# THE EFFECT OF 2-METHYLNAPHTHOQUINONE ON THE VARIOUS ELEMENTS OF THE NEUROMUSCULAR APPARATUS IN COLD-BLOODED ANIMALS

L. N. Zefirov and G. I. Poletaev

From the Department of Physiology (Head – Dr. Med. Sci. I. N. Volkova) of the Kazan Medical Institute

(Received February 20, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

2-Methylnaphthoquinone is one of the synthetic preparations of vitamin K. Several investigations [8, 9, 11] have shown that 2-methylnaphthoquinone is a powerful and, probably, a specific inhibitor of the enzyme choline acetylase, which takes part in the final stage of the synthesis of acetylcholine [6, 7].

Torda and Wolff [10] have shown that 2-methylnaphthoquinone produces an increase in the threshold of excitation of a nerve-muscle preparation, a decrease in the amplitude of the action currents of the nerve and muscle and a reduction in the rate of conduction of the excitation. These changes were attributed to disturbances in the synthesis of acetylcholine.

In addition to the purely applied pharmacological importance of vitamin K, investigations of the action of 2-methylnaphthoquinone may thus be used in order to analyze the physiological role of the chemical factor of nervous activity – acetylcholine. This latter consideration prompted the present investigation.

In this communication we give the results of myographic and electrophysiological investigations of the action of 2-methylnaphthoquinone on the various elements of the neuromuscular apparatus of the frog – the skeletal muscle, the nerve trunk and the proprioceptors.

## EXPERIMENTAL METHOD

Investigations were conducted on a nerve-muscle preparation of the gastrocnemius muscle and sciatic nerve, and partly also on the sciatic-tibial nerve trunk of the frog (R. ridibunda). The nerve-muscle preparation was immersed in a solution of 2-methylnaphthoquinone in Ringer's solution, enriched with oxygen. The concentration usually used was 1:10,000, but in some experiments smaller concentrations were used – 1:50,000 and 1:100,000. A second nerve-muscle preparation from the same frog, immersed in Ringer's solution, acted as control. In the course of the investigation the preparations were successively transferred to a humid chamber. In addition to the myographic recording, a tracing was made of the action currents of the muscle (in isometric conditions) and of the nerve by means of a two-channel cathode oscillograph with a transmission band from 0.2 to 4000 cps. The action currents of the muscle were tapped by means of silver needle electrodes, inserted into the lower part of the muscle and into the femur, and the action currents of the nerve by means of silver electrodes with an interpolar distance of 10 mm. The motor nerve was stimulated by means of an electronic stimulator. In each experiment, successive studies were made of the proprioception, the character of the action currents of the muscle in response to stimulation at various frequencies (the lability of the myoneural junction), and also the electrical activity and the functional mobility of the nerve trunk. In all 135 experiments were carried out.

## EXPERIMENTAL RESULTS

During the action of 2-methylnaphthoquinone obvious changes were observed in all the elements of the nerve-muscle preparation examined. The degree and character of these changes depended on the concentration used and the time of action of the 2-methylnaphthoquinone. The most intensive and characteristic changes,

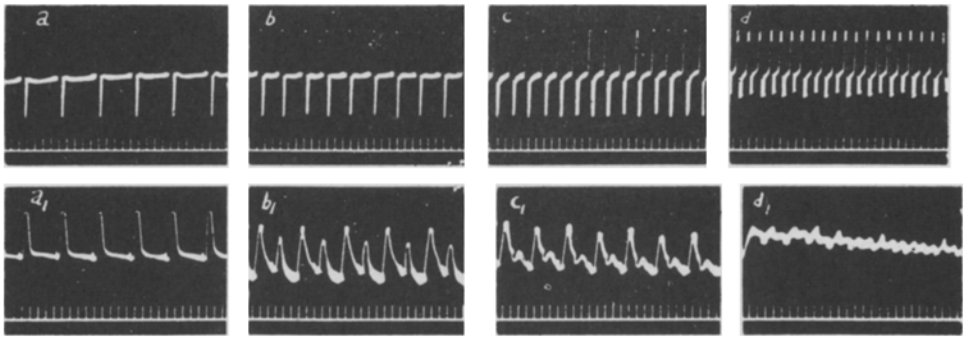

Fig. 1. Electrical activity of the muscle in response to varying frequency of stimulation of the motor nerve. Frequencies of stimulation for a, b, c and d: 20, 35, 50 and 75 per second respectively. Above) electromyograms of the control preparation; below, $a_1$, $b_1$, $c_1$, $d_1$) after 60 minutes of action of 2-methylnaphthoquinone. Time marker) 0.01 second.

