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The Study of the Pathology of the Anterior One Third of the Small Intestine of the Spontaneously Diabetic Chinese Hamster

Scot Stromsta

Western Michigan University

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THE STUDY OF THE PATHOLOGY OF THE
ANTERIOR ONE THIRD OF THE SMALL INTESTINE
OF THE SPONTANEOUSLY DIABETIC CHINESE HAMSTER

by

Scot Stromsta

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
August 1975

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ACKNOWLEDGEMENTS

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Scot Stromsta
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INTRODUCTION

According to Maugh (47), the frequency of diabetes is rapidly increasing. The number of diabetics in the United States has increased by three hundred percent in the last twenty five years, whereas the general population of this country has grown only about fifty percent in the same time span. In spite of increased life expectancy of diabetics with hypoglycemic agents, insulin and strict diets, diabetes is still the fifth leading cause of death by disease and probably the second leading cause of blindness in this country.

Diabetes mellitus is a disorder of carbohydrate metabolism. The principle symptoms of diabetes are elevated blood sugar (hyperglycemia), sugar in the urine (glycosuria), excessive thirst (polydipsia) and increased food intake (hyperphagia). The diabetic syndrome presumably results from relative or absolute insulin deficiency (47).

There are many species of animals which exhibit spontaneous diabetes mellitus. Included in this group are the dog, cat, monkey, obese mouse, KK mouse and the Chinese hamster (Cricetulus griseus) (55).

The purpose of this investigation was to examine the small intestine of the diabetic Chinese hamster for any possible diabetic lesions. The small bowel of the diabetic hamster was proposed as an interesting and logical organ to examine because of the following reasons:

1. Abnormal free fatty acid absorption, steatorrhea, hyperphagia
and hormone imbalances (14, 20, 21, 23, 53).

2. Similar embryological development of the pancreas and the small intestine (64).

3. The limited quantity of research associated with the digestive tube.

The Upjohn Company of Kalamazoo, Michigan has selectively bred spontaneously diabetic Chinese hamsters (26) for investigation of this disease and has kindly furnished the animals for this study.

It is believed that the Chinese hamster is a good animal model for the study of diabetes because of several key factors:

1. The disease shows morphological, biochemical and physiological changes which are somewhat similar to alterations associated with human diabetes (14, 25).

2. Environmental factors can be controlled to a certain extent (66).

3. Terminal studies can be performed on the animals at any time during their life history.

4. Genetic control via selective breeding is possible (27).

5. Generation time of the hamster is short.

As was previously mentioned, the Chinese hamster displays spontaneous diabetes mellitus which was presumably due to a combination of genetic and environmental factors (26). Diabetes in most laboratory animals could also be induced via drugs such as streptozotocin and alloxan (34, 38). The diabetes of the drug-induced animals was complicated due to the possibility of pathology related to the drug as well as to the diabetes (16). Diabetes, in general, is a
hereditary disease and it is possible that some pathologies associated with diabetes were genetically determined. If this was true, the chemically induced diabetic animal may not be an appropriate model to study (58). For this reason it was felt that spontaneous diabetes in the Chinese hamster was more representative of the human disease.
Structural Alteration Associated with Diabetes in the Chinese Hamster

Pancreatic insulin is diminished in diabetic Chinese hamsters (14). Beta cell granulation is decreased in the diabetic hamsters (14). The pancreas of the diabetic hamster displays reduced islet volume and Beta cell mass (14). The Beta cells of these hamsters exhibit glycogen deposits (14). Soret et al. (60) reported glycogen accumulations in the Alpha and Delta islet cells of long term glycosuric and recent onset ketonuric Chinese hamsters. Basement membrane thickening of islet capillaries has been reported (14). Orci et al. (52) reported alterations of the Beta cell plasma membrane of the diabetic hamster. They also observed an increase in the number of nuclear pores of the Alpha and Beta cells.

Alteration of testicular tissue biopsy material has been studied (59). Diabetic Chinese hamsters displayed inhibited spermatogenesis, due to a diminished thickness of the germinal epithelium and increased diameter of the seminiferous tubules. A decrease in the number of Leydig cells was also seen. It was suggested that the histological condition of the diabetic hamster testicular biopsy material resembled that of the diabetic human patient.

Pathology of the nervous system of the diabetic Chinese hamster, as depicted by light and electron microscopic examination, exhibit many similarities to those reported in human diabetic neuropathy (57).
Brains of diabetic and control hamsters have shown that diabetics display elevated numbers of vascular lesions and neuronal alterations (42). In a study of selected peripheral nerves (57), the diabetic hamster displayed:

1. Segmental demyelination.
2. Acute axonal degeneration.
3. Decreased and inconsistent internode lengths.
4. Ultrastructural indications of demyelination and remyelination.

The distal tubules in the kidney of the ketonuric Chinese hamster display accumulations of glycogen (60).

Morphological changes in the aorta of diabetic hamsters have also been reported (48).

Retinopathy of the Chinese hamster has been described by several authors (17, 59, 60, 61).
Structural Alterations Associated with the Human Diabetic Gastrointestinal Tract

Esophageal distention, delayed emptying and diminished peristaltic waves have been reported in patients with diabetes and accompanying diabetic neuropathy (29, 43). Reduced gastroesophageal sphincter pressure, weak contractions in the pharynx and esophagus and increased frequency of nonperistaltic contractions have been observed in diabetic patients by use of intraluminal manometry (44). Nondiabetic subjects matched for age and sex did not display these abnormalities (43). These disorders may be due to vagal neuropathy (29).

Kassander reported atony and delayed emptying of the stomach in patients with diabetes mellitus (39). It has been reported that gastric motor abnormalities occur in twenty to thirty percent of diabetics (16). In these cases the stomach was large and distended, and gastric retention found (39). There appears to be no evidence of obstruction; it has been hypothesized that the retention results from the diminished peristaltic contractions (67). Gastric stasis has been associated with the abnormal gastric bacterial growth and the presence of food residue (28, 30).

Autonomic neuropathy is thought to be the cause of gastric atony (40, 56). Gastric atony resembles the decreased gastric motility which follows vagotomy (40, 56, 67).

