METHODS

APPARATUS FOR PHOTOELECTRIC RECORDING OF AMPLITUDE OF CONTRACTION OF CHICK EMBRYONIC MYOCARDIAL CELLS AND EXPLANTS

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An apparatus for photoelectric recording of the frequency and amplitude of contractions of chick embryonic myocardial cells and explants is described. It is built from components of the EKS-60 Soviet scintillation electrokymograph.

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In conjunction with the engineers G. Ya. Kyandaryan and S. G. Arzumanyan, and apparatus working on the photoelectric principle has been developed for making graphic recordings of the frequency and amplitude of contraction of embryonic myocardial cells and explants in tissue culture.

The apparatus suggested for this purpose by Bucher was built from components which he constructed himself, and it is so complicated that other workers have not used it.

We have made an apparatus based on the ÉKS-60 electrokymograph, manufactured in the Soviet Union, which is intended for roentgenologic study of pulsations of the outlines of the heart, great vessels, and vessels of the lungs. Without any basic changes in design it can easily and rapidly be adapted for recording contractions of explants of the heart and of individual embryonic heart cells.

The apparatus is mounted on two movable tables and consists of the following parts: a photoelectronic multiplier, a combined amplifier and recorder, a two-channel oscilloscope, and a power unit.

The type FÉU-35 photoelectronic multiplier is mounted in a steel box, the upper edge of which is fitted hermetically on a special adapter from the set supplied with the type FMS-3 photomicrography camera. The adapter is fitted to the MBR-1 microscope instead of the monocular tube. A prism is incorporated in the body of the adapter, and is moved out of the way of the rays of light during recording by means of a handle. Observations on the object are made through the tube of the adapter.

The amplifier-recorder consists of a two-channel photoamplifer mounted along with the power unit on a movable table.

Power for the oscilloscope and also for the amplifier-recorder and photoelectronic multiplier is supplied from a power unit including a rectifier and stabilizer. The unit is connected to the 127 or 220 V ac main supply.

Because of the high sensitivity of contracting explants and cells to fluctuations of temperature, a special thermopile mounted on the stage of the microscope was used.

The source of light was a low-voltage motion picture projection lamp (12 V, 100 W) supplied through a reducing transformer. To prevent any stray light from falling on the amplifier, distorting the recording considerably, a diaphragm was placed in front of it and shut down to the required size by turning a handle.

The principle of operation of the apparatus is as follows. A beam of light passing through the microscope condenser and meeting the contracting margins of the cell or explant is set into oscillation, and this, passing through the diaphragm on to the FÉU-35, is transformed into electric pulses. These fall on the two-channel photomultiplier, where they are amplified and filtered to remove electrical interference. The

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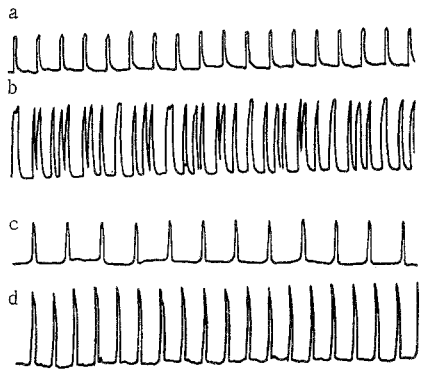

Fig. 1. Photoelectrogram of contracting chick embryonic myocardial explants. a) Initial recording (frequency of contractions 110/min); b) 5 min after addition of strophanthin in concentration of $1 \cdot 10^{-5}$ (arrhythmia); c) initial recording (frequency of contractions 75/min); d) 10 min after addition of methyluracil in concentration of $1 \cdot 10^{-5}$ (frequency of contractions 125/min).

amplified signal from the photomultiplier is fed into the end cascade of an electrocardiograph, from which it is carried to the ink-writing unit. Electric pulses are simultaneously fed from the output cascade of the cardiograph amplifier into the input of the oscilloscope.

To record contractions the explant (cell), fixed between cover slips in the perfusion chamber, is placed on the stage so that it is in the center of the diaphragm. Observations are made in the microscope with the 8× objective for explants, the 40× objective for cells, and the 10× ocular. After connecting the apparatus to the main supply, the mode of operation on the panels of the electrocardiograph and photomultiplier is switched into the "motion picture" position, while the switch on the oscilloscope is placed in the "pulse" or "respiration" position. The control on the microscope adapter is switched over to recording and observations are made on the oscilloscope screen. Clarity of the recording is controlled by changing the size of the slit of light between the contracting edge of the cell or explant and the aperture of the diaphragm. After regulation of amplification and obtaining a clear recording on the oscilloscope screen, the motor of a tape-winding mechanism is set in motion and recording carried out on tape moving at 12.5 mm/sec.

To test the effect of a substance on the spontaneous rhythm of isolated cells and explants, it is injected in the necessary concentration into the chamber through the opening in the top by means of a micropipet. The recording is then carried out in the same order and with the same amplification.

A series of tracings was made by means of this apparatus of the contractions of chick embryonic heart explants (Fig. 1). The results show that strophanthin, in concentrations of $1 \cdot 10^{-5} - 1.4\,10^{-6}$ sharply increases the amplitude and frequency of contractions and produces arrhythmia. Methyluracil in a concentration of $1 \cdot 10^{-5}$ has the same action but without arrhythmia.

LITERATURE CITED

1. O. M. Bucher, Exp. Cell Res., 13, 109 (1957).