

## READERS' FORUM

### ON THE EXISTENCE OF THIAMINE DEHYDRASE

Apropos of the Article by S. E. Shnol

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In the article "On the existence of thiamine dehydrase" S. E. Shnol [7] denies the existence of this enzyme, while, he says, using the method of A. A. Titsev to determine thiamine dehydrase. However he allowed a number of errors and distortions in his work.

According to the method we suggested, each sample should contain 0.25 millimoles of adrenaline and thiamine. S. E. Shnol added 10 times as much adrenaline and approximately 5 times as much thiamine, destroying the relationship between and concentration of these substances. Then—and this is the basic defect—instead of the 0.05 ml of plasma (extract) we recommended, he added 0.2 ml. The thiamine dehydrase effect depends not only on the content of the enzyme, but also on the concentration of inhibitors of the reaction, which diminish the oxidation of the adrenaline and thiamine, and the activity of the plasma (extract, etc.) is equivalent to these two opposing factors. As the plasma concentration increases in the sample the content of inhibitors increases and the inhibitory effect, which S. E. Shnol shows in his graphs, can also be increased. The increase in the effect of thiamine dehydrase as the enzyme source (plasma) increases is observed at low contents of inhibitors in the latter, for example, in cases of hypertension.

In order to ascertain the existence of thiamine dehydrase it is essential to use thoroughly purified enzyme [4, 5, 6] and pure oxidized adrenaline (adrenochrome according to Green) then the speed of the enzyme reaction increases in proportion to the increase in the concentration of the enzyme. The oxidized adrenaline serves as a hydrogen carrier in the thiamine dehydrase reaction and it can be replaced successfully by, for example, methylene blue, glutathione SH. By separating the enzyme, its presence can be shown even in such organs as the liver and brain, where there is much antisympathin. Experiments involving the activation and inhibition of thiamine dehydrase [2], in which have been shown the functional significance of this enzyme in the system and the changes in its activity in plasma and in organs when there are changes in the functional state of the nervous system, serve as proof of the existence of thiamine dehydrase. Another proof is the successful application of thiamine dehydrase inhibitor—antisympathin in the therapy of hypertension. Finally, in an experiment with isolated heart and with the entire organism, the formation of thiochrome has been shown during sympathetic stimulation [1].

In addition to incorrectness of method, apparently the conditions of S. E. Shnol's work were not entirely irreproachable. Thus, in the control experiments he obtained approximately 100 times as much thiochrome as should have been present (0.1-0.2 micrograms approximately). The spontaneous progress of the thiamine dehydrase reaction may be connected with traces of catalysts in water and salts (copper), so it is essential to use double-distilled water and good brands of salts in order to slow the spontaneous reaction as much as possible. The acidification of the samples, pointed out by S. E. Shnol, could be of microbiological origin. With correct work, only an insignificant increase in pH can occur. If the experiment is carried out in a phosphate buffer with a pH=6.86, then the pH is 6.85-6.87 in the control samples after incubation (24 hours at 37°), 6.89-6.90 in samples with boiled plasma, 6.88 with unboiled plasma.

Further S. E. Shnol writes: "Also described was the inhibitor of thiamine dehydrase, called antisympathin or hypotensin" and then he explains the mechanism of action of "hypotensin" [page 49]. The name "hypotensin," as well as the explanation invented by S. E. Shnol, have nothing in common with our interpretation of the physiological mechanism and therapeutic action of antisympathin [2, 3].

To check new data is necessary and important, but exact reproduction of the experimental conditions is essential for this purpose, without which the work performed can prove to be labor performed in vain.

#### LITERATURE CITED

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