Nitrogen leaching. Leaching of nitrogen into the ground water was traced indirectly by following the movement of water in the soil profile. Soil moisture curves prepared before and after each irrigation (not shown here) showed that downward movement of water was not beyond 1 m of the soil under wheat. As the movement of highly mobile NO_3 is closely associated with water, it is credible that the same could be true for nitrogen. The plant cover further reduces the amount of nitrogen available for leaching due to utilization by the plants.

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Monophosphate, the only phosphoric ester of thiamin in the cerebro-spinal fluid

G. Rindi, C. Patrini and M. Poloni

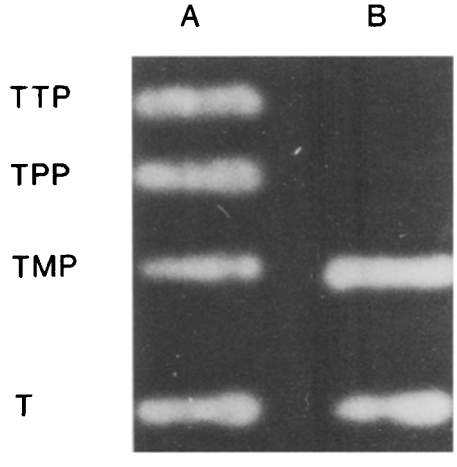
Institute of Human Physiology and Neurological Clinic, University of Pavia, Via Forlanini 6, I-27100 Pavia (Italy), 12 January 1981

Summary. With a specific and sensitive electrophoretic-fluorometric method, thiamin was found in the cerebro-spinal fluid of different mammals both in free and phosphorylated form, monophosphate being the only thiamin phosphoric ester. In humans, its amount was about 60% of total thiamin. Alcoholism greatly lowered total thiamin content, affecting both thiamin forms.

In animal tissues thiamin is present in 4 forms: free (T), mono- (TMP), pyro- (TPP) and tri-phosphate (TTP), TPP being 80–90% of the whole thiamin[1,2]. However, in rat plasma only T and TMP were found[3]. Sinclair[4,5] was unable to find thiamin phosphates in normal human cerebro-spinal fluid (CSF). Recently, Spector[6] reported that in rabbit CSF thiamin is contained mostly in an unspecified 'phosphorylated' form and that both free and phosphorylated thiamin enter the brain as well as the CSF by a saturable transport mechanism. Our current interest on the physiological functions of the thiamin phosphates in the central nervous system[7] prompted us to investigate which thiamin phosphate is present in normal CSF of humans and other mammals, as a first approach to the study of its origin and physiological meaning. The results are briefly reported here.

Materials and methods. Human CSF was obtained by lumbar puncture, in the morning, after 10–14 h of starvation; fluids containing erythrocytes or increased proteins were discarded. From other mammals the CSF was obtained by puncture of the cisterna magna, after Pentothal anesthesia. CSF was stored in the cold until the moment of chemical analysis, usually 30 min after withdrawal. After centrifugation at $350 \times g$ for 10 min in order to eliminate possible leukocytes, the samples (1–3 ml) were deproteinized with trichloroacetic acid (8% final concentration) and centrifuged again in the cold at $20{,}000 \times g$ for 10 min. The supernatant was purified and analyzed for thiamin compounds, using the electrophoretic or the direct fluorometric method described by Patrini and Rindi[8]. A comparison of the two methods for total thiamin (sum of T and phosphorylated thiamin) determination in a series of 6 CSF samples gave the following mean results (μg of total thiamin/l $\pm$ SE): 23.74 ± 1.24 (electrophoretic method) and 23.96 ± 0.98 (direct fluorometric method). The differences between paired results were not statistically significant (sign-test[9]). This allowed the electrophoretic or direct fluorometric method to be interchangeably used for total thiamin estimation (table).

Results. First a set of experiments was devoted to investigating any possible hydrolysis of CSF thiamin phosphates before and during the electrophoretic procedure. Thus, a known amount of TMP (about 50 ng) and TPP (370–400 ng) was added to a 1-ml sample of a pool of human CSFs, which was analyzed after a week's storage in a deep freezer. Another sample of the same CSF pool, but without addition of thiamin compounds, was immediately analyzed. The recoveries of the added compounds were (means of 2 experiments): TMP, 102.1%; TPP, 97.2% and total thiamin, 97.8%. This indicated the good stability of the single thiamin phosphoric esters in the CSF even after long storage at a low temperature. In another experiment only TPP was added to a sample of CSF. This was stored for



Electrophoretic analysis[8] of: *A* Thiamin compounds in pure solution (thiamin (T), 116.3; thiamin-monophosphate (TMP), 83.2; thiamin-pyrophosphate (TPP), 89.9; thiamin-triphosphate (TTP), 88.2 ng); *B* 10 ml of a pool of human cerebro-spinal fluids, carried through the entire analytical procedure[8]: only T (91.3 ng; 42.82% of total T) and TMP (121.9 ng; 57.18% of total T) were found.

