ORAL REHYDRATION THERAPY:
New Explanations for an Old Remedy

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Abstract Diarrheal diseases are among the most devastating illnesses globally, but the introduction of oral rehydration therapy has reduced mortality due to diarrhea from > 5 million children, under the age of 5, in 1978 to 1.3 million in 2002. Variations of this simple therapy of salts and sugars are prevalent in traditional remedies in cultures world-wide, but only in the past four decades have the scientific bases for these remedies begun to be elucidated. This review aims to provide a broad understanding of the cellular basis of oral rehydration therapy. The features integral to the success of oral rehydration therapy are active glucose transport in the small intestine, commensal bacteria, and short-chain fatty acid transport in the colon. The review examines these processes and their regulation and considers new approaches that might supplement oral rehydration therapy in controlling diarrheal diseases.

INTRODUCTION

Infectious diarrheas continue to be a major health problem across the world, having widespread and devastating effects in developing countries and contributing to the health burden in developed countries. The morbidity and mortality associated with infectious diarrheas result from severe dehydration. The consequences of diarrhea are more severe in the young than in the adult because a fasting child not suffering from diarrhea can lose 1 to 2% of body weight daily (1, 2). Because the young have low nutritional reserves and children in developing countries are exposed to multiple episodes of diarrhea, malnutrition in these regions becomes commonplace. The consequences of diarrhea are similarly severe in the very elderly or immunocompromised patient (2).

Although numerous pathogens can cause diarrheas with varying etiologies, the most notorious is *Vibrio cholerae*. The seminal work of the London surgeon/epidemiologist John Snow drew attention to the transmission of the disease, the
The importance of sanitation, and even the underlying cause as dehydration (3). In the century following these observations, improved sanitary conditions greatly attenuated the disease. However, in the last quarter of the twentieth century, the formulation and dissemination of oral rehydration therapy brought a tremendous improvement to the control of diarrheal diseases and in 1978 was acclaimed to be the “most important medical advance this century” (4). While there is much debate on what is an ideal oral rehydration solution (discussed below), the basic premise is that by providing the patient with an oral supplement comprised of sugar or starches, salt, and water, fluid replacement should be achieved (2, 5, 6). This seemingly simple remedy is deeply rooted in traditional medicine worldwide. Remarkably, only in the past four decades have the scientific bases of these remedies begun to be elucidated, and their success underscores why these remedies have stood the test of time.

WHAT IS ORAL REHYDRATION THERAPY?

Oral Rehydration Therapy in Traditional Medicine

The use of oral fluid replacements in the treatment of diarrhea can be documented in perhaps every culture. Diarrhea was known to Hippocrates, and elaborate treatment for diarrhea is provided in the Vedic Suśruta Samhitā (7). In rural Mississippi, for example, a mixture of flour and water is recommended, and another folk remedy suggests apple extracts as a binding agent for mild diarrhea prior to a visit to the pediatrician. In tropical countries, the formulations range from watery rice gruel to extracts of maize, yams, taro root, green bananas, the water of tender green coconuts (a sterile medium), a mixture of molasses and rock salt, and extracts of mung beans (2, 8, 9). In the developing world during the nineteenth and early twentieth centuries, there were opposing views on the value of traditional remedies vis-à-vis Western medicine. For example, in India, European colonizers often regarded tropical diseases “as diseases peculiar to India” and believed that the response of the European and the native to therapy would be different. Thus in colonial Bombay in the mid-1800s, even as Snow was making his prescient observations on the Broad Street epidemic (3), British medical and sanitation officers were of the firm belief that cholera was a poison developed in the soil (miasmatic theory) (10). Furthermore, medical officers in the Bombay Presidency considered Western medicine to be more efficacious and rejected the traditional use of “large draughts of salt and water” to treat cholera as it would cause inflammation and would work, if at all, only with Indians (10). The Indian response was equally unhelpful as local practitioners of Ayurveda claimed that cholera arose from cow slaughter.

1 As a lasting tribute to Dr. Snow, Dr. R.R. Frerichs, of the University of California at Los Angeles, has created an informative website, http://www.ph.ucla.edu/epi/snow.html, which includes access to Dr. Snow’s 1855 book.
by the Europeans! However, as deciphered by Snow, improved sanitation was critical in stemming cholera (3), and Western approaches to sanitation resulted in a reduction in the number of cholera-related deaths in heavily populated Indian urban centers from 19,996 during 1857–1865 to 10,509 during 1866–1886 (10). Recently, the common practice by Bangladeshi villagers of using old cotton saris to filter home-made drinks was put to a field test, involving 65 villages and 45,000 participants. The cotton sari folded eightfold (mesh size $\sim 20\, \mu m$) sieved out the zooplankton that harbor *Vibrio*, and filtration led to a 48% reduction in cholera (11). These improvements show that although beneficial, traditional medicines cannot be implemented in a vacuum, they are even more effective when coupled with other factors such as sanitation, education, and implementation.

**Oral Rehydration Therapy in Modern Medicine**

The terms oral rehydration salts or solutions (both abbreviated ORS) and oral rehydration therapy (ORT) are generally used interchangeably. The World Health Organization’s (WHO) original definition of ORS was confined to the use of oral rehydration salt packets dissolved in water. However, ORT encompasses both ORS as well as recommended home fluids (RHF), comprised of $Na^+$ and a source of carbohydrate, ranging from rice water to cereal-based solutions and traditional soups.

Original references to the modern history of oral rehydration can be found in a number of reports (1, 2). In the late 1940s and early 1950s, a balanced salt solution was found to help correct the acidosis in children with acute diarrhea (11a). Much like the traditional remedies, these formulations included glucose more as a source of nutrition than as the major driving force for fluid absorption. Parallel to these clinical studies, seminal work by a number of physiologists, including Ussing, Curran, Crane, Fisher, and Schultz, culminated in the demonstration that the transport of a number of solutes, in particular glucose and amino acids, was coupled to the movement of $Na^+$ across the apical membrane (reviewed in 12–14).

In 1968, the first successful clinical trials of ORS were conducted by the Cholera Research Laboratory, Dhaka, in collaboration with Johns Hopkins Institute for Medical Research and Training, Baltimore, and the All India Institute for Tropical Medicine, Calcutta (15, 16). The 1971 war on the Indian subcontinent not only resulted in the creation of the nation of Bangladesh but was pivotal in making ORS available to the general public. Hitherto, administration of ORS was believed to be the purview of medical personnel, but the rampant spread of cholera and other diseases in the aftermath of the war led the director of a medical center camp to distribute ORS to the general public for administration (17). The reported death rates in the camps using conventional intravenous fluid was 6- to 16-fold greater than in those using ORS (20–50% versus 3%) (17, 18). This reaction to

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2This laboratory was established in 1960 in East Pakistan (Bangladesh in 1971). In 1978, the Government of Bangladesh established the International Center for Diarrheal Disease Research B.
adversity was fortuitous and resulted in the general acceptance of nonmedical personnel administering ORS, a major step in providing care in regions where access to medical help is limited. Although the validity of this simple solution was questioned, these clinical efforts led to the launching of ORS as a therapy by the WHO in 1978.

A number of centers around the world contributed to the development of improved oral rehydration formulations, whereas the WHO and UNICEF were primarily and continue to be instrumental in the effective dissemination of the formulations. The number of OR packets distributed in developing countries has risen from 51 million in 1979–80 to 800 million in 1991–1992 (19). The effect of these interventions is best seen in the mortality rates; in 1978, approximately 5 million children under the age of 5 years died from acute diarrhea; these figures steadily dropped to ~4.6 million in 1980, 3.3 million in 1990, 1.5 million in 2000, and 1.3 million in 2002 (17, 19). Boosted by this success, WHO’s goals are targeted to reducing deaths attributable to diarrhea by a further 50% by 2010 (17). The success of ORT is due to the interaction of multiple variables, including caretaker compliance, adequate supplies, effective distribution, and improved sanitation. Great strides were made in the improvement of home management from 1990–1995 (19). Assessing the appropriate management of ORT is a gargantuan task as it involves surveying different regions of the world, with information from nonclinical and clinical settings, and includes multiple indicators such as accessibility to ORT, continued feeding during diarrheal episodes, and the ensuing fluid intake (19). Despite these challenges, ORT has had far-reaching repercussions globally on the management of one of the most devastating diseases. It is fitting that the first Pollin Prize for Paediatric Research was awarded to Drs. N. Hirschhorn, N.F. Pierce, D. Mahalanabis, and D.R. Nalin for their key contributions to the discovery and implementation of ORT.

Why Is ORT Lifesaving? The Composition of Oral Rehydration Fluids

The key to the success of ORT is that it replaces the fluid being lost, circumventing the need for intravenous replacement in 80 to 90% of the cases of mild-moderate diarrhea and is lifesaving in acute diarrheal diseases. It also minimizes the malnourishing effects of acute diarrhea. The composition of oral rehydration fluids has undergone some changes over the past 25 years.

