THE PATHOPHYSIOLOGY OF CHOLERA*

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INTRODUCTION

ASIATIC cholera is an acute, severe diarrheal disease caused by the organism *Vibrio cholerae*. The clinical spectrum associated with infestation with this organism ranges, however, from asymptomatic carriers to fulminant disease leading to vascular collapse within two hours of the onset of symptoms.[1] The volume of stool passed in the course of the illness may be equivalent to or even exceed the patient's body weight. In a study of 12 consecutive cholera patients in Dacca, Pakistan, in 1964, Lindenbaum and his associates found that the average duration of diarrhea was 4.7 days (range 2.7 to 6.3) and that the stool volume passed during hospitalization averaged 30.8 l. (range 5.2 to 69.1), and this did not include stool passed prior to hospitalization.[2] In some outbreaks, untreated, the mortality rate may be as high as 60%.[3] Simply replacing fluid and electrolyte losses intravenously can reduce the mortality to less than 1%.[1]

Interest in the pathophysiology of Asiatic cholera is twofold. First, cholera is a major fatal infection in many of the world's most populous underdeveloped areas. Since the public health measures which resulted in eradication of cholera from Europe and America are unlikely to become operative in the endemic areas, better understanding of the disease may lead to the development of practical preventive and therapeutic measures; further, cholera has been a stimulus to reexamine intestinal secretion, a phenomenon that since the review of Florey et al.[4] has been largely ignored or was believed not to exist. (*The Handbook*

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of Physiology, which devotes five volumes to the alimentary tract, has not even a chapter on intestinal secretion.)

The Infection

Large numbers of *V. cholerae* are found in the stool in early cholera (10^6 or greater/ml. stool). Recent studies have shown that almost as many are to be found in the upper jejunum on the first day of illness.[5] In postmortem examination of fatal cases as well as in experimental infections of laboratory animals the organisms are found in large numbers "adsorbed" to the surface of the mucosa as well as in the luminal fluid.[6, 7] The organisms do not, however, invade the epithelium or enter the crypts of Lieberkühn.[8] The number of vibrio decreases in the small bowel as the diarrhea decreases and disappears on the fourth to eighth day of illness. A few patients continue to have vibrios in the small bowel even though the diarrhea has ceased. During cholera the upper bowel is colonized by colonic bacteria and these tend to persist, even for months after the acute illness.[5] It has been demonstrated in man and experimental animals that the clinical manifestations of Asiatic cholera are solely the consequence of the massive fecal losses of fluid and electrolytes.[1, 9, 10] This massive fluid and electrolyte loss can be duplicated by giving the bacterial-free filtrate of *V. cholerae* cultures to man[11] and experimental animals.[12-14] The active principle is an exotoxin which has been concentrated and purified so that nanogram amounts have biologic activity. It is a heat-labile protein with a molecular weight estimated to be between 10,000 and 90,000.[15, 16] Purification and characterization of the exotoxin have been hampered by the lack of a precise assay of its stimulation of intestinal secretion. The most precise tests of biologic activity, though indirect, are the capillary permeability test of Craig[17] and the release of glycerol by isolated fat cells.[18]

Site and Character of Fluid Loss

The site of fluid loss in cholera is the small intestine in man and in experimental animals,[19-21] and fluid production is greater in the upper intestine, duodenum, and jejunum than in the ileum. There is no evidence that gastric, biliary, or pancreatic secretions contribute significantly to the cholera fluid. In experimental canine cholera 92.4% of the fluid produced comes from the jejunum and ileum while 5.6%

TABLE I. INTESTINAL FLUID COMPOSITION (mEq./l.)

	Jejunum				*Ileum*			
	Na	*K*	*Cl*	HCO_3	*Na*	*K*	*Cl*	HCO_3
Man[24]	148	5.6	138	15	146	5.7	121	42
Dog[20]	159	6.9	122	26	145	9.6	68	76
Rabbit[21]	153	4.3	45	92	148	4.4	48	91

comes from the alimentary tract proximal to the jejunum and 2% from the colon.[22] The colon continues to absorb fluid normally in cholera but because of its limited absorptive capacity it is unable to absorb but a fraction of the fluid delivered from the small intestine.[23]

The intestinal fluid in cholera is approximately isosmotic with plasma. Its electrolyte composition varies with the level sampled and from species to species but, more important, it is similar to the normal intestinal fluid for that species at that level (Table I).

The rice-water stool of cholera has a composition similar to ileal fluid (Na 139, K 24, Cl 106, HCO_3 48).[2] The degree to which the electrolyte composition of stool is altered from ileal fluid is determined by the volume presented to the colon and the degree of hypovolemia and sodium depletion. The concentration of protein in cholera stools is very low, 85 mg./100 ml., well below levels found in congestive failure or inflammatory bowel disease.[25] In addition, after intravenous injection, neither Evans blue dye nor I^{131} labeled polyvinylpyrolidone appear in the stools of patients with cholera.

