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

[1] H. HEARD, to be published.

[2] D. T. GRIGGS and G. C. KENNEDY, Amer. J. Sci. *254*, 722 (1956).

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lines appeared on examination of the products when precipitated basic cupric carbonate was subjected to 18000 bars and 550°C in the 'simple squeezer'.

Eventually precipitated basic cupric carbonate was introduced into a low-pressure hydrothermal bomb and subjected to 500 bars total pressure (CO_2 partial pressure = 450 bars, H_2O partial pressure = 50 bars) at 180°C for 36 h. The bulk of the products was well-crystallized malachite, but a small yield of the rhombohedral substance was also obtained.

The yield was better than before, and the diffraction lines were reasonably strong. It proved to be quite simple to subtract the known lines of malachite from the pattern. In only one case was it necessary to estimate the intensity of a line from the earlier patterns.

The X-ray powder diffraction pattern at 25°C was obtained by means of a Norelco high angle recording diffractometer, using CuK_α radiation ($\lambda = 1{\cdot}5418$ Å) and a Ni filter. The results are given in the Table.

It was found that the presumed $CuCO_3$ crystallizes in the rhombohedral system. Systematic extinctions appeared to be the same as for calcite, and it is consequently assumed that the space group is also the same, *viz.* $R\,\bar{3}c$.

The hexagonal unit-cell dimensions at 25°C, obtained by least-squares, are:

$$a_0 = 4{\cdot}796 \pm {\cdot}005 \text{ Å}$$
$$c_0 = 15{\cdot}48 \pm {\cdot}01 \text{ Å},$$

yielding an axial ratio

$$c_0/a_0 = 3{\cdot}227.$$

The dimensions of the corresponding rhombohedral unit-cell are:

$$a_{rh} = 5{\cdot}856 \text{ Å}$$
$$\alpha = 48° 11'.$$

These dimensions are well within the range covered by the other rhombohedral carbonates. While it cannot be stated with absolute certainty that the present compound is actually $CuCO_3$, the X-ray evidence is strongly in favour of this being the case.

The stability of this compound is evidently dependent not only on the CO_2-pressure and the temperature, but also on the partial H_2O-pressure. It would seem that a CO_2-pressure which is rather high in comparison with the H_2O-pressure is necessary for its stability, and this might explain why it is not found in nature.

Assuming a rhombohedral unit-cell containing 2 molecules, the calculated density of $CuCO_3$ is 3·99 g/cm³ at 25°C. This value is not dependent on the conditions of formation, as seems to be the case for $NiCO_3$[4].

Powder data

$d_{obs.}$ in Å	$d_{calc.}$ in Å	$hk \cdot l$	$100I/I_0$
3·654	3·659	01·2	35
2·819	2·831	10·4	100
2·390	2·398	11·0	30
2·161	2·174	11·3	30
1·758	1·757	11·6	25
1·538	1·539	12·2	30
1·451	1·451	10·10	20

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Zusammenfassung

In Anwesenheit von Malachit wurde kristallines $CuCO_3$ durch Behandlung des basischen Karbonates unter 450 bar CO_2-Druck und bei 180°C gewonnen. Die rhomboedrische Einheitszelle (Raumgruppe $R\bar{3}c$) besitzt die folgenden Dimensionen:

$$a_{rh} = 5{,}856 \text{ Å}; \alpha = 48° 11'.$$

[4] J. GOLDSMITH, oral communication.

[5] On leave from the National Physical Research Laboratory, Council for Scientific and Industrial Research, Pretoria (Transvaal, Union of South Africa).

Nucleic Acid Content in Mouse Epidermis Separated from Dermis by Different Methods

In various biochemical investigations of the skin, it is essential to obtain data separately for the epidermis and the dermis. This is especially important when pathological processes (e. g. the carcinogenesis) are being studied. In such cases, the interpretation of the data is difficult and often impossible if they refer to the whole skin. That is why different methods for separation of the epidermis from the dermis have been proposed: physical procedures–high vacuum[1]; splitting of the epidermis by means of a sharp razor or dermatoms[2,3]; scraping off the epidermis with a sharp safety-razor[4] or with a blunt instrument–in the last case after a preliminary loosening of the junction between the epidermis and the corium by means of tight stretching of the skin[5,6], or by heating the skin[7]; chemical reagents: unsaturated organic compounds[8,9]; acids[7,10,11]; alkali[7,12]; neutral salts[10,11]; enzymes: pepsin[13], trypsin[14–17], collagenase, esterase[15].

All methods mentioned above have different advantages and shortcomings, pointed out in the literature cited. It is most probable that for every kind of investigation a different suitable method should be used. The great importance of NA studies in connection with such pathological processes in skin as regeneration, carcinogenesis etc., makes it most desirable to specify which of the suggested methods would be applicable for nucleic acid estimation in epidermis.

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