[Chem. Pharm. Bull. 34(2) 819—826 (1986)]

Effects of Crude Drugs on Various Digestive Enzymes *in Vitro* and *in Vivo*

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(Received July 3, 1985)

The effects of cinnamon bark and zanthoxylum fruit on various digestive enzymes were investigated. Both crude drugs were without effect on starch-dextrinizing activity of diastase and protein-peptic activity of biodiastase. Starch-saccharifying and starch-dextrinizing activities of pancreatin were inhibited by both crude drugs, while the protein-peptic activity of pancreatin was inhibited only by zanthoxylum fruit to below 30% of the activity in the absence of the crude drug. Both crude drugs inhibited starch-saccharifying activity of diastase, lipid-peptic activity of pancreatin and cellulose-saccharifying activity of biodiastase by 30 to 40%.

The effects of cinnamon bark and zanthoxylum fruit on digestive enzymes in gastric and intestinal juice collected from rats were investigated. Starch-dextrinizing, starch-saccharifying and protein-peptic activities of intestinal juice were decreased by the crude drugs, zanthoxylum fruit being more inhibitory than cinnamon bark.

To investigate the effects of the crude drugs on digestive enzymes *in vivo*, 600 mg (5 μCi) of either ^{125}I-bovine serum albumin or ^{125}I-egg white albumin was orally administered to rats 10 min after administration of 100 mg of cinnamon bark or zanthoxylum fruit. Radioactivity transferred to the blood was less in the animals pre-fed with zanthoxylum fruit than in the control animals, indicating that digestion of either bovine serum albumin or egg white albumin was delayed in the presence of the crude drug.

Keywords——*Cinnamon cassia*; *Zanthoxylum piperitum*; digestive enzyme inhibition; diastase; pancreatin; biodiastase; digestibility of radio-labelled protein

Among the over the counter (OTC) drugs on the market in Japan, the total number of gastrointestinal drugs amounts to several hundred, and in most of them, digestive enzymes are prescribed together with antacids and crude drugs.[1] Enzyme activities are known to be influenced by various factors such as temperature, pH, light, coexisting substances and so on. The authors reported previously that several crude drugs such as cinnamon lowered the activities of proteolytic enzymes such as pancreatin.[2] It was reported by Murata *et al.* that cinnamon was also inhibitory to diastase.[3]

Cinnamon bark is included in 45% of the total gastrointestinal OTC drugs (nearly 350 products out of 780) and the amount taken at a time is fairly large (several dozen to 200 mg). Zanthoxylum fruit, which is prescribed in 3% of the total gastrointestinal drugs (20 products) and is used not only as an aromatic peptic crude drug but also as a spice for food, is also inhibitory to proteolytic enzymes. The authors found that there was a correlation between the inhibitory effects of these crude drugs on proteolytic enzymes and the contents of Folin-reagent-positive substances in the crude drugs.[4] Are the crude drugs inhibitory to the digestive

enzymes secreted in the gastrointestinal tract as well as to the enzymes prescribed in the products? This is an important question which has never been answered. In this study, the authors investigated the effects of crude drugs on gastrointestinal proteolytic enzymes by measuring the radioactivity transferred to the blood of rats administered either cinnamon or zanthoxylum fruit prior to ^{125}I-albumin.

Experimental

Materials——a) Enzymes: Diastase, biodiastase 2000 and pancreatin were obtained from Maruishi Pharmaceutical Co., Amano Pharmaceutical Co. and Iwaki Pharmaceutical Co., respectively.

b) Crude Drugs: Cinnamon bark (*Cinnamon cassia* BLUME: product of Kuangnan District, China) and zanthoxylum fruit (*Zanthoxylum piperitum* DE CANDOLLE; product of Yabu, Hyogo Prefecture, Japan), both of which meet the standard requirements of JP X, were used. These crude drugs were pulverized to pass through a 300 μ mesh sieve. Crude drugs, which passed through a 177 μ mesh sieve, were administered to rats through a stomach tube.

