GASTROINTESTINAL ABSORPTION AND ANTI-INFLAMMATORY EFFECT OF BROMELAIN

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The clinical utility of proteolytic enzymes is reported as anti-inflammatory agent in literature (1-3) on the therapy for traumatic or infectious diseases. The anti-inflammatory action is considered to be effectual even with oral administration. Such being the case, gastrointestinal absorption of the enzymes is essential.

Qualitative evidence of the gastrointestinal absorption of small amounts of proteins has been reported by a number of workers. Schloss et al. (4-6) employed a qualitative precipitin reaction and an indirect anaphylaxis test to demonstrate that ingested proteins were absorbed from the gastrointestinal tract, and additional studies involving complement fixation methods⁷⁾ and passive transfer tests⁸⁾ were also performed to demonstrate the absorption of intact proteins. Lamanna⁹⁾ studied the gastrointestinal absorption of botulin given rats orally in connection with its toxic reaction.

Quantitative absorption studies on bromelain were made by Smyth et al.¹⁰⁾ in details with the use of I^{131} -labelled bromelain, but questions remain as to their findings: 1) the maximum absorption level in the blood was as high as 6.3% absorption of the administered dose; 2) no radioactivity was detected of the low molecular I^{131} -tagged substances in the circulation; and (3) excretion of the absorbed I^{131} -bromelain was to the extent of 26% of the administered radio-activity in 4 hr.

This communication is primarily concerned with the anti-inflammatory effect of orally administered bromelain, and also deals with the gastrointestinal absorption of bromelain determined by a radioisotope iodine-125 tracer method in combination with immunological techniques of precipitin and Ouchterlony, gel filtration, and electrofocusing.

MATERIALS AND METHODS

Bromelain: Stem bromelain powder extracted and purified from pineapple stem was a preparation of the Green Cross Corporation (Osaka, Japan) with activity of approx. 800 caseinolytic units per mg of dry weight.¹¹⁾ For iodination by radioisotope iodine-125, the bromelain was further purified by the method of Murauchi et al.¹²⁾

Preparation of enteric-coated capsules: Enteric-coated capsules of I^{125} -labelled bromelain were prepared according to the method of Miyauchi and Noda.¹³⁾ A capsule 5.7 mm in diameter and 15.5 mm in length was used in experiments on the dog, the

TABLE 1. Composition in a capsule containing I^{125} -labelled bromelain.

Ingredient	Amount
Lactose	95.2 mg
Corn starch	90.6
Wax	10.4
I^{125} -labelled bromelain	12.75

composition being listed in Table 1. For rat experiments enteric-coated granule 1.2 mm in diameter was prepared.

I^{125} -labelled bromelain: Radioactive sodium iodine- I^{125} with more than 98% radiochemical purity was purchased from CEA CEN SORIN (France).

For oral and intraduodenal administration, bromelain was iodinated by a modification of the Greenwood method¹³. Thirty eight mg of purified bromelain were dissolved in 2.5 ml phosphate buffer (pH 7.6, 0.1 M). To the bromelain solution was added 0.5 ml NaI^{125} (1 mc) and 0.4 ml of 0.1% aqueous chloramine T, and the mixture was allowed to stand for 5 min in an ice-water bath. 0.5 ml of 0.1% aqueous sodium metabisulfite was then added to neutralize chloramine T in excess surviving the reaction. After 5 min, 0.5 ml of 5% aqueous sodium iodide was added to the mixture. The reaction mixture was gel-filtered on Sephadex G-25 to eliminate the iodine-125 unreacted.

This preparation was proteolytically active on a casein substrate at pH 7.6, 37°C, having the specific radioactivity of 4.86 μ c per mg. Caseinolytic activity remained almost unchanged during the above-described iodination. The iodinated preparation was counted using a well type scintillation counter (Packard model 88 W1).

For intravenous administration, iodination of the purified bromelain was performed by the Greenwood method¹³. One hundred μ g of the purified bromelain were dissolved in 0.05 ml of phosphate buffer (pH 7.5, 0.5 M). To the bromelain solution was added 0.1 ml NaI^{125} (1 mc) and 0.025 ml of 1.6% aqueous chloramine T. 0.1 ml of 1% aqueous sodium metabisulfite and 0.2 ml of 1% potassium iodide was immediately added to the reaction mixture. The reaction mixture was then gel-filtered on Sephadex G-25.

