THE EXISTENCE OF A THIAMINE DEHYDROGENASE

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Several years ago, A. A. Titaev announced the discovery of a new enzyme – thiamine dehydrogenase [1]. That enzyme was credited with the specific property of catalyzing the anabolism of hydroxyadrenalin through the oxidation of thiamine into thiochrome. A thiamine dehydrogenase inhibitor, identified as antisympathin or hypotensin, was likewise described. The latter name had been given on the assumption that the thiamine dehydrogenase inhibition might bring about a decrease of the quantity of anabolic adrenalin, hence a lowering of the vascular tonus.

That a vitamin should be used merely as a substrate – a source of hydrogen – so as to anabolize adrenalin, created by the organism itself, did not seem very plausible to us. That is why we sought to find an intermediate product for the oxidation of thiamine into thiochrome that would fulfill the function of a coenzyme in the thiamine dehydrogenation reaction.

But, first of all, it was necessary to reproduce Titaev's essential phenomenon – the acceleration of thiamine oxidation along with the formation of thiochrome by adding animal tissue extracts or blood plasma to the thiamine and oxidized adrenalin solution.

EXPERIMENTAL METHOD

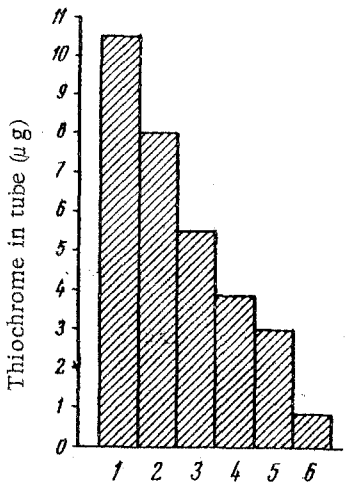
The experiments were conducted according to the description of A. A. Titaev [1]. To 2 ml of 0.1 M phosphate buffer at pH 7.6 and 6.8, or to 2 ml of physiological saline, were added the following: 0.5 ml of a 0.1% adrenalin or oxidized adrenalin solution; 0.1 ml of a thiamine solution containing 1 mg of the vitamin, 0.2 ml of tissue extracts, homogenates or plasma (or, in the case of controls, 0.2 ml of water) plus 1 drop of chloroform or chloramine-T (toluene) antiseptic. The test-tubes were placed in an incubator at 37° for different lengths of time, usually 24 hours. After incubation, 5 ml of isobutyl alcohol was added to each tube, and the test tubes were carefully shaken and centrifuged. The fluorescence intensity of the thiochrome derived from the thiamine was measured by means of a vertical-scale photometer. A PRK-4 quartz bulb with UV filter served as the source of ultra-violet radiation. Into the first photometer cell was poured a standard thiochrome solution, inasmuch as photometry by comparison with pure isobutyl alcohol is made inaccurate by color-band fluctuations. Into the other cell was placed a definite amount of the test-tube contents. Prior to each test, the curve expressing the relation of fluorescence intensity to the thiochrome concentration was newly determined. Whenever necessary, the test-tube contents were diluted with pure isobutyl alcohol, up to the level where a linear correlation was observed between the fluorescence intensity and the thiochrome concentration (usually not more than 6 μg thiochrome per 6 ml cell volume). Each time, at least two parallel tests were conducted.

EXPERIMENTAL RESULTS

As a result of repeatedly verified experiments we have established that thiamine in the absence of adrenalin undergoes practically no transformation into thiochrome. In the control tubes containing both thiamine and adrenalin always more thiochrome was formed than in the experimental ones to which tissue extracts or blood plasma had been added; but the quantity of thiochrome formed during incubation in the tubes containing pituitary and thyroid gland extracts or blood plasma was closer to the amount of thiochrome in the control tubes than in the tubes containing liver, kidney and other tissue extracts.

Therefore, neither the homogenates, the extracts – even those from the hypophysis and thyroid gland –

nor the blood plasma increased the quantity of oxidized thiamine, rather they gradually diminished it. * A typical result of such an experiment is graphically shown in the diagram.



Extent of oxidation of thiamine into thiochrome in relation to the nature of the experimental mixture (all solutions prepared in phosphate buffer at pH 6.8).
1) Control; 2) plasma and 0.5 cystein added; 3) hypophysis extract added; 4) plasma added; 5) liver extract added; 6) plasma and liver extract added. For other components, see text.

As could be expected, the pH exerted a measurable influence on this oxidizing-anabolic reaction. In two control tubes containing phosphate buffer at pH values of 7.6 and 6.8, sharp differences were noted in the thiochrome yield after incubation: almost 3 times as much at pH 7.6 as at pH 6.8. Therefore, our first thought, in order to explain the anti-thiamine dehydrogenase effect of extracts from any tissues whatsoever was that the tissues had changed the pH of the media during incubation. As a matter of fact, the pH measurement of tubes after incubation did show evidence of souring – the most acidification being observed in the test-tubes containing liver-extracts. However, we must point out that a constant ratio between the changes of pH and the quantities of oxidized thiamine was not observed. Probably this can be explained in terms of different oxidizing-anabolic potential in the various extracts and homogenates.

To verify the conclusions of our first series of experiments, we compared the capacities of extracts and homogenates, before and after boiling, to retard the oxidation of thiamine. The results of these tests, should we adopt A. A. Titaev's viewpoint, were proof that heating substantially stimulates the thiamine dehydrogenase activity of tissues. In the tests with boiled tissue extracts, the quantity of thiochrome produced reached the level encountered in the controls.

Chromatographic analysis [2] of the contents of experimental test-tubes after incubation demonstrated that during the course of thiamine oxidation, as a result of reversible reaction with the adrenalin, there were no intermediate oxidation products formed either in the experimental or in the control tubes. On the chromatograms, only the thiamine and the thiochrome were detectable. On the basis of the work undertaken, we have come to the conclusion that the claims to the existence of a thiamine dehydrogenase are unfounded. We might at most speak of an anti-thiamine dehydrogenase activity. However, it would seem that even the deceleration of thiochrome manufacture by animal tissues was non-specific: in plasma, in hypophysis and in thyroid gland tissue extracts such slowing-down was insignificant, although in other tissue extracts – especially from the liver – it was quite clear. In all our experiments, we were interested in the fate of the thiamine and not that of the adrenalin.

LITERATURE CITED

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[2] S. E. Shnol, Bull. Expt. Biol. and Med. Vol. XXXIX, No. 5 (1955).

* These results were verified on the tissues of rats (in the winter, when, according to A. A. Titaev, the thiamine dehydrogenase activity is maximal), rabbits, and dogs.