# THE EFFECT OF 2-METHYLNAPHTHOQUINONE ON THE PARASYMPATHETIC INNERVATION AND CARDIAC ACTIVITY OF COLD-BLOODED ANIMALS

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2-Methyl-1,4-naphthoquinone (Methinone [Menadione]) is a synthetic preparation of vitamin K, the role of which in the mechanism of blood coagulation is well known. 2-Methylnaphthoquinone has also been found to be a peculiar inhibitor of the acetylcholine metabolism, inhibiting the synthesis of acetylcholine by blocking the enzyme choline-acetylase [17, 18, 20]. Because of this property, Methinone can be used to demonstrate the role of acetylcholine in elements of the somatic nervous system [3, 19].

Investigation of Methinone's effect on the heart, the classic object of parasympathetic innervation, is of great interest in this connection, especially since the question of the mechanism of the effect exerted by parasympathetic nerves on the heart and the role of acetylcholine in this mechanism is still under discussion [5, 6, 10, 11, 12].

## METHOD

Experiments were performed on a frog's heart (R. ridibunda). In some cases, we used a heart isolated according to Shtraub, with intact innervation; in other cases, the heart action was studied under conditions of natural circulation. In the latter experiments, the heart and the vagosympathetic trunks were dissected out without disturbing the central nervous system. The nerves were stimulated with Kronecker's inducing apparatus, powered by a 4-volt storage battery.

We studied the effect on the isolated heart of 2-methylnaphthoquinone solutions in concentrations of 1 : 10,000-1 : 100,000; the original Methinone solution was prepared in a boiling Ringer's solution in a dilution of 1 : 5000. For intravenous administration, we used a saturated solution of 2-methylnaphthoquinone in a dose of 3 ml, i.e., 0.1 mg of the substance per 10 g of frog weight. More than 100 experiments were performed.

## RESULTS

Experiments on isolated heart. The way in which the cardiac activity and the effect of the extracardial nerves changes under the influence of 2-methylnaphthoquinone depended on the concentration of the solution used. Low concentrations (1 : 50,000- 1 : 100,000) usually did not cause changes in the frequency or force of the cardiac contractions (Fig. 1,a,b). After Methinone was introduced into the cannula, however, we observed marked changes in the parasympathetic effects upon stimulation of the vagosympathetic trunk. These changes primarily affected the inotropic component of the parasympathetic effect; it decreased quickly, then disappeared completely during the first 5-10 minutes. In this case, the amplitude of the contractions did not decrease as usual during stimulation of the nerves, but remained unchanged (Fig. 1,b). The chronotropic component of the vagal effect was more stably retained, being maintained in many cases throughout the experiment, although considerably reduced in degree.

The vagal arrest of the heart proved to be of medium stability. Over a period of 30-50 minutes, its duration gradually decreased (Fig. 1,b) until, about the end of the first hour, it usually disappeared completely. At this time, stimulation of the vagosympathetic trunk either did not cause parasympathetic effects or caused only a slight retardation of the rate of the contractions. Under these conditions, stronger stimulation did not change the character of the heart's reaction. This and the fact that the force of the threshold stimulation did not change during the experiment indicate that the disturbances observed with the action of Methinone on the isolated heart were not connected with changes in the excitability of the parasympathetic nerves.

These same concentrations of Methinone did not cause any essential changes in the sympathetic effect, which remained after the vagal effect had completely disappeared (Fig. 1, c).

When Methinone was used in a concentration of 1 : 50,000, all the changes described developed more rapidly and were more apparent than with the 1 : 100,000 concentration. Moreover, in some experiments in which the 1 : 100,000 Methinone solution was used, we observed the spontaneous restoration of the vagal effect after its disappearance, which never happened when the 1 : 50,000 concentration was used.

Washing out with a Ringer's solution restored all the parasympathetic effects except the negative inotropic effect (Fig. 1, d).

