A METHOD FOR THE DETERMINATION OF PLASMA AND BLOOD VOLUME *

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Routine red cell counts and hemoglobin determinations yield information that is valuable but very incomplete. This information concerns only the concentration of the blood. These routine methods do not furnish us absolute values for hemoglobin, for red cells, or for the oxygen-carrying capacity of the blood. With them, an anemia may be more apparent than real, as it may be dependent on or associated with a marked increase in plasma volume, while a polycythemia does not necessarily indicate an absolute increase in red cells, as it may be dependent on a decrease in plasma. In order to obtain absolute values for either red cells or hemoglobin, data relative to plasma volume or total blood volume are essential.

The volume as well as the concentration of the blood must play a rôle in pathological physiology. Absolute values are essential to the proper interpretation of certain pathological findings. Is an increase in the blood mass with overfilling of the vascular system in any way responsible for hypertension? Is the large heart frequently seen in pernicious anemia due to a large blood volume? Is the large heart sometimes seen in myocardial insufficiency (unassociated with hypertension or nephritis) due to an increase in blood mass? Questions such as these must remain unanswered until data concerning absolute values are correlated with pathological findings.

With so great variations in size and weight of individuals, the normal values for plasma and blood are best expressed in percentages or fractions of the body weight, or as the number of cubic centimeters per kilogram of body weight. Since the work of Welcker, the blood has been considered to constitute one-thirteenth of the body weight. Recently, the values obtained by Haldane and Smith (one-twenty-first), and by Plesch (one-nineteenth) have been frequently accepted. The methods affording these last values are not without serious drawbacks from the points of view of inherent technical error, complicated nature of apparatus required and the possibility of injury or discomfort attending their use.

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There is a distinct need for a simple method for the determination of plasma and blood volume, particularly for a method that can be repeatedly applied without injury to the patient.

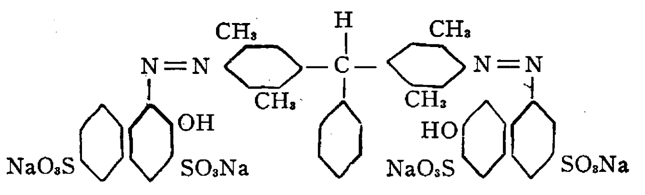
THE PRINCIPLE OF THE METHOD

The principle underlying this method is the introduction directly into the circulation of a non-toxic, slowly absorbable dye, which remains in the plasma long enough for thorough mixing, and the determination of its concentration in the plasma colorimetrically by comparison with a suitable standard mixture of dye and serum.

A dye which would fulfil these conditions was sought. Certain of the phthaleins were tried and discarded because of the rapidity with which they leave the vascular system. Dr. Herbert M. Evans, of the Anatomical Department, when consulted in regard to dyes remaining for a long time in the circulation, suggested the possibilities of vital red, and supplied us with the dye used in this work. It is with great pleasure that we acknowledge our indebtedness to him.

SOME PROPERTIES OF THE DYE

Vital red is disodium disulphonaphthol azotetramethyl triphenyl methane.



It is water soluble to the extent of 1½ per cent. In dilute solutions of 0.01 per cent., it does not dialyze against water through collodion sacs even in forty-eight hours. Under such conditions, the dye is practically adsorbed by the dialyzing membrane.

When its toxicity was determined by intravenous injection into animals, it was found that doses exceeding from 5 to 10 mg. per kilo sometimes resulted in a slight transient albuminuria. Smaller doses were harmless, so far as could be ascertained. Three mg. per kilo was chosen as the most suitable amount for our purposes. Protocols of cats and dogs studied for toxicity appear below.

TOXICITY EXPERIMENTS

PROTOCOLS

CAT 1.—Weight, 2.4 kg.; given 750 mg. intravenously; killed in three hours.

CAT 2.—Weight, 2.75 kg.; given 700 mg. intravenously; killed in twenty hours.

CAT 3.—Weight, 1.75 kg.; given 6 mg. to kilogram intravenously; no effect; urine free from albumin afterward.

Dog 5.—Weight, 7 kg. Nov. 4, 1914: Given 600 mg.; vitally stained; no toxic manifestations. One week later, 40 per cent. of dye still in circulation.

Dec. 17, 1914: Blood still contains considerable dye.

March 1, 1915: Animal still slightly vitally stained after a period of four months. On only one occasion during this whole period was a trace of albumin found in the urine.

Dog 8.—Weight, 6.85 kg. Nov. 17, 1914: Given 350 mg.

Nov. 18, 1914: Considerable albumin in urine; no casts; some red blood cells.

Nov. 22, 1914: Advanced distemper. Urine free from albumin.

Nov. 26, 1914: Died with distemper.

Dog 9.—Weight, 9.9 kg. Nov. 21, 1914: Given 99 mg. Dog was normal except for a faint trace of albumin found in the urine on first and second days.

Dog 10.—Weight, 5.5 kg. Nov. 21, 1914: Given 55 mg.; normal; no albumin.

In order to vitally stain the tissues even slightly, it was found necessary to use at least 10 mg. per kilo. If a strong solution is injected locally into the subcutaneous tissues, the tissues are stained a beautiful bright red color, which persists unaltered for many months. This accident happened with patients on two or three occasions early in this work, but since has been avoided by ascertaining with certainty that the needle was in the lumen of the vein before the dye was injected.

Traces of the dye appear in the urine and in the lymph flowing from the thoracic duct at the end of ten minutes. The dye continues to be excreted in the urine of patients for from three to four days following a dose of 3 mg. per kg. Following this amount no evidence of the dye in the tissue cells or white blood cells could be found with the oil immersion. The fate of all the dye has not been determined, but considerable amounts can be found in the plasma for three or four days following its injection.

TECHNIC

A 1.5 per cent. solution of the dye in freshly distilled water is prepared and sterilized by boiling for from five to ten minutes, and aseptically stoppered in a sterile Erlenmeyer flask. This solution is made up as required.

The patient is weighed (stripped) and the dose determined on a 3 mg. per kilo basis. The skin over the anterior surface of both elbows is cleaned up in the usual manner for venepuncture. Under aseptic technic, a small platinum-iridium needle (20 gauge) to which is attached a short piece of rubber tubing (from 2 to 3 cm. in length) is inserted into the vein. Ten c.c. of blood are withdrawn into a pipet and emptied into a 50 c.c. centrifuge tube containing just sufficient powdered sodium oxalate to prevent coagulation. The plasma so obtained is used in the preparation of the standard which is described later.[1] The rubber tubing is removed from the needle, the latter being left in the vein and a 20 c.c. Record syringe containing the exact dose[2] of the dye is attached, and its contents injected, after which the needle is withdrawn. Three minutes later, a needle is inserted into the vein of the opposite arm. Without impeding the venous return, 10 c.c. of blood are withdrawn immediately by means of a pipet; two or three minutes later, 10 c.c. more are withdrawn. The first is placed in a 50 c.c. centrifuge tube, the second in a 15 c.c. graduated centrifuge tube. To each, sufficient dry oxalate is added to prevent clotting, and the tubes are capped with rubber to prevent evaporation during centrifugalization.

The three tubes are placed in a high-power centrifuge and subjected to 3,000 revolutions per minute for twenty minutes.[3] After removal from the centrifuge, the relative proportions of erythrocytes and plasma in the graduated tube are noted. Without disturbing the red cells, the plasma is carefully pipetted off from each tube and placed in a clean test tube. We now have three test tubes, (1) containing plasma without dye; (2 and 3) containing plasma with dye in an unknown concentration. A suitable dye-plasma standard mixture of known dye content must be prepared, and with it 2 and 3 are compared.

The standard dye preparation[4] is made as follows: 0.5 c.c. of the dye solution used for the injection is brought up accurately to 100 c.c.

1. This procedure of taking plasma for mixing the standard in each case was found necessary on account of the great variation in the color of the plasma in different individuals.

2. The amount injected is 3 mg. per kg. The weight in pounds divided by eleven gives the number of cubic centimeters of a 1.5 per cent. solution to be injected.

3. To prevent hemolysis, all pipets and centrifuge tubes must be thoroughly washed with normal saline solution and dried before use. It is obvious that the slightest hemolysis will alter the color of the plasma and make accurate colorimetirc readings impossible.

4. Obviously the standard must be sufficiently intense in color to insure the patient's readings falling on the scale. Since the smaller the amount of plasma in relation to the body weight the greater will be the intensity of the dye, a standard must be chosen which will be more intense than the plasma-dye mixture obtained from normal patients having the smallest proportion of plasma in relation to body weight. The standard selected has been prepared on the following basis: The amount of drug injected is placed in the number of cubic centimeters of salt solution corresponding to the number of grams represented by 4 per cent, of the body weight, or, expressed differently, in the amount of salt solution represented by 40 c.c. for every kilogram of body weight; this amounts to 5 c.c. (7.5 mg. of dye) of the solution injected, to 100 c.c. of 0.8 per cent. sodium chlorid. For example, a man weighing 70 kilos would be given 210 mg. of dye (3 mg. per kg.), which is equal to 14 c.c. of a 1.5 per cent. solution. Four per cent. of 70 kg. is equal to 2.8 kg., and without making a correction for the difference in specific between the blood plasma and water, this would amount to, roughly, 2,800 c.c. If 210 mg. are dissolved in 2,800 c.c., then 7.5 mg. should be dissolved in 100 c.c.

with 0.8 per cent. saline. One part of this is mixed with one part of the patient's plasma and two parts of salt solution, yielding a dilution of 1 in 4. With this solution, the prism of the colorimeter (Rowntree and Geraghty's modification of the Autenrieth-Königsberger apparatus) is filled. This dilution has been found to be well adapted to colorimetric determinations. Each of the specimens (2 and 3 of the patient's plasma) is diluted with three parts saline and placed in the cup of the colorimeter and read against the above standard.

