Blood Volume I:

Critique: Spun vs. Isotope Hematocrit; ^{125}RIHSA vs. ^{51}CrRBC

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KEITH *et al.*[40] and Dawson *et al.*[20] introduced the dilution principle using color tracers to measure plasma volume (PV). As the technics developed, the problems of identification of phase dilution (curve analysis), and of extrapolation to zero time became all too evident.[2, 22, 59] The occurrence of biologic binding of indicator ("sink") and of endogenous sources of the pool solute ("cold springs") further complicated the procedures. Increasing knowledge of the dynamics of the albumin pool [1, 43, 65, 66, 69, 70] and of technics of tagging [21] led to progressive confidence in this indicator dilution technic. Thus, from extensive comparative laboratory data, one of us (H. S.) developed a mean disappearance curve of ^{131}I activity in splenectomized dogs from 10 minutes to 6 hours after injection for the purpose of studying the early plasma and blood volume responses to acute arterial hemorrhage.[63, 64]

However, many investigators [28, 31, 36, 46, 48] found that tagged albumin always yielded larger values for blood volume than simultaneous use of iron or phosphorous-tagged erythrocytes. In 1950, Sterling and Gray [60] introduced the use of radioactive chromate to tag red blood cells, a procedure subsequently developed by others.[49, 61] With this method of tagging erythrocytes available, simultaneous independent double-isotope measurements of red cell mass (RCM) and PV were possible. This led to the finding of an "isotope hematocrit," interpreted as reflecting the true whole body hematocrit (WBH), which was lower than the large vessel hematocrit (LVH) as measured by standard centrifuge technics. Thus, a physiological paradox emerged of profound over-all biological significance, i.e., WBH was measured as less than the hematocrit of blood in large vessels.

The problem has been frequently reviewed.[13, 14, 47] At fiirst the paradox was thought by Mayerson *et al.*[44] to be resolved by applying the hematocrit correction factor of Chapin and Ross [18] for trapped plasma. But major disparities of measurement continued. The known fact that the dye T-1824 appeared in lymph very shortly after injection [24] and that plasma concentration decreased rapidly for several minutes after injection [42] led to continued question of the validity of using a molecule to which the capillary wall was so readily permeable.

It was recognized that tagged albumin found its way gradually through several compartments until after about 3 days it was mixed into the body albumin pool, and plasma concentration finally conformed to an exponential disappearance rate. Thus, it was apparent that if albumin were used to measure plasma (the first dilution compartment) the concentration must be measured after mixing was complete and before a "significant" amount of the indicator left the blood stream.

Subsequent evidence accumulated proving that this was not being achieved. It

Submitted for publication August 27, 1970.

The investigation was supported by U. S. Public Health Service Grant No. HE 09667 from the National Institutes of Health.

became apparent that the smaller the molecule used to measure plasma volume, the larger became the observed values. Data from various studies produced this series:

T-1824 or ^{131}I-albumin > dextran (200,000)[39] > ^{131}I-globulin[6] > ^{131}I-myeloma protein[7] > ^{131}I-fibrinagen[8] > ^{51}Cr-RBC[8]

At the albumin end of the series, the capillary wall is readily permeable to the molecule; with increasing size it is less permeable, until at the erythrocyte end of the series it is impermeable. Renkin [55] suggests that the sieve characteristics of the capillary wall approximate 45° A, which is consistent with a steep gradient of permeability between molecular weights of 20,000 and 60,000. Baker,[8] who used albumin fibrinogen and RBC, suggested that over-estimation of plasma volume by tagged albumin is responsible for the discrepancy between the double-isotope WBH and the centrifuged LVH. He noted that the ratio WBH/LVH approached closer and closer to 1.0 the larger the tagged moiety used to measure plasma volume.

Furthermore, in clinical use, both T-1824 and ^{131}-RIHSA have proven unreliable as blood volume determinants. When tested critically by Dagher *et al.*[19] known changes in blood volume as measured by ^{131}RIHSA before and after bleeding of human donors were over-estimated by a mean of 126% (30.6% to 306.6%) with a standard deviation of ±86%.

The ratio WBH/LVH was called "ratio$_{F\ cells}$" by Reeve *et al.*[54] in 1953, and the term has persisted. For a few years it was generally thought that this ratio represented only a comparison of two measurement technics. But soon a large number of reports [3, 17, 23, 28, 29, 73] confirmed the fact that the "isotope hematocrit" was uniformly smaller than that of centrifuged blood. Thus, it became tacitly accepted that the mean whole body hematocrit actually is 8–14% less than LVH, and, as Mollison [47] states in the latest edition (1967) of his book, a correction factor of 0.86 to 0.92 is commonly applied to LVH in any computations regarding blood volume.

We resolved to reexamine this apparent paradox that the LVH is not representative of all the circulating blood. The paradox was created by measurement technics which gave different values for the same dynamic volume. In essence, the conflict is between plasma volumes as measured in two ways: a) by an albumin tag, or b) by determining blood volume using tagged erythrocytes, then applying LVH. Which is the more reliable measurement?

This report explores this question in some depth using theoretical considerations, characteristics of capillary flow, experimental data from simultaneous tagging with ^{51}Cr and ^{125}RIHSA, electrophoresis of tagged albumin, hematocrit determinations from multiple sites, and data freely borrowed from the literature.

Loose Albumin Tagging; Loss of Unbound Radioactivity in the First Minute

Because they realized some loosely bound ^{131}I was leaving the plasma very rapidly, Franks and Zizza [25] in 1958 began to seek methods to improve the strength of binding of the tag; and the uniformity and stability of the albumin to be injected continued to receive thoughtful consideration. This culminated in a more precise picture of albumin metabolism, presented by Takeda and Reeve in 1963 [66] using very carefully prepared autogenous serum albumin tagged with ^{131}I. About 4% of radioactivity had left the plasma at 10 minutes. A constant exponential slope of plasma concentration became established between the 2nd and 4th day after injection. This represented, of course, the final dilution space of albumin, not the plasma volume.

Since plasma volume calculations were based on indicator concentration in a rapid dilution compartment measured within the first half-hour or hour, a compartment

which has already lost unknown amounts of indicator, no real proof exists of the validity of the zero time intercept. Was it possible that a large and significant loss occurred in the first few minutes? If so, was it possible that the amount of such loss could be variable, and thus render the technic unreliable?

General Methods

Nine dogs from the local pound were vaccinated for canine distemper, infectious canine hepatitis, and leptospirosis. After a quarantine of 30 days, they were splenectomized, prophylacticly treated with Mepharsen HCL * for possible latent *Hemobartonella canis* infection, and allowed to convalesce for at least 14 days. Under anesthesia, catheters were placed in the thoracic duct,[50] pulmonary artery, and left jugular vein. Forty-eight hours later, a mixture of ^{51}Cr-tagged red blood cells (^{51}CrRBC), ^{125}RIHSA ** and saline was injected through the jugular vein catheter into the unanesthetized dog.

