VIPergic Innervation in the Gastrointestinal Tract of Diabetic Rats[*]

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ABSTRACT

Diabetic gastroenteropathy developed in about 70% of the rats with diabetes induced by streptozotocin. These rats were associated with diarrhea, gastric paresis, and small intestinal stasis. About 30% of the rats with diabetic gastroenteropathy had narrowed and contracted segments of the lower esophagus and 70% of the colon of the rats showed dilated and contracted segments. Changes in the distribution of VIP–like immunoreactivities in nerve plexuses of the gastrointestinal wall which were observed with destruction of the autonomic nerves (both submucosal and myenteric plexuses) could be one of the plausible causes of diabetic gastroenteropathy induced by streptozotocin, although the precise mechanism of the development of diabetic gastroenteropathy in rats remains obscure.

INTRODUCTION

Dysfuntion of the gastrointestinal tract in diabetic gastroenteropathy is known to occur in patients with long standing diabetes mellitus[12]. Diabetic gastroenteropathy has been associated with gastric paresis, small intestinal stasis, and colonic dysfunction, whose involved clinical symptoms are nausea, vomiting, diarrhea and constipation. The causes of diabetic gastroenteropathy have been searched in peripheral autonomic neuropathy[3].

Recent reviews have shown that peptidergic nerves coexist in autonomic nearves in the gastrointestinal wall. It is therefore interesting and meaningful to ascertain the changes in the peptidergic nerves of the gut wall in diabetic gastroenteropathy.

In the present study, immunocytochemical observations of the peptidergic nerves were made on the nerve plexuses of the gastrointestinal wall in rats with diabetes induced by streptozotocin.

MATERIALS AND METHODS

Experimental Animals and Diabetes

Male wistar rats, weighing 180 grams, were used in this study. Diabetes were produced by injecting 50 mg per kg of streptozotocin into the tail vein. The injection was done as quickly as possible because of the instability of streptozotocin solution. Streptozotocin solution was dissolved in citric buffer at pH 4.5 and 1 ml of the buffer containing 50 mg per kg of streptozotcin.

The rats treated in this manner were kept and fed in a conventional manner for 6 months and then were killed by decapitation. Before

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killing the rats, urine of all rats were checked for glycosuria. Blood was drawn from each rat for evaluating insulin before and after glucose instillation into the stomach.

Preparation for Morphology and Immuno-cytochemistry

The abdomen of decapitated rats was opened and examined at the macroscopic level for abnormal changes in the gastrointestinal tract from the lower esophagus to rectum. Two-tissus sample (1×1 cm) was taken from the following parts of the gastrointestinal tract and pancreas: lower portion of the esophagus, body and antrum of the stomach, pyloric sphincter, duodenum, jejunum, ileum, colon (contracted and dilated segments), and pancreas.

Conventional Histopathology

Each tissue sample of one group was fixed in Bouin's solution for 4 hours and then three micron-wax tissue sections were prepared in the usual manner. The tissue sections were stained with hematoxylin and eosin for histopathology.

Indirect Immunofluorescent method[4]

Each tissue sample of the other group was fixed in p–benzoquinone solution for 4 hours and then washed in 7% of sucrose in phosphate buffered saline at pH 7. 2. Ten micron-frozen tissue sections were prepared for immunostaining after fixation. The frozen tissue sections were left to react with the primary antiserum of vasoactive intestinal peptide (VIP) (dilution 1 : 100) for 18 hours at 4°C. The labeled second layer was applied for 1 hour at room temperature. FITC conjugated goat antirabbit globulin (MBL) was used at a solution of 1 : 100.

Control for Immunocytochemistry

In order to demonstrate that the immunocytochemical reactions were specific, the following tests were performed. (1) Prior to immuno-staining, the diluted antiserum was absorbed with synthetic VIP (10 ng). (2) Normal rabbit serum was used instead of the primary anti-serum as the 1st layer. (3) The FITC second layer was applied alone.

Evaluation of Glucose, Insulin, Pancreatic Glucagon, and Gastrin

The levels of glucose in the blood at fasting and after glucose administration into the stomach were determined by autoanalyzer. The levels of immunoreactive insulin, pancreatic glucagon, and gastrin were determined by radioimmunoassay.

The amount of intubated glucose was 0.4 g/2 ml. The rats were killed 10 and 30 minutes after glucose administration.

The total number of rats used in this study was thirty.

Control

Rats were injected with citric buffer alone.

RESULTS

Morphology

Macroscopically the pancreas appeared atrophied in rats treated with streptozotocin when compared to that in the controls. Microscopic examination of the pancreas in rats given streptozotocin showed a marked reduction in the number and size of islets. The islets were characterized by infrequent or diminished B cells and infiltration of inflammatory cells (Fig. 1.). Fibrotic changes were also found in both endocrine and exocrine pancreas.

