

consideration would not be affected.

Summary. With the aid of these methods it has been shown, in the rat, that the thia-

mine concentration in the blood parallels that in the tissues, and both reflect the intake.

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Depression of Acetylcholine Synthesis by Serum from Working Muscle. Healthy Subjects and Myasthenia Gravis Patients.*

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In the presence of serum from patients with myasthenia gravis, a persistent state of easy fatiguability,^{1,2} less acetylcholine is synthesized than in the presence of serum from healthy subjects. The purpose of the following study was to investigate the effect of exercise in both healthy subjects and patients with myasthenia gravis measuring the action of serum on acetylcholine synthesis. The results are of particular interest with respect to the mechanism of the genesis of fatigue.

Method. Experiments were performed on 12 healthy subjects and 5 patients with myasthenia gravis. The subject alternately flexed his fingers, forming a fist, and extended them fully, performing the entire action rhythmically, at the rate of one contraction per second.

A sphygmomanometer cuff was applied to the arm and inflated during the fourth to ninth contraction of the fingers to a pressure of 250 mm Hg. Ten cc blood was collected from the brachial vein. Blood collected from the other immobilized arm with occluded circulation served as control. The blood was centrifuged immediately after collection, oxygenated thoroughly, and the effect of serum on the synthesis of acetylcholine was tested by the method of Quastel, Tennenbaum, and Wheatley³ with minor modifications.² One hundred mg fresh minced frog brain, 1 cc serum, 2 cc Ringer's solution of pH 7.4, 4.8 mg glucose, and 3 mg physostigmine salicylate were incubated and shaken aerobically for 4 hours at 37°C. The amount of acetylcholine

TABLE I.
Effect of Serum Collected During Muscle Contraction on Synthesis of Acetylcholine.

Subject	No. of first formation until fatigue	Amt acetylcholine synthesized by serum from resting arm in % of healthy control serum*	Amt acetylcholine synthesized with serum from working arm in % of own control	Total decrease of acetylcholine synthesized
Twelve healthy subjects	60-105	100	60 ± 1.6	40
Patients with myasthenia gravis:				
R	15	64	85	45
L	30	72	80	43
S	50	76	74	44
V	20	74	83	39
M	40	78	76	41

* The amount of acetylcholine synthesized in the presence of serum from the control arms of healthy subjects averaged 2.08 μ .

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¹ Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 224.

² Torda, C., and Wolff, H. G., *J. Clin. Invest.*, 1944, **23**, 649.

³ Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Biochem. J.*, 1936, **30**, 1668.

TABLE II.
Short Summary of the Clinical State of the 5 Patients with Myasthenia Gravis.

Name	Sex	Age	Severity of disease	Duration (years)	Thymectomy	X-ray treatment	Symptomatology	Prostigmine Bromide (Hoffmann-La Roche) mg per day		
								Amt before thymectomy	Present amt	Achievement after medication
R	F	24	3+	10	no	yes	Moderate lid ptosis, occasional diplopia and difficulty in chewing, moderate muscular fatigability		90	Walks 1-2 blocks
L	M	46	3+	1	"	no	Severe lid ptosis, constant diplopia, mod. muse. fatig.		60	Walks
S	M	23	2+	3	yes	yes	Slight lid ptosis, rare diplopia, mod. muse. fatig.	120	15 to 30	Works as engineer
V	F	21	2+	6½	no	no	Slight lid ptosis, rare diplopia, occasional difficulty in chewing, mild muse. fatig.		45	Walks, goes to parties
M	F	28	2+	7	"	"	Slight lid ptosis, rare diplopia, occasional difficulty in chewing, mild muse. fatig.		45	Works as cashier

synthesized was assayed biologically with the sensitized rectus abdominis muscle of the frog. The amounts of acetylcholine synthesized in the presence of serum obtained from the working arm was expressed as percent of the control.

Results. The amounts of acetylcholine synthesized are given in Table I and Fig. 1. Frog brain incubated without serum synthesized on the average 1.02 ± 0.033 μ g acetylcholine per 100 mg of frog brain; mixtures of frog brain and serum from immobilized arms of healthy subjects synthesized 2.08 ± 0.080 μ g acetylcholine.² The amount of acetylcholine synthesized in the presence of serum from healthy subjects deviated from the average less than $\pm 8\%$.

