CIRCULATING RED CELL VOLUME MEASURED SIMULTANEOUSLY BY THE RADIOACTIVE IRON AND DYE METHODS [1]

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The measurement of the circulating red cell volume is of considerable value in the study of the circulation in both the normal state and in experimentally induced abnormal circulatory states, and in disease. Modern modifications employing Evans Blue (T-1824) (1 to 3) of the original dye method of Keith *et al* (4) have clearly shown that plasma volume can be measured with a high degree of accuracy in normal man and animals. Values for normal plasma volume in man determined by several workers are in general agreement both as to absolute plasma volume and plasma volume per unit of body measurement (2, 5 to 7). The method has also proved reliable in the study of intravenous crystalloid (8) and colloid (9) therapy as well as in experimentally induced (10 to 12) and clinical shock (7, 12 to 14). A recent improvement in the method described by Noble (15), in which changes in dye concentration of blood samples are corrected for variations in water content as determined by serum protein measurements, should increase the applicability of the technique.

There is no general agreement among authors that the dye plasma technique measures either the total or circulating red cell volume. The opinion that cell volume can be calculated from the determined plasma volume and the hematocrit of blood samples drawn from large arteries and veins or the auricle, is based on the assumption that the hematocrit of blood flowing through the entire vascular bed is a constant at all times and under all conditions. Smith (16) found lower values for cell volume when measured by carbon monoxide than by dye. Ebert and Stead (17) found cell volume determined by the dye method lower than the predicted volume after hemorrhage and during subsequent hemodilution. On the basis of subsequent experiments, these authors (18) concluded that the cell plasma ratio of blood contained in minute vessels is lower than that of venous blood.

Hopper (19) simultaneously measured cell volume by the dye and carbon monoxide methods in 13 normal humans and 17 normal dogs. The ratio of values by the former to the latter method averaged 1.00 in the humans and 1.08 in the dogs. The range of ratios in individual cases was from 0.91 to 1.16 in man, and from 0.72 to 1.14 in the dogs, and in each series the number of cases with ratios less than unity was about equal to those with ratios greater than unity. They found the ratios to be even more variable in abnormal subjects (20).

Root *et al* (21) made similar observations, and found little difference between the "central arterial and body hematocrit."

The red cell volume was first measured by means of radioactive iron in dogs by Hahn and coworkers (22). They found the cell volume measured by the injection of tagged cells consistently lower than the dye plasma cell volume, by as much as from 10 to 40 per cent, averaging 25 per cent.

In the course of studies on the preservation of human blood (23) we had occasion to determine the circulating red cell volume of normal young males by means of radioactive iron, and in many instances dye-plasma volumes were performed simultaneously. Similar studies were also made in a large series of normal (stray) dogs. It seemed worth while to present these data, obtained in a

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts Institute of Technology, in collaboration with the Peter Bent Brigham Hospital and the Beth Israel Hospital, Boston.

large series of cases, since no similar studies have appeared in the literature.

METHODS AND MATERIALS

Plasma volume was determined by the method of Gibson and Evelyn (24). Blood samples were taken 10 minutes, and, in duplicate, 20 minutes after the injection of dye. Total blood volume and erythrocyte volume were calculated from the determined plasma volume and the venous hematocrit. Red cell volume was determined by the radioactive iron method (25). No correction was made for the injected donor cells (which are included in the cell volume when measured by radio-iron), since in most instances the quantity given was less than 2 per cent of the circulating cell volume. Donor cells were of Group O or A, and were cross-matched with recipient's serum in each case. All recipients were Rh positive. Blood donors were prepared either with the 5-year half life isotope (Fe^{55}) or with the 47-day half life isotope (Fe^{59}) but no donor had received both isotopes. Donor blood was drawn into acid citrate dextrose (ACD-G) (23) and refrigerated until used, and in no instance was the cell volume measured with donor blood that had stood (refrigerated) for more than 36 hours. Both Rh positive and Rh negative blood donors were used. The Fe^{59} donors had received their radioactive iron less than 100 days prior to the use of their cells in all instances.

Forty male medical students between 18 and 24 years of age volunteered for these studies. All had negative histories of blood dyscrasias, malaria, jaundice and recent acute infectious disease. No reactions following the injection of donor red cells occurred.

The recipient red cell unit activity due to the administration of tagged donor red cells (cpm. per ml. of red cells referred to the activity of a suitable standard of Fe^{55} or Fe^{59} measured at the same time) varied little. In most cases sampling was continued for 5 days after transfusion. The constancy of these levels is shown in Table I, which gives recipient unit activities at 20 minutes, 1 and 4 hours after infusion, and at 24-hour intervals during the following 5 days. These data were obtained in 5 consecutive experiments. The extreme ranges of deviation were 9.5 per cent above and 14.1 per cent below the average value. Of the 40 observations in Table I, 32, or 80 per cent, were within $\pm$ 5 per cent of the averages, and only 5, or 12.5 per cent, were more than $\pm$ 10 per cent of the averages. Hence the variations observed are for the most part within the probable error of the technique.

