Jap. J. Pharmac. **19,** 418–426 (1969)

A NEW METHOD FOR THE PRODUCTION OF CHRONIC GASTRIC ULCER IN RATS AND THE EFFECT OF SEVERAL DRUGS ON ITS HEALING

KEIJIRO TAKAGI, SUSUMU OKABE AND RYUICHI SAZIKI

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo

Received for publication February 15, 1969

Several methods have been reported to produce an experimental chronic ulcer in the rat, such as thermal ulcer by Skoryna *et al.* (1, 2), clamping-cortisone ulcer by Umebara *et al.* (3), and methylcholanthrene ulcer by Lauren *et al.* (4). In these methods thermal and clamping-cortisone ulcers seem to be valuable tools in investigation of the healing process of gastric ulcer, because of the relatively long period of its persistence and closer histologic resemblance to human peptic ulcer. However, both methods include the undesirable procedure such as the incision of glandular portion of stomach in order to perform the thermocautery of the innersurface of stomach in the former method and the consecutive administration of cortisone acetate for 8 days after the gastric operation in the latter. On the other hand, Robert and Selye (5) have reported the method to evoke an ulcer by an injection of formalin solution into the rat stomach wall itself. We also tried the same procedure and found the gastric necrosis corresponding to the injected area. However, the necrotic tissue did not disappear from the stomach wall even 6 days after the operation so that the so-called gastric ulcer was not produced. Then we injected the acetic acid solution, which is well known to injure the gastric mucosa (6) or to evoke inflammation in rat paw in our laboratory (7), into the rat stomach wall and found the definite gastric ulcer having resemblances to the human peptic ulcer at gross and microscopical observation. This paper deals with the studies of the healing process of the produced ulcer and the usefulness for the assay method of the curative drugs of gastric ulcer.

METHODS

Male rats of the Donryu strain weighing 200 to 230 g at the time of operation were used in this experiment. Under ether anesthesia laparotomy was performed through a midline epigastric incision. After exposing the stomach, 1, 10, and 30% acetic acid, 0.05 ml per animal were injected into subserosal layer in the glandular part of anterior wall, with care taken not to disturb the blood vessels. At the injection of acetic acid solution a thumb was placed tightly on the inserted needle in order to avoid the leak of the solution. After the injection, the needle was pull out, but the thumb was still placed on that position for at least 30 second to prevent the leak of acetic acid by removing of the

高木　敬次郎・岡部　進・佐直　隆一

needle. The accuracy of the injection was easily confirmed by swelling of the injected portion and by blanching of the serosal coat at that area. Then the abdomen was closed and the animal was fed normally. The operation required only about 4–5 minutes per one animal. Experiments were conducted during 1.00 p.m. to 5.00 p.m. because at this time the stomach was found to be almost empty in the rats used. If the stomach is filled with the gastric content, the injection is slightly difficult and hemorrhage often occurred after removing the injection needle. Animals were sacrificed at proper intervals to assess the healing processes of the ulcer. The stomach was removed, filled with 10 ml of 1% formalin solution and put into 1% formalin solution for 5 minutes to fix the outer layer of stomach to facilitate the examination. Then the stomach was cut open along the greater curvature, spread on the pad and examined the lesions macroscopically. The ulcers produced were oval or round in shape so that the length and width were measured and the product was expressed in terms of the ulcer index. Epithelial regeneration from the edges of the ulcer and the connective tissue repair from the base of the ulcer grossly paralleled in the healing process of the ulcer. Therefore the estimation of the ulcer index appeared to be proper to appreciate the approximate value of the ulcer repair, including the connective tissue proliferation. Specimens were fixed in 10% formalin solution and stained with hematoxylin-eosin. Bilateral vagotomy was performed by painting 1% phenol solution on the esophagus at the subdiaphragmatic portion, immediately after the injection of acetic acid solution into the stomach wall. The drug used, synthetic aluminum silicate, carrageenin, atropine sulfate, chlorophyllin copper sodium salt, glycyrrhetic acid dipotassium salt, and placenta extract solution (Sauerbruch's preparation which contains 180–200 unit, King Armstrong Unit, of alkaline and acid phosphatase in 2.2 ml) were consecutively injected for 15 or 40 days beginning on the 2nd day after the operation. Synthetic aluminum silicate, carrageenin, glycyrrhetic acid dipotassium salt were suspended in 0.5% CMC solution and administered orally twice a day. Chlorophyllin copper sodium salt was suspended in 0.5% CMC solution and administered orally once a day. Atropine sulfate was dissolved in 0.9% saline solution and administered subcutaneously twice a day. Placenta extract was administered intraperitoneally twice a day. The control animals received the 0.5% CMC solution (orally) or 0.9% saline solution (subcutaneously, or intraperitoneally) for the same period. Curative ratio is expressed as follows,

