the low activities of kinase measured in extracts from animal tissues. Hydrolysis of FMN by spleen homogenates has been noted previously(11). The physiological significance of the pH 5 phosphatase may be to maintain a check on the intracellular level of FMN. This phosphatase is relatively specific as little activity is found with several organic monophosphates tested as substrates. More extensive examinations of the partially purified enzyme are being made.

Summary. Flavokinase is present in extracts from several rat tissues with activity in liver > kidney > brain > spleen > heart. Phosphatases, maximally active at pH 5, partially mask the kinase activity in these extracts. It is concluded that synthesis of FMN occurs in several organs rather than intestine alone.

1. Beinert, H., in Boyer, P. D., Lardy, H., Myrback, K., (Editors), *The Enzymes,* Academic Press, Inc., 2nd ed., 1960, v2, 338.
2. Yagi, K., *J. Biochem.* (Japan), 1954, v41, 757.
3. Yagi, K., Okuda, J., *Nature,* 1958, v181, 1663.
4. Englard, S., *Fed. Proc.,* 1952, v11, 208.
5. McCormick, D. B., *ibid.,* 1961, v20, 447.
6. Lowry, O. H., Rosebrough, N. J., Parr, A. R., Randall, R. J., *J. Biol. Chem.,* 1951, v193, 265.
7. Kearney, E. B., Englard, S., *ibid.,* 1951, v193, 821.
8. Burch, H. B., Bessey, O. A., Lowry, O. H., *ibid.,* 1948, v175, 457.
9. Kilgour, G. L., Felton, S. P., Huennekens, F. M., *J. Am. Chem. Soc.,* 1957, v79, 2254.
10. Fiske, C. H., Subbarow, Y., *J. Biol. Chem.,* 1925, v66, 375.
11. Crammer, J. L., *Nature,* 1948, v161, 349.

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Distribution of Blood Flow to the Canine Heart.* (26755)

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Total blood flow to an organ can be measured by several methods. The distribution of blood or regional flow within an organ is more difficult to quantitate. In the present investigation radioactive microspheres were used to measure regional distribution of blood in the myocardium of the dog and to estimate the size and volume flow through intercoronary anastomoses and arteriovenous shunts.

Methods. The method used is a modification of the technic of Prinzmetal *et al.*(1) developed by Grim and Lindseth(2). In these experiments glass microspheres, 20 μ in diameter, rendered radioactive by the conversion of some of the Na^{23} content of glass to Na^{24} by neutron bombardment, were injected into the coronary arteries of the dog. With the reasonable assumption that the spheres are distributed at arterial bifurcations in the same proportion as is the blood, the fraction of the injected spheres which passed through arteriovenous anastomoses could be quantitated by collecting all venous drainage and counting it in a scintillation counter. Likewise, since those spheres that remained behind in any particular tissue presumably did so because they lodged in vessels smaller than the diameter of the spheres, the "sphere count" recovered from this tissue would provide a measure of flow through vessels smaller than the spheres. If the spheres were small enough to pass through all arteriovenous shunts except capillaries, the radioactivity of the tissue would be a measure of tissue capillary flow. For the purposes of the present study tissue blood flow refers to flow through vessels 20 μ or smaller. This includes all true capillaries (less than 12 μ) and capillary-like vessels(16-20 μ).

The fraction of spheres which entered the myocardium supplied by the anterior de-

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scending branch of the left coronary artery after ligation of this vessel would provide a measure of flow through intercoronary anastomoses at least the size of the spheres used.

Preparation of microspheres. Glass microspheres‡ ranging in size from 5 to 200 μ were fractionated into homogenous samples by multiple elutriations. The spheres were subjected to neutron bombardment in the atomic pile at Oak Ridge National Laboratory. A small group of spheres was suspended in a flask of 1.5% gelatin. The mixture was stirred to keep the spheres in suspension. Ten to 20 μl of the suspension containing between 500-1000 spheres were drawn into a short section of polyethylene tubing and radioactivity within the tubing measured in a scintillation counter both before and after delivery to the dog.

Preparation of animal. Adult, mongrel dogs of either sex, anesthetized with sodium pentobarbital (33 mg/kg) and heparinized with 1.5 mg/kg, were used. The right carotid artery was exposed and cannulated with a polyethylene catheter which was advanced *via* the brachiocephalic artery into the ascending aorta. This catheter was used for coronary artery perfusion and for injection of the radioactive microspheres. Tapes were placed about the superior and inferior vena cavae and brachiocephalic artery. The azygos vein was ligated. The aortic arch was dissected to permit placement of an occluding clamp on the aorta immediately distal to the brachiocephalic artery.

