D. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 820.

9. Seligman, M., *Rev. Fran. d'etudes Clin. et Biol.*, 1958, v3, 558.

10. Robbins, W. C., Holman, H. R., Deicher, H., Kunkel, H., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 575; Carlson, L. A., Mollerberg, H., *J. Lab. Clin. Med.*, 1958, v51, 53.

11. Frious, G. J., Finch, S. C., Detre, K. D., *J. Immunol.*, 1958, v80, 324; Holman, H R., Kunkel, H., *Science*, 1957, v126, 162.

12. Rifkind, R. A., Godman, G. C., *J. Exp. Med.*, 1957, v106, 607.

Received September 9, 1958. P.S.E.B.M., 1958, v99,

Red Cell and Plasma Transit Through Spleen: the Splenic Hematocrit.* (24449)

ANTHONY J. SLIWINSKI† AND LAWRENCE S. LILIENFIELD‡
(Introduced by John C. Rose)
Cardiovascular Research Laboratory, and Depts. of Medicine and Physiology, Georgetown University Medical Center, Washington, D. C.

Considerable interest in the physiology of the spleen has been shown since Barcroft and co-workers(1-6) showed its importance as a reservoir for red blood cells. This function has been confirmed by Haam(7) and Kramer (8). Kramer concluded on effect of hypoxia on systemic hemoglobin content and spleen weight, that the spleen consists almost entirely of packed red cells. Reeve(9) postulated from P^{32} blood volume determinations that the spleen concentrates red blood cells. Everell(10) calculated splenic hematocrit in rats, after allowing 15 minutes for red cell mixing with injected labeled red cells. He cound the hematocrit to be 46.9 in female rats and 50.7 in the males. These values would raise a question when compared to Kramer's(8) conclusion that the spleen consists almost entirely of packed red cells. However, Barcroft(1) using CO. had previously demonstrated that 30 to 90 minutes elapses before the general circulation and general mass of hemoglobin in the spleen acquire the same CO content. This indicates that perhaps Everell did not allow sufficient time for red cell mixing to occur. The following experiments were designed to determine the time necessary for equilibrium to be reached between general systemic circulation and splenic venous outflow. This enabled calculation of mean transit time of plasma and red blood cells and provided data from which the splenic hematocrit could be determined.

Methods. Eight healthy mongrel dogs weighing 9 to 12 kg were anesthetized with sodium pentobarbital, 25 mg/kg. A generous midline abdominal incision was made and the spleen exposed. One of the large splenic veins was dissected free, care being taken that adequate collateral venous drainage was left intact. This vein was incised and a polyethylene catheter tied into the proximal segment. The free end of the catheter contained a stopcock. Similar polyethylene catheters were inserted into the femoral artery and femoral vein. In 6 experiments, aortic pressure was monitored with a mercury manometer connected to the femoral arterial catheter. Twelve ml of a saline suspension of the dogs' red cells previously tagged with approximately 500 microcuries of Cr^{51} and washed free of any extracorpuscular Cr^{51} activity was mixed with 125 microcuries of I^{131} tagged human serum albumin. In one experiment, I^{131} gamma globulin was used instead of I^{131} serum albumin. This mixture was then injected over an 8 to 10 second period into the femoral vein. From the start of injection until 5 minutes later, femoral arterial blood and splenic venous blood was collected continuously at 30 second intervals into paraf-

* Supported in part by research grants from Nat. Heart Inst., U.S.P.H.S., and Amer. Heart and Washington Heart Assn.

† Medical Student Fellow, Nat. Foundation for Infantile Paralysis.

‡ Established Investigator, American Heart Association.

TABLE I. Splenic Red Cell and Albumin Transit Times in 8 Dogs.

