STUDIES ON PHARMACOLOGICAL ACTIVATION OF HUMAN SERUM IgG BY CHEMICAL MODIFICATION AND ACTIVE SUBFRAGMENTS. II. INHIBITORY EFFECTS OF CARBOXAMIDE-METHYLATED L CHAIN FROM HUMAN SERUM IgG ON VARIOUS ULCER MODELS AND ITS INHIBITION MECHANISM

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In view of the strong inhibitory effects of the carboxamide-methylated L chain (Fr. I-L) from human IgG on gastric ulceration and juice secretion, we carried out various experiments to clarify the mechanism of its action and obtained the following results. Fr. I-L inhibited the gastric ulceration induced by phenylbutazone or aspirin in rats, but no appreciable effect was observed on stress or acetic acid ulceration. The distribution of ^{14}C -labelled Fr. I-L increased particularly in the stomach mucous membrane of rats after the intravenous or intraperitoneal administration.

The hexosamine content in the gastric mucous membrane, reduced by the treatment with phenylbutazone, aspirin or pylorus-ligation, was recovered by the administration of Fr. I-L to an intact level. The high total of hexosamine levels found in the gastric juice after aspirin treatment or pylorus-ligation were decreased by the injection of Fr. I-L or cimetidine.

The histochemical observation revealed that the concomitant administration of Fr. I-L with phenylbutazone or aspirin prevented the abolishment of Hematoxylin-Eosin, Periodic Acid Schiff and Alcian Blue staining polysaccharides from the stomach epithelial cells.

Keywords — carboxamide-methylation; human IgG; alkylated L chain; aspirin-induced ulcer; phenylbutazone-induced ulcer; stress ulcer; acetic acid ulcer; incorporation of ^{14}C -labelled L chain; hexosamine; histology

We found that the alkylated H and L chains obtained by reduction and alkylation of human serum IgG showed strong inhibitory effects on ulceration as well as on gastric juice secretion in pylorus-ligated rats. We reported these findings, in addition to the properties and pharmacological activities of the alkylated subfragments, in our previous paper.¹⁾

In order to further clarify the mechanisms, we examined whether Fr. I-L (corresponding to the reduced and alkylated L chain of purified human serum IgG) would be effective against various experimental ulcer models other than pylorus-ligated ulceration.

The distribution of Fr. I-L in various tissues

and its subsequent diminishment were also investigated by use of ^{14}C -labelled Fr. I-L.

On the other hand, the influence of Fr. I-L on muco-polysaccharides, a protective factor in the gastric mucous cell, was estimated by checking the content of hexosamine in the gastric mucous membrane. Furthermore, histological observation on the stomach was done in order to clarify the mechanism of Fr. I-L on the gastric mucosa.

MATERIALS AND METHODS

Experimental Ulcer Models in Rats — i) Phenylbutazone-induced Gastric Ulcer: This ulceration model was induced according to the method of Suzuki *et al.*²⁾ Male Wistar strain rats

weighing 200–250 g were fasted for 24 h, but were provided with water *ad libitum*. Each rat received 200 mg/kg of phenylbutazone suspended in 5% Gum Arabic solution perorally. Rats, deprived of solid food and water for 5 h, were sacrificed and their stomachs removed to examine the lesion in the glandular stomach area. The sum of the diameter of each lesion was used as an ulcer index. A test sample was administered intraperitoneally 30 min before the administration of phenylbutazone. Atropine sulfate (Wako Pure Chemical Industries, Ltd.) was used as a positive control reagent.

ii) Aspirin-induced Gastric Ulcer: Male Wistar rats weighing 200–250 g were deprived of food for 24 h. According to the method of Okabe *et al.*,³⁾ rats orally received 100 mg/kg of aspirin suspended in 5% Gum Arabic solution immediately after the pylorus-ligation. Each test sample was administered intraperitoneally concurrently with the pylorus-ligation. Cimetidine (Smith Klein and Fujisawa) was used as a positive control reagent. Seven h after the pylorus-ligation, rats were sacrificed and their stomachs removed to examine the lesion in the glandular portion of the stomach. The sum of the length of all lesions was used as an ulcer index.

iii) Stress-induced Gastric Ulcer: Male Wistar rats weighing 200–250 g were used as experimental animals. Rats were subjected to stress following the method of Takagi *et al.*,⁴⁾ in which animals were immobilized in the stress cage and immersed in a water bath of 23°C to the depth of the xiphoid for 20 h.

