THE UNIVERSITY OF CALGARY

The Effects of Giardiasis on Gastrointestinal Motility

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF GASTROINTESTINAL SCIENCES

CALGARY, ALBERTA

FEBRUARY, 1996

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Effects of Giardiasis on Gastrointestinal Motility" submitted by Lyse P. Déselliers in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

This study was conducted to determine the effects of giardiasis on gastrointestinal transit, motility and smooth muscle contractility. Weanling Mongolian gerbils were infected orogastrically with $2x10^5$ *Giardia lamblia* trophozoites. At the time of peak colonization, control and infected animals were infused either orogastrically or intraduodenally with ^{51}Cr. Using the geometric centre of transit as a marker, gastrointestinal transit in both the fasted and fed states, and intestinal transit in the fasted state, were all significantly ($p<0.05$) greater in the infected compared to control animals. Then, to determine whether *Giardia lamblia* has an effect on the contractility of longitudinal and circular smooth muscle, isometric tension of jejunal segments was recorded. The development of active tension with stretch and the dose-response curve to bethanechol were significantly increased in the longitudinal muscle of infected animals compared to controls. However, the circular smooth muscle did not show similar increase in contractility.

Acknowledgments

Foremost, I would like to express my gratitude to my supervisor, Dr. Merle Olson, for his guidance and friendship. I owe him a great deal of what I learned about good research and laboratory animal medicine during the completion of this thesis and residency. I would also like to thank the other members of my committee, especially Dr. Douglas Morck for his advice and good humor as well as Dr. Brent Scott, for welcoming me into his laboratory and Dr. Joseph Davison.

I am particularly grateful to Damien Tan for the patience he exercised while sharing with me some of his knowledge. My gratitude also goes out to so many people who made the last few years so enjoyable. I would like to thank Lynn Morck, Dave Kirk, Kathy Tillotson, Tyz Cyron, and all the ARC staff and GI unit members.

To my husband, Randy and my family, who supported me through all my academic endeavors, I owe my deepest thanks.

Finally, I am grateful to the Margaret Gunn's award for enabling me to complete this Master of Science.

Dedication

To Randy for his loving support.

Also, to all the gerbils that significantly contributed to my work.

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List of Abbreviations

IEL	=	Intra epithelial lymphocyte
IBS	=	Irritable bowel syndrome
IBD	=	Inflammatory bowel disease
KCl	=	Potassium chloride
DCC	=	Discrete clustered contractions
PPC	=	Prolonged propagated contractions
MAPC	=	Migrating action potential complex
RBAP	=	Repetitive burst of action potentiels
ENS	=	Enteric nervous system
MMC	=	Migrating myoelectric (motor) complex
TTX	=	Tetrodotoxin
PBS	=	Phosphate buffered saline

1. INTRODUCTION

Throughout the history of humanity, the prevalence and importance of specific pathogens responsible for inflicting infectious diseases upon people, have changed alongside the advances of medicine. Our attention has shifted from organisms that are no longer a major threat, to new or existing pathogens which are now viewed as important causes of significant health problems. One of those upcoming pathogens is *Giardia lamblia*. It was first described in 1681 by Van Leeuwenhoek, using his newly invented microscope, while investigating the etiology for his own case of diarrhea (1). It took many more years of research before *Giardia,* previously considered a commensal, was identified in 1981, as being pathogenic and recognized as such by the World Health Organization (1). Today, *Giardia lamblia* is considered the most common protozoal infection of the intestinal tract in man (1-3).

1.1. GIARDIASIS

1.1.1. Significance

Giardia lamblia is an especially important health concern for children in developing countries, since virtually all of them become infected by the age of three (4-6). Although the clinical manifestations of the infection may vary, as in adults, from asymptomatic to severe diarrhea, there is evidence that giardiasis is an important cause of growth retardation and malnutrition in some of these affected children (6, 7). Adults are also susceptible, particularly if traveling to endemic areas (8) such as Leningrad in the former Soviet Union, or hiking and camping in back country facilities (3, 9-11). Numerous outbreaks were reported

throughout North America, making *Giardia lamblia* the most frequently recognized cause of waterborne disease on this continent (3, 12). Thus, in the United States alone, over 100 outbreaks of waterborne giardiasis have occurred since 1965 with an estimated 4,600 persons hospitalized for giardiasis annually (7). In a recent survey, over 80% of surface water, taken from 66 sites in North America, contained cysts (12) and bodies of water from northern locations (Yukon), thought to be pristine, were also found to be contaminated (13). The presence of this protozoal infection in domestic animals (14-16) and wild animals (13) has also been well documented. The expression "Beaver Fever", which arose following an outbreak in Camas, Washington (1976), and has since been commonly used by the public to refer to the disease, illustrates well the zoonotic potential of this parasite (17-19). Because the possibility of cross-contamination and infection between animals and people has since been confirmed (20), the necessity of controlling *Giardia*, not only in food and water, but also in domestic animals has become evident (14, 17, 21, 22).

1.1.2. Morphological characteristics

Giardia is a protozoan which exists in two forms: the fragile trophozoites, or feeding stage, and the durable ovoid cyst stage. *Giardia* trophozoites have a pear shaped body, with a ventral adhesive disk, two oval nuclei, two central microtubular structures called median bodies, and four pairs of flagellae (3). *Giardia* taxonomy has been the subject of debate for many years. Using distinctive morphological features, the genus *Giardia* is presently divided into five species. *Giardia agilis*, *muris*, *psittaci* and *ardeae*, constitute four of these species and have host preferences which are, respectively: amphibians, rodents, budgerigar and great blue heron (1, 23). *Giardia lamblia*, also referred to as

intestinalis or *duodenalis*, is the fifth and only species known to infect humans and other vertebrates. It differs from the other species by its rather pyriform body shape with an adhesive disk shorter than one-half of the body length and more often double than single claw hammer shaped median bodies (3, 24). The *Giardia* cysts measure 5 by 8 μm, and the trophozoites are 10-12 μm long by 5-7 μm wide. Various strains or varieties of *Giardia lamblia* exist and are currently being characterized using molecular biology techniques such as electrophoretic karyotyping, DNA fingerprinting and restriction enzymes identification techniques (25, 26).

1.1.3. Epidemiology

Transmission may occur through the ingestion of infective cysts found in contaminated food or water. The cysts' transmission is greatly facilitated by its ability to survive up to several months in water, resist standard chlorination protocols and withstand a moderate range of temperature; allowing it to remain viable for more than three months in a 4°C environment (3, 4). Another important mode of transmission includes person-to-person, by fecal-oral propagation of the parasite. Studies have shown that as little as ten orally administered cysts are sufficient to induce a sustainable infection in humans (3). Moreover, up to 900 million cysts may be passed in the stools of an adult in a day. These facts contribute to explain the prevalence of the disease observed among day care children and homosexuals (24, 27-29). Following excystation, mobile trophozoites are released in the duodenum where they multiply by binary fission, colonize the small intestine by adhering to the enterocytes, and eventually encyst to be passed in the feces. The infection can be confirmed by microscopic identification of fecal cysts using various flotation and immunofluorescence

techniques (30, 31). The trophozoites may be identified in duodenal aspirates or biopsies (32-34). Recently, new immunological and enzymatic techniques have also been developed to facilitate the detection of the parasite in patients' stools and water samples (28, 35-38).

The probability of contracting giardiasis and developing the symptomatic form depends not only on the host immune response and the strain involved but also on many other risk factors. Namely, these are: (i) age; young, non-breast fed children being more susceptible, (ii) environment; poor sanitation and water supply, poverty, and fecal contamination often being linked to higher prevalence, (iii) gender; infection rates being greater in males, (iv) nutritional status; malnutrition and low protein intake often being linked to diseases. Other factors may include gastric acidity, pet ownership, life style and population density (4, 39, 40).

1.1.4. Course of infection

The prepatent period is approximately seven to nine days in humans. The clinical manifestations of giardiasis vary from an asymptomatic infection to a chronic diarrhea with malabsorption and maldigestion often leading to weight loss (6). The variability in symptoms is thought to be dependent upon the individual host factors (e.g. genetic predisposition, immunodeficiency) as well as the pathogenicity of the different *G. lamblia* isolates. Patients with symptomatic giardiasis have diarrhea with loose, foul-smelling stools, which are often accompanied by abdominal cramps and bloating, nausea, reduced appetite, malaise and weight loss (9, 32). Symptoms usually begin after an incubation period of seven days and in the vast majority of acute cases of giardiasis, the illness is self-limiting within a two to four week period. However, a proportion of

individuals (estimated at 30-50%) will go on to have chronic diarrhea for seven weeks or more (3,6). Malabsorption may take on a clinical significance for many symptomatic patients suffering from an abnormal absorption of fat, vitamin A, vitamin B_{12}, thiamine and folate. Maldigestion of disaccharides such as lactose is believed to be in part responsible for the biochemical evidences of carbohydrates malabsorption (41, 42, 45). Steatorrhea and significant weight loss may result (6). As suggested previously, clinical symptoms of giardiasis are most prominent in the undernourished and in the immunocompromized individuals during the first three years of life (4, 6, 39). Other accompanying conditions have been reported although confirmation of their respective importance and relation to giardiasis awaits further investigation. These conditions include: cholecystitis, granulomatous hepatitis, pancreatitis, urticaria, lymphoid nodular hyperplasia, arthralgia, reactive arthritis, retinitis and hypokalemic myopathy (43-45).

1.1.5. Histopathology

The histopathological description of infected human small intestinal biopsies varies greatly in nature. *Giardia lamblia* infection is associated with a variety of mucosal appearances ranging from normal villus architecture to partial or sub-total villous atrophy (33, 45, 46). The presence of jejunal crypt hyperplasia has also been found to accompany the reduction in villus height. The reason for such a wide spectrum of mucosal abnormalities is not yet fully understood. Some authors believe that it may relate to the parasitic load and the phase of infection (i.e. establishment, acute and resolution phases) (33). It has been suggested that the extent of the mucosal changes appear to be directly related to the functional impairment of the gut and to the severity of the diarrhea (32, 45). On the other hand, ultrastructural abnormalities consisting of a shortening and disruption of the

microvilli, have been reported in symptomatic patients, and this, even in the absence of microscopic mucosal alteration (47).

The inflammatory response to the parasite also appears to vary among individuals (33, 42, 46). Nevertheless, giardiasis has been frequently associated with an inflammatory cell infiltration (45, 48), characterized by intraepithelial and lamina propria lymphocytes, and occasionally, plasma cells, macrophages, neutrophils and eosinophils (41, 49, 50).

Attempts to characterize the pathological changes associated with human giardiasis were impeded by the individual variations encountered in terms of parasitic load, strain virulence, immunological status and history, infection time and localization of biopsies. Consequently, animal models of giardiasis with structural and ultrastructural abnormalities resembling those from human biopsies, were consequently developed. Studies, using these rodent animal models, showed that the infection prevailed mainly in the upper small intestine, although elongation of distal intestine villi could accompany the villus atrophy seen in the proximal small intestine (49, 51). The inflammatory response seen in human biopsies was also documented in the animal models of giardiasis. One study (49) using *G. muris* in mice, demonstrated that in the jejunum, intraepithelial lymphocyte (IEL) counts were reduced at week 1 (establishment phase) but significantly increased by week 3, when the parasite load was by then declining (resolution phase). The increase in IEL was seen after villus shortening had occurred, and when the brush border disaccharidases activity had already decreased. Another study (52), using *G. lamblia* in mice, reported that intraepithelial and lamina propria helper/inducer T cells subsets were unchanged during the establishment and the acute phase of the disease but were significantly increased, along with IgA-secreting cells, in the resolution phase of

the disease. However, this study also reported the increase of a different subset of lymphocytes (suppressor/cytotoxic T-cells and Thy 1.2 T-cells) and IgM-containing plasma cells during the initial phase of the infection. These findings, which corroborate other studies done in the gerbil and the neonatal rat (53, 54) clearly demonstrate the complexity of the host-parasite interaction, which in turn, varies throughout the course of the infection. It would be reasonable to conclude that the pathological changes observed in the gastrointestinal tract, during giardiasis, are neither pathognomonic nor consistent among individuals.

1.1.6. Host response and Immunity

The host immune response to *Giardia lamblia* remains an area of research not fully understood. Yet, the role played by the immune system in clearing the infection is now recognized as essential. The increased prevalence and severity of symptomatic giardiasis in immunodeficient patients, suffering from conditions such as hypogammaglobulinaemia, indicates the importance of humoral immunity in fighting the infection. Evidence shows that a specific anti-*Giardia* IgA response occurs following the infection. Secretory, lacteal and biliary IgA have been found to coat the surface of trophozoites (3, 55). The microbial toxicity of the secretory IgA antibody is believed to be one of the most important mechanisms responsible for the elimination of the parasite from the intestine (45, 55). However, it has been reported that as many as two-thirds of infected patients may not have measurable serum IgA levels and that the IgA response does not always clearly correlate with the clearance of the parasite (2, 3). Anti-*Giardia* IgG, which may also be found in the intestine and breast milk, are thought to participate in the elimination of the parasite by facilitating the adherence of neutrophils and macrophages to the trophozoites thus contributing to their

phagocytosis (56). The detection of serum IgG levels, however, may not be directly linked with the active state of the disease or with complete immunity as suggested by studies showing that up to half of the asymptomatic mothers from a Bangladeshi study passed cysts despite detectable serum IgG responses (4). Furthermore, symptomatic patients suffering from chronic giardiasis may have detectable levels of IgG, suggesting that circulating anti-*Giardia* IgG does not protect from the chronic form of the disease (57). Other immunogammaglobulins such as IgM and IgE have been detected in infected patients. Serum IgM may be detected at the beginning of the infection, making it a valuable though less definite diagnostic tool to differentiate present from previous infections (37). The role of IgE remains unclear but the possibility of an anaphylactic component to the immune response induced by *Giardia* has been suggested based on data showing increased counts of IgE containing cells in human jejunal biopsies and increased mucosal mast cells in murine giardiasis (58-60). The role of the immunoglobulins in either preventing attachment of the trophozoites by immobilization of the flagellae (55), or by a direct antibody dependent cellular cytotoxicity should not be overlooked and needs to be better defined.

Prolonged infections, similar to those seen with hypogammaglobulinaemia patients, were also reproduced in T-cell deficient *nu/nu* mice (61). The role of T-cell immunity was further confirmed by studies showing increased parasitic load in corticosteroid or cyclosporin A treated animals (3). In addition, because activated T-cells can produce villous atrophy (62) whereas the absence of T-cells in infected nude mice is associated with less severe villus alteration despite a prolonged infection, it was proposed that T-cells, among other mechanisms, may be responsible for some of the morphological changes observed in the infected gut (45). As previously mentioned, the increasing number of helper/inducer

(CD4+) T-cells in the intestinal mucosa, which coincides with the appearance of IgA containing cells, occurs during the elimination phase of the parasite. The importance of this particular T-cell subset is confirmed by experiments showing the defective elimination of the parasite in CD4-depleted mice (3). The exact role of T-cells in clearing the infection could be through a T-cell-dependent antibody response and/or the enhancement of other cellular defense mechanisms via the release of lymphokines. T-cell activation may occur via sensitization to specific *Giardia* antigen or through direct contact with *Giardia* lectins (45).

Other cells such as macrophages, which may act as antigen-presenting cells or phagocytes, and mast cells, possibly involved in an anaphylactic-type response, are believed to be part of the immune response to the parasite (2, 3, 52, 59, 63). In addition, the presence of non-immune factors, namely, intestinal motility, intestinal mucus layer and lipase from human breast milk, definitely contribute to the defence mechanisms in place (3, 64, 65).

1.1.7. Pathophysiology

The pathophysiology of giardiasis has been the subject of ongoing investigation. The disease's variability, in terms of the nature and intensity of symptoms, as well as in relation to the histological and laboratory findings, has made the task of characterizing its pathophysiology even more challenging. Not surprisingly, the pathophysiology of giardiasis has been described as complex and multifactorial. Nevertheless, some very valuable information, obtained through both animal and human studies, has greatly enhanced the understanding of this disease process and is therefore summarized hereafter.