Many diabetic ketoacidotic patients display acute gastritis (36). Gastric retention and an increase of detergents and urea, may break the mucosal barrier and allow diffusion of hydrochloric

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acid into the gastric mucosa to produce gastritis (11).

It has also been reported that the stomach secretes less acid and becomes atrophic at an earlier age in the diabetic than in the nondiabetic (1, 12). However, other studies contradict these findings (19, 46).

Severity and frequency of ulcers in diabetics are elevated when compared to nondiabetic patients of the same age and sex (36, 65). Hemorrhaging ulcers are likely to be severe due to the vascular changes associated with diabetes (65). It has been reported that elevated insulin levels are found in nondiabetic duodenal ulcer patients without significant changes in blood glucose (6). No explanation was given concerning the significance of this finding (6).

Diabetic diarrhea and steatorrhea have the same etiological factors in most diabetics (40). In most cases, the patient with diabetic steatorrhea could be distinguished from the diabetic with diarrhea by the fat content of the stool (40, 63). The symptom of diabetic diarrhea is not fully understood. The patient with diabetic diarrhea usually has a history of severe, poorly controlled insulin-dependent diabetes, with advanced peripheral and autonomic neuropathy (28, 50, 63).

Normal absorption of sodium, water, d-xylose and Vitamin B₁₂ have been reported in patients displaying diabetic diarrhea (40, 63). Enhanced glucose absorption has been observed in these patients (63). A diabetic malabsorption syndrome similar to the pathologies of celiac disease has been described by Ellenberg and Rifkin (15).

Diabetic diarrhea is frequently associated with the presence of
visceral neuropathy (3, 56). Diminished small intestinal tone is observed in some patients displaying diabetic diarrhea (49).

Small intestinal transit of barium is usually rapid but may be delayed (49, 50). It has been suggested that, in diabetic patients with slow transit, bacterial overgrowth in the small intestine may result in diarrhea and steatorrhea (28). In addition, heavy bacterial contamination of the stomach may contaminate the small intestine (30).

Despite significant steatorrhea in diabetic patients, the histology of the small intestine is usually normal (5, 15). However, some abnormal histology of diabetics has been reported. A jejunal biopsy with blunted villi and dense lymphocytic infiltration was attributed to bacterial contamination of the small intestine (18). It has been observed that the small intestine of diabetics display distended loops with thickened plicae circulares (40). The jejunal mucosa of some diabetics has been reported to possess long thin villi and slight infiltration of lymphocytes in the lamina propria; Periodic-acid Schiff staining revealed no abnormalities in this case (40). Exocrine pancreatic insufficiency may result in significant steatorrhea (4).

Celiac disease has been reported to occur more frequently in conjunction with diabetes (31, 45). It has been theorized that celiac disease may lead to impaired glucose tolerance due to the fact that various gastrointestinal hormones are responsible for enhanced release of insulin (9). Diminished severity of diabetes subsequent to treatment of associated celiac disease has been described (43). An absolute lack of jejunal villi has been reported in patients
with diabetes and concomitant adult celiac disease (13, 35).

Colonic atony may result in a massive quantity of fecal matter in the colon and colonic dilation (40). Visceral neuropathy may lead to an atonic colon devoid of propulsive activity and to impairment to the evacuation of colonic contents (15, 40).
Abnormalities of the Diabetic Chinese Hamster
Gastrointestinal Tract

There has been a limited amount of research connected with the gastrointestinal tract of the diabetic Chinese hamster. The one published report is by Parkinson (53) who was concerned with intestinal fat absorption in the diabetic Chinese hamster. Parkinson reported that intestinal absorption of lipids was greater in nonketotic diabetics than in nondiabetic controls and that incorporation of resynthesized triglycerides in the jejunum was equal on a unit tissue weight basis. Parkinson hypothesized that intraluminal hydrolysis did not differ between diabetic and control and that increased free fatty acid uptake in the diabetic may be a consequence of increased intestinal mass of the diabetic Chinese hamster.
MATERIALS AND METHODS

All specimens (Figures 1 and 2*) for this investigation were chosen from the Upjohn colony of Chinese hamsters described previously by Gerritsen and Dulin (24). Two types of Chinese hamsters, nonketotic diabetic and nondiabetic, were employed for the current investigation. A nonketotic diabetic hamster is one whose urine has tested plus 4 by TesTape (Eli Lilly and Company, Indianapolis, Indiana) for a minimum of two of four tests within two months but has never been positive for ketones by Ketostix (Ames Company, Elkhart, Indiana) (26). Characterization of diabetes in this animal was described by Gerritsen and Dulin (25). A nondiabetic hamster is one which has never shown a positive test for urine glucose and has not shown diabetic symptoms for at least two to three previous generations (26). This type of animal was used as a nondiabetic control animal in the current investigation.

All hamsters in the Upjohn colony are tested twice monthly for glycosuria from fifteen days of age (26). Animals that test plus 4 with TesTape are tested daily for ketonuria with Ketostix (26). It has been reported that glycosuria detected by TesTape was an acceptable procedure for detection of diabetes in the Chinese hamster (26, 53).

Care, management, housing, room temperature and handling of the Chinese hamster were described by Yerganian (66). The animals were maintained on Purina mouse breeder chow (24). The animals ranged in age from eight to eighteen months, and each diabetic was

*Figures 1 through 24 are found on pages 35 through 57.
matched with a nondiabetic control animal of the same sex and approximate age. The diabetic animals had displayed diabetes for lengths of time ranging from eight to sixteen months. All diabetic hamsters showed consistent Testape values of plus 3 to plus 4 with one exception of a plus 2 value. All diabetic hamsters displayed negative Ketostix tests for ketones in the urine. All nondiabetic hamsters showed Testape values of 0. In addition, all available blood samples were obtained and analyzed for glucose concentrations by the Upjohn Company. All blood samples reported in this investigation were obtained from the orbital sinus. Glucose concentrations were analyzed on 0.05 milliliters of blood using the Autoanalyzer microglucose procedure (25). Blood sugar concentrations of the diabetic hamsters ranged from 145 to 320 milligrams percent. The nondiabetic control hamsters showed blood sugar values from 73 to 125 milligrams percent. In Table 1 (page 18), data are summarized concerning the age and metabolic state of the animals at the time of sacrifice. The diabetic hamsters used in this study received no therapy. All hamsters, diabetic and control, were allowed food and water ad libitum.