**DEFINED FORMULATIONS** The composition of WHO packets distributed in 1978 is provided in Table 1 (17). Although highly beneficial, it soon became clear that the therapy had some limitations (20). These can be broadly categorized into compliance, questions related to osmolarity, and determination of what should

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3Survey examples include the Control of Diarrheal Diseases survey of WHO, the Multiple Indicator Cluster Surveys and Demographic and Health Surveys of UNICEF.
accompany and follow ORT. While dehydration was averted by the standard ORS (Table 1), the near-isotonic solution neither reduced the duration of the illness nor the rate of stool loss; in some cases, an increase in stool loss was even reported. In addition, there was danger of hypernatremia (6, 20). These shortcomings made the therapy less attractive to the patient and the caretaker, leading to poor compliance. As recently as 2000, there was concern about the under-utilization of ORT in developing and developed countries (5, 21, 22). In developing countries, the problem is one of misplaced expectations; i.e., that the therapy should be a “magical” cure involving a “prestigious” drug and therefore patients preferred “drugs” to the WHO version of traditional remedies. In Europe and the United States, despite the potential saving from ORT of $1 billion, in terms of hospital care and follow-up visits, the acceptance rate of ORT was disappointingly low. The causes were varied but ironically related to the poorly informed support staff, who preferred intravenous fluids to the frequent feedings (every few minutes) required in ORT, and the uncertainty over whether ORT would be reimbursed by insurance companies (5).

The criteria for improving ORS are to decrease stool output, duration of diarrhea and emesis, and to reduce the need for intravenous therapy. There has been extensive debate over the ideal osmolarity for ORS, and there may be a difference in what is needed in developed countries compared with developing countries and the nature of the diarrheas (5, 6). Fecal Na\(^{+}\) concentration in cholera is ~90 mM.

### TABLE 1  Sugar and electrolyte composition of commonly utilized oral rehydration solutions

<table>
<thead>
<tr>
<th>(CH(_2)O)(_n)</th>
<th>Standard ORS(^a)</th>
<th>Reduced pre-osmolarity ORS(^a)</th>
<th>Rice-based ORS(^b)</th>
<th>Amylase-resistant starch(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mM)</td>
<td>111</td>
<td>75</td>
<td>90</td>
<td>111</td>
</tr>
<tr>
<td>Sodium (mM)</td>
<td>90</td>
<td>75</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Chloride (mM)</td>
<td>80</td>
<td>65</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Potassium (mM)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Citrate (mM)</td>
<td>10(^*)</td>
<td>10</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Other Anion (mM)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>30</td>
</tr>
<tr>
<td>Total Osmolarity (mOsM)</td>
<td>311</td>
<td>245</td>
<td>280</td>
<td>327</td>
</tr>
<tr>
<td>Pedialyte(^d)</td>
<td></td>
<td></td>
<td></td>
<td>269</td>
</tr>
</tbody>
</table>

\(^*\)Citrate varies 10–30 mM, and earlier formulations used HCO\(_3\)\(^-\).

\(^a\) (17).

\(^b\) (24).

\(^c\) (27).

\(^d\) (11a).
and replacing this was the basis of the original ORS. However, in rotaviral diarrheas, the major type of diarrhea in children in developed countries, fecal Na\(^+\) is closer to 40 mM and therefore there is danger of hypernatremia. Pediatric societies, such as ESPGHAN\(^4\), have recommended a multistep protocol for the treatment of acute diarrheas in developed countries, including reduced osmolarity ORS (RO-ORS), fast rehydration, and rapid return to normal feeding (22). In a multicenter trial in Brazil, India, Mexico, and Peru, administration of RO-ORS (224 mOsM) decreased stool output, continued need for ORS intake, and duration of diarrhea compared with the use of WHO-ORS (23). However, a risk in reducing osmolarity in rehydrating cholera patients is hyponatremia. When RO-ORS (75 mM each Na\(^+\) and glucose, 65 mM Cl\(^-\), 245 mOsM) was tested in adults and children with cholera, it was found to be as effective as standard ORS, with a chance of symptomatic hyponatremia in adults [reduced osmolarity ORS formulation in (17)]. In a meta-analysis of noncholera diarrheas in children, the effects were more dramatic, i.e., reduced stool volume and decreased need for follow-up intravenous therapy. On the basis of such meta-analyses, the WHO-UNICEF released a new reduced osmolarity ORS formula in May 2002 (17; Table 1). However, grave reservations were expressed by pioneers in the field that this move was ill-advised as the scale of the study was not large enough and the risks of hyponatremia were not fully explored (23a).

Numerous studies on improving ORT also examine what should accompany or follow ORT. The importance of continuous feeding, especially breast-feeding, during ORT and follow-up of the treatment with a regimen to replace lost fluid (2, 5, 22) is now well accepted. Feeding is critical to promote intestinal growth and to increase net absorption. In general, breast-fed children fared better, with a 35% greater energy intake and a 250% greater protein intake than formula-fed children (2).

**RICE-BASED ORS**

Along with the strategies described above, the quest for alternate, inexpensive improved home fluid therapy continued. Answers were sought again in traditional remedies, such as use of starchy water left after over-cooking rice to treat diarrheas in India, Bangladesh, Egypt, and Mexico. In a meta-analysis of 13 clinical trials, adults and children with cholera, when fed with rice-based (50–80 gm/L) ORS (280 mOsM, Table 1), showed ~32–36% decrease in stool output (24). In other studies, ORS was shown to decrease the duration of diarrhea and rate of purging. Not surprisingly, rice-based hypoosmolar ORS was better than standard ORS or RO-ORS in cholera patients (25). Rice has the advantage of providing glucose, amino acids, and more calories than an equivalent amount of glucose and is hypoosmolar to standard WHO-ORS. It is also cheaper and readily available in cholera-endemic regions. However, for noncholera diarrhea with feeding, there was no difference between rice-based ORS and standard ORS (26).

\(^4\)European Society of Pediatric Gastroenterology, Hepatology and Nutrition.
AMYLOSE-RESISTANT STARCHES  Whereas rice is readily digested by amylase in the upper small intestine, depending on the content of pectins, dextrins, glycans, and cellulose, a significant portion of starches can be amylase resistant. Recognition of the importance of short-chain fatty acids (SCFAs) in colonic absorption has led to a heightened interest in the use of amylase-resistant starches in ORT.

In a randomized, well-designed study of 48 adolescent and adult cholera patients, the efficacy of an amylase-resistant, high-amylose maize starch (Table 1) was compared with that of rice flour and standard WHO-ORS. The starch and rice (50 g each) were resuspended in WHO-ORS, rendering them somewhat hypotonic. When compared with WHO-ORS and rice-ORT, the resistant starch significantly decreased fecal weight and reduced the duration of diarrhea; it also caused an increased fecal excretion of starch (27). Another randomized, double-blind trial involving 62 infants suffering from persistent diarrhea elegantly demonstrated the efficacy of amylase-resistant starches contained in green bananas (28). Three groups were given equivalent caloric amounts (54 kcal/dL) for 7 days of a diet comprised of rice ORS alone, or pectin or cooked banana, made up in a rice-ORS. The last two groups showed a decrease in diarrhea as early as day 2 of treatment, and by day 4, over 80% of them had no diarrhea in contrast to 20% in the rice-ORS group. Pectin- or green banana-based ORS reduced stool output and emesis and required less rehydration solutions and fewer return medical visits. Similarly, partially hydrolyzed guar gum (Benefiber®) administered in WHO-ORS reduced duration of diarrhea and stool output in children suffering from acute, noncholera diarrhea (29).

To summarize, ORT is here to stay, and exploration of amylase-resistant starches and rice as effective sources of carbohydrates to improve ORT is of tremendous therapeutic and economic benefit.

WHAT IS THE CELLULAR BASIS FOR ORAL REHYDRATION THERAPY?

The development of ORT over the past 40 years has paralleled the huge leaps in our understanding of the molecular basis of intestinal ion and fluid transport in health and disease. A brief overview is provided (for reviews, see 14, 30–34).

Intestinal Fluid and Ion Transport

DESIGN AND REGULATION  Together, the small and large intestines process >9 liters of fluid a day; approximately 7 liters come from other exocrine glands and food intake, and the intestines themselves contribute 1.5–2 liters. In a healthy individual, over 80% of this fluid is reabsorbed in the small intestine and >18% in the colon, with less than 2% being lost in the stool. The architecture of the intestines and their lining epithelia are uniquely geared to accomplish the opposing functions of absorption and secretion. There are, however, species, age, and
segmental differences along the cephalocaudal axis of the intestine in terms of morphology, expression of hydrolases, types of ion transporters, their regulation, and the luminal milieu (30, 35–37). The accepted model is that the villar/surface cells are the chief sites of absorption and the crypts are the main sites of secretion, with a few exceptions (see below) (14, 32, 38, 39). A disruption in the balance of absorption and secretion, either by decreased absorption and/or increased secretion, results in diarrhea.