Enzyme Assay——Activities of digestive enzymes were determined as follows based on the approval standards for the manufacture and import of gastrointestinal drugs.[5,6]

a) Starch-Saccharifying Activity: In a test tube, 1 ml of an enzyme solution (0.1% diastase solution or 0.05% pancreatin solution) was mixed with 5 mg of a crude drug. Incubation was started at 37°C by adding 10.0 ml of a pre-warmed potato starch solution (the pH was adjusted at 5.0 for diastase and at 7.0 for pancreatin) to the mixture. For the blank test, 10.0 ml of water was used instead of potato starch solution. The subsequent operation was as described.[6] The remaining enzyme activity was calculated according to the following equation, in which B_T and B_S stand for milliliter of 0.05 N $Na_2S_2O_3$ consumed with and without a crude drug, respectively, and A_T and A_S stand for blank values with and without a crude drug, respectively.

$$\text{the remaining enzyme activity } (\%) = (A_T - B_T)/(A_S - B_S) \times 100$$

b) Starch-Dextrinizing Activity: In a test tube, 1 ml of an enzyme solution (0.05% solution of either diastase or pancreatin) was mixed with 5 mg of a crude drug. Ten ml of pre-heated potato starch solution (1%) was added to the test tube and mixed. The mixture was incubated for 10 min at 37 °C. The subsequent procedure followed the assay method for starch-dextrinizing activity.[6] The remaining enzyme activity was calculated according to the following equation, where A_T and A_B stand for the optical densities of reaction mixtures containing a crude drug with and without enzyme, respectively, and A_S stands for the optical density of a reaction mixture containing no crude drug.

$$\text{the remaining enzyme activity } (\%) = (A_B - A_T)/(A_B - A_S) \times 100$$

c) Protein-Peptic Activity: One milliliter of an enzyme solution (0.2% biodiastase 2000 solution or 0.05% pancreatin solution) was mixed with 5 mg of a crude drug in a test tube. Five milliliters of a pre-heated casein solution (0.6%) was added to the test tube and mixed. After a 10-min incubation at 37 °C, protein-peptic activity was assayed as described.[6] The remaining enzyme activity was calculated according to the following equation. A_T and A_B stand for the optical densities of reaction mixtures containing a crude drug, in which an enzyme solution was added before and after trichloroacetic acid, respectively, and A_S and A_{SB} stand for the optical densities of reaction mixtures containing no crude drug, in which an enzyme solution was added before and after trichloroacetic acid, respectively.

$$\text{the remaining enzyme activity } (\%) = (A_T - A_B)/(A_S - A_{SB}) \times 100$$

d) Lipid-Peptic Activity: In an Erlenmeyer flask, 1 ml of an enzyme solution (1% pancreatin solution) was mixed with 10 mg of a crude drug. A pre-heated mixture of 5 ml of olive oil emulsion and 4 ml of phosphate buffer (0.05 M, pH 8.0) was added to the flask and mixed. After a 20 min incubation at 37 °C, lipid-peptic activity was assayed after the method described.[6] The remaining enzyme activity was calculated according to the following equation. B_T and A stand for milliliter of 0.05 N HCl consumed by a reaction mixture containing a crude drug, in which an enzyme solution was added before and after a 20-min incubation, respectively, and B_S stands for milliliter of 0.05 N HCl consumed by a reaction mixture without a crude drug, in which an enzyme solution was added before incubation.