The various elements involved in the pathophysiology of giardiasis have often been divided into mucosal and luminal factors. Mucosal factors include, as

previously mentioned, various degrees of villus atrophy. A reduction in villus length, as seen in other conditions such as coeliac disease (66) or viral enteritis (67), would decrease the overall surface area available for nutrient absorption. Crypt hyperplasia, also present in the *G. lamblia* infected gerbil model, is accompanied by an increased enterocyte migration rate but not an increased percentage of immature cells (54). Therefore, inadequate absorption by immature enterocytes originating from hyperplastic crypts does not, in the gerbil model, appear to contribute to the pathophysiology of the disease. Another important mucosal factor consists of the ultrastructural damage observed in the gerbil model as well as in the human biopsies (6, 47). In the gerbil, the reduced brush border surface area associated with a reduction in microvilli height is seen at a time when no inflammatory cell infiltration is detectable. These ultrastructural changes are believed to be in part responsible, along with the variable villus atrophy, for causing malabsorption and consequently, diarrhea. Interestingly, these microvillous abnormalities are not solely co-localized with adhering trophozoites, but rather appear to be generalized (51). These findings further prove that despite the ventral disc imprints left by the adhering trophozoites on the microvilli, it is unlikely that such localized events are responsible for the microvilli abnormalities seen throughout the small intestine. Furthermore, the suggestion that trophozoites may prevent nutrient absorption by physically occluding the mucosa is no longer considered plausible (47). Various experiments done in different animal models have further demonstrated that malabsorption is a key factor in the production of diarrhea. Thus, in the *in vivo* gerbil's jejunum, giardiasis causes a reduction in glucose-stimulated absorption of Na^+, Cl^-, K^+ and consequently, water . Similar findings, but for sodium only, were obtained *in vitro* (54). Although giardiasis in gerbils does not induce active

secretion, a net secretory state of Na+ and Cl- was documented in the neonatal rat model (53). The absorption of other substrates, such as 3-0-methyl-D-glucose and amino acids, have been shown to be reduced (47).

To accompany these morphological abnormalities, a significant reduction in disaccharidases activity has been documented in human and rodent giardiasis. The reduction in mucosal maltase, sucrase, lactase, saccharase, trehelase and alkaline phosphatase activity is directly linked to the maldigestion which in turn contributes to the diarrheal symptoms (47).

In order to address the luminal factors which are believed to contribute to the pathophysiology of giardiasis, it is necessary to mention bacterial overgrowth (45). The actual significance of the increased presence of enterobacteria in the proximal intestine of some patients has not been definitively determined. While some authors reject such claims, stating the inefficacy of non-giardiacidal bactericidal antibiotics to alleviate some of the symptoms of giardiasis (32), others suggest that bacterial overgrowth may be responsible for the enhanced severity of the symptoms as well as the steatorrhea observed in some patients. Their argument is based on the fact that a large number of bacteria can induce architectural abnormalities as well as deconjugate bile salts (45), thus preventing lipid solubilization and subsequent absorption of dietary fats (39). This additional luminal factor, namely, the reduction in conjugated bile salts concentration, may also follow the active, carrier-mediated uptake of the latter by *Giardia* trophozoites. The specific requirement for bile and conjugated bile salt by the parasite is confirmed by the *in vitro* demonstration of stimulated growth and reduced generation time seen in trophozoites grown in the presence of a low concentration of mammalian bile (69). However, despite what was previously believed, *Giardia* cannot deconjugate bile salts unlike many bacteria (70). Finally,

another luminal factor is linked to the abnormal pancreatic exocrine function associated with reduced trypsin, chymotrypsin and lipase activity documented in some patients (6). The clinical relevance of such findings, in light of the considerable pancreatic functional reserve, remain to be determined.

1.1.8. Pathogenesis

The first question that should be addressed before attempting to define the mechanisms involved in the production of giardiasis is the following: Is *Giardia lamblia* an invasive organism and if not, how can it produce such morphological and physiological disturbances? Although earlier reports once concluded that *G. lamblia* could invade the small intestinal mucosa in man (50), it is now generally accepted that this protozoan is a non-invasive organism (47, 71) and penetration of the mucosa is not a common occurrence. As previously mentioned, mucosal damage mechanically caused by adhering trophozoites does not appear to play a significant role in the pathogenesis of the disease. The surface mannose-binding lectin expressed by *Giardia* trophozoites may well be involved in the colonization of the proximal intestine (72). However its role in the attachment of the trophozoites and in the mucosal abnormalities encountered throughout the small intestine still remains to be confirmed.

The possible existence of an enterotoxin released by live trophozoites, or what is referred to as an excretory-secretory product (73), is presently the subject of conflicting reports (47, 64). Such a substance could originate from the lysosome-like vacuoles present on the plasma membrane of trophozoites and would be responsible, via cytopathogenic effects, for the mucosal damage associated with the infection. Hydrolases, (74) like the thiol-dependent proteinase found in these vacuoles, or other putative toxins, could participate in the

pathogenesis of giardiasis not through recognized enterotoxins secretory mechanisms, but possibly via cytopathogenic effects (47, 64).

Finally, the role of the host response in the pathogenesis of the disease still requires clarification. It is not yet known whether T-cell activation, along with the accompanying release of lymphokines, is sufficient to produce the architectural changes observed or whether intraepithelial lymphocyte multiplication is essential for such events to occur. Further research looking at the actual factors linking the decrease in villous-crypt ratio to lymphocyte activation will be necessary to better understand the role of immunity and inflammation in the morphological changes observed in some, yet not all, cases of giardiasis.

1.1.9. General considerations

Many authors, when addressing the pathophysiology of the disease, focus their attention on the mechanisms involved in causing the diarrhea which characterizes giardiasis. Thus, mucosal and luminal factors are described and their respective roles, in causing diarrhea and accompanying weight loss and/or steatorrhea, are further supported by morphological or physiological findings. However, giardiasis is not only characterized by diarrhea. Many patients also complain of abdominal pain and cramping sometimes associated with bloating and vomiting. The most common causes for similar abdominal pain in diarrheal diseases linked to malabsorption are: distension of bowel, serosal and peritoneal involvement by disease process and muscle spasm (75). Symptoms of abdominal cramping and pain, in a disease like giardiasis which is neither characterized by severe inflammation, intestinal ulceration nor significant

increase in intestinal secretion or distension, suggest that the pathophysiology of giardiasis may implicate other factors such as disturbances of intestinal motility .

Other diseases affecting the gastrointestinal tract have been known to cause motor dysfunction which in turn have been linked to various symptoms such as abdominal cramping, bloating, diarrhea, constipation and vomiting (76). For example, specific alterations of motility in patients suffering from irritable bowel syndrome (IBS) has been associated with painful episodes. Discrete clustered contractions (DCC), which are aborally propagated small intestinal mechanical manifestations of "minute rhythm", and prolonged propagated contractions (PPC), which are associated with migrating action potential complex, are both increased in frequency in patients with IBS and are both linked to painful episodes (77-79). Contrary to previous belief, the sensation of bloating in this condition, is not due to the stagnation of ileal contents, since the ileocaecal transit is actually accelerated in both patients with diarrhea and constipation (78). Such sensations may be caused by the increased visceral sensation associated to IBS (80).

Another example of dismotility associated diarrheal disease is *Clostridium difficile* enteritis. Infected patients are often presented with clinical symptoms such as abdominal distention and pain, some degree of paralytic ileus and severe diarrhea (81). It is suggested that the pathophysiology of this disease includes disordered transit since this organism is capable of inducing migrating action potential complex (MAPC) and repetitive burst of action potentials (RBAP) in the infected rabbit ileal loop (94,113,112). However, in this condition as in others where mucosal damage or gaseous and fluid intestinal distension occurs, it is difficult to attribute the pain and cramps to either a motility disorder or an accompanying factor.

Finally, the altered intestinal motility associated with the toxicity of irradiation is believed to be directly linked with the accompanying symptoms of vomiting, abdominal cramping and diarrhea which are experienced during radiotherapy (82, 83). Although symptoms such as abdominal distension and abdominal cramps and pain are not specific to any gastric, intestinal or colonic disturbances, they are some of the most common manifestations of conditions involving a motility disorder. Consequently, it is logical to explore the possibility that such motility disturbance may play a role in the pathophysiology of giardiasis.

1.2. INTESTINAL MOTILITY

1.2.1. Intestinal morphology

The small intestine is a long circular tube divided into three segments: the duodenum, jejunum and ileum. Its structure includes the mucosal layer which is characterized by numerous villi and microvilli which immensely increase (by 200 times) the overall surface area available for nutrient absorption by the enterocytes. The crypts of Lieberkühn are situated at the base of the villi and contain many dividing mucosal replacement cells. The lamina propria, in which lymphocytes and other immune cells are found, is separated from the submucosa layer by the muscularis mucosa. The vascular submucosa which consists of connective tissue, nervous plexus (i.e. submucous plexus), blood vessels and lymphatics lies on the muscularis externa layer. The latter includes the thick inner circular smooth muscle, the myenteric plexus and the thin outer longitudinal muscle. The serosal layer consists of a loose collagenous layer lined by visceral peritoneum (84).

1.2.2. Smooth muscle and the excitation contraction coupling

The intestinal smooth muscle cells are spindle-shaped, tighly packed and measure 500 to 700 μm in length when at rest (85). Electrical coupling is known to occur between the cells through structural arrangements such as gap junctions and ball-and-socket type appositions. These mechanisms, as well as others not yet well understood, allow the propagation of action potentials and the production of a synchronous response to excitation from all cells within a bundle (86). The excitation-contraction occurs in smooth muscle cells following an increase in intracellular calcium levels. This follows the depolarization of the cell in response to excitatory stimuli such as electrical currents from adjacent cells, stretch, or neurohumoral agents binding to the cell receptors. A good illustration of such excitation is the binding of a muscarinic agonist, such as bethanechol, to its M_2 receptor subtype on the surface of the smooth muscle cell (87). This binding induces the depolarization of the cell through different mechanisms such as by decreasing the probability of the ligand-gated K^+ channels to be open, thus decreasing K^+ efflux, as well as increasing the probability of cation selective channels opening, thereby increasing transmembrane cation fluxes. More importantly, activation of the muscarinic receptor induces a muscular contraction by turning on, via G-proteins, a specific phosphodiesterase, phospholipase C, which hydrolyzes phosphatidylinositol 4,5-biphosphate (PIP_2) to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. The IP_3 becomes a second messenger which is capable of releasing Ca^{2+} from the sarcoplasmic reticulum. In addition, the binding of the muscarinic agonist to the adenylate cyclase inhibitory receptor (R_i) turns off this inhibitory effector system by decreasing the cAMP production thereby preventing its inhibition of smooth muscle contraction. This is only one of

many mechanisms capable of causing cell depolarization and subsequent cell contraction. Smooth muscle contraction, initiated by an influx of calcium, occurs after the binding of four Ca^{2+} ions to one calmodulin molecule. The formation of the Ca^{2+}/calmodulin complex activates the myosin light chain kinase (MLCK), which phosphorylates the regulatory light chain (20 kDa), leading to an increase in the activity of the actin-activated myosin Mg^{2+} ATPase and subsequent cross-bridge cycling. Smooth muscle contraction is obviously much more complicated than this short series of events and many internal regulatory mechanisms are in place to allow fine adjustments (88, 89).

It is important to remember that intestinal smooth muscle cell depolarization is restricted to the depolarized phase of the slow wave. The slow wave is a slow rhythmic oscillation of the smooth muscle membrane potential which is believed to result from a combination of activation and inactivation in voltage dependant currents mainly associated with calcium and potassium channels. The intestitial cells of Cajal, which are located between the muscular layers, would be responsible for providing the pacemaker activity (90). The intrinsic slow wave frequency decreases aborally and it controls the timing of contractions at any one locus along the gut. In order for an excitatory influence to reach the critical level of membrane depolarization or threshold potential sufficient to produce an action potential and open voltage-dependent calcium channels, it must benefit from the additional oscillating depolarization of the slow wave. Because of the circumferential synchronicity of the slow wave, the bursts of action potential, which always translate into a contraction, appear as a single segmental contraction.

1.2.3. Neurohumoral regulation of motor activity

Although the slow wave and the action potential, intrinsic to smooth muscle cells, determine the timing and location of single contractions, the control of motility in terms of occurence of contractions, patterns of organization and distance of propagation, are determined by neural and humoral factors. The smooth muscle cells of the gastrointestinal tract are innervated by neurons from the myenteric plexus of the enteric nervous system (91). This intrinsic nervous system is capable of producing independent activity through the highly complex integrative and motor program circuits of the myenteric plexus (92). The integrative circuits which receive and process information from sensory and CNS neurons, control the motor neurons along with the motor program. The latter determines the sequence of events in stereotyped repetitions of motor out flow to the effector systems. The myenteric plexus, along with the sensory receptors and motor neurons, is capable of controlling effector systems such as intestinal musculature, vasculature, enteroendocrine cells and epithelial secretion and absorption. Although the enteric nervous system (ENS) is at the origin of many patterns of contractile activity such as the peristaltic and inhibitory reflexes, the migrating myoelectric (motor) complex, the fed pattern and the migrating action potential complex, the modulation or initiation of those patterns is also dependent upon the integrity of extrinsic innervation to the gut. Thus, the parasympathetic system, via the vagal and pelvic nerves and the sympathetic system, via the prevertebral ganglions, are both responsible for modulating through cholinergic (+) and adrenergic (-) pathways, the motility of the digestive tract. Acetylcholine (Ach), which serves as the final common neurotransmittor for excitatory neural control of gut motility, can act upon ENS nerves through nicotinic receptors or can directly stimulate muscle contraction via muscarinic receptors on smooth

muscle cells. Drugs such as bethanechol, which is a M_2 muscarinic cholinergic agonist, produce contraction of smooth muscle cells without activating nicotinic neuronal receptors.

1.2.4. Patterns of motor activity

The main patterns of small intestine contractile activity, often referred to by diverse nomenclatures, consist of the intestinal reflexes, the migrating myoelectric (motor) complex (MMC), the fed pattern, the migrating action potential complex (MAPC) and the repetitive bursts of action potentials (RBAPs). The intestinal reflexes include the peristaltic reflex, in which intestinal muscles contract anteriorly and relax posteriorly to a bolus to facilitate its aboral migration. The intestinointestinal, or inhibitory reflex, prevents contraction in segments adjacent to an area of marked distension. The migrating myoelectric and motor complex (MMC), is composed of three phases and is characterized by a recurrent band of myoelectric and motor activity which, in the fasted gut, propagates from the lower esophageal sphincter to the ileocecal junction. The MMC is responsible for an interdigestive cyclic "clean up" which occurs through the propulsive activity associated with its phase III, while also providing an effective defense mechanism against bacterial overgrowth. The fed pattern, initiated by the ingestion of a meal, is characterized by a continuous pattern of irregular myoelectric and motor activity. The type and duration of the associated contractions may vary according to the nature of the food ingested. The migrating action potential complex (MAPC) is an action potential discharge associated with a propulsive, aborally propagated ring contraction. Although the MAPC is a basic propulsive motor pattern intrinsic to the normal gut (93), it may also be recruited to serve as a motor defense mechanism able to clear unwanted substances,

such as non-invasive bacteria and their enterotoxins, from the intestinal lumen (67). The repetitive burst of action potentials (RBAPs), often associated with invasive or cytotoxic bacterial enteritis and mucosal necrosis, are believed to promote gut stasis and facilitate bacterial proliferation (67, 94). However, non-propagating clusters of contractions similar to RBAPs are also a feature of normal human motility. Other patterns of contractile activity may be observed in the small intestine of patients suffering from intestinal obstruction or irritable bowel syndrome (IBS). These discrete clusters of contractions or minute rhythm are similar to the MAPC and are often associated with pain (79, 80).

