

## Effect of Anti-ulcer Drugs on Gastric Mucous Hexosamine in Rats subjected to Several Ulcerogenic Conditions

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Hexosamine content of pyloric tissue of the stomach in rats exposed to fasting, water immersion and restraint stress, and cortisone treatment was determined. Water immersion and restraint stress, and cortisone treatment resulted in the decreased hexosamine content.

Anti-ulcer drugs were administered to these animals orally. In the water immersion and restraint stress the hexosamine content of pyloric tissue of the rats was increased by tyrosine, threonine, glutamine, gefarnate, synthetic aluminum silicate and sodium copper chlorophylline. Glutamine, gefarnate and sodium copper chlorophylline also induced the increase in hexosamine content of the rat pyloric tissue treated with cortisone acetate.

Several reports have shown that mucous components of the gastric mucosa were modified under fasting, restraint stress and application of ulcerogenic drugs.<sup>2-8)</sup> It might be supposed that the level of mucous components represents the mucosal resistance of the stomach in experimental animals and has an important role in the development and healing of gastric ulcer. However, few investigators have studied the effect of anti-ulcer drugs on the mucous substance of the stomach. Menguy and Masters<sup>9)</sup> have studied the effect of parathyroid extract (PTE) on gastric mucus in rats given PTE alone or with cortisone and observed that PTE caused a substantial increase in mucous content of gastric mucosa.

In the present paper the changes of gastric mucous hexosamine content were studied as an index of mucous content in the rat stomach subjected to several ulcerogenic conditions and various anti-ulcer drugs. The relationship of the hexosamine level of gastric tissue to the development and recovery of experimental gastric ulcers was also discussed.

### Material and Method

Male rats of Donryu strain were used. After treatments such as fasting, water immersion and restraint stress, and cortisone administration, animals were sacrificed by a blow on the head. Their stomachs were removed quickly and opened along the greater curvature, and any erosion was noted. The tissue of the pyloric portion was excised, and lyophilized for hexosamine assay. The hexosamine contents of the individual specimens were determined by the colorimetric method of Boas<sup>10)</sup> and calculated as glucosamine hydrochloride.

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- 2) A. Robert, R.B. Bayer, and J.E. Nezamis, *Gastroenterology*, **45**, 740 (1963).
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In order to study the effect of anti-ulcer drugs on gastric mucous hexosamine, glutamine 2.0 g/kg, sodium copper chlorophylline 0.5 g/kg, gefarnate (geranyl farnesyl acetate) 0.2 g/kg, synthetic aluminum silicate 0.5 g/kg and 4 amino acids (methionine, tyrosine, threonine, and proline) 2.0 g/kg were suspended in 0.5% CMC solution and administered orally once a day to the rats subjected to the same ulcer producing procedure. The control animals received 0.5% CMC solution. On the next day to the last oral administration of anti-ulcer drugs, animals were killed and determinations of mucous hexosamine were performed.

**1. Effect of Fasting on Gastric Mucous Content**—In the experiment of fasting alone, 60 rats weighing 210 to 240 g were divided into six groups. Animals were deprived of food with water ad libitum and every one group was subjected to three-hour pyloric ligation on each day during six days after the start of fasting. The gastric content of each rat was centrifuged and the volume of gastric juice was determined. Free and total acid were measured by titration with 0.1N sodium hydroxide using Töpfer reagent and phenolphthalein respectively as an indicator, and the pH of gastric juice was determined by means of an electrometric pH meter. Hexosamine contents of gastric juice and pyloric tissue were also determined.

In the experiment of fasting under administration of drugs, 50 fasted rats were divided into 5 groups of 10 rats. One of the 4 anti-ulcer drugs and 0.5% CMC solution was given orally for 5 days to each group, which was subjected to three-hour pyloric ligation to collect the gastric juice on the sixth day after the start of fasting. The volume and acidity of gastric juice and the hexosamine content of gastric juice and pyloric tissue were measured.

**2. The Experiments of Water Immersion and Restraint Stress**—Water immersion and restraint stress reported by Takagi and Okabe<sup>11)</sup> was imposed on 80 rats weighing 240 to 280 g. Ten rats were killed every day from the first to the eighth day after the water immersion and restraint stress and the hexosamine content of pyloric tissue was assayed.