$$\text{the remaining enzyme activity } (\%) = (A - B_T)/(A - B_S) \times 100$$

e) Cellulose-Saccharifying Activity: One milliliter of an enzyme solution (0.1% biodiastase 2000 solution) was mixed with 1 mg of a crude drug in a 25 ml volumetric flask. A pre-heated carboxymethylcellulose solution (0.625%, pH 4.5) was added to the flask and mixed. After a 30-min incubation at 37 °C, cellulose-saccharifying activity was assayed as described.[6] The remaining enzyme activity was calculated according to the following equation. A_T and A_B stand for the optical densities of reaction mixtures containing a crude drug, in which an enzyme solution was added before and after an alkaline $CuSO_4$ solution, respectively, and A_S and A_{SB} stand for the optical densities of reaction mixtures containing no crude drug, in which an enzyme solution was added before and after an alkaline $CuSO_4$

solution, respectively.

$$\text{the remaining enzyme activity } (\%) = (A_T - A_B)/(A_S - A_{SB}) \times 100$$

Diastase and pancreatin used for starch-saccharifying and starch-dextrinizing activities were equally active. Biodiastase 2000 and pancreatin used for protein-peptic activity were also of similar activity.

All the enzymes were used for assays in a concentration range where a linear relationship existed between enzyme activity and enzyme concentration.

Collection of Digestive Juice from Rats and Reaction Conditions——a) Collection of Digestive Juice: Four male rats of Wistar strain weighing 250±10 g (9 to 10 weeks old) were used for collecting digestive juice. After 24-h fasting, animals were laparotomized under nembutal anesthesia, and the cardia, the pylorus and the upper part of the small intestine (about 5 cm from the pylorus) were ligated. After an overnight fast without drinking water, the animals were decapitated and the digestive juice remaining in the stomach and the upper part of the small intestine was collected in a syringe.

b) Reaction Conditions for Digestive Juice: Protein-peptic activities of gastric and intestinal juice were assayed according to the method described.[6] As for gastric juice, 5 μl was used per reaction mixture (0.05 M lactate buffer, pH 2.0) and incubation lasted for 30 min. In the case of intestinal juice, 20 μl was used per reaction mixture (0.05 M phosphate buffer, pH 8.0) and incubation lasted for 60 min.

Starch-dextrinizing activity of intestinal juice was assayed as described.[6] At pH 7.0 (0.1 M phosphate buffer), 20 μl of gastric juice was added to the reaction mixture, which was incubated for 20 min.

As the proteolytic activity of gastric juice varied from animal to animal (C.V. = 18%), gastric juice of the highest specific activity was used throughout the experiments. Proteolytic activity of intestinal juice did not vary markedly (C.V. = 5%), and therefore intestinal juices collected from 4 rats were mixed and used.

***In Vivo* Experiments with ^{125}I-Albumin**——a) Preparation of ^{125}I-Albumin: Bovine serum albumin (fraction V) and egg white albumin (grade III) were labelled using Na ^{125}I by the chloramin T method[7] as follows. To Na ^{125}I (1 mCi/10 μl), 10 μl of 0.25 M sodium phosphate buffer (pH 7.5) was added in a vial. Albumin solution (50 μg/10 μl) and chloramin T solution (50 μg/10 μl) were added successively to the vial. The mixture was stirred for 1 min, then sodium metabisulfite solution (125 μg/10 μl) and 10 μl of 10% KI solution were added. ^{125}I-Albumin thus prepared was purified by gel-filtration on Sephadex G-100. Specific radioactivity of purified ^{125}I-albumin was 11.6 μCi/μg for bovine serum albumin and 6.8 μCi/μg for egg white albumin.

b) Oral Administration of ^{125}I-Albumin to Rats and Blood Sampling from the ^{125}I-Albumin-Administered Rats: Twelve male rats of the Wistar strain weighing 250±5 g (9 to 10 weeks old) were used. Three rats were treated as a group. Two groups of rats were administered bovine serum albumin, one group being also given a crude drug, while the other group did not receive the crude drug (control group). After 1 week, the 2 groups were switched: the group which had been administered bovine serum albumin together with a crude drug was treated as the control group and the former control group was administered bovine serum albumin together with a crude drug. Animals given egg white albumin were treated similarly.