Endocrine Function of the Pancreas

Blood sugar levels and immunoreactive insulin levels at fasting in rats with streptozotocin were 444. 8±118. 5 mg/dl and 9. 3±0. 7 μU/ml, respectively, while the levels in control rats were 120. 8±5. 5 mg/dl and 26. 8±1. 6 μU/ml, respectively (Table 1). Thus, rats treated with streptozotocin showed hyperglycemia and hypoinsulinemia. The rats had also gylcosuria. The levels of blood sugar and immunoreactive insulin of the rats given streptozotocin were 421. 5±65. 0 and 376. 0±173. 0 mg/dl, and 10. 8 ±4. 8 and 10. 2±1. 1 μU/ml at 10 and 30 minutes after glucose administration, respectively.

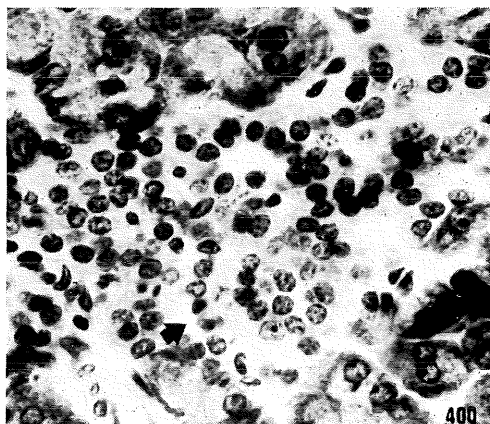These morphological and endocrine data, thus,

Fig. 1. Destroyed B cells and cell infiltration in islets of diabetic rats (HE: ×400).

Table 1. Blood sugar (BS), immunoreactive insulin (IRI), immunoreactive glucagon (IRG) and immunoreactive gastrin (IRGa) in diabetic rats and control.

	BS (mg/dl)	IRI (μU/ml)	IRG (pg/ml)	IRGa (pg/ml)
Control	120.8$\pm$5.5	26.8$\pm$1.6	47.0$\pm$18.0	80.1$\pm$4.6
DM	444.8$\pm$118.5	9.3$\pm$0.7	49.0$\pm$9.0	AGML (+) 312.5$\pm$131.6 AGML (−) 105.1$\pm$13.9

BS: Blood sugar, IRI: Immunoreactive insulin,
IRG: Immunoreactive glucagon,
IRGa: Immunoreactive gastrin

indicated that the rats treated with strepto-zotocin were diabetic.

Morphology of the Gastrointestinal Tract

Esophagus

The lower portion of the esophagus in diabetic rats, being equivalent to the lower esophageal sphincter, was contracted and narrowed, while the proximal portion from the contracted portion was relaxed and loose. Histological examination showed that nerve plexuses were decreased and/or were not observed occasionally in the contracted portion (Fig. 2). Being congruous with the changes of nerve plexuses, VIP immunoreactivities were reduced or diminished at the immunocytochemical level (Fig. 3 A and B).

Stomach

Rich VIP immunoreactivities were observed in the nerve plexuses and nerve axons of the gastric body when compared to those in control rats (Fig. 4). Somewhat reduced VIP immunoreactivities were seen in the nerve plexuses and nerve axons in pyloric sphincter muscle layer of diabetic rats, although the reactivities were not markedly different from those in the control rats (Fig. 5 A and B).

Small Intestine

Seventy percent of diabetic rats were associated with diarrhea. The small intestine of diabetic rats was, as a whole, dilated, which suggested stasis, and damaged (Fig. 6). Histological appearance, however, was almost normal.

VIP immunoreactivities were rich in the proximal intestine in these rats (Fig. 7 A and B). Also relatively rich VIP immunoreactivities were observed in the distal small intestine and ileum which were also dilated (Fig. 8 A and B).

Colon

Contracted and dilated segments in the colon
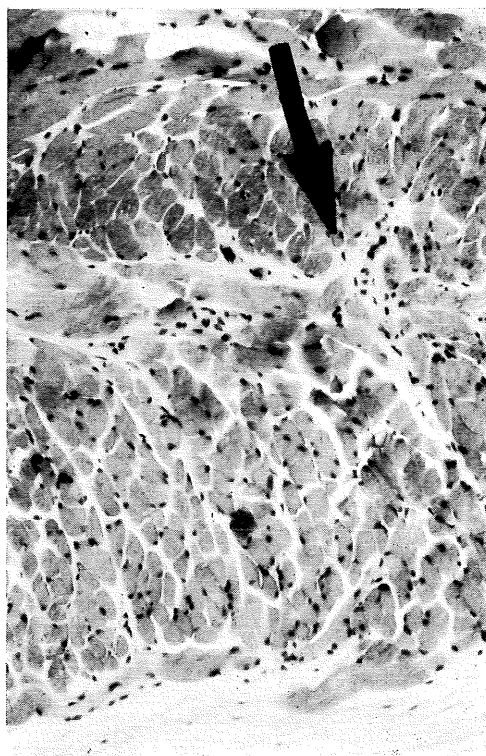
Fig. 2. A disappeared nerve plexus is noted in the contracted segment of esophagus in a diabetic rat (HE: ×400).

