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Serum Pepsinogen Levels in Normal and Experimental Peptic Ulcer Rats Measured by Radioimmunoassay

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Antiserum to the rat pepsinogen (PG) purified by us was raised in the rabbit. We established a radioimmunoassay system for PG and measured the content of it in the digestive and urinary organs. Immunoreactive PG in serum was confirmed to be PG by high-performance liquid chromatography using an ion exchange column. Serum PG levels in pregnant or lactating rats were higher than normal. The maximum level during lactation was 6 or 7 times the normal value, and began to decrease from about the 16th day after delivery. Serum PG in infant rats was elevated along with the content of mucosal PG. In the measurement of serum PG levels in experimental peptic ulcer rats, no significant change was observed in Shay and cysteamine ulcers produced in the forestomach or/and the duodenum. However, serum PG levels rose significantly in rats with indomethacin, stress and ethanol ulcers, which represent injuries on the glandular portion.

Keywords——radioimmunoassay; serum pepsinogen level; experimental gastric ulcer; pepsinogen; rat; pregnancy; lactation; stomach

We have already reported that there are two kinds of acid proteinase in the rat gastric mucosa; pepsinogens (PGs) and cathepsins, and only PGs are secreted in gastric juice.[1-5] Human PGs are classified into A, B and C type zymogens, whereas rat PGs are C type zymogens. Secreted PGs change into pepsins which hydrolyze nutritional proteins under acidic conditions. Usually, pepsins do not digest healthy gastric mucosa, but they are able to digest it in peptic ulcer. Samloff and Liebman showed that serum PG levels were decreased in patients with pernicious anemia[6] and increased in those with peptic ulcer.[7] Thus, measurement of serum PG levels may provide important information for the diagnosis of gastrointestinal diseases. However, in order to utilize serum PG level for diagnosis of gastric disease, we must know what factors affect the serum PG level. Since several kinds of experimental gastric ulcers are available in the rat, we measured the serum PG levels of normal rats and rats with experimental gastric ulcers. The results are presented here.

Materials and Methods

Chemicals——Sheep anti-rabbit immunoglobulin G (IgG) antiserum, ^{125}I and complete Freund's adjuvant were obtained from UCB Bioproducts (Belgium), New England Nuclear (Boston, Mass) and DIFCO Lab. (Detroit, Mich), respectively. Pentagastrin from Sumitomo Chem. (Osaka, Japan), histamine from Wako Pure Chem. (Osaka, Japan) and carbamylcholine, cysteamine (2-mercaptoethylamine), indomethacin and porcine pepsin from Sigma Chem. (St. Louis, Mo) were used.

Radioimmunoassay of Rat Pepsinogen——Purification of rat PG was described previously.[1] Briefly, ammonium sulfate was added to the supernatant of 10% rat stomach homogenate in 50 mM phosphate buffer (pH 7.3), and the precipitate at 25 to 80% saturation of ammonium sulfate was collected. The precipitate was purified by diethylaminoethyl (DEAE)-cellulose and DEAE-Sepharose column chromatography. Anti-rat PG antiserum was raised in New Zealand white rabbits immunized with the purified PG dissolved in saline and emulsified with an equal

volume of complete Freund's adjuvant. Radioimmunoassay (RIA) was carried out according to Samloff,[8] by the double antibody technique with sheep anti-rabbit IgG antiserum as the second antibody. Radioiodination of PG was performed by the chloramine T method; 7.3 μg of PG was iodinated with 1 mCi of ^{125}I and 2.7 μg of chloramine T for 1 min. Iodinated PG was purified by passage through a Sephadex G-50 column. For this RIA, the final incubation mixture (280 μl) contained 50 μl of antiserum at a final dilution of 1 : 560000.

Assay of Peptic Activity——Peptic activity was measured by Anson's hemoglobin method[9]; an enzyme solution (0.1 ml) was incubated at 37 °C for 10 min with 2% hemoglobin solution (pH 1.8) (1 ml), and the reaction was stopped by addition of 5% trichloroacetic acid (5 ml). The absorption at 280 nm of the supernatant of the reaction mixture was measured.

