DOES THIAMIN DEHYDROGENASE EXIST?

S. É. Shnol'

From the Central Postgraduate Medical Institute (Director — Dr. Med. Sci. V. P. Lebedeva), Moscow

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Previously published experimental results [5] have compelled us to regard as erroneous the statements of A. A. Titaev [1-3] on the existence of thiamin dehydrogenase, an enzyme acting as a specific catalyst of the transfer of hydrogen from thiamin to oxidized forms of adrenalin. According to A. A. Titaev, a measure of the thiamin dehydrogenase reaction is the amount of thiochrome formed in a medium containing thiamin, oxidized adrenalin, a buffer system and, as enzyme, an extract of the hypophysis, thyroid gland or blood plasma. According to A. A. Titaev also, liver and kidney contain a factor which specifically depresses the thiamin dehydrogenase reaction.

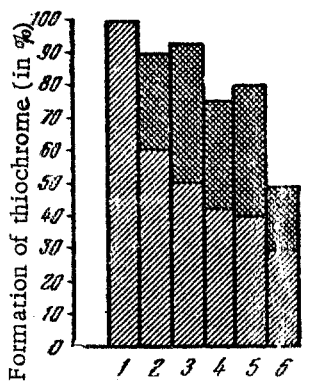
In none of the experiments previously carried out [5] have we been able to find a thiamin dehydrogenase action in any of the preparations tested. The quantity of thiochrome formed in the control tubes always exceeded that in the experimental tubes. We can agree with A. A. Titaev's conclusions only if we pay no attention to the quantity of thiochrome formed in the control tubes. In that case it may be said that perceptibly more thiochrome is formed in the tubes with extract of hypophysis or blood plasma than in the tubes with extracts of tissue homogenates of either liver or kidney.

It is, however, clear that since more thiochrome is formed in the controls than in any variant of the experiment, the relatively high thiochrome formation in the tubes with plasma and hypophysis cannot be regarded as the result of activation of thiochrome formation or as the manifest action of thiamin dehydrogenase. All the tissue preparations depress the "thiamine dehydrogenase" reaction but to a varying degree, and plasma and hypophysis less than liver. It may be thought that the action of depressing the oxidation of thiamin depends to some extent on the intensity of the thiamin metabolism in the tissues, the oxidation-reduction potential of the tissue, and so on.

Other facts which oppose the idea of enzymic catalysis of the formation of thiochrome from thiamin were the results of our experiments in which the "enzyme" preparations were preliminarily heated to 100°C. It was found [5] that this heating does not inhibit thiochrome formation in the tubes but, on the contrary, it increases it almost to the level of its formation in the control tubes and over the level in the unheated tubes.

Having obtained such unexpected results, we returned to A. A. Titaev and, following his instructions, varied the experimental conditions, in particular by altering the concentrations of the reagents. We were still unable to obtain a positive result — evidence of the existence of thiamin dehydrogenase.

After the publication of these facts [5], A. A. Titaev took up his pen [4] in sharp criticism of our work, asserting that our results were negative because we did not observe the necessary experimental conditions, that the distilled water was not pure enough (?) and so on. In fact A. A. Titaev did not reply to any of the questions asked in our paper and gave no explanation at all of the increase in thiochrome formation after heating the enzyme extracts. Being in no doubt of the correctness of our first results so far as conforming to the experimental conditions described by A. A. Titaev was concerned, (especially because we followed his personal instructions), we deemed it necessary to subject this question of the existence of thiamin dehydrogenase to fresh experimental proof.



Formation of thiochrome in several variants of experiments during incubation for 20 hours at 37°C. The extra thiochrome formed in the tubes preliminarily heated to 100°C for 15 minutes is shown cross-hatched. The ordinate axis indicates the amount of thiochrome as a percentage of the control.
1 – control; 2 – hypophysis; 3 – blood plasma; 4 – thyroid gland; 5 – kidney; 6 – liver.