MECHANISMS OF FLUID PRODUCTION

Having briefly reviewed the magnitude, composition, and site of production of the intestinal fluid that gives rise to the "rice water" stools of cholera let us review the mechanism by which it is produced. There have been four major suggestions: 1) exudation, 2) inhibition of absorption, 3) transudation, and 4) intestinal secretion.

Exudation. In view of the impressive evidence to the contrary it is surprising that this view of the pathogenesis of intestinal fluid production persisted so long. As recently as 1951 textbooks presented the views of Virchow and Koch that intestinal fluid losses were due to denudation of the intestinal epithelium; hence they could be likened to events seen after extensive burns.[26] In 1882 Cohnheim in his *Lectures on General Pathology* clearly pointed out that the choleraic stool had

such a low protein content it could not possibly be an exudate.[27] Although Goodpasture,[28] studying cholera in the Philippines, presented extensive histologic evidence in support of Cohnheim's view, the idea that morphologic alteration of the intestinal mucosa was a primary factor in the pathogenesis of cholera was not laid to rest until Gangarosa et al.[29] found normal intestinal mucosa by peroral biopsies during the course of cholera. Animal models of cholera have made it possible to follow intestinal morphology from first contact of the mucosa with *V. cholerae* or its exotoxin through to recovery.[8, 25] No light or electron microscopic alterations were found in the intestinal mucosa. In spite of the transfer of large amounts of fluid from mucosal capillaries to intestinal lumen only trivial changes if any could be found in the vascular or lymphatic vessels. These studies, as well as those in man, indicate that the intestinal fluid loss in cholera is mediated by a functional rather than an anatomical alteration of the intestine.

Inhibition of absorption. Using Visscher's calculations for the rate of clearance of sodium from plasma to gut lumen, Watten et al.[30] suggested that the explanation for the large fecal volumes produced in cholera was that the cholera organism or its products inhibit reabsorption of intestinal fluid. Support for this notion was provided by the observation that crude cholera toxin inhibited active sodium transport by frog skin.[31]

There are a number of reasons for rejecting this mechanism as the explanation for diarrhea in cholera. First, the application of Visscher's data to man probably overestimates the volume of fluid presented to the small intestine for reabsorption. It has been estimated that the small intestine absorbs 7 to 8 l. of fluid per day. Since there is no hypersecretion of saliva, bile, gastric, or pancreatic juice in cholera, complete cessation of intestinal absorption would result in a stool volume of no greater than 7 to 8 l. per 24 hours. On the other hand, the average stool volume observed in the first day of cholera was 8.3 l., and volumes as high as 16 l. have been observed.[32] Second, intestinal absorption of glucose is unaltered in experimental cholera.[33, 34] In fact, the normal glucose absorption in cholera has been put to practical use to increase fluid and sodium absorption from the intestine and to decrease the requirement for intravenous fluid replacement.[35, 36] Finally, measurements of unidirectional sodium fluxes both in man and experimental animals have shown normal lumen-to-mucosa movement of sodium with an

increased mucosa-to-lumen movement.[24, 34, 37] Some observations have suggested that absorption of sodium may be decreased in cholera[37, 38] but, if present, it is not great and is not a major factor contributing to fluid and electrolyte losses in cholera.

Transudation. Intestinal fluid in cholera has a low protein content: hence it is not surprising that increased transudation into the intestine has been considered a mechanism for production of diarrhea in cholera. For such a mechanism to be operative, the hydrostatic pressure or driving force must be increased or the permeability of the mucosa increased (or, to put it in another way, the resistance to flow must be decreased) or there must be a combination of these two factors. Recently Love has presented data which have been interpreted as favoring increased mucosal permeability as the basic defect in cholera.[40] It was found that more fluid was drawn into the intestinal lumen by a similar osmotic gradient in the cholera-infected animals than in the normals. Calculations based on net fluid movement produced by unchanged solutes of varying molecular sizes lead to the conclusion that the effective radius of the epithelial pores of the intestine doubled (increased from a normal of 6 A. to 11-12 A.). Such calculations may have validity in the study of relatively homogeneous membranes such as frog skin or toad bladder but must be interpreted cautiously when applied to a complex epithelium like the intestine which has a heterogeneous population of cells and a secretory as well as an absorptive function. If the fluid and electrolyte response to cholera and hypertonic mannitol singly and in combination are measured it appears that the individual stimuli induce fluid of differing composition. When the two stimuli are given together the volume and composition of the fluid is what would be expected if the two stimuli were acting independently and their products were mixed.[41] When "free water clearance" is calculated for the mannitol-induced fluid in response to the combined stimuli no difference is found. If, however, cholera exotoxin had caused increased permeability of the mucosa more electrolytes could pass into the lumen and the free water clearance with the combined stimuli would have been less. Finally, if the movement of labeled noncharged solutes of varying size are injected intravenously and their appearance in intestinal fluid is measured, an estimate of mucosal permeability is obtained. Cholera does not alter the ratios, hence there is no evidence that cholera increases the permeability of the intestinal epithelium.[42]