Healthy subjects contracted the fingers from 75 to 105 times before severe fatigue appeared. Approximately 40% less acetylcholine was synthesized in the presence of serum collected after performance of the contractions by the healthy subjects than in the presence of the control serum.

A short summary of the clinical state of the patients with myasthenia gravis is given in Table II. Acetylcholine synthesis in the presence of serum from the immobilized arm of patients with myasthenia gravis was less than in the presence of serum from the immobilized arm of healthy subjects. The more severe the myasthenia gravis the less the acetylcholine synthesis. The patients contracted the fingers from 15 to 50 times before severe fatigue appeared. Serum collected from patients with myasthenia gravis after performance of the contractions decreased the synthesis by 15 to 26% as compared to the serum collected from the resting arm of the same patient. Since the serum obtained from the resting arm of these patients depressed the synthesis of acetylcholine by 22 to 36% as compared to the effect of serum from the resting arm of healthy subjects, the total decrease of the acetylcholine synthesis in the patients with myasthenia gravis after performance of muscle work averaged 42%. These results suggest that when the blood changes during performance of muscle contractions as to decrease the synthesis of acetylcholine *in vitro* by 40%, performance of

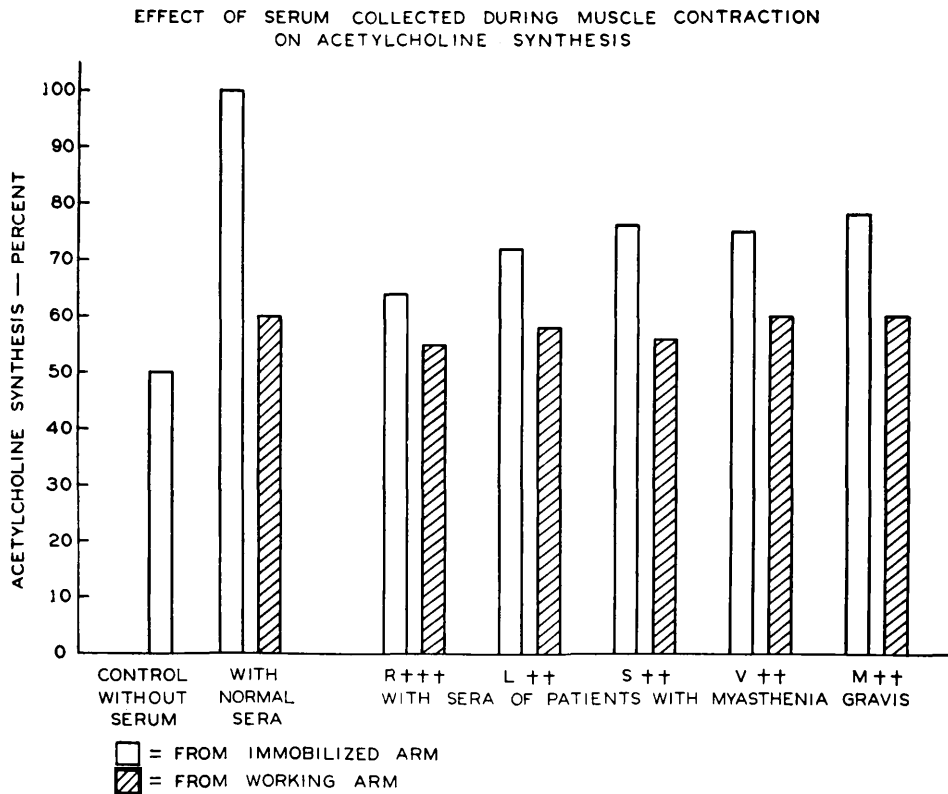


FIG. 1.

Effect of Serum Collected During Muscle Contraction on Acetylcholine Synthesis.

The average amounts of acetylcholine synthesized are expressed as percent of the amount synthesized in the presence of serum from the immobilized arm of healthy subjects.

voluntary muscle work becomes exceedingly difficult.

Discussion. The above experiments suggest that during performance of muscle work with occluded circulation changes of the physico-chemical state at the neuro-muscular junction may result in a decrease of the local synthesis of acetylcholine. It seems that voluntary muscle contraction becomes extremely difficult when the agents involved reduce the acetylcholine synthesis *in vitro* to a similar extent in both healthy subjects and patients with myasthenia gravis.

