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, In the presence of frog brain and Spinal fluid	Serum	Ringer's sol.	Total acetylcholine, 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 ±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 B_1 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 acetylcholine *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.

The thiamine pyrophosphate was kindly supplied by the Merck Research Laboratories, Rahway, N.J.

14604

Effect of Thiamine Compounds on the Striated Muscle.*

CLARA TORDA AND HAROLD G. WOLFF.

From the New York Hospital and the Departments of Medicine (Neurology) and Psychiatry Cornell University Medical College, New York City.

Minz[1] observed that cholinergic nerves liberate not only acetylcholine but also thiamine chloride when stimulated. From this observation von Muralt[2] postulated that vitamin B_1 may have some direct effect on the contraction of striated muscles following the stimulation of motor nerves. Vitamin B_1 increases the effect of acetylcholine on the intestine[3,4,5,6] and circulatory apparatus of

* This study was aided by a grant from the Josiah Macy, Jr., Foundation.

1 Minz, B., *C. R. soc. biol.*, 1938, **127**, 1251.

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

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

cat.[4] This phenomenon was explained by the fact that thiamine chloride has an affinity for choline esterase which is 26 times greater than that of acetylcholine.[7] Acetylcholine and thiamine chloride may have similar effects as both are quaternary ammonium compounds containing a free hydroxyl group. The purpose of the following investigation was to ascertain whether thiamine chloride and related compounds have a direct effect on the striated muscle of frog, and whether they modify the contraction induced by chemical agents such as acetylcholine and potassium.

Experimental. 1. *Direct effect of thiamine compounds on the striated muscle.* The rectus abdominis muscle of frog was excised and suspended in a muscle chamber containing 10 cc of Ringer's solution. The Ringer's solution was changed to one of the thiamine compounds for 3 minutes. The muscle was then washed for 20 minutes with Ringer's solution. This procedure was repeated increasing the concentration of the thiamine compounds. The pH of all solutions used here and in the following experiments was corrected to 7. The height of contraction of the muscle was registered by an isotonic lever on a kymograph.

Very high concentrations of the thiamine compounds were required to start a contraction in 3 minutes (thiamine chloride 4.10^{-2} M (average of 10 exp.), thiamine pyrophosphate 1.10^{-2} M (average of 8 exp.), acetyl-thiamine chloride 6.10^{-2} (average of 8 exp.). The sensitivity of the muscle to the thiamine compounds was not significantly increased by immersion for half an hour in a solution of physostigmine containing 2 mg physostigmine salicylate per 100 cc of Ringer's solution.

II. *Effect of thiamine compounds on effect of acetylcholine in inducing muscle contraction.* The rectus abdominis muscle of frog was prepared as before. The Ringer's solution was changed to an acetylcholine solution (50 μg per 100 cc Ringer's solution) for 2 minutes. The muscle was then washed with Ringer's solution for 10 minutes. This procedure was repeated until 3 successive exposures to the solution of acetylcholine gave similar responses. Afterwards the muscle was washed only for 5 minutes and immersed in one of the solutions of the thiamine compounds for 5 minutes. A series of solutions containing the thiamine compounds in increasing concentrations was used.

The thiamine compounds did not increase the effect of acetylcholine in inducing muscle contraction. Thiamine compounds in higher concentrations depressed the effect of acetylcholine (Table I). A similar depression was observed when the thiamine compounds were added directly to the acetylcholine instead of immersing the muscle in the solutions of the thiamine compounds for 5 minutes.

Thiamine compounds dissolved in a solution of physostigmine had a similar effect upon the sensitivity of the eserinized muscle to acetylcholine as they had on the non-sensitized muscles. (These muscles were contracted with a solution containing 10 μg acetylcholine per 100 cc Ringer's solution.)

III. *Effect of thiamine compounds on contraction of muscle induced by potassium.* The rectus abdominis muscle was treated as above, but a 20 mM solution of potassium chloride in Ringer's solution was used instead of acetylcholine. The height of contraction induced by potassium increased significantly in the presence of very low concentrations of the thiamine compounds, and increased with increasing concentrations (Table I). Thiamine pyrophosphate was the most potent agent.

