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THE DIABETIC GUT: A RISK FACTOR IN POSTOPERATIVE INFECTION

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Diabetes is acknowledged as a significant risk factor in the morbidity of post-surgical infections. Furthermore, diabetics exhibit diarrhea, which has been found to respond to antibiotic treatment. This has prompted us to explore the effect of this disease on the mucosal associated bacterial population and myoelectric activity (MA) of the small intestine. Rats were made diabetic by IV injection of streptozotocine (SZ), 45 mg/kg. Motility changes in the diabetic group became apparent by the 15th day after induction of diabetes. To characterize the mucosal aerobic and anaerobic microbial population of the diabetic small bowel multiple biopsies (proximal, middle, distal) were obtained from Sprague-Dawley SZ-induced diabetic rats, as well as from "Bio-breeding" diabetic rats. Paired biopsies were also obtained from age matched controls. Total mucosal microbial recovery was significantly greater ($p<0.05$) in all samples in the diabetic rats compared to controls. The *Bacteroides fragilis* group and the anaerobic streptococci were the predominant anaerobic mucosal isolates in the proximal bowel of diabetic animals. These observations indicate that diabetes induces time dependent changes in small intestinal MA and/or substrate availability promoting overgrowth of mucosal associated bacteria, which shifts from predominantly aerobic to anaerobic. This alteration in the mucosal flora suggests a re-evaluation in the approach to small bowel prophylaxis for upper GI surgery in the diabetic.

KEY WORDS: Diabetes, small bowel, bacteriology, anaerobes, myoelectric activity, motility, infection.

INTRODUCTION

Previous investigators have shown that few organisms colonize the proximal small bowel. The predominant organisms recovered from this region include the facultative gram negative bacteria (i.e. *Escherichia coli*) and the aerobic streptococci. Few anaerobes are recovered from this segment of the bowel and those that occur are primarily the anaerobic streptococci.[1,2] However, certain disease processes can alter the microbial ecology of the proximal gastrointestinal tract. For instance, in primary or secondary (following gastric resection) achlorhydria the jejunum becomes colonized with bacteria.[3] Also, in stagnant loop syndrome an increase in the anaerobic microbial populations in observed throughout the small bowel.[4]

Patients with longstanding diabetes mellitus exhibit a variety of gastrointestinal symptoms. Up to 75% of patients with diabetes mellitus may experience vomiting, constipation or diarrhea and early satiety.[5,6] It is generally felt that the gastrointestinal symptoms experienced by the diabetic patient are in part precipitated by alterations in intestinal motility which is a sequelae of autonomic

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neuropathy involving the gastrointestinal tract.[7] Scarpello and colleagues have suggested that bacterial overgrowth in the diabetic small bowel followed by excessive bile acid deconjugation plays a major role in the etiology of the protracted diarrhea.[8] This hypothesis is supported by the empirical observation that some patients experience relief of symptoms following antimicrobial therapy.[9] Regardless of the mechanism, the diabetic patient who presents for upper GI surgery may be viewed as being at high risk for post-surgical infection. The purpose of the present investigation is to document the effect of diabetes on microbial flora as well as on the myoelectric activity of the small intestine.

METHODS

Bacteriologic Studies

Twenty age-matched male Sprague-Dawley rats were used. In 10 of these rats diabetes was induced by a single IV injection of 45 mg/kg of streptozotocin. Streptozotocin at this dose induces mild diabetes (blood glucose 312 ± 60) not requiring the use of insulin. Diabetic rats underwent blood tests for glucose three times per week. Five diabetic Sprague-Dawley rats and their respective controls were euthanized one month after induction of diabetes and the remaining 5 with their controls at 3 months.