Each sample was administered intraperitoneally twice, 15 min prior to and 10 h after the stress treatment. Cimetidine (Smith Klein and Fujisawa) dissolved in saline was administered simultaneously to the positive control group. Animals were sacrificed 20 h after the stress treatment and the stomachs removed in order to measure the ulcer area on each animal's stomach. The result was used as an ulcer index.

iv) Acetic Acid-induced Gastric Ulcer: This ulcer was induced by the method of Takagi *et al.*,⁵⁾ namely, male Wistar rats weighing

150–200 g were lightly anesthetized with ethyl ether and the stomachs exposed following abdominal incision.

The rats received 0.05 ml of 10% acetic acid solution into the subserosa of the glandular portion of the stomach. After these treatments, the stomach was returned and the abdomen closed, and the animals were fed as usual. The sample was administered intraperitoneally twice per day for 10 d consecutively from the second day after the operation. The inhibitory effect of the sample was determined by the ulcer index obtained from calculating the area of lesion in the stomach wall.

Distribution of Fr. I-L in Rats — i) Preparation Method of ¹⁴C-Labelled Fr. I-L: After reduction with dithiothreitol, Fr. I-L was carboxamide-methylated with 1-¹⁴C Iodoacetamide (28.3 μ Ci/mmol, RCC Amersham) in accordance with the method of Inagami⁶⁾ and Heinrikson,⁶⁾ producing ¹⁴C-labelled Fr. I-L.

ii) Tissue Distribution of ¹⁴C-Labelled Fr. I-L in Rats: Male Wistar rats intravenously or intraperitoneally received the ¹⁴C-labelled Fr. I-L, prepared as previously described, at 1 μ Ci/mg Fr. I-L/100 g b.w. through the femoral vein or the abdomen. They were sacrificed at 1, 2, and 4 h after the administration.

The radioactivity in the gastric mucosa, duodenal mucosa, colonic mucosa, liver, spleen, kidney and plasma was determined by the following methods. According to Hansen *et al.*,⁸⁾ each organ was weighed and homogenized by a 4-fold volume of saline, and 150 μ l of each homogenate was transferred to a scintillation vial.

After adding 1.2 ml of Soluen 350 (Packard Instrument Company, Inc.) to each container, the vials were left overnight at room temperature in order to digest the whole sample. After digestion, each sample was neutralized with 17 N acetic acid and then decolorized with a few drops of 30% hydrogen peroxide. Then, 10 ml of scintillator (Triton X-100 500 ml; PPO 4 g; POPOP 0.1 g in 1000 ml of toluene) was added to each vial and the radioactivity was counted

by a liquid scintillation counter (Beckman Model LS-150).

Hexosamine Content in Gastric Mucous Membrane and Gastric Juice in Rats — Male Wistar rats weighing 200–250 g were subjected to gastric ulcers by treatment with phenylbutazone, aspirin, or pylorus-ligation as previously described.

They were divided into three groups; an Fr. I-L administered group, a control group, and a positive control group. In the Fr. I-L administered group, the saline dissolved sample was administered intraperitoneally 30 min before peroral administration of phenylbutazone, as in the phenylbutazone induced gastric ulceration group. The sample was administered intraperitoneally concurrently with pylorus-ligation as in the pylorus-ligated ulceration group and aspirin-induced gastric ulceration group.