of diabetic rats were observed at the macroscopic level (Fig. 6). Marked cellular infiltration and fibrotic changes were seen in colonic mucosa of diabetic rats by hematoxylin and eosin stain (Fig. 9). Furthermore, the regular architecture of the colonic mucosae of diabetic rats was scarcely observed. Neither nerve plexuses nor nerve axons were observed in the contracted segments, while a normal distribution of nerve plexuses was seen in the dilated segments of the colon (Fig. 10 A and B). VIP immuno-
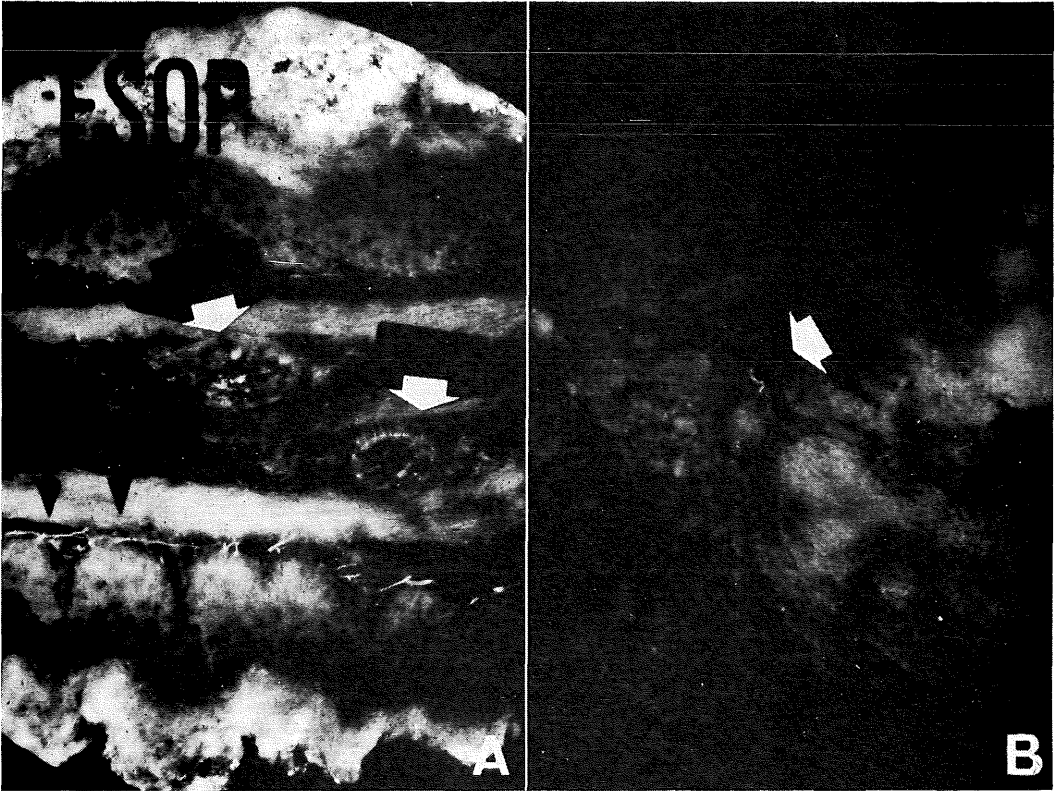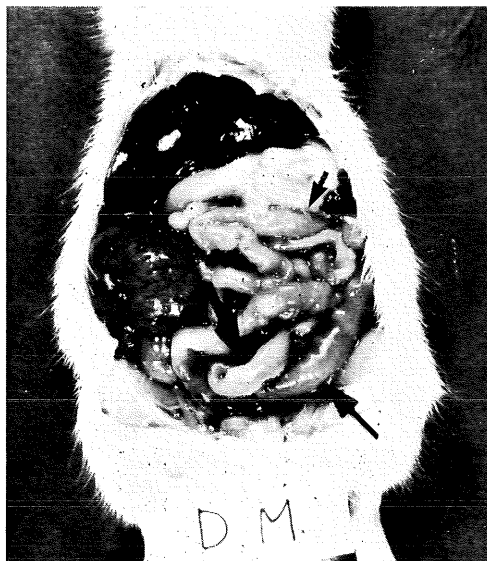
Fig. 3. A: VIP–like immunoreactive nerves in the esophagus of a control rat. B: No VIP–like immunoreactive nerves are found in the contracted segment of esophagus in a diabetic rat (IF: ×200).
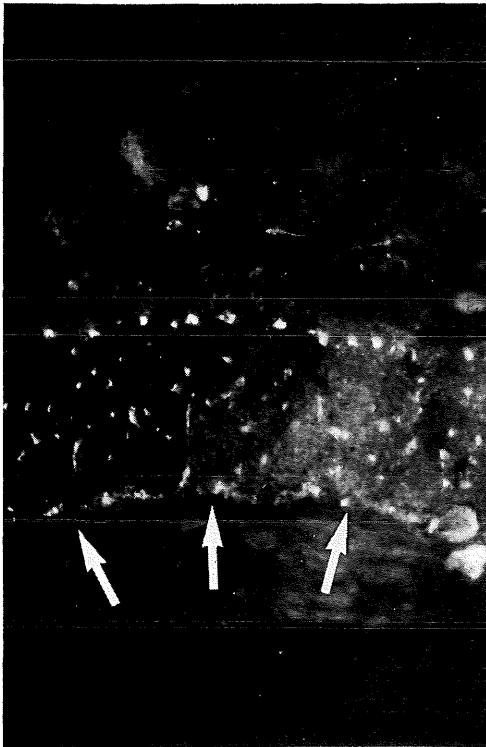