1.2.5. Motility disturbances and disease state

Intestinal motility disturbances and their associated wide range of symptoms (diarrhea, constipation, abdominal cramps, vomiting, bloating, etc.) may be brought about by various abnormalities. Thus, prolonged or accelerated transit may follow derangements of neuromuscular control affecting extrinsic nerves, intrinsic plexuses or smooth muscle cells. The pathophysiology of the various motility disorders are not all well understood. The level of disruption in the acquired conditions may vary from a derangement of the electrophysiology of the smooth muscle cell to a change in the organized patterns of contractions of the entire gut. Therefore, the study of the effect of a certain disease state on the intestinal motility may be approached from different angles. For instance, in a laboratory setting, determination of the transit time in the affected intestine of a chosen animal model may indicate the presence of a motility disorder. To further investigate the condition, the characterization of the myoelectric activity of the infected gut compared to controls, as well as the acquisition of manometric recordings, may yield important information on the patterns of intestinal electric

and motor activity. In addition, *in vitro* isometric longitudinal and circular smooth muscle contractility recordings in response to various agonist and antagonist agents would clarify the potential location of the altered mechanisms involved. Further studies investigating the molecular and electrophysiology of isolated smooth muscle cells could help to pinpoint the site of alteration in conditions affecting the cells themselves.

As mentioned previously, the levels of disturbance affecting intestinal motility vary according to the different etiologies. For instance, enteropathogenic *Escherichia coli* has been shown to cause changes in the slow wave frequency in the *in vivo* colon (94). The defect responsible for the decrease in smooth muscle force development in human colitis would be at the level of the myosin, the actin or the cross bridge formation (95) whereas in Crohn's disease, the increased contractility to carbachol in the longitudinal muscle, and to histamine in the circular muscle, appears to be receptor-mediated (96). The increased rate of aboral transit seen in the *Yersinia enterocolitica* rabbit model is not only associated with an increased frequency and duration of phase III of the MMC, but also a reduced contractility of the longitudinal muscle in response to carbachol which appears to be a receptor-independent change (97, 98). Celiac disease, which has also been associated with an increased transit rate, is linked to an augmentation in MMCs in adults and to other motor abnormalities such as DCCs, giant jejunal contractions and, in children, to nonpropagated bursts of contractions (99). Interestingly, for a specific condition, contractility changes may differ between the longitudinal and the circular smooth muscle layers. For instance, in the *Nippostrongylus brasiliensis* rat model, the contractile response to carbachol in the longitudinal muscle is increased and appears to be linked to a change in the calcium handling property of the infected muscle. Conversely, the

contractile response of the circular muscle to the same agonist is decreased and may be linked to a lesion at the cell membrane level, affecting its permeability to calcium, or to a lesion of the excitation contraction coupling mechanisms or both (100). In the *Trichinella spiralis* rat model, which is another nematode model characterized by inflammation as well as hyperplasia and hypertrophy of the muscularis externa (101), the increased contractility of the longitudinal muscle appears to be due to a change in the excitation-contraction coupling as well as a sodium pump suppression (102-104). These contractility changes are accompanied by a decreased transit time (105), a reduction in both slow-wave frequency and spiking activity, and an increased presence of MAPC (106). The enhanced frequency in MAPC has also been documented in response to *Vibrio cholerae* and choleragen (107-110), enterotoxigenic *E. coli* and its heat-labile toxin (67) and enteropathogenic *E. coli* (94). Interestingly, the presence of MAPC was also reported in the anesthetized rabbit uninfected intestinal segment, proximal to a ligated loop infected with invasive *E. coli* (111), *Shigella dysenteria* (112), *Clostridium difficile* (94, 113), *Salmonella typhimurium* (114) or *Campylobacter jejuni* (115). RBAPs have been reported in *in vivo* animal model experiments after challenge with enteroinvasive *E. coli*, *E. coli* heat-stable enterotoxin (116), *Shigella dysenteria* (112), *Clostridium difficile* (112), *Clostridium perfringens* (112) and *Campylobacter jejuni* (115). These findings regarding the presence of MAPC and RBAPs are consistent with the possibility of the local circulation of an absorbed toxin, the release of a local mediator following tissue invasion and inflammation, or the activation of a local reflex (14).

In order to determine if a change in intestinal motility may play a role in the pathophysiology of a given condition, it is essential to understand the complex cause and effect interrelations between intestinal myogenic activity, epithelial

transport and neuronal reflexes. Furthermore, the sole observation of an increase in transit rate does not necessarily indicate the presence of a motility disorder. Therefore, it is essential to further investigate such a possibility, using the appropriate experimental tools developed for such requirements.

1.3. Objectives

The aim of the present thesis was to establish if a correlation, in terms of increased transit and infection, was present in a model of giardiasis. In addition, the aim was to determine if this increased transit was associated with an altered motility, *in vivo*, an altered contractility of the intestinal smooth muscles, *in vitro*, and an inflammatory response within the intestinal mucosa. Therefore the objectives of the present study were:

1) To characterize the temporal course of *Giardia lamblia* S_2 strain infection in the male weanling mongolian gerbils.

2) To assess the gastrointestinal and intestinal transit rate in our model following infection with *Giardia lamblia*.

3) To characterize the myoelectric and motor activity in the normal and infected small intestine of our animal model.

4) To determine the nature of any changes in contractility in the infected longitudinal and circular smooth muscles.

5) To determine if an inflammatory cell infiltration was present at the time of peak infection in our animal model.

2. MATERIALS AND METHODS

2.1. ANIMAL MODEL

Giardia-free outbred male weanling Mongolian gerbils weighing 35-45 g were studied. The gerbils were obtained from the Life and Environmental Science Animal Resource Centre, University of Calgary and Harlan Sprague Dawley (Indianapolis, Indiana). They were caged in a room maintained at 18°C with relative humidity of 40-50% and provided with 12 hour light cycles. The gerbils received water ad libitum and were fed commercial rodent chow (Chow 5-L37, PMI Feeds, St-Louis, Missouri). All experimental procedures were approved by the University of Calgary Animal Care Committee and adhered to the Canadian Council on Animal Care guidelines.

2.2. GIARDIA LAMBLIA INFECTION

Infection was induced in gerbils on day zero by orogastric inoculation of $2x10^5$ trophozoites in 0.5 ml of phosphate-buffered saline. The *Giardia lamblia* S_2 trophozoites, originally isolated from a sheep in Alberta, were axenically grown in TYI-S-33 medium until their late logarithmic phase (72 hours). Then, following four successive washes and centrifugations of 10 minutes each at 500 g, the trophozoites were resuspended in phosphate buffered saline (PBS) of pH 7.4. The animals were gavaged using an 18 gauge feeding needle. The control animals received saline only.

2.3. EXTRACTION AND QUANTIFICATION OF TROPHOZOITES

Infected gerbils (n=12) were euthanized by cervical dislocation on post-infection day 4, 6, 8 and 14. One control gerbil was euthanized on day 1 to rule

out any pre-existing infection with *Giardia*. The entire small intestine was carefully removed and divided into three segments: the duodenum, located between the pylorus and the ligament of Treitz, the proximal jejunum and the distal half of the remaining small intestine. Each segment was split longitudinally, placed in 4 ml of PBS and, following an incubation period of two hours at 37°C with shaking at 120 rpm, the trophozoites in suspension were counted on a hemacytometer. The results were expressed as $\log_{10}$ of the total number of trophozoites per centimeter of gut. The results were analyzed to determine the time and location of maximal colonization.

2.4. EXPERIMENTAL DESIGN

Five separate studies were designed to examine the effects of giardiasis on intestinal transit, contractility and motility. The first two studies examined the transit rate in control and infected animals. Study 1 examined gastrointestinal transit in fasted and fed animals while study 2 evaluated intestinal transit alone, using animals with duodenal cannulas. The third and fourth studies examined jejunal contractility in the longitudinal and circular smooth muscle respectively. Finally the fifth study attempted to characterize the effect of the disease on *in vivo* motility, looking at pressure recordings in surgically prepared gerbils.

2.5. TRANSIT STUDIES

2.5.1. Gastrointestinal transit

All the animals were initially fasted for 18 hours then control (n=21) and infected (n=16) groups were again divided into two groups. The infected (n=8) and control (n=8) gerbils from the fed group received 0.5 ml of an elemental liquid

diet (Sustacal; Wead Johnson, Belleville, ON, Canada) given orogastrically with a feeding needle. This sizeable meal represented 1% of body weight for a weanling gerbil. The control (n=13) and infected (n=8) animals placed in the fasted group remained fasted. After 10 minutes, the gerbils were orogastrically infused with 2.5 μCi of ^{51}Cr, as sodium chromate ($Na_{51}CrO_4$), dissolved in 0.5 ml of water. The radioactive chromium was used as a nonabsorbable marker for measuring the quantitative movement of contents along the lumen of the bowel (136-138). A separate study (data not shown) was performed to demonstrate that this radioactive marker did not bind to trophozoites. Thirty minutes after administration of the marker, the animals were sacrificed by cervical dislocation. Immediately after euthanasia, blood was collected by cardiac puncture and placed in pre-weighed vials. The distal esophagus and distal rectum were ligated and transected to allow gentle exteriorization of the entire gastrointestinal tract. The following segments were ligated with 3-0 silk then divided without spillage into individual counting vials: the stomach, six equal small intestinal segments, the cecum and the colon. Each segment, as well as the blood and the fecal material collected during the 30 minute interval, were counted for gamma emission (LKB-Wallace). The distribution of radioactivity was graphed as a percentage present in a given segment of stomach, intestine, cecum, stool or blood. The transit was represented as the percentage of marker being present in or having gone through a given segment. The amount of radioactivity determined for the individual segments was used to calculate the geometric center (GC) of transit according to the following equation:

$$GC = \frac{\sum \text{counts per segment x segment number}}{\text{total counts}}$$

The geometric center may range from values of 1 to 9, such that a geometric center of 1 indicates that transit out of the stomach and into the small intestine was maximally inhibited, whereas a geometric center of 9 would indicate that all the marker was all in or had passed through the colon.

2.5.2. Intestinal transit

Briefly, gerbils were anesthetized with inhaled 2% halothane following an 18 hours fast. The abdomen, left side and cervical region were clipped and the skin was successively cleaned with iodine and alcohol. A cannula (polyvinyl chloride; i.d. 0.80 mm, o.d. 1.20 mm) was tunneled subcutaneously from an incision in the midscapular region and brought into the peritoneal cavity through an abdominal wall incision. The cannula was then introduced into the lumen of the proximal duodenum and secured with a purse-string suture using 5-0 silk. The abdominal wall was closed with interrupted sutures using 3-0 Dexon and the skin sutured in a similar way using 3-0 silk. The gerbils in the infected group were inoculated with trophozoites soon after their recovery from the anesthesia. The animals were allowed a postoperative recovery period of 6 days, which also allowed maximal trophozoites colonization of the infected animals. On day 6, the animals were fasted for 18 hours. Briefly, 1.25 μCi of ^{51}Cr dissolved in 0.25 ml of water was instilled directly into the duodenum via the duodenal cannula. After exactly 15 minutes, an interval which was found, from a preliminary study (data not shown) to allow optimum progression of the marker along the small intestine, the gerbils were sacrificed. The various segments were rapidly collected as described in protocol 1 and the radioactivity of each was determined in a gamma counter. The distribution of the radioactivity was determined as previously described and the geometric centers were calculated.

2.6. CONTRACTILITY STUDIES

2.6.1. Longitudinal smooth muscle

For each study, controls (n=8) and infected (n=8) animals were sacrificed by cervical dislocation six days post-infection or sham infection. The abdomen was opened and the entire small intestine exteriorized. Three 2 cm segments were resected from the mid jejunum and delicately placed in individual beakers containing warmed, oxygenated physiological saline solution (Krebs). A fourth segment was obtained to test for the presence of trophozoites. Each segment of tissue was mounted longitudinally, using 3-0 silk tread, in a 20 ml standard tissue bath maintained at 37°C and filled with continuously bubbled (95% O_2 / 5% CO_2) Krebs solution. The composition of the Krebs solution was (mM): NaCl 120.3, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, NaH_2PO_4 1.2, and glucose 11.5. The mechanical activity of the longitudinal muscle was detected by an isometric force transducer (Harvard Apparatus Ltd, Model 50-7905, Kent, UK) enhanced by a transducer amplifier (Harvard Apparatus Ltd, Model 50-7970, Kent, UK), relayed to a bioelectric amplifier (Hewlett Packard Model 8811A) and recorded on an eight-channel chart recorder (Hewlett Packard Model 7858A).

The basal and active length-stress relationships were obtained after the tissues were initially suspended and allowed to equilibrate for 30 minutes. The initial length L_i, was determined by stretching the segments until further increase in muscle length caused an increase in muscle tension. From this initial length, the jejunal segments were then progressively stretched by 10% increments. At each increment, and following an equilibration period, the segments were contracted maximally using 10^{-4} M bethanechol (carbamyl-b-methylcholine chloride; Sigma Chemical Co., St. Louis, MO, USA). After each exposure to

bethanechol, the jejunal segments were rinsed twice with fresh Krebs solution and left to equilibrate for 15 minutes. The basal pre-contraction tension was subtracted from the peak bethanechol-stimulated tension to obtain the value of the bethanechol-stimulated contraction. The muscle length at which peak active tension developed was noted as the optimal length (L_o) and was subsequently used to equilibrate the tissues in the following experiments.

The dose-response to the muscarinic agonist bethanechol was determined by exposing the jejunal segments to log molar increments of bethanechol in a non-cumulative fashion with two washes and a 20 minute equilibration period between each increment. The range of concentrations varied between 10^{-9} and 10^{-4} M. The maximum tonic tension recorded at each increment was also obtained in the presence of 10^{-6} M of the Na^+ channel blocker, tetrodotoxin (TTX; Sigma Chemical Co., St Louis, MO, USA). In the experiment using TTX, it was added immediately following the washes and the tissues were allowed a 30 minute exposure period before they were stimulated with bethanechol. This set of experiments using TTX was performed to determined if the findings we obtained were myogenic or neurogenic in origin.

A similar protocol was followed to determine the contractile response associated with non-cumulative, graded KCl concentrations (5.9, 10, 20, 30, 40, 60, 80 mM). In order to maintain the ionicity of the solution, the NaCl concentration was decreased proportionally to every increase in KCl concentration. Maximum tonic stresses in response to the KCl depolarization were determined.

Basal and active contractile responses were normalized to tissue cross-sectional area. Immediately after each experiment, the segments were removed, split, scraped free of mucosa using a glass slide, and individually weighed. The

cross-sectional area (mm^2) of each tissue was determined using the following equation (97):

$$\text{cross-sectional area } (mm^2) = \frac{\text{wet muscle strip mass}}{\text{muscle strip length (mm)} \times \text{density (mg mm}^{-3})}$$

where the density of smooth muscle was assumed to be 1.05 mg mm^{-3}. Histological studies determined the fractional thickness of the wet muscle strip attributable to the longitudinal or circular smooth muscle layer. All calculations of mass were corrected such that tension would be normalized to cross-sectional area of longitudinal or circular smooth muscle alone.

2.6.2. Circular smooth muscle.

In this series of experiments, basal and active contractile responses of control and infected jejunal circular smooth muscle were examined. This study is practically identical to the longitudinal smooth muscle study except that for each animal, three 1 cm long rings of proximal jejunum were suspended transversely in the standard tissue baths. The basal and active length-stress relationship were also determined, this time by progressively stretching the tissues by 20 % increments of L_i or initial diameter.

The dose-response to bethanechol and to graded concentrations of KCl were also determined in a similar fashion as described for the longitudinal muscle studies.

At the end of the experiments, the tissues were also split, scraped and weighed. The cross-sectional area of each tissue was determined, using the

same equation, by simply replacing the length of the muscle strip value by the initial diameter multiplied by π.

2.7. MANOMETRY STUDY

Following the halothane anesthesia, the surgical preparation and the approach as described in protocol 2, gerbils (n=4) were surgically cannulated with a triple port jejunal manometry catheter (polyvinyl chloride; i.d. 0.78 mm, o.d. 2.0 mm). Animals were allowed a postoperative recovery period of 6 days. At the time of recording, the jejunal manometry catheter was perfused with distilled water using a minimally compliant pneumo-hydraulic capillary infusion system (Mui Scientific, Mississauga, Ont., Canada) as previously described (98).

2.8. HISTOLOGY

A section of the mid-jejunum from controls (n=8) and infected (n=8) was resected and fixed using Carnoy's solution and 10% neutral buffered formalin. The sections were embedded in paraffin, sectioned and stained with haematoxylin and eosin. The histologic evaluation was done by light microscopy (Reichert, Austria). The thickness of the longitudinal, circular and total smooth muscle was measured, using a calibrated micrometer and compared between control and infected. Intra epithelial lymphocytes (IEL) were counted from 10 crypt-villus units in each specimen and the number of IEL reported per 100 enterocytes. Another set of sections were stained with alcian blue and safranine O stain and mast cells were counted from five high power fields (x400) and averaged.

