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THE ISOLATED SUPPORTED CANINE HEART: A MODEL FOR THE EVALUATION OF DRUG EFFECTS ON REGIONAL MYOCARDIAL BLOOD FLOW[1]

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ABSTRACT

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The effect of independently varying heart rate, left ventricular systolic pressure, coronary blood flow or coronary artery perfusion pressure on the transmural distribution of myocardial blood flow was determined in the isolated supported dog heart preparation by use of radioactive microspheres. The distribution of radioactivity was expressed as the epicardial/endocardial blood flow ratio (epi/endo). At a constant coronary blood flow, increases in heart rate (111-250 beats/min) or decreases in coronary artery perfusion pressure (100-46 mm Hg) resulted in an elevation of epi/endo (0.64 to 1.12 and 0.71 to 1.41, respectively). Increasing coronary artery perfusion pressure (40-130 mm Hg) and coronary blood flow from low values resulted in a decreased epi/endo (1.28 to 0.76). The absolute level of coronary blood flow had no direct effect on epi/endo but acted through changes in coronary artery perfusion pressure. The major physiological determinants of intramyocardial blood flow distribution in the isolated dog heart are coronary artery perfusion pressure and heart rate, whereas left ventricular systolic pressure and coronary blood flow are of minor importance. An equation was developed relating the mean epi/endo of the left ventricle to hemodynamic factors studied, and a highly significant correlation was found between predicted and experimental ratios. These results indicate the potential usefulness of this model for predicting epicardial-endocardial blood flow ratios based upon drug-induced changes in hemodynamic parameters.

The vulnerability of the endocardium of the left ventricle to ischemia and infarction has been well documented clinically and experimentally. Studies of tissue oxygen tension (Kirk and Honig, 1964) and percent saturation of venous blood with oxygen (Monroe *et al.*, 1975) have indicated the presence of higher oxygen extraction in endocardium compared to epicardium. Others (Brazier *et al.*, 1974) have emphasized the dependence of the endocardium on blood flow for oxygen delivery and have studied factors affecting regional myocardial blood flow. The influence of heart rate with (Neill *et al.*, 1975) and without (Neill *et al.*, 1973), coronary stenosis, intraventricular pressure (Cutarelli and Levy, 1963), coronary artery perfusion pres-

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sure (Nakamura *et al.*, 1973) and coronary occlusion (Becker *et al.*, 1973) on the transmural distribution of coronary blood flow has been examined under a variety of experimental conditions with conflicting findings. In addition various pharmacological agents have been shown to change myocardial blood flow distribution (Moir and Debra, 1967; Wilcken *et al.*, 1971; Somani et al., 1970; Gross and Winbury, 1973; Nakamura *et al.*, 1973).

The purpose of the present investigation was to isolate systematically several critical factors proposed to be involved in the distribution of myocardial blood flow and to define their role and relative importance in regional myocardial perfusion. The isolated supported heart preparation (Somani *et al.*, 1969) was chosen as the experimental model. Many variables such as heart rate, coronary artery perfusion pressure, left ventricular systolic pressure and coronary blood flow could be controlled independently, and peripheral hemodynamic factors and neural reflex activity could be eliminated. The results clearly indicate the importance of coronary artery perfusion pressure and heart rate in determining myocardial blood flow distribution when total coronary blood flow cannot increase.

Materials and Methods

Isolated supported heart preparation (ISHP). Mongrel dogs of either sex, weighing between 12 and 19 kg, were fasted overnight, anesthetized with pentobarbital sodium (30 mg/kg i.v.) and ventilated by a respirator (Harvard model 607) with room air at 10 to 15 breaths/min. Atelectasis was prevented by maintaining an expiratory pressure of 5 to 7 cm of water with a trap. Arterial blood pH was maintained between 7.35 and 7.45 by intravenous infusion of 1.5% sodium bicarbonate when necessary. After midsternal thoracotomy, the heart was suspended in a pericardial cradle. Surgical procedure for isolation and perfusion of the recipient heart has been described (Somani *et al.*, 1969). Briefly, the heart was isolated *in situ* and the coronary arteries perfused with heparin-treated (5.0 mg/kg) arterial blood at 37°C from a donor dog *via* retrograde flow through the brachiocephalic artery. Total coronary blood flow was adjusted by means of a roller pump (Sarns model 3500) to provide the desired perfusion pressure, usually 100 mm Hg. Coronary venous drainage into the right atrium and ventricle was returned to a femoral vein of the donor dog by means of a large bore cannula inserted into the right ventricle. A constant portion (10%) of the venous blood was diverted through a well-type gamma counter to monitor passage of microspheres through the myocardium. A small multihole catheter was placed in the left ventricle to drain Thebesian and aortic regurgitant flow.

A flow transducer was inserted into the arterial inflow tubing and total coronary blood flow was measured by use of a 270 Hz square wave, electromagnetic flowmeter (Carolina Medical Instruments 322). Coronary blood flow in milliliters per minute per 100 g was calculated from *in situ* calibration curves (milliliters per minute) and heart weight (grams). In addition, coronary blood flow was determined at selected intervals by timed volume collection of total venous outflow. Coronary artery perfusion pressure was measured by means of a catheter inserted into the arterial inflow cannula and a strain gauge pressure transducer (Statham P23AC). Coronary vascular resistance (CVR), expressed in resistance units, was calculated from the equation.

$$\text{CVR} = \frac{\text{coronary artery perfusion pressure (mm Hg)}}{\text{coronary blood flow (ml} \cdot \text{min}^{-1} \cdot \text{100 g heart weight}^{-1})}$$

A thin latex balloon was placed in the left ventricle through the mitral valve and was inflated with saline (5–15 ml) to provide an initial left ventricular systolic pressure of 100 mm Hg. The balloon was tied securely at the mitral valve to exclude possibility of slippage into the atrium. A Brodie-Walton strain gauge arch was sutured to the left ventricular wall perpendicular to the left anterior descendens coronary artery to determine isometric contractile force. Initially, the strain gauge arch was stretched 50% greater than resting tension and changes in contractile force were expressed as percentage of control. The electrocardiogram (limb lead II) was also monitored. Heart rate was adjusted to the desired level by right atrial pacing with a stimulator (Grass model S48). The electrocardiogram, left ventricular systolic pressure, coronary blood flow, coronary artery perfusion pressure, contractile force and donor blood pressure were continuously recorded on a polygraph (Grass model 7).

Blood pH, pO_2 and pCO_2 were measured at 37°C with an Instrumentation Laboratories model 313 blood-gas analyzer. Before and during experimental interventions, simultaneous arterial and coronary venous blood samples were withdrawn in glass syringes rinsed with 2% NaEDTA. Samples were stored on ice and were subsequently used for measurement of blood pH, pO_2, pCO_2 and oxygen content.