Absorptive and secretory functions are tightly coordinated by a plethora of neurohumoral factors. The heterogeneous epithelial cells themselves contribute modulators, and the rest of the regulatory pathways arise from either luminal stimuli or from neural, immune, or systemic stimuli from the intestinal wall (Table 2). The multiple smooth muscle layers govern transit time. The complexities of intestinal ion transport regulation have been underscored by the discovery of multiple signaling pathways and abundant cross-talk between these cascades. Multiple isoforms for all components of the canonical signal cascades, including receptors, cyclases, phosphodiesterases, protein kinases (PK), phosphatases, and target transporters, add to this complexity (30, 31, 34, 40, 41, 46, 47). Another level of regulation is achieved by compartmentalization of transporters and their regulatory molecules into subcellular domains, such as on lipid rafts (42), on scaffolding proteins (43), or in vesicular compartments (44). The pleiotropic ability of transporters

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Intestinal fluid transport regulators (categorized by intracellular mediator)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Promoters of secretion</strong></td>
<td><strong>Promoters of absorption</strong></td>
</tr>
<tr>
<td>$\uparrow$ Ca$^{2+}$</td>
<td>$\uparrow$ Cyclic AMP</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Bradykinin</td>
</tr>
<tr>
<td>ATP</td>
<td>Histamine</td>
</tr>
<tr>
<td>Bile acids</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>Vasoactive intestinal peptide</td>
</tr>
<tr>
<td>Galanin</td>
<td>E. coli LT</td>
</tr>
<tr>
<td>Gastrin-releasing peptide</td>
<td>V. cholerae</td>
</tr>
<tr>
<td>Histamine</td>
<td>$\uparrow$ Cyclic GMP</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Serotonin</td>
</tr>
<tr>
<td>Substance P</td>
<td>Substance</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>E. coli EAST</td>
</tr>
<tr>
<td>C. difficile</td>
<td>E. coli STa</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>Y. enterocolitica</td>
</tr>
</tbody>
</table>

EAST, enterosaggregative heat-stable toxin; LT, heat-labile enterotoxin; STa, heat-stable enterotoxin.
such as the cystic fibrosis transmembrane conductance regulator (CFTR) to influence the expression of other transporters adds yet another layer of complexity (reviewed in 45). Generally, stimuli that increase intracellular cAMP, cGMP, or Ca\(^{2+}\) (Table 2) cause net luminal fluid accumulation by either stimulating secretion and/or inhibiting absorption (46, 47). Agents that decrease cAMP promote absorption. Cross-talk exists between these cascades and the tyrosine kinase/phosphatase and the mitogen-activated kinase cascades (30, 34, 40, 41).

**PARACELLULAR PATHWAYS** Transepithelial transport occurs via paracellular or transcellular pathways. The tight junction at the apical end of the cells largely defines paracellular movement in terms of charge selectivity, ion and solute movement, and tissue resistance. Transepithelial resistance increases down the cephalo-caudal axis (duodenum \(< 25 \Omega \text{ cm}^2\), ileum \(\sim 90 \Omega \text{ cm}^2\), and colon \(\sim 200 \Omega \text{ cm}^2\)) (30). Intercellular contacts farther down the lateral space also contribute to the complex geometry and therefore modify flow. Whereas paracellular transport is passive, the tight junction is a dynamic lattice structure of 14 or more proteins, ranging from the transmembrane occludins and claudins to the plaque-associated zonula occludens (ZO) proteins and cytoskeletal actin (48). These are subject to regulation by cellular signals and by other membrane proteins further along the lateral space. Thus the interaction of E-cadherin with Ca\(^{2+}\) triggers a chain of events involving recruitment of catenins, vinculin, and the activation of phospholipase C and PKC and ZO proteins, resulting in tight junction formation (49). The apical perijunctional actomyosin ring is in close contact with the tight junction and can modify paracellular permeability (50). Of relevance to this review is the observation that agents that cause secretion [e.g., cAMP, *V. cholera* ZO toxin (ZOT), *Clostridium perfringens* toxin, and immune mediators], as well as those that promote absorption (e.g., glucose), increase paracellular permeability (40).

**TRANSCELLULAR TRANSPORT IN THE SMALL INTESTINE** The study of two devastating and seemingly disparate diseases, e.g., cholera and cystic fibrosis, in the 1960s–1980s revolutionized our thinking about anion secretory and solute-independent Na\(^{+}\) absorptive processes in the intestine (14, 51). This understanding followed on the heels of the discovery of the cellular basis for solute transport (see below) (13). The P-type Na\(^{+}/K^{+}\) ATPase, located on the basolateral membrane (BLM), is the sine qua non of all transepithelial active transport processes (Figure 1). Regulation of the pump is one way to modulate fluid loss. For example, diarrhea associated with T cell activation was recently shown to be because of increased epithelial permeability and decreased Na\(^{+}/K^{+}\) ATPase activity, resulting in decreased solute-dependent and electroneutral Na\(^{+}\) absorption in wild-type and CFTR\(^{-/-}\) animals (52).

**Fluid secretion** The concerted action of at least three transporters, in addition to the Na\(^{+}/K^{+}\) pump, is needed to drive secretion; regulation of any one of these
will alter net anion secretion. Na\(^+\) moves passively via the paracellular pathway (Figure 1). The pump energizes the uphill entry of Cl\(^-\) via the BLM Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter (NKCC-1) (53). NKCC-1 contains consensus sequences for a number of kinases, and, in some cell types, an increase in NKCC-1 phosphorylation is associated with a stimulation of transport (54), whereas in others, NKCC-1 activity is governed by [Cl\(^-\)]\(_i\) and/or retrieval from intracellular vesicles (53).

In NKCC\(^{-/-}\) mice, although cAMP-stimulated Cl\(^-\) secretion is impaired in the jejunum, the cGMP-stimulated fluid secretion is unimpaired, implying that other anion entry mechanisms are present (55). CFTR decreases NKCC-1 expression and activity in pancreatic cells (56) but not in the colon (57). The other essential BLM transporters are K\(^+\) channels, which repolarize the cell and maintain the driving force for Cl\(^-\) exit. There are at least two types of K\(^+\) channels in the intestine, one regulated by cAMP (KVLQTI) and the other by Ca\(^{2+}\) (14, 32).

Chloride exits the apical membrane (BBM) of the enterocyte via CFTR (reviewed in 45). CFTR expression is greater in the crypts, but there is evidence for its presence in the villus (58). Support for the critical role of CFTR in intestinal Cl\(^-\) secretion comes from the ΔF508 and CFTR\(^{-/-}\) mice, where the primary defect is in intestinal secretion, and the intestines are refractory to Ca\(^{2+}\)-dependent secretagogues (45). Cyclic AMP and cGMP, via PKA and PKGII, respectively, increase the open probability of CFTR by directly phosphorylating CFTR on the regulatory R domain. In addition, cAMP increases the recruitment of CFTR to the apical membrane from the subapical membrane compartment (45). There are at least two other classes of Cl\(^-\) channels in the intestine, the CIC family, and the Ca\(^{2+}\)-activated Cl\(^-\) channels (CLCA) (32, 59, 60). The intestine mainly contains CIC-2, and its localization to the BLM of villus enterocytes suggests a role in absorption (61). The members of the CLCA family are DIDS-sensitive Cl\(^-\) channels, and some members are chiefly found in the goblet cells and crypts of the small intestine (59). Germane to this review is whether CLCAs can play a role in rotaviral infections (see below).

Bicarbonate secretion is necessary for luminal alkalization, especially in the duodenum, and there are at least three exit routes for HCO\(_3^-\): two anion exchangers, the down-regulated in adenoma protein (DRA) (62, 63), and the putative anion transporter PAT1 (64) in the villus cells and CFTR in the crypts (65). PAT1 shows higher expression in the duodenum than in the colon, whereas DRA shows the reverse pattern. Recent evidence shows that DRA and the Na\(^+\)/H\(^+\) exchangers (NHE), NHE-2 and NHE-3, are colocalized to specific lipid microdomains and are functionally coupled (66). Entry of HCO\(_3^-\) into the enterocyte across the BLM occurs via the Na\(^+\)-HCO\(_3^-\) (NBC) family of cotransporters. The BLM also expresses the AE-2 anion exchanger proteins.

