Thiamine and its Mono-, Di-, and Triphosphoric Esters Content of Normal Rat Tissues

Up to the present, no methods have been described for the separation and determination of Thiamine[1] and its phosphoric esters in animal tissues (particularly TTP recently detected in liver[2], and in kidneys and brain[3] of rats).

Riassunto

L'uso di un nuovo metodo cromatografico per la separazione della Tiamina e dei suoi esteri mono, di, e trifosforico nei tessuti animali ha permesso di definire, per la prima volta in termini quantitativi, la presenza del trifosfato nei tessuti stessi. L'organo più ricco di questo estere è il fegato, seguito dal cuore, dal rene e dal cervello nell'ordine.

Content of T, TMP, TDP, and TTP in some rat tissues (mean ± s. e.)

Compound	Brain (5)		Liver (6)		Heart (5)		Kidney (7)	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%
Thiamine	0·11 ± 0·08	4·4	0·24 ± 0·01	3·5	0·16 ± 0·02	2·3	0·22 ± 0·02	6·2
Thiamine monophosphate	0·30 ± 0·09	11·5	0·66 ± 0·01	9·4	0·41 ± 0·05	5·9	0·35 ± 0·06	9·8
Thiamine diphosphate	2·61 ± 0·15	78·9	6·81 ± 0·44	77·9	7·44 ± 0·44	86·0	3·47 ± 0·13	78·6
Thiamine triphosphate	0·19 ± 0·03	5·0	0·92 ± 0·13	9·0	0·57 ± 0·04	5·6	0·27 ± 0·01	5·2

( ): Number of determinations; %:T, as percentage of total T found in the tissue.

On the basis of a previous research[4], in which the analytical conditions necessary for the chromatographic separation and the estimation of T, TMP, TDP and TTP in pure solutions were described, we have worked out a quantitative method for their estimation in animal tissues. The principle of this method can be summarized as follows:

The tissue is homogenized in cold 5% TCA. The extract, free from proteins, is adjusted to pH 6·7–6·8 with 40% NaOH, and passed through a charcoal column, prepared according to SILIPRANDI and SILIPRANDI[5]. After washing with H_2O, an elution with 60–70 ml of 10% *n*-propanol is carried out. The eluate is concentrated to about 5 ml in a Rinco rotating evaporator at 25–30°C, under vacuum. The concentrate and washings (15 ml), after addition of 0·8 ml 0·1 *N* HCl, are chromatographied on Dowex 1, × 8, acetate form, column, size 8 × 25 mm, and washed with 10 ml of H_2O. The percolate and washings are collected in a 25 ml volumetric flask and the T and TMP content is estimated by difference before and after Takadiastase digestion (Thiochrome method[6]). The TDP is eluted from the resin column by means of 20 ml of 0·02 *M* sodium acetate solution in 0·04 *M* acetic acid. TTP is eluted last with 20 ml of *M* acetate buffer at pH 4·5. After Takadiastase hydrolysis, the TDP content is determined directly in the eluted solution, and the TTP content after percolation through Amberlite IRC 50, buffered at pH 4·5[7].

With this method the recovery of the extracted T-compounds is about 95% with good reproducibility. A series of determinations carried out on rat tissues have given the results reported in the Table.

As can be seen, all the tissues examined contain small amounts of TMP and TTP, the biochemical significance of which is still to be elucidated. The organ richest in TTP is the liver, followed by the heart, kidney, and brain. However, by far the most abundant T compound (about 80% of the total T) is TDP.

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[1] The following abbreviations have been used: T = Thiamine; TMP = Thiamine monophosphate; TDP = Thiamine diphosphate; TTP = Thiamine triphosphate; TCA = Trichloracetic acid.

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Synthesis at High Pressure and Lattice Constants of Normal Cupric Carbonate

Normal cupric carbonate, $CuCO_3$, has not previously been prepared, although a number of basic carbonates exist, of which malachite, $CuCO_3 \cdot Cu(OH)_2$, and azurite, $Cu(OH)_2 \cdot 2\,CuCO_3$, are the best known.

Attempts were made to prepare $CuCO_3$ by subjecting dry cupric oxide to CO_2-pressures of up to 5000 bars and temperatures ranging from 100°C to 600°C in a hydrostatic bomb which is described elsewhere[1]. No reaction took place.

Subsequently a finely ground equimolar mixture of anhydrous sodium carbonate and cupric sulphate was subjected to a pressure of 20000 bars and a temperature of 550°C in the 'simple squeezer' high-pressure apparatus developed by GRIGGS and KENNEDY[2]. After 1 h under these conditions the sample was quenched at pressure. An X-ray powder diffraction examination of the products showed, in addition to the known patterns of $CuSO_4$, Na_2CO_3 and Na_2SO_4, a small number of weak lines which could be ascribed to a rhombohedral lattice with nearly the same dimensions as siderite[3]. The same but weaker

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