Myocardial oxygen consumption. Direct measurement of arterial and coronary venous blood oxygen content was made with duplicate 10-μl samples by an electrolytic cell method (Lex-O_2-Con, Lexington Instrument Co.) (Kusumi *et al.*, 1973). Myocardial oxygen consumption was calculated from the following equation:

$$\dot{M}VO_2 = \frac{(AO_2 - VO_2) \times CBF}{\text{heart weight (g)}} \times 100$$

where $M\dot{V}O_2$ = total myocardial oxygen consumption expressed as milliliters of O_2 utilized per min. per 100 g of heart weight; AO_2 and VO_2 = arterial and venous oxygen content in volumes percent (milliliters per 100 ml); CBF = total coronary blood inflow (milliliters per minute). Percent extraction of oxygen by the myocardium was calculated from the arterial-venous oxygen content difference divided by the arterial oxygen content.

Regional myocardial blood flow. The distribution of coronary blood flow was determined by use of the radioactive microsphere technique (Domenech *et al.*, 1969). Briefly, if microspheres are well mixed within the blood flow to an organ and are subsequently trapped in the precapillary microcirculation during their first transit through that organ, the distribution of radioactivity will be in direct proportion to blood flow. Carbonized plastic microspheres used in this study were 15.5 ± 1.0, 16.7 ± 2.4, 16.2 ± 2.3 and 16.2 ± 2.0 μ in diameter and were labeled with either ^{169}Yb (sp. act. 9.7 mCi/g; peak energy 63 keV), ^{141}Ce (sp. act. 11.3 mCi/g; peak energy 145 keV), ^{51}Cr (Sp. act. 27.9 mCi/g; peak energy 320 keV) or ^{85}Sr (sp. act. 13.7 mCi/g; peak energy 514 keV), respectively. Microspheres were obtained as 1 mCi of nuclide in 5 ml of saline to which one drop of Tween 80 was added to minimize aggregation. After appropriate dilution, the mixture was agitated immediately prior to injection in a Vortex mixer (Cole-Parmer model 4722) for 5 minutes. Microspheres (2-3 μCi; 75,000-150,000 spheres) were injected into the arterial inflow line in a total volume of 0.2 to 0.5 ml, 30 cm from the cannulated brachiocephalic artery. The total number of microspheres of each type injected was selected to provide a final sphere density of at least 400 spheres/tissue sample (Buckberg *et al.*, 1971). No significant change in coronary hemodynamics was seen during or immediately after microsphere injection. Several doses of 1×10^6 15 μ nonradioactive spheres have been administered to the ISHP with no detectable effect. Yipintsoi *et al.* (1973) also observed minimal effects with larger doses (8×10^6) of smaller (10 μ) microspheres in isolated hearts.

Electron microscopic examination of myocardial tissue samples revealed that microspheres used in this study were trapped in metaarterioles. Capillaries were smaller (3-7 μ) than the average microsphere diameter (13.9-19.1 μ).

Experimental design. After surgery, the ISHP was allowed to stabilize for 30 minutes and the first injection of microspheres was made. A minimal interval of 15 minutes was maintained between subsequent injections of different microspheres. The microspheres were randomly administered in a Latin square design. Group size was selected as eight to complete the square twice; thus, no isotope contributed perferentially over another to observed effects (Reneman *et al.*, 1975). After completion of the experiment, the recipient heart was excised, washed with saline, blotted dry and weighed. The heart was fixed in 10% formalin for 48 hours. After rinsing in water, epicardial fat, large vessels, atrial cap, valves, papillary muscle and chorda tendinae were removed and the ventricles were sectioned into 10 tissue areas (Fig. 1).

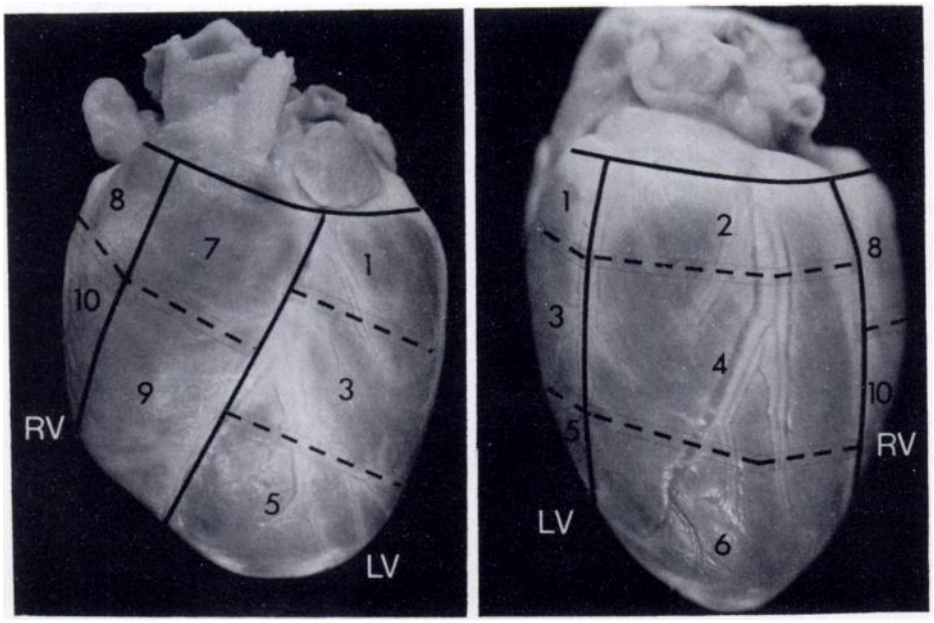

FIG. 1. Locations of the 10 tissue areas sectioned and studied for regional distribution of blood flow. Tissue areas 1 to 6 are from the left ventricle (LV), and areas 7 to 10 are from the right ventricle (RV). Note that each area was further subdivided into epicardium and endocardium.

Both ventricles were divided into anterior and posterior halves, and were subdivided into base, middle (left ventricle only) and apex. The interventricular septum was discarded. Each of the 10 tissue areas was further divided into epicardial (outer) and endocardial (inner) layers of approximately equal thickness. The 20 tissue samples were weighed, placed in plastic scintillation vials and the activity of each isotope was determined in duplicate at four energy windows (45–85 keV, 130–155 keV, 295–355 keV and 465–590 keV) in a Nuclear Chicago 1185 series autogamma spectrometer equipped with dual channel analyzer. Samples were approximately the same size (mean ± S.E. endocardial weight = 1.979 ± 0.093 g; mean ± S.E. epicardial weight = 2.057 ± 0.090 g) to reduce variation of internal absorption of gamma radiation. True activity of each isotope was separated by a preprogrammed computer (Monroe model 1860).

Four experimental groups were completed.

Group 1 (heart rate). In the first series, the effect of heart rate on the regional distribution of coronary blood flow was examined. Initially, the ISHP was allowed to stabilize at a coronary blood flow sufficient to produce a coronary artery perfusion pressure of 100 mm Hg during spontaneous beating and thereafter flow was maintained constant. Microspheres were injected at the spontaneous rate and at rates obtained by increasing heart rate in 40 to 50 beats/min increments by right atrial pacing. At each new rate, pacing was maintained for at least 15 minutes before injecting micropheres. Left ventricular systolic pressure was regulated by adjusting left ventricular balloon volume to give a peak systolic pressure of 100 mm Hg with each beat.