**Fluid absorption** Net fluid absorption largely occurs in the jejunum and ileum and is inexorably linked to the movement of Na\(^+\), either in conjunction with solutes (see below), or via NHEs. The bulk of nonsolute-coupled Na\(^+\) transport across the BBM is achieved via the NHE-2 and NHE-3 isoforms, whereas the NHE-1
isoform is localized to the BLM and is involved in pH and cell volume regulation (reviewed in 30, 67, 68). There are clear distinctions in the regulation of NHE-2 and NHE-3, and the latter appears to be responsible for the bulk of intestinal Na\(^+\) absorption because the NHE-3\(^{-/-}\), but not the NHE-2\(^{-/-}\), mouse has severe diarrhea (69). The NHEs, especially NHE-3, are inhibited by cAMP, cGMP, or Ca\(^{2+}\) and therefore contribute to net fluid loss. Glucocorticoids increase NHE-3 but not NHE-2 activity (70), but proximal bowel resection increases both NHE-2 and NHE-3 expression and activity (71). The molecular mechanisms underlying the regulation of NHEs involve sequestration in lipid rafts, vesicle recycling, binding to the regulatory proteins, and perhaps direct phosphorylation of the exchanger (72). The identification of the PDZ domain-containing NHE regulatory factors (NHERF) as proteins critical for the cAMP-mediated inhibition of NHE-3 was a major breakthrough in transport physiology (73). These proteins interact with a number of transporters critical to transepithelial salt and water movement, including CFTR and DRA, and are also linked to \(\beta\)-adrenergic receptors and to the PKA-anchoring protein ezrin. The concerted interaction of these proteins enables cAMP to simultaneously activate CFTR while inhibiting NHEs and perhaps DRA (74).

The excessive Cl\(^-\) secretion and reduced NHE activity are the bane of ORT, and interference with any of their interlinked signaling pathways would be of benefit.

TRANSCELLULAR TRANSPORT IN THE MAMMALIAN COLON  The healthy colon re-absorbs 1.5 liters of fluid/day but can triple its capacity. However, if the colon’s absorptive capacity is compromised, as seen by the neurally mediated action of cholera toxin (see below), then the diarrhea is exacerbated. Some features of colonic ion transport that are distinct from the small intestine are highlighted below (Figure 2) (reviewed in 32, 33, 75). The adult colon does not express the Na\(^+\)-dependent glucose transporter, SGLT1, and therefore Na\(^+\) glucose is not a major mechanism for Na\(^+\) uptake; rather, the distal colon expresses conductive Na\(^+\) transport. The colon is the only part of the intestine where K\(^+\) can be actively secreted and absorbed via apical K\(^+\) channels and a K\(^+\)/H\(^+\)-ATPase, respectively. Finally, the major anions in the colonic lumen are SCFA (detailed below in the section on Role of SCFA and Commensal Bacterial Microflora).

The proximal colon largely expresses NHE-2 and NHE-3, and depending on the hormonal status, the distal colon expresses NHE-2, NHE-3, and the epithelial Na\(^+\) channel (ENaC) (32). CFTR down-regulates the expression and activity of epithelial Na\(^+\) channels (60). In the NHE-3\(^{-/-}\) mice there is an upregulation of ENaC and DRA, which together probably compensate for the loss in Na\(^+\) absorption (76). Recent evidence suggests that Na\(^+\) absorption can also take place in the crypts (38, 39), perhaps even by a novel Cl\(^-\)-dependent NHE (38). Equally intriguing are the observations that, by generating a hypertonic absorbate, the crypts play a critical role in fecal dehydration (77). CIC-2 may be involved in Cl\(^-\) absorption in the rat, but in the human it is present in subapical compartments (61). The bulk of Cl\(^-\) secretion can be attributed to CFTR (33). Colonic crypts and goblets express
hCLCA1, and evidence in T-84 cells, but not in CFTR−/− mice, suggests a role for CLCAs in colonic Cl− secretion (34, 59, 60). Critical to this review is the fact that colonic absorptive processes can play a major role in rehydration therapy.

**Major Perpetrators of Diarrhea**

Space does not permit doing justice to the fascinating ways in which ingenious microorganisms adapt to hostile environments and coopt their host and their own colonizers (phages) to survive and proliferate. When microbial survival occurs at the expense of the host, the organisms are pathogenic and elicit their diarrheagenic effects by interfering with various host cell signaling cascades, leading to an inhibition of Na+ absorption and/or stimulation of Cl− secretion. For example, *Clostridium difficile* affects the cytoskeleton whereas *Shigella dysenteriae* affects protein synthesis. Enterohemorrhagenic *Escherichia coli* and *Salmonella* utilize NFκB pathways that induce the expression of galanin-1 receptors on the host cell. Endogenous galanin then activates a Ca2+-mediated Cl− secretion (78). Three examples of diseases effectively treated with ORT are given below (40, 41).

**CHOLERA AND RELATED DIARRHEAS**

The world is currently witnessing the eighth cholera pandemic, which emerged in India and Bangladesh in 1992. The different pandemics have been associated with specific strains of *V. cholerae* (serogroups O1 biotype “classical” for the fifth and sixth, O1 El Tor for the seventh, and O139 for the eighth). Substantial progress has been made in understanding the molecular basis of *V. cholerae* virulence and survival (reviewed in 79, 80), culminating in the sequencing of the genome. This gram-negative curved rod bacterium can survive in an aquatic ecosystem where it receives some protection from the hostile milieu by associating with zooplankton and forming specific biofilms. *V. cholerae* has two chromosomes. Chromosome II appears to contain most of the genes needed for survival in an aquatic system. Chromosome I contains genes needed for metabolic function and for virulence. There are two major clusters of virulence genes, the Vibrio pathogenicity island (VPI) and the filamentous phage CTXφ, that are viral in origin and integrated into the host chromosome. The VPI bears the genes for toxin-coregulated pili (TCPs) required for colonization, whereas the CTXφ bears the genes for cholera toxin A and B subunits, ZOT, and accessory cholera toxin (ACE). Although many aquatic strains of *V. cholerae* are nonpathogenic, pathogenicity can be rapidly acquired by horizontal gene transfer, first of VPI, which then provides the receptor for transfer of CTXφ. The fact that horizontal gene transfer can occur in the intestine, that *V. cholerae* utilizes quorum sensing, and that the host milieu influences the types of genes expressed in the bacteria reinforces the challenges this bacterium provides (79, 80).

Effective colonization of the bacterium in the intestine and expression of the necessary virulence genes involve unknown luminal cues and a complex regulatory dialog between VPI- and CTXφ-coded proteins and those coded for by the “ancestral” *Vibrio* chromosomes (79). However, once the bacterium has colonized
the surface cells of the small intestine, it elaborates a heteromeric toxin containing five B subunits that form a ring around the A subunit. The B subunit binds to GM1 ganglioside on the BBM and is sorted via lipid rafts into a retrograde trafficking path via the Golgi cisternae into the lumen of the endoplasmic reticulum (ER). The A subunit is then unfolded and nicked in the lumen of the ER, and the enzymatically active A1 is released via the sec61 complex. By mechanisms that are still unclear, the A1 subunit accesses the BLM G-protein, GaS, and ADP-ribosylates it. ADP ribosylation of GaS inhibits its nascent GTPase activity and therefore allows GaS to permanently activate adenylate cyclase and generate cAMP for the life of the enterocyte (reviewed in 81). Although *V. cholerae* chiefly colonize the small intestine, by activating enteric neural pathways involving release of serotonin, PGs and VIP, it can also stimulate colonic secretion (82, 83).

Other factors produced by *V. cholerae* may contribute to the diarrhea. Most intriguing of these was the observation that ZOT, via a specific receptor, ZOT-R, increased paracellular permeability in the small intestine, but not the colon, and that ACE stimulated Cl− transport. Although the plasmids *zot* and *ace* are now proven to be involved in the morphogenesis of CTXφ, recent evidence suggests that C-terminal ZOT can bind to ZOT-R (reviewed in 40). The mammalian ligand for ZOT-R, zonulin, is also a potent regulator of paracellular permeability (84). Finally, strains of enteroaggregative *E. coli* produce a heat-labile toxin (LT), which also elaborates an AB toxin, with mechanisms of action similar to CT (40, 41).

**TRAVELER’S DIARRHEA AND HEAT-STABLE ENTEROTOXINS** Enterotoxigenic *E. coli* and *Yersinia enterocolitica* (85) elaborate a heat-stable enterotoxin (STa), which acts via the cGMP signaling cascade (86, 87). The STas have 18 amino acid peptides with 6 cysteine residues and are synthesized as precursor proteins and exported via the Sec pathway. STa binds to a specific BBM receptor-guanylate cyclase (GCC) in enterocytes and colonocytes. The action of STa is reversible and involves activation of a specific cGMP-dependent PKGII (47). Whereas STa acts via PKGII in the human colon, there are also suggestions that it could inhibit a phosphodiesterase and thereby stimulate cAMP production in the colon (87, 88). Specific scaffolding proteins associated with GCC and STa signaling have been identified (89). STa-induced secretion also appears to involve the enteric nervous system (82). STa stimulates Cl− secretion and inhibits Na+ absorption (31, 90), although recently Lucas (91) suggested that in vivo STa may chiefly be affecting Na+ absorption rather than Cl− secretion. The guanylin family members are the mammalian homologues of STa (Table 2). Enteroaggregative *E. coli* produces a guanylin-like toxin, EAST-1, which interacts with GCC but has a different pathogenicity than STa (92).

