A CRYOGENIC METHOD FOR USE IN THE DIAGNOSIS AND TREATMENT OF CARDIAC ARRHYTHMIAS

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Electrical coagulation or mechanical or chemical action on an arrhythmia-generating focus are not always effective but may be accompanied by postoperative complications: aneurysms, cardiac septal defects, or thrombosis [6]. The use of cryogenic destruction of ectopic foci in ventricular tachycardias and of conducting pathways in the Wolf–Parkinson–White syndrome enables these complications to be avoided [3, 4]. However, because of the demanding parameters (acting temperature −60°C, duration of action 60-120 sec) cryodestruction is ineffective in some cases [4, 5, 7], for during operations on the heart only visual control of the size of the superficial zone of freezing can be used (L_f). Considering differences in the thickness of the myocardial wall (ranging from a few millimeters to a few centimeters), this criterion can be regarded only as a relative guide when determining the effectiveness of cryogenic destruction.

For the reasons given above it has become evident that for clinical application of the method of cryogenic destruction to be successful in the treatment of cardiac arrhythmias, further study of the optimal conditions for cryogenic action on the myocardium is necessary.

This paper describes the first attempt at establishing a theoretical and experimental basis for a program of cryogenic destruction: duration of exposure, acting temperature, depth of necrosis in experimental cardiac arrhythmias.

Two cryogenic apparatuses of expanding gas type with a working filling volume of 500 ml, operating at three different temperatures (0°C, −60°C, −150°C) for 15 min with a single filling of the apparatus with the coolant, namely liquid nitrogen, were used. In one apparatus adjustment of the two valves could change the level of heat exchange, in which case the range of temperatures at the point of contact with the myocardium could range from −60°C to −80°C or from −130°C to −150°C. The other apparatus (Fig. 1b) used an original principle of controlling the acting temperature. Using the principle of thermal resistance, the temperature at the point of contact could be changed depending on the length of a copper rod which could move freely in the working chamber, and which during filling of the apparatus with the coolant was securely fixed in the lumen of a fluorine plastic ring. Fluctuation of the tip temperature on establishment of steady-state conditions did not exceed ±5°C. The necessary length of the rod of the cryogenic apparatus was calculated by the equation of balance:

$$l_t = \frac{C_{N_2} m_{N_2} (t_o - t_{N_2}) - C_m m_m (t_m - t_c - t_o)}{\pi r_o^2 \rho_t C_t (t_o - t_t)} - Q_{PT} \ , \quad (1)$$

where l_t is the required length of the tip; C_t, C_m, and C_{N_2} are the specific heats of the tip, myocardium, and liquid nitrogen, respectively; m_{N_2} and m_m the weights of liquid nitrogen and myocardium, respectively; t_o, t_{N_2}, t_m, and t_t the temperatures of the operating theater, the liquid nitrogen, myocardium, and tip, respectively; ρ_t the density of copper; r_o the radius of the tip. Dependence of the length of the rod of the cryogenic apparatus on acting temperature is illustrated in Fig. 2a. The length of the working part of the rod for temperatures of 0°C, −60°C, and −150°C was 400, 100, and 45 mm, respectively (r_o = 3 mm).

The ratio of the depth of necrosis to the time of cryogenic action was calculated by the phase transition equation (1), and for conditions of cardioplegia this can be written in the following form:

$$L_n = \alpha \sqrt{t} \ \frac{(T_f - T_t) - \varphi) T_f - T_n)}{T_n - T_t} \ , \quad (2)$$

where L_n is the depth of necrosis; t the duration of cryogenic action; α the experimentally calculated coefficient characterizing the rate of freezing of the myocardium, namely 0.015 cm/sec$^{1/2}$ for −60°C and 0.009 cm/sec$^{1/2}$ for −150°C; T_f the