2.9. STATISTICAL ANALYSIS

For studies 1 and 2 all data are reported as means ± SEM. Results were analyzed by unpaired t-tests ANOVA.

For studies 3 and 4, results are expressed as means ± SEM. Incremental stretch of muscular tissue generates basal stress as an exponential function of the stretch. Therefore, the data on basal stress (S, in mN/mm^2) at each length (L, expressed as the percentage increment of initial length (L_i)) can be fitted to the equation:

$$S = Ae^{(mL)} + C$$

where A, m and C are constants. For the purposes of this paper, A=1, m defines the rate of exponential increase in stress per the percent increment in L_i, and C is the y-axis intercept if stress is plotted against length on rectangular coordinates with linear scales. As per definition, stress equals zero when the tissue is at its initial resting length (percentage increment of Li = 0), and therefore, by substitution C must = -1

In the bethanechol concentration-response experiments, the longitudinal muscle data on active stress (S, in mN/mm^2) at any concentration of agonist (C) can be fitted to the equation:

$$S = \frac{S_m C}{EC_{50} + C}$$

Where S_m represents the maximum stress developed and EC_{50} represents the concentration that produces half-maximum stress. This equation follows the hyperbolic Michaelis-Menten kinetics when the data are plotted on a linear (x, y)

scale. If the concentration-response data are plotted on rectangular coordinates with a log-linear (x-y) scale then the data take on a sigmoidal shape.

The circular muscle data can be better fitted to the following equation:

$$S = \frac{Min + (Sm - min)}{1 + \exp(-k(C - EC50))}$$

Which corresponds to a classic logistic function applicable to log dose versus effect kinetics.

To determine the parameters defining the basal-length stress and concentration-response relationships, a non-linear, least-squares regression analysis was performed using the statistical software package Systat (Systat; Evanston, IL, USA). To define the differences which might exist between curves and the confidence intervals for comparison of parameter estimates (m for basal-length stress data; S_m and EC_{50} for concentration-response data) a non-linear, least-squares computer analysis was utilized (117). For the KCl experiments, the active stress generated by the longitudinal and circular muscle in response to KCl concentrations of 40, 60 and 80 mM, for both the control and infected tissues are reported as means $\pm$ SEM. The results were analyzed by unpaired t-tests ANOVA. All calculations were performed using a microcomputer (Zenith, 486DX).

3. RESULTS

3.1. QUANTIFICATION OF TROPHOZOITES

All the orogastically inoculated gerbils used throughout these studies became infected with *Giardia lamblia* S_2 strain, as shown in Figure 1, but none of them died from the infection. Large numbers of trophozoites were found throughout the small intestine with an average maximum colonization of 3.2×10^5 trophozoites per cm occurring around day 5 and 6 (Fig. 2).

3.2. TRANSIT STUDIES

For the gastrointestinal transit study, the percentage of transit of ^{51}Cr in the stomach, small intestine, cecum, colon, blood and stools is shown in Figure 3 for control and infected animals in the fasted and fed states. In the fasted animals, a significantly ($p<0.05$) greater amount of ^{51}Cr was found in or had passed through the cecum in infected compared to control animals. In the fed group, a significant ($p<0.05$) increase in percentage of transit, seen in the infected animals compared to controls, was present throughout the whole small and large intestine. This increase in transit in the infected fed animals was also associated with a significantly ($p<0.05$) lower percentage of distribution of the marker in the stomach of infected compared to control animals. This would indicate that in the fed state, infection is associated with a significantly faster gastric emptying. Figure 4 shows that the mean geometric centers of transit for ^{51}Cr in fasted and fed infected animals were significantly ($p<0.05$) greater than in controls which indicates a faster linear gastrointestinal transit.

Figure 1: Light micrograph of the jejunum from a control (A) and an infected (B) gerbil, 6 days post-infection. The *Giardia lamblia* trophozoites (arrow) can be seen in the infected sample. (H & E, x400, Bar = 25 μm)

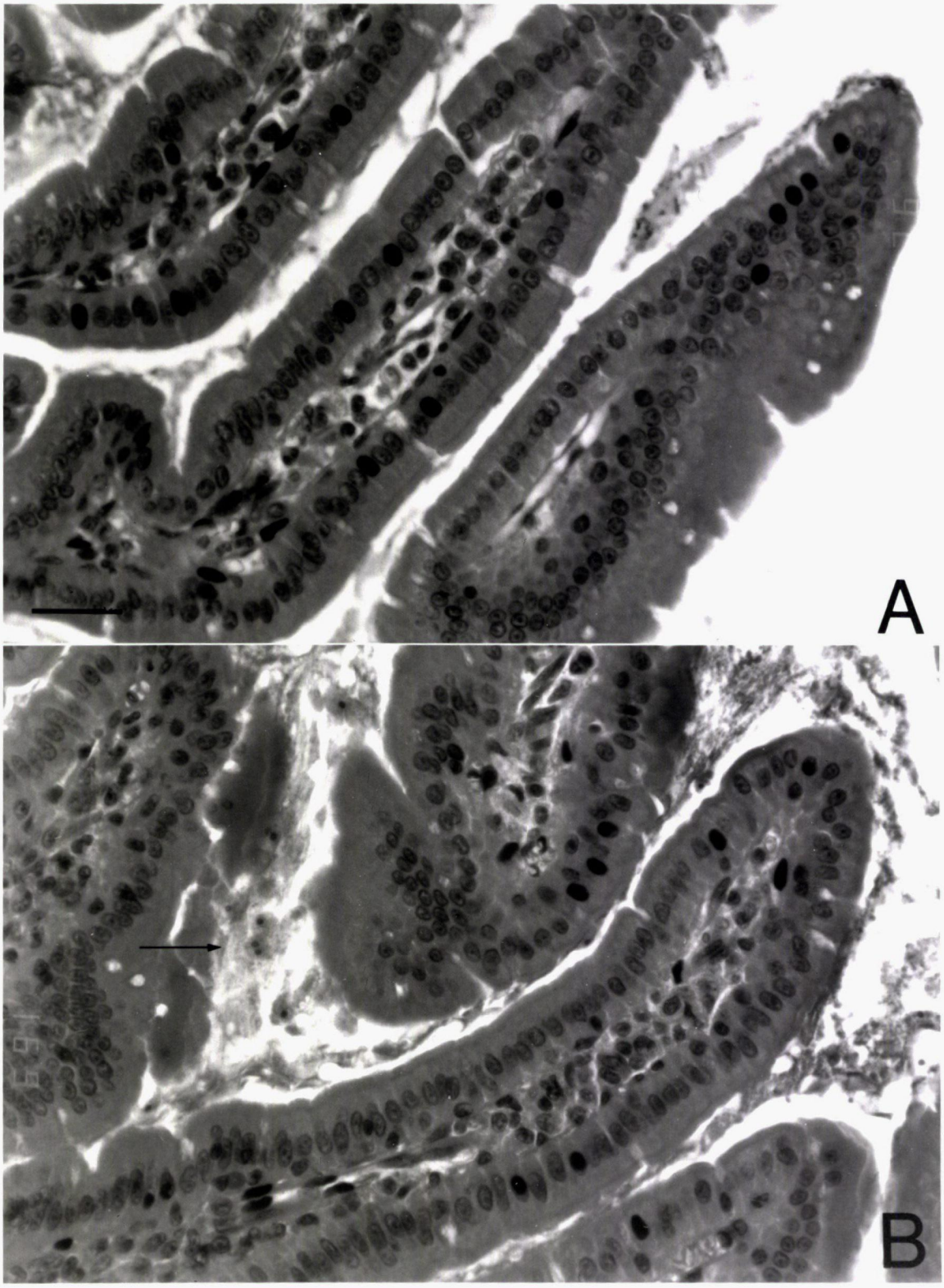

A
B

Figure 2 :Distribution of the trophozoite population along the gerbil's small intestine following oral inoculation with 2 x 10^5 trophozoites. Values are means ± SEM from 3 animals.

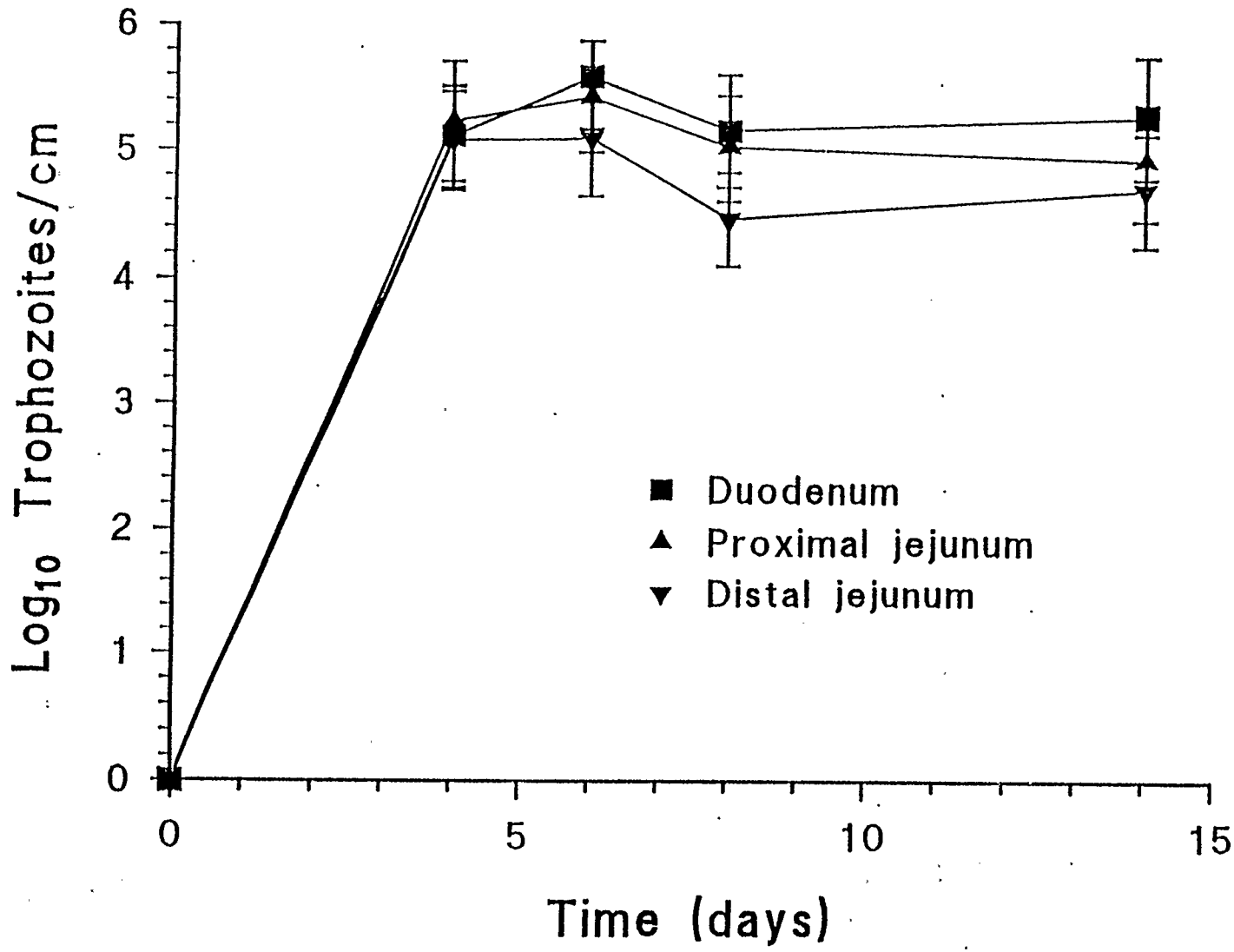
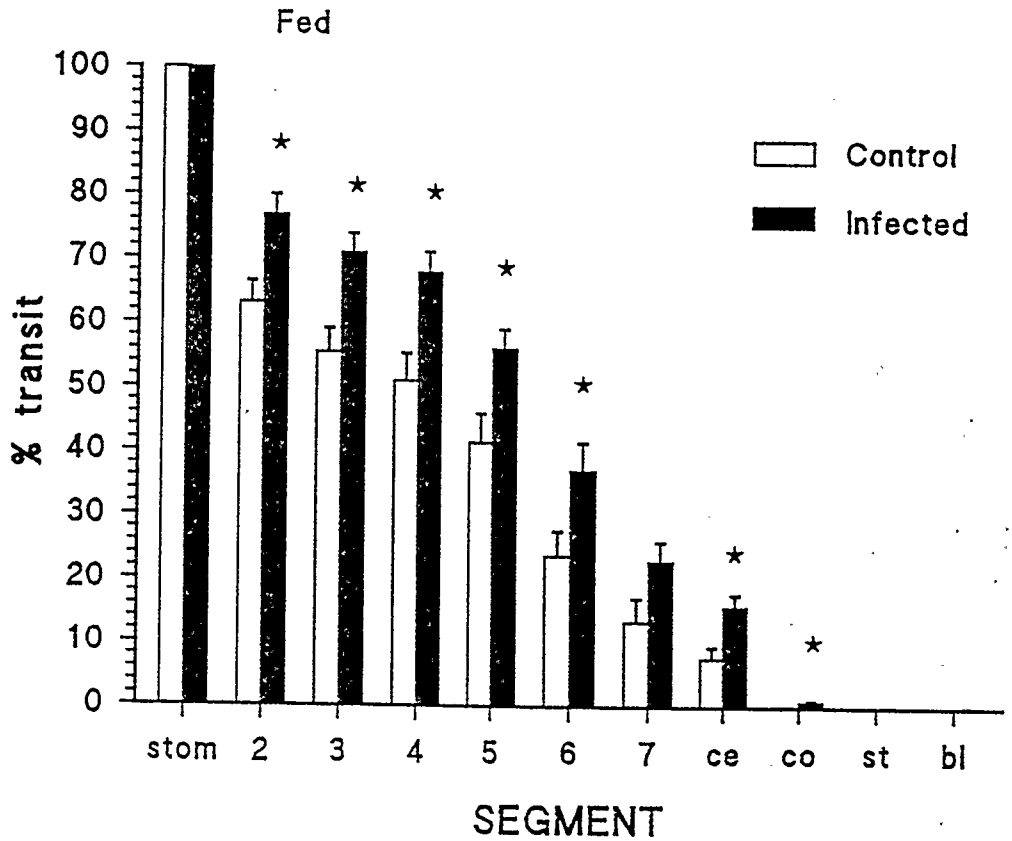

Log10 Trophozoites/cm
6
5
4
3
2
1
0
0
5
10
15
Time (days)
Duodenum
Proximal jejunum
Distal jejunum

Figure 3 : Percentage of transit of $Na^{51}CrO_4$ in fed (upper panel) and fasted (lower panel) animals for control and infected groups, thirty minutes after orogastric infusion of the radioactive marker. Values are means ± SEM; n=8 per group; * $p<0.05$ for infected vs. control.