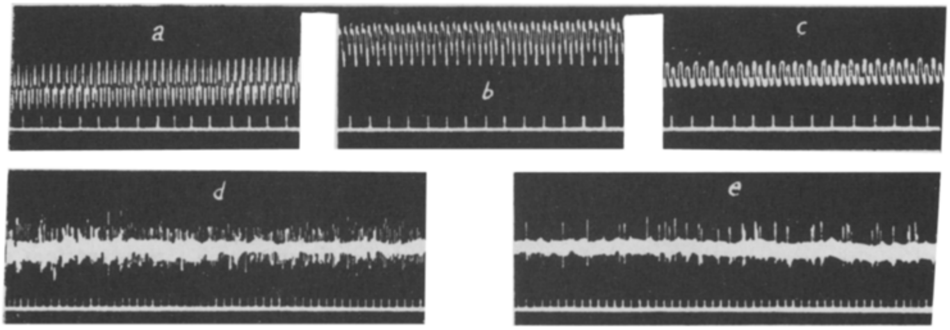

Fig. 2. Electrical activity of the nerve trunk. a) Onset of transformation of the action currents (maximal rhythm of excitation) in the control preparation (frequency of stimulation – 500 per second); b, c) the same after 90 and 120 minutes of the action of 2-methylnaphthoquinone (frequency of stimulation – 330 and 280 per second respectively); d) proprioceptive impulsation in the control preparation during stretching of the muscle with a load of 100 g; e) the same after 50 minutes of action of 2-methylnaphthoquinone. Time marker) 0.01 second.

in our opinion, were observed after 30-60 minutes of action of 2-methylnaphthoquinone. In case of longer action, exceeding 2 hours, the function of all the elements tested was sharply suppressed, in some cases to the disappearance of all activity. For this reason the changes described below will be mainly those observed in the first hour of action of the substance under examination.

Muscle and myoneural junction. In the very great majority of cases, during the action of 2-methylnaphthoquinone a persistent contraction of the muscle was observed, which began in the first minutes of immersion in the solution, and at the 45th-60th minute it amounted to 20-25% of the initial length (Fig. 1, c). This contraction was not accompanied by the development of action currents in the muscle, and its course was that of a typical contracture. Side by side with the development of the contracture, there was a fall in the indirect excitation and the magnitude of the contraction of the muscle. At the same time the residual contraction of the muscle after tetanization diminished and disappeared.

The amplitude of the action currents of the muscle in response to infrequent stimulation as a rule diminished slightly by comparison with controls, and only in two cases was the converse picture observed. The configuration of the action currents was changed: they became monophasic in character and more drawn out (Fig. 1, a, $a_1$). In response to an increase in the frequency of stimulation the action currents of the muscle diminished and became transformed, starting with a frequency of 35-75 per second (average 49 per second), whereas in the control preparations transformation arose at a frequency of 50-100 per second (average 76 per second). The lowest values and disappearance of the electrical activity of the muscle were also observed at slower frequencies than in the control preparations (93 and 110 per second; see Fig. 1, b, $b_1$, c, $c_1$, d, $d_1$). The stability of the myoneural junction was also decreased. In response to tatanization of the motor nerve at a frequency of 35 per second the action currents of the muscle completely disappeared on the average 13 seconds earlier than in the control preparations. After the more prolonged action of 2-methylnaphthoquinone (2 hours and over) a sharp suppression of the electrical activity of the muscle and the development of a complete myoneural block took place.

Nerve trunk. The amplitude and configuration of the action currents of the nerve were essentially unchanged in response to the action of 2-methylnaphthoquinone for 30-60 minutes. Only a slight depression of the second phase and some drawing out of the action currents were observed. Investigation of the optimal and maximal rhythms showed, however, that beginning with the 20th-30th minute of action of 2-methylnaphthoquinone an obvious change in these parameters was observed. The optimal rhythm of excitation, as determined by the smallest frequency to cause a decrease in the amplitude of the action currents of the nerve [5], fell on the average to 56 per second instead of 124 per second in the control preparations. The limit of transformation of the action currents, usually determined as the beginning of the appearance of an alternating rhythm, was also shifted in the direction of the slower frequencies, and averaged 234 per second instead of 390 per second in the control preparations (Fig. 2, a, b, c). It should be pointed out that the determination of the optimal and, to a lesser degree, of the maximal rhythms was the most sensitive index of the changes as they arose. Obvious movements in these parameters were thus observed in response to the action of such small concentrations of 2-methylnaphthoquinone as 1:50,000 and 1:100,000.

During the action of 2-methylnaphthoquinone the amplitude of the action currents of the nerve after 5 minutes of tetanization at a frequency of 100 per second was also more strongly diminished, which showed, in our opinion, a lowering of the functional stability of the nerve trunk. After the more prolonged action (2-3 hours and over) a progressive diminution was observed in the action currents, even to the extent of the disappearance of the electrical activity of the nerve.

Proprioceptors. After the action of 2-methylnaphthoquinone an obvious lowering of the excitation of the proprioceptors took place. At the 30th-60th minute the excitation, as determined by the value of the load applied to the muscle in order to produce minimal electrical activity of the nerve connected to the muscle, was on the average 14 g instead of 4.2 g in the control preparations. The amplitude and, in particular, the frequency of the proprioceptive impulsation were also decreased. With standard loading of the muscle of 100 g, for instance, the frequency of impulsation fell on the average to 140 impulses per second compared with 383 in the control preparations. The frequency of impulsation was most sharply lowered after 45 minutes of action of 2-methylnaphthoquinone — on the average to 52 impulses per second (see Fig. 2, d, e).

