should be noted that ANS is localized in hydrophobic regions of the membrane [12], whereas DT is bound only by membrane proteins and is localized on the outer surface of the plasma membrane [1]. It can accordingly be postulated that only the surface regions of the membrane were changed in the erythrocytes of rats with myocardial infarction.

The 7th day is thus the critical period of development of experimental myocardial infarction in rats. This period is characterized by the most marked mechanical and functional changes in the erythrocytes. The increase in erythrocyte aggregation observed at this stage of development of the infarct and the maximal changes in activity of the membrane enzymes and in the structure of the erythrocyte membrane suggest that the changes taking place in functional and mechanical processes in this disease are interconnected. The development of a myocardial infarct, especially in the acute period, not only leads to a disturbance of the rheologic properties of the blood, but also, probably, significantly changes the metabolic state of the erythrocytes.

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INVESTIGATION OF CARDIAC EXCITABILITY THRESHOLDS DURING ELECTRICAL STIMULATION

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KEY WORDS: excitability of the myocardium; pulse charge; pulse duration; pulse voltage; threshold characteristics.

The threshold characteristics of the excitability of the myocardium during its electrical stimulation are of great practical interest, for they are widely used when implanted artificial pacemakers are designed, and in particular, when the parameters of the stimulating pulse are chosen.

In this paper we examine defects of known methods of obtaining threshold characteristics of excitability of the heart and we suggest a simple method of determining them, based on direct measurements of the pulse charge.

During stimulation of the heart by square pulses [1-3] (as is used in the overwhelming majority of implanted pacemakers), to give a complete quantitative description of excitabil-

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ity of the heart it is necessary to know dependences of the following basic values on the pulse duration tu*:

Q — the pulse charge ($Q = \int_0^{tu} I_u \cdot dt$, where I_u is the momentary value of the current in the pulse;

U — the amplitude of the pulse voltage;

W — the pulse energy.

These three values are connected with each other by the equation

$$W = U \cdot Q. \tag{1}$$

It is therefore sufficient to have only two dependences, for example $U = F_1(tu)$ and $Q = F_2(tu)$ in order to characterize the excitability of the heart completely.

Usually in experimental studies of threshold characteristics of cardiac excitability only the amplitude U of the voltage pulse and its duration tu are measured directly. The pulse charge Q is determined by calculation by means of simplified equations, the impedance of the input circuit of the heart being taken to be purely active, and the shape of the pulse current to be square. Since this impedance has a considerable capacitive component [2], the shape of the pulse current will differ significantly from square.

This leads to great errors in determination of the charge, and this error increases with a decrease in pulse duration. In some cases the method of determination of the charge and energy of the pulse by calculation may actually distort the character of the relationship $Q = F_2(tu)$ and $W = F_3(tu)$.

The value of the pulse charge can be determined more accurately by determining the area of the pulse of current on the trace. However, this method is very laborious and requires special equipment.

We give below a description of a simple method of measuring the pulse charge. The method is based on measurements of the mean value of the current I_{mean} flowing through the heart, and the period T of succession of the pulses. Under these circumstances the pulse charge is determined by the equation

$$Q = I_{mean}\, T. \tag{2}$$

The convenience of investigation of the principal threshold characteristics by this method is that all the required values can be recorded directly by the measuring instruments, and this considerably speeds up the measuring process and increases the accuracy of the measurement. This is particularly important in clinical investigations, when as a rule time available for the test is limited.

Another advantage of this method is that the charge can be measured accurately whatever the shape of the voltage and current of the pulse, and this is particularly important in cases when the heart is stimulated by pulses that are not square in shape.

The experimental set-up contains a controllable voltage source, a square-pulse generator, an output transitor working under key conditions, a microammeter to measure the mean value of the current, a battery of capacitors a frequency meter to measure the period of succession of the pulses, an oscilloscope, and electrodes.

The scheme operates as follows. The output transistor periodically connects the power source to the heart. In that way the heart is stimulated by square pulses of voltage. The transitor is controlled by the generator by means of which the duration and following frequency of the stimulating pulses are changed. The amplitude of the pulse is controlled by a change in the voltage of the voltage source.

The period of succession of the stimulating pulses is measured by the frequency meter and their amplitude and duration are monitored on the oscilloscope.

The current flowing through the heart is pulsed in character and contains steady (the mean value of the current) and alternating (the higher harmonics) components. To measure the

*Here and subsequently we refer to threshold values of the parameters.