Protein concentration: The method of Lowry et al¹⁴, was employed with crystalline bovine serum albumin as standard for determination of protein concentrations. In electrofocusing, protein concentration in the eluate was monitored by measuring absorbance at 280 m μ using a Hitachi photoelectric spectrophotometer.

Caseinolytic units (CU) of bromelain: Proteinase activity of bromelain was determined by hydrolysis of casein according to the method of Hagihara et al¹⁵. To 60 μ g of the enzyme protein was added 5 ml of a casein solution in pH 7.6, 0.05 M phosphate buffer, and 30 μ moles L-cystein. Incubation was carried out at 37°C for 10 min. Absorbance at 275 m μ of the trichloroacetic acid-soluble peptides formed was measured using a Hitachi photoelectric spectrophotometer. A proteinase unit is defined as the enzyme amount which gives an absorbance at 275 m μ equivalent to 1 μ g tyrosine per min at 37°C.

Preparation of anti-bromelain serum: Bromelain in 0.15 M aqueous sodium chloride

was emulsified with an equal volume of complete Freund's adjuvant. Immunization was carried out by subcutaneous injection of 0.5 ml solution containing 4 mg bromelain into the shoulder of a rabbit, a successive second injection being given one week later. Antibody activity was tested by precipitin reactions. The animals were immunized 4 weeks after the first injection, and final bleeding was performed when sufficient antibodies were produced, usually 3 to 5 days after the last injection. The serum pool collected from individual rabbits showed precipitin reaction with an antigen suspension containing less than 0.001 % bromelain.

Administration of I^{125} -labelled bromelain. 1) *Intraduodenal administration:* A dog weighing 7.5 to 8.5 kg was fasted for 24 hr prior to the experiment, but allowed access to water ad libitum. The animal under slight ether anesthesia was incised in the abdomen according to routine surgical procedures, and immediately injected intraduodenally with 20 ml of I^{125} -bromelain solution equivalent to a dose of 55.9 mg/kg, that is radioisotopic iodine- I^{125} 140 μ C/kg. Blood samples were obtained from the femoral artery by cannulation at frequent intervals for a period of 24 hr, and counted with a well type scintillation counter. The degree of anesthesia was minimal to the extent that the animal returned to normal soon after closure of the skin.

Ten ml of 4% aqueous potassium iodide was injected intramuscularly twice, that is, 24 hr in advance and just prior to bromelain administration.

2) *Intravenous administration:* A dog weighing 7.0 to 8.0 kg was fasted for 24 hr prior to the experiment, but allowed access to water ad libitum. The animal was injected intravenously into the femoral vein with 10 ml I^{125} -bromelain solution equivalent to a dose of 11 μ C/kg, that is, radioisotopic iodine- I^{125} 5.6 μ C/kg.

Blood was withdrawn from the femoral artery at frequent intervals for a period of 24 hr, and radioanalysis was performed with a well type scintillation counter.

Aqueous potassium iodide was injected to the animal intramuscularly in advance in the same manner as above-described.

Urine was pooled in the bladder by ligation of the penis, and taken out as a 24-hour collection. At the completion of the experiment, each organ was removed, and homogenized in 0.15 M sodium chloride for determination of radioactivities in the tissues.

A dog weighing 7.5 to 9.0 kg was fasted for 24 hr prior to the experiment but allowed access to water ad libitum. The animal was given intravenously 30 ml of a low molecular fraction of the I^{125} -tagged product digested from the I^{125} -labelled bromelain by pancreatin, equivalent to a dose of radioisotopic iodine- I^{125} 1.3 μ C/kg.

Blood samples were obtained from the femoral artery at frequent intervals for a period of 24 hr. The procedures employed were exactly the same as with the intravenous administration of I^{125} -labelled bromelain.

3) *Intragastric administration of enteric coated capsule containing I^{125} -labelled bromelain:* A dog weighing 7.0 to 9.0 kg was fasted for 24 hr prior to the experiment but allowed access to water ad libitum. The animal slightly anesthetized with ether was given intragastrically 8 enteric coated capsules via stomach tube at a bromelain dose of 13.3 mg /kg, that is, radioisotopic iodine- I^{125} 17.8 μ C/kg. Blood samples were obtained at frequent intervals

for a period of 48 hr. Radioanalysis was performed with a well type scintillation counter.