In addition, ten Bio-Breeding (BB) autoimmune-mediated diabetic rats were used in this study. The BB rat is the closest animal model of human insulin dependent diabetes mellitus. Five diabetic insulin dependent BB rats, which had been treated with daily insulin, and 5 age matched diabetes resistant BB rats were euthanized one month after the verification of diabetes in the diabetic group by blood glucose determination. Following euthanasia the abdominal cavity was opened and a 5 mm tissue ring was transected from each of the proximal, middle, and distal small bowel segments. The tissue rings were placed in separate gassed-out transport vials containing 0.5 ml of Wilkins-Chalgren broth. After weight determination the samples are passed into an anaerobic chamber for processing. The tissue segments are split in half and gently rinsed twice with buffered saline (PBS, pH 7.4) to remove gross fecal debris. Previous studies in our laboratory have shown that gentle washing of bowel segments with PBS removes gross contamination and transient bacteria while preserving indigenous microbial mucosal populations.[10,11] The samples are then homogenized for 60 seconds in 1 ml glass tissue grinders and the homogenate serially plated on the following media: Trypticase soy blood agar (BBL, Cockeysville, MD), Columbia CNA blood agar, Mac Conkey agar (Difco, Detroit, MI), CDC anaerobic blood agar, phenylethyl alcohol anaerobic agar, and kanamycin-vancomycin anaerobic blood agar (Remel, Lenexa, KS). The aerobic plates were inspected at 24 and 48 hours and all isolates were characterized by standard methods.[12] The anaerobe plates were visually inspected at 48 and 96 hours and individual isolates were initially characterized by colonial morphology and gram reaction. Following oxygen tolerance testing, obligate microbial isolates were identified by conventional methodology employing PRAS II tubed media (Scott Laboratories, Fiskeville, RI).[13] Volatile and nonvolatile microbial metabolic byproducts (including acetic, propionic, isobutyric, butyric, isovaleric, lactic, and succinic) were extracted by ether-chloroform mathod and analyzed by gas liquid chromatography (Modal 580A, Hewlett, Packard, Rolling Meadows, IL) with flame ionization detector.[14] Aerobic and facultative/obligate microbial recovery was expressed as the $\log^{10}$ colony forming units (cfu)/mg tissue, wet weight.

Myoelectric Studies

Ten age matched Sprague-Dawley male rats were instrumented with five miniature bipolar electrodes. The electrodes were implanted on the mid small intestine and distributed 5 cm apart from each other.

The bipolar electrodes consist of 0.2 mm diameter silver wires, 2 mm apart, connected separately to Teflon insulated lead wires through soldered joints sealed with epoxy and embedded in silicone rubber. The two silver wires extend 0.5 mm from the silastic backing. The silastic backing of these electrode assemblies are sutured to the seromuscular layer of the gut using 6–0 prolene. The assembly was aligned in such a way that the exposed silver wires were oriented along the axis of the gut.

The leads from the recording devices were brought out through the abdominal wall and guided subcutaneously to the interscapular area and then protected in the pocket of a jacket. The leads of the cable were connected to a model 7P122 combination driver and preamplifiers and to a Grass Model 7 polygraph. These preamplifiers allow high gain amplification of the myoelectric signals. The myoelectric responses were recorded on tape using a Hewlett-Packard Model 3968A instrumentation tape recorder for later playback and analysis of the data.

Five of the rats were made diabetic by IV injection of streptozotocin 45 mg/kg and served as the experimental group of animals. Recordings of myoelectric small intestinal activity were begun five days after instrumentation of animals. Animals were recorded after an overnight fast three times per week for three consecutive months. Blood glucose was checked in diabetic animals three times per week.

Scanning Electron Microscopy Studies (SEM)

At the time tissue were collected for bacteriologic studies parallel samples were processed for SEM. Briefly, the tissue segments were prefixed in 2% glutaraldehyde buffered with 0.15M sodium cacodylate (pH 7.4) for 24 hours. The specimens are washed twice in buffer followed by postfixation in osmium tetroxide (1%). After three buffered rinses, the tissue were serially dehydrated in ethanol, critical-point dried from liquid carbon dioxide, and coated with gold-palladium. The tissues were viewed in a Philips 500 SEM at 25 kV and a spot size of 8 nm (Philips Electronic Instruments Inc., Mahwah, N.J.).