Fed
100
90
80
70
60
50
40
30
20
10
0
% transit
Control
Infected
stom
2
3
4
5
6
7
ce
co
st
bl
SEGMENT

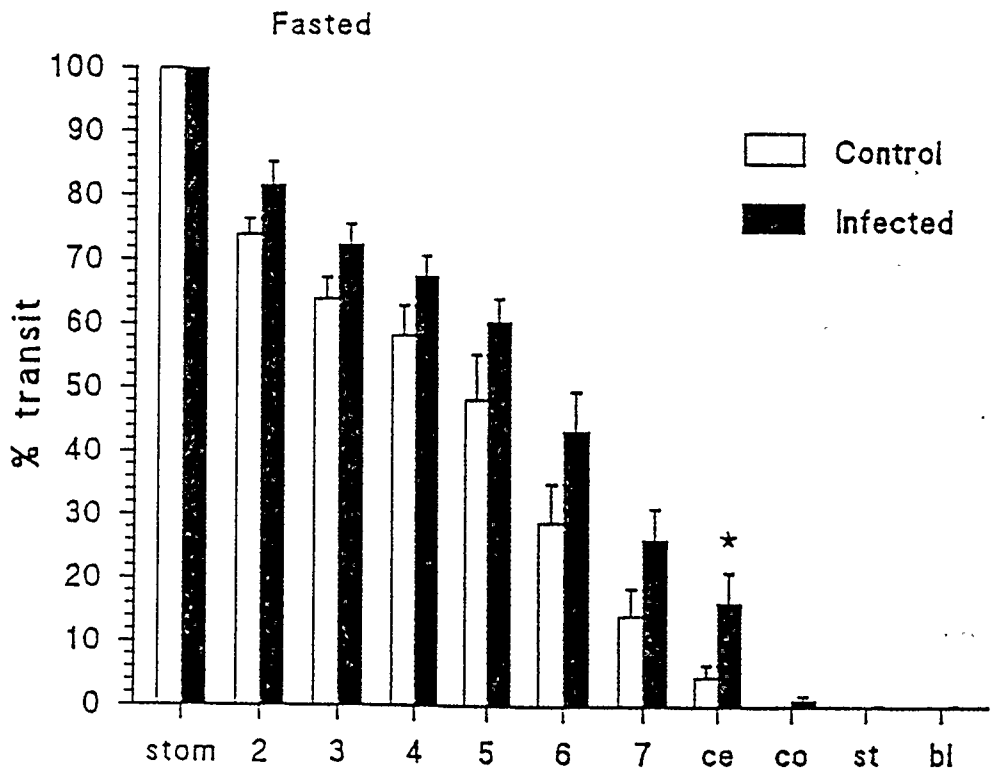

Fasted
100
90
80
70
60
50
40
30
20
10
0
% transit
Control
Infected
stom
2
3
4
5
6
7
ce
co
st
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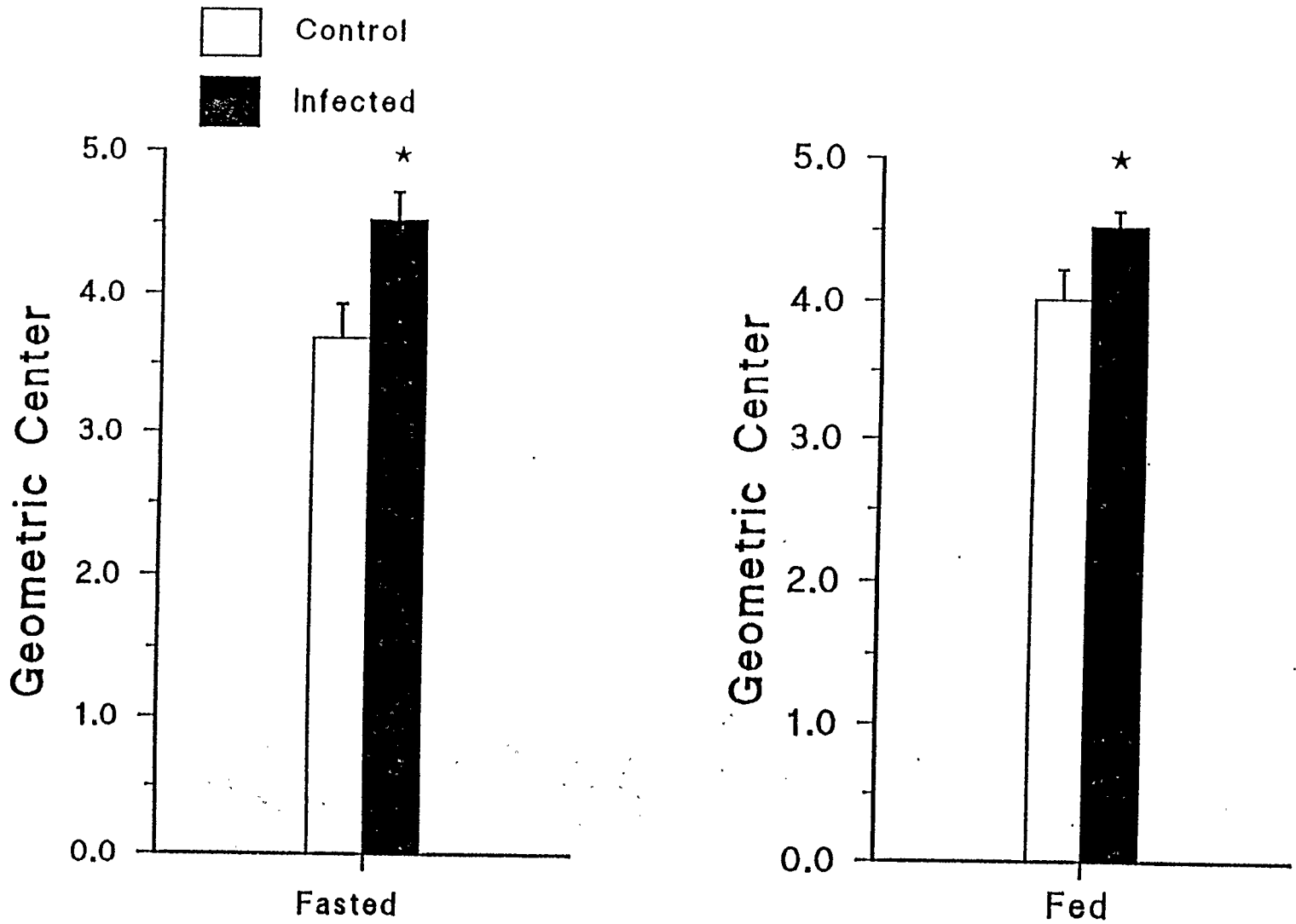
Figure 4.: Mean geometric centers of transit for $Na^{51}CrO_4$ in fasted and fed animals, for control and infected groups, following orogastric infusion of the radioactive marker. Values are means ± SEM; n=8 per group; * $p<0.05$ for infected vs. control.


Control
Infected
Geometric Center
5.0
4.0
3.0
2.0
1.0
0.0
*
Fasted
Geometric Center
5.0
4.0
3.0
2.0
1.0
0.0
*
Fed

In the intestinal transit study, there was a significantly ($p<0.05$) greater amount of radioactive marker present or having gone through the cecum in the infected group compared to the control group as shown in Figure 5. The mean geometric center of transit for ^{51}Cr was also significantly ($p<0.05$) greater in the infected animals compared to controls (Fig. 6). This would indicate that in fasted animals infection is associated with an increased rate of intestinal transit once the component of gastric emptying has been discounted.

Figure 5 : Percentage of transit of $Na^{51}CrO_4$ in cannulated control and infected animals, fifteen minutes after intestinal infusion of the radioactive marker. Values are means ± SEM; n=8 per group; * $p<0.05$ for infected vs. control.

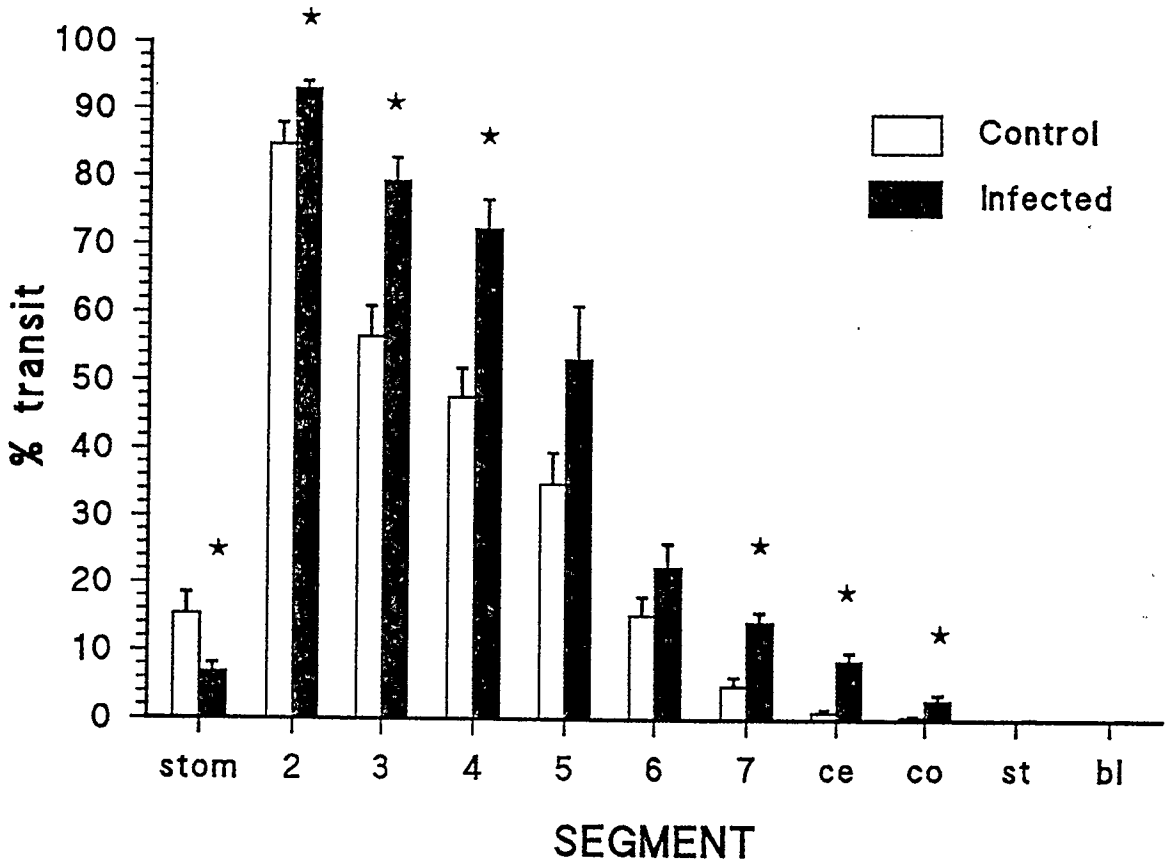
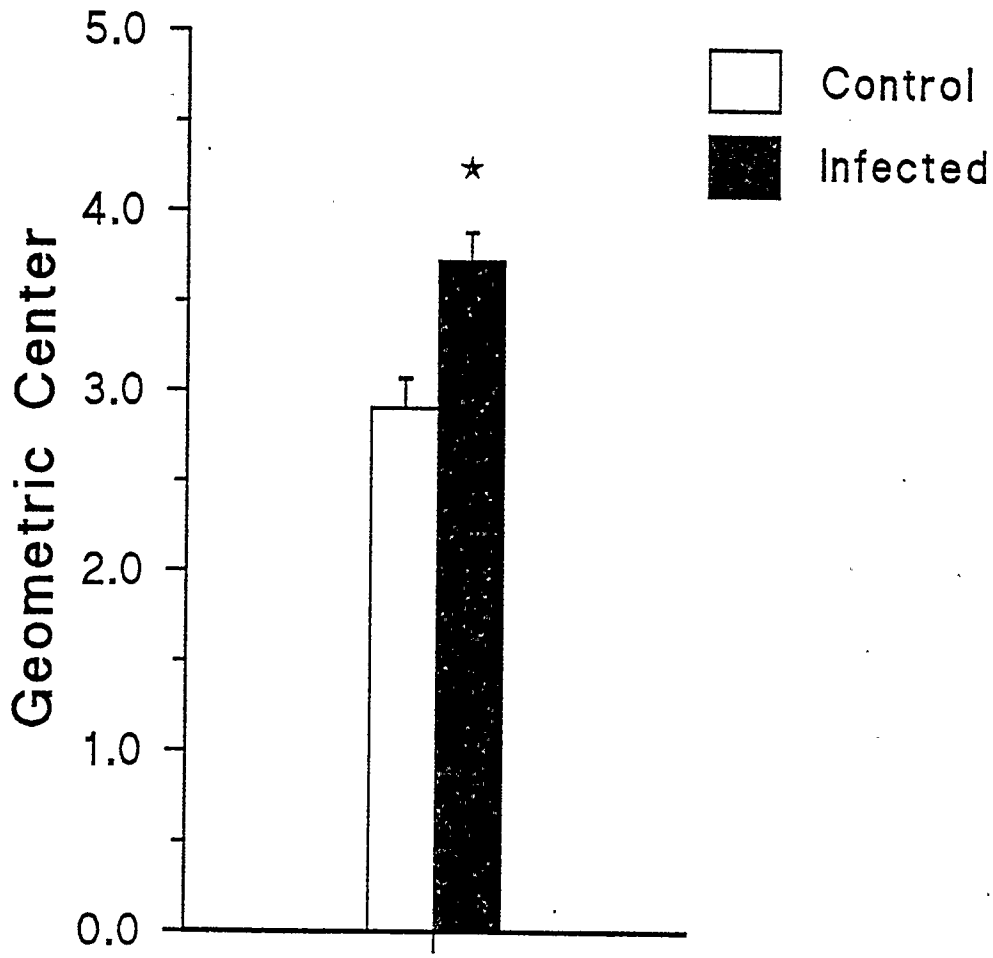

100
90
80
70
60
50
40
30
20
10
0
% transit
Control
Infected
stom
2
3
4
5
6
7
ce
co
st
bl
SEGMENT

Figure 6.: Mean geometric centers of transit for $Na^{51}CrO_4$ in control and infected animals following intestinal infusion of the radioactive marker. Values are means ± SEM; n=8 per group; * $p<0.05$ for infected vs. control.



3.3. CONTRACTILITY STUDIES

Every animal from the infected groups tested positive for the presence of *Giardia lamblia* trophozoites in the jejunum whereas all the controls tested negative. There was no significant difference in the fractional and absolute thickness of the longitudinal or circular muscle between infected and control animals (Fig. 7 and Table 1).

3.3.1. Basal and active length-stress response

Incremental stretch of the longitudinally oriented jejunal segments produced an exponential increase in basal stress. There was no significant difference between control and infected animals when comparing the estimate of the curve parameter 'M' as shown in Table 2. The optimum length (L_o) at which peak active tension developed in the longitudinal segments in response to bethanechol was 110% of L_i in control as well as in infected animals.

An exponential increase in the basal stress of the circular muscle was also observed in response to incremental stretch of transversally oriented jejunal rings. The 'M' parameters listed in Table 2 were significantly different ($p<0.05$) with the infected group being significantly lower in comparison to the control group. This lower 'M' value may well indicate the presence of an increased elasticity in the infected tissues. The optimum length (L_o) in the control tissues was 182% of L_i and was significantly ($p<0.05$) different from the L_o (202%) observed in the infected group. All subsequent experiments were done with tissues at their respective L_o.