Discussion. The thiamine compounds in concentrations about 100 times as great as found in serum induce a contraction of the striated muscle. The thiamine compounds used, even in very low concentrations, increased the effect of potassium in inducing muscle contraction. These effects are probably due to the action of thiamine compounds on the metabolism of muscle since thiamine pyrophosphate was the most potent agent.

The thiamine compounds in biological and

[4] Agid, R., Beauvallet, M., and Minz, B., *C. R. soc. biol.*, 1937, **126**, 982.

[5] Abderhalden, E., and Abderhalden, R., *Kli. Wschr.*, 1938, **17**, 1195.

[6] Abderhalden, E., and Abderhalden, R., *Pflüger's Arch.*, 1938, **240**, 647, 746.

[7] Glick, D., and Antopol, W., *J. Pharm. Exp. Therap.*, 1939, **65**, 389.

TABLE I.
Effect of Thiamine Compounds on Contraction of Muscle Induced by Acetylcholine and Potassium.

Conc. of the thiamine compounds in mols	Magnitude of contraction in % of control after immersion in the thiamine compounds for 5 min.*								
	Contraction induced by acetylcholine in muscles immersed in			Contraction induced by acetylcholine in sensitized muscles immersed in			Contraction induced by potassium in muscles immersed in		
	Thiamine chloride	Thiamine pyrophosphate	Acetylthiamine	Thiamine chloride	Thiamine pyrophosphate	Acetylthiamine	Thiamine chloride	Thiamine pyrophosphate	Acetylthiamine
0	100	100	100	100	100	100	100	100	100
3.10^{-7}	96	93	94	94	94	98	134	139	132
3.10^{-6}	93	93	92	96	93	93	149	166	162
3.10^{-5}	86	90	82	87	91	89	170	178	172
3.10^{-4}	78	89	68	76	86	64	180	232	207
3.10^{-3}	45	75	38	41	71	35	199	300	218

* Each value represents the average of 12 separate experiments. The S.E. of the mean for each value was less than $\pm 5\%$.

higher concentrations did not increase the effect of acetylcholine in inducing muscle contraction, while in high concentrations the effect of acetylcholine was depressed. The time necessary to exert this depression is very short. An adequate explanation cannot be offered at the present. It is possible that the presence of the thiamine compounds in relatively large concentrations prevents some of the receptor mechanisms from reacting to the presence of acetylcholine.

The above observations may throw some light on the mechanism of action of the thiamine compounds in regulating the contraction of muscle.

Summary. 1. The effect of thiamine chloride, thiamine pyrophosphate, and acetylthiamine in inducing muscle contraction and the effect of the thiamine compounds on the muscle contraction induced by acetylcholine and potassium were investigated. 2. Thiamine compounds did not induce a contraction of the muscle in concentrations lower than 1.10^{-2} M in a period of 3 minutes. 3. The thiamine compounds did not sensitize the muscle to acetylcholine. In higher concentrations the effect of acetylcholine in inducing muscle contraction was depressed. 4. The thiamine compounds significantly increased the effect of potassium in inducing muscle contraction.

Thiamine pyrophosphate and acetylthiamine was kindly supplied by the Merck Research Laboratories, Rahway, N.J.

14605 P

Embryonic Chick Antigens for Complement Fixation with Viruses of Eastern and Western Equine Encephalomyelitis.*

GORDON C. BROWN. (Introduced by Thomas Francis, Jr.)

From the Department of Epidemiology and the Virus Laboratory, School of Public Health, University of Michigan.

The complement-fixation test has been increasingly employed in the identification of virus infections of the central nervous system. With one exception all complement-fixing antigens thus far employed with equine encephalomyelitis have been prepared from animal brain tissue, usually that of the mouse.[1-7] Mohler,[8] using a formolized antigen

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.