At the beginning of experiment inflow, occlusion was established by drawing tight the tapes about the vena cavae. The tape about the brachiocephalic artery was tightened onto the catheter within its lumen. Perfusion *via* the catheter placed in the ascending aorta was started after 4 or 5 cardiac cycles. Heparinized blood at 38°C was injected from a calibrated siliconized glass cylinder with a pressure of 135 mmHg. Blood was collected from the right ventricle and coronary sinus by inserting a catheter *via* the right atrial appendage into the right ventricle. Holes were present in the catheter so that drainage from both ventricle and atrium was assured. When coronary flow in the beating heart was well established and after the aorta was clamped distal to the brachiocephalic vessels, the radioactive microspheres were injected into the perfusion catheter. Perfusion was continued for several minutes and volume flow was noted. Following this, perfusion was discontinued, all luminal blood was collected, and the heart was removed and weighed. The heart was divided into right ventricle, left ventricle supplied by the anterior descending coronary artery, left ventricle supplied by the circumflex artery and the septum. The ascending aorta, pulmonary artery, venous drainage from the heart and the catheters used were separately labelled for counting. All tissue was divided into 2 g samples and placed into glass vials. Radioactivity of all samples was determined using a well scintillation detector. In some experiments the left anterior descending (L.A.D.) artery was cannulated. Perfusion and sampling was carried out otherwise as described.

The distribution of microspheres throughout the heart was determined. Total flow to the heart was measured from the fall in the calibrated reservoir over a timed period. From these 2 measurements flow in ml/g/min was calculated for specific anatomic regions. Only experiments in which at least 95% of the injected spheres were recovered are reported.

Four types of experiments were performed: (1) Total perfusion of the normal dog heart; spheres 20 μ in diameter—3 dogs. (2) Perfusion of the dog heart with left anterior descending coronary occlusion; spheres 20 μ in diameter—7 dogs. (3) Perfusion of the left anterior descending coronary artery; spheres 20 μ in diameter—7 dogs. (4) Perfusion of the left anterior descending coronary artery; spheres 50 μ in diameter.

Results and discussion. The results are illustrated in Tables I to IV. In the normal dog heart microspheres 20 μ in diameter were distributed uniformly throughout the myocardial mass. Hence, the calculated mean tissue blood flow to the right ventricle was 1.36 ml/g/min, to the distribution of the L.A.D.

‡ Kindly supplied by Dr. John Ryan, Minnesota Mining and Manufacturing Co., St. Paul.

TABLE I. Fractional Distribution of 20 μ Spheres in Normal Dog Heart with Calculated Blood Flows.

Exp. No.	Fraction of spheres recovered R.V.	L.A.D.	S.&C.	R.A.	L.A.	Total flow, ml/min.	A/V shunts	Flow, ml/g/min. R.V.	L.A.D.	S.&C.	R.A.	L.A.
1	.19	.15	.55	.02	.03	83	6.0%	1.4	1.4	1.8	.48	.85
2	.29	.20	.41	.04	.03	60	3.0%	1.7	1.5	1.32	.40	.85
3	.29	.12	.49	.03	.03	103	4.0%	.94	.60	.66	.45	.45
Mean	.26	.16	.48	.03	.03		4 %	1.36	1.17	1.26	.44	.58

R.V., right ventricular myocardium; L.A.D., myocardium supplied by left anterior descending coronary artery; S., septum; C., myocardium supplied by circumflex artery; R.A., right atrium; L.A., left atrium; A/V, arterio-venous.

1.17 ml/g/min and to the septum and circumflex distribution 1.26 ml/g/min. Four percent of total flow passed through arteriovenous shunts 20 μ or greater in diameter. The distribution of spheres was: right ventricle 26%; myocardium supplied by the L.A.D. 16%; the myocardium supplied by the circumflex and the septum 48%; the right and left atria 3% to each.

There was a marked decline in number of spheres which reached the myocardium supplied by the L.A.D. after ligation of this vessel (Table II). Instead of 16% of total spheres injected (tissue flow of 1.17 ml/g/min) reaching this area of myocardium, only 3% of total flow or .29 ml/g/min reached this area. It is assumed that they traversed intercoronary anastomoses 20 μ or greater in size.

Approximately 80% of 20 μ spheres remained in the distribution of the L.A.D. when this vessel was perfused. Twenty percent of the total spheres injected passed *via* communications 20 μ or greater in size: 3.0% *via* arteriovenous shunts to the coronary sinus or lumen of the right ventricle; and *via* arterial collateral circulation 6% to the right ventricle, 8% to the septum, and 3% to the myocardium supplied by the circumflex artery (Table III).

A very similar distribution existed when 50 μ spheres were injected into the L.A.D. artery (Table IV) hence it may be concluded that the interarterial and arteriovenous shunts that exist are greater than 50 μ and that very few communications between 20 and 50 μ exist.

Microspheres were never found in the luminal blood of the left ventricle indicating competence of the aortic valve in Exp. 1 and 2. This finding casts some doubt on the existence in the dog of left arterioluminal shunts. These vessels, described by Wearn (3), are believed capable of shunting blood either into or from the ventricular lumens and thereby provide a potential source of collateral flow to ischemic muscle. Furthermore, all arterioluminal and arteriovenous shunts on the right side do not appear to exceed 5% of total flow.