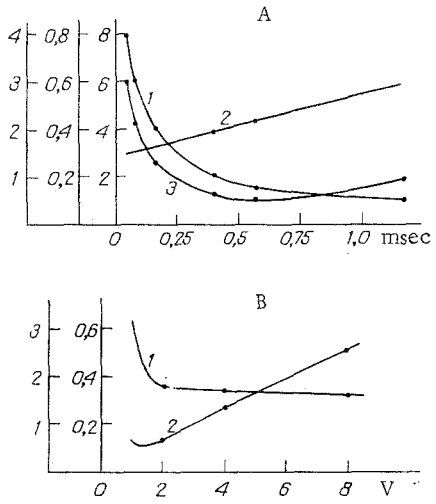

Fig. 1. Threshold characteristics of excitability of the heart. A) Dependence of threshold amplitude of charge and energy of pulse on its duration; B) dependence of threshold energy and charge of pulse on its amplitude.

mean value of the current, parallel with the microammeter, a battery of capacitors is included in the circuit. In this case the steady component of the current passes through the microammeter and the alternating component passes through the battery of capacitors. For effective filtration of the alternating component the capacity of the battery of capacitors must be chosen in accordance with the condition

$$\frac{1}{2\pi f_1 C} \ll RA, \tag{3}$$

where f_1 is the following frequency of the pulses and RA the internal resistance of the microammeter.

It will be clear from conditions (3) that for small values of RA the required capacity rises sharply. In these cases, a small additional resistance can be connected in series with the microammeter, which has practically no effect on the accuracy of the measurements.

The error of measurement of the charge in this case is determined by the error of the measuring instrument and by leakage currents of the capacitors, and it usually does not exceed 2-3%.

In order to test this method threshold characteristics of excitability of the frog's heart were studied during monopolar cathodal stimulation by square pulses.

The following measuring instruments were used for the experiments: as microammeter a type VK2-20 digital voltammeter, as the measurer of the period of succession of the pulses a type 43-33 electronic-calculating frequency meter, and as the oscilloscope an oscilloscope of type S1-55. The apparatus included a battery of capacitors of the type K53-1 with a total capacitance C = 10,000 μF.

An artificial rhythm was imposed by fixing the frequency of the stimulating pulses at a higher level than the natural frequency of the cardiac rhythm. The reference electrode was placed beneath the frog's spine. The active electrode, made from platina, was applied to the ventricle. Experiments were carried out on 12 frogs.

The experiments showed that the suggested method can be used to measure sufficiently accurately all threshold values of excitability of the heart.

Dependence of the amplitude, charge, and energy of the pulse on its duration is shown by the curves in Fig. 1A. Dependence of the charge and energy of the pulse on the amplitude of the voltage is shown in Fig. 1B.

In the working zone of stimulation (2-10 V) the pulse charge remains practically constant. This is confirmed by the results of experiments on dogs [3].

The results suggest that the threshold charge depends less on the conditions of stimulation and is therefore one of the most important characteristics of excitability of the heart. For that reason, exact determination of the charge is an additional advantage of the suggested technique.

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ERYTHROPOIETIN-INDEPENDENT STIMULATION OF ERYTHROPOIESIS IN MICE INFECTED WITH *Mycoplasma arthritidis*

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KEY WORDS: mycoplasma; erythropoiesis; erythropoietin; plethora; endogenous colonies.

Previously [2] we reported the ability of *Mycoplasma arthritidis* to stimulate endogenous colony formation in sublethally irradiated mice. A subsequent study of the stimulation of hematopoiesis induced by mycoplasmas suggested that *M. arthritidis* may perhaps act not on hematopoietic stem cells, but on already committed precursor cells, most likely of the erythroid series [3].

The object of this investigation was to attempt to shed further light on this problem.

EXPERIMENTAL METHOD

BALB/c and (C57BL/6 × A/Sn)F_1(BAF_1) mice aged 8-10 weeks were obtained from the Rappolovo nursery, Academy of Medical Sciences of the USSR.

The mycoplasmas were obtained as described previously [1] and kept at −70°C. Mice were infected intraperitoneally with a dose of 0.5 ml of mycoplasmas with a titer of 2×10^8 colony-forming units/ml. Nutrient medium for growth of the mycoplasmas was injected into control mice.

Plethora was created by the method of Curray et al. [4]. Blood of heparinized donors was washed twice and the erythrocytes resuspended in sterile physiological saline. A 60% erythrocyte suspension was injected intraperitoneally into the mice in a dose of 1 ml 4 and 2 days or 3 and 1 days before irradiation. The mice were irradiated in a dose of 550 and 920 rads. Hematopoietic cells were cloned *in vivo* in lethally irradiated mice by the method of Till and McCulloch [6]. The mice were killed on the 7th-9th day after irradiation, the spleens were fixed in Bouin's solution, and the number of visible colonies was counted macroscopically after 4 h.

The appearance of endogenous erythropoietin (EP) in intact BAF_1 mice and in mice infected with mycoplasmas 3 and 1 days before sacrifice was tested by the use of blood plasma which was inactivated (30 min at 56°C) and kept at −20°C. The test plasma was injected into mice with plethora 1, 3, and 4 days after the second transfusion, and on the 5th day 0.5 μCi ^{59}Fe in 0.5 ml physiological saline was injected intravenously. The mice were killed 24 h after injection of the isotope. Radioactivity in the spleen and blood was counted on a Nuclear Chicago Gamma-Counter and expressed relative to the activity injected. The blood volume was taken to be 5% of body weight. Plasma from anemic mice, in which blood loss was produced 48 and 24 h before removal of the plasma by taking 0.4-0.5 ml of blood from the anterior chamber of the eye, was used as the source of exogenous EP.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of *M. arthritidis* on endogenous colony

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