CALCULATION OF RESULTS

The method described above furnishes volume values for plasma and total blood. When it is desired to express values as per cent. of body weight, the formulas given below can be utilized. Since the specific gravities of the plasma and of the blood were not ascertained in this study, one of 1.050 for the whole blood and of 1.030 for the plasma have been used.

FORMULAS

Plasma Volume.—Weight of patient in kg. = W.

Standard prepared so that amount of dye injected is diluted in 40 c.c. for every kg. of body weight.

Reading of standard prepared as directed = 100.

Reading of patient's plasma against standard = R.

The number of c.c. of plasma in patient's body = 100/R × 40 W = 4,000 W/R.

Percentage of Body Weight.—Plasma volume in c.c. × Sp. G. of plasma/1,000 = weight of plasma = X.

X × 100/W = per cent. of body weight.

Cubic centimeters of plasma per kg. = No. c.c. in body/W.

Total Blood Volume.—Let hematocrit reading of the volume occupied by corpuscles = P.

Volume of plasma = 100 — P.

Total blood volume = plasma volume × 100/100 — P.

Total Blood as Per Cent. of Body Weight.—Total blood volume in c.c. × Sp. G./1,000 = weight of total blood = Y.

Y × 100/W = per cent. of body weight.

Cubic centimeters per kg. = number c.c. in body/W.

UNDESIRABLE FEATURES OF THE METHOD

Hemolysis.—Sometimes the colors do not match perfectly in quality. This is the result of hemolysis and of necessity makes the quantitative determination more difficult. It is extremely infrequent, however, to find this failure with both specimens, and on the one or two occasions when this has occurred, the determination was not attempted. Records have been kept throughout as to the matching of the colors. In a large proportion of instances the colors in both specimens were perfect. Where the matching was only slightly imperfect, readings were made and in many instances these have closely approximated readings of the other specimen in which the color was perfect. In spite of the most careful technic, inexplicable hemolysis occurred at times. It is considerably more frequent in the narrow graduated centrifuge tube used for the hematocrit values.

Lipemia.—Lipemia, if very marked, makes the determination impossible. Only once has this been encountered. In a few instances it offered some slight difficulty. By proper attention to diet and to the time of making the determination, this difficulty can be readily obviated. In our series it has not been sufficiently troublesome to require such measures.

Discomfort to Patient.—Aside from the slight pain of the venepuncture, the patient usually suffers no discomfort or inconvenience. In approximately 9 per cent. of the cases, chilly sensations occurred, while in five instances, chills, with fever reaching to 101, were seen. None of the normal cases exhibited thermic responses. They are apparently more common in cases of anemia.

CONTROLS OF THE METHOD

Controls on the method have been made in order to determine the constancy of its findings under various conditions and in order to justify certain steps in the technic. These studies are here presented under subheadings. Controls on the plasma method are first considered and later those on the hematocrit method of determining total blood volume.

A. Rate of Disappearance of Dye from Blood.—Throughout the work, readings have been made at three minute and six minute intervals. The following afford striking proof of the slowness with which the dye disappears from the circulation. In a series of thirty-six cases in which the color matching was equally good in both determinations, nine cases showed identical reading for the two determinations, nine showed higher readings for the second, the average amount above the first being 2.5 per cent., while eighteen showed higher readings for the first determination, the average amount above the second being 3 per cent. This indicates that some dye does disappear from the circulation during the time of determination, but also indicates that the loss is inconstant and small. On rare occasions, marked discrepancies in the two readings occur. Such determinations should be discarded.

Below is shown the rate of disappearance of the dye from plasma in a man over a period of twelve minutes, readings being made at approximately two-minute intervals, and the amount of dye of the first determination being considered 100 per cent.

J. C., weight 55.4 kg.; 178 mg. dye injected.

Time Elapsing Minutes[5]	Per Cent.
2	100
4	104
6	103
8	102
10	102
12	97

Rate of Disappearance of Dye from the Circulation in Dogs.—Protocols 1 and 2 indicate the slow rate of disappearance of dye from the blood of dogs, the amount present on first determination being considered as 100 per cent.

Dog 1.—Weight, 7.7 kg.; 23 mg. dye injected.

Time Elapsing Hours,	Minutes	Per Cent.
..	13	94
..	30	92
2	20	65
3	..	60
6	..	52
24	..	26

Dog 2.—Weight, 9.5 kg.; 100 mg. dye injected.

Time Elapsing Hours,	Minutes	Per Cent
..	2	93
..	4	93
..	10	86
..	12	86
..	15	81
1	6	77
1	8	73

These data demonstrate that the absorption from the blood stream is slow, and justify us in accepting determinations made within six minutes of the time of injection of the dye. Further proof of the slowness of the absorption is shown by the fact that on several occasions the plasma three to four days after injection showed a considerable amount of the dye. The urine also shows traces for some days after the injection.

B. Values Found on Repeated Determinations.—Repeated determinations on the same individual yield practically identical results, provided no appreciable change in the patient's condition has occurred. Some of these persons are normal and others are suffering from chronic disease in which no appreciable change occurred during the interim.

5. The first reading at two minutes was lower than subsequent ones. Mixing was probably not complete in two minutes.

TABLE 1.—Repeated Determinations on Men

Name	Date	Weight	Volume in c.c.	Cubic-centi-meters per Kg.
Cl.	2/ 2/15	58	2,922	50
	2/ 4/15	58	3,030	52
Fl.	2/ 2/15	67	2,969	44
	2/ 4/15	67	3,037	45
D.	3/11/15	57	2,665	47
	3/12/15	57	2,681	47
G.	3/11/15	64	3,176	50
	3/12/15	64	3,083	48
B.	1/ 7/15	61	3,036	49
	2/ 6/15	64	3,165	49
T.	2/12/15	50	1,975	40
	2/25/15	50	1,990	40
Fr.	1/20/15	62	3,056	49
	3/18/15	63	3,160	50
I.	1/12/15	81	3,172	39
	2/ 3/15	80	3,064	38
W.	4/22/15	64	3,375	53
	5/ 3/15	65	3,400	53

Dogs do not give so constant results but it is altogether probable that the dogs' plasma volume is subject to relatively greater and more sudden changes than that of man. Some interesting findings on dogs are included in Table 2.

TABLE 2.—Repeated Determinations on Dogs

No.	Date	Weight	Plasma, c.c.	C.c. per Kg.	Remarks
1	1/19	10.5	696	66.3	
	1/27	9.35	623	66.6	Distemper
	2/ 1	8.1	572	70.6	
	2/ 3	7.65	481	62.8	Following immediately a bleeding of 165 c.c.
	2/ 4	7.62	651	85.5	Next day
			958	126	After infusion of 325 c.c.
2	1/27	9.05	603	66	
	2/15	9.02	635	61	
4	1/19	15	1,202	81.1	Distemper — bled 300 c.c. six minutes before first sample
	1/21	15	1,137	76.4	

C. Plasma Volume Determined with Varying Amounts of Dye.—The same value for blood volume is obtained whether one works with 2, 3 or 4 mg. per kilogram. Three practically normal men were studied in this connection. Determinations made after injection of 2 and 3 mg. doses yielded values within 75 c.c. in two instances, while in the third, 2 and 4 mg. per kilogram were used, the results agreeing within 1 per cent. Three mg. per kg. is considered absolutely satisfactory, yielding colors well adapted to colorimetric determination.

D. Controls on the Colorimeter.—In the past, in making parallel series of readings with various phthaleins and with Folin's micro-Kjeldahl method, practically identical readings were obtained with two instruments, the Duboscq and our colorimeter. In a small series of parallel readings made in this investigation, identical readings were also obtained with both.

E. The Loss of Blood at Venesection Demonstrated.—The method was applied to three men before and after phlebotomy. The results appear in Table 3. Nos. 1 and 2 were normal donors for transfusion and No. 3 was a hypertensive patient, the subject of a therapeutic venesection.[6]

TABLE 3.—Loss of Blood at Venesection

Name	Volume Before	Amt. Bled	Volume After
1. G.	Plasma, 3,180	300	Plasma, 2,862
2. D.	Plasma, 2,790	620	Plasma, 2,573
	Blood, 5,083		Blood, 4,274
3. A.	Plasma, 3,872	800	Plasma, 3,425
	Blood, 6,143		Blood, 5,434

A decrease corresponding fairly well to the amount of blood removed is demonstrated in all three cases.