Analysis of I^{125} -labelled bromelain and I^{125} -tagged substances in serum. 1) *Gel filtration:* approx. 2.5 ml of the serum sample was subjected to gel filtration on Sephadex G-25 to separate high molecular I^{125} -tagged substances from low molecular ones in the serum, and the fractions were counted with a well type scintillation counter.

2) *Electrofocusing:* For detection of the presence of I^{125} -labelled bromelain in the serum, the isoelectric focusing method was employed using an electrolysis column (LKB 8101) of 110 ml capacity. Synthetic ampholytes used as 1% in final concentration had isoelectric points distributed between pH 3 and 10. The serum to be applied was dialyzed in advance at 4°C for 24 hr against a 1% solution of glycine, and then mixed with the less dense ampholyte solution. A focusing by electrophoresis at 4°C was performed at a voltage of 600 V for 48 hours. One ml of each fraction was then collected for radioanalysis and UV absorption, and pH measurements were made at 20°C using a Hitachi-Horiba M-5 pH meter.

Immunological identification of the bromelain absorbed: Serum samples were placed in a Visking tube about 7 mm in diameter, and subjected to dialysis under reduced pressure. Thereby, the serum sample was concentrated 3 times.

1) *Semi-quantitation of absorbed bromelain by the ring test:* the serum sample thus concentrated, and 0.01, 0.001, and 0.0001% of standard bromelain solution were carefully placed over the anti-bromelain serum in capillary tubes. After 30 min incubation at 37°C, fine lines of precipitate appeared at the interface. The visualized intensity of the precipitate for the serum sample was compared to that for the standard bromelain solution. Then, semiquantitative determination was made of bromelain contents of the serum sample.

2) *Semi-quantitation of absorbed bromelain by the Ouchterlony technique:* the serum sample, and 0.05, 0.005, 0.0025, and 0.001% of standard bromelain solution were placed in neighbouring wells in an agar plate, equidistant from a central well containing the anti-bromelain serum. The agar plate was allowed to stand overnight at room temperature in order to visualize fine lines of precipitate. Then, semi-quantitative determination was made of bromelain contents in the serum sample in the same manner as above-described.

Anti-inflammatory assay: Carrageenin-induced edema by the method of Winter et al.¹⁶⁾ was employed as an assay for anti-inflammatory action of bromelain. Male Donryu rats weighing 130 to 150 g were divided into groups of 10 animals each. Bromelain used here was either powdered or in enteric-coated form (granule 1.2 mm in diameter) and it was orally administered with 2 or 3 ml of water or saline at a dose level of 10 to 300 mg/kg. One hr after the administration of bromelain in powder or 20 hr in the enteric-coated form, 0.05 ml of a freshly prepared suspension of carrageenin (1.0% in physiological saline) was injected in the subplantar region of the right hind paw in the rat. The paw was measured in volume by the micro-pipette method (7) prior to and 3 hr after the carrageenin injection. The difference in volume measured was the volume change caused by edema. Per cent inhibition was then calculated for each dose according to the following formula:

$$\% \text{ inhibition} = 1 - \frac{T}{C}$$

where T and C are the mean volume of edema in the drug-treated groups and the control (injected with physiological saline) respectively.

RESULTS

1. Blood levels following intraduodenal administration of I^{125} -labelled bromelain

Radioactivities in the blood: Radioactivities were evident in the blood following intraduodenal administration of I^{125} -labelled bromelain (Fig. 1). A peak was attained at 4 hr, followed by a gradual decrease in radioactivity over a period of 24 hr. The measured levels are considered as the sum of the radioactivity of I^{125} -tagged bromelain absorbed as intact surviving its break-down by intestinal proteases, and that of the absorbed low

FIG. 1. Blood radioactive levels following intraduodenal administration of I^{125} -labelled bromelain.

FIG. 2. Radioactive levels in high molecular serum fractions obtained after intraduodenal administration of I^{125} -labelled bromelain. high molecular fraction, low molecular fraction.

molecular KI^{125}I -tagged products degraded from I^{125}I -labelled bromelain by intestinal proteases.

