TABLE III.
Precipitation with Malarial and Nonmalarial Hemoglobin Solutions (Employing Aged Blood Specimens).

Malarial Spec. 137		Nonmalarial Spec. 138		Nonmalarial Spec. 306	
Aged about 48 hr at room temp.				Aged 4 hr at room temp.	
No.	%	No.	%	No.	%
Precipitation in the 1:15 and 1:30 hemoglobin dilutions*					
125	91	31	24	7	2
Precipitation only in the 1:30 hemoglobin dilution					
9	7	67	28	17	4
No precipitation in the 1:15 and 1:30 hemoglobin dilutions					
3	2	30	48	282	94

* The 1:15 hemoglobin dilution was examined in ratios of 6:1, 12:1 and 18:1 with antigen suspension. The 1:30 hemoglobin dilution was examined in ratios of 12:1, 18:1 and 24:1 with antigen suspension. If precipitation occurred in one or more ratios of either dilution, the result was recorded as positive.

trols. Table III. summarizes the results obtained.

As indicated in the table, 91% of malarial hemoglobin solutions in 1:15 and 1:30 dilutions with distilled water gave positive precipitation reactions. In addition, 7% of the hemoglobin solutions gave precipitation only in the 1:30 dilution. The remaining 2% showed no precipitation. Of the nonmalarial hemoglobin solutions, 24% gave precipitation both in the 1:15 and 1:30 dilutions and 28% only in the 1:30 dilution. The remaining 48% showed no precipitation. It is thus evident that under the ageing conditions specified, the malarial hemoglobin solutions showed a greater tendency toward precipitation than the nonmalarial hemoglobin solutions.

In Table III. is also presented an experiment in which 306 nonmalarial blood specimens were aged only 4 hr at room temperature. When hemoglobin dilutions from these specimens were examined with antigen suspension, only 6% showed precipitation. Preliminary findings indicate that hemoglobin dilutions from fresh malarial blood specimens give similar precipitation results.

Studies are in progress to determine the nature of the precipitation phenomenon described in this report and if the phenomenon is applicable to the development of a practical method for differentiation between hemoglobin solutions of malarial and nonmalarial origin.

Summary. It was observed that hemoglobin solutions (laked human erythrocytes in distilled water) when mixed with tissue extract antigen (Kahn antigen suspension) under proper conditions show immediate precipitation. This phenomenon of precipitation is manifested in a relatively wide range of hemoglobin-distilled water dilutions and also in various ratios of these dilutions to antigen suspension. Hemoglobin solutions derived from malarial blood specimens show a tendency toward a wider range of precipitation than hemoglobin solutions derived from nonmalarial blood specimens. Ageing of the blood specimens at a temperature above refrigeration, such as room temperature, is an important factor in increasing the tendency toward such precipitation.

14912

Release of Curare-Like Agent from Healthy Muscle and Its Bearing on Myasthenia Gravis.*

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.

Several investigators, notably Nevins,[1, 2] have suggested the possibility of the presence in the blood or tissues of patients with myasthenia gravis of an abnormal substance or metabolite with a curare-like action which raises the threshold of skeletal muscle to the effects of acetylcholine. Walker[3] tested this

* This study was aided by a grant from the John and Mary R. Markle Foundation.

TABLE I.
Effect of serum of cat on the neuro-muscular transmission of frog.

