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Research Article

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Mechanism of Production of Intestinal Secretion by Elevated Venous Pressure

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ABSTRACT A study was carried out to elucidate the physiological mechanisms responsible for the intestinal secretion produced by venous pressure elevation. In dogs, measurements were made of the rate and composition of small intestinal secretion, rate of flow and composition of intestinal lymph, plasma composition, and mucosal water content, all in response to elevations of intestinal venous pressure. Venous pressure elevations above a threshold value of 30–35 cm H₂O produce secretion at a rate approximately proportional to the value of the pressure minus the threshold value. Above the threshold value, there were large increases in the rates of lymph flow and net sustained transcapillary filtration. These rates were also roughly proportional to the incremental venous pressure.

It is concluded that intestinal secretion produced by elevated venous pressure is almost surely secretory filtration, a passive process with the driving force for secretion an increase in mucosal tissue fluid pressures to values of only some 4–6 cm H₂O. The increased tissue fluid pressure not only provides the driving force but also produces an increase in the hydraulic permeability of the epithelium without which the driving force would be ineffective.

The transepithelial channels are large enough to permit inulin to pass freely and even plasma protein to pass in large amounts, and hence are most probably intercellular. Secretory filtration probably represents a general pathophysiological response of transporting epithelia to elevated tissue fluid pressure.

It is proposed that the threshold value for secretion and associated changes is explained by dilution of the tissue

fluid protein colloid osmotic pressure in a small subepithelial, juxtacapillary compartment.

INTRODUCTION

Intestinal secretion of unclear pathophysiology is a feature of many diseases. This paper describes an investigation of the kind of fluid secretion produced by a procedure which should increase intestinal tissue fluid pressure, namely, elevation of the venous pressure (1, 2). The observations are interpreted to strongly support the following view: This secretion is a passive filtrate, the driving force for which is a subepithelial tissue fluid pressure of only a few centimeters H₂O. However, a venous pressure of about 30–35 cm H₂O is required to reach this tissue fluid pressure, because the intestine has a buffering mechanism to prevent mucosal interstitial fluid edema in response to elevated venous pressure. It is suggested that this buffering mechanism is a reduction of the colloid osmotic pressure in a small juxtacapillary subepithelial tissue fluid compartment caused by the diluting effect of transcapillary filtrate. The modestly elevated tissue fluid pressure not only provides the driving force for secretion but also produces an increase in the hydraulic permeability (filtration coefficient, L_p)¹ of the epithelium, without which the driving force would be ineffective; and the channels are large enough to permit the relatively free passage of plasma proteins.

The production of a passive secretory filtrate of this kind in the intestine also seems to result from causes, other than elevated venous pressure, which could be expected to raise subepithelial pressure above intraluminal pressure, including negative intraluminal pressure (3), obstruction of lymphatic drainage (4), lowering of plasma colloid osmotic pressure with the lymphatics

An account of this investigation was given in more detailed form in a doctoral thesis presented to the faculty of the Graduate School of the University of Minnesota, and published in June 1973 (reference 23).

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¹ *Abbreviations used in this paper:* L_p , hydraulic permeability; L/P, lymph to plasma concentration ratios; S/P, secretion-to-plasma concentration ratios.

blocked (1), and extracellular volume expansion by saline infusion (5). Expansion of the extravascular space has been found to increase the small intestinal permeability to glucose (6), sucrose (6), and inulin (7). The intestinal fluid loss associated with hemorrhagic shock in the dog has been interpreted to be due to secretory filtration (8). This process must be seriously considered as the explanation for the fluid secretion associated with intestinal obstruction (9) and inflammation. It is almost surely responsible for the secretion frequently found in vascularly perfused intestinal preparations which develop edema (10, 11). Moreover, observations on the proximal convoluted tubule (12), gallbladder (13), and especially the stomach (14), suggest that the process may be a more or less general pathophysiological response in transporting epithelia to elevated subepithelial tissue pressure. It is also to be noted that since so-called active fluid absorption and secretion are considered to be passive processes secondary to hydrostatic pressure and osmotic driving forces established in the epithelium by active solute transport, the effect of externally imposed hydrostatic pressure differences have relevance to active fluid transport.

METHODS

Results from three types of experiments will be presented: experiments in which intestinal lymph was collected, experiments employing the Wells clamp (15), and experiments on tissue water content.

Intestinal lymph experiments. Mongrel dogs (15–25 kg) were given water but no food for about 24 h before the experiment. They were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg), supplemented by additional drug as required. During the experiment, Krebs-Ringer bicarbonate fluid was administered intravenously at a rate of about 50 ml/h.

The abdomen was opened by a midline incision. One of the larger lymphatic draining the upper jejunum was ligated about 8 cm from the intestine, where the vessel runs adjacent to one of the mesenteric veins, and any lymphatic collaterals which bypassed the tie were ligated. Since the lymphatics appear to drain 3–4 cm of intestine (16) weighing approximately 5 g, the observed lymph flow was multiplied by 20 to provide values per 100 g of intestine. After the lymphatic was ligated, a portion distal to the tie was isolated by blunt dissection from the rest of the mesentery. All lymph emerging from a small opening cut in the isolated lymphatic could be collected manually in microhematocrit tubes and weighed.

