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47. Hiroshi Moriya, Chiaki Moriwaki, Setsuko Akimoto, and Keiko Yamazaki: Studies on Kallikreins. I. Effects of the Gastro-Intestinal System and the Liver on Pancreatic Kallikrein.*1

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Effects of the gastro-intestinal system and the liver, on the hog pancreatic kallikrein were studied. The esterase and the vasodilator activities as the potency of the kallikrein were both measured. The kallikrein was quite unstable to human mixed gastric juice, however, it was not affected by the incubation with human mixed intestinal juice and the rat liver slices.

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For the purpose of elucidating the physiological roles of the kallikrein, some of the biochemical problems on kallikreins and their related substances have been studied by the authors.[1~5] At this time it is one of the important problems to clarify the stability, the behavior and other properties of the kallikrein administered in the body. Some of the chemical stabilities had already been reported by us,[6] and the present investigation was aimed to know the effects on the kallikrein activities of the gastro-intestinal system and the liver as the fundamental information for further physiological studies.

Experimental

Vasodilator Activity, Esterase Activity and Standard Kallikrein——These were the same as previously described.[3,4]

Kallikrein Preparation——The kallikrein used in this experiment was extracted and partially purified from hog pancreas in our laboratory. Its purity was 18~80 Frey Units per mg.

Human Mixed Gastric and Intestinal Juice——Pooled gastric and intestinal juices from healthy persons were kindly supplied by Dr. Kudo, Laboratory of Clinical Chemistry, Hospital of Keio-Gijuku University. Gastric juice was slightly emulsive, at pH 1.8~2.2 and after centrifugation the supernatant was used. Intestinal juice was at pH 7.8, the supernatant after centrifugation was also employed.

Incubation of the Kallikrein with Human Mixed Gastric Juice——One half to 1.5 mg. of the hog pancreatic kallikrein was incubated with 0.0125~0.5 ml. of human mixed gastric juice at 37° using citrate buffer, 0.05*M* or 0.02*M*, pH 2.0. Just after incubation, pH was immediately adjusted to 6.0 with sodium hydroxide. The kallikrein activities at incubation time, 0, 20, 30, 40, 60 and 120 min. were checked by both esterase and vasodilator activities.

Incubation of the Kallikrein with Human Mixed Intestinal Juice——The same kallikrein was incubated with 0.025~0.075 ml. of human pooled intestinal juice at 37° using phosphate buffer, 1/30*M*, pH 8.04. pH was adjusted to 6.0 with hydrochloric acid just after incubation also in all cases. The kallikrein activity was assayed by both methods at the same intervals of incubation time as in case with gastric juice. Esterase activity originally existed in the natural juice was checked before experiments.

Incubation with the Rat Liver Slices——The soft frozen liver which was isolated from normal rat was sliced with a blade, then the sliced liver was put into a saline and the blood was removed by slight

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shaking for 1 min. One milligram of the hog pancreatic kallikrein was incubated with 1 ml. of phosphate buffer, pH 7.4 and 100 mg. of the sliced liver at 37° for 3 hr. After incubation, centrifuged supernatant was employed for the determination of both vasodilator and esterase activities.

Results and Discussion

Fig. 1 summarizes the results of the incubation of the kallikrein with human mixed gastric juice, examined by the esterase method. The esterase activity when incubated with only distilled water, was slightly getting down and was a little more decreased when incubated with citrate buffer, pH 2.0 which acidity was equal to the natural gastric juice. This matter indicates that the esterase activity of the kallikrein was not stable to the acidic states, however, it was more remarkably decreased by the incubation with human gastric juice. Different volumes of the gastric juice were also employed and the same effects were observed. These findings were seemed to be concluded that natural gastric juice or enzymes in it had potent effect to diminish the esterase activity of the kallikrein. The kallikrein activity was also measured by the dog assay which gave its vasodilator activity. The activity of the kallikrein itself of varied purities can be usually represented more exactly by the vasodilator assay than by the esterase method. Table I shows the results of the incubation with human gastric juice, measured by the dog assay. Although the vasodilator activity of the kallikrein incubated with only distilled water was quite stable, it was almost perfectly diminished not only being incubated with the gastric juice but also with acidic buffer. Therefore, it was more apparently recognized that the kallikrein activity was lost by human mixed gastric