Figure 7 : Light micrograph of the jejunum from a control (A) and an infected (B) gerbil, 6 days post-infection. Neither the absolute nor the fractional thickness of the longitudinal (outer layer) and circular (inner layer) muscle differed significantly between the control and the infected groups. (H & E, x200, BAR = 50 μm)

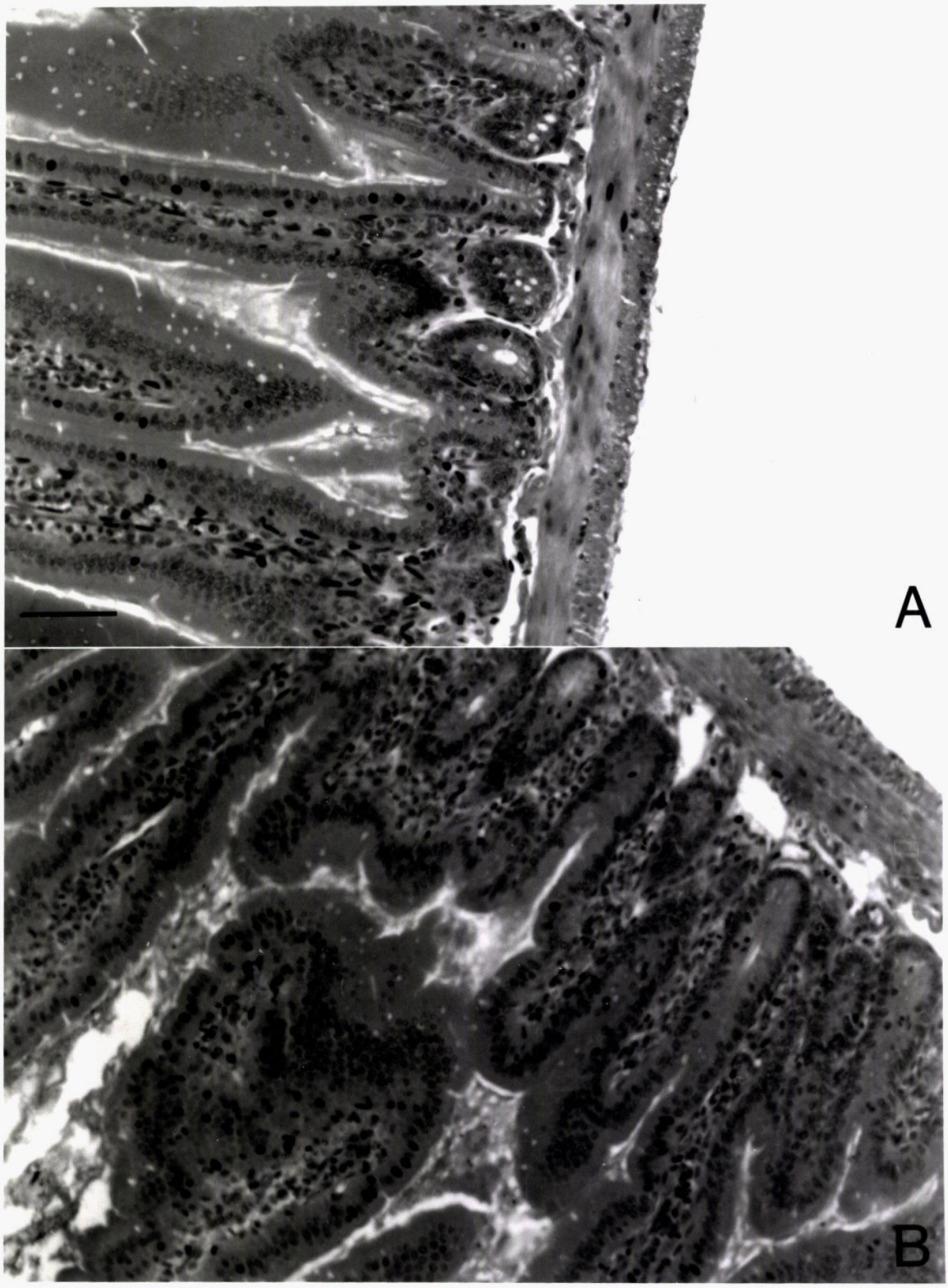

A
B

Table 1

Histological findings

	Control		Infected	
	Absolute (μm)	Fractional	Absolute (μm)	Fractional
Longitudinal muscle thickness	22.10 ± 1.88	0.42 ± 0.02	21.15 ± 1.41	0.43 ± 0.02
Circular muscle thickness	30.08 ± 1.41	0.58 ± 0.02	28.20 ± 0.94	0.57 ± 0.02
Absolute total smooth muscle thickness (μm)	52.18 ± 2.82		49.35 ± 1.41	
Intra-Epithelial Lymphocytes (per 100 enterocytes)	8.12 ± 0.44		9.25 ± 0.41	
Mast Cells (averaged from 5 high power fields)	0.91 ± 0.28		1.23 ± 0.27	

The histological findings 5 to 6 days post infection. Values are means ± SEM; $n \geq 7$ per group.

Table 2

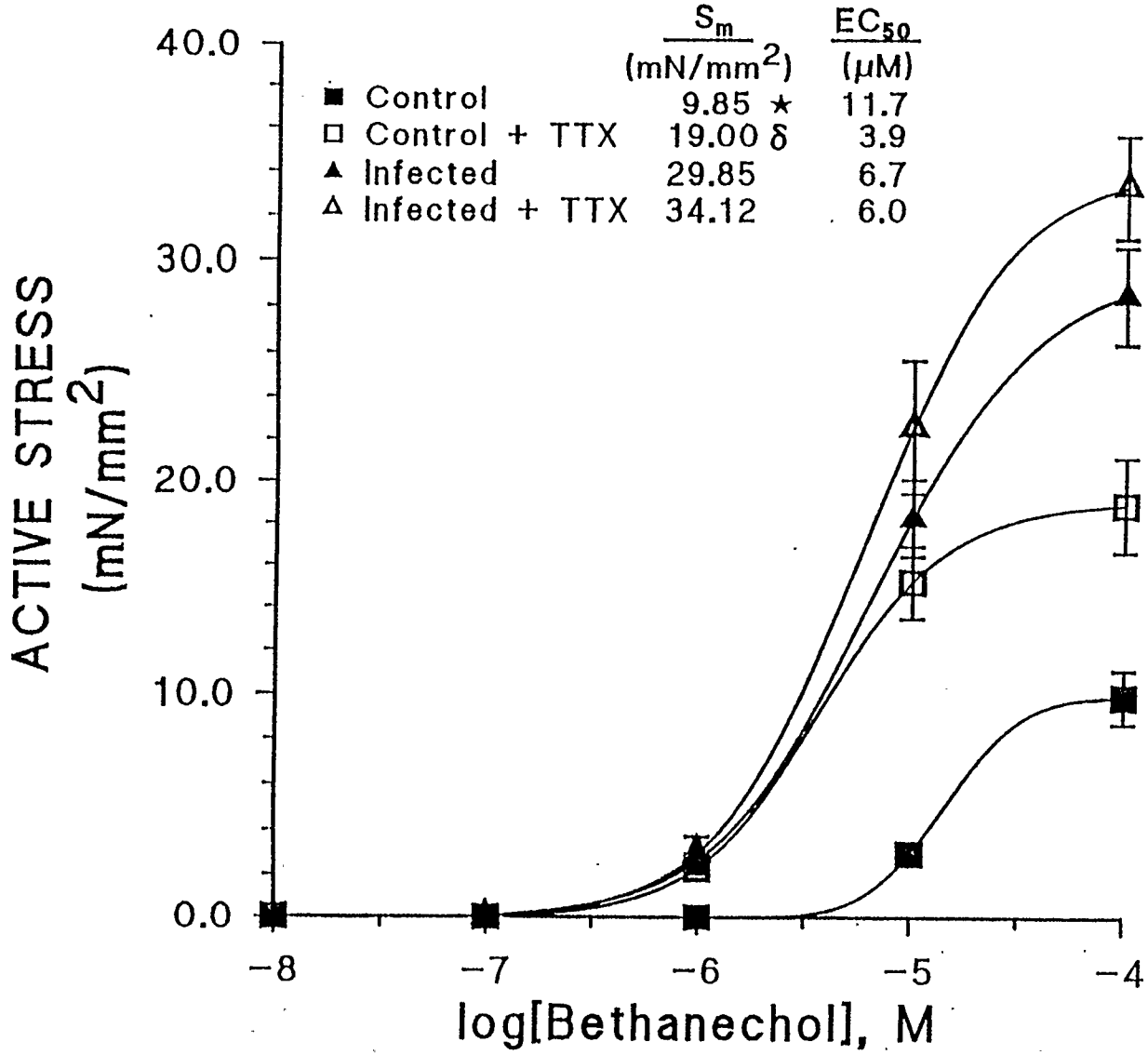
Parameter "m" for basal length-stress response.

Smooth muscle	Control	Infected
Longitudinal	119 ± 3	127 ± 6
Circular	45 ± 1	35 ± 1*

The mean ± SEM (x 10^{-3}) of the parameter 'm' for each group. n=8 per group;

* $p<0.05$ for circular infected vs. control.

Figure 8 : Longitudinal smooth muscle active stress development for control and infected tissues in the presence or absence of 10^{-6} M TTX . Values are means ± SEM; n=8 per group; *$p<0.05$ for infected vs. control. $\delta p<0.05$ for control with TTX vs. infected with TTX and control with TTX vs. control.


S_m (mN/mm^2)
EC_{50} (μM)
Control 9.85 ★ 11.7
Control + TTX 19.00 δ 3.9
Infected 29.85 6.7
Infected + TTX 34.12 6.0
ACTIVE STRESS (mN/mm^2)
40.0
30.0
20.0
10.0
0.0
−8
−7
−6
−5
−4
log[Bethanechol], M

3.3.2. Concentration-response to bethanechol

Figure 8 shows the active stress generated in response to increasing concentrations of bethanechol, by the longitudinal muscle of longitudinally oriented jejunal segments equilibrated at L_o. The stress generated by control and infected tissues in the presence or absence of tetrodotoxin was a sigmoidal function of the increasing concentration of bethanechol. As summarized in Figure 8, segments from the infected groups in the presence (n=8) and absence (n=8) of TTX generated significantly ($p<0.05$) greater maximum stress (S_m) in response to bethanechol than segments from the control groups. The EC_{50} were not significantly different between control and infected groups, and this was seen in the presence and absence of TTX. The addition of tetrodotoxin caused a significant increase ($p<0.05$) in the maximum active stress generated by the control tissues in response to 10^{-4} M bethanechol. Such an increase was not observed with the infected tissue.

Figure 9 illustrates the maximum active stress generated by the jejunal circular muscle in response to increasing bethanechol concentrations. Concentration-response curves for control and infected groups were similar as was determined in the presence or absence of TTX. The values for S_m and EC_{50} are presented in Figure 9. The addition of TTX caused a significant ($p<0.05$) increase in the maximum active stress generated by control and infected tissues. These findings would indicate the presence of an inhibitory neural pathway which is blocked by the addition of tetrodotoxin.

3.3.3. KCL depolarization

Figure 10 shows the active stress generated by the longitudinal muscle of control and infected tissues in response to graded concentrations of KCl .

Figure 9 : Circular smooth muscle active stress development for control and infected tissues in the presence or absence of 10^{-6} M TTX. Values are means ± SEM; n=8 per group; *$p<0.05$ for control vs. control with TTX. $\delta p<0.05$ for infected vs. infected with TTX.

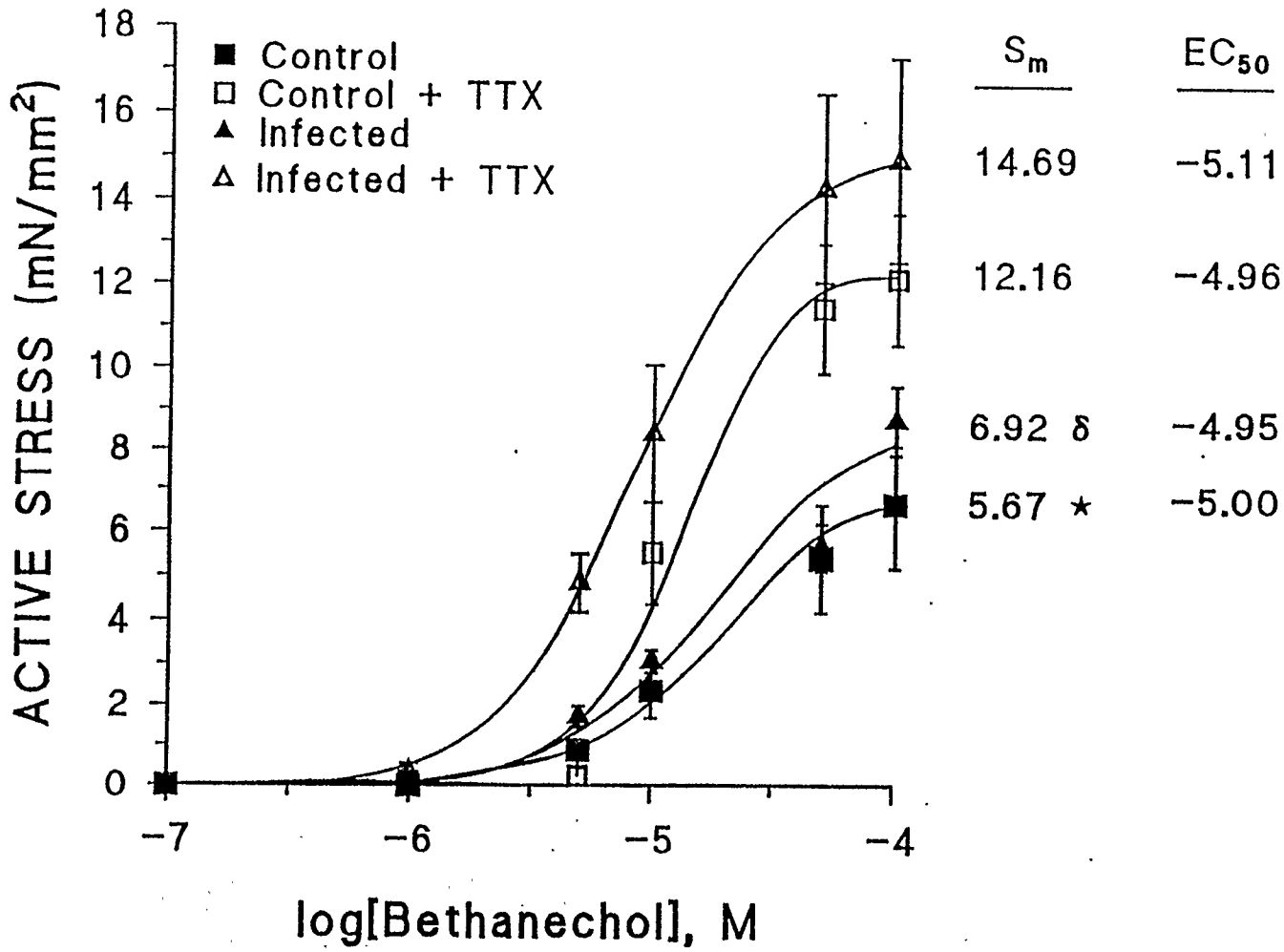
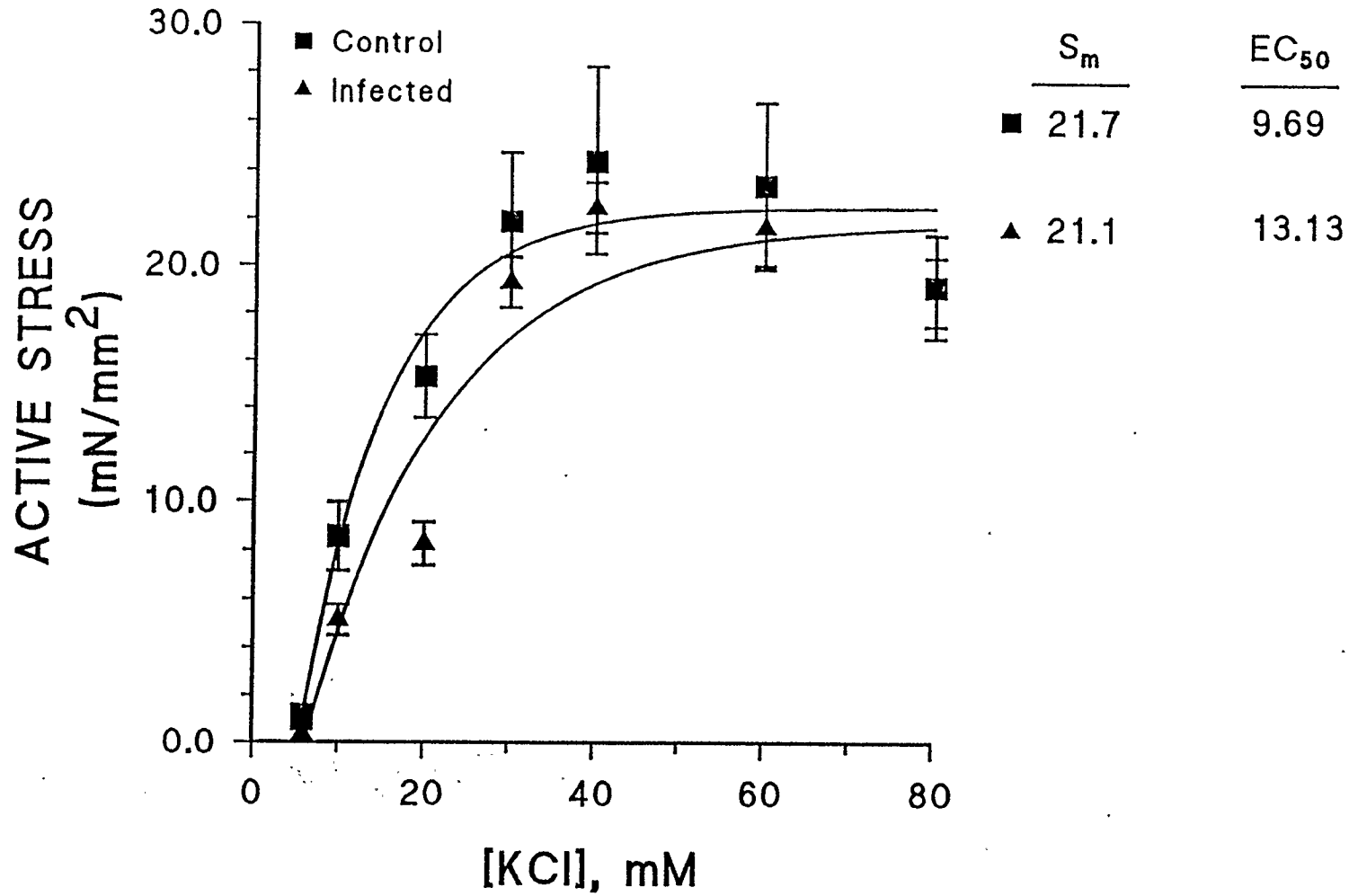

ACTIVE STRESS (mN/mm²)
18
16
14
12
10
8
6
4
2
0
Control
Control + TTX
Infected
Infected + TTX
−7
−6
−5
−4
log[Bethanechol], M
Sm
EC50
14.69
−5.11
12.16
−4.96
6.92 δ
−4.95
5.67 ⋆
−5.00

Figure 10 : Longitudinal smooth muscle active stress development for control and infected tissues in response to graded concentrations of KCl. Values are means ± SEM; n=8 per group.