$$\text{Curative ratio} = \frac{\text{Control (ulcer index)} - \text{Test (ulcer index)}}{\text{Control (ulcer index)}} \times 100\%$$

RESULTS

In general, animals withstood the procedure well and any unfavorable symptom could not be observed. The body weight of the animals with ulceration showed almost the same increase compared with the control group which was subjected to the injection of distilled water only. However, 2 out of 87 animals subjected to 30% acetic acid died by perforation on the 2nd and 3rd day after the operation. Then the production and recovery process of these ulcers were examined in relation to the concentration of acetic acid injected. The results are summarized in Table 1.

TABLE 1. Healing processes of the experimental gastric ulcers produced by injection of acetic acid solution at various concentration into the rat stomach walls.

Acetic acid Concentration (%)	Volume ml	Ulcer index (mean±S.E.), Days after operation 5	10	20	30	40	50	60	75	100	150
1	0.05	0.9 (10)	0 (10)								
10	0.05	53.7 ±4.6 (10)		8.4 ±2.5 (10)		6.0 ±2.9 (10)		6.8 ±3.6 (11)			
30	0.05	84.5 ±6.4 (8)	48.1 ±8.0 (10)	29.8 ±7.5 (10)	19.7 ±5.4 (9)	27.2 ±8.4 (10)	6.8 ±2.4 (10)	11.8 ±3.0 (10)	28.3 ±10.4 (9)	13.8 ±3.8 (9)	9.0 ±2.3 (20)

The number in the parenthesis is number of animals used.
The values following ± sign are the standard error of the mean.

1% acetic acid, 0.05 ml: Animals sacrificed on the fifth day after the operation showed small ulcers in 3 out of 10 animals and the lesions were limited to the mucosal layer, but in the other 7 rats any injury could not be found in the stomach wall. On the 10th day the innersurface of stomach seemed to be almost normal and any defect could not be observed even histologically.

10% acetic acid, 0.05 ml: On the fifth day after the operation there was already found sharply defined deep ulceration corresponding to the injected area. The ulcers were oval or round in shape and approximately 8×6 mm in size and 2 to 4 mm in depth. Necrotic debris, food residue, and hair swallowed were observed in the floor of the ulcer. The region of ulceration was frequently found to be supported by adherence to neighboring structures (mainly liver). Histological sections confirmed an ulceration which involved full thickness of the stomach wall, that is, muscle coats were completely absent in the area of the ulceration. Leukocyte exudation, edema and cellular infiltration were frequently found in submucosal layer around the wall of the ulcer.

On the 20th day 2 out of 10 animals completely healed but the remaining 8 rats showed still apparent ulceration although th depth became slightly thinner. In the floor of these ulcers there was still present the thin necrotic material but granulation tissue proliferation and mucosal regeneration were markedly advanced in each stomach. On the 40th day 2 out of 10 animals appeared completely healed from the gross observation but the location of ulceration was recognized by the difference of the mucosal color between normal and ulcerated area, and by the stellate arrangement of the mucosal folds. In 8 out of 10 animals the ulcer was still evident but became smaller in size. On the 60th day the gross and microscopical observation of the ulcer showed almost the same appearance to that of the 40 day's group. Eight out of 11 animals had a small but apparent ulcer in the central portion of originally produced ulcer.