Radioactivities in high molecular serum fractions: The radioactivity ratio was deter-

Fig. 3. Isoelectric spectrum of the serum samples obtained after intraduodenal administration of I^{125}I -labelled bromelain. (○—○) OD at $280\text{ m}\mu$, (---○) cpm, (●—●) pH.

Fig. 4. pH gradient and concentration-distribution of purified bromelain. (○—○) OD at $280\text{ m}\mu$, (---○) caseinolytic activity (CDU), (●—●) pH.

mined of the high to low molecular fraction by means of gel filtration on Sephadex G-25 of the serum sample obtained after I^{125} -bromelain administration. The results shown in Fig. 2 indicate that as little as 0.213 to 0.263 % of the administered radioactivity was detected in the high molecular fraction over a period of 24 hr, while 93 to 97 % of the radioactive dose was found in the low molecular fraction.

I^{125} -tagged bromelain in high molecular serum fractions: Fig. 3 shows the isoelectric spectrum of the serum samples, in which the position of a UV absorption peak was found at pH 9.4 almost identical to that for bromelain. The isoelectric point of bromelain was derived as pH 9.8 in the control experiment (Fig. 4), while considerably high radioactivity was found at the location of serum proteins in the isoelectric diagram (Fig. 3), particularly of albumin. This may be indicative of a reversible binding of the absorbed low molecular I^{125} -tagged product to albumin. Approx. 21 % of the whole radioactivity in the high molecular fraction accounted for that of the I^{125} -labelled bromelain absorbed as intact. On calculation, as little as 0.042 to 0.052 % of the dose of I^{125} -labelled bromelain was absorbed from the gastrointestinal tract. Assuming that the total blood volume in a dog is 8.4 % of its body weight, a bromelain concentration in the serum was calculated to be 0.46 to 0.57 μ g per ml

Immunological quantitation of the gastrointestinal absorption of I^{125} -bromelain: Table 2 shows the semi-quantitation of the absorbed I^{125} -tagged bromelain in serum by means of the ring test as well as the Ouchterlony using anti-bromelain rabbit serum. As presented in Table 2, the absorption determined as a concentration of bromelain per ml of serum was found to be lower than that measured by the radioactive iodine tracer method.

TABLE 2. Semi-quantitation of the absorbed I^{125} -tagged bromelain in serum.

A pool of serum samples obtained	
at 30 min, 1 and 1.5 hr after I^{125} -bromelain administration	0.033 μ g or less bromelain per ml of serum
at 4, 6, and 8 hr	approx. 0.033 μ g bromelain per ml of serum
at 10, 12, and 24 hr	ibid

2. Blood levels following oral administration of enteric coated capsules containing I^{125} -labelled bromelain

Radioactivities in the blood: Radioactivities were evident in the blood after a 3-hr lag phase, and reached the maximum at 10 hr, followed by a gradual decrease in radioactivity over a period of 48 hr (Fig. 5). The measured radioactivities are considered as the sum of both the radioactivity of absorbed I^{125} -tagged bromelain as intact, and that of absorbed low molecular I^{125} -tagged products degraded from I^{125} -labelled bromelain by intestinal proteases.

Radioactivities in the high molecular serum fraction: Fig. 6 shows the radioactivity ratio of the high to low molecular fraction determined by gel filtration on Sephadex G-25 of a pool of the serum samples obtained at 8 and 10 hr after I^{125} -bromelain administration.

2.3% absorption of the administered radioactivity occurred in the high molecular serum fraction, whereas 97.7% of the radioactive dose was detected in the low molecular serum fraction. This may well explain the following assumption: the enteric coated capsules of I^{125} -labelled bromelain given orally first reached the upper part of small intestine, where the capsule was disintegrated, I^{125} -bromelain discharged into the intestinal juice, and a very small quantity of the I^{125} -bromelain would pass through the intestinal wall into the circula-

FIG. 5. Blood radioactive levels following oral administration of enteric coated capsules containing I^{125} -labelled bromelain.

