EXPERIMENTAL ESTIMATION OF CHRONIC MICROSPHERE LOSS FROM THE RAT MYOCARDIUM

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Isotope-labeled microspheres (MS) are widely used to determine the cardiac output and blood flow in organs and tissues of conscious laboratory animals [3, 5]. One of the main requirements for correct use of the method to determine regional blood flow is that MS, having settled in an organ or tissue, must remain there until samples of tissue are taken in order to count the number of MS in them [5, 6]. The question of the fate of MS which have settled in the vascular bed, and of the possible loss of MS from an organ in the course of time is particularly important under chronic experimental conditions, when the time of injection of MS and the time of counting them in organs may be separated by a long time interval. There have been very few experimental studies of this problem [1, 4, 7]. The degree of loss of MS in the heart of small laboratory animals, notably rats, is not yet known, although the importance of data of this kind is very great because of the extensive use made of rats in pharmacological and physiological research.

The aim of the present investigation was accordingly to determine the loss of MS from the rat myocardium under chronic experimental conditions. To solve this problem, the method of heterotopic transplantation of the heart from a donor which had received injections of MS was used for the first time. The extent of loss of MS was calculated on the basis of two measurements of the number of MS in the heart graft: before transplantation and after 5-24 days in the recipient.

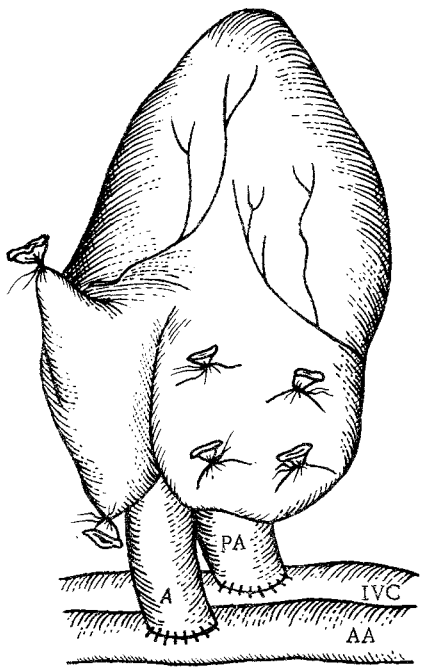

Fig. 1. Scheme of heterotopic transplantation of the heart. IVC) Recipient's inferior vena cava; AA) recipient's abdominal aorta; PA) pulmonary artery of graft; A) aorta of graft.

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TABLE 1. Loss of MS from Rat Myocardium

Parameter	Group of animals						
	1-			2-			
	1	2	3	1	2	3	4
Length of survival of animal, days	5	5	8	20	20	21	24
Initial number of MS	9703	5958	9412	5088	5010	9781	5449
Final number of MS	9155	5322	9327	4955	4831	9147	4901
Total loss of MS, %	5,65	10,67	0,9	2,61	3,57	6,48	10,06
Possible loss of MS due to leakage, %	0,75	0,75	1,20	3,00	3,00	3,15	3,60
Resultant loss of MS, %	4,90	9,92	—0,30	—0,39	0,57	3,33	6,46

EXPERIMENTAL METHOD

Male Wister rats weighing 350 ± 50 g were used. The donor rats were anesthetized with urethane (1.25 g/kg, intraperitoneally). A polyethylene catheter, welded from PP10 and PP50 tubes (Rortex, England) was passed through the right carotid artery into the left ventricle. The criterion that the catheter had entered the left ventricle was the appearance of the typical left-ventricular pressure curve on the recorder. MS 15.8-15.9 μ in diameter, labeled with ^{46}Sc (NEN, USA), normally supplied in 10% dextran solution, were used. Because of the ability of dextran to cause a prolonged fall of blood pressure (BP) in rats [2], the MS were washed with physiological saline containing 0.05% Tween-80 to remove the dextran. Immediately before injection, the MS, contained in polyethylene coils, were thoroughly shaken and exposed in an ultrasonic bath for 5 min. Between 80,000 and 100,000 MS were injected through the catheter into the donor's left ventricle in 30 sec, and during the next 10 sec the catheter was rinsed with physiological saline. After 20-30 min the animal was given an injection of 200-300 U of heparin, and under an "Olympus" operating microscope (Japan) the heart was removed, placed in cold (4°C) physiological saline, and the number of MS in it was determined. The recipients were anesthetized with ketamine (100 mg/kg, intraperitoneally). The aorta of the heart transplant was anastomosed with the recipient's abdominal aorta, and the pulmonary artery with the inferior vena cava, by the method in [9] (Fig. 1). A standard microvascular technique was used in the operation. When the asastomoses were completed, the clips were gradually removed, the distal first and the proximal next. On restoration of the coronary blood flow the graft began to fibrillate, and as a rule, after 10-40 sec, this was replaced by effective contractions. The wound was closed with interrupted sutures. The animals were killed on the 5th-8th day, and on the 20th-24th day after operation the graft was removed and the number of MS in it was again determined. The number of MS in the recipient's lungs, kidneys, spleen, liver, and heart also was measured.