Statistical Evaluation of Data

The bacteriologic data was analyzed by the Student t test and analysis of variance (ANOVO). Fasting myoelectric activity from the small bowel of controlled animals was compared to that recorded from the diabetic animals. The tracings were examined for the presence of migrating myoelectric complexes (MMC), the presence of all phases of the MMC cycle and their duration, the MMC period, and the extent of propagation of phase III as well as the migration velocity of phase III. The recordings were also examinied for the presence of ectopic phase III activities arising from different levels in the small intestine. Comparative analysis of myoelectric activity between experimental and control animals was performed using the Student t test. A cutoff of $P<0.05$ was used as a determinant of significance.

RESULTS

Aerobic and anaerobic mucosal recovery in the diabetic Sprague-Dawley rats exceeded that in their age-matched nondiabetic controls (Figure 1 and 2). In general, few anaerobes are recovered from the proximal segment of the small bowel in nondiabetic animals. Therefore, of significant note is the high mucosal recovery of anaerobic organisms in both the proximal and middle small bowel of the streptozotocin-induced diabetic animals.

In the diabetic animals there was a significant ($p<0.05$) quantitative and qualitative increase in the recovery of facultative gram negative organisms (*E. coli* and *Proteus*), anaerobic streptococci and in particular, anaerobic gram negative rods, (*Bacteroides fragilis* group), compared to non-diabetic controls (Table 1).

In the BB diabetic rats there was a significantly higher recovery of facultative and obligate anaerobic mucosal isolates in the proximal, mid and distal small intestine compared to non-diabetic control BB rats (Figure 3). It is important to note that in contrast to the animals with streptozotocin-induced diabetes, these animals required daily injections of insulin to prevent ketoacidosis. Euglycemia, however, was not strictly maintained.

As in the age-matched controls of the Sprague-Dawley group the bacterial recovery from both the proximal and mid-jejunum of the non-diabetic BB rats consisted of a minimal aerobic component and a minor anaerobic component (Table 2). In contrast, the diabetic animals demonstrated an increase in gram negative recovery (*E. coli*) and a significant increase in the anaerobic recovery which included *bacteroides, Clostridium,* and *Fusobacterium* (Table 2).