High-Performance Liquid Chromatography (HPLC) of Serum Pepsinogen——Serum (1 ml) was loaded on a TSKgel DEAE-5PW column (Toyo Soda, Tokyo, Japan) and eluted with a linear gradient of 0 to 0.8 M NaCl in 20 mM phosphate buffer (pH 7.3) using an LC-3A liquid chromatograph (Shimadzu, Kyoto, Japan). The content of PG in each fraction was measured by RIA.

Perfused Rat Stomach Preparation——Male Wistar rats weighing over 300 g were starved for a day and the perfused rat stomach preparation was obtained according to Ghosh and Schild.[10] The gastric effluent was collected at 10-min intervals, and acidity and peptic activity of each fraction were estimated as described previously.[11] An aliquot of blood was collected from the tail vein every 30 min, and the content of PG in serum was measured by RIA.

Preparation of Rat Experimental Ulcers——Male Wistar rats weighing about 200 g were used for these experiments. For Shay ulcer, the pyloric ligature was performed under ether anesthesia in rats starved for 48 h according to Shay *et al.*,[12] and sham operation was done as the control. For stress ulcer, rats were immobilized in stress cages and immersed in a water bath at 23 °C to the height of the xiphoid process according to the method of Takagi and Okabe.[13] For indomethacin ulcer, indomethacin (30 mg/kg) suspended in 0.5% carboxymethyl cellulose sodium (CMC) was injected subcutaneously into rats starved for 48 h, and 0.5% CMC was injected as the control. For ethanol ulcer, absolute ethanol (1 ml) was directly given to rats starved for 24 h and water-restricted for 19 h, through a stomach tube, according to Robert *et al.*,[14] and water (1 ml) was given as the control. For cysteamine ulcer, cysteamine (400 mg/kg) was injected subcutaneously into rats starved for 24 h, and saline was injected as the control. After a certain time, blood was collected from the inferior vena cava of each experimental ulcer rat under ether anesthesia, and serum was separated. The ulcer index was expressed as total length (mm) of injuries after fixation by instillation of 2% formalin (10 ml) into the isolated stomach for indomethacin, stress and ethanol ulcers, or as a score (0, no change; 1, erosion; 2, 1 to 4 small injuries of diameter less than 3 mm; 3, more than 5 small injuries or 1 large injury of diameter more than 3 mm; 4, more than 2 large injuries; 5, perforation) for Shay and cysteamine ulcers. The ulcer index of duodenal ulcer was expressed as an area (mm^2). The statistical significance of differences was determined by using Student's *t*-test.

Results

Calibration Curve of Rat Pepsinogen

Dose–response curves of rat PG and its analogs, measured by RIA of PG, are shown in Fig. 1. Rat pepsin competed with the binding of ^{125}I-rat PG to its antibody in a similar

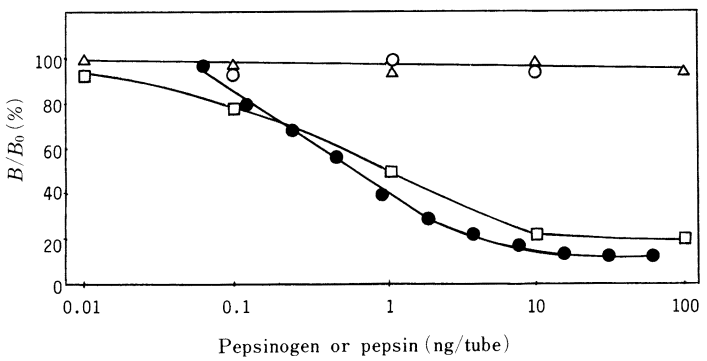

Fig. 1. Dose–Response Curves of Pepsinogen and Pepsins in Radioimmunoassay by Using ^{125}I-Rat Pepsinogen as a Label

○, heat-treated rat pepsin; △, porcine pepsin; □, rat pepsin; ●, rat pepsinogen. Rat pepsin was prepared by acid activation from rat PG on a Sephadex G-75 column.[1]

TABLE I. Contents of Pepsinogen in Various Organs, Serum and Urine

Organ	Pepsinogen immunoreactivity[a]
Esophagus	0.15±0.03
Forestomach	4.67±.3.20
Glandular portion	5016.67±1149.03
Duodenum	2.86±1.94
Pancreas	0.17±0.01
Jejunum	0.19
Ileum	0.23
Cecum	0.53±0.38
Colon	0.05
Rectum	0.05
Kidney	0.09
Urinary bladder	0.03
Seminal vesicle	0.07
Prostate	0.00
Serum	10±1
Urine	118±91

Each organ was homogenized with 50 mM phosphate buffer (pH 7.3), and a suitable amount of supernatant was used for RIA. Values are means±S.E. of 3 male rats. The values which lack S.E. include one or more cases under the limit of detection. *a*) μg/g wet weight tissue, or ng/ml for serum and urine.

