MATHEMATICAL PLANNING OF EXPERIMENTS TO DETERMINE OPTIMAL CONDITIONS FOR BIOLOGICAL CONSERVATION OF THE HEART

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By the use of a factorial experimental design a mathematical model to simulate the function of an isolated heart-lung preparation as used for biological conservation of the heart was created, and the optimal conditions for the functioning of this preparation were selected with its aid by the steep-rise method. Further experimental files of these conditions showed an almost threefold increase in the period of biological conservation of the heart.

The use of an isolated heart-lung preparation (IHLP) for biological conservation of the heart is a promising method at the present time of obtaining prolonged extracorporeal preservation of the heart under conditions close to those of its natural function [1, 2, 4–7]. However, empirical searches for optimal conditions of function of the IHLP, aimed at securing the longest possible survival of the heart, demand the performance of many experiments because of the large number of indices describing the activity of the IHLP, and determination of the optimal limits of each of these indices is extremely difficult. In the present investigation, in order to determine the optimal conditions of function of the IHLP used for biological conservation of the heart, methods based on mathematical designing of experiments [3] were used.

The object of the present investigation was to study the relationship between the output of a process, i.e., the duration of function of the IHLP (y) and the following regulatory factors (indices of IHLP activity): the systolic pressure in the arch of the aorta (x_1), the pH of the myocardium (x_2), the temperature of the heart (x_3), and the volume velocity of the return of blood into the right heart (x_4).

EXPERIMENTAL METHOD

To investigate this problem a factorial design [3] of the 2^{4-1} type was prepared, by means of which the number of experiments would be reduced sharply because of the possibility of employing a linear approximation in which combinations of the test factors could be examined at strictly fixed maximal and minimal levels in accordance with the factorial design (Table 1). The limits of the maximal and minimal values of the controlling factors were provisionally selected from the results of previous experiments [4].

Experiments were carried out on noninbred male dogs weighing 18-22 kg. To perform eight experiments in accordance with the 2^{4-1} design it was necessary to perform a further five experiments, for in some cases it was impossible to keep all of the factors concerned at the assigned level. After the completion of the 13 (8 + 5) basic experiments, a further ten experiments were performed in which the optimal conditions of function of the IHLP thus obtained were used, thus insuring the longest possible periods of biological conservation of the heart. In six of these ten experiments the conditions of function obtained were

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TABLE 1. 2^{4-1} Design for a Factorial Experiment

Systolic pressure in arch of aorta (x_1; in mm Hg)	pH of myocardium (x_2)	Temperature of heart (in °C; x_3)	Volume velocity of return of blood into right heart (in ml/min; x_4)	Duration of function of IHLP (in min; y)
60	7,35	29	90—120	295
120	7,35	29	Over 120—150	182
60	7,75	29	Over 120—150	234
120	7,75	29	90—120	356
60	7,35	33	Over 120—150	197
120	7,35	33	90—120	328
60	7,75	33	90—120	308
120	7,75	33	Over 120—150	205

TABLE 2. Design of Experiment in Coded Variables

x_1	x_2	x_3	x_4	y
—	—	—	—	295
+	—	—	+	182
—	+	—	+	234
+	+	—	—	356
—	—	+	+	197
+	—	+	—	328
—	+	+	—	308
+	+	+	+	205

combined with injection of catecholamines and glucosides into the circulating blood and also with exchange transfusions of the circulating blood.

Standard anesthesia and a standard technique of isolation of the heart-lung preparation were used in all the experiments. Heparin was injected in a dose of 3 mg/kg body weight. The surgical approach was through midline sternotomy. The epicardium was incised over the apex of the heart and a miniature selective glass electrode inserted into the substance of the myocardium to record the pH, and fixed in the heart tissues by means of a purse-string suture. A catheter was introduced through the subclavian artery into the arch of the aorta to record the pressure on an electromanometer (Orion). A wide, silicone-treated catheter introduced through the brachiocephalic artery into the arch of the aorta was used to connect the aorta with the "stabilizing reservoir", suspended at a height of 1 m above the operating table. A venous catheter, connecting the stabilizing reservoir with the superior vena cava, had a device for controlling and measuring the volume of blood entering the right heart. The blood vessels were then ligated and divided in the following order: subclavian and brachiocephalic arteries, descending aorta, both venae cavae and the azygos vein. The trachea was divided and reintubated. The IHLP was removed from the thorax and placed in a constant-temperature system on a water bath.