The mesenteric vein from a segment of intestine including the part drained by the isolated lymphatic was isolated by blunt dissection for a distance of about 2.5 cm, approximately 10 cm from the intestine, and a sleeve of 260 Intramedic polyethylene tubing, split lengthwise, was fitted around the vessel. Adjusting a plastic screw clamp placed on the tubing permitted control of the venous pressure, which was measured by means of a polyethylene catheter inserted into a side-branching vein between the clamp and the intestine. The catheter was connected to a Statham strain gauge pressure transducer (model P23AA, Statham Instruments, Inc., Oxnard, Calif.) for recording on a Sanborn Twin-Viso

recorder (Sanborn Div., Hewlett-Packard Co., Waltham, Mass.). During each experimental period the venous pressure was kept within 3 cm H₂O of the desired value. Usually only one or two adjustments of the screw clamp during a period were required.

In experiments to be referred to as loop experiments, the intestinal segment drained by the clamped vein was then cannulated at its ends with wax-coated glass cannulas which were tied in place by Penrose tubing. The loop (usually about 30 g in weight as measured at the end of the experiment) was then washed freely with Krebs-Ringer bicarbonate fluid at 37°C. During each period the loop was wrapped in warm saline-soaked gauze and rested on the abdomen of the dog, with the temperature of the loop maintained at 37°C by means of an infrared heat lamp controlled by a temperature probe placed next to the loop.

The venous pressure was adjusted to the desired initial value (which varied from 7 to 80 cm H₂O) and kept at this level during a period lasting usually 15–30 min, during all but the first few minutes of which lymph was continually collected. The duration of the period depended inversely on the lymph flow. The loop was emptied of all contents at the start and end of each period. Any secretion which formed was centrifuged after its volume was measured and the supernate stored in the frozen state. Successive periods were always conducted at the same venous pressure or at a higher one than that of the preceding period.

The experiments were terminated after four or five successive periods, or whenever the lymph became blood tinged, in which event only the results from the prior experimental periods with clear lymph were used in the present report. In most experiments, beginning about 45 min before the onset of the first experimental period, a solution of 10% inulin (mol wt 3,000 as determined by freezing point depression) and 40% L(+)-arabinose was infused intravenously through the external jugular vein. A priming dose of 18 ml of the solution was followed by a constant infusion at a rate of 0.25 ml/min for the duration of the experiment, a procedure which established fairly stable plasma concentrations of the solutes during the test periods.

A second type of experiment in which lymph was collected was performed on three dogs involving 12 lymphatics for the purpose of measuring the lymph-to-plasma concentration ratios (L/P) when the levels of venous pressure were kept constant for relatively long periods of time. After the abdomen was entered, a polyethylene catheter was tied near the ileum into the proximal end of the severed superior mesenteric vein so that venous pressure could be recorded in a manner like that described for the loop experiments. The venous pressure in the superior mesenteric vein was adjusted by means of a loose ligature placed around the vessel near the entrance of upper jejunal mesenteric veins, and all collaterals bypassing this ligature were tied off. By exerting tension on the ligature, while watching the venous pressure recording, one could continuously control the venous pressure to within 2 cm H₂O of the desired level. This is also the method used in the experiments on tissue water content to be described below. The collection of lymph was carried out as already described. The intestines were handled only to the extent necessary for these procedures; i.e., no loops were made.

Experiments employing the Wells clamp. After laparotomy as above, a segment of jejunum drained by a single mesenteric vein was inserted through the slit in the base plate of a Wells clamp (15). The segment was then cut along its antimesenteric border, spread open as a flat sheet, and clamped in place to form the floor of the clamp chamber,

reached. Thus, the rates of secretion and lymph flow at venous pressures above 30 cm were roughly similar.

On the assumption of a steady-state interstitial fluid volume, the capillary filtration rate was taken as equal to the sum of lymph flow and net secretory rate. The capillary filtration rate was, like each of its two component rates, similarly relatively unaffected by venous pressure in the range below 30 cm H₂O; but in the range above 30 cm H₂O, it was very sensitive to venous pressures, rising about 0.08 ml/min/100 g per cm H₂O. The variability of the values was similar to that in Figs. 1 and 2.

The venous pressure of some 30-35 cm H₂O at which are seen (a) the onset of intestinal secretion, (b) the abrupt increase in the rates of lymph flow and (c) increased sustained net transcapillary filtration will be referred to as the threshold pressure.

Since in each experiment successive test periods mostly of 15-30 min were always conducted at the same or a higher venous pressure, the question arises as to whether the essential observations were due to this procedural feature. It is possible that the same results would have been obtained with the passage of time, especially associated with tissue trauma. That such was not the case is indicated by grouping data on secretion according to the time at which the test period of maintenance of the indicated venous pressure was ended (Table I). Zero time was the time when the first secretion collection period was begun. At venous pressures under 35 cm H₂O, collectable secretion did not occur for at least 100 min. In a very large number of experiments in which test solutions have been repeatedly placed in and removed from canine intestinal loops, frequently involving much longer times and at least as much handling as in the present studies, it is very unusual to encounter secretion. At venous pressures above 44 cm H₂O, secretion occurred promptly even when this pressure was the initial one of an experiment. In the intermediate range of venous pres-

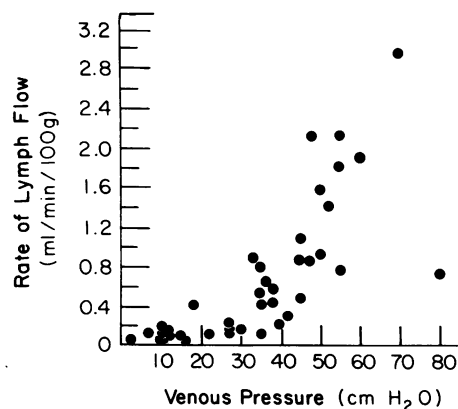


FIGURE 2 Relationship between the rate of intestinal lymph flow and intestinal venous pressure in a loop experiment.

