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THE EFFECT OF CARBON DIOXIDE ON RELATIVE RED CELL VOLUME

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In the determination of relative cell volume by any haematocrit method it is a matter of serious concern from where the blood sample is obtained. It has been claimed that differences in the relative proportions of cells and plasma occur in blood samples taken from arteries, veins or capillaries. It seems likely that these alleged differences are due either to the haemodynamic differences in flow of blood through the various vascular channels or to the carbon dioxide being carried by the blood. The former problem has been investigated by Smith, Arnold & Whipple (1921), Fåhraeus & Lindquist (1931), Ebert & Stead (1941), Stead & Ebert (1941), Hahn, Ross, Bale, Balfour & Whipple (1942), Root, Roughton & Gregersen (1946), Gibson, Peacock, Seligman & Sack (1946), Gibson, Seligman, Peacock, Aub, Fine & Evans (1946), Courtice & Gunton (1949) and Eifert (1951), producing divergent results.

Schmidt (1867), Nasse (1878), von Limbeck (1894) and Hamburger (1902) first recorded increases in erythrocyte volume, as measured by the haematocrit or specific gravity changes, in the presence of high CO_2 tensions. Further work by Joffe & Poulton (1920), Doisy & Eaton (1921), Doisy & Beckmann (1922), Mellanby & Wood (1922), Warburg (1922), Smirk (1928) and Berk (1945), using haematocrit methods, has tended to confirm these results, though the CO_2 tensions used in some of these investigations have been above the normal physiological level, and in others the cells have been washed and suspended in isotonic sodium chloride. Van Slyke, Wu & McLean (1923), using gravimetric and specific gravity methods to determine water loss from the plasma, and Henderson, Bock, Field & Stoddard (1924), using increase in refractive index as evidence of water loss, showed indirectly that there was an increase in relative cell volume on rise in CO_2 content and fall in pH of the blood; though as Henderson *et al.* point out the differences observed over the physiological range were of doubtful significance.

In this investigation an attempt has been made to measure the amount of

erythrocyte swelling in whole blood, *in vitro*, which occurs over a wide range of CO_2 tensions, and from this to predict the magnitude of changes occurring over the physiological range.

METHODS

Ox, sheep, rabbit and human bloods have been used, the anticoagulant being either 30% potassium oxalate, with a final concentration in the blood of 0·3%, or the double oxalate mixture of Heller & Paul (1933), with a final concentration in the blood of 0·2%. The blood was collected on the day of the experiment and stored at 4–6° C until required. 5–10 ml. blood samples in 150 ml. Douglas-type tonometers were equilibrated for 30 min while being rotated in a water-bath at 37° C. The gas mixtures used were a range of concentrations of CO_2 in air. In order to ascertain the effect of the state of oxygenation, blood was also equilibrated in tonometers containing room air or approximately 100% CO_2, O_2, and N_2.

Blood samples were withdrawn and placed under liquid paraffin, Meyerstein haematocrit samples were taken immediately (Meyerstein, 1942; Gilding, Meyerstein & Nutt, 1949; Jackson & Nutt, 1950). Estimations of the CO_2 content of the blood were made with as little delay as possible, using the Van Slyke manometric apparatus, and the residual gas from the tonometer tubes was analysed by the Haldane Gas Analysis apparatus.

RESULTS

CO_2 *range experiments*

Ox. Experiments were performed on blood of seven different animals, six samples being taken from each. The results from two of these animals are tabulated in detail, and those from the remainder are summarized in Table 1. Over a range of 13–700 mm Hg partial pressure of CO_2, with an increase of approximately 100 ml. in the CO_2 content of the blood, there was an increase in relative cell volume of 5–7%. If these increases in relative cell volume are graphed against the ml. CO_2/100 ml. blood or against the log. partial pressure of CO_2 there is an approximately linear relationship. Over the physiological range of 40–60 ml. CO_2/100 ml. blood the relative cell volumes would not differ by more than 1%, i.e. about 0·4 of a haematocrit unit. Since the S.D. of Meyerstein haematocrit values at 4000 *g* (i.e. 5000 rev/min in a centrifuge radius 15 cm) is 0·2–0·3 of a haematocrit unit, the differences observed are not statistically significant.