In another experiment 4 anti-ulcer drugs and 4 amino acids (methionine, tyrosine, threonine, and proline) were administered to 8 groups of ten rats for 3 days after the stress application. Animals were killed for hexosamine assay of pyloric tissue on the following day of the last oral administration.

**3. The Experiments of Cortisone Treatment**—One hundred rats weighing 220 to 240 g were divided into 5 groups of 20 animals. Every one group received orally one of 4 anti-ulcer drugs and 0.5% CMC solution as a control for 3 days. Each group was divided into 2 sub-groups of 10 rats, one of which was treated with cortisone acetate 5 mg/kg subcutaneously for 3 days and the other with 0.5% CMC solution.

All animals were fasted with water ad libitum during this period. Hexosamine assays were performed on samples of the full thickness of the pyloric portion of the stomach in all groups.

## Result

### 1. Effects of Fasting on Gastric Mucous Content

In rats fasted for more than 4 days volume, free acid, total acid and hexosamine output of gastric juice were decreased in comparison with those of the rats fasted for one day. But the hexosamine content of pyloric tissue was not changed throughout the period of fasting (Fig. 1). No lesion was found in the stomach of all fasted rats.

TABLE I. Effect of Several Anti-ulcer Drugs on Gastric Acidity, Volume and Mucous Hexosamine in Fasted Rats

	Free acid (mEq/liter)	Total acid (mEq/liter)	pH	Volume (ml/hr)	Gastric juice hexosamine ( $\mu$ g/hr)	Pyloric tissue hexosamine ( $\mu$ g/mg tissue)
Control	32 $\pm$ 8.3	99 $\pm$ 7.3	1.5 $\pm$ 0.2	1.1 $\pm$ 0.15	296 $\pm$ 29	12.9 $\pm$ 1.0
Glutamine	23 $\pm$ 7.2	70 $\pm$ 6.8 <sup>a)</sup>	1.5 $\pm$ 0.1	2.0 $\pm$ 0.12 <sup>a)</sup>	385 $\pm$ 19 <sup>a)</sup>	15.1 $\pm$ 0.5 <sup>a)</sup>
Gefarnate	37 $\pm$ 4.2	88 $\pm$ 5.8	1.3 $\pm$ 0.1	1.4 $\pm$ 0.20	408 $\pm$ 55	16.3 $\pm$ 0.7 <sup>a)</sup>
Synthetic aluminum silicate	36 $\pm$ 12.0	110 $\pm$ 6.0	1.6 $\pm$ 0.3	1.6 $\pm$ 0.19	422 $\pm$ 82	13.7 $\pm$ 1.0
Sodium copper chlorophylline	32 $\pm$ 5.6	88 $\pm$ 9.0	1.5 $\pm$ 0.1	1.0 $\pm$ 0.06	231 $\pm$ 42	18.8 $\pm$ 1.4 <sup>a)</sup>

Each value represents the mean  $\pm$  standard error.

a) statistically significant at  $P=0.05$

11) K. Takagi and S. Okabe, *Jap. J. Pharmac.*, **18**, 9 (1968).

In Table I volume, acidity, and hexosamine of gastric juice of fasted rats treated with anti-ulcer drugs for 5 days are summarized. The augmentation of gastric juice secretion in glutamine-treated rats was noted. Treatments with glutamine, gefarnate and synthetic aluminum silicate resulted in some increase in hexosamine output in gastric juice. The hexosamine content of pyloric tissue was elevated in the groups of glutamine, gefarnate and sodium copper chlorophylline, but not of synthetic aluminum silicate.

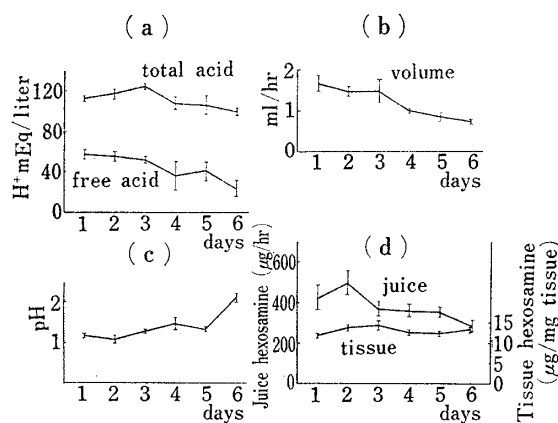


Fig. 1 Effect of Fasting on Gastric Acidity, Volume and Mucous Hexosamine

absissa: days of fasting  
 ordinate: (a) H<sup>+</sup> (mEq/liter) of free acid and total acid, (b) volume (ml/hr) of gastric juice, (c) pH, (d) hexosamine (µg/hr) of gastric juice and tissue hexosamine (µg/mg tissue). Vertical lines indicate the S. E. of the mean.