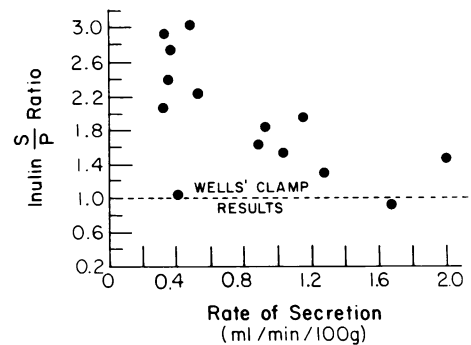


FIGURE 3 Relationship between the S/P concentration ratio for inulin and the rate of fluid secretion. The plotted points are from experiments on intestinal loops. The line represents the relationship inferred from the results with the Wells clamp (see Fig. 5B).

ures, 35-44 cm H₂O, just above threshold, secretion seems perhaps more likely to appear later in the experiment; but this feature is probably due not so much to nonspecific trauma as to the changes in the state of the tissue fluid spaces brought about by the preceding increases in venous pressure. In the Wells clamp experiments secretion did not occur at subthreshold venous pressures, but was observed almost invariably at a venous pressure of 40 cm H₂O.

Concentration relationships of solutes in the secretion, lymph, and plasma

Protein and inulin concentrations were determined in plasma, lymph, and secretions; arabinose concentrations, only in plasma and secretions.

The lymph inulin concentration was found approximately equal to the plasma inulin concentration at all venous pressures. It seems justifiable to assume that the lymph concentrations of the smaller arabinose molecule were also equal to plasma concentrations, and that the tissue fluid concentrations of inulin and arabinose were equal to their respective lymph concentrations.

Inulin and arabinose. The secretion-to-plasma concentration ratios (S/P) for inulin and arabinose in loop experiments varied with the rate of secretion (Figs. 3 and 4). At the lower rates of secretions, S/P ratios as high as 3 for inulin and 1.5 for arabinose were found. With increasing rates of secretion, the ratios tended to decrease towards or reached a value of about 1.0 at the highest rates. In contrast, as shown in Figs. 5B and 5C, the S/P values for secretion obtained from the clamp were not systematically related to the rate of secretion and averaged close to 1.0, the value approached at high rates of secretion in loop experiments. (See Figs. 3 and 4 in which horizontal lines at S/P = 1.0 are included to represent the results from the clamp studies for comparison with the plotted values from loop experiments.)

TABLE I
Values for Rate of Secretion* in Loop Experiments Grouped According to Time of End of Period of Collection†

Venous pressure	Time of end of collection period, min					
	0§-20	21-40	41-60	61-80	81-100	101-120
cm H ₂ O						
<35	0, 0	0, 0, 0, 0, 0, 0	0, 0, 0	0, 0, 0, 0	0	
35-44	0	0, 0, 0	0, 0.36	0, 0	0.37, 0.56	
45-54	1.15, 0.89	1.28	0, 0.48		0.33, 0.41	2.32
55-64		1.04		0.93, 0.33		0.53

* ml/min per 100 g of intestine.

† The number of collection periods per experiment ranged from 1 to 5 (mean 2.4).

§ Zero Time = Time of beginning of First Collection Period for Secretion.

The duration of the periods ranged from 10 to 60 min (mean 23±11 min [SD]).

Protein. The plasma protein concentration averaged 6.0±0.6 g/100 ml. In the loop experiments the secretion protein concentration varied with the rate of secretion (Fig. 6) in a manner qualitatively similar to that described above for inulin and arabinose. At low rates of secretion, the values for concentrations in the secretion range from 1.0 to 3.4 g/100 ml, corresponding to S/P ratios of 0.17-0.55. The concentration tended to decrease with increasing rates of secretion towards a limiting value near 1.0 g/100 ml, or a value of 0.17 for S/P. In the clamp experiments the protein concentration averaged 0.81±0.34 g/100 ml or a value of 0.14 for S/P, and the ratio seemed relatively independent of the rate of secretion (Fig. 5A). (In Fig. 6, a horizontal line was drawn at a protein concentration of 0.81 g/100 ml to represent the results of the Wells clamp experiments.)

The protein concentration in lymph was a function of venous pressure (Fig. 7). At normal venous pressures the concentration ranged from 3 to 6 g/100 ml, averaging 3.9 g/100 ml or close to 67% of the plasma level of

6 g/100 ml. As venous pressure was elevated to the threshold level and above, the lymph protein concentrations fell into the range of 25-50% of the plasma concentration averaging 2.34±0.84 g/100 ml or 40% of the plasma value. The open circles in Fig. 7 are the L/P values for protein in the experiments in which there was no intestinal loop and the venous pressure was maintained constant for at least an hour. These values do not

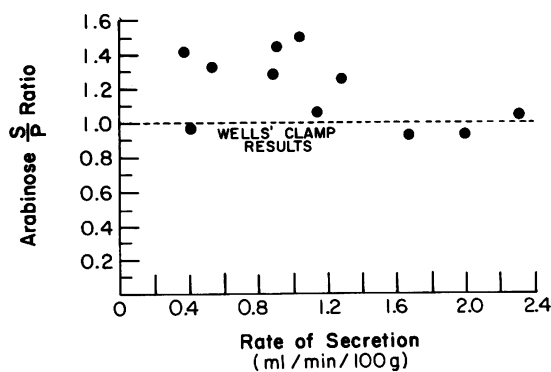


FIGURE 4 Relationship between the S/P concentration ratio for arabinose and the rate of fluid secretion. The plotted points are from experiments on intestinal loops. The line represents the relationship inferred from the results with the Wells clamp (see Fig. 5C).