After 24-h fasting, 1 ml of 10% suspension of a crude drug in water was administered orally. Control rats were given 1 ml of water. Ten minutes after crude drug administration, 3 ml of 20% albumin solution containing 5 μCi of ^{125}I-albumin, pre-heated at 37 °C, was given orally. After various time intervals, animals were fixed without anesthesia and 300 μl of blood was taken from the jugular vein.[8] Radioactivity of blood samples was determined using an auto-gamma scintillation spectrometer (type 5220, Packard).

Results

Cinnamon bark and zanthoxylum fruit, which showed rather strong inhibitory effects on proteolytic enzymes among the crude drugs tested, were chosen for this study. As shown in Table I, starch-saccharifying activities of diastase and pancreatin were lowered by the crude drugs. Starch-saccharifying activity of pancreatin was more susceptible to the crude drugs, being reduced to 21.6% of the control activity by cinnamon bark and to 6.0% by zanthoxylum fruit. Starch-dextrinizing activity of diastase was hardly affected by the crude drugs, while that of pancreatin was reduced to 29.6% of the control activity by cinnamon bark and to 4.6% by zanthoxylum fruit.

Protein-peptic activity of biodiastase was not affected by the crude drugs, while that of pancreatin was reduced to 57.2% of the control activity by cinnamon bark and to 29.4% by zanthoxylum fruit. Other preteolytic enzymes, such as trypsin, α-chymotrypsin, subtilisin and bromelin, were inhibited by the crude drugs to the same extent as or even more than pancreatin.

TABLE I. Effects of Crude Drugs on Various Digestive Enzymes

Activity tested	Residual enzyme activity (%)[a]			
	Starch saccharifying		Starch dextrinizing	
Enzyme source	Diastase[b]	Pancreatin[c]	Diastase[c]	Pancreatin[c]
pH	5.0	7.0	5.0	7.0
Control (no crude drug added)	100	100	100	100
Cinnamon bark[c]	53.2±4.5[h] (5.4)[i]	21.6±2.5[h] (1.7)[i]	99.5±3.0[h] —	29.6±4.4[h] (2.5)[i]
Zanthoxylum fruit[c]	57.2±4.9[h] (6.0)[i]	6.0±0.9[h] (0.2)[i]	97.7±0.6[h] —	4.6±2.0[h] (0.2)[i]

Activity tested	Residual enzyme activity (%)[a]			
	Protein peptic		Lipid peptic	Cellulose saccharifying
Enzyme source	Biodiastase[d]	Pancreatin[c]	Pancreatin[e]	Biodiastase[b]
pH	3.0	8.5	8.0	4.5
Control (no crude drug added)	100	100	100	100
Cinnamon bark[c]	101.1±5.2[h] —	57.2±2.5[h] (6.1)[i]	65.8±2.9[f,h] (16.0)[i]	54.4±6.1[g,h] (1.2)[i]
Zanthoxylum fruit[c]	95.5±3.8[h] —	29.4±3.0[h] (2.4)[i]	61.9±5.4[f,h] (13.5)[i]	69.8±3.5[g,h] (1.8)[i]

a) Enzyme activities were determined by the methods described in Experimental. The concentration of each enzyme solution was as follows *b*) ... 0.1% *c*) ... 0.05% *d*) ... 0.2% *e*) ... 1.0%. Five milligrams of crude drug was added per tube except *f*) 10 mg and *g*) 1 mg. *h*) Means±S.D. ($n=3$). *i*) Numbers in parentheses: IC_{50} (mg/ml).