The mixture of ^{51}CrRBC and ^{125}RIHSA was made up as follows. Thirty ml. of blood were mixed with 5 ml. of ACD solution containing 50 microcuries of $Na_2{}^{51}CrO_4$. After incubating at 37° C. for 30 minutes, the blood was centrifuged and the plasma discarded. The cells were washed twice with cold physiological saline and resuspended in about 25 ml. of physiologic saline (28° C.). To the tagged red blood cell-saline mixture, 8 microcuries of ^{125}RIHSA in 1 to 2 ml. were added, the suspension completely mixed, and exactly 20 ml. injected (during 1 minute) into the dog via the jugular vein catheter. The remaining 5 or 6 ml. of the erythrocyte-isotope mixture were retained for concentration analysis. Mixed venous blood samples were obtained from the pulmonary artery catheter at 1, 2, 3, 4, 5, 10, 15, 20, 30, 40, 60, 120, 180, and 240 minutes after injection. Lymph samples were collected on a similar time schedule when possible. A urine sample was taken at 240 minutes.

One-half ml. of the erythrocyte-isotope mixture was diluted to 2 ml. with distilled water and analyzed for radioactivity. The total amount of radioactivity of each isotope injected was thus determined.

Two ml of whole blood, plasma, lymph and urine were used for radioactivity analyses. Radioactivity of whole blood, and urine was obtained by direct analysis of the sample. Plasma activity was obtained by direct analysis converted, through use of the packed cell volume (PCV) of the sample, to plasma activity. All loss of isotope from the body via blood or urine was measured.

All counts per minute per unit volume of samples were corrected for background radiation, and isotope decay from "0" time. Isotopes were counted in a two channel RIDL gamma well scintillation counter.*

^{125}RIHSA in Lymph and Extrapolation Technics

The pulmonary artery plasma and the lymph ^{125}I concentrations were analyzed both as activity per ml. of fluid (concentration) and as activity per mg. of albumin ** (specific activity). The 10-minute concentration, a commonly accepted early moment when mixing in plasma is thought to be complete,[50] was used as a reference point. All values are expressed as a per cent of this value. These data are seen in Table 1.

As evident in column one, and as has been abundantly shown by others, the concentration of ^{125}I is constantly changing and the rate of change is also changing. This makes selection of a sample time for extrapolation to zero a matter of custom. The conditions for a solid choice, namely,

* Oxyphenarsine HCL, Parke-Davis Co., Detroit, Mich.

** Radio-iodinated human serum albumin, Abbott Laboratories, North Chicago, Ill.

* Radiation Instrument Development Laboratory, Division of Nuclear Chicago Corp., Melrose Park, Ill.

** Biuret method.

TABLE 1. *Concentration of ^{125}I in Plasma and in Plasma Albumin Relative to the Ten-Minute Concentration (Mean of 9 Dogs)*

Time of Sample (Min.)	Relative ^{125}I Concentration per ml. Plasma		Relative ^{125}I Concentration per mg. Albumin	
	Mean	SD	Mean	SD
1	1.063	0.021	1.054	0.026
2	1.047	0.028	1.039	0.032
3	1.023	0.034	1.020	0.029
4	1.021	0.023	1.031	0.020
5	1.017	0.018	1.019	0.029
10	1.000	0.000	1.000	0.000
15	0.984	0.024	0.987	0.028
20	0.979	0.025	0.987	0.019
30	0.947	0.026	0.959	0.050
40	0.919	0.018	0.941	0.034
60	0.913	0.020	0.935	0.037
120	0.839	0.014	0.837	0.036
180	0.795	0.023	0.787	0.046
240	0.755	0.022	0.756	0.052

a moment when mixing is complete and no significant loss from the system has occurred, cannot be identified from the data.

Since equilibration between plasma specific activity and lymph specific activity has not been achieved by the 4th hour—selection of an extrapolation technic on a logically defensible basis is impossible (Table 2). One could choose the 10-minute, 15-minute, or even the 30-minute point, or a retrograde extrapolation through 2, 3, 4 or 5 points on the concentration disappearance curve during the first hour, with no means of justifying the choice. The curve is not exponential, so multiple point extrapolation on semi-log paper is a curving line. Since between the 10th and 30th minute, concentration change in plasma fell about 5%, a possible spread of this degree in the computed volume is introduced by this uncertainty in methodology alone (Fig. 1).

Electrophoretic Analysis of ^{125}RIHSA

An additional factor may further complicate the use of the indicator. Many investigators have suggested that large amounts of the tag may be loosely bound to albumin and thus may be lost from plasma in the first minute or two following injection.[25] If such a loss were large, volume determinations calculated from these data would be erroneously high. Is ^{125}I loosely bound in commercial ^{125}RIHSA?

To study this possibility, an investigation was undertaken of the RIHSA itself. It was found by electrophoretic analysis that a significant per cent of ^{125}I was *not bound to albumin* in the RIHSA when purchased.

Twelve ^{125}I (RIHSA) samples taken from original isotope containers were subjected to acetate strip electrophoresis (Fig. 2). Approximately 82 to 92 per cent of the ^{125}I activity was located in the albumin band while the other 8 to 18% of the ^{125}I was outside this band. As indicated from the isotope distribution on the acetate strips, the ^{125}I was either free (distributed near the origin) or bound to some degraded polypeptide remnant of albumin (distributed between origin and albumin band). Thus, this 8 to 18% may follow an entirely different distribution pattern from albumin, be excreted (urine), bound (thyroid and reticuloendothelial system) or diluted out into the intestinal fluid (Fig. 2).

The chromium data obtained concomitantly in this investigation proved to be an accurate tool to substantiate quantitatively this early ^{125}I-RIHSA loss (see below).

TABLE 2. *Ratio of Specific Activity of ^{125} RHISA in Thoracic Duct Lymph to That in Plasma (Mean of 6 Dogs)*

Time of Sample	Mean Ratio	Standard Deviation of Mean
5 Min.	0.002	0.001
10 Min.	0.008	0.006
15 Min.	0.021	0.010
20 Min.	0.037	0.023
30 Min.	0.107	0.067
40 Min.	0.124	0.072
60 Min.	0.258	0.118
120 Min.	0.468	0.181
180 Min.	0.515	0.158
240 Min.	0.699	0.127
24 Hr.	1.001	0.065

The Red Blood Cell Dilution Space

Since normally RBC do not escape from the vascular system, *the dilution space of RBC cannot exceed the intravascular circulating blood volume.* Only in the event of blood loss by seepage into tissues, escape into urine or body cavities, or by frank external hemorrhage will the intact red cell leave the intra-vascular space. A slow circulation of erythrocytes through the splenic "reservoir" may occur, thus creating a slow compartment separate from the "circulating" blood volume. Indeed, the term "blood volume" needs to be used carefully.

Blood volume may be defined as the volume of blood rapidly circulating through the heart and vessels. This volume has been called the "effective" or "circulating" volume. It is measured by the early (for example, 10 minute) mixing time of an indicator. Or blood volume may be defined as the volume of blood which fills the vascular tree at any one moment of time, including stagnant volumes of RBC or plasma, such as in the spleen, or pooled in capacitance veins with sluggish flow or even agglutinated and "stuck" in the peripheral circulation. Indicator mixing in this "total" volume may take an hour or longer. In any one study or report, precision should be used in the use of the term "blood volume."

A figurative diagram (Fig. 3) of the vascular tree, drawn in proportion to the morphological capacities of its different parts can be used to illustrate the distribution of the "total" intravascular blood volume at any one instant of time. It may also be used for interpretation of some of the dynamic implications of blood hematocrit changes.

