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METHODS

A Method to Study Photoreactivity of Isolated Rat Heart

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A method of rat heart isolation is described. A comprehensive description is given of the apparatus to record the contractile activity of spontaneously beating heart, which makes it possible to synchronize irradiation of the heart by the low-intensity laser light with the cardiac cycle phases.

Key Words: *isolated rat heart; He-Ne laser; cardiac pacemaker; photoreactivity; chronobiology*

The chronobiological aspects of photosensitivity in living tissues [1,2] are important in the study of the effects of low-intensity laser radiation on self-excited system of isolated heart. Isolation of the heart by the Langendorff technique needs both experimental skill and adequate instrumental setup. We describe a modified technique to isolate the heart, as well as a method to record its activity and to control the modes of photostimulation.

The layout of the setup is shown in Fig. 1. In order to excise the heart from an ether-narcotized rat weighing 180-200 g, the thorax must be opened, the heart quickly cut out, and transferred to a Petri dish with ice-cold Krebs-Henseleit solution. Cardiac beats stop in few seconds. Without taking the heart from the solution, it is cleaned from adjacent tissues. After the blood is pumped out from the cardiac chambers by a light massage, the aortic arc is isolated. To perfuse the heart we used a double aortal cannula (Fig. 1, *1*). The rear (holder-attached) part of the cannula had two polished sections (for thermometer and cannulation) and two branches for introduction and disposal of the perfusate. The second branch served to maintain temperature of the solution at the steady-state level. The holder part of this cannula was fixed in a support. The demountable part of the cannula was inserted into aorta in such a way as to place its tip over the valve; thereafter, it was fixed by a serfin (a clamp) with oval drippings. Cannulated heart was taken out from Petri dish, rapidly fixed to the holder section, and placed into a double-wall glass chamber which had orifices for the transducer thread and laser beam (Fig. 1, *2*). The mounted heart was perfused with oxygenated Krebs-Henseleit solution (in mMol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; Na_2-EDTA, 0.5; glucose, 5.5; pH 7.4 [3] maintained at 37°C and 60 cm water column. Stationary pressure in the aorta was maintained with the help of a Mariotte vessel (Fig. 1). From this vessel the solution flew into a burette via a glass tube with curved tip (to prevent inflow of gaseous bubbles), and was oxygenated under a low pressure. Isolated heart started beating spontaneously after 30-60 sec. Under these conditions rat heart beats for 2-3 h. The apex of the heart was ligated and attached to the photo-transducer shutter. A 5-10-g load was applied to the heart via the ligature connecting it with the shutter. To stabilize the cardiac parameters, the heart worked during 15-20 min at the beginning of an experiment.

Cardiac contractile activity was recorded with a phototransducer consisting of a FD-24 photodiode

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mounted in the common light-tight cell with a light source (Fig. 1, *3*). The lamp power voltage (24 V) must be proper stabilized. A light-tight shutter (ligated to the heart's apex) could move between the phototransducer and the illuminating lamp. The signal from the phototransducer was fed into an amplifier and then into an analog-digital converter (ADC, Fig. 2). In this device, a quartz crystal generator sends 1.2 MHz pulses to the divider, which decreases the frequency down to 200 kHz to be fed to the clock input of an 8-bit ADC chip. The second divider produces pulses which determine the digitization frequency (60, 150, and 300 Hz) as well as the rate of data transmission to PC, which can be set by the respective switches. The pulses of the chosen digitization rate come to the "start" input of ADC chip. The data transmission rate switch feeds pulses to the input of a CT/2 binary counter (a sequencer), which rolls on the input addresses of a MX multiplexer. In order to synchronize data transmission to PC with the final moment of digitization, ADC chip generates the "ready" signal and resets the flip-flop T to zero state. This state enables operation of the binary counter to roll on the addresses of the multiplexer, thereby starting the data transmission. When transmission of a single byte is finished, the counter sends a signal to S-input of the flip-flop, which stops the counter until a new "ready" signal is generated at the output of ADC chip. PC performs a real-time calculation of the moment of signal generation and sends a single pulse to the control unit of the laser beam interrupter consisting of an electromagnetic relay, which had a light-tight blinder fixed to the armature. This pulse is amplified and fed via an OR logical element to the input of a JK flip-flop. The amplified signal of flip-flop output is applied to the armature winding of the interrupter. The control button "Phase" controls generation of a single pulse,