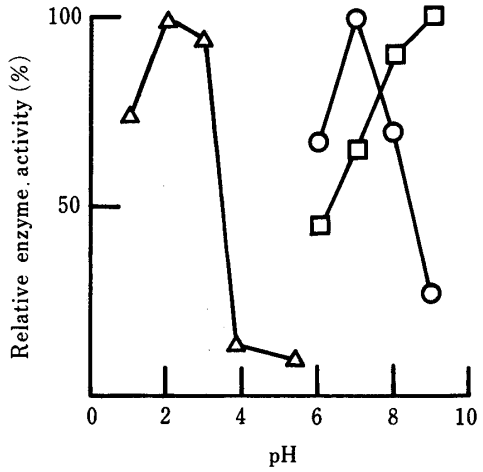

Fig. 1. pH Profile of Dextrinization and Proteolysis by Gastric Juice and Intestinal Juice

Starch-dextrinizing and protein-peptic activities of gastric and intestinal juices were assayed at various pH values according to the methods described in Experimental. As buffers, 0.05 M lactate buffer (pH 1.0—3.8) and 0.05 M phosphate buffer (pH 5.4—9.0) were used. ○, dextrinization by intestinal juice; △, proteolysis by gastric juice; □, proteolysis by intestinal juice.

Lipid-peptic activity of pancreatin and cellulose-saccharifying activity of biodiastase were also inhibited by the crude drugs to below 70% of the control activities. Considering the ratio of the amount of a crude drug to that of an enzyme, the inhibitory effects of the crude drugs on lipid-peptic activity are rather strong compared to those on starch-saccharifying and starch-dextrinizing activities and protein-peptic activity.

It was next examined whether the crude drugs, which were inhibitory to several commercial enzymes, as shown in Table I, also inhibited digestive enzymes in gastric and

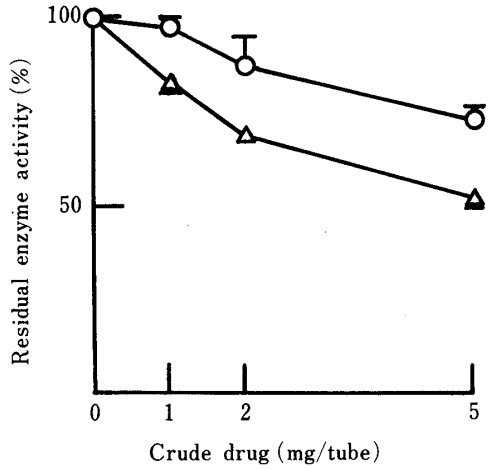

Fig. 2. Effects of Crude Drugs on Dextrinization by Intestinal Juice

Starch-dextrinizing activity of intestinal juice was assayed in the presence of various amounts of crude drugs according to the method described in Experimental. ○, cinnamon bark; △, zanthoxylum fruit. Values are the means ± S.D. ($n=3$).

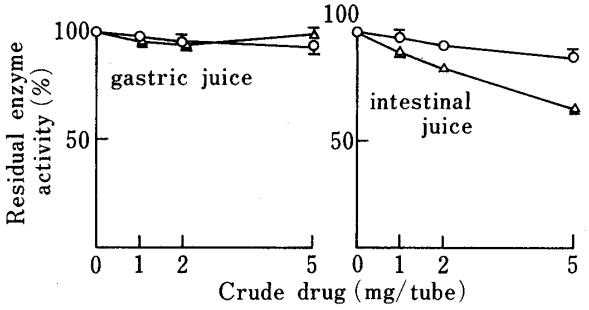

Fig. 3. Effects of Crude Drugs on Proteolysis by Gastric and Intestinal Juices

Proteolytic activity of either gastric or intestinal juice was assayed in the presence of various amounts of crude drugs according to the method described in Experimental. ○, cinnamon bark; △, zanthoxylum fruit. Values are the means ± S.D. ($n=3$).

intestinal juice. Digestive fluid, besides digestive enzymes, contains mucus, bile and various other secretions, which might diminish the inhibitory effects of crude drugs.

Optimum pH values for protein-peptic activity, for starch-dextrinizing activity and for protein-peptic activity of gastric juice were 2.0, 7.0 and 9.0, respectively (Fig. 1). As the pH in the intestinal tract rarely rises over 9.0 (data not shown), the protein peptic activity of intestinal juice was measured at pH 8.0.