The hamsters were sacrificed by exsanguination and decapitation or etherization and cervical dislocation, then the small intestine was excised (Figure 3). The first one third of the small intestine (Figure 4) was divided into two portions (anterior and posterior) and three pieces of intestine were removed from each portion. The tissues from each portion were immediately placed into one of three different fixatives, namely, Bouins (37), Zenkers (37) or cold acetone
(37) (four degrees Centigrade). Tissues fixed in Bouins fluid were stained for general morphological features. Fixation time in Bouins fluid was twenty-four hours. Tissues fixed in Zenkers fixative were stained with the Periodic-acid Schiff test. Fixation time in Zenkers was twenty-four hours, after which the tissues were washed in running water for five to eight hours to remove excess mercuric chloride. Tissues fixed in cold acetone were tested for enzyme activities of alkaline phosphatase, acid phosphatase and non-specific esterase. Fixation time in cold acetone was twenty-four hours.

Two different paraffin embedding schemes were used in this study. The first was employed for Zenker and Bouins fixed tissues. The steps were as follows:

1. 50 percent ethanol, one hour minimum, three changes, room temperature.
2. 70 percent ethanol, one hour minimum, three changes, room temperature.
3. 80 percent ethanol, thirty minutes, room temperature.
4. 90 percent ethanol, thirty minutes, room temperature.
5. 95 percent ethanol, thirty minutes, room temperature.
6. 100 percent ethanol, thirty minutes, room temperature.
7. xylene, fifteen minutes, room temperature.
8. xylene, fifteen minutes, room temperature.
9. xylene:paraffin, 50:50 mixture, one hour, 56 degrees Celsius.
10. paraffin, forty five minutes, 56 degrees Celsius.
11. paraffin, forty five minutes, 56 degrees Centigrade.

The second embedding scheme was adapted for acetone fixed tissues in an attempt to preserve enzyme activity. The steps for this procedure were as follows:

1. acetone, twelve hours, room temperature.
2. acetone, twelve hours, room temperature.
3. xylene, fifteen minutes, room temperature.
4. xylene, fifteen minutes, room temperature.
5. xylene:paraffin, 50:50 mixture, fifteen minutes, 56 degrees Celsius.
6. paraffin, thirty minutes, 56 degrees Celsius.
7. paraffin, thirty minutes, 56 degrees Celsius.

Subsequent to the last step of both the preceding paraffin embedding procedures, the tissues were allowed to harden in a wax block at 4 degrees Centigrade. The paraffin was then trimmed into small blocks for tissue orientation and mounted on pieces of wood. The blocks were sectioned at eight microns on an American Optical rotary microtome. The tissue sections were floated on warm distilled water until completely free of wrinkles and then placed on glass slides coated with Mayer’s albumin.

The following stains were used to aid in identifying and localizing structures and enzymes associated with the small intestine:

1. Harris’ hematoxylin and eosin to reveal general pathological features (37).
2. Schorr’s trichrome to differentiate blood cells, connective tissue and muscle (32).
3. Periodic-acid Schiff reaction to display mucin, glycoproteins and other polysaccharides (62).

4, 5 and 6. Alkaline phosphatase (2), acid phosphatase (2) and non-specific esterase (54) reactions to demonstrate and localize the respective enzyme activities.

The procedures of the acid phosphatase stain were modified to allow for acetone fixation and paraffin embedding. In addition, the amount of substrate, sodium alpha naphthyl acid phosphate, used in the acid phosphatase stain was doubled. There were two slight modifications in association with the non-specific esterase stain. First, the tissue sections were not counterstained, and second, a non-aqueous mounting medium was employed.

After staining was completed, a non-aqueous mounting medium was applied to the tissue and a cover-glass lowered into place, completely covering the sections. The slides were allowed to dry and then were analyzed using Wolfe and Leitz binocular light microscopes.

For all comparisons in the current investigation, the anterior portions of the proximal one third of the intestines (reported in Tables 2 through 5 as the anterior portion) of both the diabetic and control hamsters were compared. Likewise, the posterior portions of the proximal one third of the intestines (reported in Tables 2 through 5 as the posterior portion) of both the diabetic and control animals were compared.

The histological study of the diabetic and control intestines was initiated by measuring several morphological structures. One
tissue section per intestinal portion, i.e., anterior and posterior, was measured in each case, using an eye piece micrometer. The student t-test (11) was then employed to determine any statistical differences between the diabetic and control. Structures studied in this manner were the following:

1. The number of villi per cross section of intestine.
2. The cross sectional area of the small intestine as approximated by the product of the cross sectional length and width measurements.
3. The cross sectional area of the lumen of the small intestine as approximated by the product of the cross sectional length and width measurements.
4. The length of villi, ten villi per cross section were measured.
5. The width of villi at the base, middle and tip; the width of ten villi at all three positions were measured on each cross section.
6. The number of goblet cells per villus; the number of goblet cells of ten villi per cross section were obtained.

The histochemical and histopathological studies of the diabetic and control hamster intestines were undertaken by observing, comparing and recording enzyme activities and abnormal structures respectively. Six tissue sections per intestinal portion, i.e., anterior and posterior, were studied in this manner for each histochemical stain and each histopathology. Results were recorded as percent incidence of occurrence. Comparisons made in this manner were the
following:

1. Muscle thickness of the muscular coat.

2. Relative number of Auerbach's plexuses.

3. Relative amount of connective tissue in the muscular coat.

4. Lymphocyte aggregations.

5. Accumulation of fat cells between the basement membrane and the connective tissue coat of the villi.


7. Epithelial loss from the villi.

8. Intensity of the Periodic-acid Schiff positive brush border.

9. Relative enzyme activity, as shown by the staining intensity of alkaline phosphatase, acid phosphatase and non-specific esterase.

