Na_2SO_4. In cases where **1a**, **1b**, or **1c** was used, esterification with diazomethane in ether was performed in the usual manner. The bromo-ester (**2**) obtained by evaporation of the ether was purified by column chromatography on silica gel with benzene followed by vacuum distillation (oil bath temperature below 125°[4]), and was identified with the sample prepared below. Optically active **2** showed no racemization by the above purification processes.

Optically Pure Dimethyl (*S*)-Bromosuccinate ((*S*)-(−)-2)——This sample was prepared from (*S*)-(−)-bromosuccinic acid (mp 178—180°, $[\alpha]_D^{11}$ −43.0° (c=1.454, H_2O)) (reported[6] mp 177—178° (dec.), $[\alpha]_D$ −43.8° (H_2O)) by treatment with diazomethane in ether.[4b] The resulting (*S*)-(−)-**2** was purified by column chromatography on silica gel with benzene to give a colorless liquid of $[\alpha]_D^9$ −70.0° (c=1.212, benzene) in 90% yield. Distillation at bp 89—90° (5 mmHg) afforded a liquid of $[\alpha]_D^{19}$ −70.3° (c=1.208, benzene). *Anal.* Calcd. for $C_6H_9O_4Br$: C, 32.02; H, 4.03; Br, 35.51. Found: C, 32.20; H, 4.11; Br, 35.53.

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Effect of Phenolic Acids and Related Compounds on Gastric Acid Secretion in the Rat

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Response of gastric acid secretion to benzyloxycarbonyl-Trp-Met-Asp-Phe-NH_2 (tetragastrin) before and after perfusion of phenolic acids and related compounds was examined in the rat. Tannic acid and gallic acid inhibited the response to tetragastrin after perfusion of these compounds. But protocatechuic acid, *p*-hydroxybenzoic acid, salicylic acid, and aspirin stimulated the response to tetragastrin, and sometimes enhanced the basal acid secretion. Possible mechanism of ulcerative action of aspirin is discussed.

Keywords——tannic acid; gallic acid; aspirin; tetragastrin; gastric acid secretion; gastric perfusion technique; aspirin ulcer

The author previously reported that tannic acid is a potent inhibitor of gastric secretion in the Shay rat.[2] Usually tannic acid is ingested with foods, so that the gastric perfusion technique was used in this experiment. Since tannic acid is an ester of phenolic acids and a sugar, not only tannic acid but also phenolic acids, one of which is gallic acid, and their related compounds were examined in order to determine whether these compounds have an effect on gastric acid secretion.

Materials and Method

Materials——Benzyloxycarbonyl-Trp-Met-Asp-Phe-NH_2 (tetragastrin) was purchased from Nissui Seiyaku Co. All other reagents were obtained from commercial sources and used without further purification. Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, salicylic acid, and aspirin were dissolved by careful addition of sodium hydroxide to about pH 5 just before use.

Method——Wistar rats weighing about 200 g were used for the experiment by the Ghosh method.[3] The stomach cavity was perfused with 0.2 mN NaOH at a constant rate of 0.5 ml/min. Gastric effluent was collected during 10 min. After a complete end of the response to a first intravenous injection of tetragastrin, perfusate was changed to the solution of phenolic acid or related compounds for 30 min and perfused with 0.2 mN NaOH. When pH of the effluent returned to the basal level, a second dose of tetragastrin was injected, and occasionally a third dose was given. Effect of the compounds was estimated by summing the changes of pH of 10 min samples for 1 hr.

1) Location: *1-1, Keyakidai, Sakado-shi, Saitama-ken, 350-02, Japan.*
2) S. Tani, *Yakugaku Zasshi*, **96**, 648 (1976).
3) M.N. Ghosh and H.O. Schild, *Brit. J. Pharmacol.*, **13**, 54 (1958).

