

Permeability characteristics of the cholera-infected small intestine

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The concept of cholera stool being due to increased outpouring of fluid into the intestinal tract has been considered for many years (Becquerel, 1849; Saha and Das, 1952). However, the finding of an intact mucosa (Gangarosa, Beisel, Benyajati, Sprinz, and Piyaratn, 1960) and the demonstration of a sodium pump inhibitor in cholera stool (Huber and Phillips, 1960) has caused this theory to be abandoned in favour of an absorptive defect. Recent studies of water and sodium ion flux in the infected intestine (Love, 1969) and isotope studies of Mitchell (1965) showed no evidence of absorptive incapacity. The former results were more in keeping with an increase in mucosal permeability at cell membrane or vascular level.

Fordtran, Rector, Ewton, Soter, and Kinney (1965) have described a method for measuring permeability characteristics of intestinal mucosa based on earlier work of Lindemann and Solomon (1962). By using solutes of varying molecular size to produce osmotic fluid flow across the mucosa it was possible to calculate the porosity of the bowel. In the present studies this concept has been applied to the cholera-infected intestine of the rabbit in an attempt to elucidate further the mechanism of the diarrhoeal stool in this disease.

MATERIAL AND METHODS

Rabbits of local breed, weighing 1.5 to 2 kg, were anaesthetized with intravenous nembutal. Isolated loops of small intestine were constructed, leaving their blood supply intact on a mesenteric pedicle. The loops were

replaced within the abdomen for study. One loop was infected with a 1 ml suspension of cholera vibrio. A second loop acted as a control. The infected loops were studied after 10 to 12 hours' exposure to the vibrio. The cholera vibrio were either NIH type 35A3 or DRL 625/66 harvested after two hours' incubation in alkaline peptone water at 37°C. The inoculum consisted of 10^7 to 10^8 organisms suspended in 1 ml saline.

Hypertonic solutions of mannitol, urea, erythritol, and sodium chloride were instilled into the loops for 10-minute periods. Initial osmolality was between 450 and 500 mOsm resulting in a mean lumen-to-plasma osmotic gradient of 60 to 80 mOsm during the study period. To prevent fluid shifts due to chemical gradients of sodium, all solutions except hypertonic sodium solutions contained 140 mmol/l. sodium ion. The net water movement into the gut lumen was measured by input-output balance or the dilution of a non-absorbable marked polyethylene glycol (PEG).

Osmolality was determined by freezing point depression in a Fiske osmometer, PEG by the method of Hyden (1956).

RESULTS

The permeability characteristics of the normal rabbit small intestine and that infected with cholera vibrio are shown in Table I. The figures for water movement and the osmotic gradient producing it are the mean and standard deviation paired studies of control and experimental loops in 10 animals using each solution. The osmotic gradient in each study has been taken as the mean of the osmolalities of fluid instilled at the beginning and that withdrawn at the end of the test period.

TABLE I

Hypertonic Solution	Loop	Water Accumulation (ml/10 min/10 cm ²)	Mean Osmotic Gradient (mOsm)	Filtration Coefficient (ml/min/mOsm)	Reflection Coefficient (Q _x /Q _{Mannitol})	Estimated Pore Size (Å)
Mannitol	Normal	1.30 ± 0.18	68 ± 5	0.0019	—	—
	Cholera	6.32 ± 0.40	60 ± 12	0.0105	—	—
Erythritol	Normal	1.28 ± 0.10	80 ± 4	0.0016	0.84	6.5
	Cholera	3.57 ± 0.32	68 ± 10	0.0053	0.51	11.2
Urea	Normal	0.83 ± 0.11	75 ± 5	0.0011	0.58	5.5
	Cholera	1.84 ± 0.26	72 ± 6	0.0026	0.25	11.6
Sodium chloride	Normal	0.80 ± 0.12	82 ± 6	0.0010	0.53	—
	Cholera	1.46 ± 0.25	74 ± 11	0.0020	0.19	—

The filtration coefficient (ml/min/mOsm) was obtained by dividing the volume of water accumulating per minute in the loop by the mean osmotic pressure gradient producing the fluid movement. It can be seen that a much greater volume of water is attracted into the lumen of the cholera-infected loop than into the normal intestine by an equivalent osmotic gradient operative between lumen and plasma. The difference ranges from a multiple of two in the case of sodium chloride and urea up to five with mannitol. This indicates a basic alteration in permeability of the infected intestine.

