JCI The Journal of Clinical Investigation

Simultaneous Determinations of the Total Volume of Red Blood Cells by Use of Carbon Monoxide and Chromium[51] IN HEALTHY AND DISEASED HUMAN SUBJECTS

Norman Nomof, … , Kenneth Scott, Reidar Wennesland

J Clin Invest. 1954;33(10):1382-1387. https://doi.org/10.1172/JCI103015.

Research Article

Find the latest version:

https://jci.me/103015/pdf

SIMULTANEOUS DETERMINATIONS OF THE TOTAL VOLUME OF RED BLOOD CELLS BY USE OF CARBON MONOXIDE AND CHROMIUM[51] IN HEALTHY AND DISEASED HUMAN SUBJECTS[1]

By NORMAN NOMOF,[2] JAMES HOPPER, JR., ELLEN BROWN, KENNETH SCOTT, AND REIDAR WENNESLAND

(From the Department of Medicine, University of California School of Medicine, and the Radioactivity Center, University of California, San Francisco, Cal.)

(Submitted for publication March 29, 1954; accepted May 14, 1954)

During recent years several methods have been introduced for estimating the total volume of circulating erythrocytes by measuring dilution in the blood stream of cells which have been tagged with radioactive phosphorus, iron, or chromium (1–6). Values for red cell volume thus obtained may be considered more reliable than indirect estimates based on hematocrit and "plasma volume" as measured with T-1824 or other substances which label plasma proteins, because no error is introduced by the discrepancy between venous (or arterial) and "body" hematocrits (7–9). For the same reason, a closer approximation to the true total blood volume is found by adding together red cell and "plasma" volumes, as measured independently, than is found by use of the plasma volume-hematocrit methods (10).

Erythrocytes may also be labeled with carbon monoxide gas, and this is the basis of methods which have been employed widely to estimate blood volume and total circulating hemoglobin (11–17). It has long been suspected that the volume of distribution of carbon monoxide in the body (henceforth to be called "CO-available volume") may be larger than true blood volume, since the gas has affinity for pigments which are located extravascularly, such as myoglobin or non-circulating hemoglobin (11, 18, 19). Previous work has shown a discrepancy between CO-available volume and blood volume as measured with radioactively tagged cells, both in man (9) and in the dog (18). The difference in magnitude between the reported results in man and dog, and the inadequacies of the methods used in the human experiments, leave the problem open to further exploration. For these reasons, and because the CO method has certain practical advantages for physiologic and clinical work, it seemed desirable to make comparative measurements of the apparent volume of erythrocytes in healthy and diseased human subjects, using simultaneously a CO method which has been employed extensively in this laboratory and a method utilizing Cr^{51}.

MATERIALS AND METHODS

The subjects were six healthy volunteers and 29 hospitalized patients with various diseases (Table I). Determinations of CO-available volume and of the dilution of radioactively tagged cells were carried out simultaneously as a rule, but occasionally in sequence, during the same forenoon. All subjects had fasted overnight and had been recumbent for a minimum of 30 minutes before procedures were begun.

The method used for estimating CO-available volume has been described fully (14, 15). Carbon monoxide was delivered into a small, closed system into which the subject rebreathed for 20 minutes, O_2 being added as required. Samples of blood taken before and at the end of this period were analyzed for CO by the palladium reduction method (20). No correction was made for the residual CO in the rebreathing system, since this has been shown to account for an error of not more than 1 per cent of the final result (14).

The method described by Sterling and Gray for determination of Cr^{51} space (6) was modified as follows: On the afternoon prior to the study, 15 ml. of the subject's blood was drawn into a syringe containing heparin and injected into a sterile, stoppered centrifuge tube. To this was added sufficient sodium chromate solution containing Cr^{51} [3] to give a total of 25,000 to 30,000 counts per second as measured in a scintillation counter.

[1] This work was supported in part by grants from the U. S. Public Health Service, H 751 and H 1271, and by the James W. Edwards Memorial Fund, allocated by the Committee on Research of the University of California School of Medicine.

[2] While carrying out this work, Dr. Nomof was a Trainee in Cardiology of the National Heart Institute, U. S. Public Health Service.