Starch-dextrinizing activity of rat intestinal juice was decreased by the crude drugs. Cinnamon bark reduced the starch-dextrinizing activity to 71% of the control activity at the 5 mg/tube level, while zanthoxylum fruit reduced it to 52% of the control activity at the same level (Fig. 2).

Cinnamon bark hardly inhibited the protein-peptic activity of gastric juice at the 5 mg/tube level. Zanthoxylum fruit was without effect on the protein-peptic activity of gastric juice (Fig. 3). The results agreed well with our previous finding that the crude drugs were not inhibitory to pepsin.[2] Cinnamon bark reduced the protein-peptic activity of intestinal juice to 87% of the control activity at the 5 mg/tube level and zanthoxylum fruit reduced it to 64% of the control activity at the same level (Fig. 3). The effects of the crude drugs on the protein-peptic activity of intestinal juice[9] were similar to those on the starch-dextrinizing activity of intestinal juice. As the units of protein-peptic activity per milliliter of juice were 1770 and 168, respectively, for gastric and intestinal juice, intestinal juice was diluted 4 times before being compared to gastric juice.

As cinnamon bark and zanthoxylum fruit were inhibitory to various proteolytic enzymes and to the protein-peptic activity of intestinal juice *in vitro*, the effects of the crude drugs on the digestibility of orally administered albumin was investigated *in vivo* by measuring radioactivity transferred to the blood after oral administration of ^{125}I-bovine serum albumin or ^{125}I-egg white albumin with or without a crude drug.

As shown in Fig. 4, radioactivity in the blood increased with time after oral administration of ^{125}I-bovine serum albumin, and no significant difference was observed between the control and the cinnamon bark-fed group. In the zanthoxylum fruit-fed group, the time course of radioactivity increase in the blood resembled that in the control group except at 60 min after administering ^{125}I-bovine serum albumin.

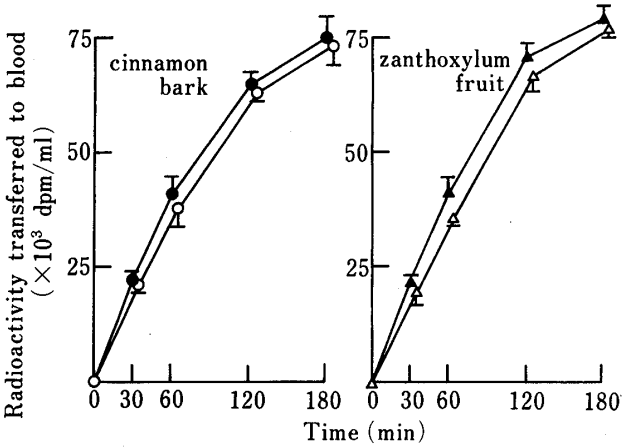

Fig. 4. Effects of Crude Drugs on the Digestion of ^{125}I-Bovine Serum Albumin

Prior to oral administration of ^{125}I-bovine serum albumin (5 μCi/600 mg), animals were tube-fed with 100 mg of either cinnamon bark or zanthoxylum fruit. At the indicated time after ^{125}I-albumin administration, blood was drawn from the jugular vein and radioactivity transferred to the blood was determined. ●, ▲, control; ○, cinnamon bark; △, zanthoxylum fruit. Values are the means ± S.D. (n=6).