Sheep. Blood of nine different animals was investigated, the results of two animals being tabulated in full and the remainder summarized in Table 1. Over a range of 6–700 mm Hg partial pressure of CO_2, with an increase of approximately 120 ml. in the CO_2 content of the blood, there was an increase of 3–7% in relative cell volume. The findings thus closely resemble those of the ox experiments.

Rabbit. Blood of seven different animals was investigated, the results of two being given in full and of the remainder summarized in Table 1. Over a range of 5–700 mm Hg partial pressure of CO_2, with an increase of between 100 and 120 ml. in the CO_2 content of the blood, the relative cell volume increased by 8–13%. The relative cell volume increase is therefore about twice that

observed for ox and sheep bloods over the same CO_2 tension range, but is not statistically significant over the physiological range.

TABLE 1. Effect of CO_2 on relative cell volume of ox, sheep and rabbit bloods

Blood	Sample	Partial pressure of CO_2 (mm Hg)	ml. CO_2/100 ml. blood	Cell volume (%)
Ox 2	1	14·80	36·30	37·50
	2	40·80	50·55	37·63
	3	49·20	54·25	37·85
	4	59·60	58·80	37·98
	5	80·20	64·50	38·35
	6	654·0	137·20	39·84
Ox 6	1	14·80	19·39	39·73
	2	31·0	26·83	39·74
	3	47·80	35·24	39·85
	4	59·40	39·62	39·84
	5	83·0	46·76	39·98
	6	525·0	121·86	41·76
Ox 1, 3–5 and 7	Means	14·35	30·91	36·35
		679·3	135·37	38·47
Sheep 3	1	12·30	34·10	36·70
	2	37·60	49·0	36·68
	3	47·30	55·20	37·03
	4	53·20	60·15	37·11
	5	67·20	64·85	37·16
	6	663·0	144·90	38·82
Sheep 5	1	13·40	29·40	38·09
	2	39·70	48·05	38·29
	3	47·20	50·05	38·66
	4	52·0	53·05	38·70
	5	62·25	58·40	39·11
	6	676·0	132·20	40·50
Sheep 1, 2, 4 and 6–9	Means	11·54	30·99	38·36
		660·50	140·85	40·63
Rabbit 4	1	6·0	10·03	31·78
	2	42·80	31·0	32·44
	3	50·80	34·20	32·55
	4	54·75	35·10	32·90
	5	60·0	36·38	32·55
	6	689·0	122·10	35·73
Rabbit 6	1	9·80	28·58	35·11
	2	32·70	46·70	35·69
	3	48·80	55·60	36·16
	4	61·0	61·19	36·49
	5	80·50	67·53	36·56
	6	675·0	151·70	38·88
Rabbits 1–3, 5 and 7	Means	7·44	17·98	32·32
		676·10	129·14	35·25

Human. Experiments were performed on samples from four subjects, and the results are summarized in Table 2. Over a range of CO_2 tensions from 10 to 655 mm Hg partial pressure, with an increase of approximately 120 ml. in the CO_2 content of the blood, the relative cell volume increased by 6–9%. These increases being similar to, though slightly more than, those for ox and sheep bloods, and slightly less than those for rabbit blood.

Lines have been fitted by the method of least squares to show the regression of the haematocrit readings on each of the variables studied, i.e. ml. CO_2/100 ml. blood and log. of the partial pressure of CO_2, in the human experiments and are shown in Figs. 1 and 2 respectively. The general equation for the lines in Fig. 1 is

$$\hat{Y} = a_1 + b_1 X_1,$$

where a_1 = the intercept, b_1 = the slope, X_1 = ml. CO_2/100 ml. blood and $\hat{Y}$ = the haematocrit value. Similarly, the general equation for the lines in Fig. 2 is

$$\hat{Y} = a_2 + b_2 X_2,$$

where a_2 = the intercept, b_2 = the slope, X_2 = log. of the partial pressure of CO_2 in mm Hg and $\hat{Y}$ = the haematocrit value. Values for a_1, b_1, a_2 and b_2 in the four experiments are shown in Table 3.