The plasma volume (Vpd), venous hematocrit, whole blood (Vwpd) and red cell volume (Vrpd) calculated from the plasma volume and hematocrit, the red cell volume determined by radio-iron (Vrr), and the sum of the plasma volume and radio-iron cell volume (Vwdr) are given in Table II. Also given is the ratio of the red cell volume as determined by radio-iron and dye-hematocrit (Vrr/Vrpd); and the body hematocrit, Vrr/Vwpd.

Red cell volume measurements were carried out simultaneously by both methods in 40 normal (stray) dogs. The dogs were of both sexes, and ranged in weight from 6.7 to 25.7 kgm. Fifteen animals were under nembutal anesthesia, and the rest were under light morphine narcosis. Results obtained are summarized in Table III.

RESULTS

In *every case* the value obtained for Vrr was less than that obtained for Vrpd. In the human series, the ratio Vrr/Vrpd showed extreme variations of from 0.70 to 0.95, the average ratio being 0.845. The standard mean deviation of the series was 0.72 per cent, the individual deviation 4.5 per cent. Seventy-five per cent of the cases had a ratio within $\pm$ 5 per cent of the average, and 90 per cent had a ratio within $\pm$ 10 per cent of the average.

In the series of dogs the ratio Vrr/Vrpd ranged from 0.62 to 0.98, averaging 0.825. The standard mean deviation of the series was 1.22, the individual deviation 7.59 per cent. Twenty-three per cent of the cases gave a ratio between .60 and .75; 39 per cent, between .75 and .85; and 28 per cent, between .85 and 1.0. The spread above and

TABLE I

Radioactivity of recipient red cells following transfusion of cells tagged with Fe^{59}*

Exp. no.	Days after transfusion								Average	Extreme deviation	
	0			1	2	3	4	5			
	20 min.	1 hr.	4 hr.							+ *per cent*	− *per cent*
70	.0207	.0191	.0214	.0205	.0203	.0201	.0206	.0206	.0204	4.9	6.4
71	.0207	.0213	.0191	.0228	.0215	.0226	.0241	.0241	.0220	9.5	13.2
72	.0234	.0243	.0246	.0233	.0219	.0242	.0223	.0222	.0231	5.3	10.9
73	.0229	.0218	.0207	.0233	.0241	.0229	.0217	.0232	.0228	5.4	14.1
74	.0403	.0377	.0425	.0433	.0433	.0408	.0405	.0408	.0413	4.8	8.7

* Expressed as Unit Activities = cpm per ml. of cells referred to cpm of a standard counted at the same time.

TABLE II

Plasma and circulating red cell volume determined simultaneously by the dye and radio-iron methods. Normal males