FIG. 6. Gel filtration of Sephadex G-25 of a pool of the serum samples obtained at 8 and 10 hr after I^{125} -bromelain administration. The column (1.0×36.0 cm) was equilibrated with 0.1 M aqueous sodium chloride. A 3 ml aliquot of serum sample was applied. The eluate in a tube is 2 ml in volume.

tion. Most of the I^{125} -bromelain administered, however, would undergo degradation into the low molecular I^{125} -tagged products by proteolytic enzymes present in the small intestine, and this low molecular radioactive fraction was absorbed in large quantities from the intestinal wall into the circulation. Assuming that the circulating blood volume in the dog is 8.4% of its body weight, the total radioactivity of the high molecular serum fraction obtained at 10 hr, when the radioactivities in the blood reached the maximum, was calculated to be 0.013% of the administered radioactivity.

Immunological identification of the gastrointestinal absorption: The ring test and the Ouchterlony using anti-bromelain rabbit serum failed to make semi-quantitation of the absorbed I^{125} -tagged bromelain in the serum, probably because of its extremely low concentration.

3. Blood levels following intravenous administration of I^{125} -labelled bromelain

Radioactivities in the blood: Fig. 7 presents the time course of the radioactivities in

FIG. 7. Blood radioactive levels following intravenous administration of I^{125} -labelled bromelain.

* % Radioactivity is expressed as % of the radioactivity measured at 10 min after intravenous administration of I^{125} -bromelain.

FIG. 8. Radioactive levels in high molecular serum fractions obtained after intravenous administration of I^{125} -bromelain.

▨ high molecular fraction, □ low molecular fraction. * Refer to Fig. 7.

serum following intravenous administration of I^{125} -labelled bromelain. The level dropped rapidly at a half-life of 50 min with increase in time, and only 20% of the initial level remained in the blood at 5 hr, followed by a slow decrease in radioactivity.

Radioactivities in the high molecular serum fraction: Fig. 8 shows the radioactivity ratio of the high to low molecular fraction determined by gel filtration of Sephadex G-25 of the serum samples, indicating the presence of low molecular radioactive fraction in the serum despite of intravenous administration of I^{125} -labelled bromelain. It is also noteworthy that rapid degradation of injected I^{125} -labelled bromelain proceeded presumably due to serum proteolytic enzymes.

Distribution of bromelain in organs, and its urinary excretion following intravenous injection: Sixty-eight % of the administered radioactivity was excreted in the urine in 24 hr after intravenous administration of I^{125} -labelled bromelain (Fig. 7). This may suggest the excretion of low molecular radioactive products degraded from the I^{125} -labelled bromelain by proteases in the system. Total radioactivity detected in the liver, kidney, and spleen at 24 hr was found 6.5%, 9.3%, and 0.6% of the administered radioactivity respectively (Fig. 7).

4. Blood radioactivities following intravenous administration of a low molecular fraction of the I^{125} -tagged digestion product from I^{125} -labelled bromelain by pancreatin

Preparation of a low molecular fraction of the I^{125} -tagged digestion product from I^{125} -labelled bromelain by pancreatin: Ten ml of I^{125} -labelled bromelain solution containing 100 μ g bromelain and 57.6 μ Ci radioactive iodine- I^{125} was added to 1 ml artificial intestinal juice (pH 7.7) containing 28 μ g pancreatin and 20 μ g sodium bicarbonate, and the reaction mixture incubated at 27°C for 24 hr. After incubation, the mixture was gel-filtered

FIG. 9. Gel filtration on Sephadex G-25 of the digestion product from I^{125} -labelled bromelain by pancreatin. The column (3.6 $\times$ 32.0 cm) was equilibrated with 0.1 M sodium chloride. An 11 ml aliquot of the digestion product of I^{125} -bromelain by pancreatin was applied. Fractions of 4.5 ml each from tube No. 53 to 74 were collected as a low molecular fraction of the I^{125} -tagged digestion product from I^{125} -labelled bromelain by pancreatin.

FIG. 10. Blood radioactive levels following intravenous administration of LMF.

* Refer to Fig. 7.

on Sephadex G-25 to separate LMF* from the intact I^{125} -bromelain (Fig. 9). As shown in Fig. 9, 16.0% of the radioactivity charged survived the digestion. The eluate in tubes numbering 53 to 74 was collected as LMF.