TABLE 2. Effect of carbon dioxide on relative cell volume of human blood

	Number of sample											
	1	2	3	4	5	6	7	8	9	10	11	12
	M.E.N., 31. iii. 52, 1. iv. 52											
Partial pressure of CO_2 mm Hg	11·0	12·7	109	127	181	216·5	291·0	359	421	504	575	655
ml. CO_2/100 ml. blood	29·4	32·0	65·2	76·8	87·9	92·0	99·9	109·5	118·5	123·0	129·0	135·2
% cells	33·34	33·25	34·98	34·99	35·08	35·18	35·45	35·91	35·65	36·03	35·67	36·23
	D.M.J., 8. iv. 52											
Partial pressure of CO_2 mm Hg	14·3	33·7	37·2	47·4	50·3	53·8	58·1	60·0	67·4	586·3	—	—
ml. CO_2/100 ml. blood	31·2	42·0	45·4	48·0	49·8	51·4	53·9	55·7	57·9	129·8	—	—
% cells	36·74	37·18	37·53	37·58	37·64	37·78	37·93	37·65	37·80	39·51	—	—
	H.P.G., 1. v. 52, 2. v. 52											
Partial pressure of CO_2 mm Hg	9·7	20·4	37·8	41·8	52·5	56·8	68·0	74·5	78·7	81·0	83·6	643·0
ml. CO_2/100 ml. blood	25·7	42·0	43·2	44·6	50·8	52·6	55·6	59·4	60·5	61·7	61·9	138·1
% cells	41·20	41·63	41·71	41·53	42·03	41·95	41·85	41·83	42·13	42·30	42·66	44·28
	M.N.N., 6. v. 52, 7. v. 52											
Partial pressure of CO_2 mm Hg	13·4	34·8	47·0	53·3	74·4	82·1	83·5	107·3	149·0	289·5	322·0	611·0
ml. CO_2/100 ml. blood	30·38	41·6	48·8	51·2	59·8	58·4	68·1	71·4	80·5	99·4	102·3	146·5
% cells	45·61	45·95	46·39	46·28	46·23	—	46·78	46·75	47·20	47·63	47·43	48·48

TABLE 3. Values of a_1, b_1. a_2 and b_2 obtained in four experiments for the lines $\hat{Y} = a_1 + b_1 X_1$ and $\hat{Y} = a_2 + b_2 X_2$ shown in Figs. 1 and 2

	a_1	b_1	a_2	b_2
M.E.N.	32·77	0·02595	31·62	1·5804
D.M.J.	36·28	0·02569	34·70	1·7347
H.P.G.	40·48	0·02782	39·12	1·6806
M.N.N.	45·0	0·02466	43·45	1·6855

When the experimental points are plotted in relation to each calculated line the agreement is within the experimental errors of the method. Lines could be fitted in a similar manner to the results of the ox, sheep and rabbit experiments.

Effect of O_2 *saturation on relative cell volume*

Results have been obtained on blood samples from sheep, and on rabbit and human bloods, exposed to high tensions of O_2, N_2 and CO_2; these are summarized in Table 4. Only in the cases of bloods exposed to high CO_2 tensions was there any significant difference in relative cell volume.