Exp. no.	Date	Age	Hght.	Wght.	Surface area	Vpd	Venous hct.	Vwpd	Vrpd	Vrr	Vwdr	Vrr/ Vrpd	Body hct.
		yrs.	*cm.*	*kgm.*	*m²**	*ml.*	*per cent*	*ml.*	*ml.*	*ml.*	*ml.*		
68	11-16-44	23	172	63.5	1.74	3110	43.4	5500	2390	1990	5100	0.83	39.1
69	11-17-44	20	180	69.0	1.86	3650	43.5	6280	2630	2100	5750	0.79	36.5
70	11-20-44	18	180	68.3	1.86	3850	40.8	6500	2450	1850	5700	0.76	32.4
72	11-24-44	21	175	72.5	1.86	3490	41.9	6010	2520	2040	5530	0.81	36.9
73	11-25-44	20	183	81.8	2.06	3930	43.5	6950	3020	2450	6380	0.81	38.4
75	11-30-44	20	172	66.0	1.77	3310	39.2	5440	2130	1810	5120	0.85	35.4
76	12-4-44	23	175	75.0	1.89	3850	42.7	6720	2870	2000	5850	0.70	34.2
77	12-5-44	22	188	78.4	2.03	3360	42.7	5860	2500	2380	5740	0.95	41.4
78	12-7-44	22	183	77.3	1.98	3330	42.1	5740	2410	2140	5470	0.89	39.1
79	12-8-44	22	173	65.4	1.77	3580	40.1	5980	2400	2040	5620	0.85	36.3
80	12-11-44	23	187	79.5	2.03	3320	40.2	5550	2230	2110	5430	0.95	38.9
81	12-12-44	22	174	63.5	1.76	3540	42.8	6190	2650	2150	5690	0.81	37.8
96	3-8-45	20	173	82.0	1.94	3520	38.5	5720	2200	1760	5280	0.80	33.3
97	3-13-45	22	173	63.3	1.76	2480	43.6	4400	1920	1610	4090	0.84	39.3
98	3-15-45	21	173	59.0	1.73	3370	43.0	5810	2440	1990	5360	0.82	37.2
99	3-20-45	20	179	65.5	1.82	2930	44.5	5280	2350	2010	4940	0.86	40.6
100	3-22-45	24	176	68.3	1.83	3070	43.4	5430	2360	2000	5070	0.85	39.6
101	3-7-45	24	186	84.2	2.08	3700	40.0	6180	2480	2260	5960	0.91	37.9
102	3-14-45	20	183	79.5	2.00	3430	42.8	6000	2570	2100	5530	0.82	38.0
103	3-12-45	23	174	70.2	1.83	3220	41.2	5750	2530	2050	5270	0.81	38.9
107	4-24-45	21	173	62.0	1.72	3180	44.0	5680	2500	2120	5300	0.85	40.0
108	4-25-45	22	173	89.0	2.06	3930	39.7	6520	2590	2200	6130	0.85	35.8
111	4-16-45	23	178	66.0	1.79	3390	42.0	5850	2460	2000	5390	0.82	40.8
113	5-7-45	23	193	80.0	2.08	5000	39.1	8200	3200	2760	7760	0.86	35.6
114	5-8-45	22	183	80.0	2.00	3850	43.3	6800	2950	2600	6450	0.88	40.3
148	8-13-45	22	183	80.0	2.00	3850	44.2	6900	3050	2480	6330	0.81	39.1
160	10-15-45	23	198	82.0	2.11	3740	42.7	6530	2790	2340	6080	0.84	38.5
162	10-22-45	20	189	84.3	2.10	4110	45.1	7480	3370	2910	7020	0.86	41.5
163	10-31-45	22	188	90.0	2.05	4180	43.0	7350	3170	2860	7040	0.90	40.7
164	10-29-45	21	174	66.0	1.82	2790	42.9	4890	2100	1870	4660	0.86	40.0
165	11-26-45	20	181	75.0	1.94	3230	45.0	5870	2640	2290	5520	0.87	41.4
167	12-3-45	23	194	84.2	2.12	4680	39.3	7710	3030	2540	7220	0.84	35.2
168	12-4-45	23	181	84.0	2.02	3400	40.0	5670	2270	1970	5370	0.87	36.7
169	12-11-45	24	194	89.0	2.18	3660	41.0	6220	2560	2160	5820	0.84	37.2
170	12-12-45	23	174	59.0	1.70	3200	45.0	5820	2620	2140	5340	0.82	40.1
172	2-27-46	20	175	66.0	1.78	2780	48.1	5360	2580	2180	4960	0.85	43.9
173	2-19-46	21	180	72.6	1.91	3980	43.2	7000	3020	2530	6510	0.84	38.9
175	2-25-46	21	173	84.3	1.97	3660	47.8	7000	3340	2920	6580	0.88	44.3
177	2-26-46	20	175	75.0	1.88	3800	40.0	6330	2530	2270	6070	0.89	37.4
178	2-21-46	20	181	76.5	1.96	3620	42.5	6300	2680	2350	5970	0.88	39.4
Average							42.44					0.845	38.44

* From Nomograms of Boothby and Sandiford.

Key: Vpd = Volume of plasma by dye method
Vwpd = Volume of whole blood by dye method
Vrpd = Volume of red cells by dye method
Vrr = Volume of red cells by radio-iron method
Vwdr = Total blood volume (Vpd + Vrr)

below the average was wider than in the human series.

This ratio, in individual cases, bore no relationship to venous hematocrit, or to absolute plasma volume or red cell volume (by radio-iron), as shown in Figure 1 for the normal males, and in Figure 2 for the dogs.

The body hematocrit (Vrr/Vwdr) in every case was lower than that of the venous hematocrit. The average of the body hematocrits was 38.3, that of the venous hematocrits being 42.5 in humans, and corresponding values were 41.6 and 46.8 in dogs. The ratio of body to venous hematocrit was 0.91 in the 2 series. Thus the body hematocrit is lower than the venous hematocrit by about 10 per cent. Since the ratio Vrr/Vrpd is an expression of the relationship of circulating cell volume to both plasma volume and hematocrit, it follows that the body hematocrit is independent of both the absolute plasma volume and hematocrit level.

Eight dogs were subjected to hemorrhages large

enough to produce considerable lowering of jugular hematocrits, but not to cause peripheral collapse over a period of a few hours to 3 days. Red cell volumes were measured by both methods simultaneously before bleeding and after hemodilution occurred. Four of the dogs were splenectomized. The data obtained are given in Table IV. The ratio Vrr/Vrpd was less than unity in all 20 determinations, ranging from 0.62 to 0.98 and averaging 0.82. Here again there was no correlation between the ratio Vrr/Vrpd and jugular hematocrit level.

Qualitatively similar observations were made in 2 patients in whom red cell volume was measured by both methods before and after transfusions of whole blood, and in 2 patients before and after phlebotomy (Table IV).

The relationship of normal blood volume to physical measurements is beyond the scope of this paper, and will be discussed in a subsequent communication.