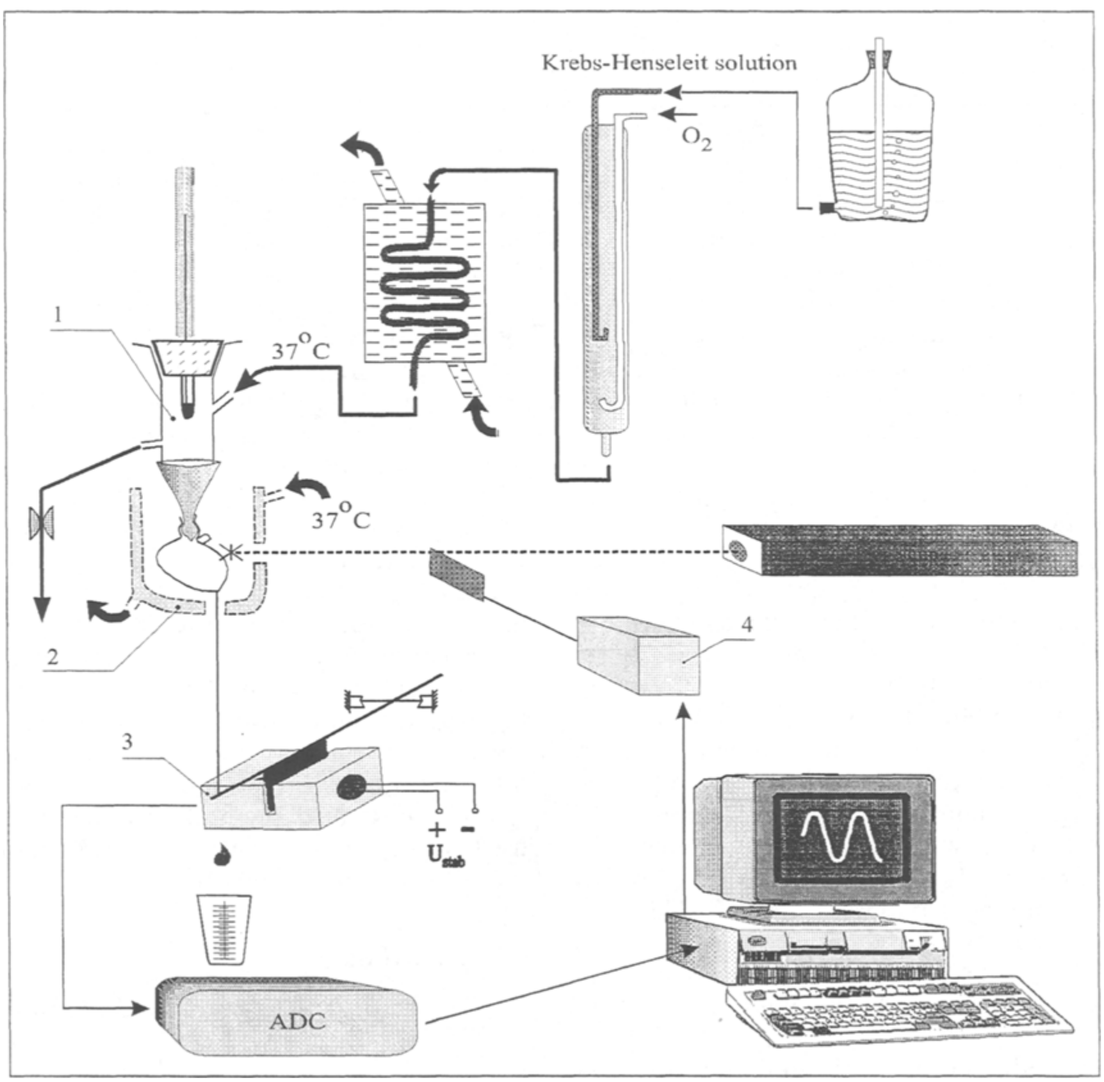

Fig. 1. A scheme of set-up for experiments on isolated rat heart.