TABLE II. Serum Pepsinogen Levels in Rats in Various States

	Serum PG (ng/ml)
Male adult	17±2 (6)
(starvation for) 24 h	44±4 (8)
48 h	69±14 (9)
72 h	36±5 (5)
Female adult	11±2 (8)
Pregnant (15—20 d)	16±3 (5)
Lactating (after delivery)	
12 d	73±3 (5)
14 d	74±3 (5)
16 d	67±4 (5)
20 d	50±4 (5)
25 d	34±2 (4)
30 d	36±3 (4)

The numbers of rats are given in parentheses. The values are mean±S.E.

manner to rat PG, but heat-treated rat pepsin (100 °C, 10 min) and porcine pepsin did not.

Contents of Pepsinogen in Various Organs

The contents of PG in serum, urine and the digestive and the urinary organs were measured (Table I). A large amount of PG was found in the glandular portion of the stomach, but only small amounts in the forestomach and the duodenum. Other organs contained negligible amounts of PG. Although the content of PG in urine varied widely, it was about 10 times that in serum.

The content of PG in the glandular portion determined by measuring peptic activities was 5.3±0.8, and that by RIA was 5.0±1.1 mg/g wet weight.

Identification of Immunoreactive Pepsinogen in Serum

We also examined immunoreactive PG in serum by HPLC. The elution profiles of PG and acid-activated PG are shown in Fig. 2a. Pepsinogen and pepsin were separately eluted at different NaCl concentrations. Elution profiles of PG in 1 ml of serum, obtained from a normal rat or ethanol (1 h after instillation) or indomethacin (25 h after administration) ulcer rat are shown in Fig. 2b, c and d, respectively. We estimated that immunoreactive PG in serum showed the same retention time as PG and no contamination by pepsin was detected.

Serum Pepsinogen Levels

Serum PG levels in fed and starved male rats, as well as normal, pregnant and lactating female rats were measured (Table II). Serum PG levels in male rats during starvation gradually rose, then decreased on the 3rd day. Those in rats on the 2nd day of starvation were 4 times as high as in fed rats.

Serum PG levels in pregnant rats were about 1.5 times those of ordinary female rats. Interestingly, serum PG levels in lactating rats were 6 or 7 times higher than that of normal rats, and they began to fall gradually from about the 16th day after delivery. However, the

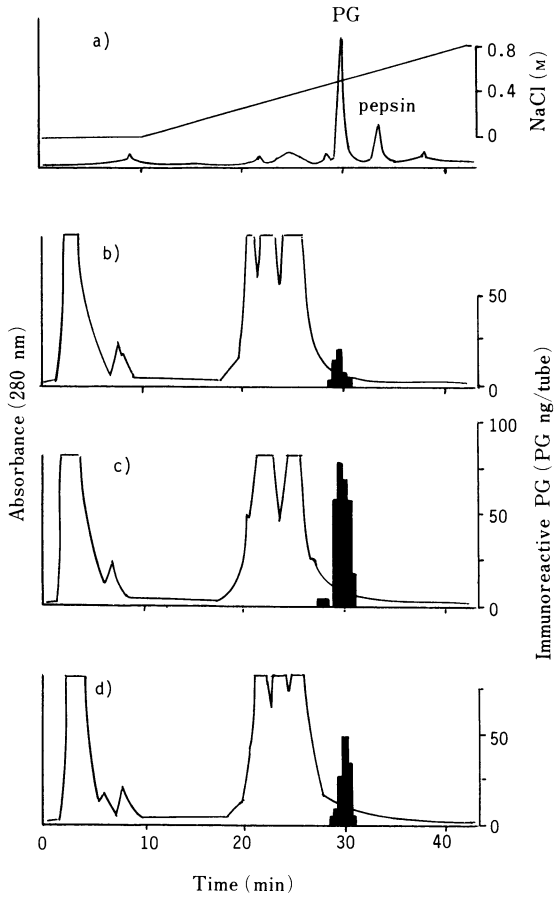

Fig. 2. Typical HPLC Elution Profiles of Pepsinogen in Sera from Normal Rats and Rats with Experimental Gastric Ulcer Induced by Ethanol or Indomethacin