[3] The Cr^{51} was made at Oak Ridge and processed by Abbott Laboratories as 0.1 per cent sodium chromate solu-

TABLE I

Comparison of apparent RBC volumes obtained by CO and CR^{51} methods used simultaneously

1 Subject	2 Age	3 Sex	4 Clinical state	5 Weight (*Kg.*)	6 Height (*cm.*)	7 Hematocrit 20 min. CO sample	8 Hematocrit 20 min. Cr^{51} sample	9 Vol. RBC by CO (*liters*)	10 Vol. RBC by Cr^{51} (*liters*)	11 Ratio CO/Cr^{51}*
Group I—No Disease										
1 D. P.	29	M	Healthy	62.0	164	49.1	46.7	2.03	1.58	1.28
2 D. G.	26	M	Healthy	68.2	171	42.4	42.2	2.07	1.69	1.23
3 S. G.	21	M	Healthy	53.8	173	46.0	46.5	2.07	1.93	1.07
4 P. S.	22	M	Healthy	73.4	178	38.4	38.7	2.65	2.03	1.30
5 J. M.	23	M	Healthy	80.6	175	42.6	41.6	2.64	2.08	1.27
6 J. H.	43	M	Healthy	77.3	174	46.2	46.6	2.66	2.40	1.11
Group II—Disease States										
7 J. B.	31	F	Psychoneurosis	66.6	161	38.4	39.2	1.34	1.04	1.29
8 M. W.	41	F	Cor pulmonale	49.0	152	46.0	46.0	1.87	1.75	1.07
9 J. C.	23	F	Cirrhosis	58.3	171	36.0	36.0	1.25	1.15	1.09
10 B. G.	39	F	Mitral stenosis	39.4	155	42.7	42.7	1.70	1.31	1.30
11 B. H.	54	F	Glomerulonephritis	73.0	161	39.7	39.7	1.62	1.47	1.10
12 J. N.	32	M	Constrictive pericarditis postop.	76.8	176	41.6	41.8	2.75	2.60	1.06
13 A. D.	31	F	Atrial sept. defect	45.0	161	47.3	46.7	2.43	2.10	1.16
14 W. K.	21	M	Hemangiomatosis	54.0	161	40.4	40.1	2.26	1.94	1.16
15 B. T.	68	M	Polycythemia vera	89.3	180	48.5	49.6	3.15	3.35	0.94
16 O. S.	19	M	Constrictive pericarditis postop.	78.0	183	45.8	46.0	2.82	2.40	1.17
17 J. P.	23	M	Congen. heart dis.	52.0	164	43.9	43.4	1.89	1.64	1.15
18 T. G.	39	M	Bronchial asthma	59.0	175	41.6	41.9	1.93	1.80	1.07
19 G. M.	43	F	Mitral stenosis postop.	60.5	157	40.7	40.7	1.64	1.47	1.12
20 R. J.	45	F	Polycythemia vera	55.0	166	60.0	60.0	2.94	2.56	1.15
21 G. D.	47	M	Rheum. heart dis.—failure	75.5	176	41.2	41.2	2.96	2.30	1.29
22 W. M.	35	M	Constrictive pericarditis postop.	74.4	181	48.0	48.0	3.24	2.69	1.20
23 M. S.	65	F	Osteitis deformans	65.5	169	44.8	43.7	2.10	1.75	1.20
24 E. S.	63	M	Cor pulmonale	65.9	165	63.5	63.5	4.90	3.70	1.32
25 A. B.	47	F	Patent ductus arteriosus	54.7	164	41.3	40.6	2.13	1.96	1.09
26 L. T.	44	F	Nephrotic syndrome	68.3	169	27.3	28.0	1.01	0.85	1.19
27 J. S.	29	F	Atrial sept. defect	46.2	168	40.8	40.9	2.33	1.86	1.25
28 L. J.	14	M	Nephrotic syndrome	59.9	177	32.0	31.9	1.55	1.35	1.15
29 M. B.	67	F	Rheum. heart dis.—failure	57.9	166	40.7	43.9	2.60	2.84	0.92
30 D. H.	25	M	Glomerulonephritis	97.3	181	44.9	44.9	3.88	3.01	1.29
31 C. S.	15	F	Patent ductus arteriosus	56.5	168	39.5	39.5	1.48	1.28	1.16
32 C. W.	49	M	Rheum. heart dis.—failure	82.8	191	40.7	40.8	3.42	3.20	1.07
33 M. P.	58	M	Polycythemia vera	88.8	171	59.4	59.9	4.43	3.85	1.15
34 G. C.	24	M	Tetralogy of Fallot	52.5	178	57.4	57.4	3.54	2.71	1.31
35 M. B.	67	F	Rheum. heart dis.—compensated	60.0	164	36.7	36.9	2.42	2.18	1.11

Mean ratio for Group I = 1.21 (S.D. 0.11)
Mean ratio for Group II = 1.15 (S.D. 0.10)
Mean of ratios for Group I and Group II combined = 1.16 (S.D. 0.10)

* Ratio of red blood cell volume as determined by CO (column 9) to red blood cell volume as determined by Cr^{51} (column 10).