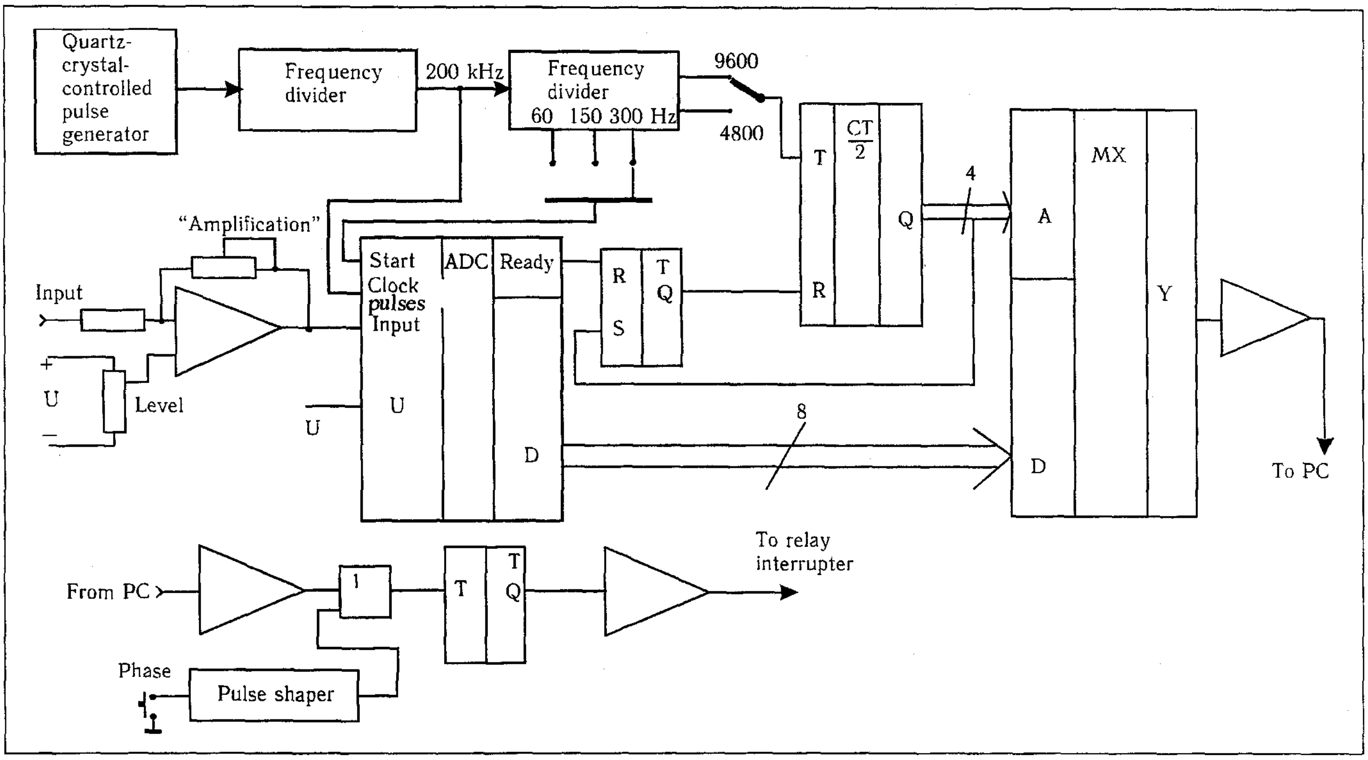

Fig. 2. A functional scheme of an analog-digital converter.

which sets the interrupter relay to the initial state when the system is turned on. When the calculated moment of the end of radiation is attained at the current cardiac cycle, PC generates a single pulse again, which resets the flip-flop to the initial state.

To tune the setup, the following parameters should be chosen: the digitization frequency 60-300 Hz; transmission rate to PC (4800 or 9600 baud); amplification coefficient, and the bias of the input signal.

To record cardiac parameters and control the laser radiation in the real time, it is recommended to use IBM PC/AT 486DX4 with at least 8 MB on-line storage.

In the experiment, the data are transmitted from ADC to PC, which processes them in the real time and displays the results as a plot, demonstrating cardiac contraction. In addition, the following parameters are shown numerically: amplitude and length of cardiac cycle, diastolic pause interval, and duration of myocardial contraction and relaxation.

The original software can chose a fragment of cardiac cycle to be shown on the display, in which the heart should be irradiated with He-Ne laser beam. The accuracy of the mode of radiation is controlled on the display by indication of the time and amplitude parameters of radiation at its start and finish.

Once the radiation period is chosen on the cardiac contraction curve, the software is switched to automatic mode. The on-line storage acquires data from ADC. The display shows mechanogram, and basic parameters of radiation and the current cardiac cycle. Simultaneously, irradiation of the heart is performed at the chosen fragment of the cardiac cycle. If the amplitude and length of the cardiac cycle vary during experimental procedure, PC corrects the mode of radiation automatically (Fig. 1, *4*).

The original software makes it possible to survey heart performance on the display, where the intervals of radiation are indicated, as well as the parameters of every cardiac cycle of previous experiments. PC calculates heart rate, diastolic pause interval, systolic and diastolic intervals, amplitude of heart beat and its minimal value, as well as the rate parameters of every cardiac cycle: maximum rate of contraction and relaxation. All data can be stored both as the raw digital values transmitted from ADC, or as calculated parameters of every cardiac cycle ready for off-line statistical processing.

The described method and the set-up can be used in any studies with pulse beam radiation directed at contractile objects.

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