Fig. 4. Relatively rich VIP–like immunoreactive nerve fibers and ganglia are found in the gastric body of diabetic rats (IF: ×200).
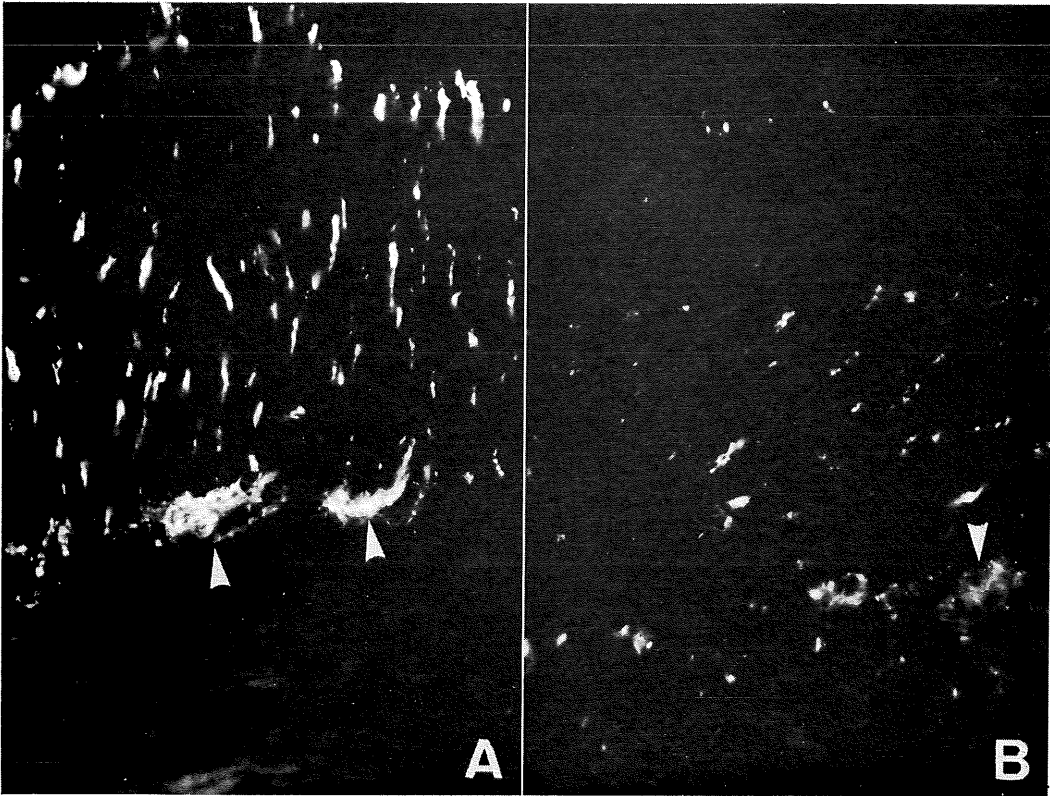
Fig. 5. A: Rich VIP-like immunoreactive nerve fibers and plexuses are found in the pylorus of a control rat. B: VIP-like immunoreactive nerve fibers and plexuses are also observed in the pylorus of a diabetic rat (IF: ×200).