Time of stimulation after inj. of serum in min.	Magnitude of contraction of the gastrocnemius muscle of the frog in % of control during electrical stimulation of the sciatic nerve followed by the S.E. of the mean. Serum collected from: immobilized limb	intermittently stimulated limb	continuously stimulated limb	passively moved limb	control Ringer's sol.	frog with excised heart
½	100±1.0	99±0.9	99±0.3	98±0.6	99±0.2	99±0.4
1	100±0.7	99±0.3	97±0.2	94±1.0	99±0.3	98±0.8
1½	100±0.8	99±0.5	96±0.4	92±1.8	100±0.4	97±0.8
2	99±0.9	98±1.2	94±0.3	90±1.7	98±0.5	98±0.9
2½	98±1.1	97±0.5	93±0.9	88±1.9	100±0.2	98±1.3
3	99±0.9	97±0.6	92±0.7	83±1.9	100±0.4	98±0.9
3½	98±0.5	97±0.3	91±1.1	85±1.8	101±0.3	99±0.6
4	98±0.6	98±0.7	90±0.5	83±2.0	100±0.7	99±0.5
4½	99±0.5	97±0.4	89±0.8	81±1.7	102±0.2	99±0.7
5	98±0.4	99±0.8	88±0.7	79±2.0	100±0.2	99±0.3
5½	98±0.7	99±0.5	88±0.8	76±1.9	101±0.4	99±0.5
6	99±0.2	98±0.2	87±0.9	74±1.6	102±0.2	98±0.2
6½	98±0.4	98±0.7	86±0.9	74±2.2	100±0.3	97±0.6
7	99±0.6	98±0.6	83±1.0	73±2.5	98±0.5	98±0.7
7½	98±0.5	98±0.5	82±0.7	69±2.6	99±0.3	98±0.5
8	99±0.4	98±0.4	82±1.3	69±2.3	100±0.7	99±0.6
8½	98±0.7	97±0.8	81±0.9	67±2.1	98±0.8	100±1.1
9	100±0.4	97±0.7	80±1.1	66±2.4	102±0.2	99±0.7
9½	99±0.5	96±0.7	78±1.4	65±2.6	100±0.4	99±0.3
10	97±0.4	96±1.2	78±1.3	62±2.5	100±0.4	100±0.9
20 min. rest period.						
30½	110±0.3	109±1.1	110±0.6	108±0.6	107±0.5	108±0.9
31	112±0.8	109±0.6	111±0.3	109±0.3	109±0.8	109±1.1
31½	112±0.7	110±0.5	111±0.5	109±0.7	110±0.2	109±0.5
32	110±0.4	109±0.9	111±0.7	110±0.4	109±0.4	107±0.7
32½	110±0.7	111±0.8	111±0.3	111±0.7	108±0.4	110±0.8
33	109±0.3	109±1.2	110±0.5	112±0.5	108±0.4	110±0.5
No. of exp.	28	15	13	23	60	10

theory by occluding the circulation in the upper limbs of patients with myasthenia gravis and exercising the muscles of the forearm. On releasing the constriction an increase in the clinical signs was observed. She concluded that muscles of patients with myasthenia gravis liberate a chemical substance which passes into the circulation and produces a neuro-muscular block in the motor end-plates of skeletal muscle. Wilson and Stoner[4] found that the serum of patients with myasthenia gravis not under prostigmine treatment collected after voluntary muscle work, when tested on the isolated nerve-muscle preparation of the frog, produces a block in neuro-muscular transmission. This effect is not obtained with serum collected from healthy subjects or patients during prostigmine treatment. These authors conclude that in contrast to the healthy subjects the blood of patients with myasthenia gravis contains a substance with curare-like action which interferes with neuro-muscular transmission.

It occurred to us that the depression of the neuro-muscular transmission in the experiments of Walker[3] and Wilson and Stoner[4] may be explained otherwise. It is likely that a dynamic equilibrium exists between stimulator substances (acetylcholine-like substance liberated at the synapse,[5-9] decomposition

[1] Nevin, S., *Brain*, 1934, **57**, 239.

[2] Nevin, S., *J. Neurol. and Psychiatry*, 1938, **1**, 120.

[3] Walker, M. B., *Proc. Roy. Soc. Med.*, Series B., 1938, **31**, 722.

[4] Wilson, A., and Stoner, H. B., *Quarterly J. Med.*, 1944, **37**, 1.

[5] Bacq, Z. M., *Ergebnisse Physiol.*, 1935, **37**, 82.

[6] Eccles, J. C., *Ergebnisse Physiol.*, 1936, **38**, 339.

[7] Brown, G. L., *Physiol. Rev.*, 1937, **17**, 485.

[8] Dale, H., *Harvey Lectures*, 1937, **32**, 229.

products thereof, some metabolites) and substances with curare-like action (other metabolites) during and after muscle contraction of healthy subjects. It is likely that agents with curare-like action are present after muscle contraction since fatigued muscles of healthy subjects behave as slightly curarized muscles as far as summation of stimuli,[10-12] post-tetanic potentiation of single twitches,[13] and response to tetanizing currents[13-15] are concerned.

Should the dynamic equilibrium be disturbed by an unusual amount of the normally occurring agent, with curare-like effect, by some unusual curare-like agent, or by a decrease of the stimulating agent present the curare-like effect appears. In the experiments of Wilson and Stoner[4] with patients not treated with prostigmine the curare-like effect could have been due to the insufficient amount of stimulator substances released into the blood during muscle work. The effect of serum of patients with myasthenia gravis observed on the neuro-muscular transmission of the frog might have been due to the lack of stimulator substances in the serum since administration of prostigmine alone was sufficient to counteract the effect of the curare-like agent. This would seem to be so unless one postulates that physostigmine has other anti-curare effects than that exerted by the decrease of the decomposition of acetylcholine and increase of the potassium content of muscle.[16, 17]

The assumption of the appearance of the curare-like effect caused by a lack of sufficient amounts of potentiator substances would be substantiated if an agent with curare-like effect could be demonstrated in the serum of healthy subjects after muscle movements.