30.0
20.0
10.0
0.0
ACTIVE STRESS
(mN/mm2)
Control
Infected
0
20
40
60
80
[KCl], mM
Sm
EC50
21.7
9.69
21.1
13.13

Possible artifacts, observed during the addition of fresh buffer to the tissue baths, were eliminated from our calculations by subtracting the tonic contraction response to 5.9 mM KCl (concentration of KCl in normal Krebs buffer) from subsequent responses. There was no significant difference between the control and infected tissues at concentrations of KCl of 40, 60 and 80 mM as shown in Table 3.

Similar findings, as shown in Figure 11 and Table 3, were obtained with the jejunal circular muscle from control and infected groups. There was no significant difference between the active stress generated by infected and control tissues in response to graded concentrations of KCl.

3.4. MANOMETRY STUDIES

Despite the use of a minimum (3 PSI) rate of infusion during the manometric recordings, we were unable to record intestinal motility for a sufficient period of time in our gerbil model. Even after only ten to fifteen minutes of recordings, the animals would develop ileus and seizures. Therefore, we were unable to obtain reliable *in vivo* recordings of the intestinal motility in our weanling gerbil model of giardiasis.

3.5. HISTOLOGY

As shown in Figure 7 and Table 1, there was no significant difference between infected and control animals, for the longitudinal and circular smooth muscle absolute and fractional thicknesses as well as for the total absolute thickness of the muscularis externa. The numbers of intraepithelial lymphocyte (per 100 enterocytes) (Fig.12 and Table 1) and mast cells (Fig.13 and Table 1) were not significantly different between control and infected animals.

Table 3

Active stress in response to graded concentrations of KCl

Smooth muscle	KCL (mM)	Active Stress (mN/mm^2)	
		Control	Infected
Longitudinal	40	24.37 ± 3.86	22.45 ± 1.05
	60	23.39 ± 3.37	21.64 ± 1.80
	80	19.15 ± 2.19	18.92 ± 1.44
Circular	40	11.76 ± 0.60	12.73 ± 0.73
	60	19.66 ± 2.36	16.86 ± 1.06
	80	20.60 ± 2.28	17.44 ± 1.18

Values are means ± SEM; n = 8 per group.

Figure 11 : Circular smooth muscle active stress development for control and infected tissues in response to graded concentrations of KCl. Values are means ± SEM; n=8 per group.

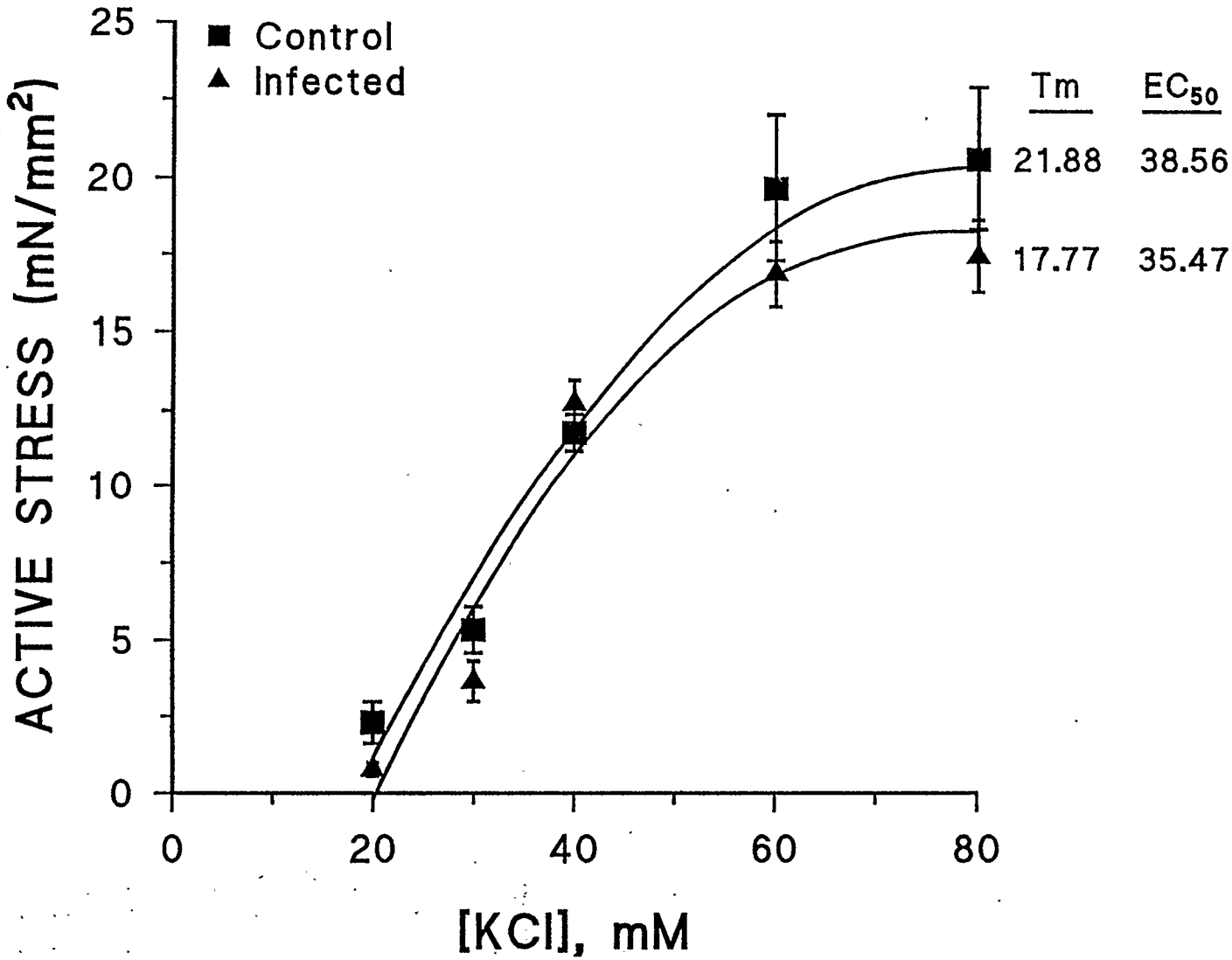

Control
Infected
Tm
EC50
21.88
38.56
17.77
35.47
ACTIVE STRESS (mN/mm2)
25
20
15
10
5
0
0
20
40
60
80
[KCl], mM

Figure 12 : Light micrograph of the jejunum from a control (A) and an infected (B) gerbil, 6 days post-infection. The number of intraepithelial lymphocytes (arrow) was not significantly different between control (A) and infected (B) tissues. (H & E, x400, Bar = 25 μm)

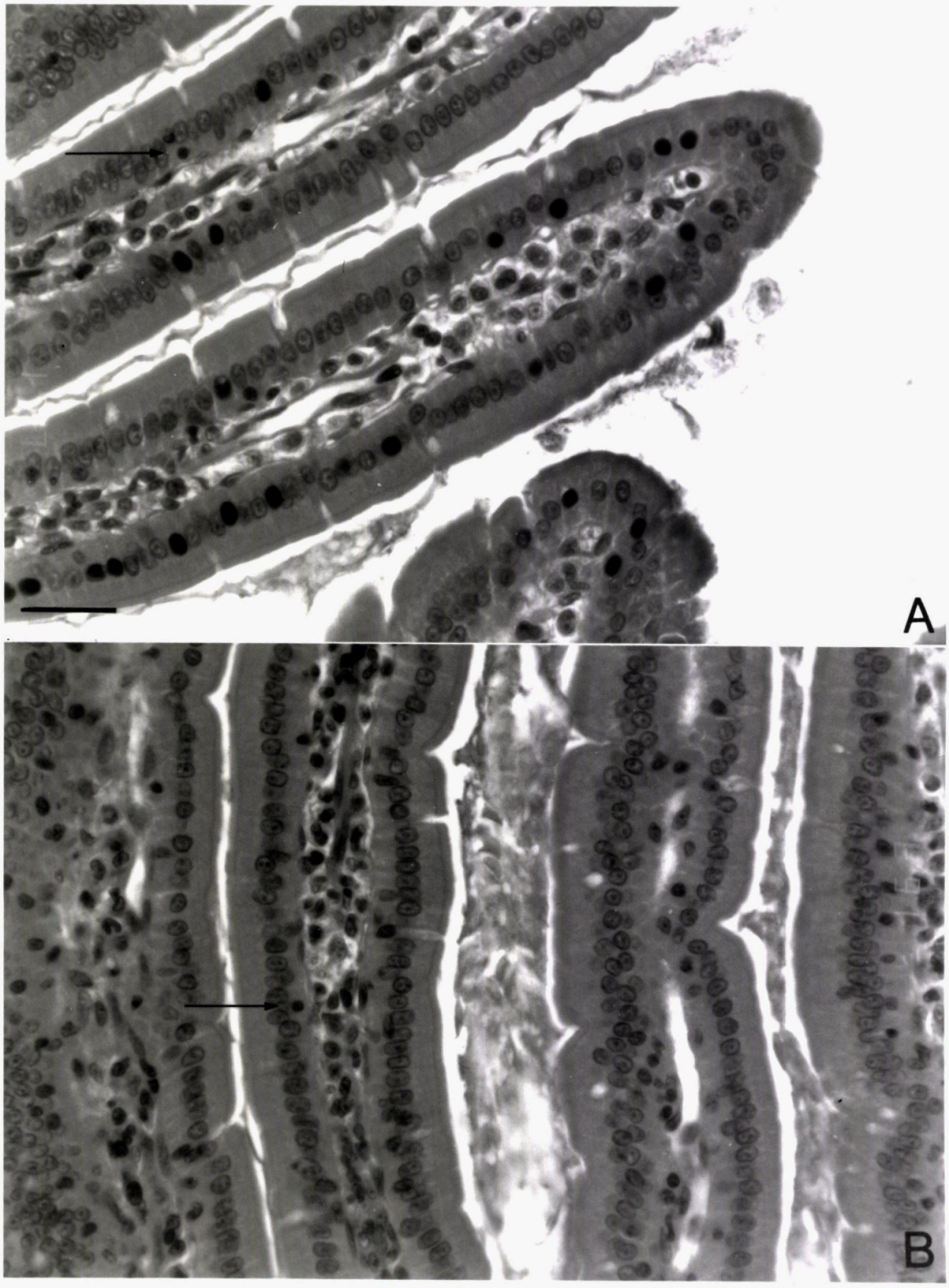
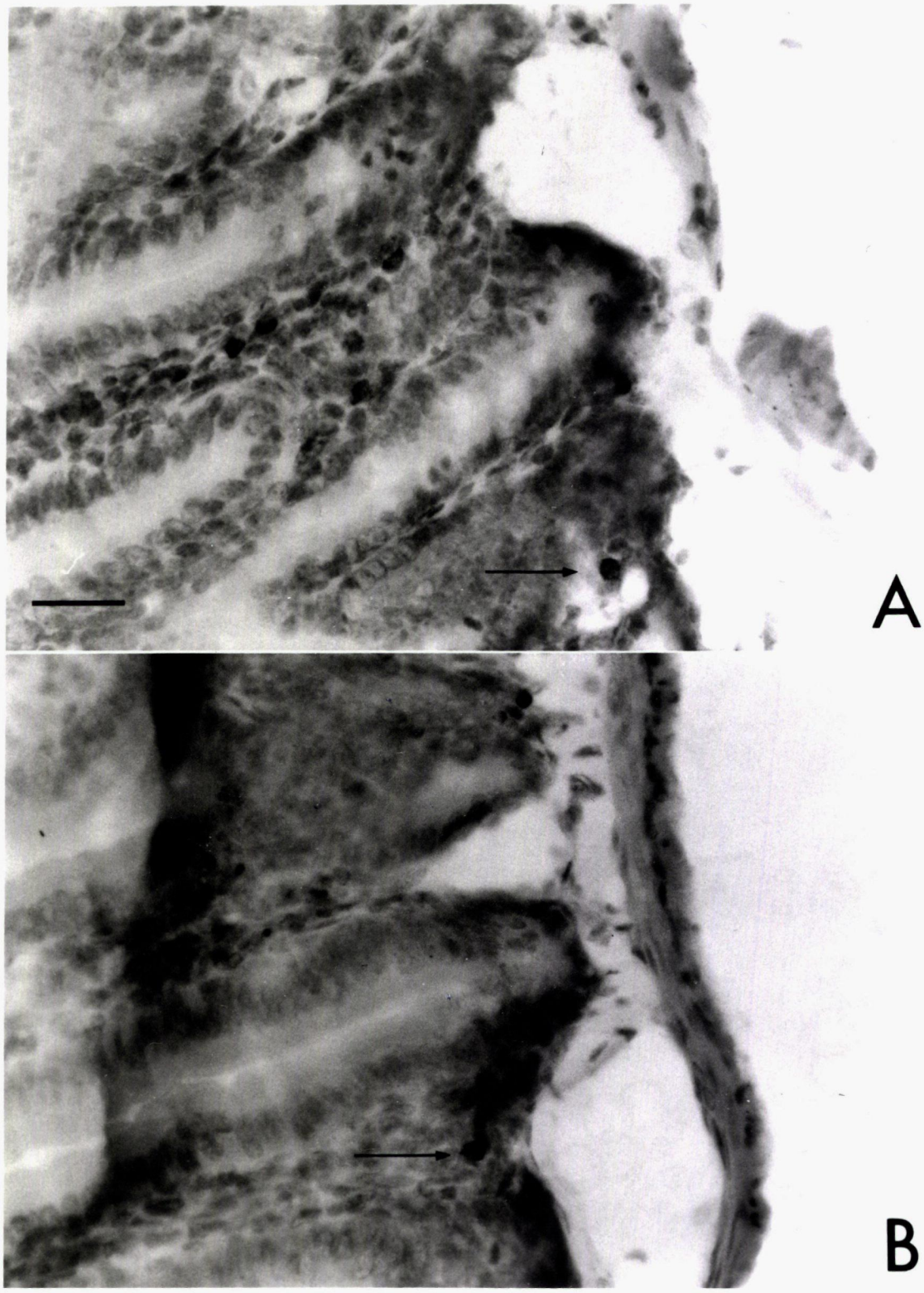

A
B

Figure 13 : Light micrograph of the jejunum from a control (A) and an infected (B) gerbil, 6 days post-infection. The number of mast cells (arrow) was not significantly different between control (A) and infected (B) tissues. (Safranine O, x400, Bar = 25 μm)


A
B

4. DISCUSSION

This study investigates the presence of a motility disorder as part of the pathophysiology of giardiasis, using a weanling gerbil animal model. The time of peak colonization of the *Giardia lamblia* S_2 strain trophozoites was on the fifth and sixth day post infection. This finding was similar to those previously published for this particular animal model (118). Although colonization was equally uptimal in all three regions of the gut, we elect to use the proximal jejunum for the contractility studies. Due to its adequate length, this region allowed appropriate sampling and furthermore, some of the changes associated with giardiasis in the gerbil model, such as the decreased glucose absorption, are only observed in this localization (54). According to previous results obtained in our laboratory, control and infected animals do not show a significant difference in daily food intake and, although weight gain is decreased in the infected animals by day 8, it remains similar to control prior to that point in time. Therefore, in the following experiments, the animals were studied on day 5 or 6, were not pair-fed and the *in vitro* studies were done using the proximal segments of the jejunum.