Arterial B.P. (mg/Hg) Zero time	Equil. time	Equil. time (min.) Red cell	Plasma	Mean transit time (min.) Red cell T_r	Plasma T_p	T_r/T_p	Hct. l.v.†	Hct. spl.	Hct. spl. / Hct. l.v.
		87.5	32.5	39.82	5.29	7.53	49.5	88	1.8
		87.5	15.0	34.03	8.28	4.1	31	65	2.1
140	118	26.0	3.8	6.8	.72	9.44	46	89	1.9
128	100	56.0	4.0	11.16	1.01	11.04	42	89	2.1
108	96	62.5	15.0	18.54	4.41	4.20	38	72	1.9
114	100	47.5	27.5	9.41	2.43	3.87	37	70	1.9
140	61	85.0	31.0	26.4	6.90	3.82	36	68	1.9
125	75	22.5	3.5	6.52	.98	6.65	38	80	2.1
Avg		59.3	16.5	19.09	3.75	6.33	39.7	77.6	1.96 ± .12‡

* I^{131} gamma globulin used instead of I^{131} serum albumin.
† Hct. l.v. was determined by centrifugation of arterial blood. ‡ ± stand. dev.

fined test tubes containing dried heparin solution. Intermittent samples were then collected every 5 minutes until 30 minutes had elapsed and thereafter every 10 minutes until 2 hours had elapsed from the time of injection. (Only experiments in which splenic venous catheter blood flow remained constant during the collection period were utilized in this study.) Following collection of the samples, 0.5 ml aliquots of whole blood were pipetted into glass vials. These were centrifuged and the red blood cells washed 3 times with normal saline, care being taken not to remove any of the red cells. The Cr^{51} activity of these washed aliquots were counted in a well-type scintillation counter, and the activity expressed as Cr^{51} counts per ml whole blood. The remaining blood in the collection tubes was centrifuged and 0.3 ml aliquots of the supernatant plasma were counted in a similar manner, the activity being expressed as I^{131} counts per ml plasma. From the Cr^{51} counts per ml in each sample and its time of collection (corrected for collecting system catheter delay and using the midpoint of the injection period as zero time), a red cell concentration vs. time curve was constructed for arterial and splenic venous blood. From the plotted curve, equilibrium time and equilibrium concentration were determined. From this data the red cell mean transit time was calculated employing a formula modified from the method of Bradley and co-workers(11) for estimating splanchnic blood volume. Mean transit time was taken as equal to the difference between mean arterial and venous concentrations from zero time to equilibrium time divided by equilibrium concentration. Similarly, plasma albumin mean circulation time was determined from the arterial and venous I^{131} counts. The hematocrit of the spleen was calculated directly from the ratio of mean transit times and large vessel hematocrit employing the formula previously derived by Lilienfield and co-workers(12,13).

Results. The time for red cell equilibrium to be reached between the splenic vein and femoral artery was longer in all experiments than the time for plasma equilibrium to occur. Average time for red cell equilibrium was 59.3 minutes (22.5 to 87.5). Average time for plasma equilibrium was 16.5 minutes (3.5 to 32.5). Red cell mean transit time as calculated by the above formula was 19.1 minutes (6.8 to 39.8). Plasma albumin mean transit time was 3.8 minutes (0.7 to 8.3).

Average hematocrit of the spleen was calculated as 77.6 (65 to 88). Ratio of splenic hematocrit to large vessel hematocrit averaged 1.96 ± .12 (S.D.).

Discussion. Sodium pentobarbital was chosen as the anesthetic agent because it causes relaxation of the spleen(14). It was thought that it would prevent the reflex contraction induced by the trauma associated with catheterizing the splenic vein and more closely simulate the physiological status of the spleen in the intact animal. For this same reason, care was taken to insure adequate splenic venous drainage when the splenic vein was catheterized.

The method of determining splenic hematocrit from transit time data requires that the blood flow remain constant. In 4 of the ex-

periments the arterial pressure fell from 125 mm Hg to 105 mm Hg. This fall was considered insignificant so that a constant blood flow during this period could be assumed.

