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ENZYME ACTIVITIES OF JEJUNAL MUCOSA IN EXPERIMENTAL BLIND LOOP SYNDROME OF RAT

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ABSTRACT

Decreased activities of jejunal mucosal enzymes, *i.e.* lactase, sucrase, maltase and gamma-glutamyl transpeptidase were found in rats with experimental blind loop syndrome.

The peroral administration of Kanamycin induced both reduction in fecal fat excretion and restoration of enzyme activities in rats with the blind loop syndrome. Similar effects of Kanamycin on fecal fat excretion and jejunal mucosal enzyme activities were also recognized in unoperated rats.

There was an inverse relationship between fecal fat excretion and activities of those enzymes which are mainly located in the brush border of the enterocyte.

From these results, significance of jejunal brush border enzymes in the blind loop syndrome was discussed.

INTRODUCTION

The blind loop syndrome is thought to be the malabsorptive state associated with abnormal overgrowth of bacteria in the stagnant loop of the small intestine. As early as 1890, White[1] reported 6 patients with macrocytic anemia, in all of whom the small intestine was abnormal. In 1924 Seyderhelm[2], from the fact that the improvement in macrocytic anemia was observed following resection of small intestinal strictures, concluded that this syndrome is caused by lesions of the small intestine. Thereafter many investigators have reported clinical cases of macrocytic anemia caused by small intestinal disorders, some of which were accompanied by other manifestations of malabsorption, such as steatorrhea, weight loss, hypoproteinemia and deficiency in calcium and vitamins.

In some diseases causing malabsorption, such as adult celiac disease[3)4)5)6)], Whipple's disease[3)4)], agammaglobulinemia[3)] and postgastrectomy sundrome[3)], decreased enzyme activities of small intestinal mucosa as well as histologic abnormalities are noted.

This study deals with activities of mucosal enzymes of the jejunum, most active site of digestion and absorption, in rats with experimental blind loop syndrome.

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MATERIALS AND METHODS

1. Animals: Male albino rats of Wistar strain weighing 150 g were reared and used for study when they grew up to approximately 300 g. The animals were divided into the following four groups:

a) Blind loop group,

b) Kanamycin treated blind loop group,

c) Unoperated group,

d) Kanamycin treated unoperated group.

2. Method of operation: Self-filling blind loop of the jejunum, 8 cm in length and 40 cm proximal to the ileocecal valve, were formed in rats according to the method described by Cameron[7]. The scheme of the operation is illustrated in Fig.1.

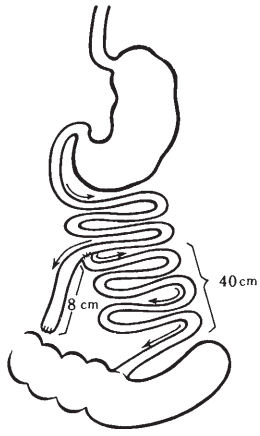

Fig. 1. Method of Blind Loop Formation. Arrows indicate the direction of peristalsis.

3. Raising of rats: Rats were kept in conventional cages and fed mouse food (MF) manufactured by Oriental Yeast Co. Japan, and fresh water *ad libitum*. During the period of fecal collection each rat was housed in an individual metabolic cage with a wire screen bottom in order to prevent the feces from mingling with urine and food.

4. Administration of Kanamycin (KM): In the fourth week after the operation rats with the blind loop were given 0.1 per cent solution of Kanamycin sulfate in place of drinking water for seven days *ad libitum*. Unoperated rats were also given KM solution by the same manner as the operated (20–40 mg of KM per day).

5. Determination of fecal fat excretion: A month after the operation each rat with the blind loop was kept in an individual metabolic cage for three days in order to collect feces. Fecal fat was measured on 3-day collections of feces according to the method described by Sobel[8]. In rats treated with KM, fecal fat was measured for three days from day 5 to day 7 after KM administration was initiated.

In unoperated group feces were collected in the same manner at the same age as the former.