Group 2 (left ventricular systolic pressure). In the second group of experiments, left ventricular systolic pressure was altered (microspheres injected at 0, 75, 150 and 225 mm Hg) by adjusting left ventricular balloon volume. Microspheres were injected 15 minutes after attaining each ventricular pressure. Coronary blood flow was adjusted initially to provide a perfusion pressure of 100 mm Hg and thereafter flow was maintained constant. Heart rate was controlled at 150 beats/min.

Group 3 (coronary blood flow and coronary artery perfusion pressure). In the third series, coronary blood flow was progressively increased by changing the output of the roller pump to provide coronary artery perfusion pressures of 40, 70, 100 and 130 mm Hg. In four additional experiments coronary blood flow was decreased to provide perfusion pressures of 130, 100, 70 and 40 mm Hg. At each level of perfusion pressure and blood flow, left ventricular systolic pressure was adjusted to 100 mm Hg. Heart rate was controlled at 150 beats/min. Coronary blood flow was maintained constant for 15 minutes at each perfusion pressure during which time microspheres were injected.

Group 4 (coronary artery perfusion pressure). In the final series of experiments, coronary blood flow was initially adjusted to provide a perfusion pressure of 100 mm Hg and thereafter flow was maintained constant. The heart was paced at 150 beats/min and left ventricular systolic pressure held constant at 100 mm Hg. The effects of changes in coronary artery perfusion pressure and coronary vascular resistance were studied by intracoronary infusion of chromonar (0.5 mg/min), a coronary vasodilator (Weiss *et al.*, 1973). The slow, steady vasodilation produced by chromonar made it possible to stop the drug infusion at selected intervals and inject microspheres at stable perfusion pressures of 80, 60 and 40 to 50 mm Hg (maximal vasodilation). Additional studies were performed in which changes in coronary artery perfusion pressure and vascular resistance were studied by a 3-minute intracoronary infusion of adenosine (50 and 100 μg/min) and nitroglycerin (5 and 10 μg/min). In these experiments coronary blood flow, heart rate (150 beats/min) and left ventricular systolic pressure (100 mm Hg) were held constant. In a final series changes in coronary vascular resistance independent of coronary artery perfusion pressure were studied by administering a 3-minute intracoronary infusion of nitroglycerin (5 and 10 μg/min). Coronary artery perfusion pressure was held constant at 100 mm Hg whereas coronary blood flow was allowed to increase. Heart rate and left ventricular systolic pressure were maintained at 150 beats/min and 100 mm Hg, respectively. Microspheres were injected at stable values of perfusion pressure.

Data analysis. Transmural distribution of coronary blood flow was expressed as the ratio of counts per gram of epicardium/counts per gram of endocardium (epi/endo). Statistical analysis of results was made by use of Student's *t* test for paired samples and linear and multiple linear regression analysis (Snedecor and Cochran, 1967). The difference between epicardial/endocardial ratios and hemodynamic or blood-gas data were considered significant when P was .05 or less.

Results

Preparations with ventricular ectopic beats or combined Thebesian-aortic regurgitant flow in excess of 10 ml/min were considered unsuccessful and not used in data analysis. A total of 54 successful experiments was completed. Summarized hemodynamic and blood-gas data are shown in table 1.

Group 1 (heart rate; $N = 8$). The effect of changing heart rate on epi/endo in different tissue areas of the left and right ventricle is shown in figure 2. During spontaneous beating (111 ± 6 beats/min, blood flow to endocardium was significantly greater than to epicardium. As heart rate was increased, blood flow was shifted

TABLE 1

Summary of hemodynamic and blood-gas data (mean ± S.E.M.)

Abbreviations used are: HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; CF, contractile force; CAPP, coronary artery perfusion pressure; CBF, coronary blood flow; CVR, coronary vascular resistance; r.u., resistance units; Epi/Endo, epicardial/endocardial blood flow ratio; $M\dot{V}O_2$, myocardial oxygen consumption.

HR	LVSP	LVEDP	CF	CAPP	CBF	CVR	Epi/Endo		$M\dot{V}O_2$
							LV[a]	RV[b]	
beats/min	*mm Hg*		*% of control*	*mm Hg*	*ml/min/100 g*	*r.u.*			*ml/min/100 g*
Group 1 (Heart Rate; N = 8)									
111 ± 6.0	100	1.4 ± 2.9	100	102 ± 3.4	95 ± 8.7	1.15 ± 0.11	0.64 ± 0.04	0.74 ± 0.04	4.1 ± 0.4
158 ± 5.0	100	3.3 ± 3.2	102 ± 1.5[c]	98 ± 3.8	95 ± 8.7	1.10 ± 0.11	0.78 ± 0.05[c]	0.85 ± 0.04[c]	5.9 ± 0.3[c]
203 ± 5.0	100	4.0 ± 2.5	105 ± 1.9[d]	94 ± 5.3	95 ± 8.7	1.05 ± 0.10	0.94 ± 0.06[c]	0.94 ± 0.03[c]	7.5 ± 0.5[c]
250 ± 6.4	100	4.7 ± 3.8	99 ± 1.5[d]	85 ± 5.8[c]	95 ± 8.7	0.94 ± 0.10[c]	1.12 ± 0.11[c]	1.09 ± 0.03[c]	8.1 ± 0.6[d]
Group 2 (Left Ventricular Systolic Pressure; N = 8)									
150	0	0	100	97 ± 2.9	90 ± 6.5	1.13 ± 0.11	0.72 ± 0.04	0.90 ± 0.07	4.9 ± 0.4
150	75	1.5 ± 3.0	101 ± 2.4	102 ± 3.7[c]	90 ± 6.5	1.19 ± 0.11[c]	0.74 ± 0.04	0.92 ± 0.06	5.3 ± 0.4[c]
150	150	1.6 ± 2.6	96 ± 2.9[c]	106 ± 5.2[d]	90 ± 6.5	1.23 ± 0.11[d]	0.78 ± 0.04	0.87 ± 0.07	5.4 ± 0.4[d]
150	225	6.7 ± 3.8	87 ± 4.1[c]	100 ± 7.2	90 ± 6.5	1.17 ± 0.13	0.88 ± 0.06[d]	0.94 ± 0.03	6.0 ± 0.3[c]
Group 3 (Coronary Blood Flow and Coronary Artery Perfusion Pressure; N = 8)									
150	100	2.7 ± 1.7	100	40	46.3 ± 4.9	0.94 ± 0.09	1.28 ± 0.04	1.11 ± 0.05	4.1 ± 0.2
150	100	1.0 ± 3.1	106 ± 3.5[c]	70[c]	73.4 ± 5.8[c]	1.00 ± 0.07	0.87 ± 0.05[c]	0.96 ± 0.04[c]	4.8 ± 0.3[c]
150	100	1.0 ± 2.5	125 ± 6.8[c]	100[c]	122.0 ± 16.6[c]	0.92 ± 0.09	0.79 ± 0.05[d]	0.85 ± 0.03[c]	6.3 ± 0.6[c]
150	100	2.0 ± 2.4	156 ± 10.1[c]	130[c]	198.3 ± 28.7[c]	0.74 ± 0.08[c]	0.76 ± 0.04[d]	0.79 ± 0.05[d]	7.9 ± 0.8[c]
Group 4 (Coronary Artery Perfusion Pressure; N = 8)									
150	100	1.0 ± 3.7	100	100	109.0 ± 10.5	0.99 ± 0.10	0.71 ± 0. 6	0.78 ± 0.04	5.5 ± 0.3
150	100	3.0 ± 2.9	95 ± 6.3	80[c]	109.0 ± 10.5	0.79 ± 0.08[c]	0.88 ± 0.04[c]	0.92 ± 0.04	5.5 ± 0.4
150	100	3.3 ± 1.3	92 ± 8.0	60[c]	109.0 ± 10.5	0.60 ± 0.06[c]	1.12 ± 0.08[c]	1.03 ± 0.04[c]	5.0 ± 0.4
150	100	3.6 ± 0.9	66 ± 7.7[c]	46 ± 1.9[c]	109.0 ± 10.5	0.45 ± 0.04[c]	1.41 ± 0.16[c]	1.21 ± 0.11[c]	4.3 ± 0.3[c]