Fig. 6. A diabetic rat shows distended small intestine (small arrow) and large intestine (large arrow).
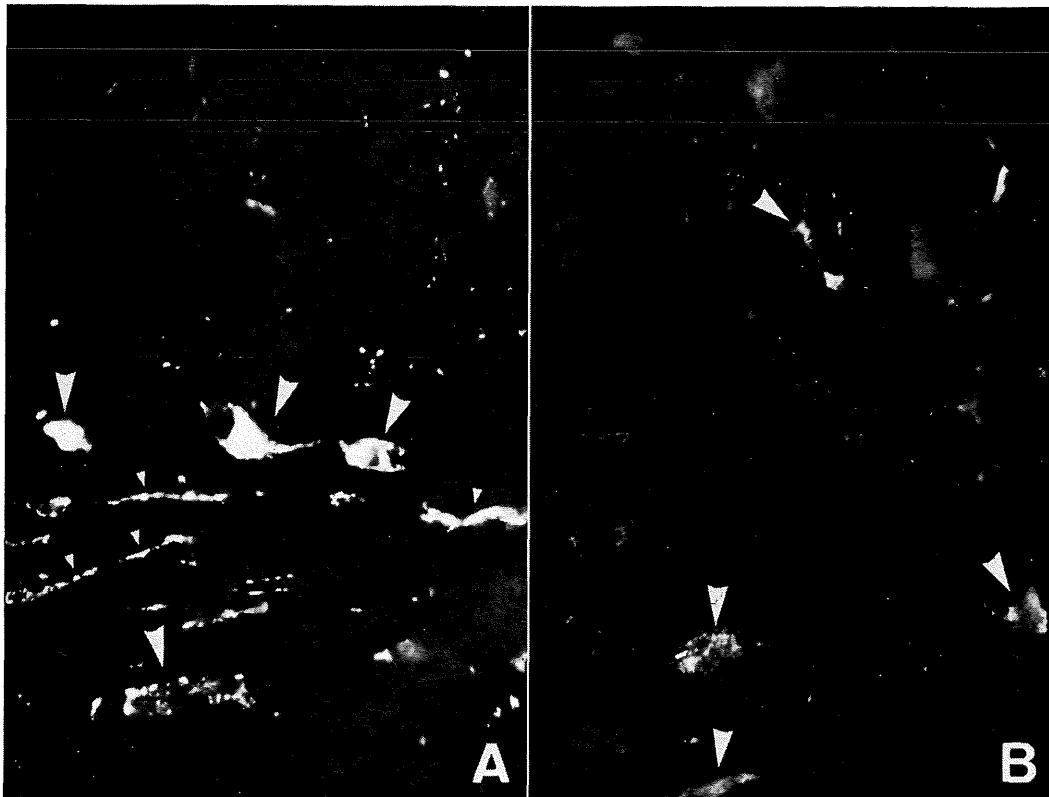
Fig. 7. VIP–like immunoreactive nerve fibers and plexuses in the duodenum of
the control (A) and diabetic rat (B).

reactivities were completely destroyed in the
contracted segments of the colon, which was
well in accord with the destruction of nerve
plexuses in the same area of the colon (Fig.
11 A and B).

DISCUSSION

Diabetic gastroenteropathy is not well under-
stood. It is said that diabetic gastroenteropathy
develops as a result of dysfunction of the
autonomic nerves, which innervated gastrointes-
tinal tract, as seen in diabetes of long
duration. However, the pathophysiology of this
impaired gastrointestinal function in diabetes
remains undefined, although several mechanisms
have been implicated. These include autonomic
neuropathy, microangiopathy, changes in insulin
and glucagon release, and metabolic disturbance.
In recent years some brain-gut hormones or
peptides including neuropeptides have been de-
scribed to play some role in the pathophysiology
of diabetic gastroenteropathy[23], although their

physiological importance remains to be deter-
mined. It is a likely fact that the metabolic
changes observed in diabetes will modify the
release of thses hormones and peptides and
alter their effects on gastrointestinal function.
The purpose in the study was, therefore, to
demonstrate on morphological and immunocyto-
chemical bases the changes in nerve plexuses
and distribution of vasoactive intestinal peptide
(VIP), one of the brain-gut peptides (peptidergic
nerves) which innervated gastrointestinal func-
tion, in rats with diabetic gastroenteropathy
induced by streptozotocin.

It is generally accepted that streptozotocin is
diabetogenic in rats[6,9,10,20]. In the present
study, hyperglycemia as well as hypoinsulinemia
have been demonstrated in rats treated with
streptozotocin. These rats, therefore, were difi-
nitely diabetic.