Figure 3 is based on the combined data of Guyton,[31, 32] Ganong,[26] McQuarrie and Humphrey,[45] Bazett,[12] Albert *et al.*,[4] Landis and Hortenstine,[41] and Wiedemann.[71] These are summarized in Table 3.

In Figure 2 the shaded areas indicate that portion from which blood samples for

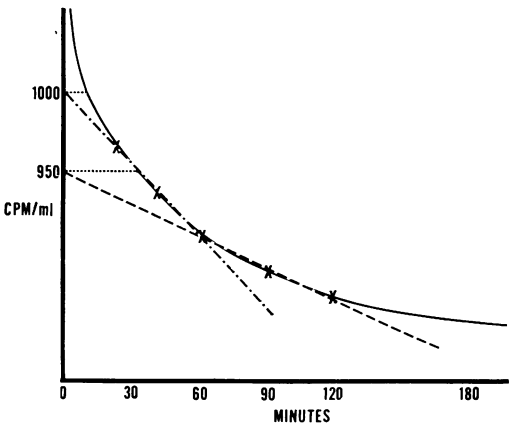

FIG. 1. A graph illustrating a typical regression curve of ^{125}I concentration (CPM/ml plasma) for 180 minutes after injection. Two possible and reasonable methods of extrapolation to zero time are illustrated. Either the 10-minute or the 30-minute ordinate intercept can be duplicated by a 3-point method. The volumes computed from these two intercepts would vary by 5%. Any point between could be established with equal justification.

accurate hematocrit determination cannot be obtained by currently available technics. It comprises approximately 15% of the total intravascular blood volume, and includes the capillary beds together with areas of sluggish circulation, i.e., spleen, bone marrow, and possibly the liver.

The unshaded areas of the diagram represent areas of the cardiovascular system that can be readily catheterized to obtain samples for hematocrit determination. To determine whether any significant variation existed in the hematocrit wherever it could be measured, the following study was undertaken.

Large Vessel Hematocrit

In four healthy dogs, of approximately 20 Kg., catheters were placed in the aorta, pulmonary artery, right jugular vein and right auricle 24 hours before the sampling procedure. The dogs had fully recovered from the anesthetic by the time the first blood samples were taken.

Samples were obtained from each dog while it was in three general physiologic states: (1) normal, unrestrained, (2) dur-

ing hypertension and splenic contraction, and (3) during mild hypotension and splenic filling. To obtain a hypertensive state with splenic contraction 2 ml. of 1/1,000 epinephrine was given intravenously during an 8-minute period. To obtain a relative state of hypotension and splenic filling, anesthesia was induced and maintained for 30 to 60 minutes with intravenous pentobarbital sodium. At least 30 minutes of anesthesia elapsed before any blood samples were taken. Three sets of simultaneous blood samples (1 ml. each) were obtained during each physiologic state (12 samples per dog). Approximately 2 to 3 minutes elapsed between each set of samples. Two weeks later the entire routine was repeated on two of the original four dogs after splenectomy.

The results obtained (Table 4) were analyzed for variance (F test at $p = 0.5$) to determine if there were any differences in LVH obtained from blood samples taken from different parts of the cardiovascular system during the normal resting state. Also, the effects of epinephrine and pentobarbital were analyzed to determine if these agents caused dissimilar hematocrits to appear in different parts of the cardiovascular system.

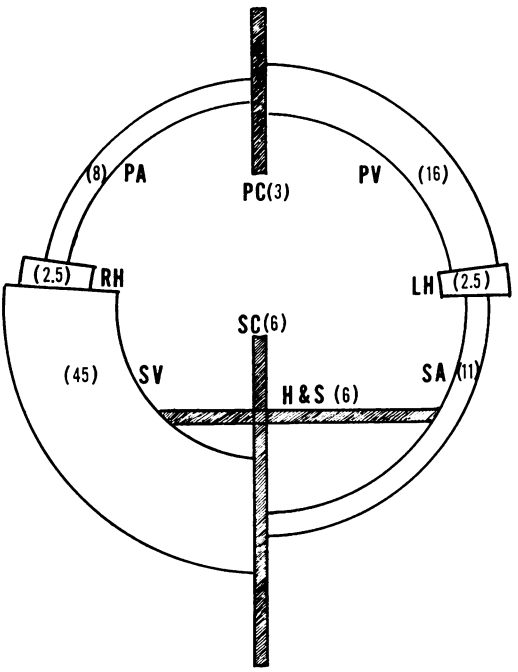

FIG. 3. A diagram to illustrate the volume distribution of blood in the vascular system. The hematocrit can be measured everywhere *except* in the cross-hatched areas, which comprise only about 15% of the total. RH = Right Heart; PA = Pulmonary Arterial Tree; PC = Pulmonary Capillaries; PV = Pulmonary Venous Tree; LH = Left Heart; SA = Systemic Arterial Tree; SC = Systemic Capillaries; SV = Systemic Venous Tree; H and S = Hepatic and Splenic Sinusoids.

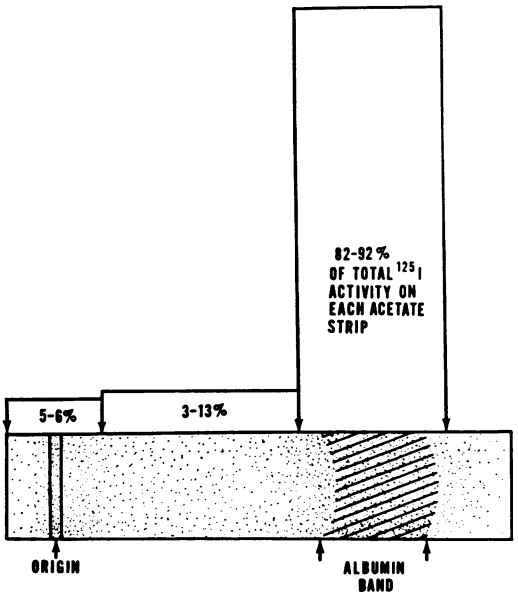

FIG. 2. Schematic representation of acetate strip electrophoresis of commercial ^{125}RIHSA. The bar graphs above indicate the distribution of radioactivity along the strip.

The following conclusions appear justified:

1. No significant difference existed in LVH simultaneously obtained from four different sampling sites in normal and splenectomized dogs.
2. There were differences in LVH between states in the non-splenectomized dogs, although sodium pentothal caused only a slight change from normal.
3. In splenectomized dogs, the capacity to change the hematocrit was apparently ablated.

This study demonstrates that, although the LVH in nonsplenectomized dogs fluctu-

ated over a range of 48.4% to 60.9%, this change was equally evident throughout all portions of the cardiovascular system that were measured.

If this LVH does not represent WBH as current theory holds, the only location in the cardiovascular system where an hematocrit could exist which is different from that measured in the large vessels would be in the capillary beds. For the WBH to have a lower value than the LVH the capillary hematocrit would necessarily be very substantially lower than the LVH since the capillary volume is only approximately 9% of the total blood volume. Is it, in fact, a realistic possibility?

The Capillary Circulation

The description of the dynamics of the capillary flow presented here is based on general physiological principles which, widely accepted, are predicated on certain specific studies of capillary circulation.[16, 35, 51, 52, 53, 54,,56, 58] This description respects the laws of Poiseuille, LaPlace, and Bernoulli, recognizes that blood is a non-Newtonian fluid, and satisfies the equilibrium constants of Donnan and the transcapillary transfers postulated by Starling (Fig. 4).