F. Hematocrit.—The whole blood volume is calculated from the plasma volume on the basis of the hematocrit findings, after centrifugalization in graduated tubes for 3,000 revolutions per minute for twenty minutes. A longer period was unnecessary, the packing always reaching its maximum within this time. The values from the same individual varied but little, whether the samples were obtained with free circulation of blood or after impeding the venous return for as long as fifteen minutes. When the venous return was cut off, the red cells increased 4 per cent. and 5 per cent. in two instances and decreased 2 per cent. and 5 per cent. in two others. Where possible, it is better to take sample for hematocrit determination from the freely

6. The Lindeman method of transfusion was used and the amount of blood measured.

circulating blood. Repeated determinations on the same normal individual give close but not identictal values. Undoubtedly some opportunity for error is here afforded.

G. Is the Dye Taken Up by the Red Blood Cells?—Early in the course of this work it became evident that the dye was not readily taken up by tissue cells or the cells in the blood stream. From the experiments with collodion sacs previously mentioned and from the fact that neither the erythrocytes nor leukocytes were stained when observed in fresh blood preparations, it seemed fair to surmise that most of the dye remained in the plasma during the short time of the determination. In order to get at this question quantitatively, it was first necessary to determine the relative amounts of plasma and red blood corpuscles in the whole blood. Several methods have been employed for this purpose, among others, the various forms of the hematocrit and the nitrogen method devised by Bleibtreu.[7] The hematocrit method described above was found to be the most satisfactory for our purpose, yielding figures that agreed closely with those obtained by other workers with this method. In two different samples of blood, the relative amounts of plasma and erythrocytes were determined by the hematocrit and by the nitrogen method. The latter gave a plasma value 4 per cent. to 9 per cent. higher than that of the hematocrit. Bleibtreu's figures were also high compared with those of other observers using the hematocrit method. In our work, the hematocrit values only were taken.

In order to determine whether the dye is absorbed by the blood cells, and, if so, to what extent, the following procedure was adopted: Fifty c.c. of blood were taken from a healthy man and rendered incoagulable with powdered sodium oxalate. Ten c.c. of this blood were used for hematocrit determination, 15 c.c. being centrifuged and the plasma pipetted off. The remainder was agitated until measured out for the experiments given below:

Experiment 1.—Centrifuge Tube 1. Fifteen c.c. of this whole blood pus 2 c.c. of dye solution plus 13 c.c. of saline.

Centrifuge Tube 2.—Five c.c. of plasma plus 1 c.c. of dye solution plus 4 c.c. of saline.

After Tube 1 was centrifugalized,[8] the plasma was pipetted off and compared with the plasma dye mixture from Tube 2, which was placed as the standard in prism of the colorimeter. The reading was 80. Therefore, in dilutions used, Tube 1 contained 80 per cent. as much dye per cubic centimeter as Tube 2. The relative amount of plasma in the blood in Tube 1, as shown by the hematocrit, is 62.5 per cent.

Then $15 \times 62.5 = 9.37$ c.c. plasma in Tube 1.

7. Bleibtreu, M. and L.: Eine Methode zur Bestimmung des Volums der körperlichen Elemente im Blut, Arch. f. Physiol. (Pfluger's), 1892, li, 151.

8. Three thousand revolutions per minute for twenty minutes.

Then 2 c.c. of dye solution is diluted in 24.37 c.c., or 1 c.c. in 12.18 c.c.
Or there should be 10/12.18, 82.9 per cent. of dye in Tube 1.
Colorimeter reading, as calculated from hematocrit data, 82.9 per cent.
Colorimeter reading observed, 80 per cent.

Experiment 2.—Experiment 1 was repeated with the blood taken from another normal individual.

Colorimeter reading, as calculated from hematocrit data, 81.8 per cent.
Colorimeter reading observed, 82 per cent.

Experiment 3.—Experiment 1 was repeated with the blood taken from a third normal individual.

Colorimeter reading, as calculated from hematocrit data, 81.4 per cent.
Colorimeter reading observed, 81 per cent.

In considering the foregoing experimental data, several factors should be noted. The hematocrit must necessarily give us the minimum value for the amount of plasma in the blood, as the corpuscles are wet and the interstices are filled with fluid. Therefore, the hematocrit findings for plasma must be slightly low, and the calculated colorimeter reading slightly high. If the dye is absorbed by or diffuses into the corpuscles, the observed colorimeter readings will be low, that is, the plasma volume large. As the calculated and observed figures agree so closely, the amount of dye lost from the plasma must be negligible. From the foregoing experiments, it would seem safe to conclude that in vitro little or none of the dye passes into the blood cells.

RELATION OF BLOOD AND PLASMA VOLUME TO BLOOD PRESSURE

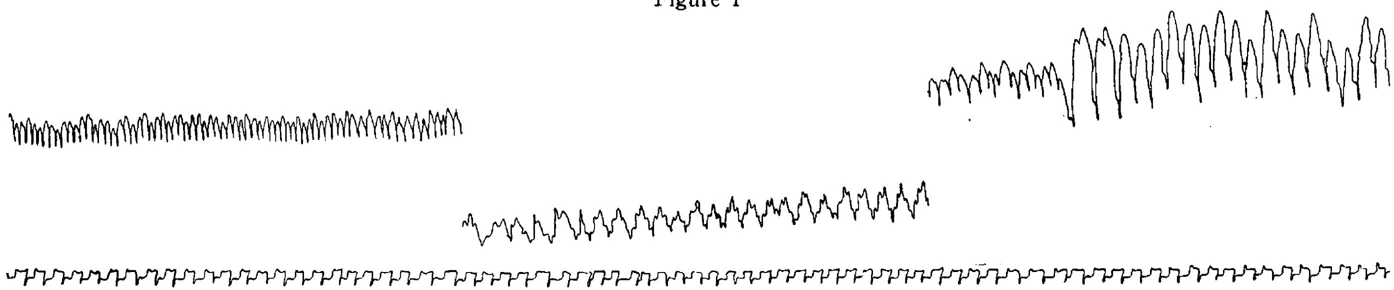
At the suggestion of Dr. Janeway, experiments were carried out to ascertain what effects changes in the caliber of the smaller blood vessels have on blood and plasma volumes. Volume determinations were made on dogs attached to a kymograph. The tracings with the blood values appear in Figures 1 and 2. The vasoconstriction was obtained by continuous administration of epinephrin, a fairly constant level being maintained for five minutes. In each determination, before injecting the dye, plasma was removed for the standard and within five minutes after the injection the samples were withdrawn for the volume estimations. The dogs weighed 14.3 and 12.7 kg. respectively.

No striking change in blood or plasma volume accompanied the fairly marked variations in blood pressure. However, these blood pressure changes were of short duration.

Dr. P. D. Lamson,[9] working in Dr. Abel's laboratory, has found that the polycythemia which develops after epinephrin injection does not reach its maximum for from fifteen to twenty minutes. Volume determinations made on two dogs after this interval showed a slight but definite decrease in plasma volume.

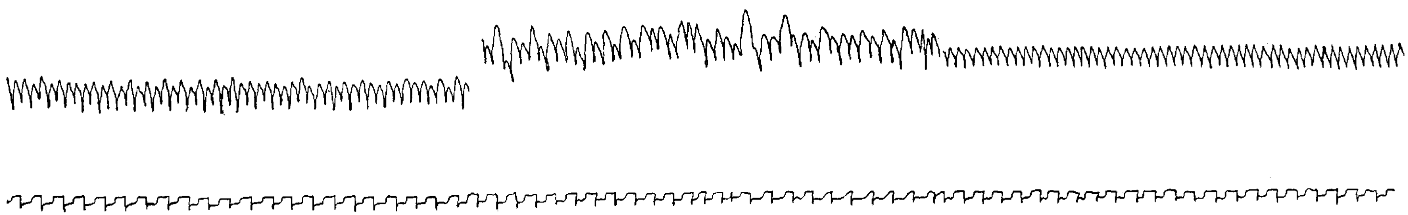
9. Personal communication.

Figure 1



Plasma	533 c.c.	591 c.c.	524 c.c.
Blood	1,278 c.c.	1,334 c.c.	1,175 c.c.
Blood pressure	126 mm.	60 mm.	170 mm.

Figure 2



Plasma	605 c.c.	610 c.c.	624 c.c.
Blood	1,440 c.c.	1,420 c.c.	1,450 c.c.
Blood pressure	120 mm.	165 mm.	160 mm.

METHODS PREVIOUSLY EMPLOYED AND VALUES OBTAINED

According to Welcker,[10] the earliest attempt to determine the blood volume was made by Haller.[11] It consisted of bleeding two criminals to death, the amounts of blood obtained being 28 and 39 pounds.

The methods fall into two general groups, only those of Group 2 being applicable for clinical purposes.

1. *Direct Method.*—Welcker, in 1854, bled animals to death, washed out the blood vessels with water, minced and washed the tissues thoroughly, brought the blood and washings together and determined their hemoglobin content. He concluded that the blood constituted one-thirteenth of the body weight in mammals. Shortly after this, Bischoff[12] applied this method to two criminals and obtained the same value. Modifications of the method were made by Heidenhain[13] and by Plesch.[14] A similar preliminary procedure was used by Cohnstein and Zuntz,[15] the calculations being based on cell counts instead of hemoglobin estimations, and by Kottmann,[16] who employed the hematocrit.

