NEW POSSIBILITIES IN THE STUDY OF THIAMINE METABOLISM

SIMPLE DETERMINATION OF THIAMINE IN BLOOD

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(Received December 16, 1955. Presented by Acting Member of the Academy of Medical Sciences of the USSR S. E. Severin)

The only widely-used methods of studying the thiamine metabolism in the system up to the present time are determinations of its concentration in the urine and blood under normal conditions or under various loads of thiamine. The method of determining thiamine in the urine is already sufficiently well developed and satisfactory in its sensitivity, but this cannot be said of the chemical methods of determining thiamine in blood and body fluid. In view of the low concentration of thiamine in the blood, quantities of blood which can only be obtained by venipuncture are required for its determination. In the case of cerebrospinal fluid, the amount of it obtained for investigation is sometimes clearly inadequate [3, 6, 7]. The determination of thiamine in blood serum is even worse. Even with the development of a micromethod for the determination of thiamine in blood and its constituents, authors (Barch and others) who used special models of microfluorophotometers reached the conclusion that existing fluorophotometric methods of determining thiamine are of little use in studying the blood serum [9]. We used the principle of luminescent analysis, first suggested by S. K. Rozental for the determination of adrenaline in the blood [4, 5] and which consists of obtaining a fluorescent ring at the point of contact between the solution under study and the corresponding reagent, and worked out on this basis a method for determining thiamine in aqueous solutions, perfusing fluids and blood. Our method permits the determination of the presence of thiamine at concentrations of 0.001 gammas per 1 ml [2]. The substance of the method consists in carefully inserting an alkaline solution of potassium ferricyanide with a Pasteur pipette [1 ml of 2% $K_3Fe(CN)_6$ per 9 ml of 30% NaOH] under the protein-free filtrate under investigation in a narrow centrifuge or Uhlenhuth test tube and the test tube is immediately examined in a narrow converging horizontal bundle of ultraviolet rays [4] for the presence of a fluorescent ring at the point of contact between the solution under investigation and the alkaline solution of potassium ferricyanide. The ring is still apparent when there is 0.001 μg of thiamine present per 1 ml of solution. Cocarboxylase,* which gives a fluorescing thiochrome pyrophosphate, is determined with like sensitivity by this method if the pure thiamine found in the cocarboxylase is taken into consideration. Thus, without preliminary enzyme action [1], the method makes it possible to determine the total thiamine in blood immediately. We tried to use our method to obtain an idea of the fluctuations of the thiamine content of blood after the administration of thiamine into the system by different means. "Thiamine curves" were obtained which can be used widely for the study of the dynamics of thiamine metabolism, by analogy with the sugar curves.

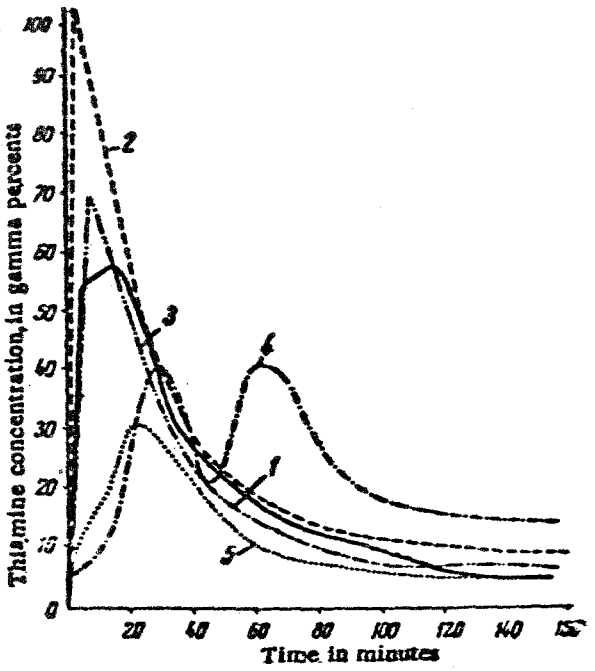
EXPERIMENTAL METHODS

To determine the thiamine content, 0.3 ml of 0.01 N HCl solution is poured into the centrifuge test tubes, then 0.2 ml of blood taken from the fingertip is poured in the same place by a micropipette. The blood is taken

*The preparation was prepared and submitted for study by the All-Union Vitamin Institute for Scientific Research.

in the morning the first time, before the system is loaded with thiamine, then in a number of other test tubes various lengths of time after loading. Then 0.5 ml of 20% trichloroacetic acid solution is added to each test tube. The contents of the test tubes are mixed well and the test tubes are centrifuged at 2000-3000 RPM for 10 minutes. Slightly over 0.6 ml of a transparent centrifugate is obtained, which is subjected to analysis (dilution 1:5).