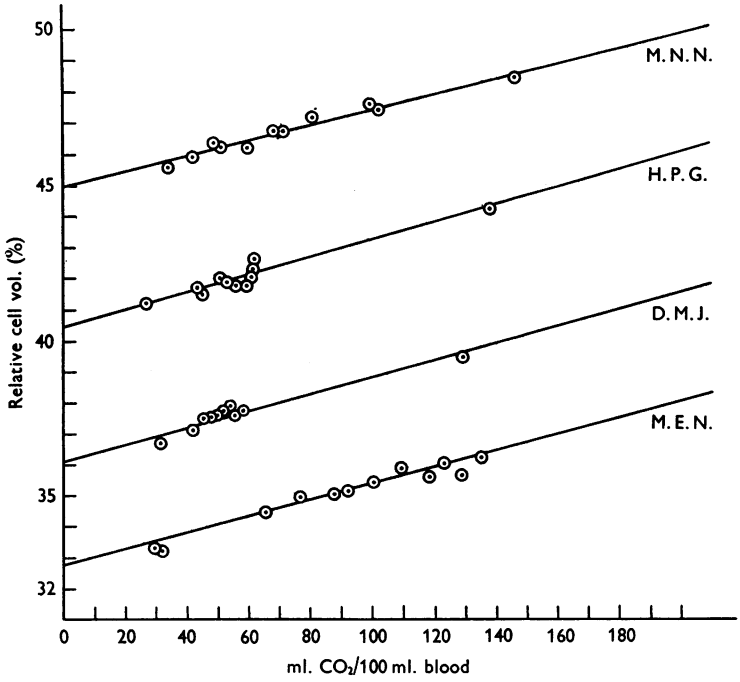

Fig. 1. Regression of relative cell volume on blood CO_2 content in four human subjects.

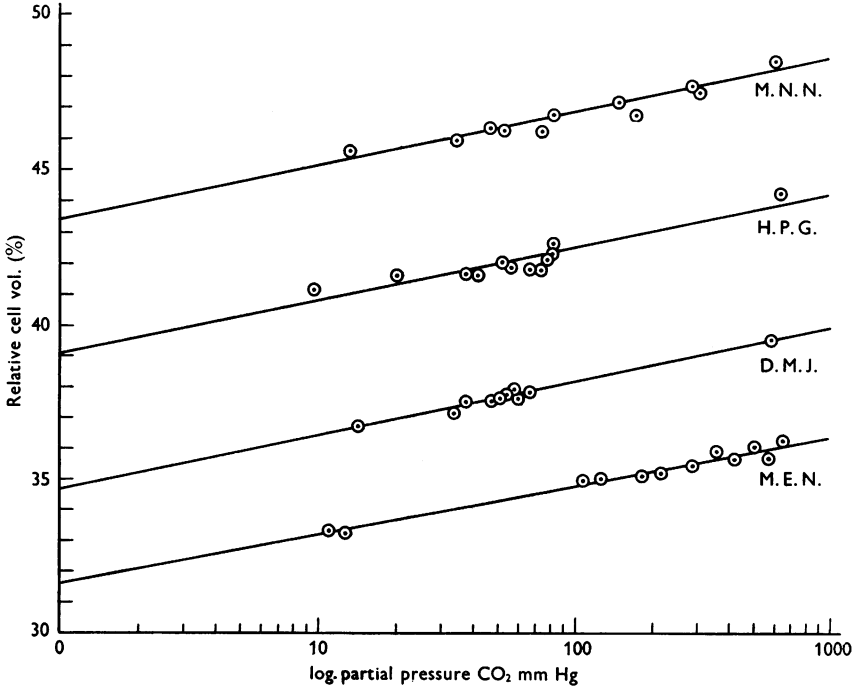

Fig. 2. Regression of relative cell volume on logarithm of blood CO_2 pressure in four human subjects.

TABLE 4. Comparison of the effect of high CO_2, O_2 and N_2 tensions on relative cell volume

Species	% cells	CO_2 %	O_2 %	N_2 %	Anticoagulant
Rabbit					
30. i. 52	34·03	91·90	2·40	5·70	Heparin
	29·65	1·20	85·25	13·55	
	29·90	1·06	7·76	91·18	
	30·03	1·23	20·38	78·39	
19. iii. 52	27·90	80·20	4·78	15·02	K oxalate
	27·80	83·70	3·97	12·33	
	25·40	1·13	1·79	97·08	
	25·45	0·99	20·61	78·40	
16. x. 52	30·70	95·20	1·41	3·39	Double oxalate
	28·54	1·16	92·65	6·19	
	28·55	0·68	1·88	97·44	
	28·38	1·55	19·90	78·55	
Sheep					
17. xii. 52	46·63	91·90	2·0	—	Double oxalate
	44·73	1·62	96·89	1·49	
	44·50	1·99	11·66	86·35	
	44·38	1·90	20·0	78·0	
Human					
31. iii. 52	35·67	82·90	3·84	13·26	K oxalate
	33·30	2·39	88·0	9·61	
	33·50	1·40	1·29	97·31	
	33·25	1·84	19·50	78·66	