2. *Indirect Methods.*—A. Infusion method: (*a*) Herbst,[17] in 1822, injected the vascular system of a corpse until the vessels seemed moderately filled. (*b*) Vierordt[18] experimented on animals. He determined the amount of blood pumped from the heart in one second and multiplied this by a number corresponding to the seconds required for a complete circulation. On the basis of certain calculations, he concluded that in man the blood constituted one-thirteenth of the body

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10. Welcker, H.: Bestimmungen der Menge des Körperblutes und der Blutfärbekraft, sowie Bestimmungen von Zahl, Maass, Oberfläche und Volumen des einzelnen Blutkörperchens beim Thier und beim Menchen, Präger Vrtljschr., 1854, iv, 11; Ztschr. f. rat. Med., 1858, iv, 145.

11. Quoted from Welcker (Note 10).

12. Bischoff, L. W.: Bestimmung der Blutmenge bei einem Hingerichten, Ztschr. f. Wissensch. Zool., 1856, vii, 331; Abermalige Bestimmung der Blutmenge bei einem Hingerichteten, ibid., 1858, ix, 65.

13. Heidenhain: Ueber die Blutmenge der Säugethiere mit besonderer Rücksicht auf Welcker's Methode der Blutbestimmung, Arch. f. physiol. Heilk., 1857, n. f., i, 507.

14. Plesch, J.: Hämodymamische Studien, Ztschr. f. exper. Path. u. Therap., 1909, vi, 380.

15. Cohnstein and Zuntz, N.: Untersuchungen über den Flüssigkeits-Austausch zwischen Blut und Geweben unter verschiedenen physiologischen und pathologischen Bedingungen, Arch. f. Physiol. (Pfluger's), 1888, xlii, 303.

16. Kottmann, K.: Ueber die Bestimmung der Blutmenge beim Menschen und Tier unter Anwendung eines neuen Prazisionshaematokriten, Arch. f. exper. Path. u. Pharmakol., 1906, liv, 356.

17. Quoted from Welcker.

18. Vierordt, K.: Das Abhängigkeitsgesetz der mittleren Kreislaufszeiten von den miltleren Puls-frequenzen der Thierarten, Arch. f. physiol. Heilkunde, 1858, n. f. ii, 527.

weight. (*c*) Valentin[19] removed a definite amount of blood (a), then injected a known amount of water into the vascular system and bled again (b). The solids of a and b were determined, the difference being due to the number of cubic centimeters of water injected. Weber and Lehmann,[20] utilizing this method, concluded that the blood constituted one-eighth of the body weight. (*d*) Quincke[21] utilized the method of Mallassez.[22] Red counts were made in cases of pernicious anemia. A known amount of blood with a known cell count was transfused, following which the patient's red cells were again counted. From these data, the volume was calculated.

B. Inhalation Method: Gréhant and Quinquaud[23] gave dogs by inhalation a measured volume of carbon monoxid and determined its percentage content in a small sample of the animal's blood. Haldane and Smith[24] modified this method and applied it clinically. The patient breathes for five minutes a measured quantity of carbon monoxid from a specially devised apparatus. A sample of blood is removed and its carbon monoxid content estimated by the carmin titration method of Haldane. Plesch[25] and Oerum[26] have employed this method, but the former determined the carbon monoxid volumetrically.

C. Antitoxin Method: Behring[27] noted that tetanus antitoxin remained in the circulation for long periods and made this the basis for the estimation of blood volume. His studies showed the blood to constitute 1/11.8 of the body weight. With it, Kämmerer and Waldmann[28] in Müller's clinic, obtained a value of 1/10.2, while Fries[29] found a value of 1/12.6.

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19. Valentin, G.: Lehrbuch der Physiologie, 1847, i, 493.

20. Weber, E., and Lehmann, C. G.: Lehrbuch der physiologische Chemie, 1850, ii, Auflage ii, 259.

21. Quincke: Weitere Beobachtungen über perniziose Anamie, Deutsch. Arch. f. klin. Med., 1877, xx, 27; ref. Plesch.

22. Malassez, L.: Nouveaux procédés pour apprécier la masse totale du sang, Arch. de physiol. norm. et path., 1874, vi, Série 2, 797.

23. Gréhant and Quinquaud: Mesure du volume de sang contenu dans l'organisme d'un Mammifère vivant, Compt. Rend. Acad. Sc., 1882, xciv, 1450.

24. Haldane, J., and Smith, J. Lorrain: The Mass and Oxygen Capacity of the Blood in Man, Jour. Physiol., 1899-1900, xxv, 331. Smith, J. Lorrain: Discussion on the Blood in Disease, Trans. Path. Soc., London, 1900, li, 311.

25. Tarchanoff, J. R.: Die Bestimmung der Blutmenge in lebenden Menschen, Arch. f. Physiol. (Pfluger's), 1880, xxiii, 548; 1881, xxiv, 525.

26. Oerum, H. P. T.: Quantitative Blutuntersuchungen, Deutsch. Arch. f. klin. Med., 1908, xciii, 356.

27. v. Behring, E.: Die Antitoxinmethode zur Blutmenge Bestimmung, München. med. Wchnschr., 1911, lviii, 655.

28. Kämmerer, H., and Waldmann, A.: Blutmengebestimmungen nach v. Behring und andere quantitative untersuchungen der Blutbestandteile, Deutsch. Arch. f. klin. Med., 1913, cix, 524.

29. Fries, H.: Ueber Veränderungen der Blutmenge in Schwangerschaft, Geburt und Wochenbett, Ztschr. f. Geb. u. Gyn., 1911, lxix, 340.

Table 4 indicates the wide variations that have been obtained by the different methods. The total number of normal cases has been small. In the report of Haldane and Smith,[24] the limits for normal are very wide. They state specifically, however, that one of the cases was very obese, that is, the one in which the blood was only one-thirtieth of his body weight.

TABLE 4.—Variations in Blood Volume by Different Methods

Author	Method	No. of Cases	Extreme Fractions	Average	Per Cent Body Weight	Remarks
Haller.......	Bled to death; weight of blood	2	....	......	....	24-30 pounds of blood. Body wt. not given
Welcker and Bischoff	Bled and washed out vessels of criminals. Compared with hematin	3	1/14.6-1/12.4	1/13	7.6	This was accepted standard from 1858 to 1881
Tarchanow*	Sweating and counting R. B. C. and Hb. before and after steam bath	7	1/11.7-1/21.8	1/15	6.6	
Vierordt....	No. c.c. put out by heart multiplied by No. of seconds required for complete circulation	....	....	1/13	....	Worked on horses and dogs. Error, 12½ per cent.
Weber and Lehmann	Infused water, washed out vessels; determined dry substance of blood	2	....	1/8	12.5	
Haldane and Smith	CO inhaled. Carmin titration	12	1/30-1/16	1/21	3.34-6.27	
Kottmann †	Infusion of salt sol. and hematocrit before and after	4	1/11.5-1/13	1/12.2	8.2	
Smith‡.......	CO method. Titration with carmin solution	6 women 12 men	....	....	4.4-6.1¶ 3.3-6.27	
Oerum.......	CO method. Titration with carmin solution	9 men 4 women	....	....	3.7-8.3¶ 2.5-6.6¶	
Plesch........	1. Infusion with Hb. determinations. 2. CO inhalation with combustion	21 4	1/21-1/16.5 1/20-1/16.5	1/19 1/18	4.69-6.05 5.02-6.05	15 % error
Kämmerer and Waldmann	Behring method.......	7	1/11.1-1/9.3	1/10.2	9-10.7	
Fries........	Behring method.......	10	1/17.5-1/11	1/12.6	7.9	Other practically normal patients gave similar values

* Arch. f. physiol., 1880, xxii, 548; 1881, xxiv, 525.
† Arch. f. exper. Path. u. Pharmakol., 1906, liv, 356.
‡ Tr. Path. Soc. London, 1900, li, 311.
¶ c.c. per 100 grams of body weight.

Results (Normal)

At least 140 determinations for plasma volume have been made on more than 100 individuals. In the earlier work, hematocrit studies were not made, so that the values for total blood volume are considerably fewer.

Normal Values.—The results here reported are for normal men, being obtained from patients attending the outpatient department of the genito-urinary clinic for some minor local condition, such as chronic urethritis, chronic prostatitis or verumontanitis, etc. The age, weight, height and blood pressure, as well as the blood values, will be seen in the accompanying tables (5 and 6).