About 70% of rats treated with streptozotocin
were associated with diarrhea and malnutrition
as well. When the abdomen of the rats was
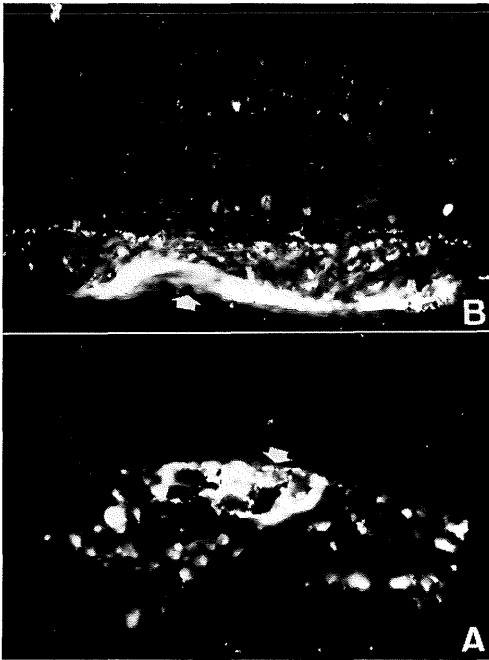
Fig. 8. Rich VIP–like immunoreactive nerve fibers and ganglionic cell bodies in the ileum of both a control (A) and a diabetic rat (B) (IF: A: ×200, B: ×400).
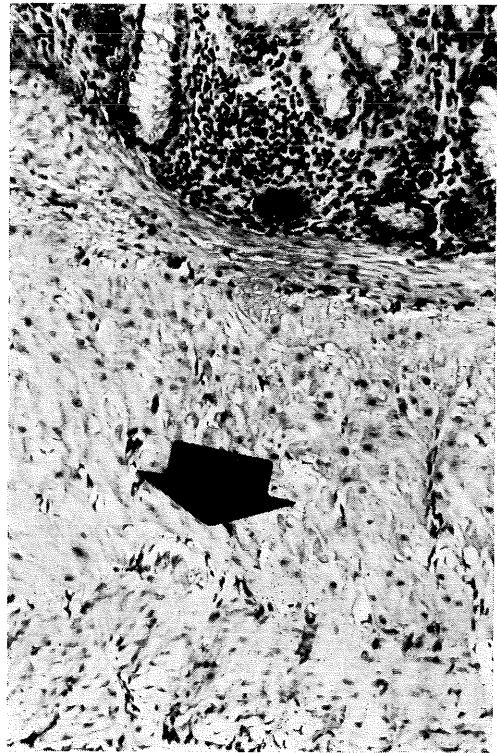
Fig. 9. Marked cell-infiltration, fibrosis and nerve plexus (arrow) are observed in the dilated segment of the colon of a diabetic rat (HE: ×700).