Should the biochemical changes responsible for the above results also occur during performance of work by muscle with adequate blood supply, muscle fatigue may at least partly be the result of the decreased local synthesis of the acetylcholine-like substance.

It is known that during prolonged activity of cholinergic systems an acetylcholine-like

substance is synthesized locally.^{4,5} A local synthesis of acetylcholine is conceivable since acetylphosphate,⁶ a possible intermediate product of the anaerobic carbohydrate and fat metabolism, may be one of the precursors of acetylcholine. Among others many intermediate products of carbohydrate metabolism^{7,8} and many amino acids^{9,10} increase the synthesis of acetylcholine (as tested by the modified method of Quastel, Tennenbaum, and

⁴ Brown, G. L., and Feldberg, W., *J. Physiol.*, 1936, **88**, 265.

⁵ Kahlson, G., and MacIntosh, F. C., *J. Physiol.*, 1939, **96**, 277.

⁶ Lipmann, F., *Adv. in Enzymology*, 1941, 199.

⁷ Torda, C., and Wolff, H. G., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 234.

⁸ Torda, C., and Wolff, H. G., in press.

⁹ Nachmansohn, D., and John, H. M., *J. Biol. Chem.*, 1945, **158**, 157.

¹⁰ Torda, C., and Wolff, H. G., in press.

Wheatley²). On the other hand decomposition products of adenosinetriphosphate^{8,11} decrease it. The local concentrations of inorganic ions may also exert some effect on the synthesis.³ Our results with serum from working muscle suggest that during prolonged muscle work the dynamic equilibrium may be shifted to cause decrease of the local acetylcholine synthesis. If such event does occur it may contribute to the genesis of muscle fatigue.

At present it cannot be stated whether the decrease of acetylcholine synthesis was due to a depressor substance released from the muscle during contractions or whether it was due to utilization of some potentiator substances ordinarily present in the blood and used by the muscle to perform work. The existence of potentiator substances in the blood can be assumed since the amount of acetylcholine synthesized *in vitro* in the presence of serum from the immobilized arm of healthy subjects is twice as much as the amount synthesized in the absence of serum.

The implications of these results regarding myasthenia gravis are: 1. In the presence of serum^{1,2} from patients with myasthenia gravis less acetylcholine is synthesized than in the presence of serum from resting healthy subjects. The factor responsible for these effects could be (I) lack of the usually occurring potentiator constituents of the serum; (II) a depressor agent being: either (a) a foreign substance;¹² or (b) an intermediate product of body metabolism;^{11,13,14,15} or (c) a sub-

stance released during muscle contraction but not adequately metabolized. (2) In patients with myasthenia gravis the depressor effect of blood that has circulated through the muscle during contraction adds to the effect of blood circulating before voluntary muscle contractions. Muscle fatigue appears as soon as the total decrease is approximately that occurring in healthy subjects at the onset of fatigue.

Summary. 1. The effect of muscle work of healthy subjects and patients with myasthenia gravis on the ability of serum to modify acetylcholine synthesis *in vitro* was investigated. 2. Acetylcholine synthesis in mixtures of frog brain and serum from immobilized arm of healthy subjects was twice as much as in the absence of serum. 3. Acetylcholine synthesis in the presence of serum from working arm was 40% less than in the presence of serum from immobilized arm. 4. Acetylcholine synthesis in the presence of serum from the immobilized arm of patients with myasthenia gravis was less than in the presence of serum from the immobilized arm of healthy subjects. The more severe the myasthenia gravis, the less the acetylcholine synthesis. 5. Acetylcholine synthesis in the presence of serum from the working arm of patients with myasthenia gravis was 15 to 26% less than with serum of their own immobilized arm, but on the average 42% less than the amount synthesized in the presence of serum from the immobilized arm of healthy subjects. 6. Muscle fatigue may be in part the result of the decrease of the local synthesis of an acetylcholine-like substance during prolonged muscle contractions. 7. Implications regarding the symptomatology of myasthenia gravis are presented.

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¹¹ Torda, C., and Wolff, H. G., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 108.

¹² Rosenow, E. C., and Heilman, F. R., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 419.

¹³ Torda, C., and Wolff, H. G., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 327.

¹⁴ Torda, C., and Wolff, H. G., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 163.

¹⁵ Torda, C., and Wolff, H. G., *in press*.