30% acetic acid, 0.05 ml: On the fifth day after the operation each animal showed an apparent ulceration which had a punched-out appearance with a steep wall and the undermining of the floor. It was approximately 8×9 mm in size and 2 to 4 mm in depth (Fig. 1, a). At gross observation the lesion became comparatively severe than that seen

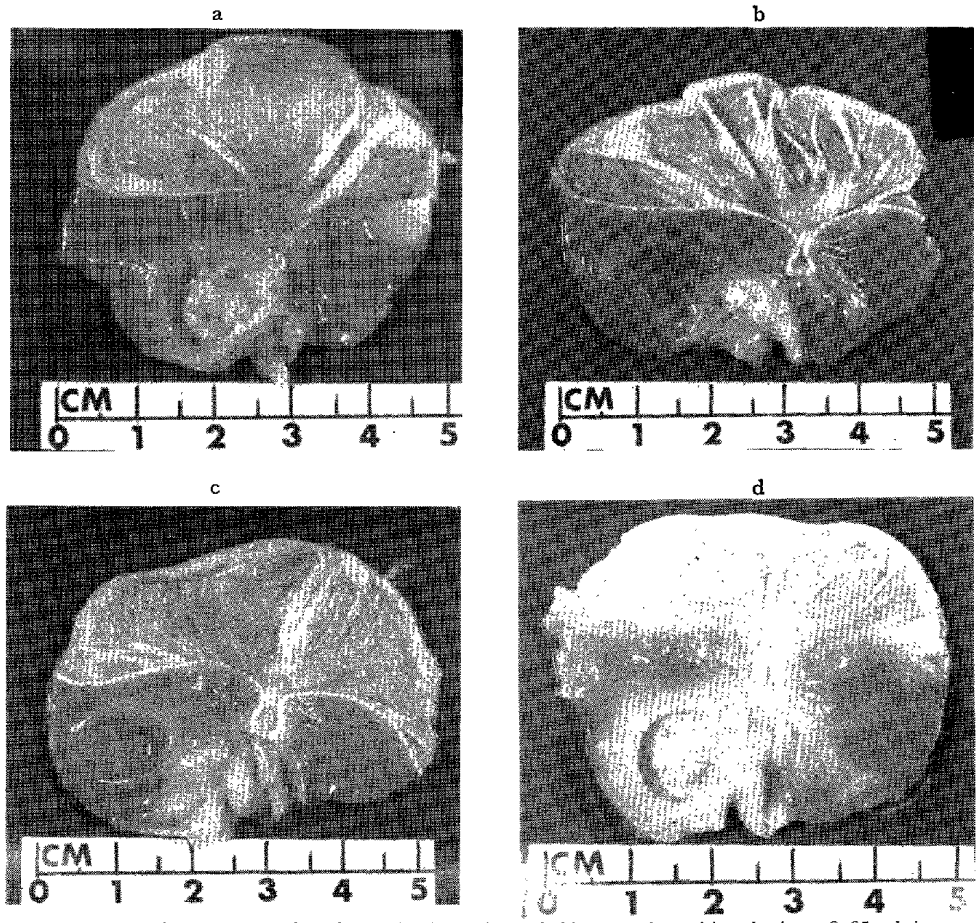

FIG. 1. Gastric ulcers produced by the injection of 30% acetic acid solution, 0.05 ml into the stomach walls in rats. Figures a, b, c, and d show the ulceration observed on the 5th, 20th, 50th, and 75th days after the operation, respectively. a, Deep and large ulcer can be seen in the glandular portion of the stomach. In the floor there are thick necrotic debris and a number of swallowed hair. The undermining of the wall was already observed in this period. b, The ulcer with slightly raised margin diminished the size and depth and the necrotic material progressively became less conspicuous. c, The size of the defect became markedly small but the mucosal regeneration comparatively delayed. d, Note the size and depth of the ulcer, comparing with that observed on the 50th day.