Radioactivities in the blood: Fig. 10 shows the time course of the radioactivities in serum following intravenous administration of LMF. The level dropped rapidly at a half-life of 1 hr and 40 min and, 35% of the initial blood level remained in the circulation at 5 hr. The elimination of LMF from the circulation was found less rapid than that of I^{125} -bromelain given intravenously.

Radioactivities in the high molecular serum fraction: Fig. 11 indicates the radioactivity ratio of the high to low molecular fraction obtained by gel filtration of Sephadex G-25 of the serum samples. Radioactivity was detected in the high molecular serum fraction in spite of LMF injection. This may suggest a reversible binding of LMF to serum proteins, particularly albumin. The radioactive high molecular fraction decreased in quantities in 10 hr.

Distribution of LMF in organs and its urinary excretion: Sixty-six% of the radioactive dose was excreted in the urine in a period of 24 hr (Fig. 10). Radioactivity detected in the liver, kidney, and spleen was 0.9%, 0.2%, and 0.1% of the administered radioactivity respectively (Fig. 10). The radioactivity incorporated in the organs was scarcely in comparison with I^{125} -labelled bromelain given intravenously.

5. Anti-inflammatory effect

The anti-inflammatory effect of orally administered bromelain was compared with hydrocortisone by means of their inhibitory action on carrageenin-induced edema in the rat. When bromelain powder was given the rat with edema with 2 to 3 ml of water, there was no significant difference in inhibition between the control (administered with saline) and the bromelain-treated group at as high dose level as 300 mg/kg (Table 3).

In the case of the enteric-coated form of bromelain, it was administered 20 hr before

Note * LMF stands for a low molecular fraction of the I^{125} -tagged digestion product from I^{125} -labelled bromelain by pancreatin.

Fig. 11. Radioactive levels in high molecular serum fractions obtained after intravenous administration of LMF. high molecular fraction, low molecular fraction. * Refer to Fig. 7.

TABLE 3. Effect of bromelain power on edema induced by carrageenin (rat).

Agents	Dose			Mean volume of edema ml $\pm$ S.E.	% inhibition	'p'
	mg/kg	C.U. kg*	route			
Bromelain powdered	30	26,190	oral	0.538 $\pm$ 0.028	0	
	100	87,300	oral	0.478 $\pm$ 0.050	1.8	
	300	261,900	oral	0.540 $\pm$ 0.030	0	
Bromelain in solution	10	8,730	I.M.	0.092 $\pm$ 0.028	81.1	<0.01
Saline (Control)	10 ml/kg	-	oral	0.487 $\pm$ 0.075	0	

* caseinolytic unit

TABLE 4. Effect of bromelain in enteric-coated form on edema induced by carrageenin (rat).

Agents administered	Dose			Mean volume of edema ml $\pm$ S.E.	% inhibition	'p'
	mg/kg (as bromelain)	C.U. kg*	route			
Bromelain enteric-coated						
6 granules	34.2	22,216	oral	0.801 $\pm$ 0.083	0	
12 granules	73.5	47,746	oral	0.683 $\pm$ 0.037	6.6	<0.05
20 granules	120.8	78,472	oral	0.445 $\pm$ 0.063	39.0	<0.01
Lactose enteric-coated (Placebo)	-	-	oral	0.892 $\pm$ 0.058	0	
12 granules	-	-				
Hydrocortisone	10	-	oral	0.460 $\pm$ 0.044	37.0	<0.01
Saline (Control)	20 ml/kg	-	oral	0.730 $\pm$ 0.049	-	

* caseinolytic unit

the injection of the phlogistic agent (carrageenin), as the enteric-coated bromelain granule was found to be retained in the stomach 8 to 10 hr following oral administration and to pass through the stomach within 12 to 20 hr. Results are shown in Table 4, where the dose-response relation seems to be evident. A single oral dose of the bromelain 120.8 mg/kg obviously suppressed development of the edema, showing 39.0% inhibition. This inhibitory effect was a little higher than that of hydrocortisone at a dose of 10 mg/kg (37.0% inhibition).

