

Gastroenterologists and gastrointestinal physiologists have become wary of dogma. Once the dogma of acid's primacy as the cause of peptic ulcer disease was destroyed by a spiral-shaped bacillus called *Helicobacter pylori*, the gastrointestinal investigator is now more open and willing to accept new ideas and to seek new evidence to support them. If "acid equals ulcer" was perhaps the most infallible of gastroenterologic dogma, i.e., our credo; then the idea that the intestinal crypts secrete fluid and electrolytes while the small intestinal villi and colonic surface cells absorb fluid and electrolytes, was perhaps one of our Ten Commandments. Thus, the article by Singh et al. in the present issue of *The Journal* (1) is important because it severely damages the dogma of a spatial segregation of absorptive and secretory processes in the intestine. The instrument of destruction, in this case, is the micropipette, long championed by the nephrologist as the best method to study the isolated renal tubule. Singh et al. have cannulated and perfused the isolated mammalian (rat) colonic crypt and have shown that, while the crypt can be made to secrete with agonists that increase intracellular cyclic nucleotide or Ca^{2+} content, the crypt cell constitutively absorbs Na^+ and water when devoid of any neurohumoral or paracrine secretory influences. Although these studies do not rule out the possibility that the crypts may be usually or often in a secretory mode, certainly the strict dogma that they can only secrete must be modified. The convincing experiments published here indicate that crypts can indeed absorb.

So how did this dogma of a spatial segregation of secretory and absorptive function develop in the first place? As is usually the case in science, the concept came from a series of experiments and observations, both direct and indirect, that were convincing at the time, but contained certain elements of ambiguity.

Much of the early evidence for the crypt secretion paradigm was indirect. For example, Field noted that tissues that did not contain crypts or gland-like structures, i.e., the mammalian gall bladder and flounder intestine, did not secrete in response to cyclic AMP-coupled agonists (2). Furthermore, Hendrix's group had shown in the 1960s that damage to crypt cells with cycloheximide inhibited cholera toxin-induced secretion, but had no effect on glucose-stimulated fluid absorption, a function of the villus cell in the small intestine (3). Conversely, these investigators also showed that damage to the villus cells with hypertonic solutions would reduce glucose absorption, but did not alter cholera toxin-induced fluid secretion (4). More direct evidence was reported in the 1970s and early 1980s through studies with micropipettes and microelectrodes. Nasset and Ju were able to collect fluid secreted from the guinea pig crypt by placing a micropipette directly in the ostia of the glands (5). Welch et al. inserted microelectrodes into rabbit colonic surface and crypt cells and showed that amiloride, a diuretic that blocks apical membrane Na^+ channels, decreased the membrane conductance of the colonic surface cells, but had no effect on the crypt cells (6). However, when an agonist such as prostaglandin E_2 that opens Cl^- channels in secretory cells was applied, there

was an increase in apical membrane conductance in the colonic crypt cells, but not the surface cells. Furthermore, these investigators showed that when the mucosa was overlaid with oil, fluid droplets could be seen to collect over the crypt ostia. It is worth noting that these experiments did not rule out Na^+ absorptive processes in the crypt cells that might not be sensitive to amiloride, nor did these experiments rule out an intrinsic capability for fluid secretion by the surface cells. For example, the lack of a Cl^- secretory response by surface cells could have also been the result of lack of PGE_2 receptors on these differentiated cells in this intestinal segment of this animal species.

Indeed a decade later, additional microelectrode studies by Stewart and Turnberg (7) showed depolarization of rat small intestinal villus cells with Cl^- channel-opening secretagogues, including PGE_2 , while Kockerling and Fromm (8) showed electrical evidence of Cl^- secretory processes in rat ileal villus and colonic surface cells. At this point, a modified dogma began to emerge: whereas both crypt and villus/surface cells secrete Cl^- and fluid, only the villus/surface cell is capable of absorbing Na^+ and water.

In 1990, Naftalin et al. began to publish both theoretical and experimental evidence that the colonic crypt might be capable of absorbing Na^+ and fluid (9–11). In these studies, the signal from a cell impermeant, Na^+ -sensitive fluorescent dye, SBFI, increased dramatically in the interstitial tissue adjacent to the basal surface of the colonic crypt cells to levels far above that in colonic crypt lumen during colonic absorption. Furthermore, the concentration of another impermeant fluorescent dye, fluorescein disulphonate, simultaneously increased in the colonic crypt lumen. These findings can only be logically explained by absorption of Na^+ and fluid from the crypt lumen. Although highly suggestive evidence for crypt Na^+ and fluid absorption, these studies lacked the power of simplicity.