TABLE 5.—Plasma Values in Twenty-Four Subjects

No.	Name	Date	Age	Weight, Kg.	Height, Ft.	In.	B. P. Systolic	Volume, c.c.	C.c. per Kg.	Fraction Body Weight	Per Cent Body Weight
1	F. J. ...	1/2	39	58.8	5	3	125	3,100	45	1/21.6	4.6
2	E. M. ..	1/6	22	69.5	5	10	...	2,775	47	1/20.7	4.8
3	G. P. ..	1/7	40	56.1	5	6	115	3,050	53	1/18.3	5.4
4	M.	1/7	39	51.8	5	11	...	2,700	52	1/18.6	5.3
5	P.	1/9	34	73.5	5	3	138	3,050	42	1/23	4.3
6	V.	1/9	23	58.6	5	7	90	3,050	52	1/18.6	5.3
7	C.	2/2	..	53.4	......		...	2,925	55	1/17.6	5.6
		2/4	..	53	......		...	3,025	57	1/17	5.8
8	F.	2/2	..	67	......		...	2,975	43	1/22	4.5
		2/4	..	67	......		...	3,050	45	1/21.6	4.6
9	S.	1/29	25	68.4	5	6	...	3,100	45	1/21.6	4.6
10	H. S. ..	12/28	33	58.9	5	5	90	2,975	50	1/20	5
11	P. B. ..	12/28	51	72	5	6	122	3,275	45	1/21.6	4.6
12	J. J. ...	12/28	22	57.5	5		...	2,800	49	1/19.8	5
13	J. S. ...	12/28	46	63.6	5	3	142	3,075	48	1/20.4	4.9
14	J. C. ...	12/29	43	71	5	6	130	3,325	48	1/20.4	4.9
15	H. O. ..	12/29	30	71	5	10	...	3,150	45	1/21.6	4.6
16	B. S. ...	12/31	32	65.3	5	6	145	3,100	47	1/20.7	4.8
17	G. D. ..	12/31	36	62.5	5	10	120	3,100	50	1/20	5
18	G.	1/2	34	69.1	5	8	130	3,300	48	1/20.4	4.9
19	F. L. ..	1/2	32	50.9	5	5	105	2,500	49	1/19.8	5
20	F. S. ...	12/30	49	66.8	6		130	3,200	48	1/20.4	4.9
21	E. J. ...	1/5	..	70.5	6	2	...	3,750	53	1/18.3	5.4
22	J. M. S.	3/11	..	52.3	......		...	2,675	51	1/19	5.2
		3/12	..	52.3	......		...	2,675	51	1/19	5.2
23	D.	1/22	..	70.9	5	11	130	3,375	48	1/20.4	4.9
24	S.	1/12	..	56.4	5	7	...	2,825	50	1/20	5

TABLE 6.—Blood Values in Eighteen Normal Subjects

No.	Name	Age	Date	Weight, Kg.	Plasma Volume, c.c.	C.c. per Kg. Body Weight	Fraction Body Weight	Per Cent. Body Weight	Hematocrit		Total Blood Volume	C.c. per Kg. Body Weight	Fraction Body Weight	Per Cent. Body Weight
									R. B. C.	Plasma				
25	H. G.	..	3/11/15 3/12/15	64.3 64.3	3,175 3,075	49.4 48	1/20 1/20.4	5 4.9	38.3	61.7	5,125	79	1/12	8.3
26	J. T.	36	4/24/15	85	3,775	44	1/22	4.5	43.4	56.6	6,675	78.5	1/12	8.2
27	W.	24	4/24/15	76.3	3,525	46	1/21	4.7	44	56	6,300	82	1/11.6	8.6
28	H. S.	..	5/ 7/15	61.8	3,225	52	1/19	5.3	45.4	54.6	5,900	95	1/10.5	9.9
29	W.	..	4/21/15	59.5	2,975	50	1/20	5.1	37.6	62.4	4,775	80	1/12	8.4
30	W—t..................	53	5/ 3/15 4/22/15	65.3 63.5	3,400 3,375	52 53	1/18.3 1/18.3	5.3 5.4	41.6 40	58.4 60	5,775 5,625	88 88	1/11 1/11	9.2 9.2
31	B.	26	4/28/15	63.6	3,025	48	1/20.4	4.9	48	52	5,825	92	1/10.4	9.6
32	J.	24	4/28/15	56.8	2,850	50	1/20	5.0	45.7	54.3	5,250	92	1/10.4	
33	G.	..	4/28/15	60.9	3,300	54	1/17.9	5.5	40.5	59.5	5,500	91	1/10.4	9.5
34	L.	28	4/28/15	60.7	3,200	52	1/18.3	5.3	46.9	53.1	6,025	99	1/19.6	10.4
35	E.	20	4/28/15	72.5	3,575	49	1/20	5.0	38.6	61.4	5,825	80	1/12	8.4
36	L.	21	5/ 2/15	66.5	2,874	43	1/22.7	4.4	49	51	5,725	84	1/11.4	8.8
37	H. B.	23	5/ 3/15	47.4	2,850	52	1/18.4	5.3	43	57	4,200	88	1/11	9.2
38	R. K.	25	5/ 3/15	72.3	3,300	46	1/21.3	4.7	45	55	6,000	83	1/11.5	
39	J. C.	33	5/15/15 5/ 7/15	55.5 56.8	2,775 2,850	50 50	1/20 1/20	5 5	45 40.4	55 59.6	5,025 4,775	90 84	1/10.5 1/11.4	9.4 8.8
40	G. P.	40	1/ 7/15	56.1	3,040	53	1/18.5	5.4	32.7	67.3	4,525	80	1/11.9	8.4
41	W. M.	39	1/ 7/15	51.8	2,700	52	1/18.7	5.3	38.2	61.8	4,375	84	1/11.3	8.8
42	E. M.	22	1/ 6/15	69.5	3,275	47	1/20.8	4.8	42.9	57.1	5,725	82	1/11.5	8.7

Plasma Values.—Forty-eight determinations were made on forty-two patients. The largest (3,775 c.c.) and smallest (2,450 c.c.) volumes were encountered in the heaviest (85 kg.) and lightest (47.4 kg.) patients (Nos. 26 and 36 respectively). The mean weight was 61.2 kg. and the mean volume 3,125 c.c.; the average weight, 63.3 kg. and the average volume 3,050 c.c. The plasma varied from 42 to 56 c.c. per kilogram, while the fraction of body weight varied from 1/23 to 1/17, the average being 1/19.4. (From this it appears that a normal man of 140 pounds has 3 liters of plasma, or 50 c.c. per kg.) The constancy in the relation of plasma to body weight in health is striking, there being no great extremes.

Blood Values.—The number of determinations of total blood values has not been so large. Twenty-one determinations on eighteen normal individuals were made, the whole blood values being calculated from the plasma volume and hematocrit findings. The plasma constitutes on the average 56.9 per cent. of the total blood, the extremes being 51 per cent. and 62.6 per cent. The largest and smallest volume (6,675 c.c. and 4,200 c.c.) were obtained in the two patients mentioned above as having the largest and smallest plasma volumes. These patients showed 78.5 and 97 c.c. per kilogram, respectively. The average blood volume was 5,350 c.c., or approximately 85 c.c. per kilogram. On a gravimetric basis, the blood constituted 8.8 per cent., or 1/11.4 of the body weight. The variations in health were not great—from 1/13 to 1/10.5 of the body weight.

The constancy of these values is in striking contrast to those of Haldane and Smith[24] and of Oerum,[26] who found the blood-body-mass relationship exceedingly variable. The results here reported support the figures of Welcker and Bischoff, Vierordt, Kottmann, Behring, Fries, and Kämmerer and Waldmann.

Clinical Studies (Pathological)

Sixty-five patients were studied, the determinations numbering eighty-eight.

For purposes of discussion the cases are grouped according to clinical conditions. It is to be regretted that the number of cases in each group is not larger. This was impossible, since the drug is of German origin and despite repeated efforts it was impossible to secure a further supply.[30] The scope of the work has been limited by the small amount of dye available.