The tube of blood was gently rotated for 40 minutes to insure fixation of the chromium in the cells. After the blood was centrifuged for 5 minutes at 3000 rpm, the supernatant plasma was removed. The cells were washed three times with Ringer's solution, suspended in the same solution to give the original volume of 15 ml., and refrigerated overnight at 4 to 5° C. Aseptic precautions were maintained throughout the *in vitro* handling of the blood.

By means of a small intravenous drip system, 10 ml. of the labeled cell suspension was injected from a cali-

tion. The volume of the solution used ranged between 0.2 and 0.5 ml. The scintillation counter had an efficiency such that 8 per cent of the gamma rays emitted from the Cr^{51} were registered as counts.

brated syringe and washed quantitatively into the subject's vein with not more than 30 ml. of normal saline solution. This amount of cell suspension gave a total of 12,000 to 20,000 counts per second in most cases. The indwelling needle was kept patent by an infusion of saline (8 to 10 drops per minute) until the required number of blood samples had been drawn. Samples for analysis were taken 10, 20, 30, and 45 minutes after the injection. In some experiments, an additional sample was taken at 24 hours. Prior to taking each sample, the first 3 ml. of blood withdrawn from the needle was discarded. The validity of taking the samples from the same needle and vein into which the tagged cells had been delivered was confirmed by finding, on several occasions, that results were essentially identical when samples were taken simultaneously from the indwelling needle and from a needle inserted in a vein of the opposite arm.

The Cr^{51} present in each sample was measured with a scintillation counter, using 2 ml. of whole blood in a dish 42 mm. in diameter. The same volume of a 1:50 dilution of the originally tagged cell suspension was used as a standard of comparison. A total of 4096 counts was made on each specimen. The counting error due to statistical fluctuations fell within ± 1.6 per cent.

The hematocrit of each sample of blood analyzed for CO or Cr^{51} content was determined in duplicate by the Wintrobe method. Tubes were centrifuged for 30 minutes at 3000 rpm at a radius of 15 cm.; no correction was made for trapping of plasma.

The total CO-available volume was calculated by the formula:

$$\text{CO space (liters)} = \frac{V_d \times P}{10(C_f - C_i)},$$

where V_d is the volume (ml.) of gas delivered into the rebreathing system, corrected to standard conditions of temperature and pressure, and P is a correction factor for purity of the gas. C_i and C_f, respectively, are the concentrations of CO (in ml. per 100 ml.) in samples of whole venous blood taken before and 20 minutes after delivery of the gas.

The total Cr^{51}-available volume was calculated similarly:

$$Cr^{51}\text{ space (liters)} = \frac{A_{tcs} \times 50 \times V_{tcs}}{1000 \times A_b},$$

where A_{tcs} is the activity in counts per second per ml. of the tagged cell suspension, V_{tcs} is the volume of cell suspension injected, and A_b the counts per second per ml. of the blood samples drawn at the end of the various time periods after delivery of the tagged cells.

The red cell volume (subsequently referred to as Vol. RBC/CO and Vol. RBC/Cr^{51}) was obtained by multiplying CO- or Cr^{51}-available space by the hematocrit of the respective sample of whole blood used in each analysis.

RESULTS

The results are summarized in Table I and Figure 1. Column 9, Table I, lists the apparent red cell volumes as determined by the CO method, and column 10, the volumes based on the 20-minute samples in the Cr^{51} method. The corresponding hematocrits are shown in columns 7 and 8.

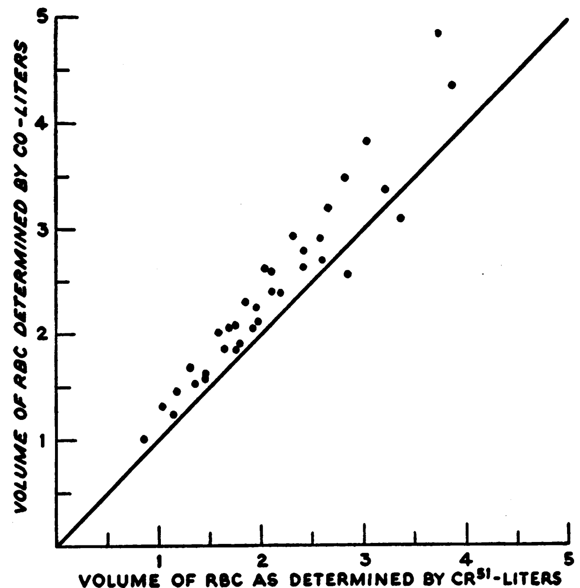

FIGURE 1.

