5. **Makoto Hayashi** : Investigations on Food Poisoning Caused by Ordinary Putrefaction. VI. Effect of Biogenic Amines on Kidney Histaminase.

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The present writer has already shown that several of the biogenic amines show synergetic effect against the pharmacological action[1] and lethal dose in mice[2] of histamine. In the pharmacological actions, agmatine, trimethylamine oxide, trimethylamine, and choline are synergetic substances that increase the histamine action by the excised uterus method of guinea pig (Magnus method), while trimethylamine oxide and phosphorylcholine were found to be synergetic substances in the cat blood pressure method. In lethal dose in mice, animal experiments have shown that the intraperitoneal injection of agmatine, arcaine, and methylguanidine cooperate with histamine action.

From such synergetic effects, it seemed of interest to examine the effect of such biogenic amines on histaminase, one of the detoxication mechanism of histamine in a living body. Clarification of such a problem would not only provide some knowledges on the mechanism of synergetic effect but would also clarify the possibility of histamine accumulation in a living body in the presence of biogenic amines.

Effect of biogenic amines on the histaminase of hog kidney was tested by the pressure method on 13 kinds of amines, i.e. putrescine, cadaverine, γ-aminobutyric acid, agmatine, arcaine, methylguanidine, trimethylamine, trimethylamine oxide, choline, phosphorylcholine, phosphorylethanolamine, betaine, and tyramine.

The experiments were carried out by measuring the oxygen uptake of histamine and these amines, each alone or in combination, by the Warburg manometer and the degree of inhibition or acceleration of the histaminase under the concurrent presence of histamine and amine was calculated. Figs. 1~13 show the amount of the oxygen uptake of each combination minus the oxygen uptake by endogenous respiration of the enzyme solution alone.

As is clear from these charts putrescine, cadaverine, agmatine, and γ-aminobutyric acid show oxygen uptake by themselves that the decrease of oxygen uptake in the

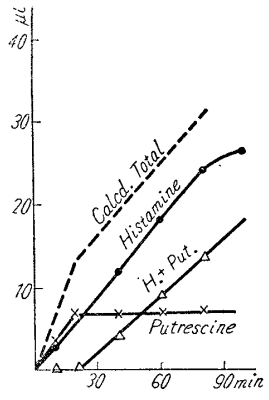

Fig. 1. Inhibition of Histaminase by Putrescine

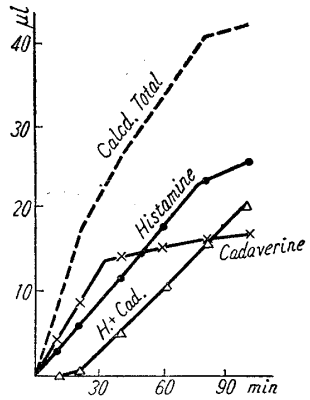

Fig. 2. Inhibition of Histaminase by Cadaverine

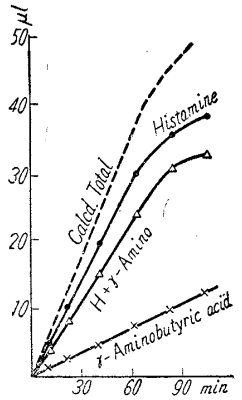

Fig. 4. Inhibition of Histaminase by γ-Aminobutyric Acid

* Okubo, Narashino-shi, Chiba-ken (林 誠).

1) M. Hayashi : J. Pharm. Soc. Japan, **74**, 1148(1954).
2) M. Hayashi : *Ibid.*, **75**, 1(1955).