Figure 4 depicts a hypothetical systemic capillary bed consisting of an entering arteriole dividing into three capillary channels then reforming a single venule, together with an arteriovenous shunt and the pericapillary intercellular space. For the purposes of discussion, it is assumed that this diagram represents both systemic and pulmonary capillary beds although this may not actually be the case. It will be noted in the diagram that one of the three capillaries contains no red cell at all, which may occur according to the early observations of Fahreus.[22]

General aspects of the flow characteristics of this system include the following: Flow is laminar in the artiolar and venular sides of the system. The flow is pulsatile on the arterial side, slows tremendously at the mid-point of the system (capillaries), then becomes continuous as it gathers speed again on the venular side. Although the rate of flow of RBC in the capillary bed is the slowest in the entire vascular system, about 0.4–0.5 mm. per sec.,[11, 15, 72] the relative disparity between the rate of RBC and of plasma flow is maximal within the area of the capillary bed as illustrated. The mean transit time from arteriole to venule for RBC's delivered to the capillaries in one ml. of blood is very much less than that for the plasma delivered in the same ml.

Plasma flow in the capillary, with its maximum surface effect in relation to its cross-sectional area (circumference varies as the square root of the area), is maximally impeded by viscosity. Laminar flow differential is extreme. The peripheral sleeve of plasma layered against the capillary wall scarcely moves; only the centrally oriented plasma between the red cells has a rate comparable to them. Even in the many capillaries which contain no erythrocytes plasma is traveling slower than the red cells in adjacent capillaries. This disparity in mean transit time of plasma through the capillary is offset to some extent by an extracapillary circulatory route adopted by the water, electrolytes, small metabolites, and albumin fraction of the plasma, i.e., the plasma annulus.[38, 51, 56, 68] These phenomena are illustrated in Figure

TABLE 3. *The Distribution of Blood in the Vascular Tree. (Sources quoted in text)*

			Average Used in Figure 2
Pulmonary circulation	25–30%		
Pulmonary arteries		7–9%	8%
Pulmonary capillaries		3%	3%
Pulmonary veins		15–18%	16%
The heart	4-6%		5%
Systemic circulation	65–70%		
Arterial tree		10–12%	11%
Capillaries		5–8%	6%
Venous tree		43–48%	45%
Unknown (liver or splenic reservoirs)			6%

TABLE 4. *Summary of Hematocrit Data Obtained from Non-Splenectomized (A) and Splenectomized (B) Dogs During Periods of Rest, Epinephrine Stimulation, and Sodium Pentobarbital Anesthesia*

IV-A	Normal				Epinephrine				Sodium Pentobarbital			
Site	1	2	3	4	1	2	3	4	1	2	3	4
Dog												
1	52.3*	52.1	52.5	52.2	62.4	61.1	62.1	61.8	54.5	54.8	54.4	54.2
2	50.2	50.0	50.2	50.0	61.3	60.9	62.1	61.0	56.3	56.5	56.0	56.0
3	48.6	48.8	48.8	48.5	66.0	66.6	66.2	66.2	52.9	52.9	52.8	52.5
4	42.4	42.9	42.7	42.4	55.6	54.6	54.4	55.3	42.7	42.9	43.1	42.9
Mean**	48.4	48.4	48.5	48.3	61.3	60.8	61.2	61.1	51.6	51.7	51.5	51.4

IV-B	Normal				Epinephrine				Sodium Pentobarbital			
Site	1	2	3	4	1	2	3	4	1	2	3	4
Dog												
3	46.9*	46.4	46.8	46.6	47.2	47.0	46.9	46.0	45.4	44.9	45.3	44.3
4	34.8	34.7	34.9	34.8	34.4	34.4	34.6	34.5	33.7	33.7	33.6	33.7
Mean#	40.9	40.6	40.9	40.7	40.8	40.7	40.8	40.3	39.5	39.3	39.5	39.8

1 = pulmonary artery, 2 = ascending aorta, 3 = right auricle, 4 = right jugular vein.
* Mean of 9 (3 microhematocrits determined on each of 3 blood samples).
** Mean of 36.
\# Mean of 18.

5, a magnified diagram of the lowest capillary in Figure 4.

A portion of the plasma entering the capillary circulation with an aliquot of RBC leaves the capillary and enters the pericapillary plasma annulus. Simultaneously, a similar volume of pericapillary plasma re-enters the capillary lumen at its low pressure end. Thus, an aliquot of RBC leaving the capillary bed is mixed with a very slightly smaller plasma volume than when it arrived there, the difference reflecting the formation of lymph.

The important concept to be derived from these considerations is that since a definite flow of plasma occurs outside the capillary the mean *intravascular* capillary hematocrit is in fact larger, i.e., definitely

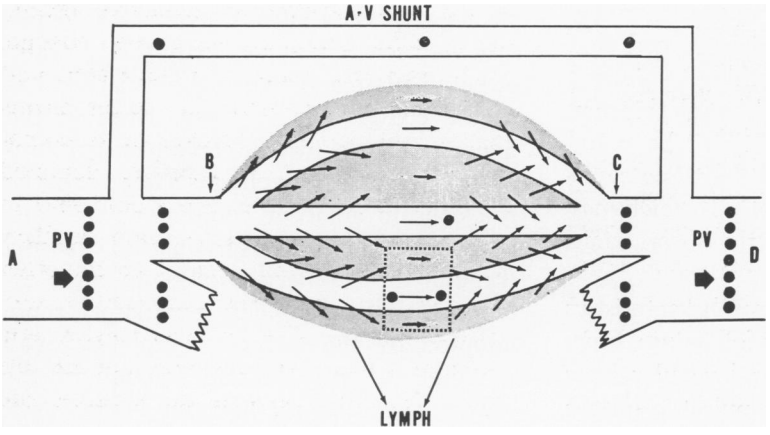

FIG. 4. Hypothetical schema of systemic capillary bed consisting of three capillaries. The stippled area comprises the pericapillary route of flow of plasma components. In the entering arteriole (A) and the departing venule (D) the hematocrit can be measured. In the capillary bed between points (B) and (C) the blood is fractionated, a true hematocrit does not exist, but a conceptual hematocrit can be constructed by application of the dimension *time*.

not smaller, than large vessel hematocrit (as would be required for WBH to be greater than LVH).

The Whole Body Hematocrit

The concept of a whole body hematocrit as representing a mean of all hematocrits through the body is a critical concept underlying all volume computations. If one measures RBC or albumin concentration in a mixed venous sample one must either assume that the entire circulating blood volume is similarly composed or if WBH is indeed less than LVH, that some blood in the tissues of certain organs or throughout the body exists in a dilute (i.e., RBC deficient) form. The concept of the plasma annulus as described in Figures 4 and 5 is critical to this conception.