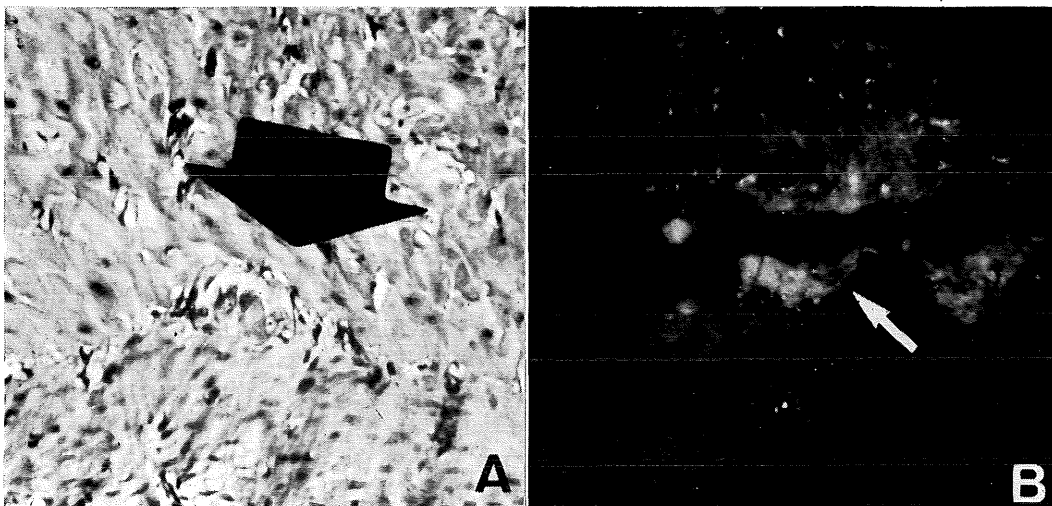
Fig. 10. One never plexus (A: HE: ×100) and VIP–like immunoreactive nerve (B: IF: ×200) in the dilated segment of the colon in a diabetic rat.
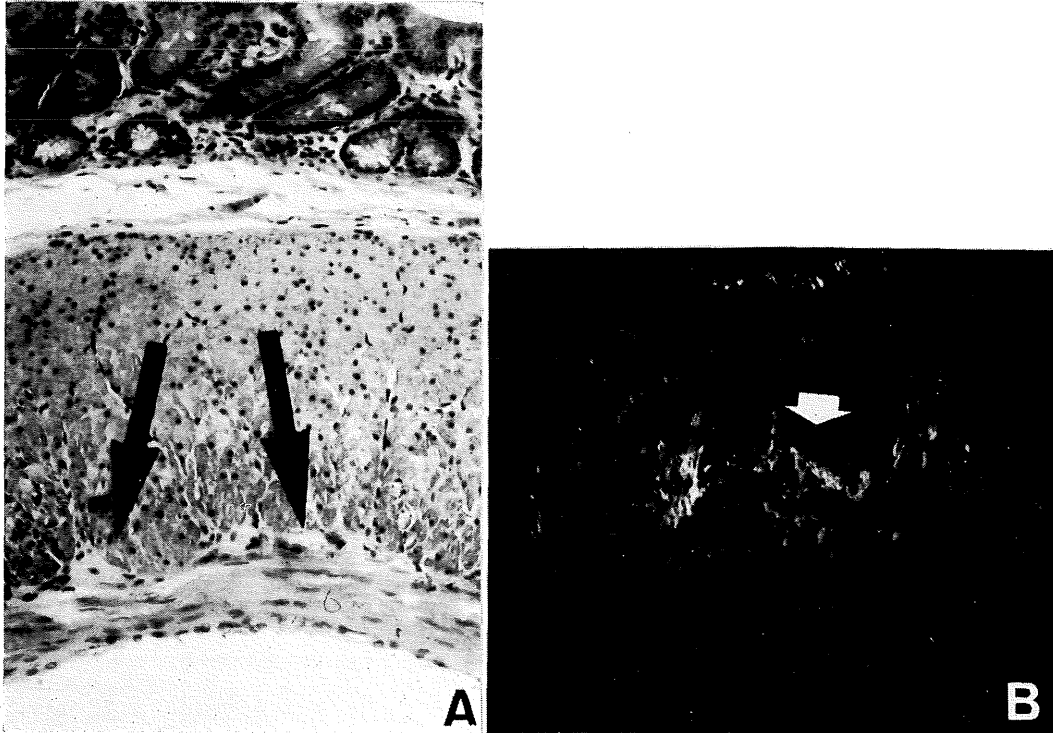
Fig. 11. Neither a nerve plexus (A: HE: ×400) nor VIP–like immunoreative nerve (B: IF: ×200) are found in the contracted segment of the colon in a diabeitc rat.
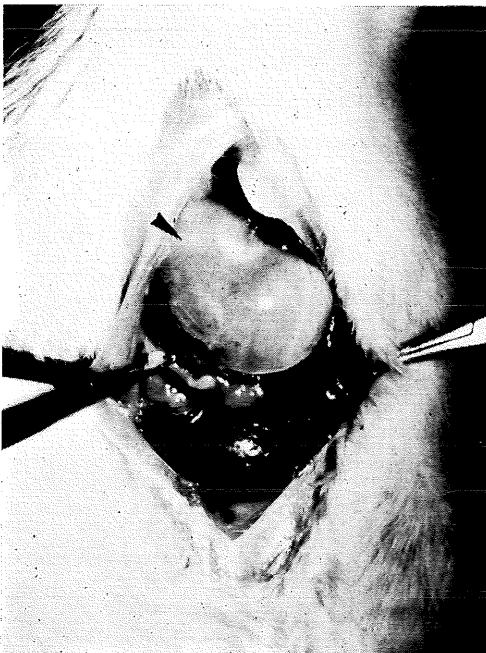

