A purified *Shigella* enterotoxin (pST) and a cell-free lysate with pST removed (CFL-pST) from the whole-cell lysate of *Shigella dysenteriae* 60 R were used to study their effect on the myoelectric activity and mucosal integrity of rabbit ileal segments. We have previously defined two myoelectric patterns: the migrating action potential complex and repetitive bursts of action potentials that occur in response to certain bacteria and their enterotoxins. The in vivo model consisted of isolated ileal segments in male New Zealand White rabbits. The segments were infused with sterile saline (1 ml/h), pST (2.4-μg injection), or CFL-pST (1 ml/h). Myoelectric activity in the segments exposed to pST was similar to that with the saline infusion, but CFL-pST induced significant alterations in myoelectric activity in the form of repetitive bursts of action potentials. The mucosa of the segments exposed to pST showed only mild inflammatory changes. In contrast, CFL-pST caused moderate to severe inflammatory changes with enterocyte necrosis. These studies show that pST, a known enterotoxin, did not alter myoelectric activity and had no significant effect on the integrity of ileal mucosa, as determined by light microscopy. CFL-pST caused both inflammation and tissue necrosis with significant alterations in motor activity. These studies suggest that *S. dysenteriae* 60 R produces a substance or substances other than pST that cause florid in vivo cytotoxicity and alter myoelectric activity.

The pathogenesis of shigellosis, a common cause of bacterial diarrhea throughout the world, depends on its ability to invade intestinal mucosa and cause enterocyte injury. The crude extract from *Shigella dysenteriae* 1, or Shiga toxin, has been shown to cause fluid accumulation in the rabbit ileum (11), to be cytotoxic in some in vitro cell preparations (24), and to alter protein synthesis in vitro (23). In addition, our laboratory has recently demonstrated that live *S. dysenteriae* 1 (strain 3818-T, a tissue invader and toxin producer, and strain 3818-O, one that does not invade but produces toxin) and *Shigella* whole-cell lysate containing Shiga toxin cause specific alterations in myoelectric activity in rabbit ileal segments (17).

It has been assumed that a single product of *S. dysenteriae* 1, Shiga toxin, is responsible for all of its biological properties. O'Brien et al. (20) have purified *Shigella* enterotoxin from the whole-cell lysate by means of anti-Shiga toxin affinity chromatography. This purified toxin (pST) has been shown to be enterotoxigenic in rabbit ileal segments, lethal to mice, and cytotoxic to HeLa cell preparations (20).

Is pST from the extract of *S. dysenteriae* 1 also responsible for alterations in myoelectric activity? Does *S. dysenteriae* produce other biologically active substances? We have previously defined two alterations in intestinal motility associated with various diarrheal states: the migrating action potential complex (MAPC) and the repetitive bursts of action potentials (RBAP). MAPC is characterized by action potential discharge activity of >2.5 s in duration, which migrates aborally over at least two consecutive electrode sites (16). This complex is associated with movement of luminal contents in the small intestine. RBAP is characterized by repetitive bursts of action potential of >1.5 s in duration, which occur on at least three successive slow waves on the same electrode site (1). RBAP activity may or may not propagate, and luminal fluid movement is not a prominent feature. The purpose of this investigation was to study by light microscopy the alterations in myoelectric activity and histology in ileal segments of New Zealand White rabbits exposed to pST and to the cell-free lysate after removal of the enterotoxin from the extract of *S. dysenteriae* 60 R (CFL-pST).


**MATERIALS AND METHODS**

The in vivo model used in all studies consisted of a ligated 15-cm segment of terminal ileum in male New Zealand White rabbits. Four monopolar Ag-AgCl electrodes were sewn to the serosa at 2.5-cm intervals. This preparation has been well described in previous publications (1, 15, 16).

Three groups of rabbits were studied and infused with: (i) sterile saline, 1 ml/h (n = 5); (ii) pST, 2.4-μg bolus in 1 ml of sterile saline (n = 5); or (iii) CFL-pST, 1 ml/h (n = 5). pST and CFL-pST were purified from whole-cell lysates of *S. dysenteriae* 60 R by means of anti-Shiga toxin affinity chromatography (20).

Activity of the ileum of each rabbit was continuously recorded for 6 h with a rectilinear physiological recorder (Beckman RM Dynograph, Beckman Instruments, Inc., Fullerton, Calif.). At the end of each experiment the animal was killed, and full-thickness segments of the ligated ileum were placed in 10% buffered Formalin fixative. The tissue was mounted on paraffin and stained with hematoxylin and eosin. The segments were examined in a blinded manner, and the histological changes were graded from 0 to 4+, with 0 (normal) being no change and 4+ equaling severe enterocyte necrosis.

Myoelectric tracings for each experiment were analyzed for the frequency of slow waves, the onset time of alterations in myoelectric activity, and the presence of MAPCs or...
RESULTS

The myoelectric recordings obtained from the ligated ileal segments in control rabbits were similar to those previously reported (1, 15–18). The myoelectric recordings consisted of brief (<1-s) and random action potential discharges associated with slow-wave activity. No deviation from this pattern was observed in any of the control recordings.

The mean slow-wave frequency among the three experimental groups did not differ significantly: the frequencies for ileal segments infused with normal saline, CFL-pST, and pST were 16.9 ± 0.1, 17.2 ± 1.16, and 17.9 ± 1.79, respectively.