6. Assay of jejunal mucosal enzyme activities: After the collection of feces was completed, rats were killed after an overnight fast. Immediately after the sacrifice small intestines of rats were quickly removed and cut open longitudinally, and then washed with ice cold saline. After saline was thoroughly blotted with filter paper, mucosa of the proximal jejunum was scraped off with a piece of glass and stored frozen until use. At the time of enzyme assay, the mucosa was homoge-

nized with a glass homogenizer equipped with teflon pestle after an addition of small amount of distilled water. Aliquots of homogenate were appropriately diluted with distilled water and used as enzyme solution.

a) Disaccharidases: Determination of activities of lactase (β-D-galactoside galactohydrolase, EC 3.2.1.23.), sucrase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26.) and maltase (α-D-glucoside glucohydrolase, EC 3.2.1.20.) was performed according to the method of Dahlqvist[9]. The activity was expressed as micromoles of substrate hydrolyzed per min per g of mucosal protein.

b) Leucine aminopeptidase (LAP, α-aminoacyl-peptide hydrolase, EC 3.4.11. 1.): Activity of LAP was determined by the method of Goldbarg[10]. The activity was expressed as micromoles of β-naphthylamine released per min per g of mucosal protein.

c) Gamma-glutamyl transpeptidase (GGTP, γ-glutamyl transferase, EC 2.3.2. 2.): GGTP activity was measured by the method of Orlowski[11]. The activity was expressed as micromoles of p-nitroaniline released per min per g of mucosal protein.

d) Protein content: Protein content of homogenate was measured according to the method of Lowry[12] using bovine serum albumin (Dai-ichi Pure Chemical Co., Ltd.) as the standard.

RESULTS

1. Effects of the blind loop formation on the growth of rats: Fig. 2 indicates the growth curves of both operated and unoperated rats. There was no difference in weight gain between both groups until the operation was made. After the

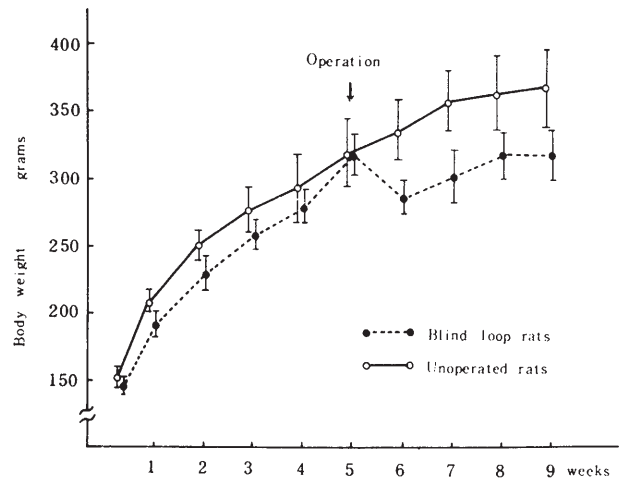

Fig. 2. Effect of the blind loop formation on the growth of rats. Each point and vertical bar indicate the mean and S.D. of body weight, respectively.

operation, the rats subjected to formation of the blind loop lost their weight temporarily and then began to grow slowly again. About three weeks after the operation, the increase in body weight ceased. On the other hand, the unoperated animals continued to increase their weight until the termination of the experiment. At the time of sacrifice body weight (mean ± S.D.) was 319.5 ± 19.5 g in the former and 369.1 ± 29.8 g in the latter. Their was a significant difference between these two groups ($p < 0.01$).

2. Fecal fat excretion: As shown in table 1, the rats with the blind loop excreted significantly increased amount of fat in stool as compared with the unoperated ($p < 0.001$). Peroral administration of KM reduced definitely the fecal fat excretion of the rats with the blind loop ($p < 0.05$) as well as that of the unoperated ($p < 0.005$).

TABLE 1. Effects on fecal fat excretion of blind loop formation and/or peroral administration of Kanamycin

Group	No. of rats	Fecal fat excretion (mean ± S.D.) mg/day
Blind loop	9	394.9 ± 75.6
Blind loop, KM treated	10	312.1 ± 19.5
Unoperated	14	242.1 ± 45.2
Unoperated, KM treated	5	162.9 ± 18.5

3. Activities of jejunal mucosal enzymes:

a) Disaccharidases: In table 2 activities of lactase, sucrase and maltase were shown. In the rats with the blind loop activities of the three enzymes were significantly reduced compared with those of the unoperated ($p < 0.001$). After the peroral administration of KM, activities of the enzymes increased significantly ($p < 0.05$), being comparable to those of normals. KM administration markedly raised the activities of these disaccharidases in the unoperated animals, too ($p < 0.05$).

TABLE 2. Effects on disaccharidase activities of the proximal jejunal mucosa of blind loop formation and/or peroral administration of Kanamycin

	No. of rats	Lactase (mean ± S.D.)	Sucrase (mean ± S.D.)	Maltase (mean ± S.D.)
Blind loop	9	11.1 ± 3.8	45.0 ± 18.5	241.4 ± 124.4
Blind loop, KM treated	10	21.5 ± 9.4	77.1 ± 15.4	434.4 ± 205.8
Unoperated	14	19.0 ± 4.3	64.5 ± 12.2	489.0 ± 153.6
Unoperated, KM treated	5	25.1 ± 7.3	81.9 ± 18.2	702.4 ± 171.5

b) LAP: As shown in table 3, there was no difference in LAP activity among those four groups.