[a] These values are the mean ± S.E.M. of the six tissue areas of the left ventricle.

[b] These values are the mean ± S.E.M. of the four tissue areas of the right ventricle.

[c] Significantly different ($P < .05$) from the preceding value.

[d] Significantly different ($P < .05$) from initial value.

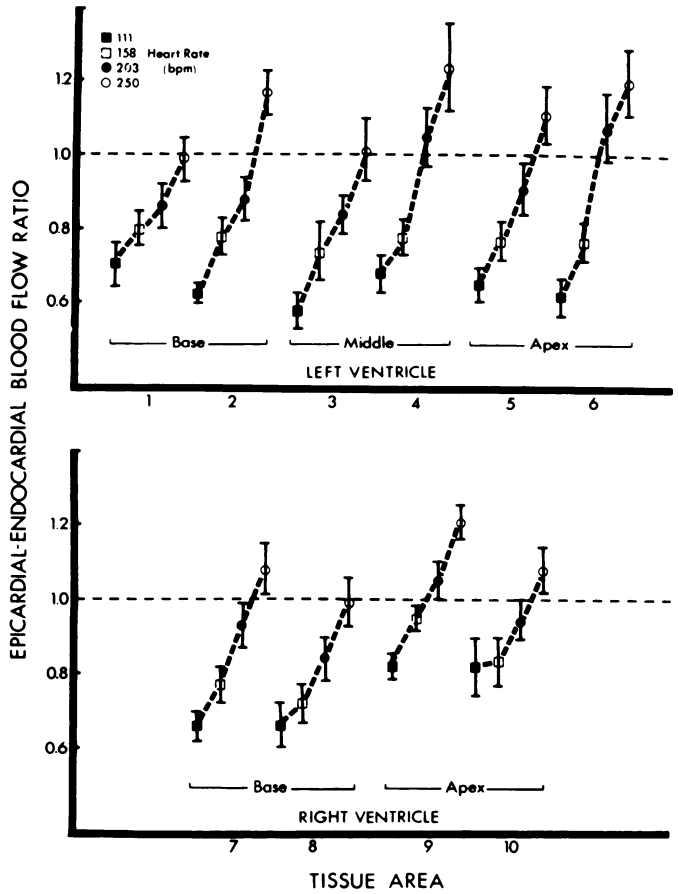

FIG. 2. Relationship between heart rate (HR) and distribution of blood flow between epicardium and endocardium in the 10 tissue areas. Each point represents mean ± S.E.M. (N = 8).

away from endocardium. Significant increases ($P < .05$) in epicardial/endocardial blood flow ratios (epi/endo) occurred in all tissue areas. The endocardium of the posterior wall of the left ventricle, particularly at the apex, appeared to be most vulnerable to the redistribution of flow produced by increasing heart rate. A significant correlation (r = 0.64; $P < .001$) was found between the mean epi/endo of the left ventricle and heart rate (table 2).

Coronary blood flow and left ventricular systolic pressure were maintained constant as heart rate was increased. The systole/diastole ratio increased at increasing heart rates (0.34 ± 0.03, 0.49 ± 0.04, 0.57 ± 0.04 and 0.70 ± 0.07). Isometric contractile force increased significantly at heart rates of 158 ± 5.0 and 203 ± 5.0 beats/min (table 1) but was not significantly different from control at the highest rate (250 ± 6.4 beats/min.) Coronary artery perfusion pressure and vascular resistance decreased in a stepwise manner (table 1). Increases in heart rate produced a rate-dependent increase in myocardial oxygen consumption (table 1).

Group 2 (left ventricular systolic pressure; N = 8). The effect of increasing left ventricular systolic pressure on epi/endo in different areas of the left and right ventricle is shown in figure 3. The mean epi/endo of the left ventricle showed small increases with elevations in left ventricular systolic pressure (table 1); however, epi/endo increased significantly from the initial control (0.72 ± 0.04–0.88 ± 0.06) only at the highest pressure (225 mm Hg). The apex of the left ventricle was most sensitive to changes in left ventricular systolic pressure (fig. 3). The epi/endo of the right ventricle remained unchanged. A positive correlation (r = 0.38) was

TABLE 2

Correlation of hemodynamic factors with the transmural distribution of coronary blood flow

Paired Factors (y vs. x)	N^a	n^b	Correlation Coefficient (r)	P	Equation of Regression Line
Epi/Endo ratios (y) *vs.*					
Coronary artery perfusion pressure					
Changing coronary blood flow	8	32	(−) 0.74	.001	$y = 1.388 - 0.0055x$
Constant coronary blood flow	8	32	(−) 0.68	.001	$y = 1.927 - 0.0125x$
Heart rate	8	32	(+) 0.64	.001	$y = 0.295 + 0.0032x$
Left ventricular systolic pressure	8	32	(+) 0.38	.05	$y = 0.699 + 0.00069x$
Coronary blood flow					
Coronary artery perfusion pressure (100–130 mm Hg)	32	32	(−) 0.26	N.S.[c]	$y = 0.851 - 0.00079x$
Coronary artery perfusion pressure (60–70 mm Hg)	16	16	(+) 0.36	N.S.	$y = 1.311 + 0.0064x$
Coronary artery perfusion pressure (40–50 mm Hg)	16	16	(+) 0.13	N.S.	$y = 1.261 + 0.0011x$

[a] Number of preparations.
[b] Number of points used in regression analysis.
[c] Not statistically significant ($P > .05$).

found between the mean epi/endo of the left ventricle and left ventricular systolic pressure (table 2). No significant change was observed in left ventricular end-diastolic pressure (table 1).