Method. Cats of 2-3 kg body weight were anesthetized with a Dial-Urethane solution (Ciba). After 2 hr 3 types of experiments were performed: (1) the femoral vein was ligated and the leg passively extended and flexed once per second for 10 min after section of the sciatic and femoral nerves; (2) the femoral vein was ligated and the sciatic nerve stimulated with an induction coil for 10 min either continuously, using a current strong enough to induce toe extension, or intermittently, alternating one second of stimulation with one second rest period using a weaker current but strong enough to induce slight muscle contraction; (3) the femoral vein was ligated for 10 min and the leg immobilized. After 10 min 3 cc blood was collected from the ligated vein, centrifuged, and the effect of serum on the neuro-muscular transmission of the frog was tested immediately.

The frog was prepared as follows: frogs of 18-23 g body weight were pithed and pinned to a board. A knee was fixed and the tendon of the gastrocnemius muscle attached to an isotonic lever. The movements of the muscle were registered by a kymograph. Electrodes were placed on the intra-abdominal branches of the sciatic nerve and the nerve was stimulated with an induction coil for 3 sec every 30 sec. After a steady height of contraction of the gastrocnemius muscle was reached, 0.2 cc serum of experimental cats were injected into the heart. Not more than 5 to 8 min elapsed between collection of blood from the cats and injection of the serum into the frog. The intermittent stimulation of the sciatic nerve of the frog was continued for 10 min as described previously and after a pause of 20 min the intermittent stimulation was resumed for 3 min. The muscle contraction in frogs injected with 0.2 cc mammalian Ringer's solution served as control.

Upon conclusion of the experiments the frogs were injected with Berlin blue to ascertain whether the blood supply of the gastrocnemius muscle remained adequate during the experiments.

Computation. The height of the tracings was measured. For each experiment 2 to 3

[9] Schriever, H., *Ergebnisse Physiol.*, 1936, **38**, 877.

[10] Bremer, F., *Comp. rend. soc. biol.*, 1927, **97**, 1179.

[11] Bremer, F., *Comp. rend. soc. biol.*, 1929, **102**, 332.

[12] Bremer, F., *Am. J. Physiol.*, 1929, **90**, 297.

[13] Rosenblueth, A., and Morrison, R. S., *Am. J. Physiol.*, 1937, **119**, 236.

[14] Hofmann, F. B., *Pfluger's Arch.*, 1903, **95**.

[15] Bouman, H. D., *Arch. Neerl. Physiol.*, 1932, **17**, 307.

[16] Thompson, V., and Tice, A., *J. Pharmacol. Exp. Therap.*, 1941, **73**, 455.

[17] Wilson, A., and Wright, S., *Quart. J. Exp. Physiol.*, 1936, **26**, 128.

frogs were injected with Ringer's solution, 2 with the serum from the immobilized leg with occluded circulation and 2 with the moved or stimulated leg. In each tracing the contraction before injection served as 100% and each following value obtained was expressed as a % of this contraction.

Results. The height of contraction of the gastrocnemius muscle of the frog during the various experimental procedures is given in Table I. Serum collected from immobilized legs with occluded circulation did not modify the contraction of the gastrocnemius muscle of the frog. Serum collected from the limbs with occluded vein after intermittent stimulation of the sciatic nerve did not modify the contraction of the gastrocnemius muscle. Serum collected after 10 min of tetanus depressed the contraction of the gastrocnemius muscle (at the end of the 10 min test period the contraction was on the average of 19% below the values of the control) and the contraction of the gastrocnemius muscle returned to its original state during the 20 min of rest. Serum collected from passively moved legs with sectioned nerves and occluded femoral vein depressed the contraction of the gastrocnemius muscle. The contraction was on the average 35% less than the control at the end of the 10 min test period. A period of 10 min was chosen for convenience. It is a period long enough to show the initiation of the depression of the muscle contraction by the serum of the cat. The depression was greater when the period of stimulation of the frog preparation was prolonged. The muscle contraction returned to its original state during the 20 min rest period and behaved similarly to the control frogs thereafter. In these experiments the liberation of stimulator substances from the nerve endings of cat may not be entirely prevented, since, during the passive movements, the motor nerve may have been stimulated to some extent.