In new experiments we attempted to take into consideration all the existing remarks of A. A. Titaev, and we used a fundamentally different method: instead of ordinary thiamine and determination of thiochrome by a fluorescent method, we used thiamin labeled with radioactive sulfur S^{35} and determined the amount of thiochrome by the radioactivity of butanol extracts or of the appropriate areas of paper chromatograms [6].

EXPERIMENTAL METHOD

In the experiments we used male white rats weighing about 200 g. The animals were killed by exsanguination after exposure of the jugular vein under ether anesthesia. We used water, doubly distilled in glass apparatus. Into each of a row of tubes was poured 0.6 ml of 1/15 M phosphate buffer (pH = 7.2), 0.2 ml of a solution of S^{35}-thiamin (360 μg and 5 μc in 1 ml), 0.2 ml of adrenalin solution (230 μg/ml), and then either 0.05 ml of a 0.9% solium chloride solution (control) or 0.05 ml of freshly prepared homogenate of an organ or of blood plasma (in preparing the homogenates to one part by weight of tissue was added 2 parts of 0.9% sodium chloride solution). Next, to the tubes was added one drop of chloroform as an antiseptic; the tubes were sealed with corks and incubated at 37°C for 20-24 hours. In another variant of the experiment thiamin and adrenalin were added to the tissue homogenate or plasma in phosphate buffer after preliminary heating on a boiling water bath and subsequent cooling.

After incubation, 0.05 ml of the contents of each tube was transferred to paper and separated chromatographically into thiamin and its conversion products by means of a mixture of butanol, methanol, formic acid and water in proportions of 110 : 80 : 15 : 10 [6]. Parallel with the chromatographic analysis the amount of thiochrome in the tubes was determined by the radioactivity of a butanol extract. The extraction was carried out by shaking up 0.5 ml of the incubated mixture with 1 ml of butanol. The tubes were centrifuged and a measured volume of the butanol extract dried on an aluminum foil dish. The radioactivity was determined by means of an end-type radiation counter with a mica window of thickness 1.9 mg/cm^2.

EXPERIMENTAL RESULTS

Only two substances – thiamin and thiochrome – could be detected on the chromatograms. Quantitative analysis of the chromatograms showed that the greatest amount of thiochrome is formed in the control tubes and that thiochrome formation in the experimental tubes is increased after preliminary heating. Similar results were given also by direct determination of the thiochrome by the radioactivity of the butanol extract.

A typical experimental result is shown in the Fig., from which it is seen in the first place that the most thiochrome is formed in the control tubes – in which about 20% of the thiamin is converted into thiochrome. The thiochrome content of the control tubes is taken as 100%.

In all the tubes containing organ homogenates or blood plasma, the thiochrome formation was significantly less than in the control tubes. Thus in the tubes with hypophysis the amount of thiochrome was 60%, in tubes with plasma – 50%, with thyroid gland – 43%, with kidney homogenate – 39% and with liver homogenate – 30%.

And so activation of oxidation of thiamin – the presence of a "thiamin dehydrogenase" system – does not appear in any of the variants of the experiment. As seen from the Fig. some activation is observed after preliminary heating of the homogenates.

The experiments confirm the results previously obtained, and we may state once again that after following A. A. Titaev's experimental procedure we cannot accept as correct his views on the existence of an enzyme thiamin dehydrogenase. All the organs and tissues tested show antithiamin dehydrogenase activity, i.e. prevent oxidation of thiamin into thiochrome. This antithiamin dehydrogenase activity is reduced if the tissue preparations are heated to 100°C.

SUMMARY

The results of experiments on the study of the reaction between thiamine and certain forms of adrenalin leading to formation of thiochromine are presented. Contrary to the views of A. A. Titaev this reaction is not catalysed by a specific enzyme "thiamindehydrase". The quantity of thiochrome formed in control tests is always greater than that in the test with the extracts or homogenates of various tissues. The tissue extracts and homogenates do not activate, but on the contrary depress the reaction. This depression is found to be considerably decreased after preliminary thermal denaturation of the preparations.

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