DISCUSSION

The data presented consistently show that the circulating red cell volume, when determined by the radio-iron technique, is some 15 per cent less than when determined by the dye-plasma-hematocrit technique. A wider spread in individual val-

TABLE III

Plasma and circulating red cell volume determined simultaneously by the dye and radio-iron methods. Normal (stray) dogs

Exp. no.	Date	Wght.	Vpd	Venous hct.	Vwpd	Vrpd	Vrr	Vwdr	Vrr/Vrpd	Body hct.	Body hct. / Venous hct.
		kgm.	*ml.*	*per cent*	*ml.*	*ml.*	*ml.*	*ml.*		*per cent*	
131-4	2-19-42		1015	43.1	1780	765	570	1585	0.75	35.9	.83
131-6	2-26-42	14.2	670	50.8	1360	690	645	1360	0.93	47.3	.93
131-7	4-16-42	13.0	620	49.3	1220	600	560	1180	0.93	47.3	.96
131-39	3-10-42	13.6	630	50.4	1270	640	600	1230	0.94	48.7	.96
131-40	3-17-42	18.2	1105	45.7	2035	930	630	1735	0.68	36.6	.80
131-41	3-25-42	25.7	1415	47.1	2670	1255	980	2395	0.78	40.7	.87
135-7	7-22-42	20.0	1500	41.0	2540	1140	1000	2500	0.88	40.0	.97
135-8	7-29-42	16.5	1100	32.1	1620	520	450	1550	0.87	29.0	.90
135-9	7-29-42	15.5	1300	40.0	2170	870	790	2090	0.91	37.8	.95
135-11	8-5-42	15.3	765	47.7	1465	700	550	1315	0.79	41.7	.88
135-15	8-19-42	17.5	780	47.4	1480	700	625	1405	0.89	44.3	.94
135-16	8-26-42	21.4	1000	47.0	1890	890	665	1665	0.75	39.9	.85
135-89	2-11-43	21.0	910	51.3	1870	960	680	1590	0.71	42.8	.84
135-90	2-11-43	14.0	730	48.0	1400	670	645	1375	0.97	46.9	.98
135-96	2-18-43	16.5	975	48.2	1880	905	675	1650	0.75	40.8	.85
135-134	4-27-43	13.5	770	44.9	1400	630	525	1295	0.84	40.6	.91
135-136	4-29-43	9.3	390	52.6	820	570	385	775	0.68	49.8	.95
21-13	5-25-42	25.0	1125	61.3	2905	1780	1090	2215	0.62	49.1	.80
21-23	5-25-42	19.0	1235	38.9	2025	790	620	1855	0.79	33.4	.86
21-29	5-8-42	7.3	370	45.0	675	305	250	620	0.82	40.3	.90
21-41	6-18-42	16.1	800	48.8	1560	760	740	1540	0.98	48.0	.98
21-42	6-18-42	14.2	810	33.4	1215	405	320	1130	0.79	28.3	.85
21-45	6-18-42	18.0	1060	39.7	1760	700	465	1525	0.67	30.5	.77
21-102	7-13-42	9.0	475	45.4	395	870	315	790	0.80	39.9	.88
21-104	7-30-42	10.7	480	44.4	880	400	325	805	0.82	40.3	.90
21-105	7-30-42	9.3	400	49.9	800	400	300	700	0.75	42.8	.86
21-109	10-13-42	8.6	380	49.0	745	365	335	715	0.89	45.4	.93
21-113	12-8-42	18.4	945	46.7	1770	825	780	1725	0.95	45.2	.96
21-114	12-22-42	18.6	795	46.4	1480	685	620	1415	0.91	43.8	.95
21-117	1-6-43	20.5	1110	46.5	2070	960	830	1940	0.87	42.7	.91
21-118	3-8-43	17.5	990	47.7	2080	1090	890	1880	0.82	47.0	.98
21-119	3-24-43	16.8	910	39.6	1600	690	550	1460	0.80	37.7	.95
21-120	3-30-43	18.0	1130	41.4	1930	800	680	1810	0.85	37.5	.91
21-121	4-1-43	12.0	615	48.7	1050	435	360	975	0.83	37.0	.76
21-129	10-8-43	10.4	570	60.2	1430	860	670	1240	0.78	54.0	.90
21-130	10-19-43	10.9	640	47.6	1220	580	440	1080	0.76	40.7	.86
21-131	11-8-43	11.8	680	41.6	1165	585	440	1120	0.75	39.3	.95
21-132	12-8-43	16.4	915	50.7	1800	885	710	1625	0.80	43.7	.86
SA-2	11-3-43	6.7	515	41.7	870	370	330	845	0.89	39.0	.93
SA-3	11-17-43	9.0	285	52.0	595	310	290	575	0.94	50.3	.97
Average				46.8					0.823	41.6	0.91