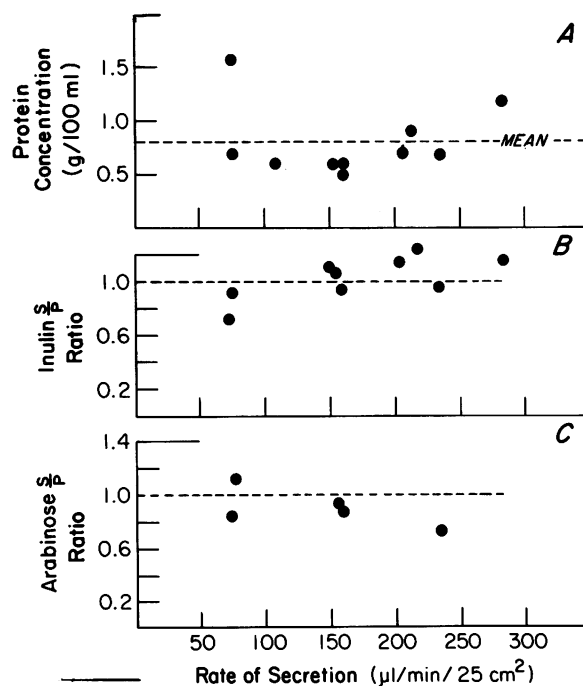


FIGURE 5 (A) Relationship between the secretion concentration of protein and rate of secretion for secretion collected from the Wells clamp. (B) Relationship between the S/P concentration ratio for inulin and rate of secretion for secretion collected from the Wells clamp. (C) Corresponding values for arabinose.

seem to differ significantly from the values obtained in the loop experiments, which are the solid circles in the figure.

Table II shows the average relative concentrations of the various protein fractions in plasma, lymph, and secretion, each concentration expressed as percentage of the total protein concentration. The lymph samples were divided into three groups, depending on the venous pressure at which they were collected: 15 cm H₂O or less, 16-30 cm H₂O, and pressures greater than 30 cm H₂O. Although the percentage of albumin in the lymph collected at venous pressures of 16-30 cm H₂O was highest, it was not significantly different from the other two lymph groups ($P > 0.02$). The distribution of protein in secretion was more like that of the lymph than of the plasma. Lymph and secretion samples showed albumin percentages which were significantly higher and gamma globulin percentages significantly lower than the corresponding simultaneously collected plasma samples. These differences are consistent with differential sieving through the capillary wall.

Osmolality and electrolyte concentrations of secretion (Table III). The sodium, potassium, and chloride concentrations of lymph in loop experiments were not significantly different from those of plasma. The osmolality and sodium concentrations of secretion were likewise not significantly different from those of plasma; however, secretion potassium and chloride concentrations were higher than the plasma concentrations. Similar results for chloride were found in the Wells clamp experiments. When the values in Table III are converted to a molal basis by adjusting for protein contents in the secretion and plasma, respectively, the sodium and chloride concentrations become nearly those one would expect for a Donnan equilibrium between the two fluids (23). A potassium concentration higher than the plasma value is

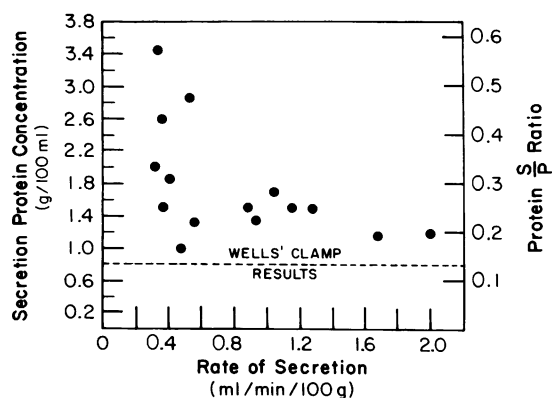


FIGURE 6 Relationship between the protein concentration in secretion and the rate of fluid secretion. The plotted points are from experiments on intestinal loops. The line represents the relationship inferred from the results with the Wells clamp (see Fig. 5A).

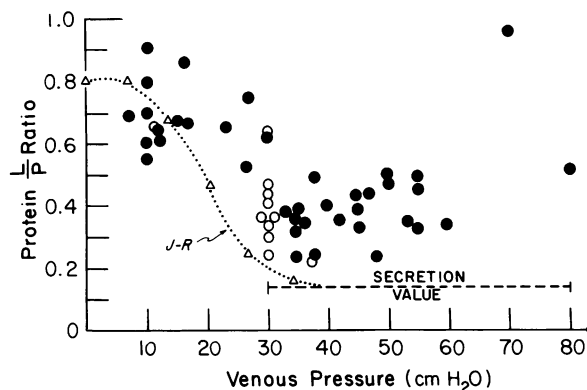


FIGURE 7 Relationship between protein concentration in intestinal lymph and the venous pressure. Closed circles are from intestinal loop experiments; open circles are from experiments in which lymph was collected for at least an hour and without a loop. The line labeled "secretion value" is the S/P protein concentration ratio from the Wells clamp experiments. The line labeled "J-R" represents the average values of Johnson and Richardson (30).

the usual finding for intestinal fluids and is attributed in part to contributions from cellular debris.

Observations on tissue water content

The ratio, experimental-to-control water content per unit dry weight, responded similarly to venous pressure elevation with both serial and simultaneous controls. Therefore, the results from experiments with both types of controls have been pooled and the ratios are plotted as a function of venous pressure in Figs. 8 and 9. The mucosal water content did not seem to increase until venous pressure exceeded about 30 cm H₂O, near the threshold value. On the other hand, nonmucosal water content increased with increasing venous pressure at all venous pressures above the initial value of some 10-15 cm H₂O.