in the group of 10% acetic acid at the same period. The external surface of the ulcerated region was strongly adherent to the liver, which formed a part of the base of the ulcer so that this separation could not be performed without perforation of the stomach. Histological sections showed almost the same configuration (Fig. 2, a) as seen in the 10% acetic acid group on the fifth day. On the 10th day the ulcer was still evident but the size and depth slightly reduced. The margin of the ulcer showed a lack of sharpness and slightly raised by submucosal edema. The floor of the ulcer was covered with necrotic material. Adherence of the ulcer base to the liver was separated by blunt dissection and thereby perforation did not occur at this period, probably due to the strengthened serosal layer

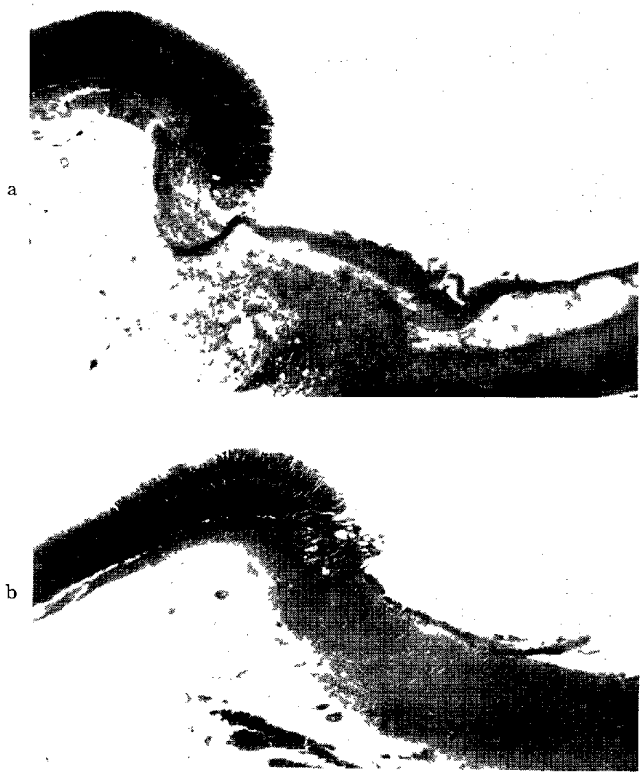

FIG. 2. Microphotographs of gastric ulcers observed on the 5th (a) and 20th (b) days after the injection of 30% acetic acid solution, 0.05 ml into the rat stomach walls (H & E, ×14). a, A large and deep ulcer can be seen. The floor is covered by a layer of necrotic debris and deep to this there is edema and granulation tissue proliferation. b, The base of the ulcer consists of a relatively wide band of granulation tissue and the epithelial regeneration is slightly seen at the margin of ulcer. A part of pancreas and liver is adherent to the serosa.

of the ulcerated region. Connective tissue proliferation further progressed but mucosal regeneration advanced to a lesser extent. On the 20th day the size and depth of the ulceration were further decreased but apparent defect existed on the mucosal surface (Fig. 1, b). Although the ulcer indices were remarkably diminished, they were attributed to the well advanced granulation tissue proliferation and not to the repair of the epithelium. (Fig. 2, b). In the floor of some ulcers there were found few hemorrhagic spots as well as necrotic debris. On the 40th day the gross appearance of ulcers was the same as before but the size of ulcers was relatively larger than those observed on the 30th day, while the depth was almost equal to them. Thin necrotic debris, hair swallowed, and some spots of hemorrhages were often recognized in the floor of the ulcer. On the 50th day the ulcer extremely diminished its size and depth and became a small spot in some cases except the delayed epithelization (Fig. 1, c). Histologic sections showed a well advanced healing phase with regards to connective tissue replacement of the necrotic wall (Fig. 3, a). The defect in the muscle layer was occupied by adult fibrous tissue and the regeneration of