The hexosamine content of the stomach mucous membrane in the Fr. I-L administered group was compared with the control groups. Cimetidine and atropine sulfate were used as positive control reagents. The hexosamine content of the individual stomach mucous membrane was determined as follows: male Wistar rats' stomachs were removed, and the gastric mucous membrane was stripped off and homogenized with 2 ml of dist. water following the assessment of their weight while wet.

According to the method of Hiroi *et al.*,⁹ the homogenate was digested with papain (Difco; 45 units) solution for 20 h at 37°C under the coexistence of 2mM EDTA and 4mM cysteine in the reaction mixture. After digestion, the reaction mixture was centrifuged at 3000 rpm for 10 min and the supernatant solutions obtained. They were hydrolyzed with 2 N HCl at 110°C for 14 h, and the hexosamine content in the solution was measured by Gunner's colorimetric method.¹⁰ We could not measure the gastric juice hexosamine content in the case of phenylbutazone-induced ulcer because of the difficulty in collecting gastric juice. We did, however, measure it for the aspirin-induced gastric ulcer and pylorus-ligation. The gastric juice

hexosamine content was measured by the method of Glass.¹¹ Proteins were removed from the collected gastric juices by TCA precipitation (5%). Distilled acetone (1.5 fold volume) was added to the TCA soluble fractions. The mixtures were then incubated for 1 h at 40°C. After incubation, the mixtures were centrifuged, the precipitates obtained, and the hexosamine content in the precipitates measured by Gunner's method.¹⁰ The calibration curve for the quantification of hexosamine was made by using glucosamine-HCl as a standard, and its content in the gastric mucous membrane or in gastric juice was expressed as glucosamine-HCl.

Histochemical Observation of the Gastric Mucous Membrane in Rats — In order to study the effect of Fr. I-L on the gastric mucous membrane from the viewpoint of histochemistry, we prepared intact rats and those with phenylbutazone-induced ulcer as experimental animals. Fr. I-L dissolved in saline was injected intravenously in each rat concurrently with the administration of phenylbutazone.

The rats were sacrificed 5 h after the treatment, the stomachs were removed, and fixed 10% of ice-cold neutral buffered formalin. Each stomach was embedded in paraffin after the fixation, sectioned 3 μ m thick with a microtome, and stretched on a cleaned microscope glass slide.

For the morphological examination, each section was stained with Hematoxylin-Eosin (HE), Periodic Acid Schiff (PAS) and Alcian Blue (AB) in the usual manner. Stained specimens were examined under an Olympus Vanox Model AHB-LB microscope, and photomicrographs were taken with Kodak Ektachrome ASA 100 film.

Statistics — Student's t-test was employed to determine the statistical significance of the data obtained in this study.

RESULTS

Anti-ulcerogenic Activity of Fr. I-L on Various Experimental Ulcer Models in Rats

i) *Phenylbutazone-induced Gastric Ulcer* — The effect of Fr. I-L on phenylbutazone-

induced gastric ulcer was examined and the results summarized in Table I. It was evident from the table that the gastric ulcers induced by phenylbutazone were suppressed by 60.9% by the *i.p.* administration of 10 mg/kg of Fr. I-L.

This differed significantly ($p < 0.05$) from the control group. Even at the dose of 5 mg/kg, Fr. I-L showed 47.4% inhibitory activity on the phenylbutazone-induced gastric ulcer. Atropine sulfate, used as a positive control, also signifi-

TABLE I. *Effect of Fr. I-L on Phenylbutazone-induced Ulceration in Rats (5 h)*

Treatment	Dose (mg/kg)	No. of rats	Ulcer index (mean $\pm$ s.e.)	Inhibition (%)
Control	—	8	32.5 $\pm$ 6.4	—
Fr. I-L	10 ^{a)}	8	12.7 $\pm$ 3.3*	60.9
	5	8	17.1 $\pm$ 3.3	47.4
Atropine sulfate	10 ^{a)}	8	6.3 $\pm$ 1.2**	80.7

a) Sample was administered intraperitoneally 30 min prior to the peroral administration of phenylbutazone. Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