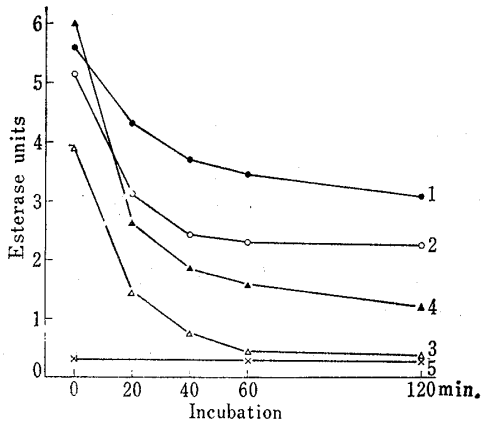

Fig. 1. Esterase Activity of the Kallikrein incubated with Human Gastric Juice.

	Kallikrein mg.	Gastric juice ml.	Citrate buffer ml.
—●— 1:	1	0	0
—○— 2:	1	0	1.0
—△— 3:	1	0.5	0.5
—▲— 4:	1.5	0.025	0.975
—×— 5:	0	0.5	0.5

One esterase unit (EU) was defined as equal to one Frey Unit for a preparation of human urinary kallikrein which had been set aside as a standard.[1] Each represents the mean of 2~4 values.

Table I. Vasodilator Activities of the Kallikrein, incubated with Human Mixed Gastric Juice, measured by the Dog Assay

Kallikrein FU[a]	Incubation (ml.)			Incubated		Activity
	HCl pH 2	Citrate buffer	Gastric juice	pH	hr.	after incubation FU[a]
11	—	—	—	7.0	0	11[b]
11	—	—	—	7.0	1	11[b]
0	—	9.75	0.25	2.0	1	no response
9	—	0.4875	0.0125	2.0	0.5	< 0.25[c]
9	—	0.4875	0.0125	2.0	1	< 0.25[c]
17	—	1.0	0	2.0	0.5	< 0.25[c]
17	1.0	—	0	2.0	0.5	< 0.25[c]
17	—	1.0	0	2.0	1	< 0.25[c]
17	1.0	—	0	2.0	1	< 0.25[c]

a) FU: Frey Units
b) Incubated with distilled water.
c) Values compared with the response of the smallest amount of the standard employed in the dog assay. Any response of these samples were not actually recognized.

juice. And the slightly decreased esterase activity measured during the incubation with only distilled water (Fig. 1), was seemed to be based on other esterase existed in the kallikrein fractions as the impurity.

In case of human mixed intestinal juice, it was observed that the intestinal juice itself contained some other esterase besides the kallikrein (Fig. 2) which could be additionally measured by the present esterase method. Fig. 3 shows the stability of the esterase potency incubated with the intestinal juice, measured by the esterase method. Esterase values at the various incubation time were seemed to include also esterase existed originally in the intestinal juice, however, only the esterase activity of the kallikrein itself was recognized to be stable, which was observed in the results using various volumes of the intestinal juice against the constant volume of the kallikrein (Fig. 3). This matter was more apparently elucidated by measuring the vasodilator activity with the dog assay. In this case the kallikrein activity was quantitatively recovered without any effect after the incubation (Table II).

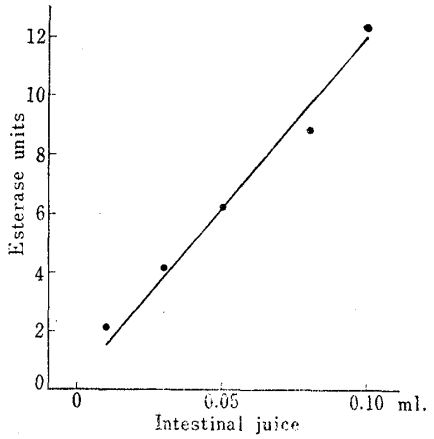

Fig. 2. Esterase Activity of Human Pooled Intestinal Juice.