Coronary blood flow and heart rate were held constant in these experiments. Increasing left ventricular systolic pressure from 0 to 150 mm Hg was accompanied by a significant increase in coronary artery perfusion pressure and coronary vascular resistance (table 1). At a left ventricular systolic pressure of 225 mm Hg, coronary artery perfusion pressure and vascular resistance were not significantly different from control. Increases in left ventricular systolic pressure produced small but significant increases in myocardial oxygen consumption (table 1).

Group 3 (coronary blood flow and coronary artery perfusion pressure; $N = 8$). In this series, coronary blood flow was varied to give perfusion pressures of 40, 70, 100 and 130 mm Hg while heart rate and left ventricular systolic pressure were held constant. The effect of changing coronary artery perfusion pressure and blood flow on epi/endo is shown in figure 4. At a perfusion pressure of 40 mm Hg (coronary blood flow of 46.3 ± 4.9 ml/min/100 g, epi/endo was the highest (lowest endocardial flow), especially at the apex of the left ventricle. Posterior areas of the left ventricle had higher ratios than analogous anterior ones. As total coronary blood flow was increased to provide a perfusion pressure of 70 mm Hg, epi/endo decreased; however, epi/endo remained relatively constant with further increases in perfusion pressure and blood flow (fig. 4). A significant inverse correlation ($r = -0.74$; $P < .001$) was found between the mean epi/endo of the left ventricle and coronary artery perfusion pressure (table 2). In four additional experiments, coronary artery perfusion pressure and coronary blood flow were decreased in a stepwise manner. The mean epi/endo of the left and right ventricle increased significantly ($P < .05$) as perfusion pressure and flow decreased (LV = 0.81 ± 0.05–1.29 ± 0.09; RV = 0.81 ± 0.04–1.23 ± 0.05). These results agree with those obtained when flow and perfusion pressure were increased (fig. 4).

The relationship between coronary blood flow and epi/endo at three ranges of perfusion pressure is represented in figure 5. Individual points are from the 32 experiments, each representing the mean epi/endo of the six tissue areas of the left ventricle. No significant correlation (table 2) was found between the mean epi/endo of the left ventricle and total coronary blood flow at perfusion pressures of 40 to 50 mm Hg ($r = 0.13$), 60 to 70 mm Hg ($r = 0.36$), or 100 to 130 mm Hg ($r = -0.26$).

No significant changes in coronary vascular resistance were seen at coronary artery perfusion pressures of 40, 70 and 100 mm Hg;

however, a significant decrease occurred at the highest perfusion pressure (130 mm Hg) and blood flow (table 1). As coronary blood flow and perfusion pressure were elevated, marked increases in myocardial oxygen consumption and contractile force were observed (table 1).

Group 4 (coronary artery perfusion pressure; $N = 8$). In this series, coronary blood flow, heart rate and left ventricular systolic pressure were held constant while coronary vascular resistance and perfusion pressure were decreased by an intracoronary infusion of chromonar. As vascular resistance and perfusion pressure were decreased, epi/endo was significantly ($P < .05$) increased in all tissue areas (fig. 6). The left ventricle responded with a greater shift in flow away from endocardium than the right ventricle, and the highest ratios were found in middle and apical regions. Again, a significant inverse correlation was found (table 2) between the mean epi/endo of the left ventricle and coronary artery perfusion pressure ($r = -0.68$; $P < .001$); however, the slope of the regression line was significantly greater ($P < .05$) than that calculated for the series in which changes in coronary artery perfusion pressure were not accompanied by decreases in coronary vascular resistance (Group 3). There was no significant change in myocardial contractile force and oxygen consumption at higher perfusion pressures, (see table 1) (100, 80 and 60 mm Hg), but contractile force and oxygen consumption decreased significantly at the lowest perfusion pressure (46 ± 1.9 mm Hg).

In two additional series of experiments, coronary blood flow, heart rate and left ventricular systolic pressure were held constant while coronary vascular resistance and perfusion pressure

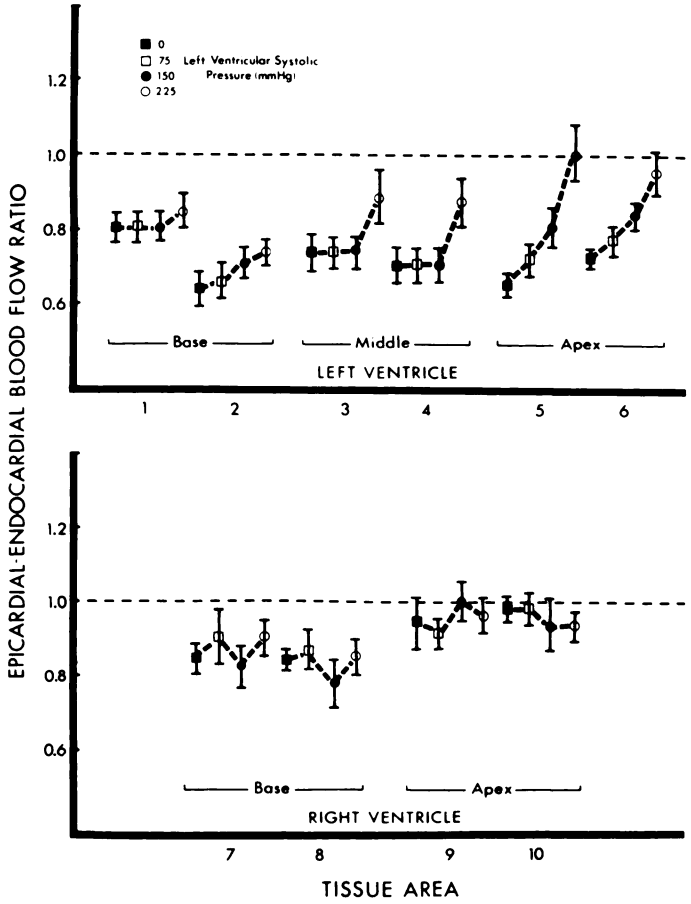

FIG. 3. Relationship between left ventricular systolic pressure (LVSP) and distribution of blood flow between epicardium and endocardium in the 10 tissue areas. Each point represents mean ± S.E.M. ($N = 8$).