TABLE 3. Effects on leucine aminopeptidase activity of the proximal jejunal mucosa of blind loop formation and/or peroral administration of Kanamycin

Group	No. of rats	Leucine aminopeptidase activity (mean ± S.D.)
Blind loop	9	79.1 ± 21.8
Blind loop, KM treated	10	80.5 ± 9.2
Unoperated	14	71.3 ± 14.6
Unoperated, KM treated	5	83.9 ± 9.2

c) GGTP: In table 4 activities of GGTP were shown. In the rats with the blind loop activity of GGTP was markedly lowered compared with that of the unoperated ($p < 0.005$) and was increased after peroral administration of KM ($p < 0.01$). In the unoperated rats activity of GGTP was also increased by KM treatment as compared with that of the control ($p < 0.05$).

TABLE 4. Effects on gamma-glutamyl transpeptidase activity of the proximal jejunal mucosa of blind loop formation and/or peroral administration of Kanamycin

Group	No. of rats	GGTP activity (mean ± S.D.)
Blind loop	7	33.4 ± 6.1
Blind loop, KM treated	4	44.9 ± 3.1
Unoperated	5	47.7 ± 6.3
Unoperated, KM treated	4	69.4 ± 14.8

4. Correlation between fecal fat excretion and enzyme activities: There was an inverse relationship between amount of fecal fat and activities of lactase (Fig. 3), sucrase (Fig. 4), maltase (Fig. 5) and GGTP (Fig. 6). On the other hand no correlation was found between fecal fat excretion and LAP activity (Fig. 7).

5. Morphology of the small intestine: Light microscopic examination was performed on histologic sections prepared from the blind loop and the proximal jejunum. There were no histologic abnormalities, such as distortion or destruction of villi and degeneration of enterocytes, in the mucosa of both blind loop and proximal jejunum.

DISCUSSION

The precise mechanism by which malabsorption is induced in the blind loop syndrome is not fully elucidated. Many studies, either clinical or experimental, have been done to make clear the pathogenesis of the blind loop syndrome. Macrocytic anemia was induced experimentally in dogs by Seyderhelm[2] and in rats by

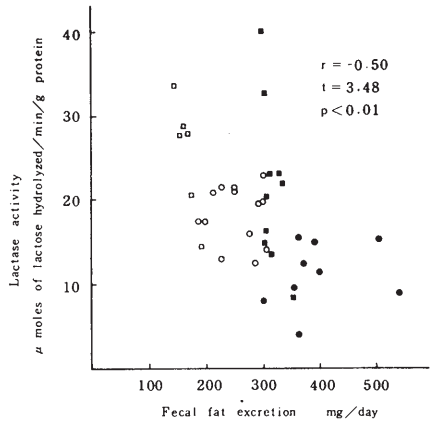

Fig. 3. Lactase activity of jejunal mucosa vs. fecal fat excretion.
● Blind loop ■ Blind loop. KM treated
○ Unoperated □ Unoperated. KM treated

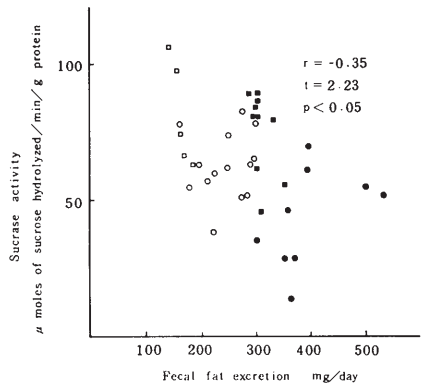

Fig. 4. Sucrase activity of jejunal mucosa vs. fat excretion
● Blind loop ■ Blind loop. KM treated
○ Unoperated □ Unoperated. KM treated

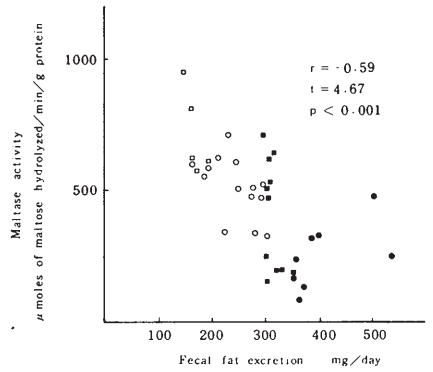

Fig. 5. Maltase activity of jejunal mucosa vs. fecal fat excretion.
● Blind loop ■ Blind loop. KM treated
○ Unoperated □ Unoperated. KM treated