TABLE 5. Effect of time of equilibration with CO_2 on relative cell volume

Species	Equilibration time (min)	% cells, exposed to 100% CO_2	% cells, exposed to Room air
Ox			
28. xi. 53	5	44·68	—
	10	44·96	—
	20	45·20	—
	30	45·28	—
	40	44·33	—
	120	—	42·31
Rabbit			
28. vii. 52	5	32·96	31·01
	10	33·88	31·63
	30	33·54	30·93
29. vii. 52	5	33·80	—
	20	34·48	31·73
	45	35·01	31·33
	60	35·44	31·15
1. viii. 52	5	32·06	—
	10	32·48	29·26
	20	32·38	—
20. viii. 52	5	27·83	24·35
	10	27·60	—
	20	27·48	—
	30	27·88	—
	93	—	24·98
21. viii. 52	5	27·63	24·73
	30	28·53	—
	40	28·13	—
	60	28·85	25·50

Effect of equilibration time on relative cell volume

Results summarized in Table 5 were obtained in experiments on ox and rabbit blood samples, and suggest that equilibration times from 5 to 40 min produce a similar effect on relative cell volume, but a slight increase is evident in two experiments on rabbit blood equilibrated for 1 hr with 100% CO_2 at 37° C.

DISCUSSION

The bloods of all species investigated showed the same general tendencies, i.e. an increase in relative erythrocyte volume proportional to the CO_2 content of each particular blood sample. Over the range 40–60 ml. CO_2/100 ml. blood, i.e. the extreme range of CO_2 content of the blood *in vivo*, there is no statistically significant change in relative cell volume as measured by the Meyerstein haematocrit in these *in vitro* experiments.

In general the results confirm those of earlier investigators who used direct measurements of relative cell volume, though the diversity of methods used makes comparisons difficult. The work of Joffe & Poulton (1920) most nearly corresponds with the present experiments on human blood. Using oxalated or defibrinated samples it was found that partial pressures of CO_2 up to 90 mm Hg did not produce any significant increase in relative cell volume and only a small volume increase thereafter. Doisy & Eaton (1921) recorded results on ox blood comparable with those of the present investigation. Over a range of 3–100% CO_2, the relative cell volume increased by 6·0–8·5%.

Smirk (1928), using human blood with potassium oxalate as anticoagulant, found that little change in relative cell volume occurred when venous blood was oxygenated by exposure to room air, but when exposed to 50% CO_2 in air relative cell volume increased by approximately 10% and by 12% on exposure to 100% CO_2 at 17° C. These results agree with the single experiment of Mellanby & Wood (1922) on defibrinated sheep blood, in which no significant difference was found in the relative cell volume when exposed to room air and alveolar air, but a difference of about 12% in relative cell volume after CO_2 saturation. Warburg (1922), using defibrinated or hirudinized horse blood, found similar, though slightly larger, relative cell volume changes over a similar range of CO_2 tensions.

Van Slyke *et al.* (1923) and later Henderson *et al.* (1924) avoided the use of an inaccurate haematocrit by substituting estimations of plasma water loss by gravimetric and specific gravity methods and by refractive index measurements respectively. From these results relative cell volume changes over the physiological range were deduced. However, as Ponder (1948) points out: 'Variations in the values obtained indicate that these methods were being used at the limit of their accuracy and have not been used to measure water shifts

over a wide range of CO_2 tensions.' Further he states that: 'Taken by themselves as they were obtained, the values for the water shift would never suggest that they are the accompaniment of ion exchanges between ideal solutions separated by a cation impermeable membrane and it is only by the selection of one value and the rejection of another that they have been made to support this conception.'

Reference to Henderson's familiar nomogram relating relative cell volume, total CO_2, CO_2 tension, pH, etc., in a specimen of human blood (blood of A.V.B.) reveals that for an increase of 5 ml. CO_2/100 ml. blood (i.e. from 48–53 ml./100 ml.) the relative cell volume increases by approximately 1%. This is a much greater effect than that observed in any of the four human experiments reported here, where increases of 9–17 ml. CO_2/100 ml. blood were required to produce a 1% increase in relative cell volume. It is noteworthy that, in line with the results of Van Slyke *et al.* on horse blood, the degree of swelling is related to the relative cell volume of the blood and is greatest where the ratio cells/plasma is least. However, there seems to be some species difference in this respect, since human and rabbit red cells swell more than those of ox or sheep.