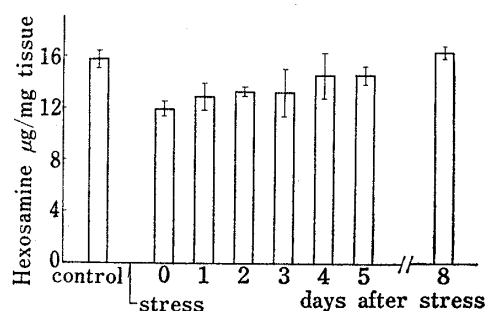


Fig. 2 Effect of Water Immersion and Restraint Stress on Hexosamine Content of Pyloric Tissue

Vertical lines indicate the S. E. of the mean.

## 2. The Experiments of Water Immersion and Restraint Stress

It is shown in Fig. 2 that the hexosamine content of pyloric tissue in the rats exposed to this stress for 20 hours was markedly lowered. But the recovery of decreased hexosamine content was fairly rapid and it was restored to the control level 8 days after the application of the stress.

The results of hexosamine determination in the pyloric tissue of stressed rats treated with oral administration of the drugs for 3 days are presented in Table II. The hexosamine content of pyloric tissue of the rats was increased by tyrosine, threonine, glutamine, gefarnate, synthetic aluminum silicate and sodium copper chlorophylline.

TABLE II. Effect of Several Anti-ulcer Drugs and Amino Acids given for 3 days on Hexosamine Content of Pyloric Tissue in Rats Subjected to Water Immersion and Restraint Stress

	Hexosamine µg/mg tissue		Hexosamine µg/mg tissue
Control	11.3 ± 1.0	Methionine	12.7 ± 1.3
Glutamine	13.8 ± 0.9	Tyrosine	15.5 ± 1.6 <sup>a)</sup>
Gefarnate	15.7 ± 0.7 <sup>a)</sup>	Threonine	14.4 ± 1.4
Synthetic aluminum silicate	13.6 ± 1.6	Proline	11.3 ± 0.5
Sodium copper chlorophylline	15.3 ± 1.6 <sup>a)</sup>		

Each value represents the mean ± standard error.

a) statistically significant at  $P=0.05$

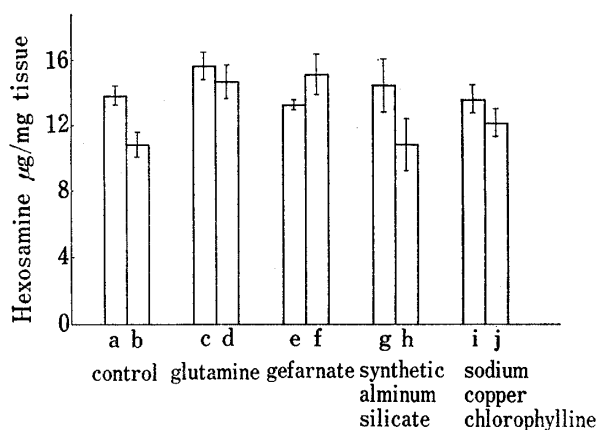


Fig. 3 Effect of Several Anti-ulcer Drugs on Hexosamine Content of Pyloric Tissue in Cortisone-treated Rats

control	a: CMC <i>p. o.</i> and CMC <i>s. c.</i>
	b: CMC <i>p. o.</i> and cortisone <i>s. c.</i>
glutamine	c: glutamine <i>p. o.</i> and CMC <i>s. c.</i>
	d: glutamine <i>p. o.</i> and cortisone <i>s. c.</i>
gefarnate	e: gefarnate <i>p. o.</i> and CMC <i>s. c.</i>
	f: gefarnate <i>p. o.</i> and cortisone <i>s. c.</i>
synthetic aluminum silicate	g: syn. al. silicate <i>p. o.</i> and CMC <i>s. c.</i>
	h: syn. al. silicate <i>p. o.</i> and cortisone <i>s. c.</i>
sodium copper chlorophylline	i: chlorophylline <i>p. o.</i> and CMC <i>s. c.</i>
	j: chlorophylline <i>p. o.</i> and cortisone <i>s. c.</i>

Vertical lines indicate the S. E. of the mean.