Tracer studies have suggested that escape of "plasma" to the pericapillary space is rapid,[68] and that a relay of an hour or more can occur before it reenters the venular end of the capillary.[54]

The theoretical and experimental arguments in favor of a circulating pericapillary plasma annulus described by Pappenheimer and Kinter [51] have been elegantly analyzed by Howe and Shaeffer.[39] It seems clear from their discussion that: (1) the existence of a plasma annulus is essential to account for known capillary dynamics; (2) the amount of plasma in an organ at any instant of time compared to the number of red cells at that instant (organ "hematocrit") will depend upon the *rate of the plasma flow* through the annulus as compared to the *rate of the RBC flow* through the capillary. A slow plasma flow would result in a big annulus. Thus, if annular plasma flow rate were faster than the RBC flow rate (which it is not) the "tissue hematocrit" would be larger than LVH: *per contra,* if it were slower, the ratio would be less than one. Considerable experimental data exist giving figures of 0.35 to 0.8 [51, 56] for the ratio of "tissue hematocrit"/LVH in

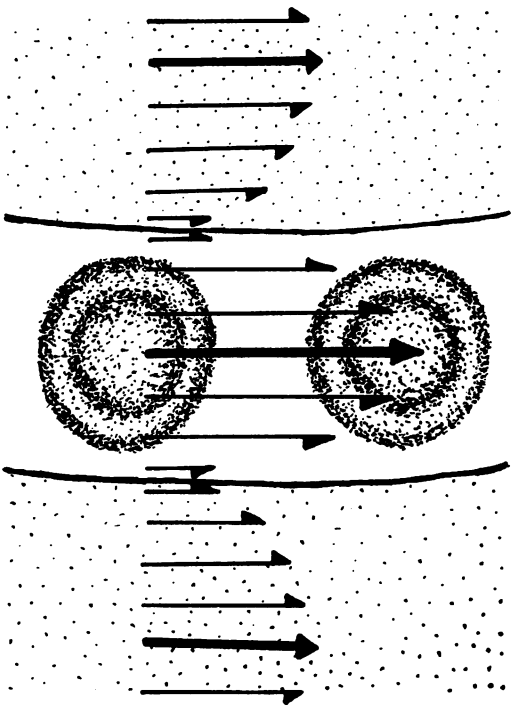

FIG. 5. An enlarged more detailed diagram of the lowest capillary of Figure 3, giving velocity profiles of intra- and pericapillary flow of RBC and plasma components. The length of the arrows indicates relative velocity of flow. The RBC travel the fastest; the plasma fractions flow at various slower rates.

liver and kidney. Thus, it appears that the organ "hematocrit" will depend upon the *rate* of plasma flow (or the total amount of intra and extravascular "plasma") in an organ or tissue. If the rate of plasma flow in the annulus relative to that of the RBC were to change in a subject, as for example, by the administration of vasoactive agents, the so-called $\text{ratio}_{F\ cells}$ would also change.

The fact that blood is fractionated and dispersed as it traverses the tissues means that a real capillary hematocrit does not exist. In Figure 4 no capillary depicted contains blood such as one would find in the heart and great vessels. One capillary has no red cell, one has one, and the other has two; and all three have lost a substantial portion of water, electrolytes, and albumin which are traveling outside the vessel. Nowhere between the arteriole and

the venule in this illustration could one draw "blood" to measure an hematocrit.

But in a steady state of the local circulation, one can describe and measure indirectly a "conceptual" hematocrit of the tissue. The hematocrit, like all physical phenomena, (Tolman, 1915),[67] has the dimention *time*. The hematocrit describes a quality of *flowing* blood. Thus, in Figure 4, in one time unit a volume of large vessel blood arrives, and in the same time unit an identical volume leaves (less the infinitesimal lymph). Since, as we have shown in Table 4, even with changing hematocrits LVH is the same wherever it can be measured, if a certain volume of blood enters a tissue with a certain LVH, and an identical volume of blood leaves in the same time interval with an identical LVH, then the "conceptual capillary hematocrit" of the blood flowing between must also have the same LVH.

In short, the centrifuged hematocrit of mixed venous blood represents the mean hematocrit of all rapidly circulating blood in the body. That WBH is less than LVH is a myth. LVH, therefore, since it measures blood typical of the entire circulating volume, should be used without any correction factor except, of course, for trapped plasma.

The remarkable accuracy of measuring blood volume and plasma volume using ^{51}CrRBC and LVH is the subject of another report. However, in the technic used in this study, the simultaneous injection of the two isotopes allowed us to reconfirm the early loss of ^{125}I from the plasma described above by using PV as measured by ^{51}CrRBC and ^{125}I plasma concentration measured simultaneously. By knowing PV by one method, one can compute total activity of ^{125}I in plasma from the concentration, and then compare this amount to that which was injected.

The Measurement of Plasma Volume Using Chromated Red Blood Cells

In the normal splenectomized dog, chromium tagged cells were found to be uniformly mixed with circulating RBC's after 10 minutes of circulation. The concentration of ^{51}Cr in RBC's at any sampling time relative to a reference concentration was computed as follows:

$$\text{RCCrC} = \frac{^{51}\text{Cr CPM/ml. RBC}}{\text{Reference } ^{51}\text{Cr CPM/ml. RBC}}$$

where RCCrC = Relative circulating ^{51}Cr concentration, CPM = Counts Per Minute, and Reference ^{51}Cr CPM/ml. RBC = the mean ^{51}Cr CPM/ml. RBC of the 10, 20 and 40 minute blood samples.

These specific times were chosen as representing the first stable half-hour period of complete mixing since at 10 minutes concentration had achieved 1.002 and then remained stable for 4 hours (Table 5). RCCrC would equal 1.0 when the ^{51}CrRBC are perfectly mixed with untagged circulating RBC. Since this figure prevailed throughout the period, any sampling times after 10 minutes could have been used, although after 120 minutes the standard deviations rose slightly.

The chromium dilution space (circulating blood volume) and red cell mass were computed as follows:

$$\text{CrDS} = \frac{\text{Total } ^{51}\text{Cr CPM Injected} - ^{51}\text{Cr CPM loss}}{^{51}\text{Cr CPM/ml. whole blood}}$$

$$^{51}\text{Cr CPM/ml. RBC} = \frac{^{51}\text{Cr CPM/ml. whole blood}}{\text{LVH corrected for trapped plasma}}$$

$$\text{RCM} = \frac{(\text{Total } ^{51}\text{Cr CPM injected} - ^{51}\text{Cr CPM loss})}{^{51}\text{Cr CPM/ml. RBC}}$$

where CrDS = chromium dilution space and ^{51}Cr CPM loss = measured loss of ^{51}Cr in blood samples.

The RCM was determined for each sampling time for each dog and the variation between RCM's of each dog was determined. The standard deviation of the mean RCM computed from the 9 samples taken 10 minutes or later from each dog was ±1.4%. The RCM was evidently stable and was being measured with accuracy.

Accepting the obvious situation, that the red cell mass remained essentially constant in these healthy, resting, untraumatized splenectomized dogs during the four hour period, the circulating blood volume (Cr DS) could fluctuate only with changes in plasma volume. Any fluctuation in plasma volume would be reflected as a change in the LVH and would be accounted for in blood volume calculations. The only significant change in RCM would be due to the loss of RBC in blood sampling and these losses were known and accounted for.