All appropriate photomicrographs were processed by the Upjohn Company.
# Table 1

Chinese Hamster Background Data

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* Age in Months
** Duration of Diabetes in Months
*** Non-fasting Blood Sugars in Milligrams Percent
' Blood Sugar Values for These Animals Were Not Available
OBSERVATIONS AND RESULTS

Normal Structure of Anterior One Third of Chinese Hamster Small Intestine

Gross anatomy

The small intestine of the Chinese hamster is a convoluted tube, extending from the pylorus of the stomach to the ileocolic valve which is located at the junction of the ileum and the ascending portion of the colon. The small bowel is approximately twenty-seven centimeters in length. It is contained in the central and lower part of the abdominal cavity and is surrounded superiorly and laterally by the large intestine. It is connected to the dorsal abdominal wall by the mesentery. The mesentery of the small intestine is fan shaped and composed of two layers between which are blood vessels, nerves, lymph vessels and lymphatic glands which service the small intestine. The portion of the small intestine just distal to the pylorus passes posteriorly and doubles back on itself, forming a loop within which the head and neck of the pancreas lie. This part of the small intestine receives the common bile duct and the pancreatic duct. Figure 3 is a photograph of the viscera in the abdominal cavity.

Histology

The wall of the Chinese hamster intestine is composed of three coats - serosa, muscularis and the mucosa.

The outer coat, the serosa, is derived from the peritoneum. The serosa completely surrounds the small intestine except along
the mesenteric border. The mesenteric border is the space where vessels and nerves pass to the intestine. Figure 10 demonstrates an example of the mesenteric border.

The middle coat, the muscularis, consists of two layers of muscle fibers, an external longitudinal layer and an internal circular layer. The fibers of the longitudinal layer cover the surface of the intestine, but are most distinct at the mesenteric border. The circular fibers form a thick uniform layer that surrounds the cylinder of the intestine. Figure 8 shows the muscular coat and both layers, the external longitudinal and the inner circular.

The inner intestinal coat of the small intestine is the mucous membrane. It is thick, vascular and consists of two subdivisions, the connective tissue coat and the epithelium. No muscularis mucosa has been observed in this animal. The connective tissue coat includes the area from the inner circular layer of muscle to the epithelium. It is composed of loose areolar tissue with blood and lymphatic vessels, nerves and numerous lymphocytes. The epithelium is of the simple columnar type and is supported by a basement membrane. Along the free border of the epithelium a brush border is observed. The mucous membrane is demonstrated in Figure 10.

The mucosal coat presents several conspicuous structures such as villi, crypts of Lieberkühn and mucous glands (see Figures 12 and 14). A lack of plicae circulares in the Chinese hamster intestine has been noted.

The essential structures belonging to the villi of the
Chinese hamster are the lacteals and blood vessels, basement membrane and the epithelium. The structures associated with the villi are arranged in the following manner: in the center of the villus is the lacteal, terminating near the summit in a blind pouch; the lacteal is surrounded by a plexus of capillary vessels and nerves; all this being enclosed by a basement membrane upon which rests a simple columnar epithelium. The matrix supporting those structures, which are enclosed by the basement membrane in the middle of the villus, is the reticular tissue of the connective tissue coat.

Crypts of Lieberkuhn are located in the connective tissue coat of the small intestine and are present in considerable numbers. They are tubular depressions of the mucous membrane, which enter into the lumen of the intestine by small circular openings. These crypts are lined by simple columnar epithelium.

The mucous glands observed in the connective tissue coat were lined by a simple cuboidal epithelium. They opened by a single duct onto the luminal surface of the intestine. These mucous glands were present in the portion of the small intestine which was just distal to the pyloric stomach.

The intestinal blood vessels, having reached the mesenteric border of the small intestine, run between the serous and muscular coats. From here, the vessels branch and supply the muscular coat and then enter the mucosa to serve the glands and villi of the mucous membrane. The lymphatic system of the small intestine begins in the lacteals as was described previously. From their
commencement, the lacteals pass into larger and larger lymphatic vessels until they finally empty into the cisterna chyli at the mid-dorsal region of the abdominal wall.

The nervous supply of the small intestine is derived from sympathetic nerve plexuses around the superior mesenteric artery and parasympathetic branches of the vagus nerve. From these beginnings, the nervous supply ramifies to become a plexus (Auerbach's plexus) between the outer longitudinal and inner circular muscle layers (see Figure 8); from this point nerve branches are distributed to the muscle coats of the intestine. From Auerbach's plexuses, nerve branches may also pass to the mucosal coat and its associated structures.

Abnormal Structures of Anterior One Third of Chinese Hamster Small Intestine

**Gross anatomical pathology**

There were two conditions which were grossly observed in the small intestine of the diabetic hamster that were not apparent in the small intestine of the nondiabetic control. First, the small intestine of the diabetic hamster displayed distention, seemingly due to an accumulation of food material. Second, the intestine of the diabetic hamster was soft and friable compared to the firmness of the intestine of the control animal.

**Histological measurements and comparisons**

The anterior portions of the diabetic intestines displayed an elevated number of villi per cross section of intestine in
87 percent of the matched pairs of hamsters. The diabetic hamsters showed a 60 percent occurrence of increased numbers of villi per cross section of intestine in the posterior intestinal portions (Table 3*). Statistical analysis showed the diabetic to have more villi per cross section at the 90 and 80 percent confidence limits for the anterior and posterior portions respectively (Table 2). In this case, only the anterior portions were considered to have a statistically significant difference.

Diabetic hamsters displayed a 73 and 53 percent occurrence of increased cross sectional area of the small intestine in the anterior and posterior portions respectively (Table 3). The cross sectional area of the diabetic was greater at the 98 percent level of significance in the anterior portions. This was considered to have been statistically significant. The increase of the posterior portions of the diabetic occurred at the 60 percent level of significance and, as such, was not considered a statistically significant increase (Table 2).

The diabetic hamsters displayed an 80 percent occurrence of increased cross sectional area of the lumen in the anterior portions when compared with the same intestinal portions of the control hamsters. Similar results were seen on only 47 percent of the matched hamster pairs in the posterior intestinal portions (Table 3). The significance levels at which measurements of the luminal area of the diabetic hamsters were larger than the measurements of the luminal area of the nondiabetics for the anterior and posterior portions were 97.5 and 30 percent respectively (Table 2). Only the

*Tables 2 through 5 are found on pages 30 through 33.
anterior portions were considered to have a statistically significant difference.