Results and Discussion

Figure 1 shows the inhibitory effect of tannic acid on the response of acid secretion, in which 8% tannic acid solution was perfused in the rat stomach for 30 min. The response to tetragastrin, after perfusion of tannic acid, gradually returned with time. Since tannic acid administered to the Shay rat binds solidly to the mucosa,[2] as reported previously, it is thought that tannic acid is effective in a low concentration and resists its emptying from the stomach. However, in this experiment a high concentration, more than 4%, of tannic acid was necessary to inhibit the response of acid secretion. Therefore, almost all of tannic acid would pass through the rat stomach with perfusate.

Gallic acid strongly inhibited the response of acid secretion when used in a lower concentration than that of tannic acid, as shown in Fig. 2. It suggested that gallic acid is an active component of tannic acid and freely permeates into gastric mucosa compared with tannic acids.

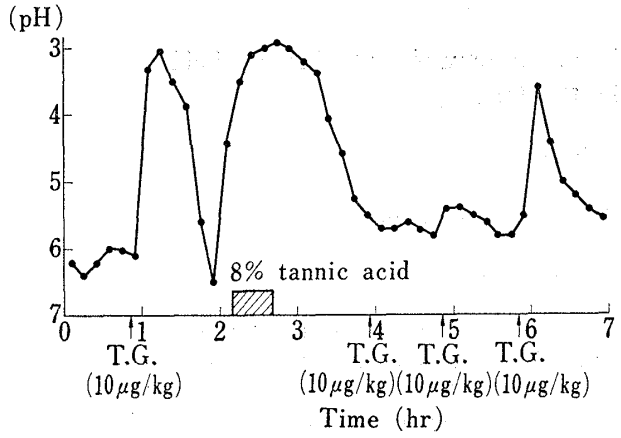

Fig. 1. Effect of Perfusion of Tannic Acid on the Response of Acid Secretion stimulated by Tetragastrin in the Perfused Rat Stomach

8% of tannic acid was perfused during the period indicated the shadow. The arrow shows the time of injection of tetragastrin (T.G.). The response to T.G., after perfusion of tannic acid, gradually returned with time.

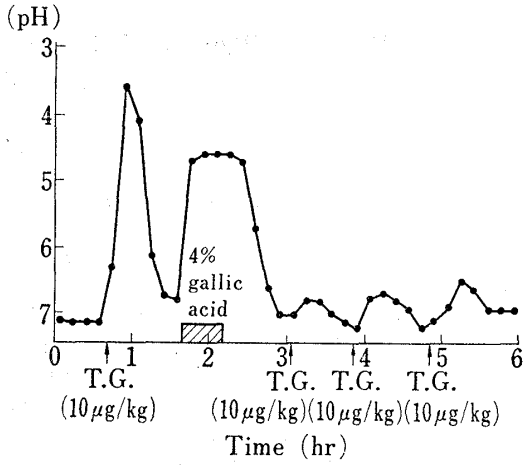

Fig. 2. Effect of Gallic Acid on the Response of Acid Secretion stimulated by Tetragastrin in the Perfused Rat Stomach

4% of gallic acid was perfused during the period indicated the shadow.

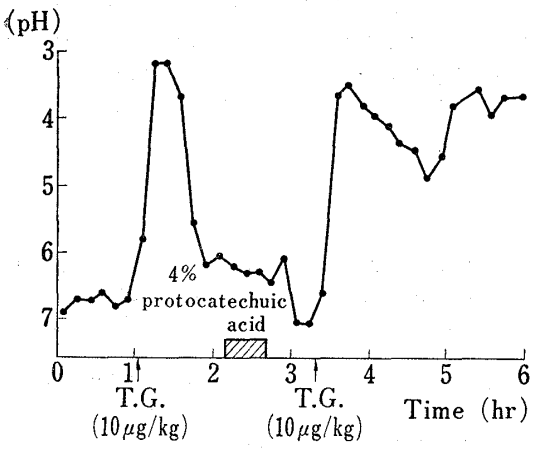

Fig. 3. Effect of Perfusion of Protocatechuic Acid on the Response of Acid Secretion stimulated by Tetragastrin in the Perfused Rat Stomach

4% of protocatechuic acid was perfused during the period indicated the shadow. Acid secretion after the response to a second dose of tetragastrin (T.G.) did not return to the basal level in this experiment.