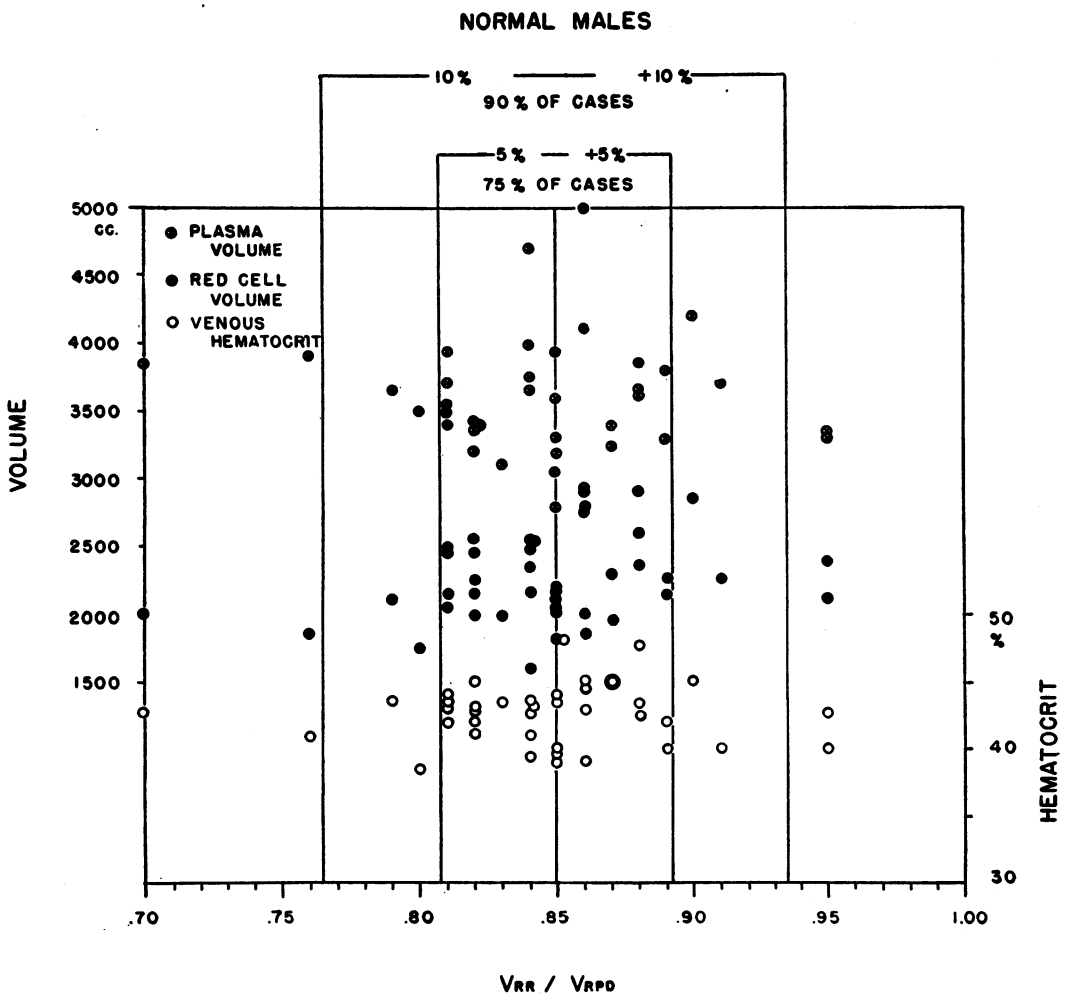

FIG. 1. THE RELATIONSHIP OF PLASMA VOLUME AND RED CELL VOLUME OF VENOUS HEMATOCRIT TO THE RATIO OF RED CELL VOLUME, AS DETERMINED BY THE RADIO-IRON AND DYE METHODS

ues was encountered in the dogs than in the humans. These dogs were in varying states of nutrition and their past histories were unknown. Hahn (26) made similar observations in 8 normal dogs, in which the ratio of radio-iron to dye plasma red cell volume ranged from 0.64 to 0.91, averaging 0.79.

Hahn and Meneely (27) more recently carried out blood volume studies by the dye and radio-iron methods in 28 hospitalized patients,[2] with venous hematocrits ranging from 27.3 per cent to 50.6 per cent. In only 2 cases was the radio-iron cell volume higher than the dye-hematocrit cell volume, the average of the ratios thereof being 0.81. The average of the body hematocrits was 31.4, and the average of the venous hematocrits was 39.7, the ratio being 0.79, considerably lower than in our series. Both of these studies are, however, in keeping with our findings.

The values obtained by the radio-iron method are independent of variations in the hematocrit of blood samples drawn from large vessels, whereas the venous or arterial hematocrit is the basis of the calculation of red cell volume in the dye method. The consistent discrepancy in results obtained by the 2 methods requires that a decision as to which method most accurately measures the true circulating red cell volume be made.

The validity of the radio-iron technique rests on 2 assumptions, (*1*) that all of the tagged donor

[2] The authors state "these patients were not normals, but people in various stages of disease or convalescence."

red cells remain intact throughout the period of significant observation, and (*2*) that all of the tagged cells become completely mixed with all of the recipient's cells within the vascular bed.

The donor cells used in these experiments were drawn in the best known blood preservative and, if not transfused immediately, were refrigerated until used. Under these circumstances little, if any, change in corpuscular measurements or in osmotic fragility occurs for at least 48 hours.

Hawkins and Whipple (28) estimated the normal life span of the canine red cell. Massive hemolysis was produced by phenylhydrazine, and this was followed by rapid regeneration of erythrocytes to a normal level over a 10 to 30 day period. Urobilinogen output fell off sharply during this period, and remained low until 100 to 120 days after cell regeneration had begun, when it rose abruptly, the rise being maintained for a period about equal to that during which regeneration had taken place. This rise was attributed to the destruction of the cells regenerated after phenylhydrazine poisoning.