Smirk (1928) found that exposure times to CO_2 of 30 min to 21 hr did not cause a significant change in the amount of swelling of oxalated human and defibrinated sheep bloods. This work on sheep blood disagrees with that of Mellanby & Wood (1922), who observed the maximum corpuscular swelling 5 min after saturation with CO_2 followed by a marked decrease within 2 hr; these changes were attributed to changes in red cell membrane permeability. However, it is not clear whether precautions were taken to prevent CO_2 loss from the blood after the preliminary saturation. Present experimental results on ox and rabbit bloods confirm those of Smirk and suggest that equilibration time is of little importance.

Under the experimental conditions used, CO_2 was mixed with room air to provide the range of CO_2 tensions; thus there was a decrease in O_2 tension with a rise in CO_2 tension. However, it can be seen from Table 4 that changes in O_2 tension are in themselves without effect on relative cell volume, though normally any decrease in oxygenation would be accompanied by an increase in CO_2. This also applies to the experiments of Warburg (1922) where changes in O_2 saturation were accompanied by changes in CO_2 tension. In the experiments recorded above, change in CO_2 tension was therefore the only factor which caused a change in relative cell volume, and a significant change in relative cell volume was only caused by CO_2 tensions above the normal physiological maximum. It seems reasonable, therefore, to conclude that changes in relative cell volume in arterial, capillary and venous blood samples recorded are due to factors other than changes in O_2 and CO_2 content.

Work on arterial, capillary and venous bloods with special reference to CO_2 content and plasma chlorides was performed by Doisy & Beckmann (1922).

The slight increases recorded in eighteen out of twenty-two experiments performed may well have been within the error of their relative cell volume determinations. More recently, Eifert (1951) found the relative cell volumes of capillary and venous human blood to be similar in ninety-seven cases, greater in capillary than venous blood in five cases and greater in venous than capillary blood in thirteen cases. In only one subject was the arterial blood significantly different from the capillary and venous samples. Other workers have noted differences, however, in the cell/plasma ratio of arterial, venous and capillary bloods and in the whole body in conjunction with blood volume determinations. Further work *in vivo* is being undertaken with these questions in mind.

SUMMARY

1. The effect of a range of CO_2 tensions from 5 to 700 mm Hg in room air on relative red cell volume, as measured by the Meyerstein haematocrit, was studied.

2. In the first series twenty-seven separate bloods were investigated, seven from ox, nine from sheep, seven from rabbits and four from humans.

3. Red cells of all species investigated swelled with increasing CO_2 tensions up to 7% of the cell volume in room air in ox and sheep, up to 13% in rabbit and up to 9% in human bloods.

4. Over the limits of the physiological range, i.e. from 40–60 ml. CO_2/100 ml. blood there was no significant change in relative cell volume.

5. A second series of experiments was performed exposing sheep, rabbit and human bloods to approximately 100% CO_2, O_2 and N_2. The high CO_2 mixture alone caused a significant change in relative cell volume. Therefore the red cell swelling observed throughout this work may be assumed to be due to CO_2 itself and not to changes in the state of oxygenation of the particular blood sample.

6. A third series of experiments, in which the effect of CO_2 saturation for times from 5 to 60 min was studied, showed no significant increase in the degree of swelling of the cells up to 40 min equilibration and only a slight increase thereafter.

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REFERENCES

BERK, L. (1945). Importance of standardizing oxygen content of blood in haematocrit determinations. *S. Afr. J. med. Sci.* **10**, 95–98.

COURTICE, F. C. & GUNTON, R. W. (1949). The determination of blood volume by the carbon monoxide and dye (T-1824) methods in rabbits. *J. Physiol.* **108**, 405–417.

DOISY, E. A. & BECKMANN, J. W. (1922). The relations existing between arterial and venous blood of the dog, with special reference to the plasma chlorides. *J. biol. Chem.* **54**, 683–691.

DOISY, E. A. & EATON, E. P. (1921). The relation of the migration of ions between cells and plasma to the transport of carbon dioxide. *J. biol. Chem.* **47**, 377–393.

EBERT, R. V. & STEAD, E. A. (1941). Demonstration that the cell plasma ratio of blood contained in minute vessels is lower than that of venous blood. *J. clin. Invest.* **20**, 317–321.