The chromium plasma volume was calculated from the chromium dilution space and red cell mass data. This measured plasma volume represents the circulating plasma volume only; it was calculated as follows:

$$\text{Circulating plasma volume} = \text{CrDS} - {}^{51}\text{RCM}$$

Zero-Time RCM

The size of the RCM before sampling had occurred ("zero time") was calculated as the mean of three RCM determinations made at 10, 20, and 40 minutes, corrected for the accumulative volume of RBC lost in sampling. With the "zero time" RCM known and the one minute LVH known, the blood volume and plasma volume could be accurately calculated *for the one minute time.* Thus, although the indicators are not yet adequately mixed, by virtue of the known stable RCM an hematocrit allows computation of blood volume and plasma volume at one minute as follows:

TABLE 5. *Relative Circulating ^{51}Cr RBC Concentration for 4 Hours Following Injection (Mean of Nine Resting Splenectomized Dogs)*

Minutes After ^{51}Cr RBC Injection	Mean Relative ^{51}Cr Concentration	Standard Deviation of Mean
1	1.021	0.021
2	1.015	0.022
3	1.015	0.021
4	1.010	0.018
5	1.009	0.015
10	1.002	0.012
15	1.005	0.015
20	0.998	0.013
30	0.997	0.010
40	1.000	0.012
60	1.001	0.012
120	1.000	0.020
180	0.995	0.025
240	1.003	0.016

"Zero time" RCM (RCM_0)
= RCM_t + total RCM previously lost in sampling.

One minute RCM (RCM_1)
= RCM_0 (At one minute there is no previous loss)

One minute BV (BV_1)
$$= \frac{RCM_1}{LVH_1} \times 100$$

One minute PV (PV_1)
= $BV_1 - RCM_1$

Loss of Intravascular Albumin Tag Quantitated

The one-minute plasma volume determined with ^{51}CrRBC becomes important when it is coupled with the one-minute plasma ^{125}RIHSA concentration. This information allows the total ^{125}I activity in the circulating plasma to be calculated and this can be compared to the total ^{125}RIHSA

TABLE 6. *Per Cent ^{125}I Remaining in Circulating Plasma Following $^{125}RIHSA$ Injection (9 Dogs)*

Minutes	Mean	SD
1	86.50	2.53
2	85.34	2.54
3	83.10	4.09
4	84.38	2.63
5	83.46	3.03
10	81.38	2.59
15	80.29	2.75
20	79.76	2.96
30	78.05	3.55
40	75.81	2.79
60	75.44	2.80
120	69.53	3.34
180	65.91	2.73
240	63.62	4.01

injected at "zero time." Thus, the loss of ^{125}RIHSA in the first minute or during any interval can be measured.

It was calculated as follows: Per cent ^{125}I in plasma =

$$\frac{PV_t \times {}^{125}\text{I CPM/ml.}_t \text{ plasma}}{\text{Total CPM } {}^{125}\text{I injected} - {}^{125}\text{I losses}} \times 100.$$

This value was computed for each sampling time of each dog and is summarized in Table 6.

These data indicate a very rapid (less than one minute) loss of a large amount of the injected ^{125}I from the circulating plasma. *At one minute only 86.5 ± 2.5% of the injected CPM of ^{125}I remains in the plasma, and at ten minutes only 81.4 ± 2.6%.* These computations match almost exactly the electrophoretic measurements of unbound ^{125}I, namely, 8–18%. If 14% of the injected ^{125}I is unbound and escapes the plasma in one minute, and if during the first 10 minutes about 4% of the injected tagged albumin would also normally leave the plasma on the basis of albumin turnover studies,[70, 71] plasma volumes based on a single ^{125}I concentration at 10 minutes would obviously suggest a dilution space computed to be about 18% too large. Variations in extrapolation technic offer the possibility of an additional 5% error. PV measurements using commercial ^{125}RIHSA are computed to be from 18 to 23% too high. This probably explains the previously mentioned gross overestimation of clinical measurements of known bleeding volumes by Dagher *et al.*[19] using RIHSA, i.e, 126% ± 86%.

Discussion

The evidence presented relates particularly to the concept of blood volume. Since Harvey (1628),[35] blood has been thought of as the cell-fluid mixture circulating in large and small blood vessels. Measure of its volume was considered to describe the capacity of the cardiovascular system. With recent stricter application of the physical laws regulating fluid flow, together with more precise understanding of the anatomy and physiology of the capillaries, it has become clear that blood cannot circulate exclusively within the vascular tree itself. Some of it must escape and then reenter. To cross the tissue beds, blood must split up into different components all of which have different transcapillary transit times. The elements are re-assembled near the venular outflow and leave in volume units of proper proportions. For this reason, no real tissue hematocrit exists.

A "conceptual hematocrit" can be identified, however, by recognizing that since rate of flow is critical an hematocrit has the dimension *time*. A certain volume of blood enters a tissue mass (capillary bed) in a certain period time; in that same period, another volume leaves. If these volumes are equal, a steady state exists. Since the hematocrits of the equal volumes per unit time which entered and left were the same, the "hematocrit" of the equal volume traversing the intervening circuit must also have been the same. An LVH truly does characterize WBH, when the latter is defined as a quality of a mean sample of the circulating blood.

That ^{51}CrRBC equilibration time is prolonged during experimental hypovolemia

has been clearly shown.[5, 9, 27] Baker *et al.*[10] used early and late mixing times of ^{51}CrRBC to differentiate "effective" from "total" blood volumes, the difference interpreted as a measure of sluggish or pooled blood compartments.

However, in the normal splenectomized dog in a comfortable environment the red cell mass is the most constant component of the entire circulation. Accurate measure of it using ^{51}Cr, together with an LVH which does in fact measure the mean blood hematocrit permits the most accurate measurement of plasma volume currently available. Using this method together with measurement of plasma concentration of ^{125}I simultaneously injected confirms with remarkable quantitative agreement the electrophoretic evidence of unbound ^{125}I, and shows that about 18% of the ^{125}I activity in the commercial indicator has left the plasma by 10 minutes. Thus plasma volumes computed by this method are too large, and "isotope hematocrits" using radioiodine will be computed too low.

Summary

Commercial iodinated human serum albumin (^{125}RIHSA) and chromium-tagged erythrocytes (^{51}CrRBC) were simultaneously injected in 9 splenectomized dogs. Frequent blood and thoracic lymph samples were collected for four hours. Acetate strip electrophoretic analyses were made of 12 samples of commercial ^{125}RIHSA. In 4 normal and 2 splenectomized dogs determination of large vessel hematocrit (LVH) was made on simultaneous samples from 4 sites during 3 different hematological states.

1. Electrophoresis demonstrated 8–18% of ^{125}I in commercial RIHSA is not bound to albumin.

2. Simultaneous LVH's from different sites were always identical.

3. Since blood fractionates as it traverses tissue and the rate of the components vary, no true capillary hematocrit exists, but a conceptual hematocrit can be established using the dimension *time*. Since it is equal to LVH, the whole body hematocrit (WBH) is accurately measured by LVH.

4. Using plasma volume (PV) derived from ^{51}CrRBC-blood-volume (BV) and LVH, total circulating ^{125}I was computed. About 14% of the ^{125}I injected is lost from plasma at one minute.

5. ^{125}RIHSA measurement gives BV values 18–23% too high; ^{51}CrRBC remain entirely within the vascular system, and coupled with LVH, provide the best indicator available for blood, red cell, and plasma volume measurement.