Scanning electron micrographs obtained from control Sprague-Dawley rats

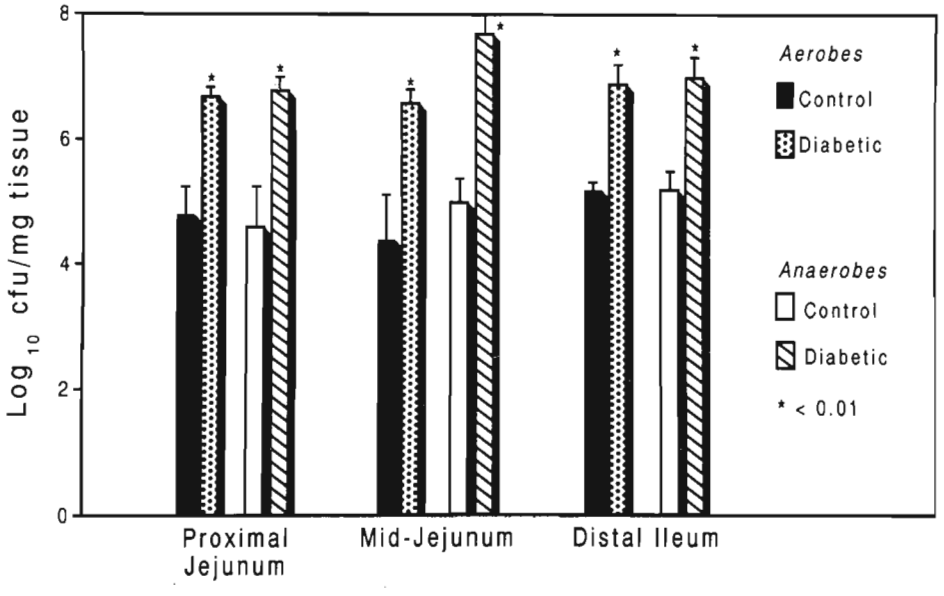

Figure 1 Mean mucosal aerobic/anaerobic microbial recovery (±SE) from the small bowel of streptozotocin-induced diabetic Sprague-Dawley (1 month) rats and age-matched controls.

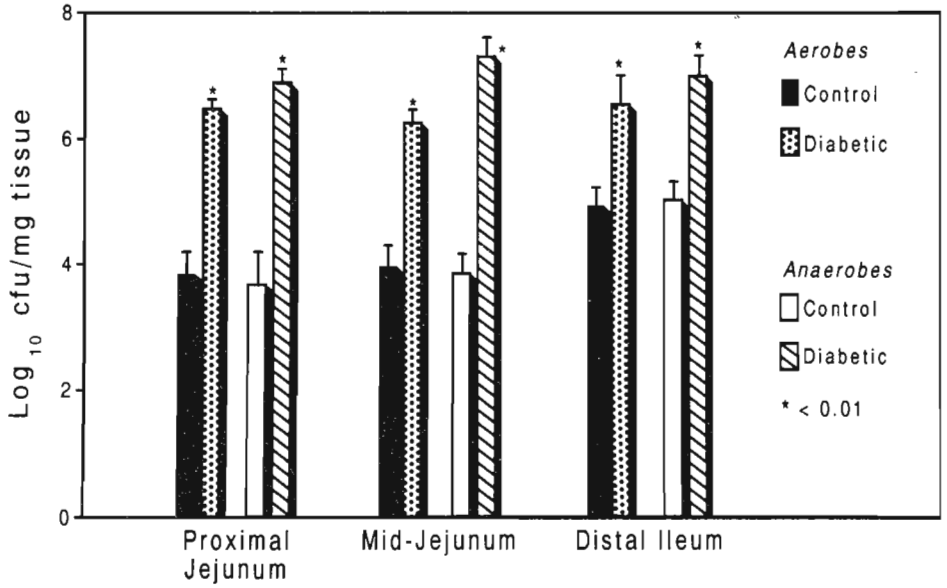

Figure 2 Mean mucosal aerobic/anaerobic microbial recovery (±SE) from streptozotocin-induced diabetic Sprague-Dawley (3 months) rats and age-matched controls.

Table 1 Predominant mucosal microbial recovery in diabetic and nondiabetic sprague-dawley rats.

	Proximal Jejunum	*Mid-Jejunum*
Control	*Streptococcus* – 4.5[a]	*Streptococcus* – 3.1
	Lactobacillus – 3.0	*E. coli* – 3.0
	Peptostreptococcus – 3.9	*Yeast* – 3.2 *Peptostreptococcus* – 4.4
Streptozocin	*Streptococcus* – 3.5	*Streptococcus* – 6.0
Treated	*E. coli* – 5.9	*E. coli* – 6.0
	Proteus – 4.8	*Eubacterium* – 4.9
	Peptostreptococcus – 5.9	*Peptostreptococcus* – 7.4
	Bacteroides – 6.1	*Bacteroides* – 6.4

[a] (Log_{10} colony forming units (cfu)/mg tissue)