Diabetic hamsters displayed a 60 percent occurrence of longer villi in the anterior portions of intestines when compared to control hamsters. Similar results, at a 53 percent occurrence rate, were observed in the posterior intestinal portions (Table 3). Table 2 shows significance levels for greater diabetic villi length of 90 and 40 percent for the anterior and posterior portions respectively. 40 percent in this case was not considered statistically significant, whereas 90 percent was considered a significant increase.

Villi width measurements at the base of the villi of the anterior portions of the diabetic and control hamsters revealed a larger mean value for the nondiabetic hamsters. The diabetic hamster measurements were larger in 60 percent of the hamster pairs (Table 3). The significance level of the control being larger than the diabetic in the anterior portion was 10 percent (Table 2). This increase was not considered statistically significant. Villi width measurements at the base of the villi using the posterior intestinal portions of intestine showed the diabetic was wider in 53 percent of the pairs of hamsters (Table 3). The significance level of the diabetic having a wider base measurement was 80 percent, and this was not considered a statistically significant increase (Table 2).

Diabetic hamsters displayed a 47 and a 60 percent occurrence of wider villi at the middle measurement when compared with paired
control hamsters in the anterior and posterior portions respectively (Table 3). The diabetic hamsters showed wider measurements at the middle of the villi in the posterior portions at the 75 percent significance level (Table 2). The statistical differences between measurements of the diabetic and control were not considered significant in either the anterior or posterior intestinal portions.

The diabetic hamsters displayed a 67 and a 53 percent occurrence of wider measurements of villi tips when compared to nondiabetic controls in the anterior and posterior portions respectively (Table 3). The diabetic hamster displayed wider villi tips at the 60 percent significance level in the anterior portion and at the 80 percent level in the posterior portion (Table 2). Neither portions, anterior or posterior, were considered to show a statistically significant increase.

The diabetic hamsters showed an increased number of goblet cells per villus in both the anterior and posterior portions. In the anterior portion, the diabetic showed an 87 percent occurrence of more goblet cells per villus and this was statistically significant at the 99 percent level (Tables 2 and 3). The diabetic hamsters displayed a 73 percent occurrence of more goblet cells per villus in the posterior portions (Table 3). This increase was significant at the 99.5 percent level (Table 2).

The results of the microscopically measured structures are summarized in Tables 2 and 3. In Table 2, listed by anterior and posterior intestinal portions and structures measured, the mean and standard deviations are recorded for diabetic and nondiabetic
control hamsters. t-values, p-values and significance levels in percent are listed to illustrate the statistical difference between the diabetic and control hamsters. Table 3 is a summary of frequency of occurrence of those structures measured in Table 2.

**Histopathology**

The control hamsters displayed a 47 percent occurrence of greater muscle thickness of the muscular coat in the anterior and posterior intestinal portions when compared to the matched diabetics (Table 4, Figures 5 and 6).

In the anterior portion of the intestines, the control hamsters were determined to have a greater number of Auerbach's plexuses in 33 percent of the hamster pairs when compared to the control animals (Table 4, Figures 7 and 8). Control hamsters displayed a greater number of Auerbach's plexuses in 47 percent of the hamster pairs in the posterior intestinal portions (Table 4).

The anterior portions of diabetic hamsters showed a 20 percent occurrence of connective tissue in the muscular coat of the hamster pairs (Table 4). In this portion of the intestine, the control hamsters displayed no examples of increased connective tissue in the muscular coat. In the posterior intestinal portions, the diabetic and control hamsters both showed a 13 percent occurrence of increased connective tissue in the muscular coat (Table 4).

Lymphocyte aggregations (Figures 9, 10, 13 and 14) were a mass of lymphocyte cells in the connective tissue coat. Accompanying the lymphocyte infiltration were large, nucleated cells containing
peripherally located chromatin masses. These cells appeared to be plasma cells or wandering macrophages. The villi which were situated on the lymphocyte aggregations were generally reduced in size, and sometimes reduced in number.

Diabetic hamsters displayed a 40 percent occurrence of lymphocyte aggregations in the anterior intestinal portions whereas the controls showed a 20 percent occurrence of lymphocyte aggregations in these cases (Table 4). Diabetic hamsters displayed a 33 percent occurrence of lymphocyte aggregations in the posterior intestinal portions whereas no lymphocyte aggregations were found in the posterior portions of the controls (Table 4).

The blood vessels in conjunction with the blood vascular lesions were extremely distended with accompanying irregularity of the vessel wall. All blood vascular lesions were found in association with the previously described lymphocyte aggregations. All of these lesions contained eosinophilic material in the center of the blood vessels (Figures 13 and 14).

No blood vascular lesions were seen in either the diabetic or the control in the anterior intestinal portions (Table 4). Likewise, no blood vascular lesions were seen in the posterior portions of control hamster intestines (Table 4). The diabetic hamsters, however, displayed a 27 percent occurrence of blood vascular lesions in the posterior intestinal portions (Table 4).

Decreased numbers of epithelial cells at the tips of villi were seen. Concomitant villi tip deformation was also observed. In these cases, it appeared that the connective tissue at the center
of the villi was also damaged (Figures 15 and 16).

The anterior intestinal portions of the diabetic hamsters showed a 27 percent occurrence of epithelial loss (Table 4). The posterior portions of the diabetic hamsters showed similar results in 13 percent of the hamster pairs (Table 4). In both intestinal portions, anterior and posterior, the control hamsters displayed increased intestinal epithelial loss in 7 percent of the hamster pairs (Table 4).

Diabetic hamsters displayed a 53 percent occurrence of increased PAS brush border staining intensity (Figures 17 and 18) in the anterior intestinal portions when compared to the control hamsters (Table 4). The nondiabetic hamsters, in contrast, showed no examples of increased PAS brush border intensity in the anterior portions (Table 4). In the posterior intestinal portions, the diabetic and control hamsters displayed a 27 and 13 percent occurrence of increased PAS brush border intensity respectively (Table 4).

The histopathological results are summarized in Table 4. This table is organized by structures examined and intestinal portions. Frequency of histopathological occurrences (in percent) as displayed by diabetic versus control hamsters are recorded under the appropriate headings.

Histochemistry

Alkaline phosphatase enzyme activity as shown by the staining intensity (Figures 19 and 20) was greater in the control hamsters in 67 percent of the hamster pairs, and was increased in the diabetic
hamsters in 33 percent of the pairs. These results were found in both the anterior and posterior intestinal portions (Table 5).