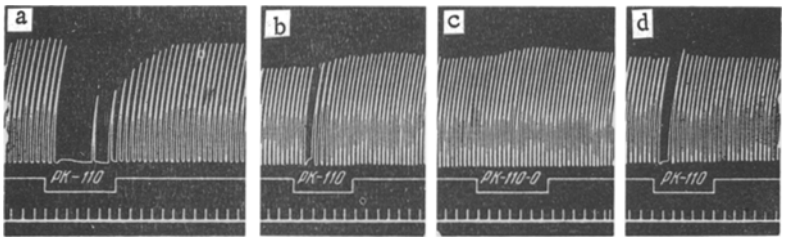


Fig. 1. Effect of Methinone (1 : 50,000) on the parasympathetic effects of an isolated heart. a) Before action of Methinone; b) 25 minutes after introduction of Methinone solution into cannula; c) after 45 minutes; d) 15 minutes after start of washing out the heart with a Ringer's solution. Curve (from top to bottom) show: recording of heart contractions, indication of stimulation of vagosympathetic trunk, time indicated in 5-second marks.

The changes caused by the higher concentrations of Methinone (1 : 10,000-1 : 20,000) were primarily the same in type and sequence as those caused by the lower concentrations, but were quicker to develop. The inotropic component of the vagal effect disappeared almost immediately after the introduction of Methinone, during the first few minutes of its action. The chronotropic component and cardiac arrest were observed for a somewhat longer period, up to 10-20 minutes. In contrast to the preceding experiments, the prolonged action of 2-methylnaphthoquinone in the higher concentrations caused irreversible depression of the parasympathetic effects. Another peculiarity of the effect of these concentrations was the fact that after the disappearance of the vagal effects, the sympathetic effects also became depressed.

The disturbances which occurred in the extracardial innervation with the action of the higher Methinone concentrations took place on a background of acute change in the heart's contractility and automatism. The amplitude of the contractions decreased, and their rhythm became progressively slower (Fig. 2, a,b). Subsequently,

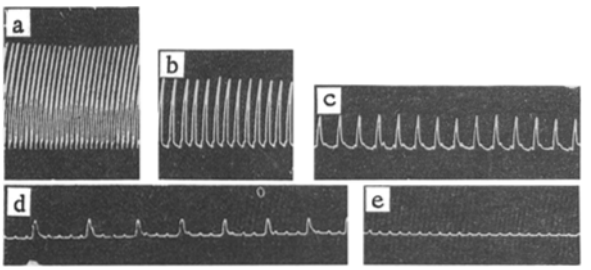


Fig. 2. Change in the activity of an isolated heart caused by high concentrations (1 : 10,000) of Methinone. a) Before introduction of Methinone; b,c,d) 40, 60, and 80 minutes respectively, after introduction of Methinone solution into cannula; e) the same after 100 minutes (auricles only contracting). Cylinder rotation rate the same as in Fig. 1.

acute disturbances in the conduction of automatic impulses developed, causing disaaciation of the auricular and ventricular contractions,and, later, the development of atrioventricular block (Fig. 2,c,d,e). Ventricular arrest usually occurred during this period, and while the less frequent sinal automatism was preserved; in some cases, the automatism of the sinus and ventricle each had an independent rhythm.

Experiments introducing 2-methylnaphthoquinone into the blood. The introduction of Methinone into the blood also disturbed the parasympathetic effects. The vagal cardiac arrest usually disappeared five minutes after the administration of the preparation. Sometimes it was replaced by a negative chronotropic effect, which persisted longer and disappeared gradually (Fig. 3, b,c,d). In all the experiments of this series, the vagal effects were spontaneously restored in an order opposite to that of their disappearance.* The development rate and degree of the disturbances and the restoration of the parasympathetic effects specifically depended upon the amount of Methinone introduced into the blood: the changes caused by the smaller doses were slower to develop and less complete, and restoration occurred more rapidly than with the administration of the larger doses. In these experiments, there was usually no change in the contractility of the heart.

In the control experiments, in which similar amounts of a Ringer's solution were (3 ml) introduced into the frog's blood, neither the activity nor the parasympathetic effects of the heart were affected.