Each point represents the apparent volume of RBC found by the CO method (CO-available space times the hematocrit of the sample analyzed for dilution of CO at 20 minutes), related to the apparent volume of RBC found by the Cr^{51} method (Cr^{51}-available space times the hematocrit of the sample analyzed for dilution of Cr^{51} at 20 minutes). The solid line represents a 1:1 relationship. (See Table I.)

As shown in Figure 1, Vol. RBC/CO was larger than Vol. RBC/Cr^{51} in both healthy subjects and patients with various diseases, with only two exceptions. The ratio of the CO to Cr^{51} values ranged from 0.92 to 1.32 for the entire series of 35 pairs of determinations. The mean ratio was 1.16, with a standard deviation of 0.10. In patients with a variety of pathologic states, the relationship between CO and Cr^{51} spaces was similar to that found in healthy subjects.

Considered in terms of absolute volumes, the average discrepancy of 16 per cent between the results of the two methods is of some magnitude. For example, the smallest and largest red cell volumes found by the chromium method were 0.85 and 3.85 liters. Assuming the average discrepancy of 16 per cent, these volumes would be 136 and 610 ml. greater, respectively, if determined

TABLE II

Red cell volume as determined by Cr^{51} and serial sampling

Subject*	Time of sample after injection of tagged cells: 10 *min.*	20 *min.*	30 *min.*	45 *min.*	24 *hr.*	Per cent deviation between result of 24 hr. sample and 20 min. sample
1	—	1.58	1.69	1.69	1.76	+10.1
4	—	2.08	2.10	1.98	2.26	+ 8.0
5	—	1.97	2.01	2.01	2.10	+ 6.2
11	1.46	1.47	1.46	—	1.53	+ 3.9
15	3.30	3.47	3.51	3.47	3.36	− 3.2
18	1.93	1.90	—	1.97	1.94	+ 2.1
21	2.33	2.30	2.32	2.35	2.39	+ 3.9
22	2.63	2.69	2.83	2.70	2.92	+ 8.5

* Subjects are referred to by same numbers as in Table I.

by CO. Variations of this degree would be important in the application of these methods to physiologic and pathologic problems.

DISCUSSION

Whenever the measurement of blood volume or fractions thereof is based on the principle of dilution, two criteria must be satisfied. First, the substance which is introduced as indicator must have reached uniform concentration in the cell mass or plasma occupying all portions of the vascular compartment before the sample to be measured is taken. Second, the substance must not leave the blood stream during the mixing period which precedes the sampling. (In methods for determining "plasma" volume which are in use currently, the second criterion is not satisfied, but it is assumed that loss of the indicator occurs at a constant rate during the entire time required for mixing and for collection of serial samples.)

The first criterion probably is met adequately by both methods under consideration here. It has been demonstrated that with the method used in these experiments, CO gas becomes equilibrated with the blood in less than 20 minutes, and remains at essentially constant concentration in the blood for periods of an hour or more. This occurs with subjects at rest, and also during massive venous congestion of the extremities (15).

In our experience, maximum distribution of Cr^{51}-tagged red cells is also reached in 10 to 20 minutes in both normal and pathologic states, although it has been reported that in rare cases of severe congestive heart failure, longer periods may be required for complete mixing (21, 22). The results of serial sampling, which was done in each experiment reported here, showed no instance of delayed mixing in our subjects.

Table II and Figure 2 give the results of eight representative experiments in which samples were taken 10, 20, 30, and 45 minutes, and 24 hours after introduction of radioactively tagged cells. In every case the concentration of tagged cells in the blood had reached plateau levels within 20 minutes after the injection. Even after 24 hours, when some loss of tagged cells from the circulation was to be expected (5, 6, 22), the apparent increase in Vol. RBC/Cr^{51} averaged only 5 per cent and never was more than 10 per cent of the volume calculated from 20-minute samples. Thus, it appears unlikely that the discrepancy between results of red cell volume determinations with CO and Cr^{51}, when based on 20-minute mixing pe-