TABLE II
Mean Relative Composition of Proteins of Secretion,
Lymph, and Plasma

	Percentages of total proteins present				
	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Plasma (18),	40.3	9.4	13.8	23.1	13.4
Lymph (7),	45.0*	7.1	13.4	24.1	10.3*
VP 0-15, †					
Lymph (6),	49.7*	5.3*	12.8	23.5	8.7*
VP 16-30,					
Lymph (14),	46.5*	7.4*	11.4*	23.7	11.0*
VP >30					
Secretion (13),	44.5*	7.4*	13.8	23.3	10.8*

Numbers in parentheses indicate the number of samples.

* Indicates values which differ from their simultaneously collected plasma values with a statistical significance of $P = 0.02$ or better.

† VP, venous pressure.

TABLE III
Osmolality and Electrolyte Concentrations in Plasma,
Lymph, and Secretions at Venous Pressures
above Threshold (30 cm H₂O)

	Plasma	Lymph	Secretion
Osmolality, mosmol	306 ± 5 (4)		294 ± 13 (6)
Sodium, meq/liter	148 ± 5 (4)	149 ± 6 (5)	148 ± 6 (5)
Potassium, meq/liter	3.8 ± 3 (4)	3.8 ± 1 (4)	6.4 ± 7 (5)
Chloride, meq/liter	111 ± 3 (11)	106 ± 5 (4)	126 ± 11 (11)

The measure of variability is the SD.
The numbers in parentheses are the number of observations.

The changes in mucosal water content, even at venous pressures above 30 cm H₂O, were small relative to the total net capillary filtrate formed during a period of measurement. This provides support for the assumption of a steady-state interstitial fluid volume (though not necessarily composition) which was the basis for estimating the capillary filtration rate to be the sum of lymph flow and intestinal secretion.

DISCUSSION

As stated in the introduction, we interpret the findings as favoring a filtration mechanism for the observed secretion, a recurring idea associated most closely in the physiological literature with Wells (1). A more detailed description of the probable mechanism will be given later after certain other aspects of the observations have been discussed.

S/P ratios should be 1.0 for arabinose and inulin in a filtrate without molecular sieving. Actually values greater than 1.0 were found in most of the secretion samples in loop experiments. Such values were unexpected because, whatever the mechanism of fluid secretion, active secretion of these solutes seems highly improbable. Almost surely the explanation of this finding is that fluid absorption occurred from a secretion originally containing these solutes at a concentration no higher than the tissue fluid concentration, which in turn was close to the plasma concentration.

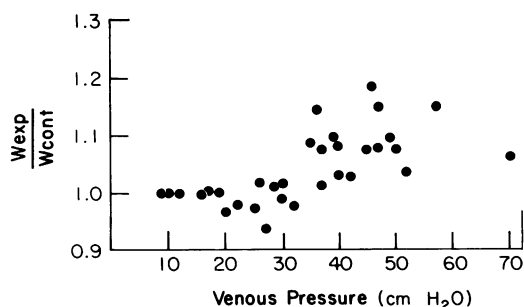


FIGURE 8 Relationship between the ratio, water content of experimental mucosal tissue (W_{exp}) to control mucosal tissue water content (W_{cont}), and venous pressure. W is the water content per unit dry weight.

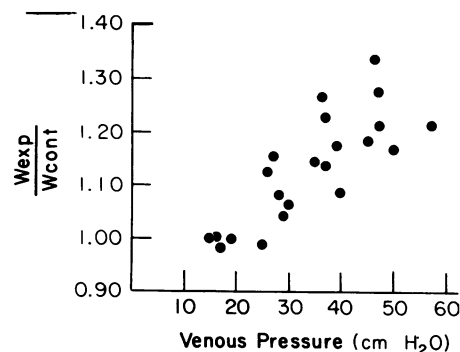


FIGURE 9 Relationship between the ratio, water content of experimental nonmucosal tissue water content (W_{exp}) to control nonmucosal tissue water content (W_{cont}), and venous pressure. W is the water content per unit dry weight.

It would be surprising if fluid absorption did not occur, since if fluid of the electrolyte composition of the secretion were placed in the lumen, it would normally be actively absorbed. The absorbate would be expected to contain arabinose and inulin at much lower concentrations than in the luminal fluid, thereby increasing the concentration in the collected net secretion. Moreover, higher S/P values were associated with the lower rates of secretion when the magnitude of the fluid absorptive stream relative to fluid secretion should be large; conversely, lower S/P values would be expected at the higher secretory rates, as was found to be the case. Similarly, the protein concentration in the collected secretion decreased as the net secretory rate increased (Fig. 6). The evidence seems convincing to us that a fluid circuit consisting of simultaneous secretory and absorptive 'streams' (i.e. convective volume flows) existed during the loop experiments, with the probably active absorptive stream relatively constant and independent of the level of the venous pressure. The smaller concentrating effect in the case of arabinose would be expected, according to this explanation, in view of the relatively higher intestinal permeability of arabinose compared to inulin and protein.

The findings of the experiments in which the Wells clamp was used supply more direct evidence for a fluid circuit. In the Wells clamp experiments oil was used to minimize the effects of absorption. As would be expected, the values of S/P for inulin and arabinose and for the secretion protein concentration were independent of the rate of secretion (Fig. 5). Furthermore, in the Wells clamp experiments the average values (1.0 for the S/P of inulin and arabinose and 0.81 g/100 ml for the secretion protein concentration, shown as horizontal lines in Figs. 3, 4, and 6, respectively) are those approached at high net secretory rates in the loop experiments, as the fluid circuit theory would predict. The results are given elsewhere (23) of an analysis of the relationships between the magnitudes of the two streams on the assump-

tion that the intestinal clearance of inulin measures the secretory volume flow.