The results obtained showed that 2-methylnaphthoquinone possesses peculiar cholinolytic properties and that it depresses the effect of the parasympathetic nerves on the heart. However, the disturbances in the parasympathetic effects caused by Methinone are not due to a decrease in the excitability of the vagus nerves or to a decrease in

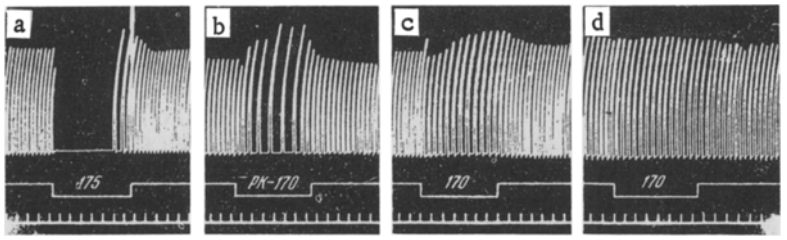


Fig. 3. Change in the parasympathetic effects after the intravenous injection of Methinone. a) Before injection; b, c, d) 5, 10, and 40 minutes respectively after the injection. Curves the same as in Fig. 1.

the heart's sensitivity to acetylcholine. In special experiments, we found that Methinone in a concentration of 1 : 50,000, which causes the gradual disappearance of the parasympathetic effects on an isolated heart, does not alter the sensitivity of the heart to pharmacological acetylcholine.

The literary data on the anti-choline-acetylase activity of 2-methylnaphthoquinone suggest that the explanation of the results obtained is disturbance of the acetylcholine metabolism and liberation of acetylcholine in the parasympathetic nerve endings. The results of the experiments with low concentrations of 2-methylnaphthoquinone acting on an isolated heart indicates that their effect is specific and is not attended by any change in the muscular elements.

The research on the effect of 2-methylnaphthoquinone permits certain conclusions about the physiological significance of acetylcholine in the parasympathetic innervation of the heart. The experiments described above demonstrated that the vagal effects are depressed in a definite order with the action of Methinone: the inotropic component is the first to disappear, followed by the vagal arrest of the heart and, lastly, by the chronotropic effect. This sequence evidently reflects the degree to which acetylcholine participates in the development of these effects. It is interesting that the changes caused by atropine in the effect of the vagus on the heart occur in an opposite order [4].

On one hand, the material obtained confirms the fact that the mechanisms of the inotropic and chronotropic effects of the parasympathetic nerves on the heart and the degree to which the chemical factor, acetylcholine, participates in these mechanisms differ considerably [5, 6, 8, 11, 12]. On the other hand, however, our experiments showed that acetylcholine plays a specific part in the formation of the chronotropic effect and, particularly, the cardiac arrest. We believe the hypothesis can be expressed that acetylcholine is not the direct cause for the development of inhibition in the automatic center in the case of arrest and a negative chronotropic effect, but only promotes the effect of the excitatory, electro-ionic component of the parasympathetic pulses; acetylcholine to some extent determines the direction and stability of the polarization changes, which are evidently caused by inhibition of the "automatism" center [7, 11, 12, 13 et al.]. In our opinion, acetylcholine plays the same role in the latter case as in the elements of the somatic nervous system [ 1, 2, 3].

The effect of higher concentrations of Methinone on the heart must be evaluated somewhat differently. The disturbance we observed in the automatism and the function of the conduction system could be caused by more profound disorders of the acetylcholine metabolism. There are data which indicate that acetylcholine plays an important part in the origin of cardiac automatism and in the function of the conduction system [9, 14, 15 et al.]. However, we are inclined to think that the disturbance of cardiac activity caused by high concentrations of Methinone is due not only to disturbance in the acetylcholine metabolism, but to the non-specific, alterative effect of 2-methylnaphthoquinone as well, which is further confirmed by the clearly expressed depression of myocardial contractility observed with the action of high concentrations.

## SUMMARY

When acting upon the isolated heart or administered into the blood flow, concentrations of 2-methylnaphthoquinone (1: 50,000-1: 100,000) provoke a decrease and disappearance, first of the inotropic component of the parasympathetic effect, then the vagal arrest, and, lastly, the inhibition of chronotropic component. These changes are caused by the disturbance of the acetylcholine metabolism and the liberation of this substance in the nerve endings of the vagus. Besides, large concentrations (1: 10,000) render a nonspecific altering effect on the cardiac elements.

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* The spontaneous restoration of the vagal effects, sometimes observed with the action of weak Methinone solutions (1 : 100,000) on an isolated heart and always observed with the introduction of Methinone into the blood, seems to be due to a gradual decrease in the active concentration of the vitamin as a result of its fixation by protein substances [16].

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