30. It is probable that many dyes will answer the purpose equally well. Studies along these lines are now in progress.

TABLE 7.—Blood Observations Before and After Delivery

No.	Name	Age	Para	Relation of Observation to Labor, Days		Blood Lost	Weight of Child	Body Weight	Plasma Volume	C.c. per Kilo	Blood Volume	Per Cent. of Body Weight	Per Cent. of Estimated Weight	Estimated Decrease in Blood Volume	Hematocrit Volume R.B.C.
				Before	After										
1	L. V.	21	0	2	...	210	4,165	55.5	3,213	57.9	5,637	10.66	11.16	.....	43
					5	...	.....	48.2	2,487	51.6	4,363	9.50	.....	1,275	43
2	G. Z.	30	1	19	...	100	4,150	61	3,343	54.8	5,306	9.13	10	.....	37
					4	...	.....	52.7	3,057	58	4,700	9.36	.....	506	35
					11	...	.....	53.9	2,641	49	4,192	8.1	.....	1,286	37
3	M. B.	16	0	13	...	150	3,315	51.8	2,621	50.6	3,971	8.05	8.5	.....	34
					4	...	.....	45.4	2,243	49.4	3,299	7.6	....	672	32
4	E. J.	20	0	9	...	325	3,100	57	3,329	58.4	5,044	9.03	9.6	.....	34
					1½	...	.....	50	3,030	60.6	4,734	9.94	....	310	36
					9	...	.....	50.5	2,525	50	3,945	8.2	....	1,099	36
5	C. O.	23	3	6	...	250	3,970	65.6	3,870	59	5,691	9.11	9.7	.....	32
					4	...	.....	55	2,695	49	4,022	7.68	....	1,569	33
6	F. S.	25	2	4	...	800	3,085	63.9	3,623	56.7	6,039	9.92	9.5	.....	40
					3	...	.....	57.3	2,974	51.9	4,721	8.65	....	1,318	37
7	E. T.	22	1	39	...	200	4,075	64.5	4,689	72.7	7,105	11.55	12.5		
					9	...	.....	60	3,078	51.3	4,809	8.41	....	2,296	36
8	L. L.	22	2	4	...	100	3,640	61.4	3,899	63.5	5,262	8.98	10.2	.....	28
					7	...	.....	59.3	2,894	48.8	4,904	8.68	....	358	41
9	B. W.	18	0	11	...	225	3,420	61.7	2,974	48.2	4,647	7.89	9.4	.....	36
					5	...	.....	59.1	2,216	37.5	3,821	6.79	....	826	42
10	A. McD.	20	0	1	...	675	3,420	63	3,717	59	6,300	10.5	11.4	.....	41
					8	...	.....	57	2,519	44.2	3,760	6.9	....	2,640	43
11	M. F.	16	0	6	...	200	1,040	47.1	1,795	38.1	3,042	6.78	....	.....	41
					10	...	.....	43.6	1,548	35.5	2,669	6.43	....	373	42
12	E. Z.	17	0	61	...	...	.....	54.5	3,461	63.5	5,408	10.42	....	.....	36
13	M.	19	0	14?	...	...	.....	57.3	3,266	57	5,184	9.51	....	.....	37

Pregnancy.—Observations have been made before and after delivery in eleven cases (Table 7). In the latter months of pregnancy, the blood mass in proportion to the body weight is definitely increased. Whereas, normally, blood constitutes 8.8 per cent., in twelve pregnant women prior to labor the blood averaged 9.56 per cent., which corresponds to the highest limits of normal value. In four instances, the blood values rose considerably above normal limits. The plasma volumes were correspondingly increased, averaging 58.4 c.c. per kilogram, instead of 50 c.c. Determinations made a week to ten days after labor showed that a great decrease in the blood mass had occurred which could not be accounted for by loss of weight (child, placenta and amniotic fluid) or loss of blood at parturition. After labor, the blood and plasma formed 9 per cent. and 4.9 per cent. of the body weight, respectively. The average loss of blood, according to the obstetrical report of these cases, was only 300 c.c., whereas blood volume studies showed an average loss of 1,100 c.c.

Efforts have been made, but without success, to find the dye in the placenta, in the amniotic fluid and in the child's urine, following the administration of the dye intravenously to the mother during labor. Abnormal absorption of the dye would not seem to be the explanation of the high blood values recorded in pregnancy. This opinion is based on the fact that the decrease in blood mass is not found immediately after parturition, for in two cases (2 and 4) it will be seen that determinations made thirty-six hours in one case, and four days after delivery in the other, showed the same high values for plasma and blood. Subsequent determinations, ten days after labor, showed in both cases the marked decrease recorded in the other cases of the series. The consideration of blood volume in relation to pregnancy will form the subject of a separate communication in collaboration with Dr. J. R. Miller, with whom this part of the work was carried out in the wards of Prof. J. Whitridge Williams.

These findings confirm the widespread belief among obstetricians that pregnant women stand hemorrhage unusually well. Comparatively very large bleedings in eclampsia are well borne. It would appear that Nature has here a factor of safety, the large blood volume, safeguarding against the effects of hemorrhage before, during or after labor. Or it may be that the larger blood supply is necessary for proper nutrition of the child.

Case 11 was complicated by secondary lues and nephritis. It does not accord with the other cases of the series.

Emaciation.—Two cases (1 and 2, Table 8) are included: The first is in a woman who had lost 12 to 15 kg. through voluntary starvation. The plasma volume is absolutely normal, and since red cells were 4,000,000, her blood volume was, in all probability, not much below normal. The second patient, very tall and thin, also showed strictly normal values.

Obesity.—Six cases (Nos. 3, 4, 22, 26, 31 and 32, Table 8) showed in every instance a plasma volume decidedly below that of the average normal. The average was 37.1 c.c. per kg., or 3.8 per cent. of body weight, which is less than three-fourths of the normal value. In the one case (32) in which a hematocrit reading was obtained, 51 c.c. per kilogram of blood was found. This patient had only as much blood per kilogram as the normal individual has plasma per kilogram. Case 3 is of extreme interest, inasmuch as the plasma formed less than 3 per cent. of the body weight. These findings are in harmony with those of Haldane and Smith.

Emaciation and Anemia.—Ten cases (Nos. 5 to 14, inclusive, Table 8) exhibiting emaciation, in combination with moderate or severe secondary anemia, were studied. In three of these, malignant neoplasms were present. In six, the plasma volume was higher than the average normal, and in three, abnormally high. The average in eight cases was 56 c.c. per kilogram, which indicates a serous plethora. In four, the total blood volume was studied and three gave low values. It is evident, therefore, that in such conditions loss of plasma does not go hand in hand with loss of weight and decrease of red blood cells. Unfortunately, hematocrit studies were not made in the carcinoma cases. With the degree of anemia indicated in the table, it is evident, however, that the total blood volume must have been approximately normal.

Since the average blood value was 78 c.c. (1/13.2 of body weight), instead of 85 c.c. per kg., it becomes apparent that the anemia is fairly accurately indicated by the blood count. In Cases 8 and 11, there is a decrease in the volume, as well as in the cell content of the blood.

Pernicious Anemia.—Only three cases (Nos. 15, 16 and 17, Table 7) were studied. No. 15 showed an extremely high plasma volume of 72 c.c. per kg., the highest encountered outside of pregnancy. The blood volume was normal, but slightly below the average. In the other two, plasma values were normal, and the total volume considerably decreased.

With so great an increase in plasma volume in Case 15, it was thought that the whole blood mass might be larger than normal and be brought into causal relationship with the enlarged heart sometimes seen in pernicious anemia. The two subsequent cases showed low blood values and no cardiac enlargement. Further data in this connection are necessary.

Polycythemia.—Only two cases (Nos. 18 and 19, Table 8) with high blood counts were studied. Patient 18, throughout several weeks' stay in the wards, presented a persistently high red cell count (8,000,000). Two determinations, a week intervening, yielded identical results, that is, both plasma and blood volumes were decidedly smaller than normal. This patient cannot, therefore, be considered plethoric, since he has only 73 c.c. of blood per kg., instead of the normal 85 c.c. per kg. The total number of cells and the absolute hemoglobin were probably about normal. The apparent plethora was caused by the low plasma content of the blood, 39 c.c. instead of 50 c.c. per kg.

Patient 19 showed a red blood cell count of 5,600,000, a count slightly above normal. This is an instance of true plethora. The plasma volume was also considerably above the average normal, being 60 instead of 50 c.c. per kilogram. The blood pressure was 135/90, while the blood was 113 c.c. per kilogram, instead of 85 c.c. per kilogram.

These cases strikingly demonstrate the necessity of total value for the proper interpretation of the routine methods of studying the blood.

Three other cases showed counts somewhat above the normal. In two of these, the plasma volume was low and the blood volume consequently not increased.

Diabetes.—Of the four patients whose cases were studied (Nos. 20 to 23, inclusive, Table 8), three were moderately thin men and one an obese woman. The three men showed a sugar content in the blood between 0.18 per cent. and 0.45 per cent., and all showed plasma and blood volumes that were strictly within the limits of normal. The obese patient (No. 22) showed blood volume decidedly below normal, so that the obesity exerted a greater influence on the blood-body-mass relationship than did the glycosuria. From these four cases it appears that an increase in the blood volume does not result from or accompany hyperglycemia.

Myocardial Insufficiency.—Nine plasma determinations were made on seven cases (Nos. 24 to 30, inclusive, Table 8) of myocardial insufficiency. Nephritis was absent, or played a very minor rôle in this series. Several of these patients had marked edema, and the average volume, 47 c.c. per kilogram, was slightly low, so that, in general, edema is not associated with an increase in plasma corresponding to the increase in weight. High values may occur, as is seen in Patient 30. The lowest plasma value (Case 27) is associated with a high red blood count.