However, in 2 experiments, the fall was from 130 mm Hg to 70 mm Hg at the time of red cell equilibrium. The results obtained from these experiments did not significantly differ from the others, therefore, the data were considered valid. It appears that the splenic blood flow remains rather constant over a wide range of blood pressure variations in a pentobarbital anesthetized dog.

The wide range of times (22.5 to 87.8 minutes) required for red cell equilibrium to occur agrees well with Barcroft's(1) determinations that 30 to 90 minutes are required for red cell equilibrium in the spleen. This would indicate that a longer time than the usual 20 minutes should be used when determining blood volumes in dogs with Cr^{51} tagged red blood cells. It would probably be best to use the upper limit of 90 minutes to insure adequate time for red cell mixing. The low splenic hematocrit figures obtained by Everell(9) were most likely the result of not permitting sufficient time for equilibrium.

It is notable that in this study the splenic hematocrit was very nearly twice the large vessel hematocrit (average 1.96 ± .12 S.D.) over a range of arterial hematocrits from 31 to 49.5. This supports the contention that the dog spleen contains red cell concentrated blood.

Summary. Five-hundred microcuries Cr^{51} tagged red blood cells and 125 microcuries I^{131} tagged serum albumin was injected into 8 sodium pentobarbital anesthetized dogs and blood samples collected simultaneously from the femoral artery and splenic vein. Time for red cell equilibrium was 59.3 minutes (22.5 to 87.5 minutes). Plasma equilibrium time was 16.5 minutes (3.5 to 32.5 minutes); red cell mean transit time was 10.09 minutes (6.8 to 39.82 minutes). Plasma mean transit time was 3.75 minutes (0.72 to 8.28 minutes). Average hematocrit of the spleen was 77.6 (65 to 88). Ratio of splenic hematocrit to large vessel hematocrit was 1.96 ± .12 S.D. over a range of arterial hematocrits from 31 to 49.5.

The authors gratefully acknowledge technical assistance of Mr. Thomas Doyle, Mr. Francis Farrell and Miss Jean Pietras.

1. Barcroft, J., Barcroft, H., *J. Physiol.,* 1923, v58, 138.

2. Barcroft, J., Murray, C. D., Orahovats, D., Sands, J., Weiss, R., *ibid.,* 1925, v60, 79.

3. Barcroft, J., Harris, H. A., Orahovats, D., Weiss, R., *ibid.,* 1925, v60, 443.

4. Barcroft, J., Flory, H. W., *ibid.,* 1928, v66, 231.

5. ———, *ibid.,* 1929, v68, 181.

6. Barcroft,, J., *Proc. Staff. Meet. Mayo Clin.,* 1929, v4, 302.

7. Haam, E. V., PROC. SOC. EXP. BIOL. AND MED., 1934, v31, 1206.

8. Kramer, K., Luft, U. C., *Am. J. Physiol.,* 1951, v165, 215.

9. Reeve, E. B., Gregersen, M. I., Allen, T. H., Sear, H., *Trans. Assn. Am. Physicians,* 1953, v175, 195.

10. Everell, N. B., Simmons, B., Lasher, E. P., *Circ. Research,* 1956, v4, 419.

11. Bradley, S. E., Marks, P. A., Reynell, P. C., Meltzer, J., *Trans. Assn. Am. Phycns.,* 1953, v66, 294.

12. Lilienfield, L. S., Kovach, R. D., Marks, P. A., Hershenson, L. M., Rodman, G. P., Ebaugh, F. G., Freis, E. D., *J. Clin. Invest.,* 1956, v35, 1385.

13. Lilienfield, L. S., Rose, J. C., Porfido, F. A., *Circ. Research,* 1957, v5, 64.

14. Goodman, L. S., Gilman, A., *Pharmacological Basis of Therapeutics.* 2nd Edit., MacMillan Co., N. Y., 1955.

Received September 11, 1958. P.S.E.B.M., 1958, v99.