TABLE II. *Effect of Fr. I-L on Aspirin-induced Ulceration in Rats (7h)*

Treatment	Dose (mg/kg)	No. of rats	Ulcer index (mean $\pm$ s.e.)	Inhibition (%)
Control	—	8	29.3 $\pm$ 2.1	—
Fr. I-L	10 ^{a)}	8	17.6 $\pm$ 2.0**	39.9
	5	8	22.8 $\pm$ 4.0	22.2
Control	—	8	20.2 $\pm$ 4.7	—
Cimetidine	100 ^{a)}	8	3.2 $\pm$ 1.1**	84.1
	50	8	6.4 $\pm$ 1.0*	68.4

a) Sample was administered intraperitoneally after pylorus ligation. Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

TABLE III. *Effect of Fr. I-L on Stress-induced Ulceration in Rats (20h)*

Treatment	Dose (mg/kg)	No. of rats	Ulcer index (mean $\pm$ s.e.)	Inhibition (%)
Control	—	8	28.7 $\pm$ 1.7	—
Fr. I-L	10 $\times$ 2 ^{a)}	8	24.9 $\pm$ 1.4	13.4
	5 $\times$ 2	8	30.6 $\pm$ 1.8	0
Cimetidine	100 $\times$ 2 ^{a)}	8	14.1 $\pm$ 1.9**	50.9
	50 $\times$ 2	8	22.8 $\pm$ 1.6*	20.6

a) Sample was administered intraperitoneally after twice 15 min prior to stress exposure and 10 h later. Significantly different from the control group: * $p < 0.05$, ** $p < 0.01$.

FIG. 1. Gel Filtration of ¹⁴C-Carbox-amide-methylated Fr. I after Reduction by Dithiothreitol on Sephadex G-200 Column

Column size: 10×300 mm, solvent: 40 mM SDS-50 mM phosphate buffer (pH 8.0), tube volume: 0.5 ml.

cantly decreased the ulcer index at 10 mg/kg *i.p.*

ii) *Aspirin-induced Gastric Ulcer* — As shown in Table II, in the animals treated with Fr. I-L at 10 mg/kg *i.p.*, the ulcer index was significantly less (about 40%) than that of the control group. However, no significant differences were found in the rats receiving Fr. I-L administration at 5 mg/kg. Cimetidine used as a positive control reagent, significantly decreased the ulcer index at the dose of 50 and 100 mg/kg.

iii) *Stress-induced Gastric Ulcer* — Even when Fr. I-L was twice intraperitoneally administered at 10 mg/kg, it failed to inhibit ulcer formation induced by the stress of water immersion as shown in Table III. On the contrary, cimetidine significantly suppressed the ulcer at 50 and 100 mg/kg *i.p.*

iv) *Acetic Acid-induced Gastric Ulcer* — Fr.

TABLE IV. Specific Radioactivity of Various Tissues at Different Intervals following Intravenous or Intraperitoneal Injection of ¹⁴C-Labelled Fr. I-L