**ROTA VIRUS-INDUCED DIARRHEAS** Rotavirus is the major cause of diarrhea in children in developed countries, but is also prevalent in developing countries, contributing (20%–25%) to overall mortality. The virus mainly affects the small intestine, is self-limiting, and shows a strong age-dependence in a variety of species, causing diarrhea in the very young and often being asymptomatic in the adult. The
A double-stranded RNA virus has at least six viral proteins and five nonstructural proteins (NSP), of which one, NSP4, is the first known viral enterotoxin (reviewed in 93). The mechanisms underlying rotaviral infections appear to be a potpourri of the processes utilized by various bacterial pathogens. Thus in the early stages, the virus, NSP4, and its C-terminal peptide (114–135) increase [Ca\(^{2+}\)] in the enterocytes of all ages, but elicit only Cl\(^{-}\) secretion in the young animal. A pertussis toxin–sensitive activation of PLC increases [Ca\(^{2+}\)] initial from extracellular stores and later from the ER. NSP4, but not cAMP or carbachol, stimulates Cl\(^{-}\} secretion only in young CFTR\(^{-/-}\) mice (94). This finding led to the suggestion that NSP4 is activating CLCA in the young. The increase in [Ca\(^{2+}\)] unleashes a host of other effects ranging from a decrease in junctional resistance to actin reorganization and a disruption in cell morphology, thereby reducing the absorptive surface area. A second stage of infection involves the host enteric system and is associated with villus damage, although not necessarily with inflammation. Although rotaviral infections curtail Na\(^{+}\)-glucose absorption, ORT is effective, perhaps by promoting residual glucose absorption.

Why Does ORT Work?

The cellular underpinnings of ORT rest in the digestion and absorption of nutrients; the faster the absorption of sugar, the faster the absorption of water and Na\(^{+}\).

TRANSPORT OF GLUCOSE AND OTHER NUTRIENTS  Carbohydrates, which constitute 45–60% of the Western diet, are comprised of polymers of glucose, amylose (\(\alpha\)-1,4), amylopectin (\(\alpha\)-1,6), complex polysaccharides, and disaccharides. Salivary \(\alpha\)-amylases start the process of digestion, but the bulk of the common polysaccharides is hydrolyzed by the pancreatic \(\alpha\)-amylase, at the \(\alpha\)-1,4 linkages, to release oligosaccharides. The oligosaccharides are broken down further by surface hydrolases such as maltase-glucoamylase and isomaltase, which cleave the \(\alpha\)-1,4, and \(\alpha\)-1,6, linkages, respectively. Similarly, surface disaccharidases break down sucrose, lactose, and maltose. Pancreatic proteases and enteral brush border peptidases cause the breakdown of proteins to release oligopeptides, dipeptides, and amino acids. There are adequate hydrolases to ensure complete digestion of amyloses, with the surface hydrolases constituting >10% of the total BBM protein. Although pancreatic \(\alpha\)-amylase may be low in the infant, salivary \(\alpha\)-amylase and BBM glucoamylase appear sufficient for digestion (reviewed in 36, 95, 96).

The elucidation of the mechanism of glucose absorption in the small intestine has revolutionized the field of transport physiology from many perspectives. The key features are that hexoses can be transported both actively and passively and that active glucose transport is coupled to Na\(^{+}\) movement at the BBM and to an ATP-independent step at the BLM (reviewed in 13). These observations led to the tenet of secondary active transport and vectorial movement, not only of sugars, but also of amino acids, vitamins, and ions. The major route for BBM amino acid entry now appears to be the dipeptide carrier (see Daniel, this volume; 97).
Marking another milestone in the field were the yeoman efforts of Wright and colleagues (98), leading to the molecular identification of the Na\(^+\)-glucose transporter, SGLT1, on the BBM, both by protein purification techniques and expression cloning. SGLT1 transports Na: \(\alpha\)-glucose or \(\beta\)-galactose with a stoichiometry of 2:1. Site-directed mutagenesis and structure-function studies of SGLT1 have mapped the regions of Na\(^+\) and glucose binding to the N- and C-terminal transmembrane domains, respectively (99). Mutations in SGLT1 result in the rare and potentially fatal congenital diarrhea of glucose-galactose malabsorption, which emphasizes the importance of SGLT1 as the major mechanism for active glucose uptake (100). Cloning of SGLT1 led to the identification of other distinct sugar transporters in the enterocyte; GLUT5 on the BBM, which facilitates fructose entry, and GLUT2 on the BLM, which facilitates glucose, galactose, and fructose exit from the cell (Figure 1). SGLT1 activity may influence other routes of glucose entry. Transport through SGLT1 activates PKC\(\beta\)II and recruits GLUT2 to the BBM; this apical GLUT2 then functions as a high-capacity, low-affinity route for sugar entry (101, 102). Activation of SGLT1 was also shown to alter perijunctional actomyosin contraction and increase tight junctional permeability and thereby paracellular glucose transport (50). The relative roles of these pathways to overall glucose transport remain to be elucidated. A role for SGLT1 as a conduit for water is discussed below under water transport.

Regulation of hexose transporters have been extensively studied, especially during development and in response to dietary changes (103). The most critical finding was the observation that elevation in cAMP (104, 105) or cGMP (106) does not alter Na\(^+\)-dependent glucose or amino acid transport. These important observations form the linchpin of the success of ORT. Other features of carbohydrate digestion also contribute to the efficacy of this treatment. First, neither SGLT1 nor GLUT2 is rate limiting; the hydrolysis of polysaccharides is the rate-limiting step. This explains the advantage of rice-based ORTs. Rice provides more molecules of glucose per osmole, but does not add substantially to the osmotic load because as the oligosaccharides are hydrolyzed, the glucose does not accumulate in the lumen, but rather is rapidly transported via SGLT1 and GLUT2. Short-chain glucose polymers reduce cAMP-induced water secretion much better than \(\alpha\)-glucose in vitro (107). Second, carbohydrates stimulate SGLT1 expression and therefore ORT treatment will help increase glucose absorption. Third, SGLT1 and GLUT2 are expressed in late gestation, whereas GLUT5 appears at weaning (103). Thus, young infants, the most adversely affected age group in diarrhea, have the necessary cellular machinery to respond to ORT.

**WATER TRANSPORT** The key element to ORT is rehydration and therefore water movement. The human small intestine can absorb up to 8 liters of water a day in the absence of any overt hydrostatic or osmotic gradients. The molecular mechanisms and physical forces underlying water transport across epithelia are hotly debated and have spurred an international symposium (108). Study in gastrointestinal epithelia is confounded by heterogeneous cell types, complex geometry of
lateral spaces, and increasing tissue resistance from the leaky small intestine to the
tighter colonic epithelium (6, 109). The relative contributions of the paracellular
and the transcellular routes to net water movement have not been resolved (109).
Paracellular transport of solutes and water across the tight junction is thought
to be governed by passive driving forces, although the nature of these forces is
subject to debate. A complicating feature is the dynamic nature of the tight junction
and the lateral spaces discussed earlier. Transcellular water transport could occur
either via water diffusing across the lipid bilayer or via membrane water transport
molecules. A favored explanation for transepithelial water transport is the modified
standing gradient hypothesis (based on the earlier compartment model of Curran &
Macintosh and standing gradient model of Diamond & Bossert, whereby the cre-
ation of relatively small osmotic gradients is sufficient to drive water movement
(reviewed in 108, 109).

Another subject of debate is whether transcellular movement of water always
involves transporters (108). Two discoveries of the past decade have revolutionized
our thinking of water transport in the intestine. First was the seminal discovery in
1991 of the ubiquitous aquaporin (AQP) family of integral membrane proteins with
a high water selectivity (110). Permeation through aquaporins occurs in response
to osmotic and hydraulic gradients, and at least 10 aquaporins have been identified
in mammals (110). Considerable information is available regarding AQPs local-
ization in a variety of tissues, regulation, ability to transport CO₂ and glycerol,
association with disease states, and the atomic structure of AQP1. The identifi-
cation of AQPs in the intestine have been slower, and although AQP2, AQP3,
AQP4, AQP7, AQP8, and AQP10 have now been localized to various regions of
the intestine (111–113), their role in diarrheal diseases remains to be elucidated.
AQP10 is abundantly found in the duodenum and jejunum, and AQP4 is present
on the BLM of colonic surface but not crypt epithelia (112, 113). The AQP4−/−
mouse shows a slight increase in fecal stool water content but no impairment in the
ophylline-mediated Cl− secretion, suggesting a role in transcolonic water perme-
ability but not in secretion (113). AQP3 has been localized to the apical membrane
of colonocytes, and VIP/cAMP upregulate AQP3 mRNA expression in HT29 cells
(111). If borne out in in vivo studies, this finding may have an implication for the
role of cAMP in increasing water transport in diarrhea.