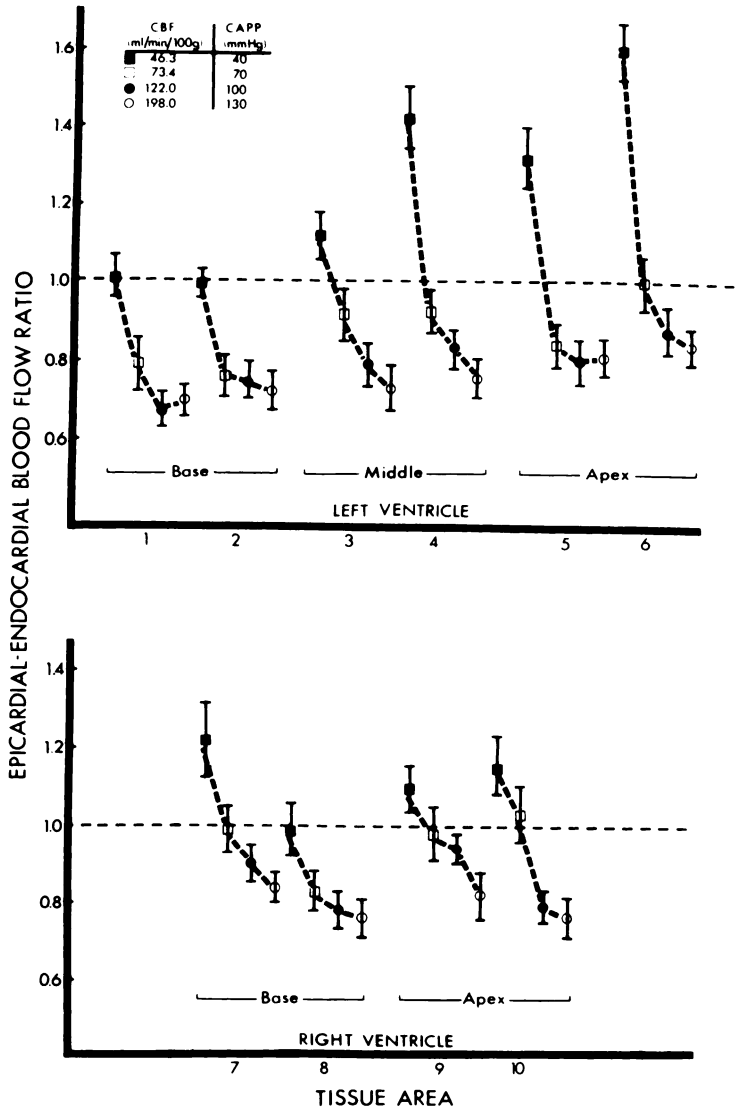

FIG. 4. Relationship between coronary artery perfusion pressure (CAPP) and distribution of blood flow between epicardium and endocardium in the 10 tissue areas. Perfusion pressure was increased by elevation of coronary blood flow (CBF). Each point represents mean ± S.E.M. (N = 8).

were decreased by a 3-minute intracoronary infusion of adenosine or nitroglycerin. As vascular resistance and perfusion pressure were decreased, the mean epi/endo of the left ventricle significantly ($P < .05$) increased with both vasodilators (table 3). Although maximal coronary vasodilation was not attained with these two vasodilators, the results were similar to those obtained with chromonar. In a final series of experiments, coronary artery perfusion pressure, heart rate and left ventricular systolic pressure were held constant while coronary vascular resistance was decreased and coronary blood flow increased by a 3-minute intracoronary infusion of nitroglycerin. As coronary vascular resistance decreased and blood flow increased, the mean epi/endo of the left ventricle showed no significant change. These results further suggest the importance of changes in coronary artery perfusion pressure in determining epi/endo in the ISHP.

Prediction index. With all the data from the

four experimental groups (N = 128), The epi/endo (R) of the left ventricle can be related to heart rate (HR), coronary artery perfusion pressure (CAPP), left ventricular systolic pressure (LVSP) and coronary blood flow (CBF) by the multiple regression equation: R = −0.07226 + 0.00221 Hr + 0.00067 LVSP + 0.00053 CBF + 40.01146 (1/CAPP), multiple correlation coefficient = 0.67; $P < .0005$. Further analysis of the multiple regression equation indicated that coronary blood flow ($P < .2$) and left ventricular systolic pressure ($P < .1$) were minor contributing factors to epi/endo whereas heart rate and coronary artery perfusion pressure were highly significant ($P < .001$). Results of the predictability of the multiple regression equation are illustrated in figure 7 where predicted epi/endo are plotted *vs.* experimental epi/endo. The 16 data points in figure 7 represent average predicted and experimental epicardial/endocardial ratios of the left ventricle at each level of heart rate, left ventricular systolic pressure, coronary artery perfusion pressure and coronary blood flow in the four groups (table 1).

A highly significant correlation (0.98; $P < .001$) was found between experimentally obtained ratios and those predicted by use of the multiple regression equation. The data obtained in the studies in which adenosine or nitroglycerin were infused into the ISHP further confirms the predictability of the multiple regression equation. There was no significant difference in the predicted *vs.* the experimentally obtained changes in epi/endo in any of the three series (table 3).

Discussion

Transmural perfusion gradient. Under similar hemodynamic conditions (heart rate of 150–158 beats/min, left ventricular systolic pressures of 75–100 mm Hg, coronary artery perfusion pressures of 98–102 mm Hg), the mean epi/endo of the left ventricle in the four experimental groups was similar and indicated a 25% greater flow to endocardium. Wüsten *et al.* (1974) and Flameng *et al.* (1975) by use of 8 to 10 μ spheres, and Monroe *et al.* (1972) and Neill *et al.* (1973) by use of 15 μ spheres have also found greater endocardial flow. Other investigators (Brazier *et al.*, 1974; Becker *et al.*, 1973) have reported equal flow between epicardium and endocardium. A number of factors may be responsible for the wide variations in epi/endo seen from different laboratories.

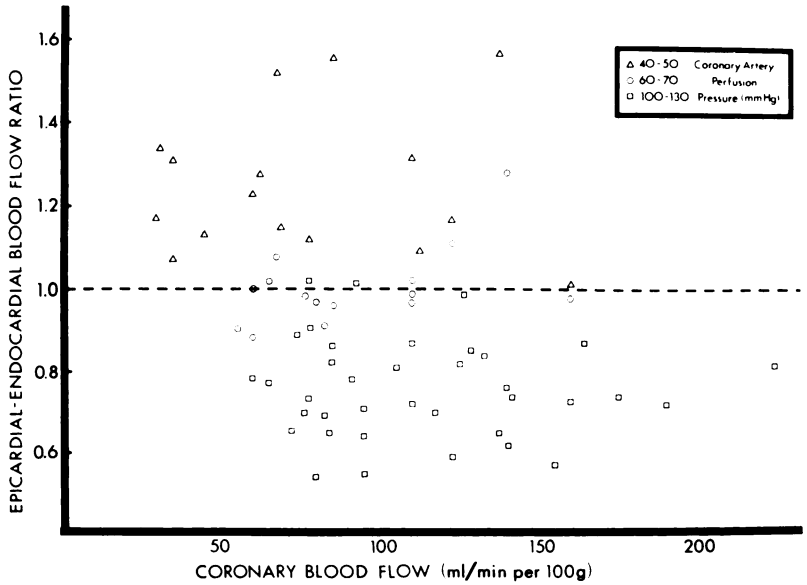

FIG. 5. Relationship between total coronary blood flow and the epicardial/endocardial blood flow ratio at three ranges of perfusion pressure. Each point represents the mean epicardial-endocardial ratio of the left ventricle. Individual points are from the four groups. At each point left ventricular systolic pressure and heart rate were 75 to 100 mm Hg and 150 beats/min, respectively.