Fig. 12. A marked dilated stomach in a diabetic rat

opened, gastric paresis of varying degree (to gastric atony) (Fig. 12) and small intestinal dilatation and stasis were observed. These signs and morphological findings observed macroscopically were similar those observed in patients with diabetic gastroenteropathy. Furthermore, it was interesting that the dilated and constricted segments were found in the colon of the rats. These findings suggest that rats associated with these signs and morphological fiindings similar to diabetic gastroenteropathy observed in human cases have diabetic gastroenteropathy. The remaining 30% of the rats did not show such signs and findings, although the rats were diabetic because the rats had glycosuria, hyperglycemia and hypoinsulinemia.

The lower esophagus of about 30% of the rats with gastroenteropathy was contracted and narrowed, while the proximal esophagus from the contracted area was dilated. Nerve plexuses in the wall of the contracted area of the esophagus were destroyed and VIP immunoreactivities were not observed concomittantly in the same area. Since the esophageal smooth muscle

seemed to be normal at the microscopical level, these nerve changes may influence the pathogenesis of contraction of the lower esophagus. As the esophagus is largely innervated by the vagus nerve, esophageal motor disturbance is considered to be a dysfunction of esophageal innervation[21,25]. In addition to this, the present study showed that destruction of VIPergic innervation may play a role in esophageal contraction. The mechanism may be similar to that observed in cases of Hirschsprung's disease and Chagas disease[19]. On the contrary, autonomic and VIPergic innervation of the esophageal wall was almost normal in a large number of rats with diabetic gastroenteropathy without esophageal contraction. Contraction of the esophagus was not observed in any of the diabetic rats without gastroenteropathy and in control rats as well.

Gastric retention (paresis) as well as delayed gastric emptying have been considered to be late complications of diabetes[8,11,16,26]. The enlarged stomach with retention in rats with gastroenteropathy observed in the present study was similar to those (gastric paresis) in human diabetic cases. Rich VIP-like immunoreactivities were found in the mucosa and muscle layers of glandular stomach in these rats, suggesting that VIP in the nerves may be one of the pathophysiological causes of gastric retention and delayed gastric emptying because one of physiological actions of VIP is relaxation of smooth muscles[22]. Abnormality of nerve plexuses in the glandular stomach was not noted. VIP-like immunoreactivities were not different in pyloric sphincter from rats with gastroenteropathy and without gastroenteropathy as well as control rats.

The etiology of gastric stasis observed in diabetes, however, remains unresolved. In view of the frequent association with peripheral autonomic neuropathy which is a similar condition found after vagotomy[27], it is likely that visceral neuropathy is important in the etiology. Actually, in the another study, immunocytochemical VIP immunoreactivities were normal or rich in the gastric body mucosa and muscle layers in truncal vagotomized rats with marked gastric stasis[14]. This finding supports the role of VIP in gastric stasis. Hyperglycemia and glucagon have also been shown to play a role in delayed gastric emptying in non-diabetic human cases

with duodenal ulceration[2,7]. A high concentration of blood glucagon was not observed in rats with gastric stasis in the present study. Therefore, pancreatic glucagon does not seem to play any role in the mechanism of delayed gastric emptying in rats with gastric paresis in the present study. Hypergastrinemia has been observed in rats with gastroenteropathy. This many be due to gastric retention and atrophic gastritis[13].

Small intestinal dilation and stasis were observed in rats with diabetic gastroenteropathy. However, no specific lesions except thin and fragile wall were observed in enteric ganglia, mucosa, muscle, and microvasculature at light microscopic level. These support that there is no evidence of histological abnormalities which affect the intestinal sympathetic or parasympathetic nerves and the submucosal or myenteric plexuses in human cases[1,5,15,26]. It is very interesting that there were rich VIP-like immunoreactivities in almost all mucosa, muscle, and ganglia of the small intestine in rats with gastroenteropathy in the present study. The evidence also suggests that VIP may participate in the pathogenesis of small intestinal stasis in rats with diabetic gastroenteropathy as in the case of gastric paresis. The mechanism why VIP immunoreactivities increased, however, remains uncertain.

Constipation has been reported to be a common feature of diabetic neuropathy in human cases[17,21]. In the present study, however, 70% of the rats with diabetic gastroenteropathy had diarrhea. Macroscopic findings showed dilated and contracted (narrowing) segments in the colon of these rats. Microscopical evidence, however, was very interesting. Thickened muscle layer was found in the contracted segments of the colon where neither nerve myenteric plexuses nor VIP nerves existed, while flattened mucosa associated with fibrotic changes and cellular infiltration were observed in the dilated segments where myenteric plexuses and VIP nerves were found. These findings were very similar to those observed in the diseased segments of Hirschsprung's disease and Chagas disease[19] as well. These suggest that VIP existing in the nerve neurones of the colon may play an important role in the pathognomonic changes in the colon of rats with diabetic gastroenteropathy. In the contrast with these

observations, little changes were observed in rats without diabetic gastroenteropathy and no abnormal changes were observed in the colon of control animals. These support the pathognomonic role of VIP mentioned above in rats with diabetic gastroenteropathy.

Finally, the mechanism of development of macroscopical and histological abnormalities including destroyed myenteric plexuses observed in the gastrointestinal tracts remains obscure. The direct toxic action of streptozotocin on the autonomic nerves in the gastrointestinal tract[18] could be denied since 6 months had passed after the injection.

The results observed in the present study were the chronic effects of streptozotocin-induced diabetes on the gastrointestinal tract in rats. The results suggest that one of the brain-gut hormones (peptides), VIP, in the gastrointinal tract of the present study modified the release and exerted its effect on the gastrointestinal tract. Furtheremore, metabolic disturbances[18] induced by diabetes after streptozotocin administration may be a more peausible cause of the development of diabetic gastroenteropathy.

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