On the basis of the inulin, arabinose, and electrolyte composition of the secretion, the secreted fluid was passing through pores at least as large as inulin, molecular radius 12-15 Å (24). The protein analyses suggest that the channels are at least tens of Angstroms in radius and may well be much larger.

By use of a modification of the isogravimetric technique of Pappenheimer and Soto-Rivera (25), it has been found that not until venous pressure exceeds about 30 cm H₂O does sustained net capillary filtration occur at significant rates in the intestine (26, 27). That is, the intestine has mechanisms which limit edema in the face of elevations of venous pressure up to about 30 cm H₂O, and this applies, over time intervals such as studied here, even to the denervated organ with its lymphatic drainage obstructed. Our experiments yielded a similar threshold. The results of two markedly different procedures are thus completely consistent with one another in this particular respect.

In vitro, a transmural pressure difference of only 4-6 cm H₂O results in a rather abrupt increase in the hydraulic permeability of the mucosa and the onset of a passive secretory stream (28). It seems probable that a similar process occurs in vivo. If so, the absence of secretion indicates that the mucosal interstitial fluid pressure remains less than 4-6 cm H₂O at values of venous pressure below threshold for secretion. If lymph flow is roughly proportional to interstitial fluid pressure, the plot in Fig. 2 further implies that a substantial increase in tissue fluid pressure did not occur until venous pressure reached or exceeded the threshold value. This interpretation is also supported by the finding that an increase in mucosal water content (representing interstitial edema) did not become detectable below the threshold venous pressure. These results strongly suggest that the chief compensatory factor is not elevated tissue fluid pressure, and hence that most likely it is decreased tissue fluid colloid osmotic pressure, as concluded by others (29, 30).

However, Wallentin (26) has pointed out that when intestinal venous pressure is elevated, not enough fluid is filtered to dilute appreciably the interstitial fluid protein; and the small increases in mucosal interstitial fluid water content at venous pressure elevations below threshold (Fig. 8) are in agreement with this view. But this argument neglects the possibility that the mucosal interstitial fluid may be heterogeneous. Reduction in tissue colloid osmotic pressure by dilution could still be the chief compensatory factor if the mucosal interstitial fluid is compartmentalized into a small juxtacapillary compartment and a larger compartment comprising the bulk of the mucosal interstitial fluid, and if transcapillary

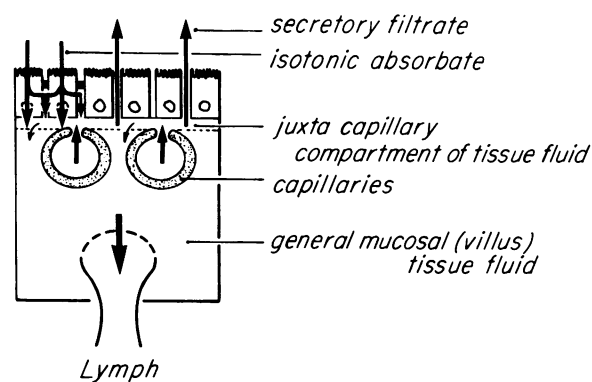


FIGURE 10 Diagram showing the relatively isolated small juxtacapillary subepithelial tissue fluid compartment proposed to reconcile the apparent discrepancy between (a) reduction in tissue fluid colloid osmotic pressure as the main safety factor against edema as venous pressure is raised, and (b) the relatively small increases in net capillary filtrate. The arrows denote the secretory filtrate and isotonic absorbate streams making up the fluid circuit which during net secretory filtration is very probably responsible for S/P ratios above 1.0 for arabinose and inulin. As seems probable, secretory filtration is shown as passing through the lateral intercellular spaces and isotonic absorption through areas with relatively intact tight junctions. The secretory filtrate would contain inulin close to the plasma value. The absorbate with a concentration less than this value would raise the luminal concentration, yielding an S/P value greater than 1.0 in the collected secretion. When absorption is largely prevented by use of high density oil in the Wells clamp, the S/P ratio approaches 1.0.

exchange in the juxtacapillary compartment dominates the observations. With the volume of the juxtacapillary compartment small, only correspondingly small amounts of transcapillary filtrate would be required to dilute or wash out its colloids without appreciable accumulation of fluid in the bulk of the mucosa.

There is evidence that the mucosal interstitial fluid is in fact compartmentalized in this way, permitting very prompt changes in the value of tissue fluid colloid osmotic pressure adjacent to the mucosal capillaries in response to changes in capillary pressure. The villus capillaries lie about 0.5 μm from the basement membrane of the mucosal epithelial cells and their diaphragmed fenestrae are on the side of the capillary wall which faces the epithelium (31, 32). This is the portion of the capillary wall across which filtration would be expected to occur (32), and the thin layer of the interstitial fluid lying between the capillary and the epithelium would be the compartment of interstitial fluid with which villus capillary fluid exchange would occur. Even if its thickness were as large as 5 μm, its volume would be of the order of only 1 ml/100 g of intestine (calculated by taking 2 cm²/g of dog jejunum and a mucosal-to-serosal area ratio of 10 [33]).

The compartmentalization of the mucosal interstitial fluid would also provide the intestine with a very efficient

mechanism for water absorption. The absorbed fluid would be delivered directly into the small compartment lying between the mucosal capillaries and the epithelial cells. There would be a large decrease in the protein concentration of this compartment, providing a large net driving force for passive flow of water into the capillaries to be carried away by the blood. Without compartmentalization, the proteins of the entire villus interstitial fluid space would need to be diluted to increase the osmotic driving force for passive fluid absorption into the villus capillaries. This would be inefficient and tend to make the villi edematous during fluid absorption.

