38. *Allithiamine, a Newly Found Compound of Vitamin B₁.*

By Motonori FUJIWARA and Hiroshi WATANABE.

Department of Hygiene, Faculty of Medicine, Kyoto University.

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Having found the fact that, when thiamine is added to the extract of garlic (*Allium sativum*) in alkaline medium, it turns into a compound which exerts the action of thiamine on animals in spite of its missing the qualitative reaction of thiamine, the authors studied the biochemical properties and physiological action of this compound. They came to the conclusion that it is a new compound hitherto unknown, and assigned the name "allithiamine" to it.

Preparation of Allithiamine.

The extract of garlic is mixed with thiamine solution, the pH being adjusted to 8 with sodium hydroxide. Then this mixture allowed to stand for 30 minutes in the water bath at 60°C.

In case the extract is made from the cloves of garlic previously heated in boiling water, allithiamine cannot be obtained. But, addition of protein extracted from other cloves of garlic makes this extract capable of producing allithiamine. Therefore allithiamine is considered not to be formed directly by constituents of garlic, but by a substance secondarily formed out of some substrate during grinding by enzymic action.

Biochemical Properties of Allithiamine.

1. It is negative to the qualitative reactions of thiamine, thiochrome and diazotized *p*-aminoacetophenon reactions.
2. When reduced by cysteine or sodium hydrosulfite, the above qualitative reactions are restored.
3. It is adsorbed by fuller's earth in acid medium like thiamine, but not adsorbed by permutit.
4. It is not decomposed by thiaminase or by thermostable thiamine inactivating substances which are contained in various plants such as leaves of sweet potato, burdock, etc.
5. It is more stable than thiamine in alkaline medium.

Actions of Allithiamine on Living Organisms.

1. In the test on growth of rats or rice-birds (*Uroloncha domestica*), the effect of allithiamine is not inferior to thiamine.

2. When administered through mouth, allithiamine is excreted in urine in the form of free thiamine. Thereby no displeasing feeling occurs.

3. To observe the manner of the absorption and excretion of allithiamine in human being, the urinary excretion of thiamine was measured after oral administrations of thiamine and of allithiamine. It was found that allithiamine was absorbed from the intestinal canal much more rapidly and to a greater amount in comparison with thiamine.

Fig. 1 shows the hourly amounts of thiamine found in urin in the first, second and third hour after an oral administration of 10 mg. of thiamine and of allithiamine respectively.

Fig. 1. Urinary Excretion of Thiamine

Fig. 2. Urinary Excretion of Thiamine

Fig. 2 shows the amount of the urinary excretion of thiamine for 24 hours after oral administrations of 1, 3, 5, 10, 20 and 40 mg. of thiamine and of allithiamine respectively.

It has generally been believed and is also confirmed by our experiments (fig. 2) that the absorption of thiamine through the alimentary canal is limited to an extent so that no further increase in its urinary discharge can be found even when the amount of intake is augmented over 5 to 10 mg. On this point the distinction of allithiamine from thiamine is striking. As can be seen in figure 2, the urinary discharge of the former increased linearly with the increase in the amount of intake, suggesting that its absorption grew almost endlessly in proportion to the amount of intake.

Extraction of Allithiamine.

Two hundred grams of garlic is thoroughly ground, extracted

with 400 ml. of absolute alcohol, filtered with suction. The residue is further extracted with 400 ml. of absolute alcohol, the filtrate thus gained is mixed with the previous filtrate. The combined filtrate is condensed at a low temperature under reduced pressure. 400 ml. of absolute alcohol is added to this condensed extract, then centrifuged to make the liquid clear. 1.5 g. of thiamine is added to this clear liquid, the pH is adjusted to 8 with sodium hydroxide, then this mixture is put in the water bath at 60°C. for 60 minutes to facilitate the process of reactions with occasionally adjusting of pH to 8. After the liquid is condensed at a low temperature under reduced pressure, 500 ml. of ether or ethyl acetate is added. By shaking this mixture vigorously allithiamine shifts to the ether or ethyl acetate phase. The ether or ethyl acetate solution is condensed at a low temperature under reduced pressure until the substance becomes solid. Benzol is added to this, and stirred thoroughly until the feebly yellow rough crystal is formed.

The fact that the extract of garlic changes thiamine into a compound easily absorbable from the intestinal canal, appears interesting in its relation to the immemorial and world-wide custom of taking garlic as a tonic food. The action of the plant on thiamine seems to be not characteristic of the garlic only. We have also found a similar action in *Allium odorum*, *Allium Bakeri*, *Allium nipponicum*, and further, though weakly, in plants of allium species such as *Allium Cepa* and *Allium fistulosum*. The resultant compounds of these plants may, however, not necessarily be quite identical¹ with allithiamine, although, assumably, they have a resemblance to each other. On these problems, the study is now in progress in cooperation with Dr. T. Matsukawa, a staff investigator of the Research Laboratory attached to the Takeda Pharmaceutical Ind. Ltd.

Recently Dr. D. Matsukawa and his coworker¹⁾ in the Niigata Medical School have presented reports that they have found the fact that garlic has a capacity to prevent the thiamine deficiency in human being, and came to the conclusion that garlic increases the bacterial synthesis of thiamine in the intestinal canal. Their findings seem to have a close connection with ours, and further investigations are required.

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Reference.

- 1) D. Matsukawa and Y. Sato: Proceedings of the Committee on Research of Vitamin B, **42**, 11, 1950; **43**, 14, 1950; **48**, 3, 1951 and **49**, 18, 1951.