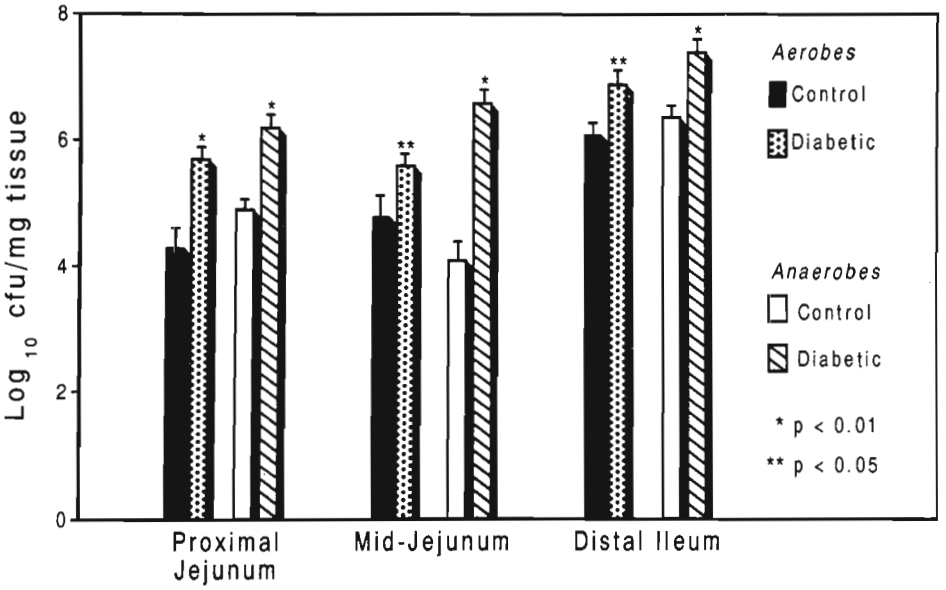

Figure 3 Mean mucosal aerobic/anaerobic microbial recovery (±SE) from small bowel of diabetic BB rats and age-matched (non-diabetic) controls.

Table 2 Predominant mucosal microbial recovery from BB diabetic and non-diabetic rats

	Proximal Jejunum	*Mid-Jejunum*
Control BB	*Streptococcus* – 3.7[a]	*Streptococcus* – 4.0
	Lactobacillus – 2.7	*Lactobacillus* – 3.1
	Peptostreptococcus – 4.1	*E. coli* – 3.5 *Peptostreptococcus* – 3.4
Diabetic BB	*Streptococcus* – 4.1	*Streptococcus* – 4.7
	Lactobacillus – 4.0	*Lactobacillus* – 4.4
	E. coli – 4.8	*E. coli* – 5.0
	Peptostreptococcus – 5.1	*Peptostreptococcus* – 5.5
	Bacteroides – 5.8	*Bacteroides* – 6.2
	Clostridium – 4.4	*Fusobacterium* – 5.0

[a] (Log_{10} colony forming units (cfu)/mg tissue)

demonstrated a thin mucin sheath covering the mucosal surface of the proximal small intestine which had a fragile feather-like consistency. A few micro-organisms were observed embedded in this mucus (Figure 4). In contrast, micrographs obtained from the proximal jejunum of Sprague-Dawley diabetic rats demonstrated a thick mucous sheath covering the mucosal surface in which a large heterogeneous microbial population, including both rods and cocci, were embedded (Figure 5).

Motility changes in the diabetic group became apparent by the 15th day after induction of diabetes (Table 3). The first myoelectric parameter to demonstrate a change was MMC cycling duration; a significantly prolonged MMC cycle was noted (25 ± 3 vs 16 ± 2.3 min) by the end of the first month as compared to controls. Phase III duration and migration velocity were not affected at this time period. During the following month, however, these parameters also showed significant changes compared to control recordings. As shown in Table 3, MMC cycle duration was significantly prolonged (42 ± 9 vs 16 ± 2 min) and Phase III duration was significantly diminished (2.1 ± 0.6 vs 3.4 ± 0.6 min). Furthermore, the mean Phase III migration velocity was significantly increased (2.12 ± 0.25 vs 4.3 ± 0.42 cm/min). Complete disappearance of MMC's was noted in two of five rats during the third month after induction of diabetes. On a number of occasions Phase III occurred independently at different electrode sites and it did not migrate in an organized fashion. Long duration, high amplitude, rapidly migrating spike bursts were observed in the small