When transcapillary filtration rates are very large, as at high venous pressures, one might expect the interstitial fluid and lymph protein concentrations to approach at least roughly that of the transcapillary filtrate. The protein L/P ratio at venous pressures above threshold averages 0.4 (range 0.2-0.5, with one exception), ratios which imply that at these higher venous pressures the sieving coefficient for protein across the capillary wall is similarly in the range of 0.20-0.50 (reflection coefficient 0.50-0.80). Therefore, it at normal venous pressures the intestinal capillaries have the usually assumed low permeability to protein (reflection coefficient near 1.0), it seems likely that not only the epithelial but also the capillary wall channels at the elevated venous pressures are larger than normal. To account for an L/P protein concentration ratio of 0.33, only about 0.1% of the capillary fenestrae need become diaphragm free by rupture at the elevated venous pressures to produce the observed high lymph protein concentrations (23).

According to the proposed explanation offered for the threshold, the protein L/P ratio might be expected to have decreased progressively as the venous pressure was elevated toward threshold. The corresponding plotted points in Fig. 7 (solid circles) suggest only a slow decline in the ratio until near threshold, when a much more abrupt fall occurs. A plausible explanation for the failure to find a progressive decrease in concentration is that at the slow rates of subthreshold lymph flow the dead space between the site of lymph formation in the villi and the cannula in the lymphatic had not been adequately washed out. At the high rates of lymph flow beginning at threshold the dead space effect would be less important. Johnson and Richardson (30) collected small intestinal lymph from cannulated lymphatics until stable protein L/P values were obtained for a given venous pressure, in the range 0-34 cm H₂O. At the highest venous pressures up to an hour was sometimes necessary to reach a stable value. Their average results for the relationship between the protein L/P ratio and venous pressure, plotted in Fig. 7 (line labeled "J-R"), differ from our findings in that they actually show the kind of definite progressive decrease in L/P we expected as venous pressure is

raised toward threshold. Thus at a venous pressure of 25 cm H₂O the L/P ratio of Johnson and Richardson has fallen to about 0.25 while the value we have found is still about 0.6. Because of this discrepancy we carried out the experiments on the collection of lymph at venous pressures sustained for an hour or longer. Stable L/P values were reached. The results, as already noted, fall in the range of values obtained in the loop experiments with collections of shorter times. Hence, the discrepancy between our findings and those of Johnson and Richardson do not seem to be due to the shorter time periods employed in our loop experiments, but to other possible procedural differences: The Johnson and Richardson preparation was a completely isolated loop autoperfused via a polyethylene circuit, and the perfusing blood contained an anticoagulant and an anticholinergic agent; whereas the lymph we collected was from the upper jejunum studied *in situ*.

The values of Johnson and Richardson, if taken as the more applicable ones, would provide better evidence for the model of secretory filtration we offer than do our own results. Not only does the protein L/P show the more pronounced decline as venous pressure is raised, but the value reached at threshold is the same as the S/P protein concentration ratio of the Wells clamp experiments (line labeled "secretion value" in Fig. 7). Equality of lymph and secretion protein concentrations would be consistent with the view that the secretion is a filtrate through channels large enough to permit the proteins to pass without restriction.

Conclusion: Secretory filtration hypothesis for mechanism of production of intestinal secretion by elevated venous pressure

The following characteristics of intestinal secretion resulting from venous pressure elevation suggest that this type of fluid secretion is primarily a passive filtrate for which the driving force is elevated tissue fluid pressure: (a) The rate of secretion responded to venous pressure elevation as though it were a passive process. Once secretion began, the rate of secretion was roughly proportional to the incremental venous pressure (Fig. 1). (b) The secretion rate (Fig. 1), lymph flow rate (Fig. 2), capillary filtration rate, and mucosal water content (Fig. 8) all showed practically the same threshold venous pressure, and increased approximately linearly with venous pressure above threshold. This seems strong evidence that increased mucosal tissue fluid pressure, due to the accumulation of interstitial fluid, was the driving force for the passive flow of lymph and secretion. (c) When the plasma contains inulin or arabinose the concentrations of these solutes in the secretion is very probably equal to the tissue fluid concentration. Additional points favoring a filtration process are: (d) the osmo-

lality and concentrations of Na^+ and Cl^- are consistent with those of a protein-poor filtrate of interstitial fluid; (e) an analogous type of intestinal secretion is produced *in vitro* by elevated serosal fluid hydrostatic pressure (28); and (f) cholera-like secretion, the best documented examples of an active intestinal secretion, differs in important respects from the secretion induced by elevated venous pressure. Cholera-like secretion contains little plasma protein (34) and practically negligible concentrations of so small a molecule as L(-)-glucose, when the latter is administered intravenously (35).

Elevated tissue fluid pressure associated with raised venous pressure could conceivably stimulate active secretion of a fluid of the observed composition. Or the initiating stimulus could be some hemodynamic factor, such as reduced blood flow, leading to almost any number of alterations, including metabolic ones, in the perfused tissues, which might initiate active secretion. However, in the absence of evidence for these possibilities, the case for secretory filtration seems most likely the correct one.

To provide a more detailed description of the proposed mechanism, we begin with normal venous pressures and an empty lumen. Net capillary filtration and lymph flow are very small; tissue pressure is negligible; and the protein concentrations in mucosal tissue fluid and lymph are relatively high. As venous pressure is raised, capillary pressure is also raised an approximately equal amount (36); and net capillary filtration initially increases into the small juxtacapillary compartment across intact capillary fenestrae (Fig. 10). But continued augmented net filtration is prevented by the decrease in the compartmental tissue fluid protein concentration (colloid osmotic pressure) due to dilution and washout. The tissue fluid pressure is elevated, but only slightly; and the increase in mucosal water content is also very small. Hence there is no secretion and lymph flow remains at low rates.

