FURTHER OBSERVATIONS ON THIAMIN DEHYDROGENASE

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The existence of thiamin dehydrogenase, previously described by A. A. Titaev [1-3], has recently been put in doubt by the work of S. É. Shnol' [5,6], who investigated extracts of various organs and tissues and also the blood plasma and came to the conclusion that an antithiamin dehydrogenase activity was present and that the thiamin dehydrogenase reaction was not enzymatic in nature. The main argument against A. A. Titaev's findings is the observation by S. É. Shnol' that increased thiochrome formation is present in the plain control tube and the control tube preliminarily inactivated by boiling, by comparison with the experimental tube. In his reply to S. É. Shnol's findings as described in the latter's first paper [5], A. A. Titaev [4] pointed out the incontestability of the presence of thiamin dehydrogenase in the blood of patients with hypertension. S. É. Shnol's second investigation [6], using S^{35}-thiamin and keeping to the technique used by A. A. Titaev, failed to reveal the enzymatic nature of the thiamin dehydrogenase reaction, although in this paper the author did not reply to A. A. Titaev's claim that thiamin dehydrogenase activity could clearly be detected in the serum of patients with hypertension. The question of the enzymatic nature of this process under consideration thus remains unresolved.

In order to shed light on this problem, we investigated the thiamin dehydrogenase activity of the cerebrospinal fluid and the serum in various diseases.

EXPERIMENTAL METHOD AND RESULTS

The thiamine dehydrogenase activity of the cerebrospinal fluid was investigated in 40 children suffering from tuberculous meningitis. Assuming the enzymatic nature of this reaction, and in view of the relatively low protein content of the cerebrospinal fluid, in each experiment we took 1 ml of fluid, and we adhered to all the molar proportions of the ingredients of the incubation mixture recommended by A. A. Titaev.

In all cases, after incubation the thiochrome content was higher in the control than in the experimental sample. Boiling one of the experimental tubes slightly increased the formation of thiochrome therein, but the quantity of this compound was equal to that found in the control tube without CSF in only two of 20 cases.

The results obtained relating to the "antithiamin dehydrogenase" action of the CSF are given in Table 1.

It may be seen that the "antithiamin dehydrogenase" activity of the CSF varied between relatively wide limits.

During the investigation of the thiamin dehydrogenase activity of the serum obtained from healthy subjects and patients with different diseases, the experiment was always performed in three tubes with different volumes of serum (0.05-0.1-0.2 ml) and two control tubes were used, one without serum and the other containing 0.1 ml of serum, inactivated on a water bath. In the majority of samples tested (in 14 of 15 healthy subjects; in five patients with hepatitis; in eight with gastritis; in 8 of 9 with peptic ulcer; in 15 with malignant neoplasms, and in 18 of 20 with other diseases), the enzymatic nature of the thiamin dehydrogenase reaction could not be demonstrated, for the quantity of thiochrome found in the controls was greater than or equal to that in the experimental tubes.

Two groups of patients, however, gave reproducible and quite definite results suggesting activation of the thiamin dehydrogenase reaction by the blood serum. This refers to patients with hypertension and, more especially,

TABLE 1. Thiochrome Content after Incubation of Tubes for 20 Hours (as % of that Found in Controls)

Tube no.	TC*	Tube no.	TC*	Tube no.	TC*
1	72	15	69	29	70
2	80	16	92	30	48
3	58	17	81	31	69
4	60	18	84	32	92
5	66	19	74	33	84
6	90	20	87	34	71
7	74	21	71	35	80
8	75	22	64	36	80
9	80	23	80	37	59
10	56	24	92	38	62
11	52	25	91	39	90
12	74	26	57	40	85
13	78	27	99		
14	39	28	65		

*TC = Thiochrome content.

TABLE 2. Quantity of Thiochrome Formed in a Period of 20 Hours (in γ)

Patient	Diagnosis	Test I (0.05 ml)	Test II (0.1 ml)	Test III (0.2 ml)	Control I (without serum)	Control II (boiled serum)
B-r	Uremia	24	25	36	24	25
M-a	"	39	38	48	30	36
K-ch	Tumor of the kidney	66	72	64	54	59
I-j	Uremia	66	78	84	70	80
S-k	"	45	49	58	40	49
A-a	"	72	79	89	66	70
K-a	Hypertension (stage III)	42	43	74	51	50
M-v	" "	38	45	62	34	32
Ts-v	" "	55	72	60	57	49
A-o	" "	60	67	83	52	54
P-v	" "	41	39	59	38	32
S-i	" "	29	37	44	35	33
Ya-ya	Hypertension (stage II)	62	66	52	50	52
L-m	Hypertension (stage I)	34	36	33	45	38
B-o	" "	47	58	46	45	44

to patients with uremia developing as the result of chronic nephritis. Another noteworthy fact was that patients with far-advanced forms of hypertension gave an increased thiamin dehydrogenase activity. In these experiments the thiochrome formation increased obviously with an increase in the volume of serum used in the test, and preliminary boiling of the serum partially or completely suppressed activation of thiamin dehydrogenation. It must be mentioned that isolated sera, taken from patients with stage I or II hypertension, often gave the opposite—i. e., "antithiamin dehydrogenase"—effect. These findings are given in Table 2.

In a proportion of the tests depression of the thiamin dehydrogenase reaction was observed, beginning with the tubes containing 0.2 ml of serum. It is probable that the relationship between the activators and the inhibitors of the thiamin dehydrogenase reaction is highly complex, so that their interaction varies even after the simple further dilution of the experimental sample with phosphate buffer. Thus, when five experiments were carried out with equal volumes of all the components, but with different final volumes of incubation fluid (3 and 5 ml), a difference was observed in the results. Two tests out of five, which did not show thiamin dehydrogenase activity in the more concentrated medium (3 ml), did show such activity when diluted to 5 ml.

The results obtained suggest a possible connection between the thiamin dehydrogenase activity and an enzymatic mechanism provided that the thermal denaturation of proteins at pH=7.4 can be considered to be a sufficiently reliable criterion. The relationship between the activators and inhibitors of thiamin dehydrogenation in the serum is not yet clear and requires special investigation. Attention is directed to the slight increase in thiochrome formation in the samples of serum taken in uremia and inactivated by boiling. This points to the presence of certain nonprotein activators of the thiamin dehydrogenase reaction in the serum.

SUMMARY

Examination of the cerebrospinal fluid and blood serum in persons with different diseases in most cases failed to reveal any thiamin dehydrogenase activity. It was only in uremia and, for a time, in hypertensive vascular disease that the blood serum possessed a pronounced ability to activate the dehydrogenation of thiamin in the presence of adrenalin.

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