The analysis consists of the preparation of a definite series of dilutions, beginning with 1:20 for the test before loading and with 1:100 for most of the tests after loading with thiamine. In view of such great dilutions, and also due to the fact that 0.2-0.3 ml of the diluted solution of the protein-free infiltrate is sufficient for the determination itself, 0.2 ml of blood is completely adequate to obtain all the necessary dilutions.



Thiamine concentration in the blood various lengths of time after thiamine loading.
1) After intramuscular administration of 50 mg of Vitamin B_1 to patients with pulmonary tuberculosis; 2) the same after intravenous administration to one patient with pulmonary tuberculosis; 3) the same to a healthy person; 4) after giving 500 mg of Vitamin B_1 per os to a healthy person; 5) after intramuscular administration of 30 mg of cocarboxylase to a tuberculous patient. (Thiamine concentration in gamma-percents).

The technique of obtaining the required dilutions [8] using automatic pipettes takes little time of itself. It is enough, for example, to indicate that not over 30-40 minutes (preparation of dilutions and 10 thiamine determinations) is required to obtain a "thiamine curve" if the time required for taking the samples and settling the proteins is neglected. The amount of thiamine in the sample under investigation in μg% is determined by multiplying the last dilution at which the fluorescing thiochrome ring is still found by 0.1 (0.001 x 100). First it is necessary to subtract the dilution at which the fluorescent ring is formed when pure alkaline solution is inserted (control) from the dilution which is found. Practically, it is convenient to carry out the determination in the following order: in the first test tubes of all the series of dilutions is placed a layer of pure alkali; if no fluorescing ring is present, which is found in the majority of cases, alkaline potassium ferricyanide solution is added to all the following test tubes until the dilution is found at which the fluorescent ring begins to disappear. The dilution preceding this moment is recorded for the calculation of the thiamine concentration. If a fluorescent ring is formed with pure alkali in the first test tube of a number of dilutions, alkali is added to the subsequent test tubes until this "control" ring disappears, and then the addition of the alkaline potassium ferricyanide solution is begun. In such cases the control dilution is subtracted before calculating from the experiment. There is no need to prepare another series of dilutions for the control, since the fluorescing ring from pure alkali never appears at dilutions greater than those at which the ring appears with alkaline ferricyanide. The thiamine curves of two healthy persons have been studied by us, in one case with loading through the mouth with 500 mg of thiamin hydrochloride and in the other with intravenous administration of 50 mg of Vitamin B_1. Also investigated were the reactions of 3 patients with pulmonary tuberculosis to intravenous loading with 50 mg of Vitamin B_1, who received phthivazide and of another such patient, only after intramuscular administration of 30 mg of cocarboxylase.

The data obtained, in the form of curves indicating the thiamine content of the blood, are shown in the drawing.

Examination of the drawing draws attention to the considerable difference in the initial rise of the curve at the same intravenous thiamine load of a healthy person and of patients with pulmonary tuberculosis. A distinct

difference is apparent in the duration of the hyperthiamine state 120 minutes after the thiamine is given. The curve which is obtained with a load of a 10-fold dose of thiamine by mouth differs sharply from the other curves not only in the thiamine level of the blood but also in its unique shape with two distinct peaks. The presence of such different curves already indicates that they can be used successfully to study the conditions of the absorption of thiamine by tissues under various physiological and pathological conditions of the system.

The data we obtained agree well with the data of a number of authors who carried out similar investigations by more complicated and laborious methods [10, 11].

The results of three cases of intravenous administration of thiamine to patients with pulmonary tuberculosis gave such close values that the curves can almost be superimposed, which testifies to the good reproducibility of the data obtained by this method.

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* In Russian.