The adaptation of the receptor elements was also changed. The frequency of impulsation at the end of the first minute of stretching of the muscle with a standard load of 100 g was diminished to 44.2% of the initial value, and in the control preparations, to 56.6%. Some degree of acceleration of adaptation thus took place, which was also particularly clearly revealed after 45 minutes of action of 2-methylnaphthoquinone.

The results obtained showed that the dominant feature of the action of 2-methylnaphthoquinone, starting with the very first minimal changes, was the drawing out of the waves of excitation and of the subsequent processes, as expressed by the marked widening of the action currents of the muscle, the more rapid onset of transformation of the spikes (lengthening of the refractory period) and the appearance of monophasic action currents. Evidence of these was provided by the considerable and steady decrease of the optimal and maximal rhythms, i. e., the diminution of lability of the nerve trunk.

Another characteristic feature of the developing changes was the lowering of the endurance or working capacity of the elements that were examined; this was expressed too, by the more rapid onset of transformation, the regression and disappearance of the muscle currents and the considerable decrease in the amplitude of the action currents of the nerve in response to an accurately measured 5-minute stimulus.

During the more prolonged action of 2-methylnaphthoquinone, the most prominent feature was the marked depression and disappearance of electrical activity. This depression of the tissue functions did not take place simultaneously in the different elements, the functions of the myoneural junction and the proprioceptors were more rapidly disturbed, followed, much later, by those of the nerve trunk.

In view of the findings previously reported in the literature, the changes described were evidently to a large extent connected with interference with the synthesis of acethylcholine and with a deficiency of this chemical agent. This concerned particularly, in our opinion, the first period of action of 2-methylnapthoquinone. The character of the changes observed under these circumstances suggested that in the elements investigated, acetylcholine is a factor responsible for processes of recovery reducing the duration of stimulation and the subsequent phenomena, and hence increasing the lability of the nerve, muscle and myoneural junction.

It must be emphasizedthat similar conclusions on the physiological role of acetylcholine were made by one of us after the use of another method of interference with the acetylcholine metabolism of the body, namely by the preliminary exclusion of the pancreas by operation [2-4].

The changes in proprioception demand special discussion. There are reports [1] that a contracture of the muscle may itself cause cessation of the activity of the proprioceptors. The question thus arises, whether the changes of proprioception in our experiments were not due to the simultaneous development of a contracture. However the fairly considerable load which we used (100 g), and also the absence of any parallel between the development of contraction of the muscle and the disturbance of proprioceptive impulsation, suggest that 2-methylnaphthoquinone itself caused the disturbance of function of the proprioceptors. This provides evidence that acetylcholine plays a definite part in the process of proprioception, which is confirmed also by the negative influence of atropine on this process [1].


## SUMMARY

The authors studied the effect of 2-methylnapthoquinone on the frog's skeletal muscle, the nerve stem and the proprioceptors. A reduction of contractions and development of muscle contracture resulting from the effect of this preparation was noted. The action currents become monophasic in character and their wave length increases. Their transformation occurs with stimuli of lower frequencies. The lability of the nerve stem is also reduced. The excitability of the proprioceptors, as well as the frequency of the proprioceptive impulsation is decreased. "Prolongation" of the excitation waves and reduction in stability of the elements under investigation is, evidently, connected with disturbance of the acetylcholine synthesis and deficiency in this chemical agent.


## LITERATURE CITED


[1] G. V. Gershuni, S. P. Narikashvili, Doklady Akad. Nauk SSSR, 36, No. 3, 123-128 (1942).

[2] L. N. Zefirov, A. V. Kibyakov, Fiziol. Zhur. SSSR, 40, No. 2, 183-190 (1954).

[3] L. N. Zefirov, A. V. Kibyakov, Fiziol. Zhur. 42, No. 6, 470-476 (1956).

[4] L. N. Zefirov, Yu. G. Shapiro, Byull. Éksptl. Biol. i Med., 43, No. 1, 23-28 (1957).

[5] L. V. Latmanizova, Vvedenskii's Laws in the Electrical Activity of Excited Units, Leningrad, 1949 [In Russian].

[6] D. Nachmansohn, R. T. Cox and A. L. Machado, J. Neurophysiol., 1943 v. 6, pp. 383-396.

[7] D. Nachmansohn and I. B. Wilson, Advances in Enzymol., 1951, v. 12, pp. 259-339.

[8] C. Torda and H. Wolff, Proc. Soc. Exper. Biol. a. Med., 1944, v. 57, pp. 236-239.

[9] C. Torda and H. Wolff, Science, 1946, v. 103, pp. 645-646.

[10] C. Torda and H. Wolff, Am. J. Physiol., 1949, v. 158, pp. 465-469.

[11] M. S. Weiss, A. Voripaieff and D. Nachmansohn, Federation proc., 1948, v. 17, pp. 198.


*Original Russian pagination. See C. B. Translation.