The diabetic and control hamsters showed the same acid phosphatase activity in the anterior portions. In the posterior portions, the diabetic hamsters displayed a 67 percent occurrence of increased activity when compared to the controls (Table 5). The control hamsters in the posterior intestinal portions showed a 33 percent occurrence of elevated activity (Table 5, Figures 21 and 22).

The diabetic hamsters displayed a 67 percent occurrence of increased non-specific esterase activity in the anterior intestinal portions (Table 5). The control hamsters in this case had greater activity in only one pair (Table 5). Both the diabetic and control animals showed a 50 percent occurrence of increased non-specific esterase activity in the posterior intestinal portions (Figures 23 and 24, Table 5).

Table 5 is organized by enzymes and intestinal portions. Summarized in this table are the enzyme activities as displayed by staining intensities of the diabetic and control hamsters. These activities are recorded as incidences of occurrence (in percent).
TABLE 2
Microscopic Measurements

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<tr>
<th>Structures Measured</th>
<th>Intestinal Portion</th>
<th>Mean and Standard Deviation Values</th>
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<td>Number of Villi/Cross Section</td>
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<td>Cross Sectional Area of Small Intestine (mm²)</td>
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<td>Cross Sectional Area of Lumen of Small Intestine (mm²)</td>
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<td>Length of Villi (u)</td>
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<td>Length of Villi (u)</td>
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* t-values
** P-values
*** Significance Levels in Percent
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<th>Structures Compared</th>
<th>Intestinal Portion</th>
<th>Incidence of Occurrence or Absence Displayed by Diabetic and Control Hamsters (in percent)</th>
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<tr>
<td>Number of Goblet Cells/Villus</td>
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<td>73.3</td>
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* Increased Size or Number Displayed by Diabetic Hamster When Compared to Control Hamster
** Increased Size or Number Displayed by Control Hamster When Compared to Diabetic Hamster
*** No difference in Size or Number Displayed by Diabetic or Control Hamster
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<td>* 26.7 ** 46.7 *** 26.7 **** 0.0</td>
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<td>Epithelial Loss</td>
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<td>Epithelial Loss</td>
<td>Posterior</td>
<td>* 13.3 ** 6.6 *** 6.6 **** 73.3</td>
</tr>
<tr>
<td>PAS Brush Border Intensity</td>
<td>Anterior</td>
<td>* 53.3 ** 0.0 *** 46.7 **** 0.0</td>
</tr>
<tr>
<td>PAS Brush Border Intensity</td>
<td>Posterior</td>
<td>* 26.7 ** 13.3 *** 60.0 **** 0.0</td>
</tr>
</tbody>
</table>

* Increased Frequency or Amount Displayed by Diabetic Compared to Control
** Increased Frequency or Amount Displayed by Control Compared to Diabetic
*** No Difference in Frequency or Amount Displayed by Diabetic or Control
**** Was Not Observed
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Intestinal Portion</th>
<th>Incidence of Occurrences of Relative Histochemical Activities as Displayed by Diabetic and Control Hamsters (in percent)</th>
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</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase</td>
<td>Anterior</td>
<td>33.3 66.7 0.0</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>Posterior</td>
<td>33.3 66.7 0.0</td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>Anterior</td>
<td>50.0 50.0 0.0</td>
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<tr>
<td>Acid Phosphatase</td>
<td>Posterior</td>
<td>66.7 33.3 0.0</td>
</tr>
<tr>
<td>Non-specific Esterase</td>
<td>Anterior</td>
<td>66.7 16.7 16.7</td>
</tr>
<tr>
<td>Non-specific Esterase</td>
<td>Posterior</td>
<td>50.0 50.0 0.0</td>
</tr>
</tbody>
</table>

* Increased Activity Displayed by Diabetic Compared to Control
** Increased Activity Displayed by Control Compared to Diabetic
*** No Difference in Activity Displayed by Diabetic or Control
Explanation of figures

Figure 1. Photograph of the Chinese hamster, *Cricetulus griseus*.

Figure 2. Photograph of *Cricetulus griseus* showing its approximate length of 80 millimeters.
Explanation of figures

Figure 3. Photograph of the anatomy of the viscera in the abdominal cavity. The respective arrows point to the following:
1-stomach, 2-large intestine, 3-small intestine and 4-bladder (which is distended in this diabetic hamster).

Figure 4. Photograph of the digestive tube from the stomach through the cecum and large intestine. The markers indicated the anterior one third of the small intestine.
Explanation of figures

Figure 5. Cross section of the posterior portion of the diabetic intestine showing the reduction of the muscular coat (arrow). Hematoxylin and eosin. 400X

Figure 6. Cross section of the posterior portion of the control hamster illustrating the normal thickness of the muscular coat (arrow). Hematoxylin and eosin. 400X
Explanation of figures

Figure 7. Cross section of the posterior portion of the diabetic displaying the relative absence of Auerbach's plexuses in the thin muscular coat. The arrow points to the muscular coat. Hematoxylin and eosin. 400X

Figure 8. Cross section of the posterior portion of the control displaying the rich supply of Auerbach's plexuses (arrows 1, 2 and 3) between the inner circular (arrow 4) and outer longitudinal (arrow 5) muscle layers. Hematoxylin and eosin. 400X
Explanantion of figures

Figure 9. Cross section of the posterior portion of the diabetic illustrating a massive lymphocyte aggregation occupying approximately one half of the entire connective tissue coat. The lymphocyte aggregation is located between the markers as indicated by the arrows. Note the abnormal villi that project from the lymphocyte aggregation. Hematoxylin and eosin. 100X

Figure 10. Cross section of the posterior portion of the control illustrating the normal connective tissue coat. The mesenteric border (arrow) is the expanded portion which contains vessels coursing to and from the intestine. The mucous membrane is the area from the inner muscle layer up to and including the epithelial cells of the villi. Hematoxylin and eosin. 100X
Explanation of figures

Figure 11. Cross section of the anterior portion of the diabetic intestine demonstrating the accumulation of adipocytes (arrow) between the basement membrane and the connective tissue stroma of the villi. Hematoxylin and eosin. 400X