FIG. 3. Microphotographs of the gastric ulcers observed on the 50th (a) and 75th (b) days after the injection of 30% acetic acid solution, 0.05 ml into the rat stomach walls (H & E, ×14). a, The epithelization is well avanced, but the mucosal erosion can be seen in the central portion of the originally produced ulcer (H & E, ×14). The regenerated mucosa lies directly on the connective tissue without the interposition of muscularis mucosae. b, Deep ulceration is manifested and the floor is covered by thin necrotic materials.

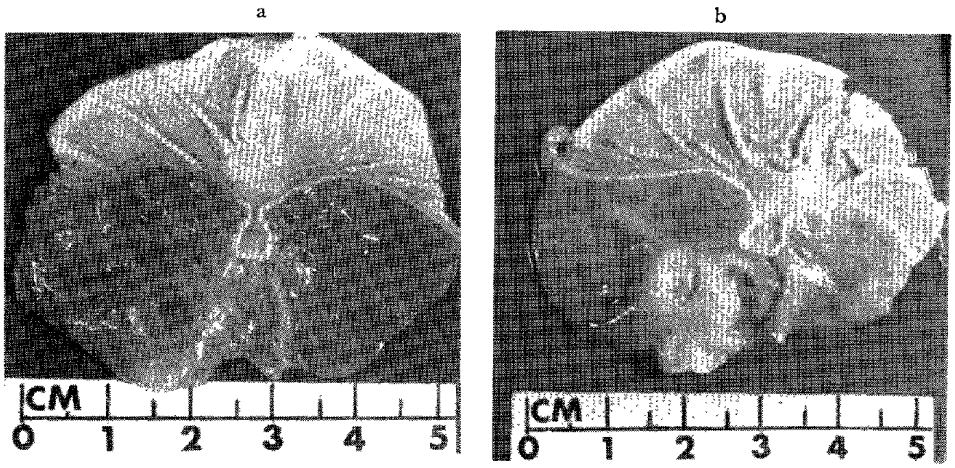

FIG. 4. Gastric ulcers produced by the injection of 30% acetic acid solution, 0.05 ml into the stomach walls in rats. Figures a and b show the ulceration observed on the 100th and 150th day after the operation, respectively. a, Black area in the floor of the ulcer shows the relatively severe hemorrhages. b, A clearly defined ulcer without any hemorrhages can be seen in the glandular portion of the stomach.

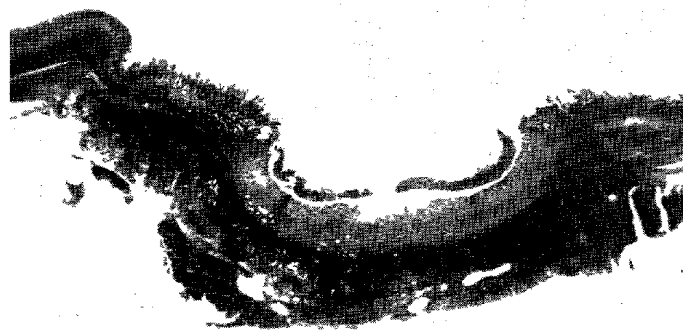

FIG. 5. Microphotographs of gastric ulcers observed on the 100th day after the injection of 30% acetic acid solution, 0.05 ml into the stomach walls (H & E, ×10). A typical penetration into the fibrous tissue wall of the healed ulcer can be seen in the ulcers.