Treatment	Specific radioactivity		
	1 a)	2 a)	4 a)
A) Intravenous injection			
Liver ^{b)}	525.3 ± 13.5	400.6 ± 24.3	278.7 ± 10.4
Gastric mucosa ^{b)}	116.3 ± 10.4	153.8 ± 32.1	178.0 ± 49.5
Duodenal mucosa ^{b)}	276.5 ± 31.9	264.5 ± 45.4	187.5 ± 13.3
Colonic mucosa ^{b)}	151.8 ± 23.2	148.3 ± 34.5	95.4 ± 11.2
Kidney ^{b)}	1129.2 ± 28.5	507.5 ± 33.7	261.3 ± 7.3
Spleen ^{b)}	225.6 ± 17.0	193.1 ± 29.2	144.8 ± 3.5
Plasma ^{c)}	36.2 ± 1.6	13.4 ± 1.5	5.0 ± 0.2
B) Intraperitoneal injection			
Liver ^{b)}	344.3 ± 15.2	288.6 ± 14.3	268.2 ± 11.1
Gastric mucosa ^{b)}	95.7 ± 6.4	109.2 ± 13.4	136.5 ± 19.6
Duodenal mucosa ^{b)}	234.4 ± 16.3	219.1 ± 17.6	150.2 ± 10.3
Colonic mucosa ^{b)}	116.5 ± 10.1	113.2 ± 14.7	102.2 ± 10.2
Kidney ^{b)}	336.6 ± 29.1	202.6 ± 10.3	125.7 ± 13.4
Spleen ^{b)}	177.2 ± 16.1	149.3 ± 6.2	79.3 ± 11.1
Plasma ^{c)}	20.3 ± 1.9	19.7 ± 2.1	9.0 ± 0.8

a) Time after injection (h).

b) dpm/10 mg tissue wet weight.

c) dpm/100 μl plasma.

¹⁴C-labelled Fr. I-L was administered intravenously or intraperitoneally at the dose of 1 μCi/mg Fr. I-L/100 g body weight. All values represent mean ± s.e. (n=5)

I-L showed no inhibitory activity in the rats having acetic acid-induced gastric ulcer (which was one of the chronic ulcer models) even after administration (*i.p.*) twice per day for 10 d of Fr. I-L at 5 mg/kg.

v) *Distribution of ^{14}C -Labelled Fr. I-L in Rats*

—Fr. I was carboxamide-methylated with ^{14}C -labelled iodoacetamide following reduction by dithiothreitol, and separated from the reaction reagents by column chromatography on Sephadex G-200. The absorbing pattern of the alkylated H and L chains of Fr. I at 750 nm completely agreed with the pattern of the radioactivity as shown in Fig. 1.

^{14}C -Labelled Fr. I-L was used for the tissue distribution in intact rats. As shown in Table IV, the radioactivity in the gastric mucosa increased during the periodic observation, while that of other organs examined decreased, in both groups of rats (those who received ^{14}C -labelled Fr. I-L intravenously and intraperitoneally, respectively).

Effect of Fr. I-L on hexosamine content in

both gastric mucosa and gastric juice; Treatment with phenylbutazone caused a marked reduction in hexosamine content in the stomach mucous membrane compared with that of the intact rats, as shown in Table VA). However, the *i.p.* administration of Fr. I-L at 10 mg/kg prevented the effect of concurrently administered phenylbutazone and the loss of hexosamine content in the gastric mucosa was obviously prevented. Atropine sulfate, used as a positive control drug in the case of phenylbutazone induced ulceration, also prevented the loss of hexosamine content in the gastric mucous membrane.

The effect of Fr. I-L on the hexosamine content in the mucous membrane of the stomach of intact rats was also examined. Four h after the *i.p.* administration of Fr. I-L, rats were sacrificed, the hexosamine content in the gastric mucosa of each rat was examined, and the results summarized in Table VB).

The hexosamine content significantly increased in the Fr. I-L injected group as low as 5 mg/kg. The hexosamine content in the gastric

TABLE V. *Effects of Fr. I-L on Hexosamine of Mucous Components in the Glandular Portion of the Stomach in Intact Rats and Rats subjected to Gastric Ulcer by Phenylbutazone*