References

1. Abdou, I. and Tarver, H.: Plasma Proteins. II. Relationship between Circulating and Tissue Protein. J. Biol. Chem., **190**:790, 1960.
2. Agress, C. M., Rosenburg, M., Schneiderman, A. and Brotman, A. J.: Blood Volume Studies in Shock Resulting from Myocardial Infarction. I. Studies with Evans Blue Dye (T-1824). J. Clin. Invest., **29**:1267, 1950.
3. Albert, S. N. and Albert, C. A.: Blood Volume Methodology. Clinical Scintillator, **9**, 1965.
4. Albert, S. N., Jain, S. C., Shibuya, J. and Albert, C. A.: The Hematocrit in Clinical Practice. Springfield, Ill., Charles C Thomas, 1965.
5. Andersen, D. and Shoemaker, W. C.: Exchange of Injected Labelled Red Cells in Liver Tissue during Hemorrhagic Shock. Proc. Soc. Exp. Biol. Med., **125**:30, 1967.
6. Andersen, S. B.: Simultaneous Determinations of Plasma Volume with ^{131}I-labelled Gammaglobulin. ^{131}I-labelled Albumin, and T-1824. Clin. Sci., **21**:221, 1962.
7. Andersen, S. B. and Gabuzda, T. G.: Simultaneous Determination of Plasma Volume with T-1824 and ^{131}I-labelled Autologous and Homologous Paraprotein. Clin. Sci., **24**:40, 1964.
8. Baker, C. H.: Cr^{51} Labelled Red Cell, I^{131} Fibrinogen, and T-1824 Dilution Spaces. Am. J. Physiol., **204**:176, 1963.
9. Baker, C. H. and Wycoff, H. D.: Time Concentration Curves and Dilution Spaces of T-1824 and I^{131} Labelled Proteins in Dogs. Amer. J. Physiol., **201**:1159, 1961.
10. Baker, R. M., St. Ville, J. M., Suzaki, F. and Shoemaker, W. C.: Evaluation of Red Cell Equilibration in Hemorrhagic Shock. AMA Arch. Surg., **90**:538, 1965.
11. Band, P. (ed.): Medical Physiology. St. Louis, C. V. Mosby, pp. 43, 54, 1961.
12. Bazett, H. C.: Quoted by A. C. Burton. Physiology and Biophysics of the Circulation. Chicago, Year Book Med. Pub., p. 64, 1965.
13. Berson, S. A.: Blood Volume in Health and Disease. Bull. N. Y. Acad. Med., **30**:750, 1954.

14. Berson, S. A. and Yalow, R. S.: The Use of K^{42} or P^{32} Labelled Erythrocytes and I^{131} Tagged Human Serum Albumin in Simultaneous Blood Volume Determinations. J. Clin. Invest., **31**:572, 1952.
15. Best, C. H. and Taylor, N. B.: Physiological Basis of Medical Practice. Baltimore, Williams and Wilkins, pp. 163, 176, 1955.
16. Bosley, P.: Concept of a Capillary Unit and the Probable Relationship of a Nerve Fiber, and Related Tissues, to the Capillary in the Human Nailfold. Bibliotheca Anat., **7**:314, 1965.
17. Chaplin, H., Jr., B. L. Mollison, and Vetter, H.: The Body-venous Hematocrit Ratio: Its Constancy over a Wide Hematocrit Range. J. Clin. Invest., **32**:1309, 1953.
18. Chapin, M. A. and Ross, J. F.: Determination of True Cell Volume by Dye Dilution, by Protein Dilution aind with Radioactive Iron. Error of the Centrifuge Hematocrit. Amer. J. Physiol., **136**:447, 1942.
19. Dagher, F. J., Lyons, J. H., Finlayson, D. C., Shamsai, J. and Moore, F. D.: Blood Volume Measurement: A Critical Study. Prediction of Normal Values: Controlled Measurement of Sequential Changes: Choice of a Bedside Method. Advances in Surgery, **1**:69, 1965.
20. Dawson, A. B., Evans, H. M. and Whipple, G. H.: Blood Volume Studies, III. Behavior of Large Series of Dyes Introduced into the Circulating Blood. Amer. J. Physiol., **51**:232, 1920.
21. Donato, L., Matthews, C. M. E., Nosslin, B., Segre, G. and Vitek, F.: Proc. Pisa Conference on Problems Connected with the Preparation and Use of Labelled Proteins in Tracer Studies. Round Table on Applications of Tracer Theory to Protein Turnover Studies. Euratom. J. Nucl. Biol. Med., **9**:2, 1966.
22. Fahreus, R.: The Suspension Stability of the Blood. Physiol. Rev., **9**:241, 1929.
23. Fairbanks, V. F. and Tauxe, W. N.: Plasma and Erythrocyte Volumes in Obesity, Polycythemia, and Related Conditions, Compartments, Pools, and Spaces. U. S. Atomic Energy Comm., Oak Ridge, Tenn., pp. 283–298, 1967.
24. Ferrebee, J. W., Leigh, O. C. and Berliner, R. W. Passage of the Blue Dye, T-1824, from the Blood Stream into the Lymph. Proc. Soc. Exper. Biol. Med., **46**:549, 1941.
25. Franks, J. J. and Zizza, F. Simultaneous Measurement of Plasma Volume in Man with T-1824 and an Improved ^{131}I Albumin Method. J. Appl. Physiol., **13**:292, 1958.
26. Ganong, W. F.: Medical Physiology. Los Altos, Lange Medical Publications, p. 456, 1967.
27. Gelin, L. E.: Studies in Anemia of Injury. Acta Clin. Scand. Suppl., **210**:5, 1956.
28. Gibson, J. G., 2nd, Peacock, W. C., Seligman, A. M. and Sack, T.: Circulating Red Cell Volume Measured Simultaneouslv by the Radioactive Iron and Dye Methods. J. Clin. Invest., **25**:838, 1946.
29. Gibson, J. G., Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J. and Evans, R. D.: The Distribution of Red Cells and Plasma in Large and Minute Vessels of the Normal Dog, Determined by Radioactive Isotopes of Iron and Iodine. J. Clin. Invest., **25**:848, 1946.
30. Gregersen, M. I. and Rawson, R. A.: Blood Volume. Physiol. Rev., **39**:307, 1959.
31. Guyton, A. C.: Medical Physiology. P. Bard (ed.). St. Louis, C. V. Mosby, pp. 46 and 267, 1961.
32. Guyton, A. C.: Textbook of Medical Physiology. Philadelphia, W. B. Saunders, p. 264, 1966.
33. Hahn, P. F., Ross, J. F., Bale, W. F., Balfour, W. M. and Whipple, O. H.: Red Cell and Plasma Volumes (Circulating and Total) as Determined by Radio Iron and by Dye. J. Exper. Med., **75**:221, 1942.
34. Harvey, W.: De Motu Cordis. (Courtesy Chapin Library, Williams College, Williamstown, Mass.), 1628.
35. Heimberger, H.: Kontraktile funktion and anatomischer basa der menschlichen kapillaren. Z. Zellforsch., **4**:713, 1926.
36. Hevesy, G., Koster, K. H., Sorensen, G., Worburg, E. and Zerahn, K.: The Red Cell Corpuscle Content of the Circulating Blood as Determined by Labelling the Erythrocytes with Radio Phosphorous. Acta Med. Scand., **116**:561, 1943–44.
37. Howe, J. T. and Shaeffer, Y. S.: Analysis of a Recent Hypothesis of Plasma Flow in Percapillary Spaces. Circ. Res., **21**:925, 1967.
38. Howe, J. T. and Shaeffer, Y. S.: On the Dynamics of Capillaries and the Existence of Plasma Flow in the Pericapillary Lymph Space. NASA TN D-3497, 1966.
39. Jaenike, J. H., Schreiner, B. F. and Waterhouse, C.: The Relational Volumes of Distribution of ^{131}I-tagged Albumin and High Molecular Weight Dextrans in Normal Subjects and Patients with Heart Disease. J. Lab. Clin. Med., **49**:72, 1957.
40. Keith, N. M., Rowntree, L. G. and Geraghty, J. J.: A Method for the Determination of Plasma and Blood Volume. Arch Intern. Med., **16**:547, 1915.
41. Landis, E. M. and Hortenstine, J. C.: Functional Significance of Venous Blood Pressure. Physiol. Rev., **30**:1, 1950.
42. Lawson, H. C., Overby, D. T., Moore, J. C. and Shadle, O. W.: Mixing of Cells, Plasma and Dye T-1824 in the Cardiovascular System of Barbitalized Dogs. Amer. J. Physiol., **151**:282, 1947.
43. Matthews, C. M. E. and Freeman, T.: Compartments, Pools and Spaces. U. S. Atomic Energy Commission, Oak Ridge, Tenn., pp. 299–319, 1967.
44. Mayerson, H. S., Lyons, C., Parson, W., Nieset, R. T. and Trautman, M. V., Jr.: Comparison of Results of Measurement of Red Cell Volume by Direct and Indirect Techniques. Amer. J. Physiol., **155**:232, 1948.
45. McQuarrie, D. G. and Humphrey, E. W.: Vasopressors and Vasodilators in Surgery. Surg. Clin. N. Amer., **48**:877, 1968.
46. Meneely, G. R., Wells, E. B. and Hahn, P. F.: Application of the Radioactive Cell Method for Determination of Blood Volume in Humans. Amer. J. Physiol., **148**:531, 1947.