Figure 12. Cross section of the anterior intestinal portion of the control demonstrating a lack of fat cells in the villi. Note the simple columnar epithelium (arrow 1) and the goblet cells (arrow 2) of the villi. Hematoxylin and eosin. 400X
Explanation of figures

Figure 13. Cross section of the posterior portion of the diabetic displaying a blood vascular lesion. Note the dense eosinophilic material in the lumen of the vessel (arrow 1), the highly abnormal vessel wall (arrow 2), the lymphocyte (arrow 3) infiltration (small dark-staining nuclei) and the macrophages or plasma cells (arrow 4, large light-staining nuclei). Hematoxylin and eosin. 400X

Figure 14. Cross section of the posterior portion of the control displaying normal blood vessels (arrow 1) near the crypts of Lieberkühn (arrows 2 and 3). Hematoxylin and eosin. 400X
Explanation of figures

Figure 15. Cross section of the posterior portion of the diabetic showing epithelial loss and villus tip destruction. Note the epithelial cells that are seemingly being sloughed (arrow) and the pyknotic appearing nuclei contained in these cells; also note the debris in the lumen. Hematoxylin and eosin. 400X

Figure 16. Cross section of the posterior portion of the control showing healthy villi and epithelial cells. Note the red blood cells (arrow). Hematoxylin and eosin. 400X
Explanation of figures

Figure 17. Cross section of the posterior portion of the diabetic demonstrating the intense staining of the PAS positive brush border (arrow). Periodic-acid Schiff. 400X

Figure 18. Cross section of the posterior portion of the control demonstrating the light staining of the PAS positive brush border (arrow). Periodic-acid Schiff. 400X
Explanation of figures

Figure 19. Cross section of the anterior portion of the diabetic illustrating alkaline phosphatase activity in the brush border (arrow). Alkaline phosphatase. 400X

Figure 20. Cross section of the anterior portion of the control illustrating alkaline phosphatase activity in the brush border (arrow). Note that the activity seems to be similar to that of the diabetic. Alkaline phosphatase. 400X
Explanation of figures

Figures 21 and 22. Photomicrographs of cross sections of posterior intestinal portions demonstrating similar acid phosphatase activity (arrows) in the brush borders of the diabetic and control hamsters respectively. Acid phosphatase. 400X
Explanation of figures:

Figures 23 and 24. Photomicrographs of cross sections of posterior portions illustrating similar non-specific esterase activities (arrows) in the diabetic and control hamsters respectively. Non-specific esterase. 400X
DISCUSSION

Pathologies associated with diabetes, in humans and animals, are quite variable. For example, all human diabetics are not afflicted with retinopathy, and in those patients where retinopathy is present, it may be different in both eyes of the same patient. In addition, some pathologies, including vascular lesions in the Chinese hamster, correlate with length and severity of diabetes (60). A further complication in this particular study was the fact that several sublines of diabetic hamsters were employed (Table 1). Because diabetic hamsters are highly inbred, one might expect similar complications and manifestations of diabetes within a subline only, not throughout the entire colony (14).

One must temper the data of this and similar studies with judgement because of the following reasons:

1. The current investigation employed several sublines of diabetic animals.
2. The severity and duration of diabetes in these animals were somewhat variable.
3. The possibility of sampling error.
4. The variable nature of diabetic pathology.

Histologically, it was determined that some diabetic Chinese hamsters had a thinner muscular coat and a reduced number of Auerbach's plexuses in both intestinal portions (Figures 5 and 6, Table 4). The innervation responsible for contraction of the muscular coat and subsequent tone and peristalsis of the intestine is through the...
parasympathetic nervous system (33). The parasympathetic postganglionic perikarya are observed as the large ganglion cell bodies in Auerbach's plexuses (41). Decreased numbers of Auerbach's plexuses and diminished muscle thickness may be manifested as intestinal atony and reduced segmentation and peristaltic contractions. In addition, if, in fact, there is reduced intestinal tone, this may also explain the mushy texture of the intestines (previously described in gross anatomical pathology) and the increased cross sectional area of the intestines of the diabetic hamsters (Tables 2 and 3). Diminished segmentation and peristaltic contractions may reduce the efficiency of digestion and absorption of food material in the small intestine and cause delayed emptying which results in excess food residue. An accumulation of food material in the intestine of diabetic hamsters was discussed in the description of the gross anatomical pathologies of the intestines of diabetic hamsters. Atony, distention, hypomotility and delayed emptying have all been observed in the gastrointestinal tract of human diabetics, in particular, those patients who displayed associated neuropathy (39, 40, 56, 67).

The results of the luminal area measurements (increased luminal area in diabetics) show marked similarities to the previously described results for cross sectional areas of the entire intestinal tube (Tables 2 and 3). The similarity of these two results tend to indicate that the entire wall of intestine, i.e., the serosa, muscularis and mucosal coats, were of the same approximate thickness in the diabetic and control, varying little even with decreased
muscle thickness of the diabetics. If it is true that the wall of the intestine is of the same approximate thickness for diabetic and control animals, then one would expect to observe increased luminal area with an increased area for the entire intestinal tube. The data support this hypothesis (Tables 2 and 3).

Observations of connective tissue in excessive amounts in the muscular coat of the small intestines of the diabetic and nondiabetic hamsters were too few and variable to allow analysis (Table 4). Berge et al. reported connective tissue replacement of the outer longitudinal muscle layer of the small intestine in a human diabetic patient with diabetic diarrhea (3). The data of this investigation are insufficient to support any hypothesis concerning connective tissue in the muscular coat.

Lymphocyte aggregations are observed in some diabetics, more often in the anterior portion of the intestine of diabetic hamsters (Table 4). Lymphocyte collections may indicate sites of inflammation due to irritation from possible abrasion due to increased chyme passage, bacterial overgrowth, vascular lesions and/or intestinal stasis. Bacterial overgrowth due to intestinal stasis and vascular lesions has been reported in studies with the gastrointestinal tracts of human diabetic patients (28, 30).