	Treatment	Dose (mg/kg)	No. of rats	Hexosamine ($\mu\text{g}/100\text{ mg tissue wt.}$)	Increase (%)
A) Phenylbutazone-induced ulceration					
Intact	Saline	—	8	347.6 ± 13.8	—
Phenylbutazone	Saline	—	8	250.5 ± 11.4	—
	+Fr. I-L	10 ^{a)}	8	300.0 ± 13.6	19.8
	+Atropine sulfate	10 ^{a)}	8	$470.6 \pm 64.8^{***}$	87.9
B) Intact rats					
	Saline	—	8	304.8 ± 14.1	—
	+Fr. I-L	10 ^{b)}	8	$377.8 \pm 18.3^{**}$	24.0
		5	8	$340.2 \pm 8.0^*$	11.6
	+Cimetidine	100 ^{b)}	8	$370.5 \pm 17.5^*$	21.6

a) Sample was administered intraperitoneally 30 min prior to the peroral administration of phenylbutazone.

b) Sample was administered intraperitoneally 4 h prior to determination of hexosamine.

All values represent mean $\pm$ s.e.

Significantly different from the saline group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

mucosa clearly depended on the dose of Fr. I-L.

Cimetidine, 100 mg/kg *i.p.*, also increased the hexosamine content in the gastric mucosa in intact rats.

In the case of the experimental ulcers induced by the aspirin or pylorus-ligation, the total hexosamine content in the gastric juice was decreased in the Fr. I-L administered group compared with the saline group. The effect of 10 mg/kg of Fr. I-L was very much similar to that of 100 mg/kg of cimetidine which was used as a positive control. A marked reduction of hexosamine content in the stomach mucous membrane caused by the aspirin induction or pylorus-ligation was prevented by the administration of Fr. I-L or cimetidine and maintained at almost the same level of that in the intact rats.

Effect of Fr. I-L on the Histochemical Observation of the Gastric Mucous Membrane in Rats

The PAS stained stomach mucous membrane of intact rats was shown in Fig. 2 a).

PAS staining positive muco-polysaccharides were observed from the epithelial cells in the surface mucous membrane of the stomach to the

whole epithelial cells in the fovea gastrica. However, the phenylbutazone-induced gastric ulcer showed not only a remarkable disappearance of the epithelial cells from the surface of the stomach mucous membrane, but also a severe destruction of the lamina propria mucosae, as shown in Fig. 2 b) (HE staining). It was also observed that in the ulcer induced by phenylbutazone, a remarkable decrease in the PAS staining positive substances from both of the epithelial cells in the surface mucous membrane of the stomach and the lamina propria mucosae (Fig. 2 c)).

Although the mucous layer was not found in the HE staining specimen, the concomitant administration of Fr. I-L prevented the histochemical disorders and the epithelial cells were lined up almost normally (Fig. 2 d)). It was also found that the decrease of the PAS staining positive muco-polysaccharides was inhibited by the administration of Fr. I-L, and showed almost the same figures as those of the intact rat (Fig. 2 e)). These histochemical changes were observed in the AB staining specimens as well. Almost all of the AB staining positive substances disappeared

TABLE VI. *Effects of Fr. I-L on Hexosamine Contents in Gastric Juice and Mucous Membrane of Stomach in the Ulcerated Rats induced by Aspirin or Pylorus-ligation*

Experiments	Treatment	Dose (mg/kg)	No. of rats	Gastric volume (ml/100g b.w.)	Total hexosamine (μ g/gastric juice /100 g b.w.)	Hexosamine (μ g/100 mg tissue wet wt.)
Intact	Saline	—	8	—	—	347.6 $\pm$ 13.8
A) Aspirin-induced ulceration (pylorus-ligated for 7 h)	Saline	—	8	6.28 $\pm$ 0.27	95.4 $\pm$ 10.9	267.8 $\pm$ 18.2
	+Fr. I-L	10 ^{a)}	8	3.40 $\pm$ 0.36***	75.3 $\pm$ 11.9	318.6 $\pm$ 15.2
	+Cimetidine	100 ^{a)}	8	3.54 $\pm$ 0.26***	78.5 $\pm$ 8.7	325.3 $\pm$ 26.5
	Saline	—	8	—	—	356.2 $\pm$ 25.4
B) Pylorus-ligated for 4 h	Saline	—	8	2.75 $\pm$ 0.34	57.2 $\pm$ 14.3	281.6 $\pm$ 26.2
	+Fr. I-L	10 ^{a)}	8	1.10 $\pm$ 0.10***	30.1 $\pm$ 4.1	390.6 $\pm$ 54.9
	+Cimetidine	100 ^{a)}	8	1.11 $\pm$ 0.16***	29.2 $\pm$ 5.2	498.7 $\pm$ 45.0**

a) Sample was administered intraperitoneally immediately after pylorus ligation.