The second breakthrough in water transporters was the demonstration that
SGLT1 expressed in Xenopus oocytes can transport water with a rate of 249
molecules of water:2 Na:1 glucose (reviewed in 114). This could account for
the transport of 4.5–5 liters of water a day in the intestine, which therefore may not
need AQPs. Based on sensitive cell-volume and voltage measurements, Loo et al.
contend that this “solute-water cotransport” is specific and distinct from transport
due to osmotic gradients and that both “secondary active” transport and osmotic
gradients contribute to water transport (114). However, this explanation has been
questioned by others who arrive at similar stoichiometries of water influx into
oocytes but attribute it to osmotic flow (115). If SGLT-mediated water transport
is demonstrated in epithelia, it might explain rehydration via ORT in the small
intestine, but not in the colon. Perhaps in the colon, where water needs to be absorbed against larger osmotic gradients, AQP5s may play a more prominent role, as suggested by their prevalence in surface colonocytes. Thus the conduit for water along the cephalocaudal axis of the intestine needs to be identified, but osmotic gradients clearly play a major role in driving water flow.

THE GENERATION OF SCFA AND ROLE OF COMMENSAL/SYMBIOTIC BACTERIAL MICROFLORA

Although the devastation caused by enteropathogens is great, symbiotic and commensals microflora, largely anaerobes, play a critical role in water conservation in the gut and are essential for the efficacy of complex polysaccharides in ORT. Bacterial fermentation of undigested carbohydrates and proteins releases aliphatic SCFA. Four decades ago, SCFA were considered to contribute to carbohydrate-induced diarrhea, but their importance as a fuel source in herbivores and ruminants led to a detailed examination of their role in human and in laboratory models (116, 117). These studies reveal that SCFA, the major luminal anions, could well be the “wonder molecules” of the colon affecting a variety of functions from fluid absorption to growth, differentiation, and defense.

**Source and generation**

Most SCFA are generated in the cecum and proximal colon. Considering that the colon has $10^{11}$–$10^{12}$ bacteria/gm tissue, with ~40 species making up the microbiota, the nature of the fermentation depends on the microflora as well as on the composition of the undigested carbohydrates. The carbohydrates range from plant polysaccharides, such as cellulose, glycans, pectin, psyllium, and lignins, to amylase-resistant starches, and sugars, such as stacchyose. In the human colon, over 90% of pectin but only 20% of cellulose are digested. Undigested dietary fiber contributes to fecal bulk. The colonic bacteria also salvage nutrients from the sloughed off contributions of the intestinal lumen including mucins, cell debris, and secretions and synthesize vitamins (37, 116–118).

In humans on a Western diet, resistant carbohydrates make up 10 to 12% of the daily diet, yielding 0.5–0.6 moles of SCFA, which contribute 10% of the total daily energy requirement (compared with 70% in ruminants and horses) (31, 119). Acetate, propionate, and butyrate, in a ratio of 69:21:10 (70–100 mM) contribute up to 90–95% of total SCFA, with the rest being made of isobutyrate, valerate, isovalerate, and caproate (116). The colon derives 60 to 70% of its metabolic energy from SCFA, with butyrate providing >50% (120). Propionate is chiefly utilized in the liver and acetate in the muscle and peripheral tissues.

How are SCFA formed? Elaborate functional studies culminating in the recent cloning of the complete genome (121) of the abundant commensal anaerobe *Bacteroides thetaiotaomicron* illustrate how SCFA may be generated via host-commensal microbial interaction, which is briefly described here (reviewed in 37).

*5Symbiosis implies benefit to both host and microbe, and commensals implies benefit to one without detriment to the other, but the distinction can be blurry.*
The bacterium has distinct metabolic pathways for different nutrients and conserves energy by expressing the requisite genes only when it senses the presence of the nutrient. For example, to digest complex polysaccharides, the bacterium expresses a set of eight genes, termed the starch utilization system (sus) (susA–G and susR). SusR is a transcriptional regulator, which senses di- or oligosaccharides and turns on the susA–G genes. The gene products, SusC–SusF, reside in the outer bacterial membrane and bind to and harness complex polysaccharides. SusG, an outer membrane α(1,4, or 1,6)−amylase, starts the process of hydrolysis to oligosaccharides, which are then rapidly transported through the outer membrane porins into the periplasmic space. Here, the oligosaccharides encounter SusA, another α−amylase. Hydrolysis is completed by the SusB protein, an α-glucosidase in the cytoplasm. The sugars are utilized by the bacterium for its own energy needs and through anaerobic fermentation release pyruvate and ATP. The pyruvate can be further fermented to SCFA with the release of an additional ATP. The Bacteroides similarly has evolved different factories to effectively utilize host-derived glycans and mucins, the benefits of which are reaped by itself and the host (37, 122).

Mechanisms of transport  Mammals have evolved mechanisms to absorb SCFA rapidly; this process is linked to Na⁺ absorption and therefore fluid conservation in the colon. Butyrate is the favored substrate, and SCFA can traverse the epithelial layer either paracellularly or transcellularly via diffusion or carrier-mediated routes. SCFA are weak electrolytes and can exist in the protonated (SCFA-H⁺) or anionic forms (SCFA⁻). The relative contributions of the transcellular routes are hotly debated because small changes in the transmembrane pH can shift the protonated:anion equilibrium (117, 123, 124). There is, however, clearly a lumen-to-serosa pH gradient and SCFA gradient that helps in net SCFA absorption. Elegant studies have tried to dissect the contributors to and the role of the pH microclimate around the apical membrane (AM) (123, 125). Protonated SCFA are lipophilic and can diffuse into the colonocyte by a process that is not saturable, not HCO₃⁻ dependent, is pH dependent, and increases with SCFA chain length (117, 123, 125).

To understand carrier-mediated SCFA transport, a brief summary of anion absorptive processes in the colon is appropriate (32, 75, 117, 123, 124) (Figure 2). Despite species and segmental variability, at least four carrier-mediated transporters for anions have been identified functionally on the AM of surface colonocytes and at least two of these are related to SCFA transport. The apical transporters are Cl⁻/HCO₃⁻, Cl⁻/OH⁻, Cl⁻/SCFA⁻, and HCO₃⁻/SCFA⁻ exchangers, distinguishable on the basis of their kinetics, ion specificities, and sensitivity to inhibitors such as stilbenes (DIDS, SITS), niflumic acid, or α-cyano-4-hydroxycinnamate. There is evidence for Cl⁻/SCFA but not HCO₃⁻/SCFA transporters in the crypt (124). Thus SCFA is transported into the cell, perhaps in exchange for HCO₃⁻, resulting in intracellular acidification. Once inside the cell, a major portion of SCFA is metabolized. However, some could be recycled to the AM and some is transported across the BLM into the portal circulation. There is evidence for HCO₃⁻/SCFA⁻ transport...
across the BLM (124, 126), which may account for the transepithelial absorption of SCFA as well as SCFA-dependent HCO$_3^-$ secretion and luminal alkalinization. The BLM also has a Cl$^-$/HCO$_3^-$ exchanger and a Na$^+$-HCO$_3^-$ symporter (32, 124).

There are promising leads as to the molecular identity of these transporters. First, the Cl$^-$/HCO$_3^-$ anion exchanger AE-1 has been localized to the AM of surface colonocytes, and AE-2 has been localized to the BLM of surface and crypt cells. Second, the DRA protein, defective in congenital chloridorrhea, is highly expressed in the colon and can function as a Cl$^-$/HCO$_3^-$, Cl$^-$/Cl$^-$, and/or SO$_4^-$/Cl$^-$ exchanger (63). Its role in fluid absorption is underscored by the fact that it is upregulated in NHE-3$^{-/-}$ mice (76). Finally, the H$^+$-monocarboxylate transporter MCT1 in the colon, which transports butyrate and lactate and is reduced in carcinoma states, has recently been identified (127). Butyrate transport is Na$^+$-independent, DIDS insensitive, and blocked by antisense mRNA inhibition of MCT1 (128). Thus MCT1 could be a major SCFA transporter in colonocytes.

Also of note is the profound effect SCFA absorption has on other mechanisms, which results in net fluid absorption. First, acidification of the cell by SCFA entry activates apical NHE, thereby increasing Na$^+$ absorption. SCFA can also promote Cl$^-$ absorption either directly by recycling to the apical membrane via Cl$^-$/SCFA$^-$ exchangers or indirectly by being metabolized to HCO$_3^-$, which then exits the cell. The net result is an increase in NaCl and therefore fluid absorption (117, 129). Second, the effects of SCFA on Na$^+$ absorption are not blocked by cAMP and therefore, much like Na$^+$-dependent glucose transport in the ileum, SCFA transport is beneficial when cAMP levels are pathologically elevated, as in cholera. Third, SCFA inhibits basal and cAMP-, but not Ca$^{2+}$-, stimulated secretion (130, 131). Different steps in the cAMP to Cl$^-$ secretion signaling cascade from an inhibition of adenylate cyclase (131) to that of NKCC-1 (132) or of apical membrane Cl$^-$ transport (123) have been suggested to be the sites of SCFA action. Fourth, SCFA play a role in volume regulation and activate volume-sensitive Cl$^-$ channels on the BLM (123, 133). Fifth, SCFA acts via PYY to alter colonic motility and increase transit time (134). Taken together all these processes promote fluid absorption.

