestine removed, lymph flow measured in our preparations must reflect total lymph flow from the ileal segment. Lymph flow was measured at 15-min intervals during the entire time course of all experiments reported in this work.

**Determination of Secretion Rates**

After both plexiglass plugs were secured in the ileal lumen, the intestinal contents were removed by slow infusion of Tyrode solution into the inflow cannula of one plug, whereas the outflow cannula of the opposite plug remained open for drainage. Once the intestinal contents became clear, a gentle stream of air was applied to aid in the removal of residual fluid. At 15-min intervals thereafter, the air stream was applied, and the luminal fluid was collected in a 10- to 25-ml graduated cylinder. The luminal fluid collected during these time intervals was assumed to represent secreted fluid.

**Experimental Protocol**

**Decreased plasma colloids.** After establishment of extracorporeal lymph flow, Tyrode solution was infused into the femoral vein at a rate of approximately 2.5 ml/kg per min. Every 15 min thereafter, the following measurements were made: lymph flow, filtration secretion rate, plasma colloid osmotic pressure (Pratt-Guyton oosmeter), and hematocrit. Arterial and venous pressures were constantly monitored throughout the entire time course of the experiment.

**Elevated intestinal venous pressure.** Once control lymph flow was determined at 10 mmHg venous pressure, the intestinal venous pressure was rapidly increased to 30 mmHg by raising the height of the venous reservoir. At 5 min intervals for 30 min and 15 min intervals thereafter following venous pressure elevation, the following measurements were made: lymph flow, filtration secretion rate, plasma colloid osmotic pressure, and hematocrit.

**Cholera toxin challenge.** The cholera toxin solution was prepared by dissolving 10 g of a freeze-dried crude filtrate of *Vibrio cholera* (Wyeth Laboratories, courtesy of the National Institutes of Health Cholera Advisory Committee) in 100 ml Tyrode's solution. The crude toxin solution was dialyzed against a Tyrode's solution for 48 h at 4°C in order to remove low molecular weight impurities and retain isotonicity. After measuring control lymph flow at a set venous pressure of 10 mmHg, the cholera toxin solution (10 g/100 ml) was introduced into the ileal lumen. This solution was replaced during the first hour because previous experiments have indicated that approximately 1-2 h are required before secretion begins. The same parameters were measured during the remainder of the experimental period as measured in the increased venous pressure and decreased colloid groups.

**RESULTS**

The results of the present study are summarized in Table 1.

**Decreased Plasma Colloids**

Figure 1A is from a typical experiment showing the changes in intestinal lymph flow and secretion rate during continuous infusion of Tyrode solution (2.5 ml/min per kg). Control values for lymph flow, plasma colloid osmotic pressure, hematocrit, and venous pressure measured at 15 and 30 min prior to beginning the infusion averaged 0.6 ml/min per 100 g, 21.5 mmHg, 50%, and 4 mmHg, respectively. Lymph flow increased immediately after the infusion began and attained a maximum measured flow of 1.70 ml/min per 100 g approximately 75 min after beginning the infusion. Note
that secretion was not observed until 45 min after starting
the infusion. At the maximal observed lymph flow, a
secretion rate of .55 ml/min per 100 g was recorded,
whereas plasma colloid osmotic pressure, hematocrit,
and venous pressure were 3 mmHg, 20%, and 9 mmHg,
respectively. After the 75-min measurements, lymph
flow progressively fell, whereas secretion rate in-
creased. At the 160-min measurement lymph flow and
secretion rate were equal (1.10 ml/min per 100 g).

TABLE 1. Lymph flow and secretion rates after
elevation of intestinal venous pressure to 30
mmHg and decrease of plasma colloids by
Tyrode solution infusion, and during
cholera toxin secretion