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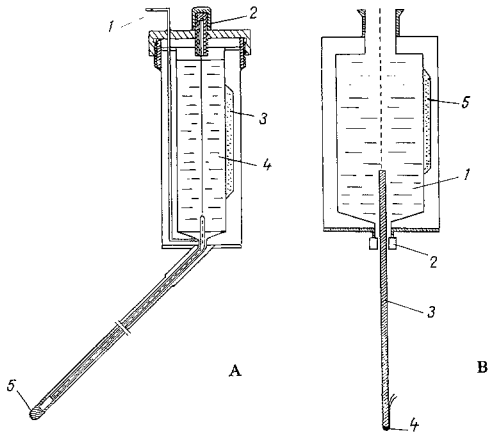

Fig. 1. Diagrams of cryogenic apparatuses: two-valve type (A), and model working on thermal resistance principle (B). A: 1, 2) Valves, 3) carbon getter, 4) container for cryogenic agent, 5) tip; B: 1) container for cryogenic agent, 2) fluorine plastic ring, 3) tip (thermal resistance), 4) thermocouple, 5) carbon getter.

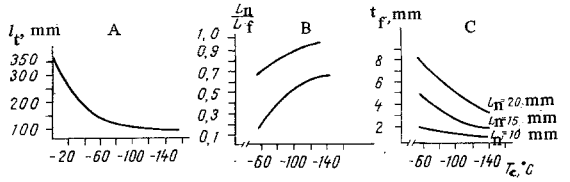

Fig. 2. Graph showing dependence of length of tip of thermal resistance l_t (A), depth of necrosis L_n (B, 1), depth of freezing L_f (B, 2), and time t_n (C) required to obtain different depths of necrosis (L_n = 10, 15, 20 mm) on temperature of cryogenic action T_c (°C). A and C are calculated values; B shows experimental results for different values of L_n/r_o:

$$1-\frac{L_n}{r_o}=3,0,\quad 2-\frac{L_n}{r_o}=1,5.$$

temperature of freezing; T_t the temperature of the tip; T_n the temperature of necrosis (about −20°C); $\varphi = L_n/r_o$. Dependence of the depth of decrosis on the duration of cryogenic action under conditions when the heart is working was determined by the equation:

$$L_n=\beta\sqrt{t}\ \frac{(T_f-T_t)-\varphi)T_f-T_t)}{T_n-T_t}, \tag{3}$$

where β is the experimentally calculated coefficient characterizing the rate of freezing under conditions of the working heart, namely 0.01 cm/sec$^{1/2}$ for −60°C and 0.007 cm/sec$^{1/2}$ for −150°C. Data showing dependence of the destruction time on the acting temperature for different depths of necrosis, calculated at levels (2) and (3), are given in Fig. 2c.

The effectiveness in practice of the theoretically calculated cryodestruction programs was tested experimentally on simulated cardiac arrhythmias. Experiments were carried out on eight adult mongrel dogs of both sexes weighing 12-18 kg. For premedication, a 2% solution of trimeperidine was injected in a dose of 1-3 mg/kg body weight. Endotracheal anesthesia was maintained by fractional injection of 5% hexobarbital solution (total dose 25-30 mg/kg). Access to the heart was obtained through a midline sternotomy. Supraventricular arrhythmia was induced by application of acetylcholine to the right or left atrium or by stimulation of various zones of atrium by means of a Biotronik EDP-10 stimulator. Ventricular arrhythmias were simulated by ligation and subsequent reperfusion in the middle third of the anterior interventricular branch