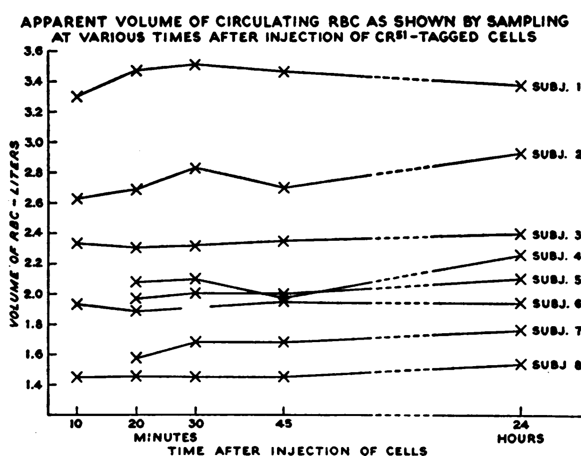

FIG. 2. THE APPARENT VOLUME OF RBC AS SHOWN BY ANALYSIS OF BLOOD SAMPLES OBTAINED AT 10, 20, 30, AND 45 MINUTES, AND AT 24 HOURS AFTER INTRAVENOUS INJECTION OF CR^{51}-TAGGED CELLS, IN EIGHT HUMAN SUBJECTS. (SEE TABLE II.)

riods, can be attributed to delayed mixing of the chromium-tagged cells or to failure of these cells to reach portions of the circulatory system which were accessible to CO during the first 20 minutes after delivering either agent into the blood stream.

The discrepancy in results can be attributed more easily to failure of the CO method to fulfill the second criterion stated above. The finding of a CO-available space which is consistently larger than the space which is available to cells labeled with chromium might conceivably be attributed to 1) fixation of CO early in the mixing period by hemoglobin or other compounds which are located elsewhere than in the circulating blood, 2) metabolism or excretion of the CO, or 3) leakage of gas through faulty connections.

The first explanation is the most acceptable one. The characteristics of the time: concentration curve of CO in the blood are not consistent with continuing loss of CO by metabolism or excretion after the first 20 minutes of rebreathing (15). Care was taken to prevent loss of gas through leaks in the apparatus during each experiment. Furthermore, errors due to leakage would be expected to produce less consistent discrepancies between the results of the two methods than were actually found.

There is close quantitative agreement between these results in man and the observations of Root, Allen, and Gregersen in splenectomized dogs (18). These investigators found the red cell volume of the splenectomized dog to be 12 per cent greater when measured with cells tagged with CO than with cells tagged with P^{32} (18). On the other hand, Hevesy, Köster, Sørensen, Warburg, and Zerahn (9) reported an even greater discrepancy (24 per cent) between cell volumes measured with CO and with P^{32}-tagged cells. The procedures used by them, however, permitted errors tending to produce low values with the P^{32} method and high values with the CO method. These possible errors included: 1) Premature sampling when using P^{32} (5 to 10 minutes), whereas the CO samples were taken after a longer interval (15 minutes); and 2) use of a large rebreathing system.

No information is available as yet concerning the sites, presumably extravascular, where 12 to 16 per cent of the CO accumulates when introduced *via* the airways or by infusion of CO-tagged cells. According to Haldane and Smith (19), dissolved CO in plasma and body fluid can account for not over 0.2 per cent of all the CO distributed throughout the body. The fact that equilibrium is reached within 20 minutes after delivery of the gas, after which the concentration of CO in the blood remains nearly constant (15), suggests that the CO combines with compounds having an avidity for CO similar to that of hemoglobin. Experiments are in progress to determine the predominant sites of such combinations.

SUMMARY AND CONCLUSIONS

In 35 human subjects, 6 of whom were healthy and 29 suffering from a variety of diseases, simultaneous measurements were made of the apparent volume of red blood cells, using CO gas and cells tagged with Cr^{51}. The volume of distribution of CO 20 minutes after delivery of the gas was found to be consistently larger than that of the radioactively tagged cells at an equal interval after injection. The mean difference amounted to 16 per cent of the volume as measured with Cr^{51}-tagged cells. It is concluded that in this brief interval, significant quantities of CO leave the blood stream. The CO probably combines rapidly with compounds for which it has high affinity, but the sites where this occurs remain unknown.

Addendum: Recently we have found that better tagging of red cells with Cr^{51} is obtained when A.C.D. solution is used as anticoagulant instead of heparin during the *in vitro* handling of the blood.

ACKNOWLEDGMENT

We wish to acknowledge the able technical assistance of Mrs. Tillie Leake, Miss Vivian Ashford, and Miss Barbara Bradley.