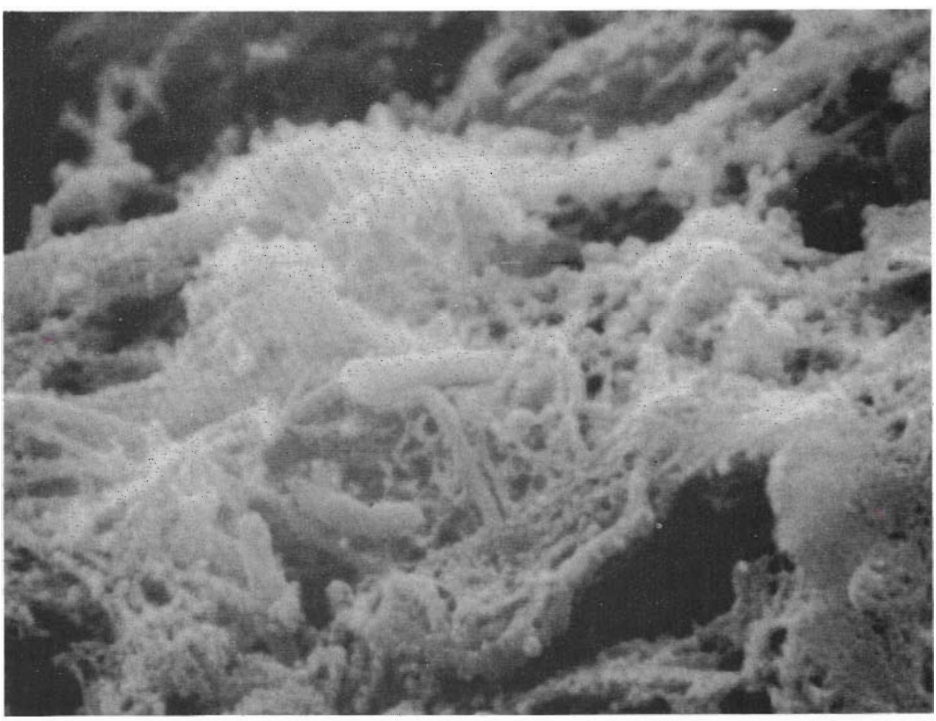

Figure 4 Scanning electron micrograph obtained from the proximal small intestine of control (non-diabetic) Sprague-Dawley rat. Note the thin mucin sheath covering the mucosal surface. A few micro-organisms are embedded in this mucus (Mag. 7500 X).