If the volume of water moved by different solutes is compared with that produced by mannitol then a measure of the reflection coefficient of that solute can be made (Fordtran *et al*, 1965). The results show that in the normal intestine the smaller molecules of sodium chloride and urea exert a lower osmotic effect than the larger molecule of erythritol. This is an indication of solute entering through the mucous membrane. The results in the cholera-infected loops indicate that all solutes were unable to exert as great an osmotic drag for water as they did in the normal loop, the difference being greatest with the smaller molecular solutes.

Using the figures of Schultz and Solomon (1961) and Lindemann and Solomon (1962), in which curves have been drawn relating the reflection coefficient of non-lipid soluble solutes to the pore radius of the intestinal membrane, it has been possible to arrive at an approximate size of the pores of the intestinal mucous membrane in the present studies. The normal mucosa appears to have a pore radius of 5.5 to 6.5 Å and the cholera-infected intestine a pore radius of 11.2 to 11.6 Å.

DISCUSSION

The present studies indicate that the bulk flow of water produced across the intestinal mucosa by an osmotic gradient is much greater in the cholera-infected bowel than in the normal intestine. The filtration coefficient (ml/min/mOsm) was approximately five times that in the normal intestinal loop. This difference indicates an essential change in the permeability characteristics of the intestine produced by the cholera vibrio.

These changes might be due to differences in surface area, blood flow, or characteristics of the actual membrane. In acute experiments comparing similar regions of bowel, surface area disparity is most unlikely. To differentiate between blood flow and membrane permeability it is helpful to compare the effects of different solutes used in the production of the osmotic gradients.

The reflection coefficients of urea, erythritol, and

sodium chloride differ markedly from each other both in the normal and the infected intestine. This coefficient is dependent on the molecular size of the solute in question relative to the size of the water-filled pores of the membrane (Solomon, 1961). If the change in permeability were due to alterations in blood flow then it would be expected that the reflection coefficients of all solutes would be equally altered in the infected loop. This is not supported by the results as the coefficients are unequally affected by vibrio infection. It seems reasonable, therefore, to suppose that the present studies support a basic change in mucosal permeability rather than a change in blood flow which would have affected all solutes approximately equally.

The intestinal mucosa appears therefore to behave as a porous membrane (Lindemann and Solomon, 1962; Fordtran *et al*, 1965). If it is assumed that these pores are right-cylinders of uniform size then the formulae of Renkin (1954) and Solomon (1961) can be used to calculate the pore radius. The reflection coefficients of the solutes studied in the present experiments indicate a pore radius of approximately 6 Å in the normal intestine. This figure is similar to that found by Lindemann and Solomon (1962) in the rat and by Fordtran *et al* (1965) in man. In the cholera-infected bowel the radius is 11 to 12 Å. This would support the concept of increased intestinal permeability in this disease.

It must be stressed that the concept of a water-filled channel as a right cylindrical pore of uniform dimensions is probably a gross over-simplification of the true situation. The intestinal barrier to fluid movement is at least composed of three membranes, the luminal and basal epithelial cell membranes and the capillary membrane. It is impossible to decide from the present studies at which level the overall permeability is increased in the cholera-infected bowel. The work of Huber and Phillips (1960) on other epithelial tissues may indicate a contribution of the epithelial cell membranes. They propounded the theory of sodium pump inhibition as an explanation of their results but Ling (1965) has suggested that all this means is that the cell is a barrier to mass solute movement. Other studies (Craig, 1965; Love, 1965; 1969) would support a vascular element in the production of fluid in the vibrio-infected intestine as the only other studies showing similar changes in unidirectional sodium ion flux were produced by venous occlusion (Shields and Code, 1961). It may at this time be dangerous to lay emphasis on one or other mechanism as the sole factor in the accumulation of fluid in the cholera-infected intestine, as it is likely that the vibrio produces multiple toxins which may each produce different effects (Leitch, Burrows, and Stolle, 1967).

SUMMARY

The movement of water into the intestine produced by osmotic gradients of various solutes has been studied in the normal and cholera-infected rabbit intestine.

More water was drawn into the intestinal lumen in a similar osmotic gradient in the infected than into the normal intestine.

The bulk fluid movement produced by solutes of varying molecular size allowed theoretical pore sizes to be calculated for the normal and infected intestines.

The findings support the concept that the cholera vibrio produces an increase in intestinal permeability.

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