

Effect of Vitamin B₁ and Cocarboxylase on Synthesis of Acetylcholine.*

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Thiamine chloride and the pyrophosphate ester of thiamine chloride are potent inhibitors of choline esterase.^{1,2,3,4} The concentrations required for such inhibition are higher than those found in the serum, but probably do not exceed the concentrations present in some tissues. Yet, thiamine chloride, even in higher concentrations, does not help patients benefited by other inhibitors of choline esterase (myasthenia gravis). The above observations suggested the investigation of the effects of thiamine compounds on the synthesis of acetylcholine.

Method. The synthesis of acetylcholine was studied following the method of Quastel, Tennenbaum, and Wheatley⁵ with minor modifications.^{6,7} Varying amounts of thiamine chloride (from 2.7 μ g to 2.7 mg) and of thiamine pyrophosphate (from 2.8 μ g to 2.8 mg) were added to mixtures containing enzyme (100 mg minced frog brain), substrate (1 cc serum or spinal fluid or Ringer's solution), physostigmine salicylate (3 mg) glucose (4.8 mg), and Ringer's solution (2 cc), and the pH of the mixtures was adjusted to 7.4. Identical mixtures without the thiamine compounds served as controls. The mixtures were shaken and incubated aerobically for 4 hours at 37°C. After incubation the free and total

acetylcholine synthesized was determined biologically using the sensitized rectus abdominis muscle of frog. Since the thiamine compounds, in the concentrations used, did not induce muscle contraction, and since they did not significantly modify the effect of acetylcholine in inducing muscle contraction, the use of the rectus abdominis muscle was a valid means of determining the acetylcholine content of the mixtures.

Results. The amounts of acetylcholine synthesized in the presence of the thiamine compounds are summarized in Table I. Both thiamine chloride and thiamine pyrophosphate in biological concentrations ($3 \cdot 10^{-6}$ M) increased the synthesis of acetylcholine slightly (average 10%). Mann and Quastel,⁸ using thiamine chloride, found a similar increase. Both thiamine chloride and thiamine pyrophosphate, in higher than biological concentrations, decreased the synthesis of acetylcholine. Thiamine chloride was a more potent depressor than was thiamine pyrophosphate. Changes in the synthesis of acetylcholine (as expressed in percentage) due to the presence of the thiamine compounds were similar in mixtures containing only frog brain, frog brain and serum, and frog brain and spinal fluid.

Discussion. An adequate explanation of the mechanism through which the thiamine compounds increase and depress respectively the synthesis of acetylcholine cannot be offered. It is possible that the slight increase of synthesis is due to the direct effect of cocarboxylase on the carbohydrate metabolism, resulting in a better supply of the acetylradical. It is possible that the decrease of synthesis of acetylcholine is due to a direct reaction of large amounts of the thiamine compounds with the enzyme involved in the synthesis of acetylcholine. Such reaction cannot

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¹ Glick, D., and Antopol, W., *J. Pharm. Exp. Therap.*, 1939, **65**, 389.

² Sullmann, H., and Birkhauser, H., *Schweiz. Med. Wschr.*, 1939, **69**, 648.

³ Massart, L., and Dufait, R., *Naturwissenschaften*, 1939, **27**, 567.

⁴ Glick, D., and Antopol, W., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 396.

⁵ Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Bioch. J.*, 1936, **30**, 1668.

⁶ Torda, C., and Wolff, H. G., *J. Clin. Invest.*, 1944, in press.

⁷ Torda, C., and Wolff, H. G., in press.

⁸ Mann, J., and Quastel, J. H., *Nature*, 1941, **145**, 856.

TABLE I.
Effect of Thiamine Compounds on Synthesis of Acetylcholine *in vitro*.

Substance	Conc. in mols	No. of exp.	Amounts of acetylcholine synthesized in % of control*					
			Free acetylcholine			Total acetylcholine		
			In the presence of frog brain and Spinal fluid	Serum	Ringer's sol.	In the presence of frog brain and Spinal fluid	Serum	Ringer's sol.
(Control) †			100 (1)	100 (2)	100 (3)	100 (4)	100 (5)	100 (6)
Thiamine chloride	3.10 ⁻⁶	8	114	108	117	116	109	120
	3.10 ⁻⁵	8	87	93	90	73	80	70
	3.10 ⁻⁴	8	60	68	71	66	65	55
	3.10 ⁻³	8	40	44	38	40	37	36
Thiamine pyrophosphate	2.10 ⁻⁶	4	108	104	110	105	108	111
	2.10 ⁻⁵	4	87	90	89	81	84	90
	2.10 ⁻⁴	4	72	77	72	67	72	68
	2.10 ⁻³	4	56	54	56	59	60	55

* The S.E. of the mean for each value was less than $\pm 10\%$.

† The amounts of acetylcholine synthesized in μg per 100 mg frog brain are: (1) 2.11 ± 0.053 , (2) 1.45 ± 0.011 , (3) 0.41 ± 0.013 , (4) 3.16 ± 0.049 , (5) 2.08 ± 0.029 , (6) 1.02 ± 0.033 .

be excluded since both the thiamine compounds and acetylcholine are quaternary ammonium compounds containing a free hydroxyl group, and since the thiamine compounds also have a higher affinity for choline esterase than does acetylcholine. Concentrations of vitamin B₁ and cocarboxylase inhibiting choline esterase also inhibit the synthesis of acetylcholine. Hence, no significant aid can be expected from these substances for patients with disorders resulting from decreased synthesis of acetylcholine.

Summary. 1. The synthesis of acetylcho-

line *in vitro* in the presence of thiamine chloride and thiamine pyrophosphate was investigated. 2. Both thiamine chloride and thiamine pyrophosphate, in concentrations of 3.10⁻⁶ M and 2.10⁻⁶ M respectively, slightly increased the synthesis of acetylcholine (average 10%). 3. The thiamine compounds in higher concentrations depressed the synthesis of acetylcholine. Thiamine chloride is a more potent depressor than thiamine pyrophosphate.

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Effect of Thiamine Compounds on the Striated Muscle.*

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Minz¹ observed that cholinergic nerves liberate not only acetylcholine but also thiamine chloride when stimulated. From this observation von Muralt² postulated that vita-

min B₁ may have some direct effect on the contraction of striated muscles following the stimulation of motor nerves. Vitamin B₁ increases the effect of acetylcholine on the intestine^{3,4,5,6} and circulatory apparatus of

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² von Muralt, J., *Naturwiss.*, 1939, **27**, 261.

³ Binet, L., and Minz, B., *Arch. int. Physiol.*, 1936, **42**, 281.