REFERENCES

1. Hahn, L., and Hevesy, G., A method of blood volume determination. Acta physiol. Scandinav., 1940, 1, 3.
2. Reeve, E. B., and Veall, N., A simplified method for the determination of circulating red-cell volume with radioactive phosphorus. J. Physiol., 1949, **108**, 12.
3. Hahn, P. F., Ross, J. F., Bale, W. F., Balfour, W. M., and Whipple, G. H., Red cell and plasma volumes (circulating and total) as determined by radio iron and by dye. J. Exper. Med., 1942, **75**, 221.
4. Gibson, J. G., 2nd, Weiss, S., Evans, R. D., Peacock, W. C., Irvine, J. W., Jr., Good, W. M., and Kip,

A. F., The measurement of the circulating red cell volume by means of two radioactive isotopes of iron. J. Clin. Invest., 1946, **25**, 616.

5. Gray, S. J., and Sterling, K., The tagging of red cells and plasma proteins with radioactive chromium. J. Clin. Invest., 1950, **29**, 1604.
6. Sterling, K., and Gray, S. J., Determination of the circulating red cell volume in man by radioactive chromium. J. Clin. Invest., 1950, **29**, 1614.
7. Smith, F. P., Arnold, H. R., and Whipple, G. H., Blood volume studies. VII. Comparative values of Welcker, carbon monoxide and dye methods for blood volume determinations. Accurate estimation of absolute blood volume. Am. J. Physiol., 1921, **56**, 336.
8. Gibson, J. G., 2nd, Peacock, W. C., Seligman, A. M., and Sack, T., Circulating red cell volume measured simultaneously by radioactive iron and dye methods. J. Clin. Invest., 1946, **25**, 838.
9. Hevesy, G., Köster, K. H. Sørensen, G., Warburg, E., and Zerahn, K., The red corpuscle content of the circulating blood determined by labelling the erythrocytes with radio-phosphorus. Acta med. Scandinav., 1944, **116**, 561.
10. Gregersen, M. I., Effect of circulatory states on determinations of blood volume. Am. J. Med., 1953, **15**, 785.
11. Arnold, H. R., Carrier, E. B., Smith, H. P., and Whipple, G. H., Blood volume studies. V. The carbon monoxide method—its accuracy and limitations. Am. J. Physiol., 1921, **56**, 313.
12. Chang, H. C., and Harrop, G. A., Jr., The determination of circulating blood volume with carbon monoxide. J. Clin. Invest., 1928, **5**, 393.
13. Asmussen, E., On the determination of the blood volume by the CO-method. Acta physiol. Scandinav., 1942, **3**, 156.
14. Hopper, J., Jr., Tabor, H., and Winkler, A. W., Simultaneous measurements of blood volume in man and dog by means of Evans blue dye, T-1824, and by means of carbon monoxide. I. Normal subjects. J. Clin. Invest., 1944, **23**, 628.
15. Brown, E., Hopper, J., Jr., Sampson, J., and Mudrick, C., Venous congestion of the extremities in relation to blood volume determinations and to mixing curves of carbon monoxide and T-1824 in normal human subjects. J. Clin. Invest., 1951, **30**, 1441.
16. Courtice, F. C., and Gunton, R. W., The determination of the blood volume in man by the carbon-monoxide and dye methods. J. Physiol., 1949, **108**, 142.
17. Sjöstrand, T., A method for the determination of the total haemoglobin content of the body. Acta physiol. Scandinav., 1948, **16**, 211.
18. Root, W. S., Allen, T. H., and Gregersen, M. I., Simultaneous determinations in splenectomized dogs of cell volume with CO and P^{32} and plasma volume with T-1824. Am. J. Physiol., 1953, **175**, 233.
19. Haldane, J., and Smith, J. L., The mass and oxygen capacity of the blood in man. J. Physiol., 1900, **25**, 331.
20. Wennesland, R., A new method for determination of carbon monoxide in blood. Acta physiol. Scandinav., 1940, **1**, 49.
21. Nylin, G., and Hedlund, S., Weight of the red blood corpuscles in heart failure determined with labelled erythrocytes during and after decompensation. Am. Heart J., 1947, **33**, 770.
22. Reilly, W. A., French, R. M., Lau, F. Y. K., Scott, K. G., and White, W. E., Whole blood volume determined by radiochromium-tagged red cells. Comparative studies on normal and congestive heart failure patients. Circulation, 1954, **9**, 571.