Equally relevant to ORT are the long-term effects of SCFA. A well-designed study (135) demonstrated that long-term exposure to SCFA in vitro (in Caco2/bbe cell lines) or in vivo (colon of pectin-fed rats) results in the specific activation of NHE-3 protein and activity, but not of NHE-2 or other marker proteins. Pectin feeding did not alter ileal NHE-3, suggesting SCFA specifically promote colonic Na$^+$ absorption. The small intestine, however, exhibits SCFA/HCO$_3^-$ transport (136), and SCFA stimulates ileal proglucagon mRNA as well as Glut-2 expression in a total parenteral nutrition rat model (137). Long-term treatment with SCFA increases expression of its own transporter, MCT-1 (138), which in turn promotes further Na$^+$ absorption.

**Other benefits of commensal bacteria and SCFA** Commensal bacteria and SCFA have potent effects on numerous other colonic functions, and although probably not all the microbial effects are due to SCFA, the actions attributable to SCFA
are more easily delineated. There is a strong correlation between lower levels of butyrate and disease states of the colon, including cancer, and therefore with growth and differentiation (116, 139). Both SCFA and commensal bacteria alter blood flow, stimulate the enteric nervous system, and alter gene expression in the colonocyte (37). SCFA combat oxidant-mediated injury by stimulating the production of heat shock proteins (140). Equally intriguing is the increasing evidence that microorganisms can influence and change protein expression in the host cell [e.g., SGLT1 (122)] and that the host can direct bacterial growth to the distal intestine by restricting expression of the host glycoproteins needed for colonization to that region (37). Such a symbiotic relationship has an evolutionary advantage: The anaerobe is able to survive in a hostile and competitive milieu, and the host is able to salvage nutrients from ingested material that it could not digest on its own. These relationships are being exploited for therapeutic use (see section below on Prebiotics and Probiotics).

Further Considerations

Integral to the success of ORT are active glucose transport in the small intestine, commensal bacteria, and SCFA transport in the colon, and the fact that cAMP and cGMP do not affect glucose or SCFA transport. Because a major target for ORT is children under the age of 5, understanding developmental physiology is crucial. Extensive information is available on the developmental regulation of nutrient digestion and absorption (96, 141), but less is known about ion transport processes (35, 36). The neonate appears more susceptible to diarrhea, as seen in the rotaviral studies (93), but are there any inherent defense mechanisms? The newborn rabbit colon is refractory to cGMP (142) and Ca\(^{2+}\)-dependent secretagogues in terms of Cl\(^-\) transport (143), suggesting that there are inherent pathways to prevent excess fluid loss in the neonate. The gut flora change from facultative anaerobes in the weanling to obligate anaerobes in the adult; how this influences host cell responses awaits further examination.

RECENT DEVELOPMENTS AND FUTURE DIRECTIONS

The effectiveness of ORT rests in the ability to distribute it to wide segments of the affected population, even when health care is not readily accessible. Any simple additives to this formulation that would help control diarrhea would clearly be of further benefit.

Naturally Occuring Compounds as Clues for Therapy

**RICE FACTOR** Mathews et al. (144) demonstrated that a specific low M\(_r\) (<1.5 kDa) factor in rice, termed RF, has potent antisecretory effects. RF is neither a peptide nor a glycoprotein and is hydrophobic with a net negative charge. RF inhibits intestinal cAMP-mediated Cl\(^-\) secretion by specifically decreasing the open probability
of CFTR and has no effect on volume-sensitive Cl\(^-\) channels. Thus rice-based ORT could present a triple advantage: increased glucose availability, increased production of SCFA, and a factor that inhibits Cl\(^-\) secretion.

**HERBAL AND OTHER NATURAL MEDICATIONS** In traditional medicine such as Ayurvedic (7) and Yunani of the Indian subcontinent and the Kampo formulations of China and Japan, the use of herbal extracts to treat diarrhea is recommended in addition to oral fluid replacement. An understanding of the biochemical basis of such formulations may help develop potent antidiarrheal additives to ORT. In Eastern Europe and in France, smectite, an unabsorbable clay, has been shown to reduce the duration of diarrhea (reviewed in 5). The efficacy of poppy seeds as an antidiarrheal agent was recognized by the ancient Egyptians long before their analgesic properties were appreciated. Opium, camphor, and asafoetida were recommended in the traditional treatment of cholera in India (10) (see next section for description of their use in modern therapy). Extracts of different parts of tropical plants ranging from Tamarindus indica to Curcuma longa have been used as antidiarrheal agents in traditional Thai medicine (8). The latex of the ornamental tree Croton lechleri has been used in the indigenous communities of South America to treat watery diarrheas. This latex contains proanthocyanidin oligomers, of which one, SP-303, was shown to be effective in inhibiting cholera toxin and cAMP-mediated Cl\(^-\) secretion in a mouse model and in cell lines (145).

Recently, Oi et al. (146) identified the active ingredients of Daio-kanzo-to, a Kampo medication, as coming from Rhei rhizoma (Daio). The most effective compound was identified as rhubarb galloyltannin and was demonstrated to inhibit three important actions of cholera toxin: ADP ribosylation, elongation of Chinese hamster ovary cells, and fluid accumulation in a rabbit ileal loop assay. Equally important, chemically synthesized analogs of the galloyltannin had similar effects. The natural and chemically synthesized analogs of the galloyltannin attenuated cholera toxin–induced fluid secretion when given before, with, or after the toxin. Given their effect on ADP ribosylation, the galloyltannins may be of benefit only in the treatment of LT- and CT-associated diarrheas.

**Prebiotics and Probiotics**

The past decade has seen an explosion in the exploitation of beneficial bacteria, in the form of prebiotics and probiotics, to treat diseases ranging from inflammatory bowel disease (147), antibiotic-associated and acute diarrheas (148), to colon cancer (149). The origins of this therapy also lie in traditional medicine, but the scientific bases are being elucidated (149). Prebiotics are defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health” (150). Prebiotics such as lactulose and fructo-oligosaccharides, which cannot be digested or absorbed in the small intestine, make their way to
the colon where they promote the growth of commensal species, as well as suppress the growth of pathogens. The therapeutic use of prebiotics in acute infectious diarrheas has not been extensively investigated. Probiotics are defined as “live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance” (150). Probiotics have been used to study acute diarrheas. The most popular of the probiotic bacteria are Lactobacillus species, Bifidobacterium bifidum, Streptococcus thermophilus, and the yeast Saccharomyces boulardii.

The efficacy of probiotics in treating acute diarrheas is clearly dependent on the species used and on the etiology of the diarrhea. For example, Lactobacillus GG (L-GG) had a positive effect on adults with traveler’s diarrhea, whereas in a smaller study, Lactinex (L. acidophilus + L. bulgaricus) had no beneficial effect (148). With respect to ORT, one of the most detailed studies performed on children with acute diarrhea was a multicenter, randomized, placebo-controlled, double-blinded study sponsored by ESPGHAN (151). Lactobacillus GG given ad libitum significantly decreased duration of acute diarrhea. Interestingly, the effect was most profound on children with rotavirus and not with bacterial infections, and L-GG may have prophylactic effects (151, 152). Antibiotic-associated diarrhea is often (∼33%) caused by C. difficile, and both S. boulardii and L-GG have independently proven to be effective in reversing this diarrhea (152). Much attention is being paid to the potency and reliability of commercial formulations. VSL#3TM (VSL Pharmaceuticals, Inc.), for example, contains four strains of Lactobacilli, three of Bifidobacteria, and one of S. thermophilus. In double-blind, placebo-controlled trials, the efficacy of VSL#3 as a prophylactic and therapeutic in pouchitis was demonstrated (153). The VSL#3 formulation restored mucosal epithelial barrier function in IL-10−/− mice, and a factor from these bacteria provided resistance to Salmonella infection (154).

With the development of probiotics of known composition, their mechanism of action is being elucidated. Probiotics could produce antibacterial agents; they could compete with the endogenous microflora for adhesion, for receptors on the epithelial cells, and for essential nutrients; they could stimulate and/or suppress the gut-associated lymphoid tissue; and they could alter the types of mucin secreted by goblet cells to interfere with host-pathogen interactions. Because microbial ecology varies with geographical location, of relevance to ORT is the question whether a globally applicable, inexpensive probiotic can be developed.