At the threshold (about 15 cm H_2O above normal venous pressure) the buffering capacity against edema formation of the tissue fluid colloid osmotic pressure of the pericapillary space has been exhausted. Further increases in venous (and capillary) pressure now cause sustained increases in the capillary filtration rate, which in turn raises tissue pressure to 4-6 cm H_2O . The consequences are: the onset of secretory filtration, increased lymph flow with low protein concentration, and increased mucosal tissue water content. The effect of the tissue fluid colloid osmotic pressure in opposing secretion is negligible, because the protein concentration is small and the epithelial channels are large enough to make the reflection coefficient (37) of the proteins small. The composition of the secretion is that of a filtrate of tissue fluid through channels large enough to permit proteins to pass more or less freely (secretory arrows of Fig. 10). The value of 30-35 cm H_2O for the threshold is ap-

proximately accounted for by the sum of normal venous pressure and normal intestinal lymph osmotic pressure plus 4-6 cm H_2O .

With further progressive elevation of venous pressure, tissue fluid pressure also rises, producing augmentation of secretory and lymphatic flow. Both of these flows, roughly equal in rate, moderate the rise in tissue fluid pressure, which would otherwise increase to a level such as to bring the Starling forces into balance and no doubt be associated with massive edema. Elevation of venous pressure may cause increased leakage of plasma proteins from the capillaries across a small fraction of the fenestrae which have broken. With sufficiently high venous pressures, capillaries rupture and gross breaks occur in the epithelium with blood in the tissue spaces, lymph, and secretion. According to a recently published abstract the responses of the cat and dog intestines to elevated venous pressures appear to be practically identical (38). "Secretory filtration" seems a better term for the process than the more customary terms, transudation or exudation, because it is explicit as to driving force (hydrostatic pressure) and it avoids the ambiguity of the distinction between the two more conventional terms.

Suggestions as to the identity of the channels for secretory filtration include, besides the most likely one of the intercellular spaces (28, 39) as shown in Fig. 10, the defects left at the tips of the villi by shedding cells (28), and large areas of individual villi denuded of epithelium (8). Under direct visualization of the *in vitro* canine mucosal membrane preparation we have seen blister-like blebs appear on the villi under serosal fluid pressure, and the blebs can burst. The channels are almost surely extracellular but a pathway could occur through goblet cells opened by rupture of their supranuclear cell membranes. It may be that a large part of the variability in the secretion protein concentration, especially at low secretion rates, is due to protein released from cellular debris. The nature of the channels undoubtedly depends on the stage and severity of the process. At threshold the channels could be intercellular spaces and tight junctions which are dilated, but otherwise normal ("shunt pathway"; for recent discussion refer to reference 40). In any event, our attempts to identify the routes of secretion by ordinary light or scanning electron microscopy have thus far failed.

The simultaneous presence of fluid absorption and secretion (fluid circuit), for which the evidence seems strong, implies that the sites of secretory flow are separated by areas of perhaps normal fluid absorptive processes, the latter represented by the absorptive arrows in Fig. 10.

It is evident that two crucial features of the proposed mechanism are the relatively distinct mucosal juxtacapillary compartment and the application of the *in vitro* ob-

servation that when subepithelial tissue fluid pressure reaches a value of only some 4-6 cm H₂O, secretory filtration occurs. Secretory filtration results because, in addition to providing a driving force for secretion, the elevated tissue fluid pressure increases the effective hydraulic permeability of the epithelium by widening existing channels or opening new similarly wide ones.

For heterogenous membranes such as the intestinal epithelial sheet the value of L_p when measured with osmotic pressure as the driving force may be much less than the L_p when measured with hydrostatic pressure as the driving force. For any membrane the measured values may be falsely low if there are unstirred layers adjacent to the membrane. The L_p of the intestinal membrane in a variety of preparations as measured osmotically is of the order of 10^{-4} ml/min per 100 g per cm H₂O (33, 41, 42). The observed rates of secretion in this study are of the order of 1 ml/min per 100 g. If the hydrostatic pressure driving force is 10 cm H₂O, the L_p during secretion is thus 1,000 times larger than the normal osmotically measured one. Even so great a discrepancy could conceivably occur due to differences in the conditions of measurements. However, the appearance in the secretion of large molecules such as inulin and even plasma protein gives additional support for a real increase in the L_p in response to the elevated venous pressure.

More work needs to be done on the reversibility of the process. Damage, only slowly repairable, seems to be produced by only 15 min of elevated portal venous pressure in the dog, since intestinal transport of water was found not to return to control values for hours (2).

The question arises as to why diarrhea is not in fact a prominent feature in conditions such as cirrhosis of the liver and constrictive pericarditis characterized by portal venous hypertension (43). Perhaps because of the slowly progressive nature of these diseases there is time for developments which counteract the effect of elevated venous pressure, such as increased lymphatic drainage, decreased capillary permeability, and increased mucosal resistance to the effects of elevated tissue fluid pressure. Astaldi and Strosselli (44), who studied small intestine mucosal biopsies from advanced cirrhotics, found marked edema of the mucosa; however, among other abnormalities they reported a loss of the normal intercellular space between mucosal epithelial cells with apparent fusion of adjacent cells. If the intercellular space is the route of secretion in pressure induced secretion, as Hakim and Lifson postulated (28), this might explain the absence of obvious secretion in cirrhosis.

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