To rule out any effect of drugs (catecholamines, glucosides, etc.) on the period of function of the IHLP, no drugs were injected into the circulating blood (except in the six experiments mentioned above), although their deprivation substantially limited the period of function of the IHLP.

To keep the pH of the myocardium within the assigned limits, 5% sodium bicarbonate solution was injected into the circulating blood and the level of supply of O_2 and CO_2 into the inspired mixture was changed (indices of the acid-base balance of the blood, determined by Astrup's micromethod, were measured simultaneously. The pressure in the arch of the aorta was regulated by changing the height of the stabilizing reservoir. The temperature of the heart was kept at a stable level by means of a constant-temperature system.

EXPERIMENTAL RESULTS

Any process in a system can be described as a certain function of the output of the process (y) of the factors x_1, x_2, x_3, and x_4 acting on the system.

In the present case the operator R is approximated by a linear polynomial

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4, \quad (1)$$

where x_1, x_2, x_3, and x_4 are variable and β_0, β_1, β_2, β_3, and β_4 are regression coefficients. This equation describes a certain surface in multidimensional space called the response surface.

To facilitate the calculations, the factors were coded [3] in accordance with the equation

$$\tilde{x}_i = \frac{x_i - x_{i0}}{\lambda_i}, \quad (2)$$

where $\tilde{x}_i$ represents the value of the factor in coded variables; x_i the value of the factor at one level in natural variables; x_{i0} the value of the factor in natural variables at a basic level, calculated as the arithmetic mean between the chosen levels of the factor; λ_i the unit of variation (the half-difference between the upper and lower levels).

The design of the experiment in coded variables is shown in Table 2, in which the minimal values of each factor are designated by the sign (–) and the maximal by the sign (+).

After the design of the experiment had been followed the regression coefficients were calculated by the equation

$$b_i = \frac{\Sigma x_{iu} y_u}{N}, \tag{3}$$

where b_i represents the estimate of the regression coefficient β_i obtained experimentally; x_{iu} the value of the variable in the corresponding column of the design matrix; y_u the result of the u-th experiment; N the total number of experiments. The following values were obtained for the regression coefficients: $b_0 = 263$; $b_1 = 4.6$; $b_2 = 12.6$; $b_3 = -3.6$; $b_4 = 58.6$.

Statistical verification of the significance of the regression coefficients [3] and analysis of the experimental results showed the advantage of an inflow of blood into the right heart not exceeding 90-120 ml/min over a larger inflow of blood. Since the variable x_4 (volume velocity of the inflow of blood into the right heart) was estimated qualitatively and not quantitatively, it was not included in the mathematical model of the process.

The mathematical model of the process is of the form

$$y = 263 + 4.6x_1 + 12.6x_2 - 3.6x_3. \tag{4}$$

The search for optimal conditions of function of the IHLP giving the longest possible period of biological conservation of the heart was carried out by the "steep-rise" method [3] using the mathematical model of equation (4).

The regression coefficients b_i in the equation of the linear model (four) are at the same time the coefficients in equation (5) for the gradient of the response function and, consequently, they can be used in the search for the extremal region. In the present case, in the search for the maximum period of function of IHLP, the gradient Δy is

$$\Delta y = 4.6i + 12.6j - 3.6k, \tag{5}$$

where i, j, and k are single vectors in the direction of the axes of coordinates.

Movement in the direction found led to the region of the maximum for equation (4), where the systolic pressure in the arch of the aorta (x_1) was 100 mm Hg, the pH of the myocardium (x_2) was 7.6, and the temperature of the heart (x_3) was 30°C, while the return of blood to the right took place at a velocity of 90-120 ml/min.

Keeping the pH of the myocardium at such a high level is justified because respiratory and metabolic alkalosis is known to increase the contractility of the myocardium. So far as the temperature obtained for the heart (30°C) is concerned, it probably is also justified in the IHLP, for it enables tachycardia to be avoided, which would led to the rapid exhaustion of the energy reserves of the myocardium. For instance, whereas at 37°C the heart rate of the IHLP was 130-150/min, at 30°C it was only 70-90/min. So far as the volume velocity of the return of blood into the right heart is concerned, values of 90-120 ml/min are evidently the optimum ensuring stable function of the specimen, freeing the pulmonary circulation from the risk of hypo- or hypervolemia.

The use of the conditions thus obtained for function of the IHLP prolonged the duration of its activity in four consecutive experiments to 600 min. Supplementary administration of catecholamines and glucosides and exchange transfusions of the circulating blood, used in the other six experiments, enabled stable function of the IHLP to be obtained for 16 h, and there is no reason to suppose that the limit has yet been reached.

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