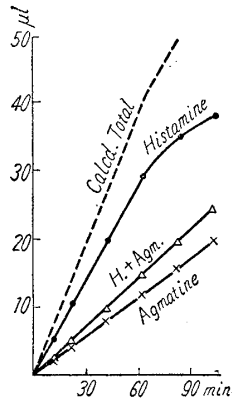

Fig. 4. Inhibition of Histaminase by Agmatine

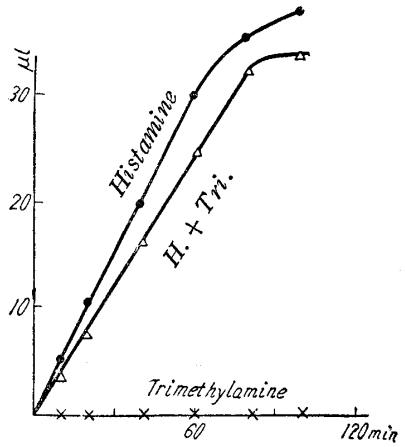

Fig. 5. Inhibition of Histaminase by Trimethylamine

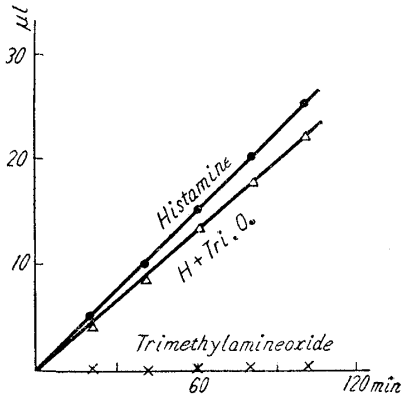

Fig. 6. Inhibition of Histaminase by Trimethylamine Oxide

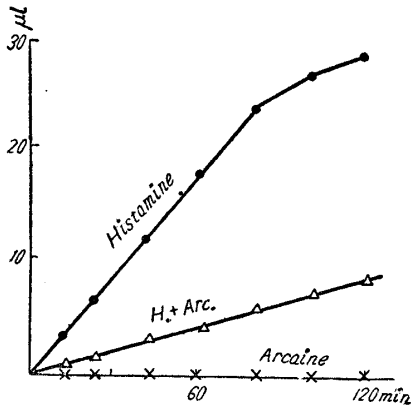

Fig. 7. Inhibition of Histaminase by Arcaine

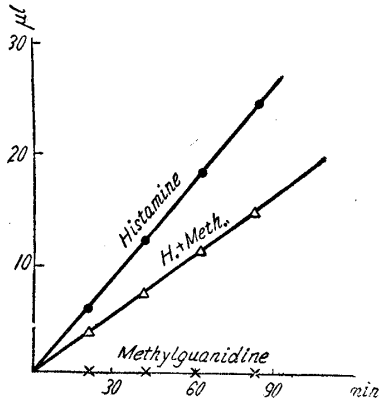

Fig. 8. Inhibition of Histaminase by Methylguanidine

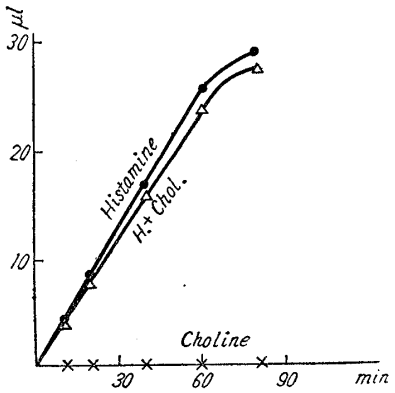

Fig. 9. Inhibition of Histaminase by Choline

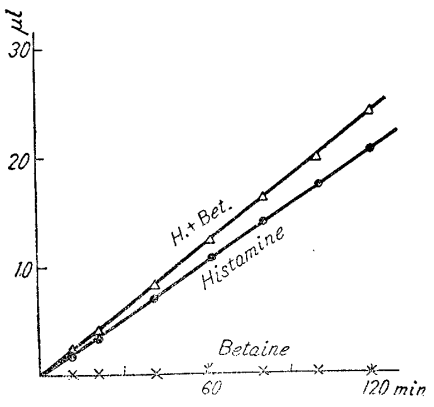

Fig. 10. Activation of Histaminase by Betaine

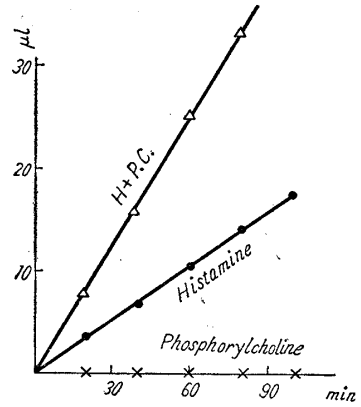

Fig. 11. Activation of Histaminase by Phosphorylcholine

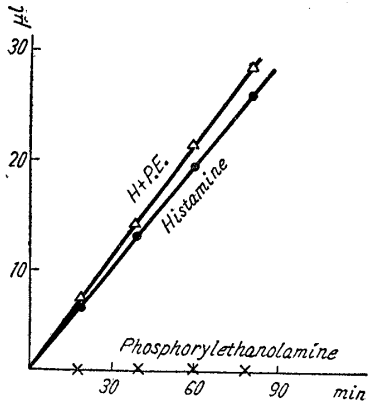

Fig. 12. Activation of Histaminase by Phosphorylethanolamine

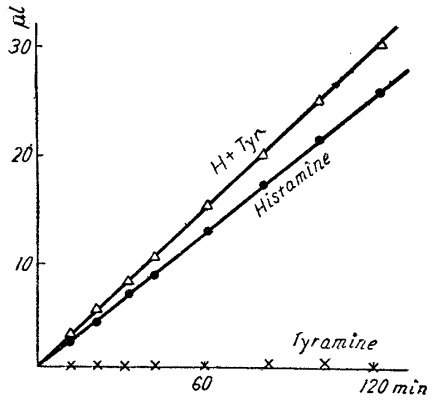

Fig. 13. Activation of Histaminase by Tyramine

TABLE I. Inhibition and Acceleration of Kidney Histaminase by Biogenic Amines

Putrescine	−100%	(cross inhibit.)
Cadaverine	−100	(cross inhibit.)
Agmatine	− 65	(cross inhibit.)
γ-Aminobutyric acid	− 40	(cross inhibit.)
Arcaine	− 74	
Methylguanidine	− 42	
Trimethylamine	− 17	
Trimethylamine Oxide	− 13	
Choline	− 3	
Phosphorylethanolamine	+ 8	
Betaine	+ 9	
Tyramine	+ 18	
Phosphorylcholine	+144	

presence of histamine must be due to the mutual inhibition of each amine with histamine. This phenomenon has been designated by the writer as cross inhibition.