Functional characteristics of the transplanted heart were investigated in two animals 3 weeks after the operation. Under pentobarbital anesthesia (30 mg/kg, intraperitoneally) the left ventricle of the recipient's heart, and abdominal aorta were catheterized through the left femoral artery and the left ventricle of the transplanted heart was catheterized through the apex. The catheters were connected to Statham D23ID electromanometers, and BP, the pressure in both ventricles, the heart rate, and the index of contractility (dP/dt) of both hearts were recorded on a Graphitec (Japan) automatic writer.

The number of MS was counted on a "Compugamma 1282" gamma counter (LKB, Sweden). The results were subjected to statistical analysis bu Student's t test for paired samples and by linear regression. The data are presented in the form M ± m.

EXPERIMENTAL RESULTS

The functional characteristics of the donor's and recipient's hearts indicate that the graft contracted regularly, but at a lower frequency, and it had a lower index of contractility than the recipient's own heart. On average the systolic pressure in the left ventricle of the transplanted heart was 115 mm Hg, compared with 125 mm Hg in the recipient's heart. HR was 275 and 430 beats/min, respectively, and the index of contractility was 2750 and 7750 mm Hg/sec. These results are close to those obtained in other investigations in which the heart was transplanted into the abdomen of rats [8].

Data showing the number of MS in the transplanted hearts are given in Table 1. The average loss of MS was 5.71 ± 1.39% of the initial number ($P < 0.01$). Regression analysis

showed that the loss of MS was independent of the length of the survival of the animal after the operation, for it is described by the equation $y = 0.005x + 5.627$, where x is the number of days after implantation. The coefficient of correlation was 0.012. Mean values of the loss of MS in two groups of animals (with survival times of 5-8 and 20-24 days) were 5.74 ± 2.82 and 5.68 ± 1.68 respectively, and they did not differ from each other significantly.

According to data in the literature losses of MS during the first 2-10 min after their injection into the animal (known as "early losses") can be distinguished from those arising later, after a period of days ("late losses") [1, 4, 5]. The conditions of these experiments stipulated removal of the heart from the donor 20-30 min after injection of MS, so that for the first time we were able to determine the true magnitude of the "late losses" of MS. The decrease in the number of MS in the transplanted heart which we recorded could be a reflection of two processes. The first is a true decrease in the number of MS in the heart on account of their migration from the arterial into the venous part of the coronary vessels, the second an apparent decrease in the number of MS due to leakage of radioactive material from them. To investigate the first of these processes, the number of MS was determined in the lungs of all the recipient rats. In no experiment were MS found in the lungs. In certain other organs with a rich blood supply, namely the heart, kidneys, spleen, and liver, no MS likewise were found. The absence of MS in the lungs suggests that losses of radioactivity by the myocardium take place, not through slipping of MS as such out of the heart, but escape of the radioactive element from them. The value of the "leakage criterion," quoted by the firm (NEN) in the specification of MS, is 0.3% during incubation *in vitro* with plasma at 38°C for 48 h. In the course of 20-24 days, losses of radioactivity could amount to 3.0-3.6%, which is less than the mean value determined in the animals of the 2nd group in the present experiments, namely 5.68%. MS *in vivo* are probably characterized by a higher "leakage criterion" than during incubation with plasma *in vitro*.

The results of this investigation thus demonstrate that the degree of preservation of MS in the rat myocardium is high for 5-24 days. In none of the experiments did the loss of radioactivity exceed 10%. Thus MS can be used with success to study the coronary blood flow in rats under chronic experimental conditions.

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