DISCUSSION

Smyth et al. (10) reported the gastrointestinal absorption of I^{131} -labelled bromelain, in which the maximum radioactive levels in the blood were demonstrated to the extent of 6.3% absorption of the administered radioactivity in 1 hr following intraduodenal administration of I^{131} -labelled bromelain, and 12% of the radioactivity dose was excreted in the urine in 5 hr, and they concluded that this absorption and excretion occurred in the intact form of I^{131} -bromelain. We consider however that this absorption reported by Smyth et al. (10) to be too high if I^{131} -bromelain is absorbed and excreted as intact. In our experiment on the gastrointestinal absorption of I^{125} -labelled bromelain, radioactive levels reached the maximum 4 hr after intraduodenal administration of I^{125} -bromelain equivalent to 9.2% of the administered radioactivity (Fig. 1). Gel filtration of the serum samples obtained at intervals, however, evidenced that the radioactive levels in high molecular serum fractions were as little as 0.21 to 0.26% of the administered radioactivity over a period of 24 hr. In addition, electrofocusing of the serum samples revealed that about 21% of radioactivity of the high molecular serum fraction was responsible for the bromelain absorbed from the gastrointestinal tract as intact. Accordingly, the serum radioactivity level for bromelain was estimated as 0.042 to 0.052% of the administered radioactivity. Assuming a circulating blood volume in the dog is 8.4% of its body weight, blood levels for the bromelain absorbed as intact were calculated to be 0.46 to 0.57 μ g per ml of serum. Immunological quantitation of the bromelain indicated that its serum concentration was 0.033 μ g or less per ml of serum.

In the gastrointestinal absorption of I^{131} -labelled bromelain, Smyth et al., (10) no identification of a radioactive substance in serum as I^{131} -tagged bromelain was made. In consequence, they may have concluded that as high as 6.3% absorption of the administered radioactivity could be responsible for the bromelain absorbed as intact as a TCA-precipitable, non-dialyzable electrophoretically characterized substance.

When enteric coated capsules containing I^{125} -labelled bromelain were given to a dog by stomach tube, radioactive levels were evident in the blood at 4 hr following a lag phase of 3 hr, and reached the maximum at 10 hr equivalent to 6.6% of the radioactivity administered (Fig. 5). Radioactivity in the higher molecular serum fraction obtained at 10 hr was determined as 0.013% of the administered radioactivity (Fig. 6), although immunological identification failed to evidence the presence of the absorbed bromelain in serum.

These results can be summarized briefly :

Radioactivity measured at the maximum blood levels	Intragastric administration of enteric coated capsules containing I^{125} -bromelain	Intraduodenal administration of a solution of I^{125} -bromelain
Total radioactivity in the blood	0.6% of radioactive dose	9.2% of radioactive dose
Total radioactivity in the high molecular serum fraction	0.013%	0.21—0.26%
Radioactivity in the bromelain fraction		0.042—0.052%

In intraduodenal administration of a solution of I^{125} -labelled bromelain, I^{125} -tagged substances were presumably retained at intestinal absorption sites in higher concentrations than those produced when enteric coated capsules containing I^{125} -bromelain were given by stomach tube, and consequently larger absorption occurred, which was evidenced by the subsequent high radioactive levels in the blood.

In intraduodenal and intragastric administration, it is true that rapid breakdown of I^{125} -bromelain by digestive proteases occurred in the gastrointestinal tract; for eg., in intraduodenal administration of a I^{125} -bromelain solution, as much as 93 to 97% of the radioactivity in serum were found attributable to that of the low molecular serum fraction, whereas only 3 to 7% to that of the high molecular serum fraction, 21%, of which was responsible for the I^{125} -bromelain absorbed as intact.

In intravenous administration of I^{125} -bromelain, the radioactive levels rapidly dropped at a half life of nearly 50 min. A 24 hour urinary collection revealed 68.0% of the administered radioactivity, suggesting the probability that the substance excreted in the urine could be I^{125} -tagged products degraded from I^{125} -bromelain. The total radioactivity detected in the liver, kidney, and spleen at 24 hr was 6.5%, 9.3%, and 0.6% of the administered radioactivity respectively, indicating the rather high accumulation of radioactive substances in tissues compared to that obtained in intravenous administration of a low molecular fraction of the I^{125} -tagged digestion product from I^{125} -bromelain by pancreatin; approx. 10% of the radioactivity administered persisted in the blood at 24 hr, 70% was excreted in the urine, and 17% distributed into the tissues including the liver, kidney, and spleen (Fig. 7). Radioactivity was detected in the low molecular serum fraction as well, although I^{125} -bromelain with a molecular weight of 33,000 was given intravenously. This is due to the fact that I^{125} -bromelain in the circulation underwent rapid breakdown by serum proteases.