There are other reasons for rejecting transudation as an important mechanism in the pathogenesis of cholera. In the absence of an osmotic gradient between the lumen and the lamina propria, the driving force for filtration through the epithelium is hydrostatic pressure. In cholera in man purging continues even when the patient is hypotensive and hypovolemic. In experimental canine cholera the rate of intestinal fluid production was not impaired when mesenteric blood pressure was decreased to 30% of control values by a clamp on the mesenteric artery.[43] The studies of Hakim and Lifson[44] are often quoted in support of the notion that hydrostatic filtration can explain fluid production in cholera. In these *in vitro* studies, net secretion was produced by increasing the pressure on the "serosal" surface. In this preparation net glucose movement was reversed when net fluid movement was reversed. In cholera, in contrast, glucose absorption continues at a normal rate as net fluid movement changes from absorption to secretion.[33]

Observations describing altered permeability and fine structure of the intestinal capillaries have also been used to support the hypothesis of transudation.[44-46] It has been reasoned that since permeability of the vessels of the skin is increased by injection of cholera toxin into the skin[17] cholera toxin may reach the intestinal vessels and produce similar changes. On the other hand, other observers have found no changes in the mucosal vasculature in experimental cholera.[8, 25] It seems reasonable to expect some functional changes in the mucosal capillaries in cholera since the large quantities of fluid appearing in the lumen are delivered to the epithelium by the mucosal capillaries. The likely interpretation of all this is that the vascular changes described are the response to and consequence of the massive intestinal secretion rather than the cause.[47]

Finally, neither increased epithelial permeability nor increased hydrostatic driving force can explain the differing anion concentrations found in the jejunum and ileum, which are similar to those found in the uninfected state and are characteristic of the fluid found in jejunum and ileum. Even if increased permeability were the explanation for the large volume of fluid entering the intestine, it would be necessary to postulate other mechanisms to maintain the constancy of the anion concentrations in the jejunum and ileum at concentrations differing considerably from plasma.

Increased secretion. Little has been written on intestinal secretion during the last three decades and, until recently, all fluid and electrolyte

movements into the intestinal lumen had been assumed to be passive.[48] This view of normal intestinal physiology has, of course, greatly influenced the nature of hypotheses proposed to explain the pathogenesis of cholera. Physicians of earlier generations, however, held a different view. In 1855 Dr. John Snow suggested: "It would seem that the cholera poison, when produced in sufficient quantity, acts as an irritant on the surface of the stomach and intestine, or, what is still more probable, it withdraws fluid from the blood circulating in the capillaries, by a power analogous to that by which the epithelial cells of the various organs abstract the different secretions in the healthy body."[49] A similar view was held by Cohnheim, who concluded, after presenting extensive clinical, pathologic, and experimental data, that ". . . the process of cholera may be interpreted by supposing that first, under the influence of the virus, which has probably entered the intestine from without, there takes place, *an extra-ordinary profuse secretion from the glands of the small intestine.*" [27] Much evidence supports these suggestions that cholera fluid is the secretary product of the intestine.

First, in spite of the reversal of net fluid movement from "absorption" to "secretion" by cholera exotoxin, unidirectional flux of labeled sodium from intestinal lumen to mucosa as well as glucose absorption is unaltered from control values, whereas the flux of sodium from mucosa to lumen is greatly increased.[23, 34, 37] Such findings are difficult to reconcile with a passive process in the absence of any changes in physical driving forces such as hydrostatic and osmotic pressure.

Such consideration leads us to look for support for Cohnheim's hypothesis that cholera fluid originates in the crypts of Lieberkühn. If "absorption" and "secretion" are anatomically separated, cholera exotoxin might stimulate the "secretory area" while leaving the absorptive area unaffected. If cholera exotoxin stimulates secretion by the crypts of Lieberkühn without altering the absorptive function of the villi, agents which damage the crypts preferentially would be expected to modify the secretory response to cholera exotoxin. This notion was substantiated by studies of the effect of cycloheximide, an inhibitor of protein synthesis. On exposure to cycloheximide the epithelial cells of the crypts of Lieberkühn, having a higher protein synthetic rate, are effected earlier and at a smaller dose than are the columnar absorptive cells of the villi. The earliest morphologic evidence of the reversible inhibition of the synthesis of protein is disappearance of mitotic figures from the