Significant alterations in myoelectric activity occurred only in the ileal segments perfused with CFL-pST. The predominant pattern was RBAP. RBAP activity either was limited to a single electrode site or migrated to subsequent electrode sites (Fig. 1).

The ileal segments exposed to saline or pST had an RBAP frequency of 0.46 ± 0.3 or 0.0 per h, respectively. The RBAP frequency for CFL-pST-exposed segments was 4.4 ± 0.3 per h (P < 0.01 compared with the groups exposed to saline and to pST; Fig. 2).

The onset time for the appearance of alterations in RBAP activity was 3.3 ± 0.65 h in the CFL-pST group. This lag period for altered motor activity was similar to the onset time of other toxins (1, 10, 16–18).

No significant MAPC activity was observed in the ileal segments exposed to pST, CFL-pST, or saline.

The histological sections obtained from the ligated ileal segments were examined by light microscopy. Routine light microscopy revealed no pathological changes in the segments infused with sterile saline. Ileal segments exposed to pST showed only minimal inflammation and no enteroctye necrosis (Fig. 3A and B). All biopsy specimens obtained from segments infused with CFL-pST had considerable inflammatory changes (infiltration of the submucosa with polymorphonuclear cells) and evidence of enteroctye necrosis with villus distortion (Fig. 4A and B).

DISCUSSION

We have previously shown that two specific alterations in intestinal motility occur in response to certain bacteria or their enterotoxins (1, 16). The MAPC occurs in response to *Vibrio cholerae*, the whole-cell lysate, or the purified enterotoxin (choleragen) (15, 16). MAPC activity and the secretion of water and electrolytes occur as the result of activating the adenylate cyclase system (4, 5, 12). However, choleraigenoid, a second purified protein, which may also attach to GM1,
receptors on the enterocyte but fail to induce secretion or activate adenylate cyclase (6, 7, 14), also induces MAPC activity (21). Thus, the MAPC may be induced in the absence of active secretion.

Prior studies with Clostridium difficile have suggested that other substances may stimulate RBAP activity (10) through mechanisms independent of the cyclic nucleotides, just as choleragenoid stimulates MAPC activity. Products of the whole-cell lysate of C. difficile, which included a high-molecular-weight fraction (>50,000), a low-molecular-weight fraction (<50,000), the purified enterotoxin, and the purified cytotoxin were investigated in the rabbit model (10). The purified cytotoxin, which causes in vitro cytotoxicity but only mild secretion with no inflammatory changes in vivo, caused no changes in motor activity. The purified enterotoxin, which causes no changes in vitro but induces florid cell damage in vivo, also caused no changes in motility. RBAPs occurred only with the whole-cell lysate or with the high-molecular-weight fraction from C. difficile. These studies suggested that the high-molecular-weight fraction contains an additional substance that alters motility in the absence of cell damage or active secretion.

In the present investigation of pST (which causes in vitro cytotoxicity for some mammalian cell preparations [24]) and CFL-pST, only CFL-pST induced RBAPs. pST induced only minimal inflammatory changes in vivo as determined by light microscopy and no alterations in motor function. Alterations in the villus tips by pST have recently been shown by electron microscopy (A. D. O'Brien, unpublished data).

These data, however, also suggest that some other substance may exist in CFL-pST that causes florid in vivo cell damage and alters motor activity.

Previous studies (10, 21) have shown by light microscopy that bacteria can produce substances that specifically alter the motor function of the small intestine without producing fluid secretion or enterocyte injury. A similar condition may exist for the products of S. dysenteriae 60 R. RBAP, a nonpropulsive complex, may be an adaptive feature of bacteria that results in stasis, enhances multiplication of the organism, and results in invasion of the bacteria into the enterocyte to cause disease. In contrast, MAPC may represent a defense mechanism of the host to clear unwanted luminal contents.

At present, we have no evidence that defines the substance or substances secreted by the bacteria that alter motor function. Neurosensory stimulation in the lumen by 5-hydroxytryptamine (serotonin) (2, 3) results in active secretion. Recently, McGowan et al. (19) identified serotonin in the lysate of Entamoeba histolytica, a parasite that causes enterocyte injury and active secretion. They suggest that the serotonin secreted by the organism contributes to the host’s secretion caused by this organism. The mucosa and submucosa are richly innervated by both 5-hydroxytryptamine and adrenergic receptors (9). Unlike the situation with serotonergic nerves, stimulation of the adrenergic receptors results in absorption (8). We hypothesize that altered motor function from substances in the lumen of the intestine may be modulated through these sensory neurons that synapse at
FIG. 4. Histological sections from an ileal segment exposed to CFL-pST from *S. dysenteriae* 60 R. (A) There was intense inflammation of the mucosa and submucosa (×14); (B) the villus tips were sheared off by the inflammatory response (×28).

the myenteric plexus. Altered motor activity has been shown to occur through an intrinsic neural reflex arc by the use of neural antagonists (15) and occurs independent of the central nervous system in an in vitro bath system (18). These substances remain to be defined.

In summary, these studies show that pST from *S. dysenteriae* 60 R does not alter myoelectric activity and does not induce any significant histological changes in rabbit ileal segments as determined by light microscopy. In contrast, CFL-pST alters myoelectric activity in the form of RBAP activity and significantly alters the histology of the intestinal mucosa. The histological alterations consist of inflammation and enterocyte necrosis. Therefore, we conclude that *S. dysenteriae* produces biologically active substances that may cause in vivo cytotoxicity and alter motor function.

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LITERATURE CITED