**Pharmacological Approaches**

Attempts to identify high-potency antagonists of intestinal fluid secretion have met with varying success.

**ANTIBIOTICS AND VACCINES** Although prescribed for bacterial pathogens, antibiotics are ineffective against viruses. Attempts to develop vaccines have met with
some success, but the challenges are compounded by the ingenuity of the microorganisms and the complexities of horizontal gene transfers (80).

**SIGNAL TRANSDUCTION BLOCKERS** An alternate strategy is to develop drugs targeted to various steps of the fluid transport process, ranging from a direct inhibition of Cl\(^-\) channels to blocking proximal steps of the signal transduction cascade, including neural pathways. Several promising agents were effective in animal model and in vitro studies, but performed poorly in clinical trials. For example, recombinant growth hormone was a potent proabsorptive agent in vivo in the rat but failed to have the same effect in humans (155). Although serotonin receptor antagonists show some promise because serotonin release appears to be pathogen- and toxin-specific, these drugs are not applicable to all forms of diarrhea. Blockers of VIP-mediated secretion such as igsines have greater possibilities of success because they can block CT- and LT-mediated secretion (156).

**AGENTS THAT PROMOTE ABSORPTION** Much attention has focused on developing agents that promote absorption. Somatostatin, enkephalins, and \(\alpha\)-adrenergic agents promote absorption largely by acting via the G\(_{\alpha i}\) cascade to decrease cAMP production (30). Alpha2-adrenergic agonists such as clonidine have limited clinical value because of their adverse side effects. Greater success has been obtained with octreotide, the long-acting somatostatin analog. In the treatment of secretion related to VIPomas, octreotide appears to act by inhibiting release of VIP and fluid secretion (156). Recently, octreotide was demonstrated to increase net epithelial absorption rate in the jejunum and ileum of human volunteers, largely by inhibiting secretion (157). Opiate agonists such as loperamide are widely used in the treatment of mild diarrheas, largely for their effects on decreasing motility and increasing transit time. However, in severe diarrheas, loperamide can cause enteropooling. Therefore, targeting another arm of this cascade has proven to be more effective in the treatment of severe diarrhea. Met- and leu-enkephalin-containing neurons can be localized in the lamina propria in the vicinity of the enterocytes and act via \(\delta\)-receptors to promote Cl\(^-\) and fluid absorption. However, enkephalins are rapidly degraded by endogenous membrane-associated metalloproteinases. A potent enkephalinase inhibitor, racecadotril, has proven to be effective in the treatment of acute diarrheas in adults and children without adversely affecting motility (156). Equally important, it has been demonstrated to be a good adjunct to ORT in a clinical trial in Peru (158).

**Cl\(^-\) CHANNEL INHIBITORS** A direct inhibitor of CFTR would be ideal as the bulk of intestinal Cl\(^-\) transport is driven by this channel. Pharmacological inhibitors of Cl\(^-\) channels (45) such as 5-nitro-2(3-phenylpropyl-amino)benzoate (NPPB) and glibenclamide are not useful because they can be nonspecific. The most exciting advances in this regard are the recent reports from Verkman et al. on the development of compounds to activate CFTR as a therapeutic approach to CF (159),
and on compounds that will inhibit CFTR, to combat diarrheal diseases. These authors have developed a highly sensitive method to measure CFTR activity in intact epithelial cells, using a halide-sensitive modified yellow fluorescent protein (YFP-H148Q) (160). Through combinatorial chemistry they developed 50,000 compounds, which were analyzed by high-throughput screening (161). Screening was carried out in the presence of a cocktail of forskolin, isobutylmethyl xanthine, and apigenin to ensure that screening was restricted to compounds affecting CFTR directly rather than the signaling cascade. This screening yielded six thiazolidinone compounds that inhibited cAMP-activated CFTR function. The most promising was CFTRinh-172, which inhibited CFTR with a \( K_i \) of 300 nM, but had no effect on \( K^+ \) channels, \( Ca^{2+} \)-activated \( Cl^- \) channels, or the multidrug-resistant protein, MDR-1 (161). Intraperitoneal infusion of this nontoxic compound blocked cholera toxin–induced \( Cl^- \) secretion in a mouse model. Although direct blockers of CFTR may not be effective against rotaviral infections, such screening processes for specific drugs are nevertheless a very promising approach.

**SUMMARY**

Analysis of the cellular and molecular basis of time-honored remedies for the effective, accessible, and inexpensive treatment of devastating diseases such as diarrhea can be very useful. The serendipitous phenomena of glucose and SCFA transport being unaffected by secretagogues that elevate cAMP and cGMP is the crux of the success of ORT. The simplicity of ORT also contributes to its success, but can the one-size-fits-all formula continue? Issues of hypo- versus hypernatremia, hypo- versus isoosmolar, and the fact that rotavirus and cholera toxins affect different types of \( Cl^- \) channels must be considered in designing additives to ORT. Clearly, improving the formulations with either inhibitors of secretion or probiotics, as long as they are inexpensive and readily available, would be highly beneficial. Its successes will be heightened with accessible education and improved sanitation. The tremendous global impact of ORT is due to the ingenuity of numerous investigators in unraveling the secrets of the microorganisms and determining the molecular basis of secretion and absorption, as well as performing complex clinical studies under challenging conditions. With the continued multiprong collaboration of pharmacognocists, ecologists, epidemiologists, microbiologists, cell physiologists, and physicians, strategies such as oral rehydration therapies will continue to be a marvel of modern medicine.

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PHYSIOLOGICAL BASIS FOR ORT C-1

See legend on next page
Figure 1  Transport mechanisms in the small intestine. Transepithelial transport is driven by the BLM Na⁺/K⁺-ATPase pump [1]. In the villus, apical NHE2 and NHE3 [2 and 3] and Cl⁻/HCO₃⁻ exchangers [4; DRA, PAT1] drive NaCl entry and HCO₃⁻ exit. Basolateral K⁺ channels [5], Cl⁻ channels [6, CLC-2], and the basolateral NHE1 [7] regulate volume and cell pH. Digestion of nutrients occurs by the action of luminal α-amylases and proteases and surface hydrolases such as isomaltase [8] and peptidase (not shown). Apical entry of glucose occurs mainly via SGLT1 [9] and recent reports also suggest GLUT-2 [10], whereas amino acid entry occurs via Na⁺-coupled cotransporters [11] and H⁺/dipeptide cotransporters [12]. Glucose exits the BLM via GLUT-2 [10] and amino acid via various amino acid transporters [13]. Cl⁻ and glucose transport can occur via paracellular routes (*). Absorption of water can occur either via paracellular routes (*), via SGLT1 [9] or perhaps via aquaporin10 [14]. In the crypt, secretion of fluid is governed by the BLM Na⁺/K⁺-ATPase [1] and maintained by basolateral K⁺ channels [5], the basolateral NKCC-1 [15], and CFTR [16]. Movement of Na⁺ and water occurs via paracellular routes (*). Extracellular modulation of epithelial function results from the actions of systemic factors, immune cells, nerve cells, and the endocrine system; intracellular regulation of transport may be mediated by cAMP (cA), cGMP (cG), and calcium (Ca²⁺).
See legend on next page
Figure 2  Transport mechanisms in the colon. Transepithelial transport is driven by the BLM Na⁺/K⁺-ATPase pump [1]. On the surface cells, NHE2 and NHE3 [2 and 3] and ENaC channels [4] drive Na⁺ entry, whereas apical Cl⁻/OH⁻ exchangers [8] and Cl⁻/HCO₃⁻ exchangers [9; DRA] drive Cl⁻ entry. Basolateral NHE1 [5], K⁺ channels [7], and Na⁺/HCO₃⁻ cotransporters [10] help maintain absorption. Water can move paracellularly (*) and via BLM aquaporins [15]. Commensal bacteria (colored ovals) break down undigested polysaccharides and proteins (chains of solid circles) and generate SCFA such as butyrate, propionate, and acetate. The SCFA are absorbed by surface colonocytes via apical Cl⁻/butyrate exchangers [11], HCO₃⁻/butyrate exchangers [13], and monocarboxylate cotransporter MCT-1 [12] and BLM HCO₃⁻/butyrate exchangers [14]. In the crypt, secretion of fluid is governed by the BLM Na⁺/K⁺-ATPase [1] and maintained by basolateral K⁺ channels [7], the basolateral NKCC-1 [6], and apical CFTR [16]. Na⁺ and water follow Cl⁻ exit via paracellular routes (*). The apical Cl⁻-dependent NHE [17] and Cl⁻/OH⁻ exchangers [8] may play a role in crypt absorption. The crypt cells extrude SCFA via apical Cl⁻/butyrate exchangers [18]. Apical Cl⁻/HCO₃⁻ exchangers [9] and basolateral Na⁺/HCO₃⁻ cotransporters [10] may contribute to HCO₃⁻ extrusion.
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