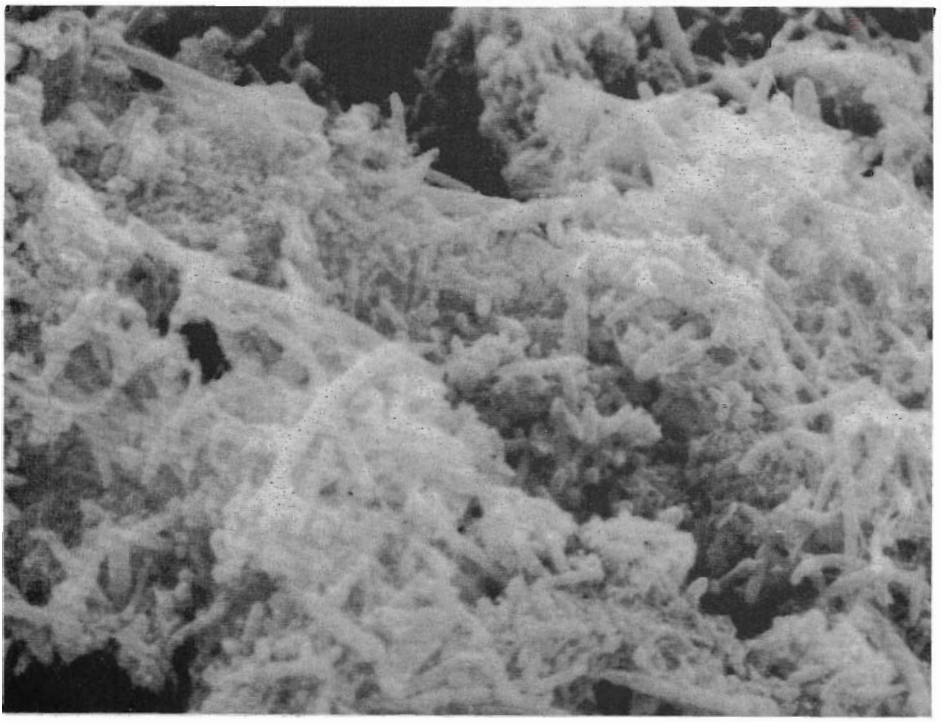

Figure 5 Micrograph obtained from the proximal small intestine of streptozotocin-induced diabetic Sprague-Dawley rat. Note the thick mucous sheath covering the mucosal surface in which a large heterogenous microbial population is embedded (Mag. 7500 X).

Table 3 MMC Parameters in control and diabetic rats (Means ± SE)

	MMC Cycle Duration	*Phase III Duration*	*Phase III Migration Velocity cm/min*
Control	16±2.3	3.4±0.6	2.12±0.23
Diabetic 1 month	25±3.1*	2.8±0.8	2.5±0.30
Diabetic 2 months	42±9.2*	2.1±0.6*	4.3±0.42*
Diabetic 3 months	Complete absence of Phase III or stationary Phase III occurring at different levels in the small intestine. High amplitude and long duration fast migrating spike bursts dominate. Animals develop severe diarrhea.		

* $P<0.05$

intestine of the diabetic rats. This type of activity was more intense during the second and third post-streptozotocin month and occurred every 10–15 minutes. During this period of time the animals developed severe diarrhea.

DISCUSSION

These observations in diabetic rats indicate that diabetes induces time-dependent changes in small intestinal myoelectric activity. These events are likely to be associated with perturbations of intestinal function and homeostasis which is manifested in part, as a microbial overgrowth in the proximal small bowel. We found the greatest change in mucosal microbial flora in the streptozotocin induced diabetic rats which were not treated with insulin. The BB diabetic animals which were treated with insulin showed less bacterial overgrowth than the streptozocine induced diabetic non-insulin treated rats. It may be that the presence of intermittent insulin decreases the available glucose stores present in the proximal bowel or normalizes gastrointestinal motility, thereby preventing excessive microbial overgrowth. These hypotheses, however, remain to be tested. In diabetic animals the microbial mucosal population in the proximal and middle small bowel shifted from a predominantly aerobic gram positive flora to a facultative gram negative population with a significant increase in the recovery of obligate gram negative micro-organisms. In both the Sprague-Dawley and the BB diabetic animals, *Bacteroides fragilis* group was a major mucosal isolate in the proximal small bowel. This is a significant finding, since the recovery of *bacteroides fragilis* is exceedingly rare in the proximal bowel of normal animals.

Diabetes is a major metabolic defect which effects the hosts ability to respond to infectious challenge. For instance, defects in neutrophil chemotaxis, adherence and phagocytic function has been shown in patients with hyperglycemia.[15,16] It is generally recognized that patients with uncontrolled diabetes are more susceptible to infection and that the sequelae associated with these infections are often life threatening. Because of this, every effort is usually made to resolve any existing infection or source of infection in diabetic patients undergoing elective surgery.

If the present animal studies are a valid indicator of proximal bowel mucosal colonization in the human diabetic, a re-evaluation of our current prophylaxis for upper GI surgery is warranted. Such prophylaxis should be targeted to include both facultative and obligate gram negative coverage; especially coverage against the *bacteroides fragilis* group.

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