The samples were eluted with a linear gradient of 0 to 0.8 M NaCl in 20 mM phosphate buffer (pH 7.3) at 0.94 ml/min on a TSKgel DEAE-5PW (7.5 × 75 mm) column; fractions were collected every 30 s. The samples were (a) a mixture of PG and acid-activated PG, (b) normal serum (1 ml), (c) serum from ethanol ulcer rat, (d) serum from indomethacin ulcer rat. Each serum (1 ml) was eluted under the same conditions as in (a), and immunoreactive PG is indicated by closed columns. Each elution profile was obtained in one of three experiments.

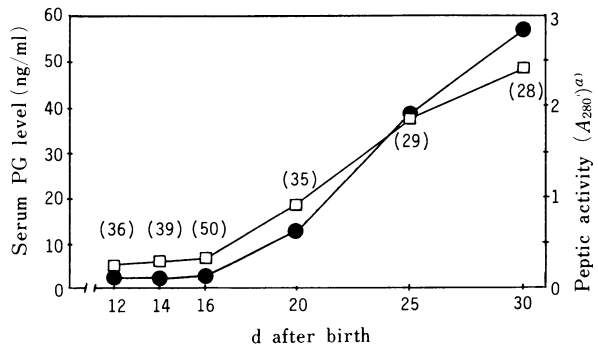

Fig. 3. Changes of Serum Pepsinogen Levels and Mucosal Peptic Activities in Rats after Birth

□, mucosal peptic activity; ●, serum PG level. The numbers of rats are given in parentheses. *a*) Absorbance (280 nm) of the reaction mixture obtained as described in Materials and Methods.

content at the 30th day after delivery remained 3 times higher.

In regard to changes of PG content during development, the peptic activities of gastric mucosa and serum PG levels in immature rats of both sexes from the 12th to the 30th day after birth are shown in Fig. 3. No significant difference in serum PG levels between male and female immature rats was observed. The contents of mucosal and serum PGs similarly increased on about the 20th day after birth. Serum PG levels at the 30th day after birth were about 4 times those of adult male rats, but mucosal PG content was about half.

Effects of Pentagastrin, Histamine and Carbamylcholine on Serum Pepsinogen Levels in Perfused Rat Stomach Preparation *in Vivo*

The correlation between PG secretion and serum PG levels was examined. Submaximal doses of three secretagogues, pentagastrin, histamine and carbamylcholine, were injected

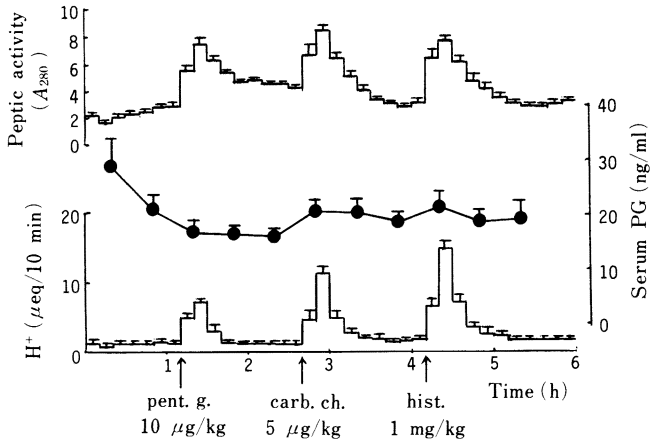

Fig. 4. Effects of Pentagastrin, Carbamylcholine and Histamine on Serum Pepsinogen Levels and Acid and Pepsin Secretions in Perfused Rat Stomach *in Vivo*

Pentagastrin (pent.g.), carbamylcholine (carb.ch.) and histamine (hist.) were intravenously injected at the indicated times and doses. Acid secretion and peptic activity in gastric effluent and serum PG level (●) are mean values±S.E. from 5 rats.