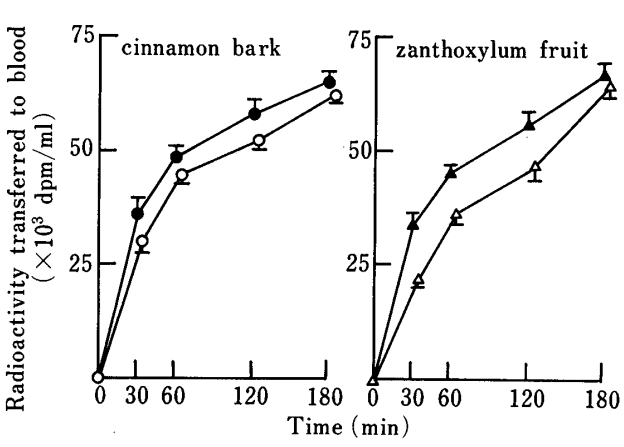

Fig. 5. Effects of Crude Drugs on the Digestion of ^{125}I-Egg White Albumin

Animals were treated as in Fig. 4 except that ^{125}I-egg white albumin (5 μCi/600 mg) was administered instead of ^{125}I-bovine serum albumin. ●, ▲, control; ○, cinnamon bark; △, zanthoxylum fruit. Values are the means ± S.D. (n=6).

When ^{125}I-egg white albumin was administered instead of ^{125}I-bovine serum albumin, the radioactivity increase in the blood was delayed by co-administered crude drugs (Fig. 5). Thirty minutes after administering ^{125}I-egg white albumin, radioactivity in the blood of rats fed with cinnamon bark was 83% and that of rats fed with zanthoxylum fruit was 64% of the radioactivity attained by the control rats. After 3 h, the radioactivity in the blood of rats previously fed with zanthoxylum fruit reached 96% of the value attained by the control group, and no difference in radioactivity in the blood was observed between the 2 groups thereafter.

Discussion

The ratio of amount of crude drug to that of enzyme used in Table I exceeds the ratio in commercially available gastrointestinal drugs by several fold. Usually several to 10 different kinds of crude drugs are combined in a gastrointestinal OTC drug. Frequently combined stomachic crude drugs are glyccyrhiza, phellodendron bark, coptis rhizoma, ginger, ginseng, fennel, clove, gentian, swertia herb, scopolia extract and rhubarb. These crude drugs are combined in gastrointestinal drugs as often as or even more frequently than cinnamon bark. Among them, glyccyrhiza, phellodendron bark and rhubarb inhibit the protein-peptic activity of pancreatin,[10] and coptis rhizoma, ginger, clove, gentian and rhubarb inhibit the starch-peptic activity of pancreatin (data not shown). When these crude drugs are combined in OTC gastrointestinal drugs, it is, therefore, necessary to design prescriptions to avoid lowering of the activities of digestive enzymes. Essential oil of aromatic stomachic crude drugs, such as cinnamon bark and zanthoxylum fruit, may be prescribed instead of the crude drugs themselves, since the authors confirmed previously that essential oil from cinnamon bark was without inhibitory effect on protein-peptic activity.[2,4]

Murata *et al.*[3] reported that amylase-like activity of diastase was lowered by several crude drugs by using the method of Somogi and Nelson. In this method, blank values increased in proportion to the amount of crude drugs. The method used for starch-dextrinizing activity in this report requires no blank test with a crude drug and therefore is

more straightforward. In addition, this method gives more precise data than the cup method. The method used for lipid-peptic activity also requires no blank test with a crude drug.

It was investigated whether cinnamon bark and zanthoxylum fruit, which were found to inhibit the proteolytic activity of pancreatin *in vitro*, might have any effect on digestive enzymes *in vivo*. Though the increase of radioactivity due to ^{125}I-tyrosine in the blood after oral administration of ^{125}I-bovine serum albumin was not affected by co-administered crude drugs, the increase of radioactivity in the blood after ^{125}I-egg white albumin was delayed by co-administered crude drugs. This difference in the effect of crude drugs on the digestibility of bovine serum albumin and egg white albumin seems to be explained by the results obtained by Iwai.[11] Using loss of antigenicity of the gastrointestinal contents to specific antibodies as a measure of digestibility after oral administration of various proteins to rats, he tried to identify regions of the gastrointestinal tract where specific proteins are digested. It was found that bovine serum albumin was digested in the stomach (and lost its antigenicity), while egg white albumin was not digested till it reached the middle and the lower part of the small intestine.[11] In our *in vivo* study, crude drugs came into contact with pepsin and possibly with pancreatic juice prior to their contact with albumin. The crude drugs used, however, do not inhibit pepsin but do inhibit pancreatin. Therefore it seems that digestibility of only egg white albumin, which is digested and absorbed in the small intestine, was inhibited by the crude drugs. The authors confirmed that a portion of crude drugs reached the middle part of the small intestine within 10 min after oral administration.