The value derived by subtracting the oxygen uptake by endogenous respiration from that by histamine or amine alone is taken as the calculated total, and this value was taken as the standard in calculating the degree of inhibition by comparing the initial velocity. Other amines did not show any oxygen uptake by themselves that the comparison was made of the oxygen uptake at the time of histamine alone and that in the presence of the amine.

The degree of inhibition or acceleration of kidney histaminase by various amines is shown in Table I. Miyaki and others[3] have shown that several amines inhibit the action of bacterial amine oxidase. The effect of the amines on bacterial histaminase is different from tissue histaminase and decrease in the order of tyramine, cadaverine (cross inhibition), agmatine (cross inhibition), trimethylamine, arcaine, and putrescine (cross inhibition). There is no acceleration by phosphorylcholine.

As for the effect of biogenic amines on tissue histaminase, Zeller[4] showed that putrescine and cadaverine show strong inhibition. The present experimental results have shown that putrescine, cadaverine, agmatine, and γ-aminobutyric acid effect marked cross inhibition against tissue histaminase, that arcaine and methylguanidine effect strong inhibition against histaminase but is not affected by histamine, and that trimethylamine and trimethylamine oxide effect a weak inhibitory action. It was also shown that

3) K. Miyaki, M. Hayashi, S. Ando, H. Yasuda (Unpublished).
4) Zeller : Advances in Enzymol., **2**, 93(1942).

choline gives hardly any effect while betaine and phosphorylethanolamine show a weak accelerating effect and tyramine and phosphorylcholine, a markedly strong accelerating effect.

The fact that putrescine, cadaverine, agmatine, methylguanidine, and γ-aminobutyric acid strongly inhibit the action of tissue histaminase may indicate the accumulation of histaminase in a living body in the presence of these amines and the variation of physiological action accompanying it.

Some time ago, the writer assumed the synergetic effect of histamine and agmatine, arcaine, or methylguanidine as the cause for mass food poisoning from seasoned dried fish ("Samma Mirin-boshi") that occured in various parts of Japan during 1952 to 1954.[2,5] The histamine content in such dried fish was found to be about 450 mg./100 g. The present series of experiments have not only added some evidences for such assumption but also offered a basis for further studies on the cause of poisoning by putrefying foods in general. Further, these phenomena are thought to have a direct bearing on hepato-cerebral syndrome that are recently being taken up and on the cause of autotoxicosis that the problems are being studied in cooperation with workers in this field.

The writer takes this opportunity to express his deep gratitude to Prof. Miyaki of this Institute for his unfailing guidance throughout the course of this work, to Prof. Akiya of the University of Tokyo for his valuable advices, and to Mr. Yasuda for his assistance in these experiments.

Experimental

Warburg Manometric Method

Main chamber : Enzyme suspension, 1.0 cc., 0.05 *M* Phosphate buffer (pH 7.2), 0.8 cc. (0.6 cc. in case other amine was mixed).

Center well : 10% KOH, 0.2 cc.

Side arm : 0.01 *M* Amine-phosphate buffer solution 0.2 cc. (Same 0.2 cc. in case of mixing other amines).

The oxygen uptake was measured by horizontal shaking in a thermostat of 37°.

Preparation of the Enzyme Solution—Fresh hog kidney was ground in a homogenizer, under ice-cooling, degreased and dehydrated with acetone, and dried *in vacuo*. This dried powder was extracted by 0.05 *M* phosphate buffer (pH 7.2) in the amount of 100 mg. of the powder per cc. of the buffer, by stirring for 1 hour at a room temperature and then the extract was centrifuged. Under ice-cooling, the supernatant was dialysed by 0.02 *M* phosphate buffer (pH 7.2) for 3 hours. During this time, external liquid was changed several times. After dialysis, the mixture was heated at 60° for 10 minutes to inactivate the monoamine oxidase system and other respiration enzyme systems. Such purification procedures will not give a single enzyme system of histaminase and the enzyme shows a fair amount of endogenous respiration but was found to be utilizable for the present series of experiments.

Summary

Effect of biogenic amines on the hog kidney histaminase was examined by the Warburg manometric technique and it was clarified that putrescine, cadaverine, agmatine, and γ-aminobutyric acid effected strong cross inhibition, arcaine and methylguanidine a strong inhibition, and trimethylamine and trimethylamine oxide effected a weak inhibition, while choline showed practically no effect. Phosphorylethanolamine and betaine, on the other hand, indicated a slight acceleration and tyramine and phosphorylcholine effected a strong acceleration.

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5) K. Miyaki, M. Hayashi : J. Pharm. Soc. Japan, **74**, 1145(1954).