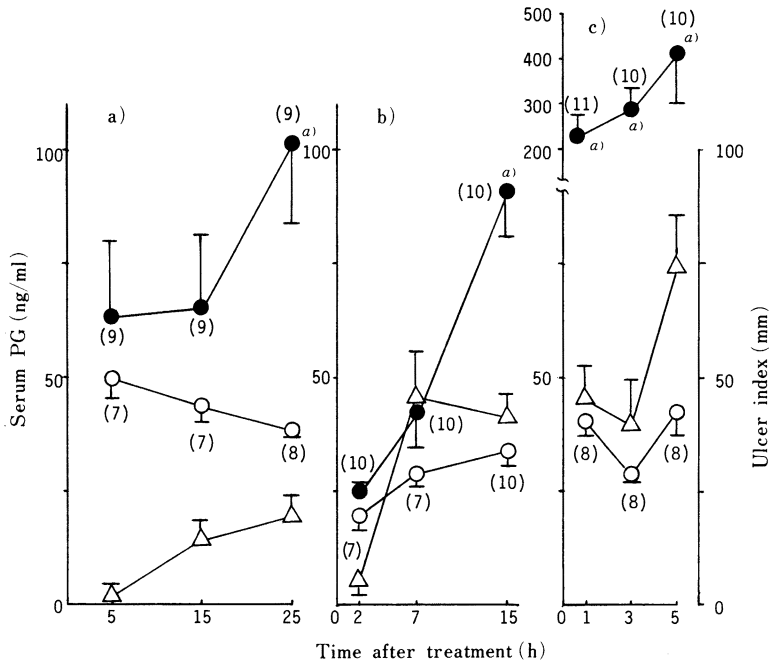

Fig. 5. Changes of Serum Pepsinogen Levels and Ulcer Indexes in Indomethacin (a), Stress (b), and Ethanol (c) Ulcer Rats

Serum PG levels in rats with experimental gastric ulcer (●) and corresponding control rats (○) are mean values±S.E. from the numbers of rats indicated in parentheses. Ulcer indexes (△) in experimental gastric ulcer were obtained from the same rats. *a*) $p<0.01$.

TABLE III. Changes of Serum Pepsinogen Levels and Ulcer Indexes in Shay and Cysteamine Ulcer Rats

	Shay ulcer		
Time (h)[a]	2	5	10
Serum PG		Sham operation	
(ng/ml)	26±2 (6)	26±2 (7)	39±3 (7)
		Shay ulcer	
	38±5 (10)	37±4 (10)	52±9 (9)
Ulcer index[b]	0	0.3±0.2	3.0±0.2

	Cysteamine ulcer		
	Control	Duodenal	Duodenal and gastric
Serum PG (ng/ml)	34±3 (8)	25±8 (4)	53±16 (10)
Ulcer index			
Duodenal[c]	—	9±4	11±3
Gastric[b]	—	—	2.3±0.2

The values are means±S.E. from the numbers of rats given in parentheses. *a*) Time after ligation. *b*) Scores. *c*) mm^2.

intravenously at 90 min intervals (Fig. 4). Acid and pepsin secretion were stimulated by these secretagogues, whereas serum PG levels were only slightly elevated by histamine and carbamylcholine.

Serum Pepsinogen Levels in Experimental Gastric or/and Duodenal Ulcer Rats

The experimental gastric or/and duodenal ulcers were divided into two groups, one consisting of glandular injury induced by indomethacin, ethanol or stress, and the other of forestomach or/and duodenal injury induced by cysteamine or pyloric ligature. Serum PG levels in these experimental ulcer rats are summarized in Fig. 5 and Table III.

Serum PG levels of starved rats at 25 h after injection of indomethacin were about 2.5 times the control. Clear injuries were seen at 15 and 25 h judging from the ulcer indexes. Serum PG levels of rats under restraint and immersion stress at 15 h were about 4 times the control and significant changes in ulcer indexes were observed, even at 2 h after the stress. After instillation of ethanol through a stomach tube into starved rats, great increases in serum PG levels were seen; within 1 h, the level increased about 5 times, and at 5 h, about 10 times. However, the ulcer indexes determined at 5 h were only twice those at 1 h.

On the other hand, ulcer indexes in rats with experimental forestomach ulcer caused by pyloric ligature were large at 5 and 10 h, but serum PG levels showed no significant difference from those of the sham operation. In cysteamine ulcer, serum PG levels and ulcer indexes were measured at 18 h after cysteamine administration. Cysteamine ulcer was divided into two groups, one in which the duodenum is injured and the other affecting both the duodenum and the forestomach. Serum PG levels in cysteamine ulcer rats with injuries of both the duodenum and the forestomach were higher than those of the duodenum only. However, neither group showed a significant difference from the control.