This method provides a quantitative measurement in the heart of flow *via* arterial collaterals and arteriovenous shunts. The size of the vessels in question can be precisely de-

TABLE II. Total Perfusion Dog Heart with L.A.D. Coronary Artery Occlusion Injection Glass Microspheres 20 μ in Diameter.

Exp. No.	Fraction of spheres recovered R.V.	L.A.D.	S.&C.	R.A.	L.A.	Total flow, ml/min.	A/V shunts	Flow, ml/g/min. R.V.	L.A.D.	S.&C.	R.A.	L.A.
5	.28	.01	.60	.03	.04	73	4.0%	1.20	.03	1.50	.46	.35
6	.21	.02	.58	.08	.07	93	4.0%	.67	.19	.85	.86	.61
7	.28	.01	.60	.06	.04	43	1.0%	.44	.02	.34	.36	.28
8	.25	.08	.56	.03	.05	118	3.0%	2.20	.99	2.38	1.04	1.43
9	.23	.02	.64	.02	.06	72	3.0%	1.50	.23	1.51	.72	1.00
10	.18	.02	.74	.02	.02	108	2.0%	1.55	.30	2.47	.73	.71
11	.18	.05	.69	.01	.05	130	2.0%	.74	.28	1.20	.38	.86
Mean	.23	.03	.63	.04	.05		2.6%	1.18	.29	1.46	.65	.75

TABLE III. Perfusion L.A.D. Coronary Artery Injection Glass Microspheres 20 μ in Diameter.

	Fraction of spheres recovered						Flow, ml/g/min.			
Exp. No.	R.V.	L.A.D.	S.	C.	Total flow, ml/min.	A/V shunts	R.V.	L.A.D.	S.	C.
29	.06	.86	.04	.01	60	3.0%	.27	2.60	.24	.07
30	.01	.92	.02	.02	20	3.0%	.01	.72	.03	.04
31	.07	.82	.04	.05	33	2.0%	.26	1.40	.13	.12
32	.02	.83	.08	.03	20	4.0%	.02	.68	.11	.01
33	.05	.66	.21	.04	115	4.0%	.21	1.40	.83	.16
34	.10	.73	.06	.07	48	4.0%	.08	.62	.14	.06
35	.09	.73	.13	.02	65	3.0%	.23	1.56	.06	.02
Mean	.06	.79	.08	.03		3.3%	.17	1.3	.22	.07

TABLE IV. Perfusion L.A.D. Coronary Artery Injection Glass Microspheres 50 μ in Diameter.

	Fraction of spheres recovered						Flow, ml/g/min.			
Exp. No.	R.V.	L.A.D.	S.	C.	Total flow, ml/min.	A/V shunts	R.V.	L.A.D.	S.	C.
50	.04	.78	.02	.13	18	3%	.01	.70	.03	.17
51	.06	.78	.03	.07	50	6%	.04	.76	.04	.07
52	.04	.92	.01	.01	15	2%	.04	.67	.15	.15
53	.01	.90	.04	.02	24	3%	.01	.60	.06	.05
Mean	.04	.84	.03	.06		3.5%	.03	.68	.07	.11

termined and the value of operative or other procedures or factors believed to increase intercoronary anastomoses might be more quantitatively assessed.

The beads used in the present study were completely spherical. The small number injected did not change the flow, pressure, resistance relationships that existed in the heart prior to their injection. Preliminary studies revealed that long periods of perfusion after injection of the microspheres did not increase the number of spheres which passed through arteriovenous shunts over that which existed with very short periods of perfusion.

Summary. A known quantity of radioactive glass microspheres either 20 or 50 μ in diameter were injected into the coronary system of the dog heart being perfused at a known and physiological rate with oxygenated blood at 38°C. With the assumption that spheres were distributed at arterial bifurcation in the same proportion as blood, the fraction which appeared in the venous drainage was taken as a measure of arteriovenous shunts the size of or larger than the spheres injected. Those spheres which remained in the tissue provided a measure of flow through vessels smaller than the spheres. Since the spheres were small enough to pass through all arteriovenous shunts except capillaries, the radioactivity of the tissue was a measure of tissue capillary flow (less than 20 μ for the purposes of the present study). There was a uniform distribution of spheres in the normal dog heart. A calculated tissue blood flow of approximately 1.2 cc/g/min existed in the right ventricle and throughout the left ventricle. This flow diminished to approximately .30 cc/g/min in the distribution of the left anterior descending coronary artery after ligation of this vessel. Evidence is presented to suggest that arteriovenous shunts account for 2-4% of total flow across the heart and that these vessels are probably greater than 50 μ in diameter. No evidence for arterioluminal shunts 20 μ or greater in size communicating with the left ventricle was found.

1. Prinzmetal, M., Simkin, B., Berman, H., Kruger, H., *Am. Heart J.*, 1947, v33, 420.

2. Grim, E., Lindseth, E. O., *Med. Bull. Univ. of Minn.*, 1958, v30, 138.

3. Wearn, J. T., Mettier, S. R., Klumpp, T. G., Zschiesche, L. J., *Am. Heart J.*, 1933, v9, 143.

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