Smyth et al. (10) reported the fairly rapid renal clearance of intravenously given I^{131} -bromelain, equivalent to 26% urinary excretion of the I^{131} -bromelain in the intact form in 4 hr, and commented that the renal route of excretion for bromelain well offer opportunity for its clinical application in urinary tract inflammations and infections.

Our experiments, in which a very small gastrointestinal absorption of I^{125} -bromelain

was evidenced may rule out such large renal excretion of intact I^{125} -bromelain.

According to Lamanna⁹⁾, bacterial toxins given rats intraduodenally are absorbed from the gastrointestinal tract into the lymphatic system; gastrointestinal absorption of botulin given orally was demonstrated in his experiment by collecting the absorbed toxin in lymphatic fluids by cannulation into the thoracic duct, and the total absorption was found to be in the order of 1/100,000 of the oral dose.

Our data demonstrated the absorption percentage of 0.042 to 0.056 for I^{125} -labelled bromelain in the intact form, which was slightly higher in percentage as compared with botulin.

If the intact bromelain absorbed acts as an anti-inflammatory agent, the gastrointestinal absorption, even though the blood level was low, may give a possible explanation of the inhibitory effect of bromelain on the edema induced in the rat paw, as evidenced in the present experiment.

SUMMARY

Gastrointestinal absorption of bromelain was studied in the dog by means of a radioisotope iodine-125 tracer method combined with gel filtration, electrofocusing, and immunological techniques.

The anti-inflammatory effect of orally administered bromelain (enteric-coated form) was also investigated using the rat.

1. In intraduodenal administration of a solution of I^{125} -labelled bromelain, a blood radioactive peak was attained at 4 hr equivalent to 9.2% absorption of the administered radioactivity, but, over a period of 24 hr after I^{125} -bromelain administration, 0.213 to 0.263% absorption of the radioactive dose was found in the high molecular serum fraction, 21% of which was responsible for I^{125} -tagged bromelain absorbed as intact, equivalent to 0.042 to 0.052% absorption of the administered radioactivity. Gastrointestinal absorption of bromelain immunologically proved the same.

2. In intragastric administration of enteric coated capsules containing I^{125} -labelled bromelain as much as one eighth of the intraduodenal radioactive dose, a blood radioactive level reached the maximum at 6 hr following a 3 hr lag phase equivalent to 0.6% absorption of the administered radioactivity, and 0.013% absorption of the radioactive dose was attributed to the radioactivity in the high molecular serum fraction. In this case, the presence of bromelain absorbed was not evidenced immunologically.

3. In intravenous administration of I^{125} -labelled bromelain, I^{125} -bromelain was eliminated from the circulation at a half-life of 50 min, and low molecular I^{125} -tagged substances were detected immediately after I^{125} -bromelain administration; it is indicative of the rapid break-down of I^{125} -bromelain by serum proteases. A binding of these low molecular I^{125} -tagged substances to serum proteins was also revealed. A 24 hr urinary collection was found 68% of the radioactive dose.

4. The anti-inflammatory effect of bromelain was demonstrated on the carrageenin-induced edema in the rat hind paw. Bromelain, when orally administered in enteric-coated

form, showed 6.6% inhibition of the edema at a dose of 73.5 mg/kg (47,746 caseinolytic units/kg), and 39.0% inhibition at a dose of 120 mg/kg (78,472 caseinolytic units/kg). This inhibitory effect was highly significant and almost equivalent to that obtained with hydrocortisone given at a dose of 10 mg/kg, while powdered bromelain orally administered did not show any inhibitory effect on the edema at a dose level ranging from 20 to 300 mg/kg.

5. The data obtained here indicate that orally administered bromelain is absorbable from the gastrointestinal tract and exhibits fairly potent anti-inflammatory activity on the exudative phases provided it is in the form of enteric-coated capsules.

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