<table>
<thead>
<tr>
<th></th>
<th>Exp</th>
<th>Control* Lymph Flow, ml/ min per 100 g</th>
<th>Mean ± SE</th>
<th>Maximum Lymph Flow (Lmax), ml/min per 100 g</th>
<th>Mean ± SE</th>
<th>Secrecion Rate at Lmax, ml/min per 100 g</th>
<th>Mean ± SE</th>
<th>Maximal Secrecion Rate (Secmax), ml/min per 100 g</th>
<th>Mean ± SE</th>
<th>Lymph Flow at Secmax, ml/min per 100 g</th>
<th>Mean ± SE</th>
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<tr>
<td>Decreased plasma colloids</td>
<td>N1</td>
<td>0.060</td>
<td>1.700</td>
<td>0.500</td>
<td>2.000</td>
<td>0.000</td>
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<td>N9</td>
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<td>1.500</td>
<td>0.300</td>
<td>2.200</td>
<td>0.030</td>
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<tr>
<td>N4</td>
<td>0.020</td>
<td>1.600</td>
<td>0.400</td>
<td>2.300</td>
<td>0.000</td>
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<tr>
<td>Mean ± SE</td>
<td>0.043 ± 0.009</td>
<td>1.500 ± 0.100</td>
<td>0.340 ± 0.090</td>
<td>2.450 ± 0.290</td>
<td>0.330 ± 0.020</td>
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<tr>
<td>Increased venous pressure</td>
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<td>1.000</td>
<td>0.100</td>
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<td>0.100</td>
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<td>1.250</td>
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<tr>
<td>M4</td>
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<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
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<td>0.900</td>
<td>0.150</td>
<td>1.300</td>
<td>0.080</td>
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<tr>
<td>Mean ± SE</td>
<td>0.020 ± 0.009</td>
<td>1.100 ± 0.090</td>
<td>0.35 ± 0.07</td>
<td>1.250 ± 0.047</td>
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<td>Cholera toxin</td>
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<td>0.000</td>
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<td>0.007</td>
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<td>1.150</td>
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<tr>
<td>B17</td>
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<td>1.700</td>
<td>0.150</td>
<td>1.140</td>
<td>0.000</td>
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<tr>
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<td>0.900</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>Mean ± SE</td>
<td>0.080 ± 0.009</td>
<td>1.43 ± 0.018</td>
<td>0.02 ± 0.028</td>
<td>0.90 ± 0.028</td>
<td>0.02 ± 0.002</td>
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*Net secretion rates were not detected during the control state. †Each control lymph flow represent the average of 3 steady-state measurements.

Lymph flow in this experiment ceased approximately 210 min after the infusion began. The values for secretion rate, plasma colloid osmotic pressure, venous pressure, and hematocrit during this time period were 2.00 ml/min per 100 g, 3.0 mmHg, 11 mmHg, and 15%, respectively. At 240 min, secretion rate was 2.2 ml/min per 100 g, whereas lymph flow, plasma colloid osmotic pressure, venous pressure, and hematocrit were unchanged from measurements recorded at 210 min.

For this group of animals, the mean ± SE values for control lymph flow and maximum lymph flow were .04 ± .01 and 1.50 ± .11 ml/min per 100 g, respectively. These results indicate that decreasing plasma colloids by the methods used in our preparation caused an approximate 38-fold increase in lymph flow. The mean values for secretion rate at maximum lymph flow, maximum secretion rate, and lymph flow at maximum secretion rate were .34 ± .09, 2.45 ± .29 and .03 ± .02 ml/min per 100 g, respectively.

**Elevated Venous Pressure**

The changes in intestinal lymph flow and filtration secretion rate that occurred during a typical experiment during which venous pressure was elevated to 30 mmHg are shown in Figure 1B. Control lymph flows of .08 ml/min per 100 g were determined at 15 and 30 min prior to venous pressure elevation. Only 15 min were required for lymph flow to attain a maximum value (1.20 ml/min per 100 g) after the increase in venous pressure. Filtra-
tion secretion was first observed approximately 20 min after elevation of venous pressure. Lymph flow, after reaching its maximum value, progressively decreased as the secretion rate increased. The highest secretion rate observed in this particular experiment was 1.05 ml/min per 100 g. No significant changes in plasma colloid...
osmotic pressure, hematocrit, and venous pressure were observed throughout the experimental period. The mean control (.05 + .01) and maximum lymph flow (1.04 ± .06) values for this group indicates that a 20 mmHg increase in intestinal venous pressure resulted in a 20-fold increase in lymph flow. Mean values of .14 ± .11, 1.23 ± .05, and .07 ± .02 ml/min per 100 g were calculated for secretion rate at maximum lymph flow, maximum secretion rate, and lymph flow at maximum secretion rate, respectively.