47. Mollison, P. L.: Blood Transfusion in Clinical Medicine. Oxford, Blackwell, Fourth Ed., Chapter 3, 1967.
48. Nachman, H. M., James, G. W., III, Moore, J. W. and Evans, E. I.: A Comparative Study of Red Cell Volumes in Human Subjects with Radioactive Phosphorus Tagged Red Cells and T-1824 Dye. J. Clin. Invest., **29**:259, 1950.
49. Necheles, T. F., Weinstein, I. M. and LeRoy, G. V.: Radioactive Sodium Chromate for the Study of Survival of Red Blood Cells. J. Lab. Clin. Med., **42**:358, 1953.
50. Nelson, A. W. and Swan, H.: Long-term Catheterization of the Thoracic Duct in the Dog. AMA Arch. Surg., 98:83, 1969.
51. Pappenheimer, J. R. and Kinter, W. B.: Hematocrit Ratio of Blood within Mammalian Kidney and Its Significance for Renal Hemodynamics. Amer. J. Physiol., **185**:377, 1956.
52. Prothero, J. W. and Burton, A. C.: The Physics of Blood Flow in Capillaries. I. The Nature of the Motion. Biophysical J., **1**:566, 1961.
53. Prothero, J. W. and Burton, A. C.: The Physics of Blood Flow in Capillaries. II. The Capillary Resistance to Flow. Biophysical J., **2**:199, 1962.
54. Reeve, E. B., Gregersen, M. I., Allen, T. H. and Sears, H.: Distribution of Cells and Plasma in the Normal and Splenectomized Dog and Its Influence on Blood Volume Estimates with P^{32} and T-1824. Amer. J. Physiol., **175**:195, 1953.
55. Renkin, E. M.: Physiological Aspects of Capillary Permeability. The Microcirculation. Winters, W. H. and Brect, A. N., eds.) Springfield, Ill., Chas. C Thomas, 1969, Chap. 10, pp. 136–143.
56. Sapirstein, L.: Macromolecular Exchanges in Capillaries. Microcirculation. Urbana, U. of Ill. Press, p. 47, 1958.
57. Sapirstein, L. A., Buckley, N. M. and Ogden, E. O.: Rate of Extravasation of Intravenously Injected Thiocyanate in the Dog. Amer. J. Physiol., **183**:178, 1955.
58. Skalak, R. and Branenmark, P. I.: Deformation of Red Blood Cells in Capillaries. Science, **164**:717, 1969.
59. Smith, H. E., Arnold, H. R. and Whipple, G. H.: Blood Volume Studies. VII. Comparative Values of Metallic, Carbon Monoxide, and Dye Methods for Blood Volume Determinations. Accurate Estimation of Absolute Blood Volume. Amer. J. Physiol., **56**:336, 1931.
60. Sterling, K. and Gray, S. J.: Determination of the Circulating Red Cell Volume in Man by Radioactive Chromium. J. Clin. Invest., **29**:1614, 1950.
61. Strumia, M. M., Taylor, L., Sample, A. B., Colwell, L. S. and Dugan, A.: Use and Limitations of Survival Studies of Erythrocytes Tagged with ^{51}Cr. Blood, **10**:429, 1955.
62. Swan, H.: Experimental Acute Hemorrhage. Arch. Surg., **91**:390, 1965.
63. Swan, H., Montgomery, V., Jenkins, D. and Marchioro, T. L.: A Method for the Continuous Measurement of Plasma Volume in the Dog. Ann. Surg., **151**:319, 1960.
64. Swan, H. and Nelson, A. W.: Canine Trapped Plasma Factors at Different Microhematocrit Levels. J. Surg. Res., **8**:551, 1968.
65. Takeda, Y.: Metabolism and Distribution of Autologous and Homologous Albumin ^{131}I in the Dog. Amer. J. Physiol., **206**:1223, 1964.
66. Takeda, K. and Reeve, E. B.: Clinical and Experimental Studies of the Metabolism and Distribution of Albumin with Autologus ^{131}I Albumin in Healthy Men. J. Lab. Clin. Med., **61**:183, 1963.
67. Tolman, R. C.: The Measurable Quantities of Physics. Phys. Rev., **19**:237, 1917.
68. Vidt, D. G. and Sapirstein, L. A.: Distribution Volumes of T-1824 and Chromium-51 Labelled Red Cells Immediately Following Injection. Circ. Res., **5**:129, 1957.
69. Wetterfors, J.: Albumin. Acta Med. Scand. Suppl., **403**:9, 1965.
70. Wetterfors, J.: Catabolism and Distribution of Serum-Albumin in the Dog. Acta Med. Scand., **177**:243, 1965.
71. Wiedemann, M. P.: Dimensions of Blood Vessels from Distributing Artery to Collecting Vein. Circ. Res., **12**:375, 1963.
72. Winton, F. R. and Bayliss, L. E.: Human Physiology. Philadelphia, Blakiston Co., p. 45, 1948.
73. Wood, G. A. and Levitt, S. H.: Simultaneous Red Cell Mass and Plasma Volume Determinations Using ^{51}Cr Tagged Red Cells and ^{125}I Labelled Albumin. J. Nucl. Med., **6**:433, 1965.