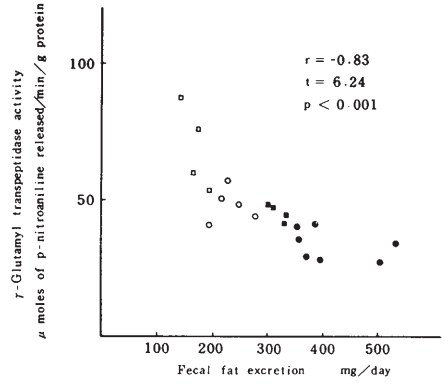

Fig. 6. γ-Glutamyl transpeptidase activity of jejunal mucosa vs. fecal fat excretion.
● Blind loop ■ Blind loop. KM treated
○ Unoperated □ Unoperated. KM treated

Cameron[7]. Since Aitken *et al.*[13] succeeded in producing steatorrhea in rats, steatorrhea induced in animals have been reported by Panish[14] and Hoet[15]. In this study rats with a self-filling loop developed the blind loop syndrome as shown by the increased amount of fecal fat and retarded growth.

Abnormal proliferation of intestinal bacteria was found in the blind loop syndrome[16][17]. Improvement of clinical states in the blind loop syndrome by

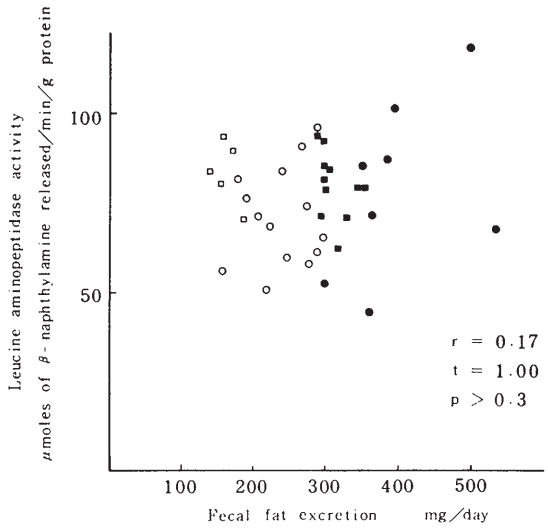

Fig. 7. Leucine aminopeptidase activity of jejunal mucosa vs. fecal fat excretion.
- ● Blind loop
- ■ Blind loop. KM treated
- ○ Unoperated
- □ Unoperated. KM treated

antibiotic therapy suggests that intestinal bacteria may play an important role in the development of steatorrhea[16)17)18)19)]. Amelioration of steatorrhea observed after the peroral administration of KM in this study supports the concept.

It is widely accepted that impaired fat absorption in the blind loop syndrome is caused by the altered bile acids metabolism, deconjugation of bile acids induced by bacterial overgrowth in the small intestine[19)20)21)22)23)24)]. Dawson *et al.*[20)] found that there were not only reduced absorptions of fat and sugar but also severe mucosal injuries including destruction of villi in the rat intestine which had been incubated in a deoxycholate solution. From these facts, they inferred that steatorrhea in the blind loop syndrome might be due to intestinal injuries which were caused by free bile acids, *i.e.* products of deconjugation of bile acids by intestinal bacteria. On the contrary, no light microscopic abnormalities in the intestinal mucosa were found in the blind loop syndrome by many investigators[15)19)23)25)26)27)]. In this study there was no light microscopic abnormality in histologic sections prepared from the blind loop and the proximal jejunum. Kim *et al.*[21)] and Tabaqchali *et al.*[22)] could not find any histologic abnormality in the intestinal mucosa in both clinical and experimental blind loop syndrome, but they observed a decreased concentration of conjugated bile salts in the intestinal juice and improvement in steatorrhea by peroral administration of conjugated bile salts. From these

results they presumed that steatorrhea in the blind loop syndrome might not be caused by the impaired ability of an injured enterocyte to absorb fat but by an inadequate concentration of conjugated bile salts for micelle formation. On the contrary, Rosenberg *et al.*[23] found that there was an adequate concentration of bile acids for micelle formation and that high level of free bile acids was also present in the intestinal juice of patients with a stagnation syndrome and stated that free bile acids might play an important role in the development of steatorrhea in the blind loop syndrome.