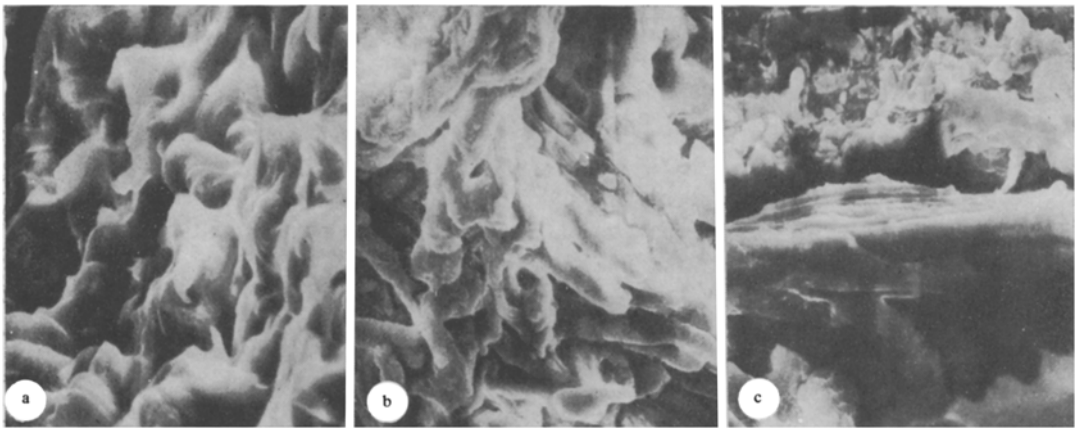

Fig. 3. Myocardium of dog under normal conditions (a) and after single (b) and repeated (c) cryodestruction. In b, temperature of cryogenic action −60°C, duration 4 min; in c, temperature −150°C for 2 min and −60°C for 3 min. Magnification 1000 X.

of the left coronary artery or by injection of a 30% solution of NaCl into the myocardium to different depths. The heart was then mapped in accordance with specially worked out schematic maps by means of detector electrodes and a 16-pole needle electrode. After localization of the arrhythmia-generating focus, a temperature of 0°C was applied to the ectopic focus, leading to restoration of the sinus rhythm for 30-40 sec, after which the arrhythmia was resumed (diagnostic test). The test confirmed the results of the electrophysiological investigation and, by inducing reversible changes in the myocytes, the possibility of effective cryodestruction of the ectopic focus was next determined. If the arrhythmia-generating focus was located in the left atrium, the left atrium was isolated electrically from the rest of the heart [2]. One of the key stages of the operation was cryodestruction of the region of the coronary sinus from the side of the left atrium. Cryodestruction was carried out at temperatures of −150°C and −60°C consecutively, with a time interval of 5 min, both on the working heart and under conditions of combined cardioplegia, produced by a solution made up at the A. N. Bakulev Institute of Cardiovascular Surgery. The temperature was monitored by means of copper-constantan thermocouples, soldered into the tips of the apparatuses, and a needle thermocouple 400μ in diameter, which could measure the temperature in the substance of the myocardium. Temperature curves were recorded on three KSP-4 potentiometers and a TZ-213S automatic writer. Deviation of the calculated data from the experimental did not exceed 5-7%. This error can be taken to be perfectly satisfactory for the first stage of the investigation.

It was shown in [7] that repeated cycles of cryogenic application is a highly effective method for the destruction of biological tissues. Application of a temperature of −150°C in the first stage of the procedure enabled the myocardium to be quickly frozen to a great depth and the ratio of the zone of necrosis to the zone of freezing (L_n/L_f) to be increased for large zones of destruction. Application of low temperatures (−150°C) led to crystallization of the intracellular and extracellular fluids, causing alteration of the cell membranes and intracellular structures. Under these circumstances anesthesia plays a negative role, for it reduces enzymic lysis of the cell structures. After spontaneous reheating of the myocardium, cryodestruction was repeated at −60°C, which caused predominantly extracellular crystallization and manifestation of factors of enzymic lysis. A disadvantage of this temperature program is that a longer duration of exposure is required and the ratio n/f is reduced, i.e., the zone of freezing is increased (Fig. 2b); this must be taken into account during cryodestruction of arrhythmia-generating foci located near the sinoatrial or atrioventricular nodes, to prevent any possible injury to them. For this purpose tips with a larger area of contact with the myocardium ($r_o \geqslant 10$ mm) were used. In such cases, with the assigned depth of necrosis, the value of L_n/L_f was under 1.5 (Fig. 2c).