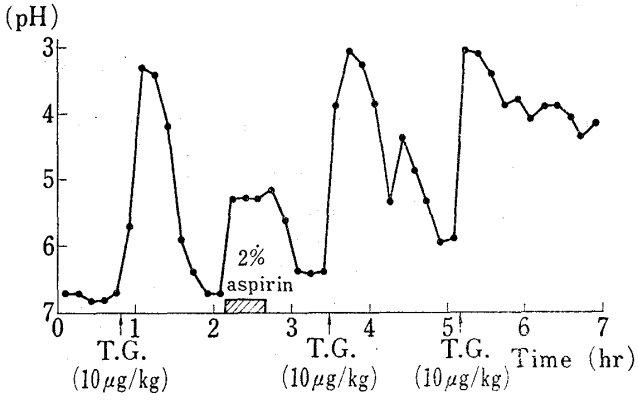

Fig. 4. Effect of Perfusion of Aspirin on the Response of Acid Secre tion stimulated by Tetragastrin in the Perfused Rat Stomach

2% of aspirin was perfused during the period indicated the shadow.

On the other hand, phenolic acids, which have less hydroxyl groups than gallic acid, such as protocatechuic acid, *p*-hydroxybenzoic acid, and salicylic acid, did not inhibit the response of acid secretion, but rather stimulated it. Figure 3 shows the effect of protocatechuic acid on acid secretion. The basal acid secretion was sometimes enhanced after perfusion of these compounds, and also the gastric mucosa had erosions by visual inspection after the experiments.

Aspirin, a derivative of salicylic acid, also stimulated the response of acid secretion, as shown in Fig. 4. There are many reports which mentioned that aspirin caused gastric ulcer.[4] Davenport[5] found that aspirin facilitated H^+ back-diffusion to gastric mucosa, and Kuo and Shanbour[6] suggested that initial action of aspirin was an inhibition of ion transport which was followed by increase in the permeability. Aspirin was dissolved with sodium hydroxide to about pH 5 in this experiment, so that almost all of aspirin existed as the dissociated form. Although the undissociated form of aspirin is absorbed faster than the dissociated form, it is thought that dissociated form of aspirin affects acid secretion. Taking other reports into consideration, these experiments suggested that aspirin injured the gastric mucosa; inhibition of ion transport, facilitation of H^+ back-diffusion, production of erosion, and then increased response to stimulants. All of these would lead to the aspirin ulcer.

TABLE I. Effect of Perfusion of Phenolic Acids and Related Compounds on Gastric Acid Secretion stimulated by Tetragastrin in the Rat

Treatment	(%)	Per cent of control
Tannic acid	8	6.9± 6.0
	4	30.3±22.4
Gallic acid	8	7.0± 3.9
	4	9.8± 2.2
Protocatechuic acid	8	50.2±20.0
	4	133.9±11.9
p-Hydroxybenzoic acid	8	106.7±10.1
	4	131.6±47.7
Salicylic acid	8	222.9±35.7
	4	155.8±46.6
Aspirin	8	133.1±31.7
	4	111.5±14.8
	2	141.9±27.5

Each value expresses as per cent of the response stimulated by tetragastrin after perfusion of these compounds to that before perfusion. The values represent the mean ± standard error for 3 to 4 animals.

Results of these experiments are summarised in Table I. The relationship between structure and effect or magnitude is not seen. The inconsistent results, such as gallic acid inhibiting and protocatechuic acid, *p*-hydroxybenzoic acid, and salicyclic acid stimulating the response of acid secretion, suggest that the number of hydroxyl groups is an important factor for their pharmacological effect. There are many biologically and pharmacologically active substances among phenolic acids and related compounds. In oral administration of these compounds as a medicine, their direct effect on gastric mucosa should be considered carefully.

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5) H.W. Davenport, *Gastroenterology*, **46**, 245 (1964).
6) Y. Kuo and L.L. Shabour, *Am. J. Physiol.*, **230**, 762 (1976).