As shown in Fig. 5, radioactivity in the blood of rats fed crude drugs increased with time, approaching the value of the control group. This is probably because crude drugs combined with egg white albumin to lose their inhibitory effects on proteolytic enzymes. This is also supported by our previous finding that inhibitory substance(s) in cinnamon bark combined with protein non-specifically.[2] Egg white albumin was digested and absorbed faster than bovine serum albumin (*cf.* Figs. 4 and 5), possibly because 20% egg white albumin solution is less viscous than 20% bovine serum albumin solution and therefore moves more easily to the small intestine.

The experimental conditions employed in our study, namely administration of crude drugs prior to albumin after 24-h fasting, differ from the situation under which gastrointestinal drugs are actually used. However, the effects of the crude drugs on various enzymes in the body should be investigated widely, as crude drugs may be taken as foods or herbal medicines, not just as gastrointestinal drugs.

The method that we used in this report for investigating the effect of crude drugs on the digestibility of protein *in vivo* is quite new, and it is very significant that the crude drugs inhibitory to various proteolytic enzymes *in vitro* were actually shown to be inhibitory to proteolysis in the gastrointestinal tract of living animals. This method may be useful to investigate *in vivo* the effects of various other compounds reported to be inhibitory *in vitro* to various proteases, such as antacids, metal ions, salicylate, *etc.*[12]

References and Notes

1) "Drugs in Japan (OTC Drugs)," 3rd ed., Yakugyojiho-sha, Tokyo, 1982, p. 152.
2) K. Yamasaki, H. Yokoyama, Y. Nunoura, C. Umezawa and C. Kawasaki, *Chem. Pharm. Bull.*, **29**, 2966 (1981).
3) K. Murata, M. Yokoshi, A. Yoshida and S. Hayashi, *Shoyakugaku Zasshi*, **38**, 90 (1984).
4) K. Yamasaki, H. Yokoyama, Y. Nunoura, C. Umezawa and K. Yoneda, *Shoyakugaku Zasshi*, **36**, 11 (1982); *idem, ibid.*, **37**, 422 (1983).
5) The approval standards for the manufacture and import of gastrointestinal drugs were specified in Notification No. 520 of the Pharmaceutical Affairs Bureau dated 22 April, 1980.
6) Notification No. 523 of the Evaluation and Registration Division, Pharmaceutical Affairs, dated 22 April, 1980.
7) W. M. Hunter and F. C. Greenwood, *Nature* (London), **194**, 495 (1962); F. C. Greenwood, W. M. Hunter and J.

S. Glover, *Biochem. J.*, **89**, 114 (1963).
8) T. Kanetake, *Nippon Saikingaku Zasshi*, **37**, 943 (1982).
9) Commentary on JPX, Hirokawa Shoten, Tokyo, 1981, D-715.
10) Presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982 and at the 29th Annual Meeting of the Japanese Society of Pharmacognosy, Sapporo, September 1982.
11) K. Iwai, *Nippon Eiyo-Shokuryogaku Kaishi*, **36**, 403 (1983).
12) K. Okazaki, J. Yamamoto and Y. Chiba, *Yakuzaigaku*, **22**, 98 (1962); A. Ichiyanagi, M. Hasegawa, K. Nishi, H. Fujimoto and H. Shino, *Byoin Yakugaku*, **1**, 152 (1975).