Free thiamin (T), thiamin-monophosphate (TMP) and total thiamin (T+TMP) content of cerebro-spinal fluid of different mammals (means ± SE)

	n	Total T (μg/l)	T alone (μg/l)	% total T	TMP alone (μg/l)	% total T
Normal humans	20[a]	21.17±0.66	8.55±0.34	40.39	12.62±0.48	59.61
Alcoholic humans	15[b]	11.82±1.14	5.24±0.69	44.33	6.58±1.08	55.67
Rabbits	4[c]	119.43±8.70	44.23±4.00	37.03	75.20±5.91	62.97
Pigs	4[c]	58.37±0.97	31.74±0.30	54.38	26.63±1.14	45.62
Cats	4[c]	39.51±2.67	13.59±1.74	34.40	25.92±1.83	65.60
Dogs	5[c]	8.55±0.43	3.43±0.44	40.26	5.12±0.17	59.74

n, Number of cases. [a] 12 electrophoretic and 8 direct fluorometric determinations. [b] 4 electrophoretic and 11 direct fluorometric determinations. [c] electrophoretic determinations.

30 min at room temperature and then electrophoretically analyzed for thiamin phosphates together with another CSF sample of the same pool without addition of TPP. 95.5% of the TPP was recovered, suggesting that this phosphate, if present, did not undergo any significant hydrolysis in a reasonable time interval and could certainly be detected as the unmodified compound. Finally, a set of recovery experiments were carried out by adding to 7 1-ml samples of human CSF known amounts of T, TMP, TPP and TTP ranging from 10 to 20 ng/ml. After the electrophoretic procedure, these amounts were recovered as follows (means ± SE): T, 90.5% ± 1.01; TMP, 91.8% ± 0.73; TPP, 90.2% ± 0.88; TTP, 85.8% ± 0.69. The recoveries were of the same order of magnitude as those from rat tissues using the same method[8]. Thus, the preliminary experiments as a whole showed the complete reliability of the analytical procedure used in the present investigation as far as the sensitivity and the preservation of the pattern of thiamin compounds present in CSF are concerned.

Results and discussion. In 12 CSF samples obtained from different healthy subjects, without any clinical disorder involving thiamin status, the only thiamin compounds detected after electrophoretic separation were constantly T and TMP (fig.), their mean percentage being 40.7% and 59.3% of total thiamin content respectively.

This preliminary result allowed the direct fluorometric method, quicker and easier in execution, to be used for routine determinations. All results we obtained are collected in the table, which includes also some human alcoholic CSFs as well as those of different mammals. The contents of total thiamin we found in normal human CSF are in good accordance with those (22.7–30.5 μg/l) recently reported by Baker et al.[10-12] on normal American subjects, using a biological method with *Ochromonas danica,* a protozoan equally sensitive to all thiamin compounds. However, our values are higher than those (12 μg/l) previously obtained by Sinclair[4,5], using *Phycomyces blakesleanus,* by Sobotka et al.[13] (10–20 μg/l), using a microbiological method and by Dastur et al.[14] (12.9 ± 0.73 μg/l), using *Ochromonas danica* on Indian subjects. In accordance with Dastur et al.[14], we found that alcoholic CSFs have a greatly lowered total thiamin content, reflecting an almost identical decrease of both T and TMP (table). The CSF of non-human mammals contained different amounts of total thiamin: the highest was that of the rabbits, where our results are in keeping with those of Spector[6], the lowest that of the dogs. However, in every instance, the only thiamin phosphate present in CSF was TMP, the content of which ranged from 45 to 65% of total thiamin.

Since only TMP, together with free T, is present in blood plasma[3], in milk[15] and in the CSF (table), the conclusion can be reached that the extracellular fluids contain a considerable amount of phosphorylated thiamin, but only in the form of monophosphate. This suggests that TMP, as well as T, can go in and out of the cells.

It has been reported that the electrical stimulation of rat sciatic nerve[16] and spinal cord[17], or exposure to neuroactive agents of spinal cord and sciatic nerve[18,19] as well as of neuronal subcellular membranes[20] release only TMP together with T. Since the thiamin originally present in rat nervous tissue was mostly in the form of TPP and TTP[1,2], TMP seems to be an extracellularly circulating product of the catabolism of the intracellular thiamin polyphosphates, although it is actively transported into, and probably used by, neuronal cells[6]. Actually TMP seems to be excreted in urine[21,22]. However, its origin and physiological significance deserve further investigation.

The results of some experiments we carried out by incubating human CSF with TMP or TPP at 37 °C for 10–40 min, showed that the CSF was provided with both thiamin-monophosphatase and thiamin-pyrophosphatase activities.

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