crypts of Lieberkühn. This reversible inhibition of maturation has been shown to be caused by inhibition of the synthesis of protein necessary for cells to go from prophase to metaphase. With increasing doses of cycloheximide the crypt epithelium shows irreversible damage, and eventually the epithelium of the villi show morphologic and functional abnormalities. At a level causing only disappearance of mitotic figures from the crypts, cycloheximide inhibits the outpouring of intestinal fluid that normally follows exposure of the intestinal mucosa to cholera toxin.[50] Calculations of bidirectional fluxes indicate that the cycloheximide effect is due entirely to inhibition of the exotoxin-induced increase in mucosa to lumen flux.[51]

Once the production of intestinal fluid has been instituted by cholera exotoxin, no inhibition by cycloheximide is evident until 2½ to 3 hours after administration of the drug.[52] After cycloheximide the intestine does not regain its responsiveness to cholera exotoxin until protein synthesis recovers sufficiently to permit crypt cells to go into mitosis.[53] These findings suggest that cholera exotoxin induces the production of intestinal fluid through a process dependent upon protein synthesis. Once initiated, the secretion persists for several hours in the absence of further protein synthesis.

Another observation supporting the concept that the crypt and villous epithelia respond differently to cholera exotoxin is provided by measurement of the transmembrane potential by micropuncture of intestinal epithelial cells. The major changes induced by cholera toxin and theophylline (see below) were on the intervillous cells adjacent to the crypts and not on the villous cells.[54]

If the intestine is exposed to hypertonic sodium sulfate the villous epithelium is damaged, glucose absorption is impaired, but responsiveness of the mucosa to cholera exotoxin is unimpaired.[55] Finally, the newborn rat has no crypts and is unresponsive to cholera toxin. Production of intestinal fluid after exposure to cholera exotoxin appears only when the crypts of Lieberkühn are fully developed.[56]

Strong evidence that the production of intestinal fluid in cholera is the consequence of an active secretory process has been shown by studying intestinal mucosa in modified Ussing chambers. This permits measurement of unidirectional fluxes isolated from the effects of hydrostatic, chemical, and electrical gradients. In these experiments cholera exotoxin produced active secretion of chloride with a change in secre-

tion of another anion, assumed to be bicarbonate, and with decreased sodium absorption.[39,57] The differences between the *in vivo* and *in vitro* studies have yet to be resolved.

Most exciting is the observation that the *in vitro* effects on ion fluxes induced by cholera toxin were similar to those produced by cyclic AMP and by theophylline which causes endogenous cyclic AMP to accumulate by inhibiting its degradation by phosphodiesterase.[58] In addition, prostaglandins (PGA_2 and PGE_1) which also elevate tissue levels of cyclic AMP also lead to intestinal secretion. Intestinal mucosal levels of cyclic AMP have been found to be elevated by cholera exotoxin.[60] Recent studies have shown that cholera exotoxin activates adenyl cyclase in mucosal epithelial cells,[61] and it has been found that the level of intestinal adenyl cyclase in intestinal biopsies taken during acute cholera were twice the levels found in the convalescent period.[62]

Some of the pieces of this cholera jigsaw puzzle are beginning to fit together. The binding of cholera toxin to the intestinal epithelium sets off a sequence of events that results in the secretion of a characteristic isotonic fluid. One step in the sequence appears to be the activation of adenyl cyclase which, in turn, produces cyclic AMP; this provides the energy for the active ion transport which carries the fluid into the lumen. It also appears that there is an anatomic separation of absorptive and secretory function. The suggestion is that the choleraic fluid is produced by the crypts of Lieberkühn. There are, however, many missing pieces to find and fit into the puzzle. Where is the toxin bound? How are the secretory orders transmitted to the cell? Is activation of adenyl cyclase the primary event or is it the consequence of other events? Present information suggests that the onset of secretion precedes cyclase activation.[61, 63] What is the secretary process that is turned on? Is it the same in the jejunum as in the ileum? If chloride is indeed the ion that is secreted into the lumen in response to cholera toxin how are we to explain the excess bicarbonate in the luminal fluid?[21, 39, 64] The list can be easily extended manifold.

Finally, is the secretion of cholera a pathologic process or is it instead the extreme stimulation of a normal function? Is it a manifestation of what Florey et al.[4] suggested in their review of intestinal secretions:

> It may be necessary for a constant secretion of fluid to take place from the crypts of Lieberkühn to keep food particles in suspension while they are attacked by pancreatic enzymes, and

as the products of digestion are absorbed water and salts go with them. One may envisage a circulation of fluid during active digestion, the secretion passing out from the crypts of Lieberkühn into the lumen and back into the villi.[65]

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