Shemin and Rittenberg (29) fed glycine tagged with N^{15} and found it resulted in the formation of heme with a high concentration of the isotope. They followed the N^{15} concentration of heme in human red cells for several months and concluded that the average life time of the erythrocyte is about 125 days.

Ashby (30) determined the life span of fresh red cells as being from 100 to 130 days by the agglutination method, in which Group O cells are injected into Group A recipients. These results have been repeatedly confirmed (31 to 35). There can be little doubt that freshly drawn compatible donor cells have their full life expectancy when administered to a recipient.

The second assumption is supported by the data presented in Table I. The recipients of these infusions of fresh Group O cells were leading normal daily lives: eating, exercising, and sleeping. The

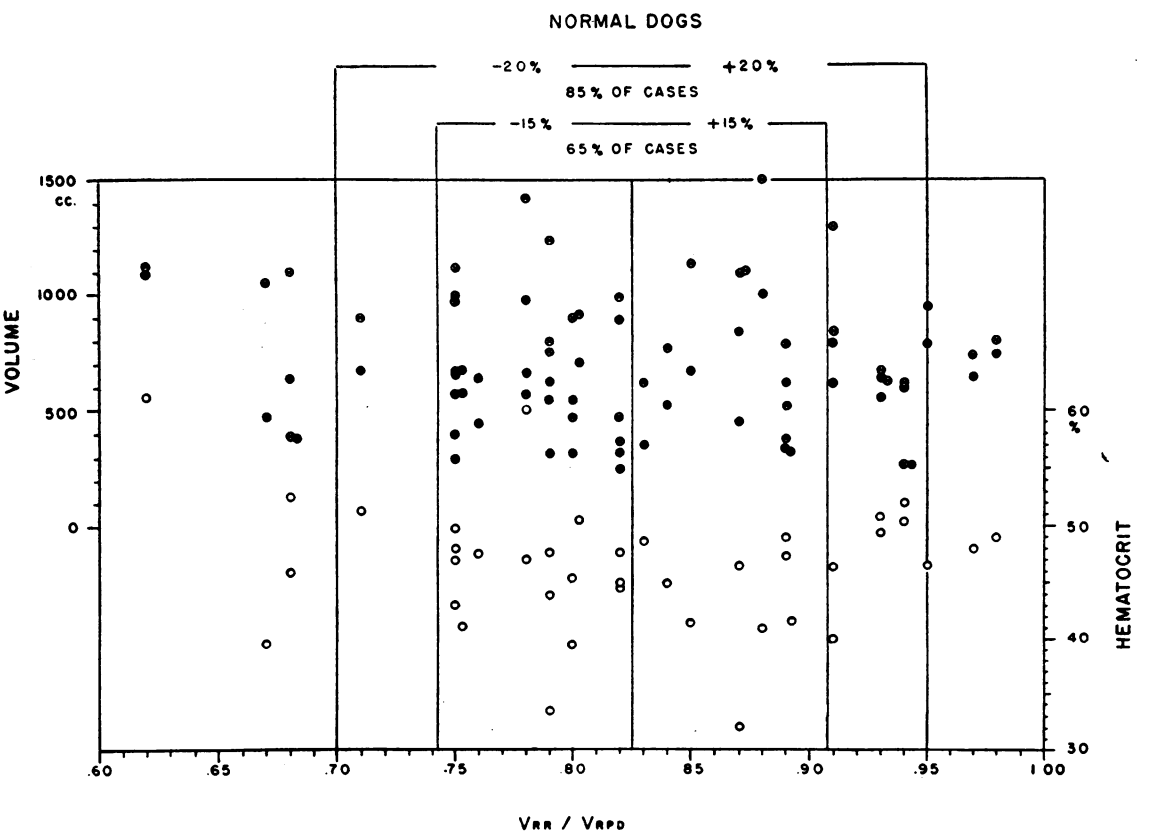

FIG. 2. THE RELATIONSHIP OF PLASMA VOLUME AND RED CELL VOLUME OF VENOUS HEMATOCRIT TO THE RATIO OF RED CELL VOLUME, AS DETERMINED BY THE RADIO-IRON AND DYE METHODS

TABLE IV

Red cell volume measured by the radio-iron and dye method before and after bleeding and transfusion. Eight dogs undergoing repeated hemorrhage