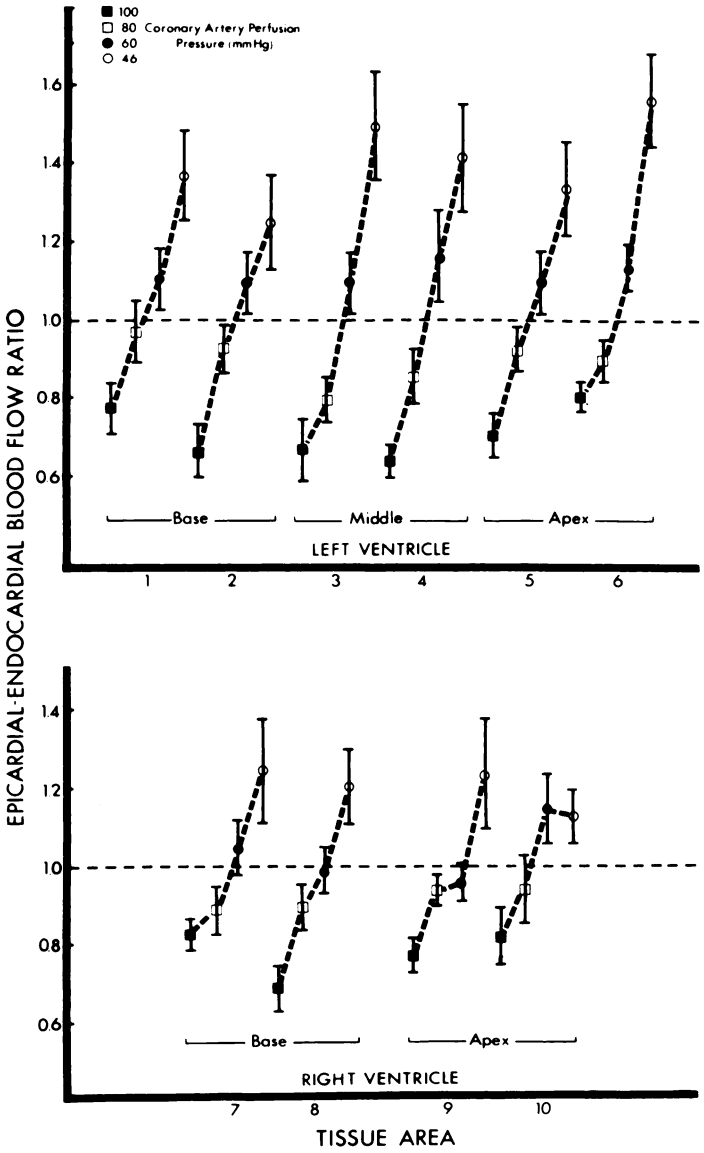

FIG. 6. Relationship between coronary artery perfusion pressure and distribution of flow between epicardium and endocardium in the 10 tissue areas. Perfusion pressure was decreased by intracoronary infusion of chromonar. Each point represents mean ± S.E.M. (N = 8).

Microsphere size, anesthesia, type of preparation (isolated heart, open-chest animal, closed-chest animal) and the hemodynamic state of the heart (perfusion pressure, heart rate, degree of autonomic tone) would markedly influence regional myocardial blood flow. Although many of these factors may affect the epi/endo, this would not invalidate our use of the microsphere technique to measure relative changes in tissue perfusion.

Heart rate. The effect of tachycardia on epi/endo has been studied. Several reports indicate that the epi/endo remains homogenous over a wide range of heart rates (Buckberg *et al.*, 1975; Gross and Winbury, 1973). A criticism of previous work is that spontaneous heart rates were abnormally high and not physiological (Neill *et al.*, 1973). The ISHP has the advantage of lower spontaneous rates within the physiological range.

Results of the present study are in agreement with those of Neill *et al.* (1973, 1975) who found increased epi/endo during ventricular pacing in normal unanesthetized dogs and those with a proximal coronary stenosis, and Flameng *et al.* (1975) who reported decreased endocardial perfusion produced by pacing during maximal coronary vasodilation.

In the ISHP, coronary blood flow was maintained constant by a pump. In addition, oxygen extraction (25.5–43.8%) was sufficiently low at heart rates of 111 to 203 beats/min that compensatory vasodilation (indicated by decreases in coronary artery perfusion pressure and coronary vascular resistance) was small except at the highest heart rate. Therefore, tachycardia was unaccompanied by major hemodynamic changes, although the fall in coronary artery perfusion pressure at the highest heart rate probably contributed to the increased epi/endo.

Downey and Kirk (1974) have shown a large gradient in regional perfusion during systole. With ^{86}Rb to measure regional blood flow, the epicardium was found to have a blood flow twice that of the endocardium during systole. To maintain equal or greater perfusion of endocardium, endocardial flow should be greater than that to epicardium during diastole. Thus, any factor which decreases diastolic perfusion time might be expected to increase epi/endo. In the present series of experiments, the systole/diastole ratio increased from 0.34 ± 0.03 to 0.70 ± 0.07. Therefore, tachycardia shortened diastole relative to systole and thereby decreased endocardial perfusion.

Left ventricular systolic pressure. Previous reports suggest that the level of intraventricular pressure has no effect on epi/endo, if coronary artery perfusion pressure is maintained (Cutarelli and Levy, 1963; Moir and Debra, 1967). On the other hand, Gamble *et al.* (1974) and Monroe *et al.* (1972) have reported transient decreased endocardial perfusion after abrupt increases in left ventricular systolic pressure. In the present study, as left ventricular systolic pressure was elevated there was a small increase in coronary artery perfusion pressure due to increased extravascular compression. Appar-

TABLE 3

Effect of adenosine and nitroglycerin on regional myocardial blood flow (mean ± S.E.M.)

Abbreviations used are: CAPP, coronary artery perfusion pressure; CVR, coronary vascular resistance; LV Epi/Endo, left ventricular epicardial/endocardial blood flow ratio.