**Cholera Toxin Challenge**

Figure 1C represents a typical experiment showing the changes in intestinal lymph flow and secretion rate after cholera toxin treatment. The control lymph flow was .08 ml/min per 100 g. After treatment with cholera toxin, no significant changes in lymph flow were observed until 45 min. A maximum lymph flow of .15 ml/min per 100 g was recorded at approximately 60 min after the intraluminal administration of cholera toxin. At 120 min, secretion began and lymph flow ceased. Secretion rate increased rapidly, reaching a maximum value of 1.10 ml/min per 100 g at the end of 240 min. No significant changes in plasma colloid osmotic pressure, hematocrit, or intestinal venous pressure occurred during the course of the experiment.

Mean values for control lymph flow, maximum lymph flow, secretion rate at maximum lymph flow, maximum secretion rate, and lymph flow at maximum secretion were .08 ± .01, .14 ± .02, .04 ± .04, .99 ± .08, .02 ± .02, respectively. The change in lymph flow observed after cholera toxin challenge averaged only 75% above control values.

**DISCUSSION**

Figure 2 is a schematic representation of the intestinal interstitium, capillary exchange vessels, and mucosal membrane. The volume of the interstitium (V) can be described in the following fashion

\[
V_T = \int [J_{V,MA} - J_{V,SP} - J_{V,SA} + J_{V,L} - J_{V,L}] \, dt + V_{T,0}
\]  

where \(J_{V,MA}\) is the volume moved across the mucosa as a result of active solute transport; \(J_{V,SP}\), the passive bulk movement of fluid into the intestinal lumen; \(J_{V,SA}\), the volume movement into the intestinal lumen caused by an active process, e.g., choleragenic secretion; \(J_{V,L}\), lymph flow; \(V_{T,0}\), the initial interstitial fluid volume; and \(J_{V,L}\), the volume flow across the capillary caused by imbalances in Starling forces.

Volume flow across the capillaries can be described as

\[
J_{V,L} = KFC \left[ (P_t - P_v) - \sigma (\pi_t - \pi_c) \right] 
\]

where \(KFC\) is the filtration coefficient of the capillary membrane; \(\sigma\), the osmotic reflection coefficient of the plasma proteins at the intestinal capillary (\(\sigma = 1\) if the proteins are impermeable and \(\sigma = 0\) if the proteins are freely permeable); \(P_t\), the capillary hydrostatic pressure; \(\pi_t\), the plasma colloid osmotic pressure; \(\pi_c\), the tissue oncotic pressure; and \(P_v\), the interstitial fluid pressure.

Under normal conditions, passive bulk movement of fluid into the intestinal lumen \((J_{V,SP})\) is essentially zero because of the very low hydraulic conductance of the mucosal membrane (10, 19). Also, the volume movement into the intestinal lumen caused by active processes \((J_{V,SA})\) is normally zero relative to volume movement across the mucosa; however, certain intestinal transport systems can decrease the net volume movement of fluid across the mucosa into the tissues, e.g., increase in chloride backflux associated with galactose transport (21). Using these assumptions, equation 1 simplifies to

\[
V_T = f \left[ J_{V,MA} + J_{V,L} - J_{V,L} \right] \, dt + V_{T,0}
\]

Equation 3 states that the intestinal interstitial volume is equal to the difference in lymphatic and capillary removal of the volume associated with active solute transport.

When intestinal capillary pressure is elevated or plasma colloid osmotic pressure is decreased, fluid moves from the vascular system into the intestinal interstitium. The fluid accumulation within the interstitium causes tissue pressure and lymph flow to increase and tissue colloid osmotic pressure to decrease; these changes oppose further filtration out of the capillaries. If the alterations in tissue forces are not sufficient to prevent filtration, then tissue pressure continues to increase, and, if elevated to values between 4-7 mmHg, filtration secretion will occur. An increase in the hydraulic conductance of the mucosal membrane was observed when 4-7 cm H2O pressure was applied to the serosal side of the intestine (12). Thus it appears that as tissue pressure increases to some critical level the normal tight junction between villus epithelial cells disrupts and provides a low resistance pathway for fluid movement into the intestinal lumen (11, 12). This observation can be mathematically described by placing \(J_{V,SP}\) into equation 3; once \(J_{V,SP} > J_{V,MA}\), then net filtration secretion occurs into the intestinal lumen.

The results of the present study indicate that when filtration secretion occurs, lymph flow also decreases.
This suggests that changes altering tissue forces and resulting in increased mucosal conductance may also be responsible for the observed decrease in lymph flow.