All values represent mean $\pm$ s.e.

Significantly different from the saline group: ** $p < 0.01$, *** $p < 0.001$.

FIG. 2. *Histochemical Improvement by Administration of Fr. I-L on Gastric Mucous Membrane in Phenylbutazone-induced Ulcerative Rats*

a) intact (PAS staining; $\times 150$), b) phenylbutazone-induced ulcer (HE staining; $\times 150$), c) phenylbutazone-induced ulcer (PAS staining; $\times 150$), d) phenylbutazone-induced ulcer + Fr. I-L (HE staining; $\times 150$), e) phenylbutazone induced ulcer + Fr. I-L (PAS staining; $\times 150$).

from the upper layer of the gastric mucosa in the control group rats, but remained in the whole gastric mucosa of the Fr. I-L administered group. Almost the same effects were observed in the specimens of the Fr. I-L administered group, with respect to the aspirin-induced gastric ulceration.

DISCUSSION

We found that the alkylated L chain fraction (Fr. I-L) resulting from the reduction and alkylation of human serum IgG strongly inhibited gastric juice secretion and ulceration induced by pylorus-ligation.⁹⁾ We conducted various studies in order to clarify the mechanism of the induction of the pharmacological activity of Fr. I-L.

Fr. I-L was found to have a significant inhibiting effect on phenylbutazone-induced ulcer and aspirin-induced ulcer, but no appreciable inhibiting effect on stress ulcer or acetic acid ulcer.

Regarding tissue distribution of Fr. I-L estimated by using ¹⁴C-labelled Fr. I-L, a progressive increase of radioactivity was observed exclusively in the stomach relative to the lapse of time, following *i.v.* and *i.p.* administration. There were no differences in the distribution of radioactivity in the stomach with respect to rats injected intravenously or intraperitoneally with ¹⁴C-labelled Fr. I-L.

Recently, more attention has been paid to the gastric mucous membrane barrier concept for better understanding of the developmental mechanism of acute pathological changes in the mucous membrane of the stomach. The decrease in PAS staining positive polysaccharides present in the cortical epithelial cells of the gastric mucous membrane during the initial stage of ulceration¹²⁾ suggests that neutral polysaccharides may play an important role in the protection of the gastric mucous membrane.¹³⁾ Cimetidine was known to have a hexosamine content increasing activity in the gastric mucosa of ulcerated rats.⁹⁾ Therefore we examined the effect of Fr. I-L on the hexosamine content in the gastric mucosa and compared to that of cimetidine. It was found that Fr. I-L decreased the gastric juice

secretion and prevented the loss of the hexosamine content in the gastric mucous membrane in the pylorus ligated rats (Table VI). The total hexosamine content in the gastric juice was also decreased by the administration of Fr. I-L. These results led us to an assumption that Fr. I-L prevented the disappearance of hexosamine, which is one of the protective factors for ulceration, into the gastric juice and maintain the hexosamine content in the gastric mucosa in normal level. In other words, we assumed that the anti-ulceration activity of Fr. I-L was based on the intensification of the protective factors. This assumption was also supported by the histological observation of the stomach. However, the mechanisms of the intensification of the protective factors by Fr. I-L still remains an open question at the present time. Further investigations, such as the effects of Fr. I-L on the biosynthesis and disintegration of muco-polysaccharides in the gastric mucous membrane, must be done in order to evaluate the protective effect of Fr. I-L on gastric mucous membrane.

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