A study of cardiac myocyte membranes (sarcolemmas) after various programs of cryodestruction also confirmed the efficacy of repeated cryodestruction cycles (Fig. 3a-c). Samples were taken from the subendocardial layer of the myocardium of the left ventricle 8 h after cryodestruction, processed in the usual way in glutaraldehyde, and examined in the J-JSM-SI scanning electron microscope with a resolving power of 25 nm.

The results of this investigation thus showed that it is possible to calculate theoretically the programs of cryodestruction of arrhythmia-generating zones and foci, located in different parts of the myocardium. Application of repeated cycles of cryodestruction enables the number of unsuccessful cases of such treatment to be reduced. The cryogenic apparatus with adjustable thermal resistor rod can be effectively used for diagnosis and surgical treatment of cardiac arrhythmias.

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PLANNING OF AN INVESTIGATION BASED ON STATISTICAL COMPARISON OF MEANS

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A considerable, if not the major, part of all medico-biological research consists essentially of the comparison of mean values of some features in experimental and control groups of objects. However, there is as yet no general agreement regarding the relative sizes of the groups (samples) for comparison of means (I am not discussing comparison of pairs), although most workers prefer them to be of the same size, on the grounds that this enables some simplification (in the case of equal groups) of the mathematical equations used to analyze the data [5].

Nowadays the soundness of this argument is questionable: Computer techniques have made everything much easier and, in any event, in all experimental research the work of mathematical calculation is only a small fraction of the work involved in the investigation as a whole.

Nevertheless, if dispersions in groups are not known beforehand and if the number of experimental and control groups is equal, it is best to distribute the obejcts studied equally among all groups: The symmetry of the scheme of such an experiment gives no grounds for any other distribution. However, in real investigations, the number of experimental groups as a rule will be greater than the number of control groups (most often of all there is only one control group), and in that case the control groups should preferably be larger than the experimental groups.

The reasons for this will be clear from the following example. Let us assume that an experimenter has available 100 animals and that he needs to have one control and nine experimental groups. In that case, with the traditional equal division of the animals each group would contain 10, and in each comparison of means 10 experimental and 10 control animals, a total of 20 animals, would be considered. But if, breaking with tradition, we remove one animal from all the experimental groups and transfer it to the control group, in each comparison 9 + 19 = 28 animals will be considered, so that the attainable level of significance of the difference between means will be increased. Yet this is achieved without any change in the total number of animals used in the experiment!

The control group also can be enlarged at the expense of the experimental groups. The answer to the question of what is the optimal number of animals in the groups is given by the equations presented below.

The mean level of significance $\bar{\gamma}$ obtained by a certain comparison will, of course, be increased if the corresponding difference between the general mean values exists objectively. If, however, the general means are equal (this, of course, happens extremely rarely in biology or medicine), no redistributions of the animals can help to prove the opposite.

The rational choice of group size is usually effective in medicine when clinical material is in short supply. Examples (see Fig. 2) when the use of an optimal scheme of investigation instead of the traditional scheme enables the number of patients in experimental groups to be reduced by almost half, and with the same number of patients six more experimental groups can be organized, are given in [3]. Clearly this represents both economy of work and a significant reduction in the time of the investigation. The situation when the switch from an optimal to a traditional experiment involves lowering of the mean level of significance of the results from $\bar{\alpha} = 0.05$ to 0.11, i.e., it actually reduces their value, is illustrated in Fig. 1 (it will be recalled that the significance of the results of the investigation $\gamma = 1 - \alpha$, so that a maximum of γ corresponds to a minimum of α).

Let us turn to the mathematics. We shall consider that each of μ experimental groups of animals will be compared with each of ν controls. When such investigations are planned, one of the folllowing problems will have to be solved.

1. The total number L of animals taking part in the experiment is known. How should this number be distributed among groups so as to obtain the highest level of significance $\bar{\gamma}$ of the experimental results (Fig. 1)?

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