If intestinal venous pressure is elevated to a level at which no filtration secretion occurs (20 mmHg), the increased lymph flow (4-5 x normal) is maintained for several hours (Fig. 3). The results obtained at 20 mmHg venous pressure from four animals support the contention that the decreased lymph flow observed concomitant to filtration secretion is initiated and maintained by the filtration secretion process per se.

Lymph flow is known to be influenced by several factors, e.g., external compression forces acting on lymphatic vessels, intrinsic lymphatic contractility, and factors that promote lymphatic filling (pressure gradient and lymphatic wall conductance). It is possible that the decreased lymph flow, occurring concomitantly to the onset of filtration secretion, may be due, in part, to changes in one or more of these lymphatic propulsion factors. The contribution of external compression forces is probably insignificant under the conditions of these experiments because atropine was administered to prevent the gut motility-lymph flow effect.

It is likely that a decrease in lymphatic contractility and/or filling accounts for the incompetence of the lymphatics in these filtration secretion experiments. One cannot help but entertain the possibility that the decrease in lymph flow observed after the onset of filtration secretion results from the opening of a low resistance fluid pathway in the mucosa. Fig. 4 is a schematic representation of the mucosal interstitium. Panel A (Fig. 4) represents the mucosal interstitium under normal conditions in which the filling pressure \( P_f \) (i.e., in which case would be equal to tissue pressure) acts as the driving force for \( J_l \) (lymph flow), and \( J_{V,SP} \) (volume flow across the mucosa) equals zero. Panel B illustrates the effect of opening the mucosal membrane on \( J_l \) given the same filling pressure. This illustrates that simply opening a low conductance pathway \( J_{V,SP} \) will not alter the preexisting flow unless opening the new channel causes an increased resistance in the former pathway \( J_l \). A likely explanation for the resulting decrease in lymph flow after the onset of filtration secretion observed in our experiments is presented in Panel C; i.e., a decrease in filling pressure occurs, resulting in a greater volume flow through the mucosa with its higher conductance. There is certainly a distinct possibility that the volume flow across the mucosal membrane decreases lymphatic filling because the volume movement into the lumen may decrease tissue hydration and compliance that in turn decreases tissue pressure. In a recent study of Starling force balance in an isolated intestinal preparation (17), the authors suggest that tissue pressure may decrease once the filtration secretion process commences. Unfortunately, no direct experimental measurements of intestinal tissue pressure have been made during the filtration secretion process, and we must await such evidence to firmly support our hypothesis. Another possible explanation for the decreased lymph flow is that some endogenous humoral agent (e.g., villikinin) may normally stimulate lymphatic contractility, and this agent is either washed out of the interstitium or diluted by the filtration secretion process.

The secretion process that occurs after exposure of the gut lumen to cholera toxin differs markedly from filtration secretion. Unlike filtration secretion that occurs at the villus epithelium (11), choleragenic secretions are believed to result from active transport processes in the crypts of Lieberkühn (1, 9). Contrary to the large increase in mucosal permeability observed after elevated venous pressure and decreased plasma colloids, no increase in the mucosal membrane permeability has been observed during choleragenic secretions (5). Considerable evidence exists indicating that the driving force for intestinal secretion in cholera is active chloride or sodium chloride transport by crypt epithelium. Therefore, it is generally believed that choleragenic secretion is independent of alterations in tissue forces (9). Our results (Fig. 1C) suggest that an active process is responsible for choleragenic secretion because there appears to be no correlation between secretion rate and lymph flow.
flow, which is opposite to the observations seen with filtration secretion. A decrease in intestinal lymph flow should occur when an active process pumps solutes out of the mucosal interstitial space because fluid accompanies the solutes and would lower tissue pressure. However, in each cholera toxin experiment, lymph flow increased by 50-115% before approaching the final zero flow. The increased lymph flow observed in the cholera toxin experiments suggests either a slight alteration in tissue forces and/or some minor direct or indirect stimulatory effect of the exotoxin on intestinal lymphatics. The possibility of direct stimulation seems highly unlikely because, even by the most sensitive bioassay techniques, no cholera toxin appears to permeate the mucosal membrane (2). The possibility also exists that the early increase in lymph flow may have been secondary to impurities in the toxin preparation, such as amino acids in the culture medium, that may have stimulated absorption prior to the onset of the toxin effect.

REFERENCES


6. Dobbs, W. A. The flow of lymph to the cardiac node following saline infusion in the dog. Microvascular Res. 8: 14, 1974.