No.	Name	Date	History No.	Age	Diagnosis	Red Blood Cells	Hgb. Sahli.	Weight	
								Actual	Best
1	A. E.	1/15	33581	32	Chronic pancreatitis. Malnutrition. Visceroptosis	4,022,000	82	36.8	48
2	E. J.	1/5	33514	24	Accident. Pain in side..........	5,100,000	100	70.5	...
3	R. M.	1/15	33583	48	Syphilis. Obesity	4,200,000	84	95.4	100
4	E. N.	1/6	33506	58	Obesity. Myocardial insufficiency, hypertension	5,000,000	80	107.7	159
5	R. B.	1/6	33467	66	Carcinoma stomach	2,600,000	45	38.8	...
6	J. H. C.	1/12	33559	72	Achylia gastrica; malnutrition. Arteriosclerosis	4,000,000	80	52.7	68
7	J. H.	1/6	33474	65	Carcinoma prostate. Metastases	2,120,000	45	48.6	...
8	A. E.	3/18	33818	31	Secondary anemia. Diarrhea. Psychosis. Migraine	3,600,000	61	43.9	52?
9	J. N.	1/15	33586	48	Carcinoma stomach	3,800,000	65	55.9	68
10	H. W.	4/9	.....	25	Balantidium coli. Secondary anemia	1,072,000	20	.....	...
11	J. S.	....	33040	22	Secondary anemia. Leg ulcers	1,500,000	31	50	...
12	R. A.	1/8	33522	54	Anacidity. Malnutrition. Secondary anemia	4,600,000	70	44.5	75
13	F. M.	3/12	33829	39	Duodenal ulcer. Secondary anemia	3,500,000	75	59.5	70
14	P. S.	2/22	33714	34	Acute nephritis. Syphilis. Secondary anemia	3,500,000	65	63	87
15	P. J. B.	1/5	33433	42	Pernicious anemia	1,192,000	28	68.2	...
16	T. A.	2/25	33770	38	Pernicious anemia	1,500,000	25	64.1	...
17	W. M.	1/23	C. H. I.	69	Penicious anemia. Aplastic type	1/17, 1,300,000; 1/28, 800,000	.. 22 15	53.6	...
18	G. T.	2/17	33642	22	Peritoneal adhesions. Polycythemia	8,000,000	100	50.5	55
		2/25	.....	..	....	....	..	.....	...
19	F. O.	1/5	33526	25	Mitral insufficiency. Left hemiplegia	5,600,000	101	69.1	72
20	G. V. H.	3/18	33876	23	Diabetes mellitus. Pulmonary tuberculosis. Coma	5,400,000	98	42.5	53
21	E. N. V.	3/11	33787	60	Diabetes mellitus. Pulmonary tuberculosis. Arteriosclerosis. Aortic insufficiency	4,700,000	82	58.2	76
22	M. B. J.	1/21	33487	37	Diabetes mellitus. Obesity......	....	85	80.9	80
23	L. W.	3/12	33817	29	Diabetes mellitus	5,500,000	89	54.1	67
24	B. S.	1/12	33571	44	Myocardial insufficiency. Pulmonary tuberculosis .	5,200,000	100	78.2	84
25	O. O. M.	2/17	33735	60	Arteriosclerosis. Myocardial insufficiency	4,700,000	94	87.3	...
		2/25	.....	..	....	....	..	73.4	...
26	W. J. R.	1/15	33584	70	Myocardial insufficiency	5,000,000	84	89.5	...
27	J. S.	1/6	G. No. 98729	48	Syphilis. Aortic insufficiency. myocardial insufficiency	6,500,000	107	68.8	...
28	W.	1/23	......	..	Myocardial insufficiency	....	..	73.2	...
29	J. S. M.	1/9	33521	59	Arteriosclerosis. Angina pectoris. Myocardial insufficiency	5,000,000	80	89.5	112
30	J. F.	1/12	33531	42	Syphilis of aorta. Aortic insufficiency	....	..	60.4	...
		1/22	.....	..	....	....	..	59.1	...
31	E. W. S.	2/4	33679	62	Pulmonary tuberculosis. Arteriosclerosis. Chronic nephritis	5,000,000	75	90.2	...

Edema	Blood Pressure	Cardiac Enlargement	Plasma c.c.	C.c. Per Kg.	Hematocrit Per Cent. Plasma	Blood Volume	C.c. per Kg.	Per Cent. Body Weight	Fraction Body Weight	Remarks
None	100/ 60	None	1,850	50						
None	120/ 80	None	3,750	53	60	6,250	89	9.3	1/10.9	6 feet 3 inches in height
None	120/ 70	None	2,725	28.6						
Slight	210/110	Moderate	4,950	45.8						
None	100/ 60	None	2,575	66	75	3,425	88	9.2	1/10.8	
None	150/100	None	2,375	45						
Right leg sacrum	130/ 65	None	3,150	64	77.2	4,075	84	8.8	1/11.4	
None	100/ 65	None	2,175	49	65.3	3,325	76	8	1/12.5	
None	110/ 65	Slight	3,725	66						
Moderate	105/ 45	Slight	3,425	60	85.6	4,025	70	7.3	1/13.6	
None	....	None	2,900	57	78.8	3,675	72	7.5	1/13.2	
None	120/ 90	Slight	2,120	47.6	67.3	3,150	71	7.4	1/13.5	
None	100/ 65	Slight	3,400	57	70.6	4,800	88	9.2	1/10.8	
None	120/ 75	Slight	3,225	51	64.3	5,025	80	8.4	1/12	
None	100/ 55	None	4,950	72	90	5,500	81	8.5	1/11.7	
None	120/ 70	Slight	3,450	54	84	4,250	66	6.9	1/14.4	Improved
None	....	None	2,850	53	.....	.....	...	....	....	Died
None	120/ 70	None	1,975	39	53.2	3,725	73	7.7	1/13.5	
....	....	....	2,000	39	53.2	3,750	73	7.7	1/13.5	
None	135/ 90	Slight	4,125	59.7	52.5	7,880	113	11.9	1/8.4	
None	140/ 90	None	2,175	51.3	57	3,800	89	9.3	1/10.7	Blood glucose 0.25-0.3 Necropsy
None	160/ 60	Slight	3,275	56	62.1	5,375	92	9.6	1/10	Blood glucose 0.45-0.18
None	120/ 70	None	2,825	35	.....	.....	...	....	....	Blood glucose 0.18
None	120/ 80	None	2,775	51	.....	.....	...	....	....	Blood glucose 0.4-0.34
Marked	90/ 50	Moderate	3,900	50						
Marked	120/ 80	Marked	4,375	50	54.3	8,050	92	9.6	1/10.3	Necropsy
Slight	....	Marked	3,900	53						
Moderate	170/120	Moderate	3,800	42.5						
Moderate	130/ 90	Marked	3,025	40	45.6	6,650	87	9.1	1/11	
....	....	....	3,175	43						
Moderate	150/115	Marked	3,975	43	.....	.....	...	....	....	Necropsy
Slight	130/ 90	Slight	3,385	56	.....	.....	...	....	....	Necropsy
....	115/ ?	....	2,841	48						
Slight	160/ 90	Slight	3,300	36.7	.....	.....	...	....	....	Weight 130 lbs. in 1903

TABLE 8.—CLINICAL (PATHOLOGICAL)—

No.	Name	Date	History No.	Age	Diagnosis	Red Blood Cells	Hgb. Sahli.	Weight	
								Actual	Best
32	L. J.	....	33777	47	Hypertension. Chronic nephritis. Osteo-arthritis of spine	5,000,000	90	79.4	...
33	E. F.	1/20	33757	36	Syphilis. Arteriosclerosis. Hypertension. Chronic nephritis	3,500,000	65	62.3	65
		3/18	.....	..	....	....	..	.....	...
34	E. O.	2/21	33697	47	Arteriosclerosis. Chronic nephritis. Hypertension. Polycythemia	5,900,000	105	72	80
		2/27	.....	..	....	....	..	.....	...
35	G. L.	1/20	33510	53	Syphilis. Hypertension	5,000,000	83	60.7	70
36	R. A.	2/17	33643	45	Chronic nephritis. Hypertension. Myocardial insufficiency	3,000,000	60	65.9	...
37	F. W.	2/17	33722	47	Chronic nephritis. Hypertension. Cerebral hemorrhage	5,800,000	92	56.6	...
38	M. C.	1/23	C. H. I.	..	Hypertension	....	..	42.7	...
39	J. E. A.	2/12	36283	49	Chronic nephritis. Hypertension. Arteriosclerosis. Hematuria	a. m., 4,000,000;	80	78.3 76.4	... ...
		2/18				p. m., 3,700,000	75	.....	...
		2/25	.....	..	....	....	..	75.9	...
40	H. O. C.	1/14	36082	55	Arteriosclerosis. Hypertension. Enlarged prostate	....	..	70.1	...
41	N. P. P.	3/18	36551	51	Arteriosclerosis. Hypertension. Carcinoma of bladder	3,800,000	72	98.4	...
42	E. S. H.	2/12	36298	71	Arteriosclerosis. Hypertension ..	....	..	59.1	...
43	M. P.	1/12	33549	32	Chronic nephritis. Hypertension. Myocardial insufficiency	4,400,000	63	87.3	...
		2/3	.....	..	....	....	..	76.3	...
44	W. L. I.	1/12	33424	38	Chronic nephritis	4,000,000	82	80.9	...
		2/3	.....	..	....	....	..	79.5	...
45	T. K.	1/12	33544	29	Chronic nephritis	4,200,000	93	62.7	73
46	A. T.	1/3	33472	22	Chronic prostatitis	4,300,000	100	52.3	61
47	T. W.	1/7	33479	71	Chronic infectious arthritis......	4,800,000	75	72.7	73
48	D. K. S.	1/12	33572	51	Cerebral arteriosclerosis. Brain tumor?	....	..	56.4	...
49	F. N.	1/20	33390	40	Emphysema. Chronic bronchitis	5,000,000	90	54.8	...
50	B.	1/7	33333	43	Syphilis. Aneurysm of thoracic aorta	4,200,000	92	61.4	...
		2/2	.....	..	....	....	..	63.6	...
51	F. T.	1/14	33545	53	Chronic appendicitis	5,000,000	90	48.6	...
52	M.	1/14	33461	18	Typhoid fever	4,500,000	85	60	...
53	S. B.	2/27	36426	49	Carcinoma bladder. Secondary anemia	3,300,000	33	58	...
		3/11	.....	..	....	....	..	58.4	...