Thus the present studies of Singh et al. (1) with micropipette perfusion represent direct evidence that is incontrovertible: the isolated, cannulated colonic rabbit crypt was shown to absorb fluid from an Na^+ -containing perfusion solution and to secrete fluid when Na^+ was removed from the solution. Furthermore, secretion could be induced by dibutyryl-cyclic AMP and by vasoactive intestinal polypeptide, an agonist that induces intracellular cyclic AMP formation. Secretion was also induced by acetylcholine, an agonist that increases intracellular Ca^{2+} content. Most will now agree: the dogma is dead; the crypt can absorb water; and the process is Na^+ dependent. Furthermore, there is some evidence that Na^+ absorption may be due to a novel Cl^- -dependent $\text{Na}^+ - \text{H}^+$ exchange process (12) perhaps in conjunction with conventional $\text{Na}^+ - \text{H}^+$ exchange isoforms.

What are the ramifications of crypt absorption? These findings strengthen the idea that the colonic crypts are the final and most important arbitrator of stool fluidity. Colon secretion from the crypts has long been considered a cause of liquid stools, and the secretagogue action of drugs, bacterial toxins, inflammatory mediators, neurotransmitters, and hormones, perhaps augmented by prostaglandin secretion from pericryptal myofibroblasts, are likely causes of more liquid feces in health and disease. Naftalin (9–11) suggests that colonic crypt Na^+ and fluid absorption creates osmotic-induced "suction" apparatus of the millions of colonic crypts that allows them to virtually suck

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/95/11/2102/02 \$2.00

Volume 96, November 1995, 2102–2103

the fluid from solid colonic waste, thus creating hard feces. The perfused colonic crypt techniques will allow further study of this hypothesis and will clarify the mechanism(s) of Na^+ absorption.

The micropipette technique described here represents a method of studying the transport processes in an isolated colonic crypt with maintained polarity. Just as these techniques have unlocked the secrets of the renal tubule, they can help unravel the mysteries of the intestinal crypts. For the gastroenterologist and gastrointestinal physiologist, these are exciting times. New techniques promote new paradigms and destroy old dogma.

Don W. Powell
Department of Internal Medicine
University of Texas Medical Branch at Galveston

References

1. Singh, S. K., H. J. Binder, W. F. Boron, and J. P. Geibel. 1995. Fluid absorption in isolated perfused colonic crypts. *J. Clin. Invest.* 96:2373–2379.
2. Field, M. 1980. Regulation of small intestinal ion transport by cyclic nucleotides and calcium. In *Secretory Diarrhea*. M. Field, J. S. Fordtran, and S. G. Schultz, editors. American Physiological Society, Bethesda, MD. 21–30.
3. Serebro, H. A., F. L. Iber, J. H. Yardley, and T. R. Hendrix. 1969. Inhibition of cholera toxin action in the rabbit by cycloheximide. *Gastroenterology*. 56:506–511.
4. Roggin, G. M., J. G. Banwell, J. H. Yardley, and T. H. Hendrix. 1972. Unimpaired response of rabbit jejunum to cholera toxin after selective damage to villous epithelium. *Gastroenterology*. 63:981–989.
5. Nasset, E. S., and J. S. Ju. 1973. Micropipet collection of succus entericus at crypt ostia of guinea pig jejunum. *Digestion*. 9:205–211.
6. Welch, M. J., P. L. Smith, M. Fromm, and R. A. Frizzell. 1982. Crypts are the site of intestinal fluid and electrolyte secretion. *Science (Wash. DC)*. 218:1219–1221.
7. Stewart, C. P., and L. A. Turnberg. 1989. A microelectrode study of responses to secretagogues by epithelial cells on villus and crypt of rat small intestine. *Am. J. Physiol.* 257:G334–G343.
8. Kockerling, A., and M. Fromm. 1993. Origin of cAMP-dependent Cl^- secretion from both crypts and surface epithelia of rat intestine. *Am. J. Physiol.* 364:C1294–C1301.
9. Naftalin, R. J., and K. C. Pedley. 1990. Video enhanced imaging of the fluorescent Na^+ probe SBFI indicates that colonic crypts absorb fluid by generating a hypertonic interstitial fluid. *FEBS Lett.* 260:187–194.
10. Bleakman, D., and R. J. Naftalin. 1990. Hypertonic fluid absorption from rabbit descending colon in vitro. *Am. J. Physiol.* 258:G377–G390.
11. Pedley, K. C., and R. J. Naftalin. 1993. Evident from fluorescence microscopy and comparative studies that rat, ovine and bovine colonic crypts are absorptive. *J. Physiol. (Lond.)*. 460:525–547.
12. Rajendran, V. M., J. P. Geibel, and H. J. Binder. 1995. Chloride dependent Na-H exchange: a novel mechanism of Na^+ transport in colonic crypts. *J. Biol. Chem.* 270:11051–11054.