Experiment no.		Vpd	Venous hct.	Vwpd	Vrpd	Vrr	Vrr/Vrpd	Body hct.	Body hct. / Venous hct.
		ml.	*per cent*	*ml.*	*ml.*	*ml.*		*per cent*	
131-4	Spleen intact	1015	43.1	1780	765	570	0.75	32.0	.74
		985	33.7	1480	495	535	0.76	26.0	.77
131-6	Spleen intact	670	50.8	1390	720	645	0.89	46.3	.91
		725	41.3	1240	515	500	0.97	40.3	.98
131-7	Spleen intact	620	49.3	1220	600	560	0.93	45.8	.93
		630	28.3	880	250	240	0.96	27.3	.97
		780	27.6	1080	300	240	0.81	22.2	.81
21-13	Splenectomized	1125	61.3	2900	1775	1090	0.62	37.6	.61
		785	56.5	1810	1025	770	0.75	42.3	.75
		1110	42.9	1940	830	730	0.88	37.6	.88
21-18	Spleen intact	870	49.5	1720	850	620	0.73	37.1	.73
		1255	36.3	1970	715	480	0.67	24.3	.67
21-23	Splenectomized	1235	38.9	2020	785	620	0.79	30.3	.78
		1140	37.6	1825	685	530	0.78	28.9	.77
		930	31.3	1350	420	410	0.98	30.4	.97
21-42	Splenectomized	810	33.4	1210	400	320	0.80	26.4	.79
		750	32.5	1110	360	265	0.74	23.9	.74
		875	27.0	1200	325	265	0.82	22.1	.82
21-45	Splenectomized	1060	39.7	1760	700	465	0.67	26.4	.67
		590	31.6	865	275	200	0.73	23.1	.73
Average							0.81		0.77
Two polycythemic patients undergoing phlebotomy *									
CR-1		2510	59.8	6270	3760	2930	0.78	53.8	0.90
		2750	55.2	6130	2510	2510	0.75	47.7	0.87
CR-4		3230	55.2	7340	4110	3640	0.89	53.3	0.97
		2800	54.7	6160	3360	2630	0.79	48.3	0.89
Average							0.80		0.91
Two patients receiving whole blood transfusions									
O-O	Acute hemorrhage	4920	10.7	5500	580	510	0.88	9.3	0.87
		4680	15.4	5530	850	800	0.94	14.4	0.94
A-D	Hemolytic anemia	2780	24.5	3690	910	825	0.91	22.3	0.91
		2600	41.4	4440	1840	1590	0.87	36.8	0.89
Average							0.90		0.91

* These experiments were carried out in collaboration with Dr. A. Cournand at Bellevue Hospital, N. Y. C.

constancy of their red cell radioactivity levels precludes the possibility that any considerable portion of their own cells (with the possible exception of immature cells in marrow) were in vascular areas into which the tagged cells had not entered, since the influx of any considerable quantity of such cells would have lowered the circulating red cell radioactivity levels of these individuals. The fact that initial radioactivity levels closely approximated the averages of the levels over a 6-day period is proof that mixing of tagged with nontagged cells was complete within 20 minutes after injection.

Further proof of complete mixing of tagged

with all of the non-tagged cells lies in the observation that the unit activity of a recipient's red cells is neither raised nor lowered by the brisk removal of a large quantity (20 per cent) of total circulating red cell volume, and that the amount of cells removed is accurately measured (to within 5 per cent) by a subsequent volume determination involving the injection of a further quantity of tagged cells. Likewise, radio-iron cell volume determinations, before and after large transfusions of non-tagged red cells, do accurately measure amounts of cells given (25).

Our findings are in agreement with those of Hahn (22), who found little change, over periods of from 3 to 8 days, in the red cell radioactivity of red cells in dogs who had received tagged erythrocytes.

If it be accepted that the true circulating red cell volume is accurately measured by the radio-iron technique, then the consistently higher volume found by the dye plasma technique must be due to differences in the hematocrit of blood within large and small vessels.

We have found the body hematocrit to be about nine-tenths of the large vessel hematocrit. This implies that the hematocrit of some portion of the total vascular content must have an hematocrit even lower than the body hematocrit.

Direct observations on the hematocrit of minute vessel blood are few, largely because of the technical difficulties of obtaining true capillary blood. Ebert (18) obtained blood from both large and small vessels of the forearm of human subjects. Arterial flow to the extremity was first

