

A Note on the Chemistry of Enterogastrone

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From the upper part of the small intestine a substance can be extracted that inhibits the histamine induced gastric secretion. This substance has been given the name *enterogastrone*. Papers concerning the chemical properties of enterogastrone are very few, but according to Harris, Gray and Ivy¹ it might be of a protein nature, as they found an inactivation of their preparations by proteolysis with pepsin.

Recently one of us² showed that the active principle in our preparations had *no electrophoretic mobility between pH 2.06 and 7.42*. Furthermore it was very *easy to dialyse* through a cellophane membrane³.

As these observations do not accord with the idea that enterogastrone might be a protein it was found necessary to reinvestigate the effect of proteolysis on our preparations.

EXPERIMENTAL

The preparation process of the enterogastrone was mainly that recommended by Gray, Bradly and Ivy⁴. The extract obtained was dialysed through a cellophane membrane, thus giving the active principle in the dialysate. (The method will be described in detail in a forthcoming paper.)

The activity was tested on dogs with denervated gastric pouches according to Heidenhain. Histamine dihydrochloride was injected intravenously at a slow constant rate thus giving a constant secretion rate of gastric juice⁵. Then a 50 mg dose of the enterogastrone preparation was given intravenously causing an inhibition of the secretion, which was sometimes complete and lasted for several hours⁶.

RESULTS

Effect of pepsin

In this experiment 50 mg of a very active preparation were incubated for 75 minutes at 37° C with 1 ml gastric juice from a dog and 4 ml citrate buffer,

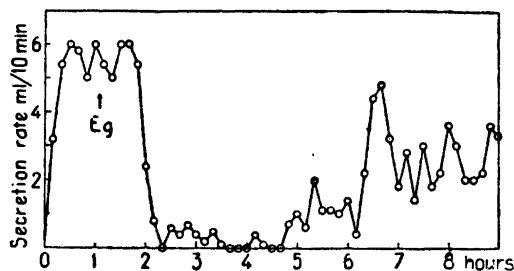


Fig. 1. The effect of a trypsin-treated enterogastrone preparation on the histamine-induced gastric secretion.

pH = 1.5. As a control experiment the peptic activity of this gastric juice was determined by the method given by Riggs and Stadie⁶. It was found that 1 ml of the juice could easily cause a »complete» proteolysis — as obtained with this method — of an egg-white solution containing 50 mg protein at the given conditions.

The pepsin-treated enterogastrone preparation was then given to a dog and found to possess the same inhibiting effect as the untreated one.

An injection of a mixture of 1 ml gastric juice and 4 ml citrate was ineffective.

Effect of trypsin

50 mg of the same enterogastrone preparation were dissolved in 4 ml borate buffer, pH 8.5, and incubated with 4 mg crystalline trypsin (Difco) for 14 hours. After digestion the same inhibiting effect characterized the preparation when injected into a dog. The result is given in Fig. 1.

A control injection of the buffer substance containing only the trypsin (4 mg) did not have any influence on the secretion from the gastric pouch.

Another 125 mg enterogastrone were dissolved in water free of CO₂ and adjusted to pH 8.0 with 0.1 N NaOH. The total volume was made 10 ml. When 10 mg trypsin had been added 1 ml was withdrawn, given one drop of toluol and titrated according to the wellknown Sørensen formol titration. The starting point was chosen pH 7.00 and after addition of 2 ml formalin (40 %, pH 8.25) the titration was continued with 0.04 N NaOH to pH 8.25.

The remaining 9 ml were divided into 1 ml samples each given one drop of toluol and incubated at 37° C. Every day the pH was controlled to be about 8.0. Only after the first day an adjustment with NaOH was necessary. Four of these samples were formol titrated at various times and the results are given in Table 1.

Table 1. The titration results after digestion by trypsin.

Substrate: 12.5 mg enterogastrone preparation in a water solution adjusted to pH 8.0.
Trypsin: Crystalline trypsin (Difco) 1 mg.
Temperature: 37° C.
Starting point: pH 7.00.
End point: pH 8.25.

Sample no.	Digestion		Formol titration ml 0.04 N NaOH	Difference ml
	time hours			
1	0		0.53	0.00
2	26		1.08	0.55
3	51		1.21	0.68
4	91		1.26	0.73
5	140		1.28	0.75

It was found that the digestion by trypsin was almost finished in 4—6 days.

The preparation used could be precipitated by trichloroacetic acid before but not after digestion by trypsin. The biuret reaction remained, however, positive even after the proteolysis.

At the same time as sample 5 was titrated (*i. e.* after six days digestion) 4 ml (50 mg) of the same digested mixture was tested on dog and found to retain its entire inhibiting properties.

DISCUSSION

The experiments performed show that the inhibiting power on the histamine induced gastric acid secretion of an intestinal extract — enterogastrone — is unaffected by both pepsin and trypsin. In addition to the findings that the active principle of the enterogastrone preparation is easy to dialyse and has no or a very small electrophoretic mobility between pH 2.06 and 7.42 this may suggest that enterogastrone is no protein.

The tryptic action did not, however, give a complete hydrolysis of the existing peptides but left some unsplit which may be of a relatively low molecular weight.

SUMMARY

It was impossible to verify the suggestion that enterogastrone could be inactivated by pepsin. It was also shown that the inhibitory effect on the histamine induced gastric secretion was unaffected by trypsin action.

These observations suggest, that the enterogastrone inhibition active principle may be of a non-protein nature. This view may be confirmed by the demonstration that the substance can easily be dialysed and that its electrophoretic mobility is very small and unaffected by large pH-variations.

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