### 3. The Experiments of Cortisone Treatment

The decrease in hexosamine content of pyloric tissue of fasted rats by cortisone acetate is shown in Fig. 3 a and b. This decrease was reduced under the treatment with glutamine or sodium copper chlorophylline, and even reversed with gefarnate as shown in Fig. 3 c, d, e, f, i and j. In normal fasted rats without cortisone, 4 anti-ulcer drugs showed no significant increase in hexosamine content of the rat pyloric tissue compared with the control (Fig. 3 a).

### Discussion

Gastric mucus is changeable by various treatments. Robert, *et al.*<sup>2)</sup> reported that during the development of ulcer in fasting rats the gastric mucus content of gastric juice and mucosa was lowered peculiarly in rats with ulcers and that the volume and acidity of gastric juice was

also declined. Lambert, *et al.*<sup>3)</sup> confirmed the finding that incorporation of radiosulfate in the gastric mucosa of rats was prevented by restraint stress. Hakkinen, *et al.*<sup>4)</sup> observed that the aminosugar content of acid polysaccharides in the gastric wall of the glandular stomach were slightly increased in the "pre-ulcerous" groups and fell sharply to below the control values in the ulcer groups in rats subjected to restraint stress. There are a number of studies on the alteration of gastric mucosubstance by the application of ulcerogenic drugs such as cortisone, ACTH, phenylbutazone, indomethacin, and cinchophen.<sup>5-8,12)</sup>

In the present investigation the hexosamine content of gastric juice and tissue was determined as an index of mucosubstance. This is a valid assumption since hexosamine in tissues is always a component of protein bound polysaccharides. It is likely that the increased mucous content of gastric mucosa comes from the serum glycoproteins.<sup>9)</sup> The finding of the increased mucous content is interesting but it is difficult to interpret the mechanism at the present moment. But, whatever basic mechanism is underlying, hexosamine content of gastric juice and mucosa may represent the condition of mucosal resistance.

Hexosamine content of pyloric tissue was lowered by water immersion and restraint stress (Fig. 2) or by cortisone treatment (Fig. 3 a and b) as indicated by other investigators. Fasting resulted in the decreased hexosamine output of gastric juice (Fig. 1) but hexosamine content of pyloric tissue was maintained during the period of fasting.

In the study of drug treatments we found that administration of anti-ulcer drugs such as glutamine, gefarnate and sodium copper chlorophylline to the rats exposed to the ulcerogenic treatments induced the increased hexosamine content of pyloric tissue compared with the control group. Anti-ulcer drugs are mainly divided into agents affecting gastric acid and pepsin secretion and agents promoting the regeneration of injured gastric tissue. Glutamine, tyrosine, gefarnate and sodium copper chlorophylline are supposed to belong to the latter group.<sup>11)</sup> Synthetic aluminum silicate did not alter the hexosamine content of pyloric

12) R. Menguy and L. Desbaillets, *Annal Surgery*, **168**, 475 (1968).

tissue in fasted rats and cortisone-treated rats. From these findings hexosamine determination of pyloric tissue may be capable of distinction of mechanisms of action of anti-ulcer drugs. But it is possible that drugs without curative action on experimental gastric ulcers elevate the hexosamine content of gastric tissue.<sup>12)</sup> Of threonine and proline which are amino acids not having good curative ratio reported by Takagi and Okabe,<sup>11)</sup> threonine increased hexosamine content in stressed rats (Table II). In other cases the increase in hexosamine content of pyloric tissue seemed to induce cure of existing stress ulcer.

Consequently, it is necessary in the precise evaluation of mucosal resistance to study other factors of mucosubstance than hexosamine content of gastric tissue.