The injection of 0.2 cc serum induced an increase of the volume of blood of the frog, a temporary injury to the heart, and, if sera from passively moved limbs and continuously stimulated limbs were used, the cat serum induced a short block of the frog heart followed by a prolonged increase of the heart rate. To ascertain whether the changes of blood supply of the gastrocnemius muscle during and after the injection of the cat serum contributed to the decrease of the contraction of gastrocnemius muscle, experiments were performed using frogs with excised hearts. The height of contraction of the gastrocnemius muscle after stimulation of the sciatic nerve was very similar to that found in control frogs with intact hearts.

For purpose of further control the gastrocnemius muscle was directly stimulated through electrodes inserted into the tendons of the muscle. Since the extent of contraction was not significantly modified by the injection of cat sera into the frog it may be assumed that the decrease of the contraction of the gastrocnemius muscle during the stimulation of the sciatic nerve was due to the decrease of the neuro-muscular transmission.

Discussion. The above experiments suggest that during movements substances that depress the neuro-muscular transmission are passed into the blood. It may be asumed that in healthy animals the effect of these substances is counteracted by the presence of stimulator substances released at the synapse during the stimulation of the motor nerve since serum collected after intermittent stimulation of the motor nerve did not modify the neuro-muscular transmission of frog.

It is likely that the passive movements of the leg resulted only in stretching the muscles without inducing any contraction. It has been shown, however, that stretching increases the metabolism, oxygen consumption, and heat production of muscle. Just as in the case of muscle contraction, these processes depend perhaps on the organic phosphate content of the muscle, since the metabolic effects of stretching are not modified by poisoning the muscles with monoiodoacetic acid.[18, 19] Stretching induces pH changes similar to those induced by contraction.[20] Since continuous stimulation of the sciatic nerve for 10 min induced the release of some substances that depressed the neuro-muscular transmission of the frog it is possible that the substances released

[18] Feng, T. P., *J. Physiol.*, 1932, **74**, 441.

[19] Feng, T. P., *J. Physiol.*, 1932, **74**, 455.

[20] Margaria, R., *J. Physiol.*, 1934, **82**, 496.

during the passive movements are also released during active movements.

The possibility that blood and tissues of patients with myasthenia gravis contain an abnormal substance or metabolite with curare-like effect cannot be excluded at present. However, the above experiments support the postulate that the symptoms of patients with myasthenia gravis and the demonstrated decrease in synthesis of acetylcholine in the presence of the serum[21, 22] and spinal fluid[23] of these patients are intimately related.

During prolonged stimulation of the preganglionic fibres of sympathetic ganglia acetylcholine synthesis takes place since the total amount of acetylcholine liberated may be several times that obtained from an unstimulated ganglion by extraction.[24, 25] If a similar synthesis occurs at the neuro-muscular synapse it is conceivable that decreased acetylcholine synthesis may lead to a decreased release of acetylcholine-like substances at the synapse during performance of work. It may be assumed that in patients with myasthenia gravis the decreased synthesis of acetylcholine leads to a disturbed dynamic equilibrium during performance of work resulting in a dominance of the agent with curare-like effect. The decreased liberation of acetylcholine-like substance at the synapse by itself, the dominance of the agents with apparent curare-like effect, and perhaps also other not yet identified processes manifest themselves clinically in the easy fatiguability of patients with myasthenia gravis.

Summary and Conclusions. (1) The effect of serum collected after passive movements of limbs of cats with occluded circulation on the neuro-muscular transmission of the frog was compared to the effect of serum obtained from immobilized limbs with occluded circulation, and from limbs with occluded circulation after intermittent or continuous electrical stimulation of the motor nerve. (2) Serum collected from passively moved limbs depressed the neuro-muscular transmission of the frog, whereas serum collected from immobilized limbs and limbs after intermittent stimulation of the motor nerve did not modify the neuro-muscular transmission. Serum collected from limbs after continuous stimulation of the motor nerve depressed the neuro-muscular transmission of the frog to a lesser extent. (3) The following postulate is offered: (a) that during performance of muscle work agents with curare-like effect are passed into the blood; (b) that in healthy subjects the effect of these substances is counteracted by sufficient amounts of stimulator substances or decomposition products thereof; (c) that in patients with myasthenia gravis the decrease of the synthesis of acetylcholine-like substances may manifest itself by the decrease of stimulator substances during the performance of work; and (d) that the lack of stimulator substances allows the usual agents with curare-like effect to manifest their presence during performance of work. The decreased release of acetylcholine-like substances at the synapse by itself, the dominance of substances with apparent curare-like effect, and perhaps also other not yet identified processes may lead to the easy fatiguability observed in patients with myasthenia gravis.

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

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

23 Torda, C., and Wolff, H. G., *Science*, 1944, **100**, 200.

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

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