Drug	CAPP	CVR	LV Epi/Endo	LV Epi/Endo	
				Expt. Δ	Predicted Δ
Adenosine (constant CBF) (N = 6)					
Control$_1$	98.4 ± 1.3	1.09 ± 0.07	0.71 ± 0.06		
50 μg/min	82.0 ± 1.9[a]	0.91 ± 0.06[a]	0.80 ± 0.06[a]	0.09 ± 0.03[a]	0.08 ± 0.01[a]
Control$_2$	98.6 ± 2.4	1.03 ± 0.06	0.72 ± 0.06		
100 μg/min	71.6 ± 3.4[a]	0.79 ± 0.06[a]	0.93 ± 0.06[a]	0.21 ± 0.04[a]	0.19 ± 0.02[a]
Nitroglycerin (constant CBF) (N = 6)					
Control$_1$	94.3 ± 1.0	1.00 ± 0.10	0.77 ± 0.06		
5 μg/min	85.8 ± 2.0[a]	0.90 ± 0.08[a]	0.83 ± 0.07[a]	0.06 ± 0.02[a]	0.05 ± 0.01[a]
Control$_2$	92.5 ± 1.8	0.97 ± 0.09	0.78 ± 0.06		
10 μg/min	80.2 ± 2.9[a]	0.84 ± 0.07[a]	0.88 ± 0.07[a]	0.10 ± 0.02[a]	0.08 ± 0.01[a]
Nitroglycerin (constant CAPP) (N = 6)					
Control$_1$	100	1.34 ± 0.16	0.65 ± 0.07		
5 μg/min	100	1.20 ± 0.15[a]	0.65 ± 0.08	0.00 ± 0.03	0.00 ± 0.00
Control$_2$	100	1.31 ± 0.17	0.71 ± 0.10		
10 μg/min	100	0.89 ± 0.06[a]	0.71 ± 0.11	0.00 ± 0.04	0.00 ± 0.00

[a] Significantly different ($P < .05$) from control by paired comparison.

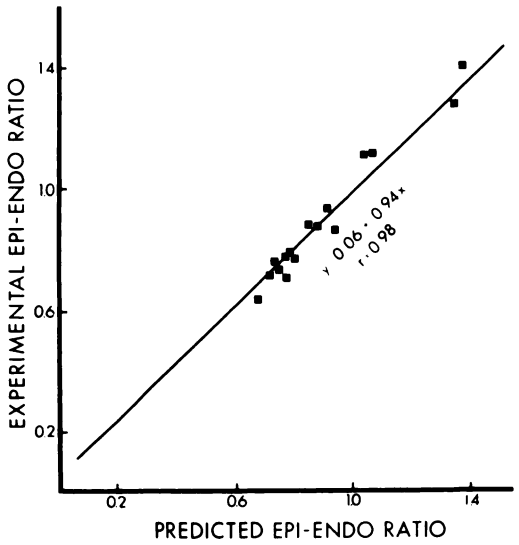

FIG. 7. Comparison of mean epicardial/endocardial (epi/endo) blood flow ratios of the left ventricle calculated from the multiple regression equation [R = −0.07226 + 0.0022 Hr + 0.00067 LVSP + 0.00053 CBF + 40.01146 (1/CAPP)] with values obtained experimentally.

ently, the increase in extravascular compression was small and the maintenance of the normal coronary artery perfusion pressure was adequate to prevent a redistribution of blood flow away from the endocardium. In addition, since blood flow to endocardium occurs primarily in diastole, increasing left ventricular systolic pressure would be expected to have a minimal effect on epi/endo. A significant decrease in endocardial perfusion occurred at the highest left ventricular systolic pressure; however, the epi/endo was still less than 1.0. Although alterations in heart rate and coronary artery perfusion pressure affected epi/endo similarly in the right and left ventricle, no changes in epi/endo of the right ventricle were observed during changes in left ventricular systolic pressure (table 1).

Coronary artery perfusion pressure. In the present investigation, perfusion pressure was varied by either changing coronary blood flow (group 3) or by infusing a coronary vasodilator at a constant blood flow or perfusion pressure (group 4). The absolute level of coronary blood flow had no significant effect on epi/endo (fig. 5), but acted through changes in coronary artery perfusion pressure (tables 2 and 3). Although low coronary artery perfusion pressure was associated with a high epi/endo in both groups, important differences existed. At a reduced coronary blood flow and perfusion pressure (group 3), flow to epicardium and endocardium was low. In addition, there was a heterogenous transmural gradient in blood flow, the endocardium receiving less flow than epicardium. As perfusion pressure and blood flow were increased, the proportion of total flow to endocardium increased. Above 100 mm Hg, the distribution of blood flow between epicardium and endocardium remained similar (table 1). Coronary vascular resistance was maintained near control until a high flow and perfusion pressure were attained. In group 4, coronary vascular resistance and perfusion pressure decreased in parallel during intracoronary infusion of chromonar. Chromonar has been shown to produce maximal dilation of the coronary arterioles and to abolish reactive hyperemia in dogs (Weiss *et al.* 1973). At maximal coronary vasodilation (perfusion pressure of 46 mm Hg) the epi/endo was higher than that obtained by a reduction of blood flow to give a perfusion pressure of 40 mm Hg in group 3. As vascular resistance was decreased in parallel with perfusion pressure, there was a marked redistribution of flow away from the endocardium in spite of an adequate total coronary blood flow. This phenomenon resembles coronary steal (Wilcken *et al.*, 1971). Similar results were obtained when either adenosine or nitroglycerin were administered by intracoronary infusion to the ISHP perfused at a constant coronary blood flow.

Clinical implications. Under normal conditions, coronary blood flow increases to meet increased myocardial oxygen demands (Berne, 1964) because oxygen extraction is already high. Since the endocardium extracts more oxygen than epicardium (Monroe *et al.*, 1975; Gamble *et al.*, 1974), the inner layers of the heart are especially flow-dependent for oxygen delivery. Factors modulating flow to the endocardium may have some influence on the pathophysiology of myocardial infarction and angina pectoris.

The coronary shunt hypothesis was developed by Somani *et al.* (1970) to explain decreased clearance of ^{86}Rb by the heart during administration of catecholamines or during increases in heart rate (Somani *et al.*, 1969) or left ventricular systolic pressure. The decreased clearance of ^{86}Rb was explained: either flow to endocardium could be diverted to epicardium or anatomical arterial-venous shunts could be opened. The present study differentiates between the two

hypotheses. No significant difference was found in the content of radioactivity (microspheres) in coronary venous blood, before or after any of the experimental interventions. However, this does not preclude the possibility that arterial-venous shunts less than 15 μ in diameter exist in the myocardium. More likely, functional shunting of flow occurs between epicardium and endocardium in response to various hemodynamic alterations.

The ISHP perfused at constant CBF has similarity to three-vessel coronary artery disease where under normal conditions blood flow may be adequate, but during stress flow cannot increase to meet increased oxygen demand, and angina pectoris or myocardial infarction results. Subendocardial infarction is common in man (Guy and Eliot, 1970) and canine infarcts are exclusively endocardial (Shaper, 1971). We have defined several physiological alterations that may lead to endocardial ischemia, *e.g.*, decreased diastolic coronary perfusion gradient, increased heart rate (decreased diastolic perfusion time) and, of lesser importance, increased left ventricular systolic pressure. Previously, Buckberg *et al.* (1972) showed the importance of the diastolic perfusion gradient and the diastolic duration in determining subendocardial perfusion during maximal coronary vasodilation.

In contrast, therapeutic approaches to the problem of endocardial ischemia might involve increasing diastolic coronary perfusion gradient, decreasing heart rate (increasing diastolic perfusion time) and left ventricular systolic pressure or a combination of the three. Further studies with drugs are needed in this model to determine the applicability of the multiple regression equation to predict changes in coronary blood flow distribution initiated by hemodynamic changes after administration of pharmacological agents.

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