Studies on Blood Volume—(*Continued*)

Edema	Blood Pressure	Cardiac Enlarge-ment	Plasma c.c.	C.c. Per Kg.	Hema-tocrit Per Cent. Plasma	Blood Vol-ume	C.c. per Kg.	Per Cent. Body Weight	Frac-tion Body Weight	Remarks
Slight	180/100	Moderate	2,700	34	66.7	4,050	51	5.3	1/18.7	
None	200/150	Slight	3,050	49						
....	....	....	3,150	51						
None	200/150	Slight	3,050	42	56.1	5,475	76	8	1/12.5	Blood glucose 0.12
....	....	....	.....	48	61	5,675	78	8.2	1/12	
None	185/105	Slight	3,025	50						
Slight	240/130	Slight	3,175	48	63	4,913	74	7.8	1/12.7	Blood glucose 0.14. Died
None	195/130	Slight	2,950	52	54.3	5,425	96	10	1/10	
None	180/ ?	Slight	1,825	43						
None	245/140	Moderate	4,650	59						
....	235/135	....	3,875	51	63	6,150	84	8.8	1/11.9	800 c.c. blood removed
....	235/155	....	3,425	45	63	5,425	80	8.4	1/13.1	
....	220/125	....	4,150	55	75	5,500	72	7.6	1/13.1	
None	175/110	Slight	3,500	50	60.8	5,750	81	8.5	1/11.7	
None	195/110	Slight	4,325	44	66.4	6,525	65	6.8	1/14.7	Died
None	210/ 90	Moderate	3,150	53						
Consider-able	180/100	Marked	3,875	44	64.4	6,025	69	7.2	1/13.9	
Slight	115/ 75	Slight	4,047	53						
Moderate	130/ 80	Slight	3,172	39						
....	....	....	3,064	38.4						
None	100/ 85	None	2,925	46						
None	140/ 90	Slight	2,420	45						
None	140/ 85	None	3,450	47.6	63.7	5,425	75	7.8	1/12.8	
None	120/ ?	Slight	2,825	56.4						
None	110/ 70	None	2,450	45						
None	120/ 70	Slight	3,025	49	60	4,900	78	8.1	1/12.2	
....	....	....	3,175	50						
None	110/ 70	None	2,200	46						
None	95/ 60	None	3,050	50						
None	168/ 88	Slight	3,500	61	70.6	4,550	78	8.2	1/12.2	Died
....	....	....	3,350	57						

Although in anasarca the number of cubic centimeters of blood per kilogram may not be increased above normal, the total blood volume may be large in relation to the normal body weight. Case 25 had a blood mass of over 8 liters. With the disappearance of his edema, he lost 14 kg. Considering his weight at this time in relation to the blood volume, 8,050 c.c., found during edema, he had 109 c.c. per kilogram, which is an extremely high value. The patient died six weeks later. At necropsy, the kidney showed numerous small scars throughout, with some atrophy of the tubular cells, and also an acute inflammatory process, as evidenced by infiltration with polymorphonuclear leukocytes. There was a slight chronic nephritis with an acute terminal process. Myocarditis was extensive and fibrous in type, with hypertrophy of muscle and small scattered scars everywhere. The heart was of tremendous size. It is quite possible that in this instance the large blood mass stood in causal relationship to the large heart.

Hypertension and Chronic Nephritis.—In nineteen determinations on thirteen cases (Nos. 31 to 43, inclusive, Table 8), a plasma volume greater than normal was found only once, while the total blood volume was not found increased in nine determinations on six cases. This would appear to demonstrate that in these cases the hypertension was not dependent on an increase in blood mass. In hypertension, the blood volume is normal and frequently low. The increased tension would appear, therefore, to be dependent on a vascular system that is too small, rather than on a blood volume that is too large. The average value for plasma was 42.8 c.c. per kilogram, and for the whole blood, 75 c.c. per kilogram, in both instances the lower limit of normal. The small values are not dependent on edema, as will be ascertained from the table, since in only three cases was even slight edema present, and in only one was there great cardiac enlargement. The results are in striking contrast with those of Plesch.[14]

Several of these cases are of peculiar interest. Patient 39, who, on one determination showed an increased plasma volume, had a marked hematuria so that hemorrhage may have accounted for this finding. Subsequent determinations showed the plasma volumes to be normal. Following venesection with removal of 800 c.c., there was no decrease in blood pressure, but immediate decrease in plasma volume. A week later, there was an increase in plasma to above that found before the venesection, but without a corresponding increase in the blood volume.

That hypertension can exist in association with a low blood volume is clearly indicated by Case 32, the patient showing a systolic blood pressure of 180 and diastolic of 100. The plasma was 34 c.c. per kilogram and the total blood, 51 c.c. per kilogram. The hypertension existed despite the fact that the patient's total blood mass was not greater than the plasma volume is normally. Obesity and a slight edema were in part responsible for these low values.

Case 43 is also of great interest. The patient is the only one of the series presenting marked edema at the time of determination. At this time, he had 40 c.c. of plasma per kilogram. Following a loss of 11 kg. (disappearance of edema), the plasma volume was found to be slightly larger, 4,050 c.c., compared with 3,875 c.c. It is evident that the plasma volume remained the same throughout, not being influenced by the presence or disappearance of the edema. This does not always occur, as will be seen by contrasting with Case 25.

From these cases, it would appear that true plethora is not present in hypertension, and that a small or contracted vascular system and not an increase in blood mass is responsible for the increased blood pressure.

Chronic Nephritis Without Hypertension.—Only two cases (Nos. 44 and 45, Table 8) fall into this group. Patient 44 was an instance of chronic parenchymatous nephritis, with slight edema and very marked albuminuria. The plasma was estimated twice, three weeks intervening. Following a 1.5 kg. decrease in weight, the plasma decreased 100 c.c. Both cases gave values below the normal average for plasma.

Miscellaneous Group.—A small series of miscellaneous unrelated cases (Nos. 46 to 53, inclusive, Table 8) were studied. Case 50, an instance of aneurysm of the ascending arch of the aorta, and with a moderate grade of anemia, showed identical results on two occasions—an average normal value for the plasma. Patients 49, suffering with chronic bronchitis; 51, with chronic appendicitis; 46, a neurasthenic, and 48, with cerebral arteriosclerosis, all showed values within the limits of normal.

COMMENT

Except in pregnancy, hypertension and obesity, the number of cases studied in any one condition has been so small that far-reaching conclusions are impossible. Sufficient evidence has been adduced to convince us of (*a*) an increase in blood volume just before term in uncomplicated pregnancy, (*b*) absence of a causal relationship between increased blood volume and hypertension, and (*c*) of a relatively small blood mass in obesity. Further blood volume studies in these and in other clinical conditions is contemplated.

From this study, it is apparent that a great variation in the blood mass in relation to body weight may occur in any one disease. Complications of any kind may also exert a great influence on the blood volume, as evidenced by Case 2 in Table 7. It becomes evident that we cannot take for granted that the blood volume is increased or decreased in any given disease, but must actually determine it in each individual case. In the present state of the subject, generalizations are undesirable.

SUMMARY

1. A method for the determination of plasma volume is described. From the plasma volume and hematocrit values the blood volume can be calculated.

2. With this method, duplicate determinations on normal subjects yield identical values.

3. The amount of decrease in blood volume as the result of hemorrhage and of increase following intravenous infusion of saline has been demonstrated.

4. Changes in blood volume are insignificant in experimentally induced hypertension and hypotension, and cannot be brought into causal relationship with them.

5. The plasma normally constitutes approximately 5 per cent. or one-twentieth of the body weight. Normal individuals have approximately 50 c.c. of plasma per kg., the extremes being from 42 to 56 c.c. per kilogram.

6. The normal hematocrit values obtained were approximately 43 per cent. for erythrocytes and 57 per cent. for plasma.

7. The blood normally constitutes 8.8 per cent., or 1/11.4 of the body weight. Normal individuals have approximately 85 c.c. of blood per kilogram, the extremes being 78 and 97 per kilogram.

8. In pregnancy, before term, the blood and plasma volumes are increased. A condition of serous plethora exists. Within a week or two after delivery, the blood values return to normal.

9. In obesity, the plasma and blood volumes are relatively small.

10. Many cases of anemia exhibit a relatively large plasma volume.

11. Polycythemia in the sense of a high blood count may be dependent on a low plasma volume. It may be associated with a large plasma volume, in which case true plethora is indicated.

12. Hyperglycemia exists without increase in blood volume.

13. In anasarca accompanying myocardial insufficiency the blood volume may be absolutely increased.

14. A small volume is shown in many cases of marked hypertension. Therefore so far as these studies go it would appear that hypertension is not dependent on a large blood volume.