epithelium was partly present on it. On the 75th day the ulcers became larger and deeper than those observed on the 50th day (Fig. 1, d). The largest one showed almost same size and depth as seen on the 5th day after the operation, except the rolling margin of the ulcer. Moreover, in these ulcers apparent hemorrhages resulted in the bottom or on the edge of the ulcer (Fig. 3, b). On the 100th day the ulcer was still evident in each animal and its size was found to be a little smaller than that observed on the 75th day but the depth was almost in a same degree. The floor of the ulcer was covered by thin necrotic material and in some cases remarkable hemorrhages were found (Fig. 4, a). The epithelium of the originally ulcerated area was not repaired at all even on the regenerated connective tissue. Histologically the overall configuration of the ulcer resembled that seen on the 75th day (Fig. 5). On the 150th day, the ulcer with a considerable necrotic debris was clearly observed in 17 out of 20 animals (Fig. 4, b). In 3 out of 20 animals, the lesions were completely repaired with regenerated connective tissue but epithelization did not occur at all on it.

The effects of several drugs and bilateral vagotomy on the healing of the produced ulcer

As above mentioned, the ulcer produced by 30% acetic acid solution persisted for a long period so that we administered several drugs listed in Table 2 for 15 or 40 days in order to estimate the curative effect on the ulcer. Synthetic aluminum silicate, an antacid agent, had little effect on the gastric ulcer healing compared with the control group. Carrageenin, an antipeptic agent, showed a relatively high curative ratio but was not significant. Atropine sulfate, a potent anticholinergic agent, showed a little effect but was also not significant. Chlorophyllin copper sodium salt, placenta extract, and glycyrrhetic acid dipotassium salt showed a significant healing of the ulcer by the 15 days administration. However, although placenta extract also showed a significant healing of the ulcer by 40 days administration, the curative ratio was slightly decreased compared with that of the 15 days group. On the other hand, bilateral vagotomy resulted in significant delay of the ulcer healing.

TABLE 2. The effect of several drugs and vagotomy on the healing process of the ulcer produced by the injection of 30% acetic acid solution into the rat stomach wall.

Treatment	No. of animals		Dose (per kg)	Administration		Curative ratio %	P value*
	Control	Test		Days	Routes		
Synthetic aluminum silicate	15	10	1 g	15	p.o.	11.5	>0.10
Carrageenin	15	15	600 mg	15	p.o.	32.0	>0.10
Atropine sulfate	15	15	20 mg	15	s.c.	26.7	>0.05
Vagotomy	15	15	—	15	—	−30.6	<0.01
Chlorophyllin-(Cu-Na)	11	12	500 mg	15	p.o.	39.8	<0.05
Glycyrrhetic acid (K_2)	1	12	700 mg	15	p.o.	33.7	<0.05
Placenta extract	11	10	5 ml	15	i.p.	43.6	<0.05
Placenta extract	16	17	5 ml	40	i.p.	30.9	<0.05

* Level of significance was calculated using student's *t*-test.

DISCUSSION

We have previously reported the healing process of the stress ulcer induced in the rat by immersing into a water bath and curative effects of several drugs on this ulcer (8). The stress ulcer has occurred in the glandular portion of the stomach and took about 28 days for their recovery, but the lesions were limited only in the mucosal layer and did not extend into the submucosal layer. Then we devised a new method to produce a gastric ulcer similar to a human chronic ulcer, that is, the defect involves muscle coat and takes much time to heal. The ulcer produced by the injection of acetic acid solution into the rat stomach wall was assumed to be a so-called "intractable ulcer" which is frequently seen clinically but is seemed to be very difficult to produce in experimental animals. Two reasons were thought. 1, The ulcer persisted more than 150 days without any additional procedure. 2, The enlargement of the ulceration and the appearance of hemorrhages in the floor or on the edges of the lesions was confirmed during the healing process. Skoryna *et al.* (1, 2) described the ulcer production in rats by thermocautery method and the detailed chronological study over 100 days of its healing process. However, they did not confirm any penetrating re-ulceration without the occurrence of mucosal erosion in the central portion of the healed ulcer. On the other hand, they evoked the definite re-ulceration by means of the cortisone administration following the complete healing of the produced ulcer (9). Umebara *et al.* (3) reported that ulcer produced by clamping of the rat stomach wall naturally healed within 28 days, whereas it persisted more than 7 weeks with the intramuscular administration of the cortisone acetate following the operation. Hence, these findings seem to give some clue to solve the mechanism of chronicity including the spontaneous re-ulceration seen in our experiment.