The present findings demonstrate that *Giardia lamblia* can induce, in a gerbil animal model, an increase in the rate of intestinal transit. This increased rate was seen in fed and fasted animals, five and six days post infection. Furthermore, this increase was accompanied, in the infected fed animals, with a faster gastric emptying rate compared to controls. These findings demonstrate the presence of an overall faster gastrointestinal transit rate in the infected fed animals. Using a surgical method to by-pass the stomach and therefore eliminate gastric emptying, we confirmed that the intestinal transit rate was increased as

well, in infected fasted animals compared to controls. Knowing that the rate of transit of a given meal is not directly related to gastric emptying unless this is very rapid or very slow (119) and due to technical restrictions, we limited our comparison of the intestinal transit, using a cannula, to fasted animals only (119).

Other models of intestinal parasitism, such as the *Trichinella spiralis* (120), and *Nippostrongylus brasiliensis* (121) infections, as well as the *Yersinia enterocolitica* (97) model of bacterial enteritis, reported similar increases in transit. However, the present study is the first to report an increase in intestinal transit associated with this non-invasive organism (47).

This accelerated transit suggests that giardiasis may be associated with a motility disorder. However, it is important to keep in mind that theoretically, any given transit time is equal to the capacity of the bowel divided by the flow rate of the intestinal content. Moreover, flow rate is directly proportional to the pressure developed in the intestinal segment on the luminal content and inversely proportional to the resistance encountered along the way. Thus, a condition causing a decrease in transit time, such as giardiasis, could bring about this accelerated transit via i.) a reduction in resistance (decrease in segmental contractions), ii.) an increase in pressure (increase in propulsive contractions or increase in intestinal content due to decreased absorption or increased excretion), and/or, iii.) a decrease in capacity (increase in smooth muscle tone or muscle hyperplasia/hypertrophy with subsequent decrease in lumen diameter) (122). It is important to remember that transit time may also be influenced by factors other than those directly linked to intestinal motility. Hence, disorders affecting intestinal secretion and absorption may influence motility by inducing an increase in luminal fluid content and intra-luminal pressure. Agents such as lactulose and metoclopramide both accelerate small bowel transit. However,

while metoclopramide stimulates intestinal contractions by its direct effect on smooth muscle, lactulose, which is a nonabsorbable disaccharide, reduces transit time by retaining fluid in the lumen, via its osmotic activity, therefore stimulating peristalsis (123).

All of these factors that directly affect transit rate have to be examined while taking into consideration what is known of the pathophysiology of giardiasis. Previous findings indicate that giardiasis, studied using this particular animal model, is not associated with an increased fluid secretion (47). The possibility, however, that the presence of malabsorption may induce, via an osmotic effect, an increase in intestinal bulk and intraluminal pressure leading to an accelerated transit, remains to be determined. According to the histopathological findings from the present study and from human biopsies, and conversely to the *T. spiralis* and *N. brasiliensis* enteritis models, in giardiasis there is no increase in the thickness of the infected jejunum muscularis externa. Although this lack of muscle hyperplasia or hypertrophy in giardiasis would not be expected to alter intestinal capacity, the presence of an alterd muscle tone is possible. Moreover, one could speculate that such a possible increase in smooth muscle tone could contribute to a decreased rate of water absorption (124) although such a conclusion would require further evidence. In addition, while increased intestinal bulk is capable of influencing motility, the reciprocal effect of an increased transit rate, associated with decreased contact time, may well prevent adequate absorption. Hence the difficulty to isolate either effect, and study the significance of each one independently.

An accelerated transit might also arise following a modification at higher levels of organized patterns. Thus, in giardiasis, a reduction in segmental contractions or an increase in propulsive contractions could act to decrease

intestinal transit time. Whether or not a proximal increase in propulsive motility, alone or accompanied by an increased secretion and/or decreased absorption, will result in diarrhea greatly depends on two control mechanisms. The "ileal brake", which causes, in the presence of fats and fatty acids in the ileum, inhibition of jejunal motility, and the colonic salvage, which is responsible for the absorption of increased ileal effluent (135).

In our model of giardiasis, the gastrointestinal transit time was decreased not only in the fasted animals but also in the fed ones. These findings differ from the published results on the fed state motor activity in the *Trichinella spiralis* and *Cholera* toxin dog models (105, 125). Thus, the increased mean transit times reported in those two models, both mainly due to a reduction in the frequency and distance of propagation of the migrating contractions, were believed to result from a compensatory mechanism adopted to allow more contact time for absorption. However, considering that this beneficial effect is overcome by the presence of giant migrating contractions (also referred to as MAPC by some authors (135)) in the fed state of the Trichinella model, it is too early to conclude that such compensatory mechanism may exist and be effectively recruited in any given diarrheal state.

Our attempt to evaluate *in vivo* motility in our gerbil model of giardiasis failed due to technical difficulties associated with the size and physiology of this particular animal model. Thus, the weanling gerbil, which has been well characterized as a model for giardiasis (11, 54, 126) is not an appropriate laboratory animal to obtain manometric or myoelectric recordings. This is due to its poor ability to recover from either the extensive surgery involved or the techniques associated with manometric recordings. The gerbil's intestinal tract is adapted to desert conditions and a gerbil's daily water intake is proportionally

much smaller than that of rats (gerbil: 0.05 ml/g BW, rat: 0.15 ml/g BW). This explains why a continuous intestinal infusion of water or saline, although minimal, is so detrimental to their normal intestinal physiology. Nevertheless, the characterization, in another model, of the intestinal activity associated with giardiasis, in terms of myoelectric and motor patterns, would yield essential information allowing us to link the increased transit with possible myoelectric and motor disturbances. It is unlikely that RBAPs are associated with giardiasis since this organism is non-invasive and RBAPs are generally believed to promote gut stasis, not accelerated transit. However, the involvement of MAPCs, which are not solely associated with secretory diarrhea, is plausible. To hypothesize that a putative *Giardia* secretory-excretory product could modulate the reflex arcs within the enteric nervous system is certainly as tempting as the suggestion that such "toxin" may affect the intestinal mucosa. In any case, stronger evidence will be necessary before such an idea should even be discussed.

Usage of diagnostic techniques such as perfused tube and ambulant manometry as well as radiotelemetry in human volunteers suffering from symptomatic giardiasis would greatly enhance our knowledge of the disease. Thus, such an approach would not only document the presence of a motility disorder, but could also enable us to gain a better understanding of the origin of some of the symptoms of giardiasis, such as abdominal pain, cramping, vomiting as well as bloating sensations. Furthermore, the role played by bacterial overgrowth in the pathophysiology of the disease could also be evaluated. Although bacterial overgrowth has been associated with similar nonspecific gastrointestinal symptoms as giardiasis (127), the impaired MMC activity in patients suffering from bacterial overgrowth syndrome (128, 129) hardly correlate with the possibility of an accelerated transit as suggested by our findings.

To further investigate the causes for this increased transit rate, the effects of the infection on the *in vitro* contractility of the longitudinal smooth muscle was examined. The present study demonstrates that the longitudinal muscle from infected jejunal segments shows an altered contractile function. Although there is no difference between the control and the infected groups in terms of basal stress development and optimum active stress (L_o), there is a significant increase in the tonic response stimulated by the muscarinic agonist, bethanechol, in the infected animals compared to controls. This would indicate that the increased responsiveness to bethanechol observed in the infected tissues is not likely caused by possible alterations in the tissue elasticity or composition. Moreover, this increased contractility was not caused by a modification in the muscle architecture since our histological measurements showed that there was no significant difference between controls and infected segments, for both the longitudinal and circular smooth muscle, in both the fractional and total thickness. Finally, this increased contractility was reproducible in the presence of the sodium channel blocker, tetrodotoxin which inhibits axonal conduction primarly along the S / type 1 neurons of the enteric nervous system. This indicates that the alteration is most likely myogenic and not neurogenic in origin. The overall increased tone generated by the control tissues in the presence of TTX confirmed the presence of a neural inhibitory pathway. The fact that infected tissues did not show a similar significant increase in tone in the presence of TTX suggest that the infection may affect this inhibitory pathway in the longitudinal muscle. The fact that there was no significant difference in the active stress between the infected and control groups in response to graded depolarizations of KCL indicates that the site of alteration associated with the increased contractile ability of the infected tissues is probably proximal to the activation of the

contractile apparatus. While characterization of the precise mechanisms involved still requires further investigation, this may reflect changes in either the muscarinic receptors (number or subtype expression), the signal transduction pathways or perhaps the Ca^{2+} storage/mobilization.

Similar increases in isometric force generation by the jejunal longitudinal muscle were reported in other models of intestinal parasitism (104, 130). However, it appears that the potential mechanisms involved in this present model of giardiasis would differ from those implicated in the *Trichinella Spiralis* and *N. brasiliensis* rat models (102, 104, 130, 131). In any case, altered receptor-mediated contractions have been reported in at least one other model of enteritis as shown by studies done on small intestine smooth muscle from Crohn's patients (96). Moreover and conversely from these nematode models (101, 103), our model of giardiasis, when examined at the time of peak colonization, is neither characterized by smooth muscle hyperplasia nor by gross evidence of inflammation as indicated by our histological findings. Thus, in keeping with previous results from our laboratory (51), at the time of the study (day 5 and 6), when both villus and microvillus height as well as dissacharidase activity are known to be significantly decreased in the gerbil model (54), there is no significant increase in intraepithelial lymphocytes in infected tissues. These findings are similar to the *Giardia muris* mouse model, in which intraepithelial lymphocyte numbers were found to only increase after villus shortening and after brush border disaccharidases reduction had occurred (49). Furthermore, there was no evidence of mast cell infiltration at this time of infection.

Despite these experimental findings, the role played by T-lymphocyte activation in the pathogenesis of giardiasis remains controversial. The reduced severity in villus architecture abnormalities observed in infected nude (athymic)

mice compared to infected euthymic animals (61) along with the induction of crypt cells hyperplasia and villous atrophy associated with the activation of T-lymphocytes, both strongly support the idea that mucosal inflammation is involved in the pathogenesis of the disease. As far as contractility is concerned, there is evidence to further support such a possibility. Thus, T-cell activation has been linked to the development of the increased muscle contractility seen in the inflamed intestine of the *T. spiralis* infected mouse (132) and Sprague-Dawley rat (131). Although further confirmation is needed, it is believed that such muscle changes would be associated with helper T lymphocytes, not CD8+ intestinal intraepithelial lymphocytes. The mechanisms involved in the interaction between lymphocytes and smooth muscle cells in the inflamed *T. spiralis* infected intestine is unclear but may implicate cytokines and possibly phenotypic transformation of the smooth muscle cells as seen in the vascular smooth muscle (131). These additional results imply that the causative role of mucosal inflammation involving T-lymphocyte activation, in the structural, functional and contractile abnormalities observed during the course of the disease may be of some significance. Further characterization of the mucosal inflammation present in our model, in terms of its nature and location, over the time course of the disease, will shed some light on the actual role of the host response in the pathophysiology of the disease.

The increased contractility observed in the longitudinal smooth muscle of our model is consistent, in terms of the possibility of an augmented tone leading to a decreased transit time, with our *in vivo* findings. However, to obtain a more comprehensive understanding of the *in vitro* smooth muscle contractility in our model of giardiasis, and to allow further correlation between our *in vivo* and *in vitro* studies, we also examined the circular smooth muscle layer. Our findings show that in the circular smooth muscle, the basal stress development in the

infected tissues was significantly lower than in the control tissues. This finding was accompanied by a greater optimum length in the infected tissues. This could indicate a modification in the composition or elastic property of the infected circular muscle. These findings were, however, not associated with altered contractility of the circular muscle. Actually, there was no significant difference between control and infected animals in response to graded concentrations of KCl or to log molar increments of bethanechol, alone or in the presence of TTX.

As mentioned earlier, similar differences between the longitudinal and the circular smooth muscle responses were reported in other models of enteritis. Thus, the circular muscle strips from *N. brasiliensis* infected rats showed a decreased responsiveness to cholinergic and adrenergic agonist (day 8-10 post-infection) whereas the longitudinal muscle from infected animals generated a significantly greater tension than control tissues (day 7-9) (100, 130). The physiological importance of such altered contractility in either the circular or longitudinal smooth muscle of this nematode model remains difficult to ascertain. Although there was a slight and transient increase in transit on day 8 of infection, little change was noted on days 10, 12 and 14 while a small decrease in transit was found on day 6. The reasons for the variability in contractile response between both muscle layers is unknown. However, it has been recognized that inflammatory mediators such as prostaglandins may induce variable responses in the longitudinal and circular muscle within a given region of the gut. Thus, PGE_2 produce *in vitro* contractions in the longitudinal smooth muscle of the rat stomach and the guinea pig colon whereas the same prostaglandins cause the circular smooth muscle of the same tissues to relax (133, 134). However, since the absence of overt histopathological inflammation characterizes the infected gerbil small intestine, such factors may not serve to explain the discrepancy observed

between the contractility of the two smooth muscle layers in our model of giardiasis. In addition, the fact that isometric studies cannot always correlate with an increased isotonic function, as it was demonstrated in the sensitized canine airways (88), implies that the *in vivo* situation might actually differ from the *in vitro* findings. Nevertheless, our findings demonstrate the importance of examining both layers in the study of a disease state.

In summary, our findings show that *Giardia lamblia* is capable of accelerating intestinal and gastrointestinal transit rate in the gerbil model. This modified transit is seen in connection with an increased contractility in the longitudinal smooth muscle of the infected jejunum. The question of whether an increase in contractility in the longitudinal muscle alone would be sufficient to cause an acceleration in transit during giardiasis still remains unanswered at the present time. Nevertheless, these findings suggest that the abdominal cramping, bloating and pain as well as the diarrhea observed during giardiasis may be due in part to alterations of normal gastrointestinal transit and smooth muscle contractility. The possibility of host factors involvement in these gastrointestinal disturbances still needs to be demonstrated.

5. CONCLUSION

In giardiasis, symptoms such as abdominal cramping, bloating and vomiting are often reported along with complaints of diarrhea (9, 32). Since similar symptoms have been described in other gastrointestinal diseases which are known to implicate motility disorders (77, 79, 81, 82, 83), the possibility that altered motility may play a role in the pathophysiology of giardiasis was investigated. Using the weanling Mongolian gerbil as an animal model, the following findings were obtained.

The oral inoculation in weanling Mongolian gerbils of $2x10^5$ *Giardia lamblia* trophozoites results in a peak colonization occurring 5 to 6 days post- infection and mainly localized in the proximal small intestine. At this time the gastrointestinal transit in both fed and fast states, and the intestinal transit in the fasted state, were significantly greater in the infected compared to control animals. The contractility studies performed at the same time post-infection, indicate that in the longitudinal smooth muscle of the proximal jejunum, the development of active stress in response to increasing concentrations of bethanechol was significantly increased in the infected animals compared to controls. This significant increase was still seen in the presence of TTX but was not observed in response to KCl depolarization. Therefore, this altered contractility of the infected tissues may reflect receptor-dependent changes in the smooth muscle function. However, such increased contractility in response to a muscarinic agonist was not seen in the circular smooth muscle. Furthermore, unlike the longitudinal smooth muscle, the basal stress response and the optimum length value of the infected circular muscle differed significantly from control tissues. This may indicate a modification in the composition or elastic property of the infected circular muscle. However, no change was observed in

the thickness of the infected circular nor longitudinal smooth muscle compared to control tissues. These transit and contractility findings occurred in the absence of any significant increase in the number of intra-epithelial lymphocytes or mast cells.

It is concluded that the increased transit and contractility changes observed in this animal model of giardiasis indicate that a motility disorder may play a role in the pathophysiology of the disease.

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