All observations of blood vascular lesions were made only in the posterior intestinal portions of diabetic hamsters (Table 4). These peculiar results may be due to the fact that several highly inbred strains of diabetic hamsters were used in this investigation and varying durations of diabetes were shown by the diabetic
population (Table 1). The animals that displayed blood vascular lesions in this investigation were the diabetic hamsters of pairs 1, 4, 5 and 12 (Table 1). These hamsters had displayed diabetes prior to sacrifice for 16, 10, 9 and 10 months respectively.

The vessel dilations seen in association with the blood vascular lesions may be due to weakening of the vessel wall. The lymphocyte aggregations, which surround all blood vascular lesions (Figure 13), may indicate an inflammatory response due to the presence of these lesions. The observations, however, did not give any direct indication of possible cause of the inflammation of the connective tissue coat surrounding the blood vascular lesions.

The eosinophilic material previously described in association with the blood vascular lesions may be a thrombus. This would tend to indicate some sort of hemorrhage of the vessel wall. The eosinophilic material, because of its location, may impede the flow of blood through the vessel by blockage of the lumen. In addition, if hemorrhaging had occurred in these vessels, this could be a serious impediment to the processes of transport and therefore assimilation of previously absorbed food materials.

Some diabetic hamsters displayed greater frequency of accumulations of adipocytes between the connective tissue coat and the basement membrane in the villi than did the matched controls in both the anterior and posterior intestinal portions (Figures 11 and 12, Table 4). All diabetics did not demonstrate this abnormality (Table 4). Increased adipocytes in diabetic hamsters would tend to correlate with previously observed increased free fatty acid
absorption in the small intestine of nonketotic diabetic Chinese hamsters (53). However, current investigation reveals no data to support or refute a hypothesis of this nature.

The previously described epithelial loss was observed more often in the diabetic hamsters than in the controls in both the anterior and posterior intestinal portions (Figures 15 and 16, Table 4). Again this observation was quite variable.

Partially digested material is absorbed in the small intestine by concomitant digestion and transport through the epithelial cells of the villi to the subepithelial space (the connective tissue coat). From the subepithelial space, the nutrients enter the appropriate vessel, blood or lymphatic, for transport away from the small intestine (33). If epithelial cell numbers are decreased because of abnormal loss, it is possible that fewer nutrients can be absorbed. In addition, if the connective tissue coat is exposed to the luminal environment, nutrients already in the connective tissue coat may be expelled into the lumen rather than transported into one of the vessels of the villi. As a result, it seems possible that the intestinal villi burdened with the previously described epithelial loss and villi tip destruction may lose their ability to absorb food material at an efficient rate. This would seem to correlate with observations of Gerritsen and Blanks (22), who suggest that the hyperphagic diabetic Chinese hamster retains similar caloric quantities to nondiabetics.

Sprue (33, 40) and malabsorption syndrome (15) of the small intestine display similar villi abnormalities as those demonstrated...
in Figure 15. Both sprue and the malabsorption syndrome display villi that are deformed at the tip, blunted or entirely absent. Vast lymphocyte infiltration occasionally accompanies these diseases.

The diabetic hamsters displayed increased number of villi in both the anterior and posterior intestinal portions (Tables 2 and 3). The villi of the diabetic intestine were also wider, but only in the posterior portions (Tables 2 and 3). The increased intestinal surface area resulting from these villi may be a necessary physiological compensatory mechanism if there was as previously discussed, a reduced absorption efficiency in the diabetic hamsters.

The anterior portion of the intestine, studied in the current investigation, is the portion of the intestine that is commonly termed the duodenum. Physiologically, the duodenum is primarily concerned with digestion of food rather that absorption. The posterior intestinal portion, would normally be considered part of the jejunum, which in contrast to the duodenum, is functionally concerned with absorption. If the posterior intestinal portions functionally represent primarily absorptive areas, this may help explain the increased villi width and increased villi surface area in these portions.

Guyton (33) states that all small bowel secretions occur in response to chyme presence in the intestine. He explains that greater food quantities elicit greater intestinal secretions. In addition, irritative or tactile stimuli can also elicit greater intestinal secretion.
An increased number of goblet cells per villus in both the anterior and posterior intestinal portions and an increase in the intensity of the PAS positive brush border in the posterior portion were seen in the diabetic hamster (Figures 17 and 18, Tables 2, 3 and 4). Diabetic Chinese hamsters consume more food than matched control animals. In addition, excess food residue in the small intestine of diabetic hamsters has been observed (see gross anatomical pathologies). In light of these two observations, it is not surprising to see increased mucus and glycoprotein in and along the epithelial border of the diabetic hamsters. It is conceivable that the irritation which is responsible for the increased lymphocyte aggregations exacerbates the PAS positive observations in the manner that Guyton describes (33).

Significant differences were not found to exist in the activities of the transport enzymes studied between the diabetic and control intestines (Figures 19, 20, 21, 22, 23 and 24, Table 5). Possible explanations for these findings are that spontaneous diabetes mellitus in the Chinese hamster does not significantly alter the activities of these enzymes in the intestine, or that the histochemical methods used for detection of these enzymes were not sensitive enough to display any changes. Caspary et al. (8) reported normal activities of several disaccharidases, peptide hydrolases and alkaline phosphatase along the brush border of small bowel biopsies of human diabetics. They hypothesized that their results could be due to the fact that the diabetic patients received insulin therapy and displayed good metabolic control at the time the biopsies were
taken. They also suggested that diabetes was not associated with changes in brush border enzyme activity.

Streptozotocin (7) and alloxan-diabetic (51) rats have been reported to show increases in brush border hydrolase activities. However, these increases may be a result of damage to the exocrine pancreas by the previously mentioned drugs (8).
SUMMARY

1. Decreased muscle thickness of the muscularis externus and diminished numbers of Auerbach's plexuses were observed in some diabetic hamsters.

2. Diabetic hamsters showed increased cross sectional area of the small intestine.

3. Lymphocyte aggregations and blood vascular lesions were observed in some diabetic animals.

4. Increased numbers and size of intestinal villi were found in some diabetics.

5. Increased numbers of goblet cells and increased PAS positive brush border intensity were seen in diabetic hamsters.

6. Significant differences in activities of brush border enzymes were not detected between the diabetic and control hamsters.
REFERENCES


52. Orci, L., M. Amherdt, F. Malaisee-Lagae, A. Perrelet, W. Dulin,