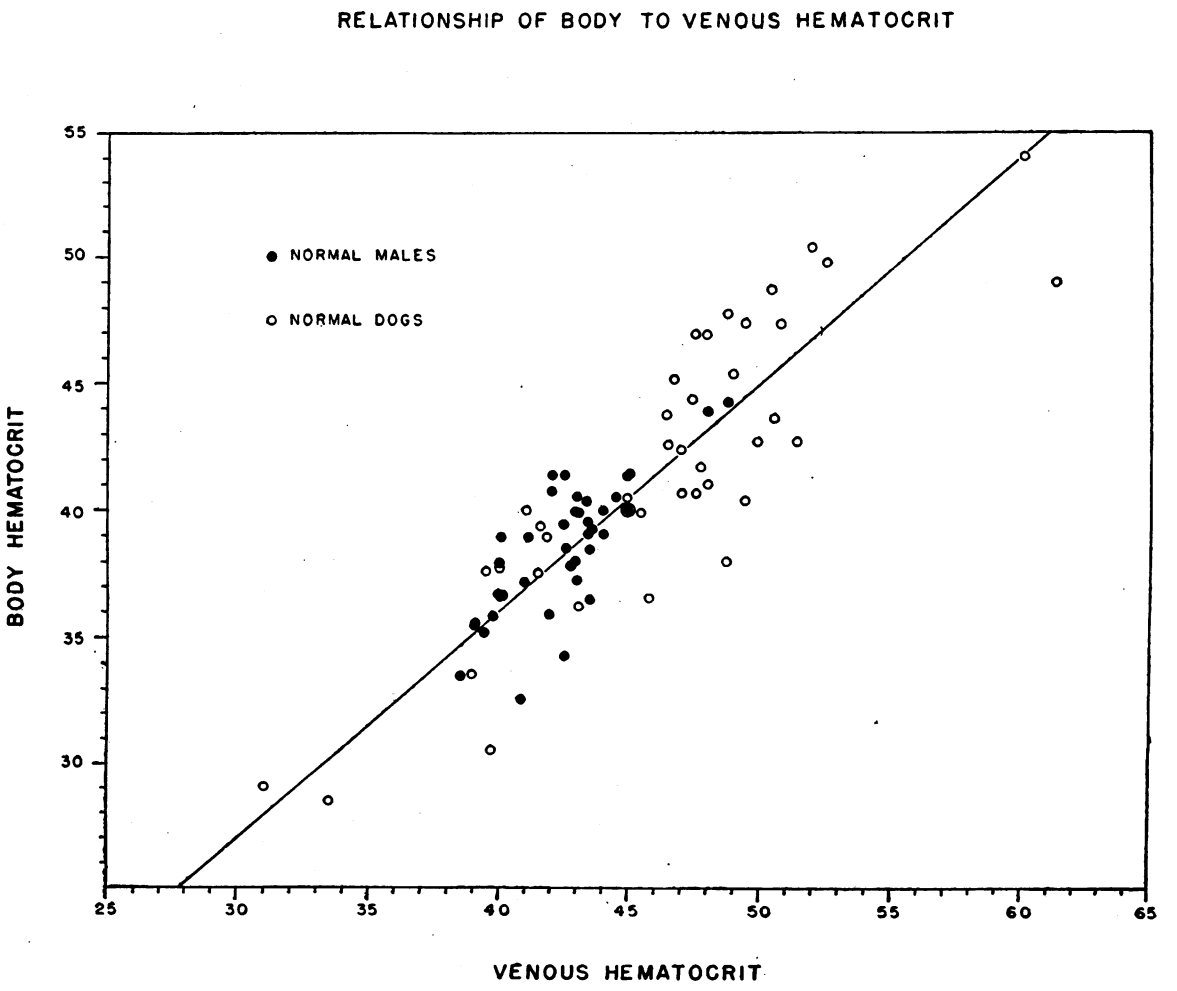

FIG. 3. THERE IS A LINEAR RELATIONSHIP BETWEEN THE HEMATOCRIT OF ALL THE BLOOD OF THE BODY AND BLOOD OBTAINED BY VENOUS SAMPLING

occluded. Blood was then removed from a large vein until no more could be obtained. An Esmarch's bandage was then applied and additional blood from minute vessels was squeezed out. A comparison of the cellular content of the large and minute vessel blood was made on the basis of hemoglobin content. In 15 experiments the average hemoglobin content of the minute vessel blood was 13.2 grams per 100 ml., while that of the large vessels was 14.3 grams per 100 ml., the ratio being 0.9. Since some of the blood obtained by the final squeezing may have come from vessels larger than true capillaries, the hematocrit of very minute vessel blood may well be lower than the above ratio would indicate.

It is of interest to speculate to what extent the ratio of body to large vessel hematocrit remains constant at varying hematocrit levels. Hahn (36) found a linear relationship between jugular hematocrit and circulating red cell mass as determined by radioactive iron in individual dogs, within a range of from 11 to 57. This relationship may be expressed as the ratio of body to venous hematocrit. In both of our series there is a good correlation between body and venous hematocrit, within a range of from 38 to 48 for humans, and from 32.1 to 61.3 for dogs, as shown in Figure 3.

Thus it appears probable that the proportion of blood in large and minute vessels, and the hematocrits of the blood flowing through those compartments both remain fairly constant, within fairly narrow limits, under normal conditions. Direct evidence that this is the case will be presented in a further publication (37).

The significance of these findings is worthy of comment. The actual quantities of red cells involved in the intrinsic error of the dye plasma technique are not inconsiderable, ranging from 100 to 600 ml. in individual cases (Table II). This discrepancy is probably not too serious from a clinical diagnostic point of view, since the significant changes of cell volume in disease are frequently of a greater order. They do, however, become significant in clinical investigation, particularly in circulatory disturbances where the normal distribution of cells in large and minute vessels may be considerably disturbed.

CONCLUSIONS

1. Circulating red cell volume was determined by both the radioactive iron and dye-plasma methods in 40 normal males and 40 normal (stray) dogs.

2. The ratio of the radio-iron to the dye-plasma red cell volume averaged 0.85 in humans and 0.82 in dogs.

3. The ratio of average body hematocrit to large vessel hematocrit averaged 0.91 in both series.

4. There is no relationship of either ratio to absolute plasma volume or large vessel hematocrit.

5. There is a linear relationship of body hematocrit to large vessel hematocrit.

6. The probability that the hematocrit of minute vessel blood is less than the body hematocrit is discussed.

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