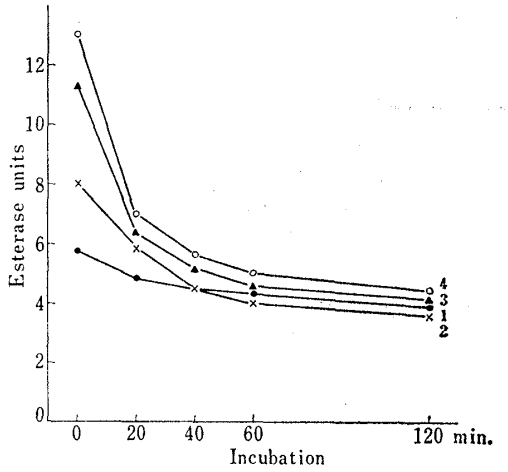

Fig. 3. Esterase Activity of the Kallikrein incubated with Human Intestinal Juice.

	Kallikrein mg.	Intestinal juice ml.	Buffer ml.
—●—	1 : 1	0	1.000
—×—	2 : 1	0.025	0.975
—▲—	3 : 1	0.050	0.950
—○—	4 : 1	0.075	0.925

Table II. Vasodilator Activities of the Kallikrein, incubated with Human Mixed Intestinal Juice, measured by the Dog Assay

Kallikrein FU[a]	Incubation (ml.)		Incubated		Activity recovered FU[a]
	Phosphate buffer	Intestinal juice	pH	hr.	
0	—	—	7.0	1	0
11	—	—	7.0	1	11
0	0.95	0.05	7.8	1	0.6
11	0.95	0.05	7.8	1	11[b]
11	0.95	0.05	7.8	2	11[b]

a) Frey Units
b) Vasodilator value originally existed in the intestinal juice itself (0.6 FU/0.05 ml.), was already reduced.

On the effects of the rat liver slices, both esterase and vasodilator activities were not affected with the incubation at the present experimental condition (Fig. 4 and Table Ⅲ). The liver slice itself did not give any esterase or vasodilator activity.

From these results, it was concluded that a kind of macromolecular enzymes, the kallikrein, was unstable to human gastric juice, but quite stable to the intestinal juice and also stable to the rat liver slices. Therefore, it might be possible to be referred that the kallikrein, when orally administered to the animal or human body, must be readily destroyed in the stomach, however, if some protection to the digestion in the stomach is given, it would be stable enough in the intestinal system. Behavior and properties of the kallikrein after given in the intestinal system, for instance, the passage of it across the intestinal wall, are now studying by us.

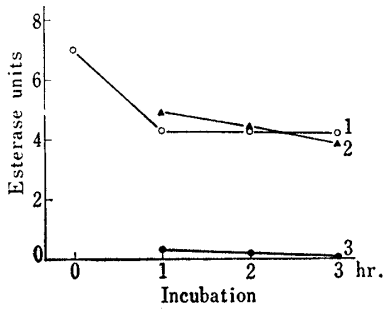

Fig. 4. Stability of the Kallikrein incubated with the Rat Liver Slices, measured by Esterase Method.

	Kallikrein mg.	Liver slices mg.	Phosphate buffer ml.
—○—	1 : 1	0	1.0
—△—	2 : 1	100	1.0
—●—	3 : 0	100	1.0

Table Ⅲ. Stability of the Kallikrein, incubated with the Rat Liver Slices, measured by the Dog Assay

Kallikrein FU[a)]	Incubation hr.	Activity recovered FU[a)]
0	1	no effect
11	1	11
0	2	no effect
11	2	11
0	3	no effect
11	3	11

100 mg. of the rat liver slices was employed in all cases.
a) Frey Units]

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