There have been many reports on the inhibitory effects of free bile acids on the absorption of water, amino acids and glucose in the small intestine[28)29)30)31)32)]. In the study on rats with a self-filling loop, Gracey *et al.*[33)34)] noted a rise in the free bile acids level in jejunal juice, decreased monosaccharide absorption and morphological changes, which could not be detected by light microscopy, *i.e.* distortion and destruction of microvilli, swelling and vacuolation of mitochondria and retention of fat droplets in the intestinal epithelium on electron microscopic observation. From these facts they considered the deleterious effect of free bile acids on intestinal mucosa as the cause of the blind loop syndrome. In patients with intestinal stasis syndrome, several defects in mucosal fat absorption, *i.e.* decreased numbers of fat particles in absorptive cells, decreased numbers of absorptive cells participating in fat absorption and impaired transport of chylomicrons out of the absorptive cells into the lamina propria, were also demonstrated by electron microscopic examination of jejunal biopsies[35)]. Moreover, this study revealed decreased activities of jejunal disaccharidases and GGTP associated with steatorrhea in rat blind loop syndrome. Gracey *et al.*[36)] also reported that feeding sodium deoxycholate orally to rats caused depression of the activity of the small intestinal enzymes.

In the process of digestion and absorption of sugar and protein, they are primarily hydrolyzed into oligosaccharides and peptides by the action of enzymes secreted by the stomach and the pancreas in the lumen of the digestive tract, and finally almost all of them are taken up into enterocytes by the energy-requiring system, partly related to a sodium expenditure pump, after broken down into monosaccharides and amino acids, respectively, by enzymes present in the brush border facing the intestinal lumen[37)38)39)]. Enzymes assayed in this study are mainly located in the brush border of the enterocyte. Disaccharidases play a fundamental role in digestion of sugars[40)]. GGTP also participates in absorption of amino acids[41)].

After the administration of KM, decreased activities of disaccharidases and GGTP were raised with concomitant improvement in steatorrhea in rats with the blind loop. It may be inferred, therefore, that malabsorption of sugar and protein as well as fat exists in the blind loop syndrome.

In the unoperated rats, administration of KM decreased the fecal fat and elevated the activities of jejunal disaccharidases and GGTP. Growth stimula-

tion[42)43)] and/or enhanced absorption of calcium[44)] and amino acids[45)46)] were induced by antibiotic feeding in conventional animals but not in germ-free ones[42)43)]. These phenomena may show that mild malabsorption caused by subclinical infection of the intestine was improved by antibiotics[47)]. Results of this study support the concept that intestinal infections may have inhibitory effects on its digestive and absorptive abilities.

Presence of an inverse relationship between fecal fat excretion and activities of lactase, sucrase, maltase and GGTP suggests that lowered activities of these enzymes are closely related to the malabsorption in the blind loop syndrome. Steatorrhea and decreased activities of jejunal disaccharidases and GGTP observed in the rats with the blind loop syndrome may indicate that an impairment in absorption of sugar and protein as well as fat develops in the blind loop syndrome.

LAP, whose activity was not affected either by blind loop formation or by KM administration, may show a different response to some cytotoxic agents from the other enzymes. This possibility is partly supported by the fact that LAP activity was even increased histochemically in the idiopathic steatorrhea accompanying severe intestinal mucosal damages on a light microscopy[48)].

SUMMARY

Determinations of fecal fat excretion and activities of upper jejunal mucosal enzymes, *i.e.* lactase, sucrase, maltase, leucine aminopeptidase and gamma-glutamyl transpeptidase, were performed in rats subjected to self-filling blind loop formation in the jejunum.

1) Rats with the blind loop showed increased fecal fat excretion and retarded growth as compared with the unoperated.

2) Activities of jejunal mucosal disaccharidases and gamma-glutamyl transpeptidase were lowered in rats with the blind loop, while no change was recognized in leucine aminopeptidase activity.

3) In rats with the blind loop, no light microscopic abnormalities were noted in the mucosa either of the blind loop segment or upper jejunum.

4) In rats with the blind loop, peroral administration of Kanamycin induced both decrease in fecal fat excretion and increase in activities of jejunal mucosal disaccharidases and gamma-glutamyl transpeptidase. The similar effects of Kanamycin on the jejunal mucosal enzyme activities and fecal fat excretion were also recognized in the unoperated. Leucine aminopeptidase activity, however, was not affected by peroral administration of Kanamycin.

5) There was an inverse relationship between fecal fat excretion and activities of lactase, sucrase, maltase and gamma-glutamyl transpeptidase, but no relationship between fecal fat excretion and leucine aminopeptidase activity was noted.

6) Impaired digestion and absorption of sugar as well as of protein seem also to be present due to lowered activities of brush border enzymes in the blind loop syndrome.

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