Usually the thickness of stomach wall of rats was approximately 1 mm but the depth of the ulcer showed 2–4 mm in an early phase of ulceration, because the penetration of gastric wall, inflammation of the submucosal layer and rapid proliferation of connective tissue occurred. The recovery processes of the gastric ulcers consisted of the proliferation of connective tissue from the base of ulcer and regeneration of mucosal epithelium from

the edges of ulcer. The regeneration of epithelium showed a comparative delay compared with that of connective tissue in the later phase of the healing. During the period up to 150 days after the injection of 30% acetic acid solution, the muscularis mucosae and muscle coats of the stomach were not repaired at all. It was also tried to produce duodenal ulcer by this technique, but it could not be accomplished due to the technical difficulty of injection of acetic acid solution into duodenal wall in rats. From the results of several drugs on ulcer healing, we considered that the inhibition of gastric acid or pepsin did not accerelate the healing the ulcer and that the agents stimulating the epithelization or granulation of the stomach tissue exerted a significant accerelation of the repair in the early phase of the ulceration. Concerning the influence of the vagotomy on the healing, we can not comment nowadays, but it seemed that the inhibition of gastric motility may result in the aggravation. In subsequent reports we will discuss the influence of cortisone acetate administration or stress confliction on the healing process of acetic acid ulceration.

SUMMARY

A simple method was described to produce a penetrating gastric ulcer by means of the injection of an acetic acid solution into gastric wall of the rat. The ulcer produced at 1% acetic acid solution healed within 10 days because of the weak injury limited in the epithelial layer, but at 10 and 30% acetic acid solution the ulcerated regions involved of full thickness of gastric wall and persisted more than 60 days. Especially in the group of 30% acetic acid the ulcer was clearly recognized even on the 150th day after the operation. The repair process closely resembled to that of the human peptic ulcer from the macroscopical and histological observation. Chlorophyllin copper sodium salt, placenta extract, glycyrrhetic acid dipotassium salt showed significant repairing effect on the produced ulcer. Synthetic aluminum silicate, carrageenin and atropine sulfate did not show a significant accerelation on ulcer healing. Bilateral vagotomy indicated the aggravation of the ulcer significantly.

Acknowledgement: The authors wish to thank Dr. Mamoru Saito, of the Department of Cancer Immunology of the Institute of Medical Science of the University of Tokyo, for his help in the histological studies.

REFERENCES

1) Skoryna, S.C., Webster, D.R. and Kaiin, D.S.: *Gastroenterology* **34,** 1 (1958)
2) Phillips, M.J., Skoryna, S.C., Webster, D.R. and Kahn, D.S.: *Can. J. Surg.* **4,** 239 (1961)
3) Umebara, S., Tabayashi, T., Shibata, E., Ito, H., Shimizu, M. and Okiishi, Y.: *J. Therap.* **47,** 89 (1965) (*in Japanese*)
4) Lauren, P. and Holmberg, B.: *Ann. Med. exp. Fenn.* **44,** 40 (1966)
5) Robert, A. and Selye, H.: *Ann. Endocrinol.* **13,** 846 (1952)
6) Johnson, L.R. and Overholt, B.F.: *Gastroenterology* **52,** 505 (1967)
7) Takagi, K. and Kayaoka, S.: *Yakugaku Zasshi* **88,** 14 (1968) (*in Japanese*)
8) Takagi, K. and Okabe, S.: *Jap. J. Pharmac.* **18,** 9 (1968)
9) Kahn, D.S., Phillips, M.J. and Skoryna, S.C.: *Am. J. Path.* **38,** 177 (1961)