Discussion

We have shown that anti-rat PG antiserum raised in the rabbit gave a positive Ouchterlony test with mouse and hamster PG, but a negative one with porcine PG.[2] The antiserum also reacted to rat pepsins. Using this antiserum, we quantified the contents of PG in the digestive and urinary organs, serum and urine by RIA. A very large amount of PG was found in the glandular portion of the stomach. Immunoreactive PG was not detected in the prostate gland, which is known to produce gastricsin.[15,16] Therefore it seems probable that immunoreactive PG in serum and urine comes from the glandular portion of the stomach, and anti-rat PG antiserum does not react with intrinsic proteins other than rat PGs and pepsins. The content of PG in urine varied so widely that we concluded that the measurement of PG in serum would be more useful.

Proceeding to the measurement of serum PG levels, we examined whether or not immunoreactive PG in serum is PG itself. If serum PG diffuses back from the gastric lumen, immunoreactive PG in serum should be pepsin. However, the elution profile of immunoreactive PG in serum on HPLC with a TSKgel DEAE-5PW column showed that the serum PG obtained from normal or ulcer rat had the same retention time as PG derived from the gastric mucosa. The following experiments were done on the premise that immunoreactive PG in serum is PG itself.

First of all, serum PG levels in normal rats of different age, sex and condition were measured. We found that serum PG levels changed during starvation; they increased first and fell later. These results suggest that serum PG levels in man depend upon the time after eating. Waldum *et al.* reported that there was no significant difference of mean human serum PG I levels at different stages during pregnancy.[17] However, slightly higher levels during pregnancy were obtained in the rat. Furthermore, serum PG levels during lactation were much higher than those during pregnancy. Needless to say, the content of mucosal PG and the size of the stomach during pregnancy or lactation are almost normal, so that higher serum PG levels might be due to enhancement of the stomach function. It was confirmed that the high serum PG levels during lactation began to decline from the 16th day, that is around the weaning period.

On the other hand, in immature rats mucosal PG began to be synthesized from around the weaning period and serum PG levels paralleled the increase. Much PG was found in the circulation at the 30th day after birth, even in healthy immature rats. These results also suggest that serum PG levels reflect stomach function. It is not clear why an exocrine enzyme should be released into the circulation as a result of an enhancement of stomach function in normal conditions.

We examined whether or not serum PG levels were elevated in perfused rat stomach *in vivo* by the stimulation of PG secretion. Serum PG levels were slightly elevated by carbamylcholine and histamine, whereas peptic activities in gastric effluent were about twice the basal ones. Similar results in man were observed by Waldum,[18] *i.e.*, vagal and hormonal stimulation increased serum PG levels.

There are many reports about the relationship between serum PG levels and gastric diseases in man, and high serum PG levels were described in gastric and duodenal ulcer patients. We were uncertain why high serum PG levels were observed in duodenal ulcer patients, though only a very small amount of PG existed in the duodenal mucosa. We separately examined serum PG levels in two kinds of experimental peptic ulcer in the rat, in relation to the amount of PG in the injured part, *i.e.*, the glandular portion of the stomach and the forestomach or/and the duodenum. High serum PG levels were observed in the experimental injury at the glandular portion, but not at the forestomach or/and the duodenum in rats. High serum PG levels were found in rats with experimental ulcers in the

glandular portion. However, there is a big discrepancy in serum PG levels between duodenal ulcer patients and experimental duodenal ulcer in the rat. It is known that hereditary factors are similar in each type of experimental rat, whereas this would not be the case in patients. As we have suggested that serum PG levels might reflect stomach function, it seems likely that enhanced stomach function causes duodenal ulcer in most cases in man. Serum PG levels and ulcer indexes in the same ulcer model were not correlated, and this may suggest that serum PG does not originate from the injured mucosa but from the repairing mucosa. Moreover, among the experimental ulcers of the glandular portion of the stomach, there were marked differences in serum PG levels between stress and ethanol ulcers, even if they showed similar ulcer indexes (which represent the degree of injury). Different types of mucosal injuries may occur in stress and ethanol ulcers.

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