EIFERT, M. (1951). Das relative Erythrocytenvolumen des capillären, venösen und arteriellen Blutes. *Z. klin. Med.* **147**, 437–442.

FÅHRAEUS, R. & LINDQUIST, T. (1931). The viscosity of the blood in narrow capillary tubes. *Amer. J. Physiol.* **96**, 562–568.

GIBSON, J. G., 2nd, PEACOCK, W. C., SELIGMAN, A. M. & SACK, T. (1946). Circulating red cell volume measured simultaneously by the radioactive iron and dye methods. *J. clin. Invest.* **25**, 838–847.

GIBSON, J. G., 2nd, SELIGMAN, A. M., PEACOCK, W. C., AUB, J. C., FINE, J. & EVANS, R. D. (1946). The distribution of red cells and plasma in large and minute vessels of the normal dog, determined by radioactive isotopes of iron and iodine. *J. clin. Invest.* **25**, 848–857.

GILDING, H. P., MEYERSTEIN, W. & NUTT, M. E. (1949). An instrument for accurate reading of an improved Meyerstein haematocrit tube. *J. Physiol.* **108**, 32*P*.

HAHN, P. F., ROSS, J. F., BALE, W. F., BALFOUR, W. M. & WHIPPLE, G. H. (1942). Red cell and plasma volumes (circulating and total) as determined by radio iron and by dye. *J. exp. Med.* **75**, 221–232.

HAMBURGER, H. J. (1902). *Osmotischer Druck und Ionenlehre in den medicinischen Wissenschaften*, **1**, p. 442. Wiesbaden: J. F. Bergmann.

HELLER, V. G. & PAUL, H. (1933). Changes in cell volume produced by varying concentrations of different anticoagulants. *J. Lab. clin. Med.* **19**, 777–780.

HENDERSON, L. J., BOCK, A. V., FIELD, H. JR. & STODDARD, J. L. (1924). Blood as a physico-chemical system. *J. biol. Chem.* **59**, 379–431.

JACKSON, D. M. & NUTT, M. E. (1950). The accuracy of the Meyerstein haematocrit. *J. Physiol.* **111**, 150–159.

JOFFE, J. & POULTON, E. P. (1920). The partition of carbon dioxide between plasma and corpuscles in oxygenated and reduced blood. *J. Physiol.* **54**, 129–151.

VON LIMBECK, R. (1894). Ueber den Einfluss des respiratorischen Gaswechels auf die rothen Blutkörperchen. *Arch. exp. Path. Pharmak.* **35**, 309–334.

MELLANBY, J. & WOOD, C. C. (1922). The influence of carbon dioxide on the interchange of ions between the corpuscles and the serum of blood. *J. Physiol.* **57**, 113–128.

MEYERSTEIN, W. (1942). Simple sealed haematocrit tube. *J. Physiol.* **101**, 5*P*.

NASSE, H. (1878). Untersuchungen über den Austritt und Eintritt von Stoffen (Transsudation und Diffusion) durch die Wand der Haargefässe. *Pflüg. Arch. ges. Physiol.* **16**, 604–634.

PONDER, E. (1948). *Hemolysis and Related Phenomena*, p. 112. London: Churchill.

ROOT, W. S., ROUGHTON, F. J. W. & GREGERSEN, M. I. (1946). Simultaneous determinations of blood volume by carbon monoxide and dye (T-1824) under various conditions. *Amer. J. Physiol.* **146**, 739–755.

SCHMIDT, A. (1867). Ueber die Kohlensäure in den Blutkörperchen. *Ber. sächs. Ges. (Akad.) Wiss.* **19**, 30–54. Cited by Henderson, L. J. in *Blood. A Study in General Physiology*, 1928, p. 92. New Haven: Yale University Press.

SMIRK, F. H. (1928). The accurate measurement of the proportion of corpuscles and serum in blood. II. The volume of red blood corpuscles in venous and oxygenated blood after exposure to various saturations of carbon dioxide. *Brit. J. exp. Path.* **9**, 81–89.

SMITH, H. P., ARNOLD, H. R. & WHIPPLE, G. H. (1921). Blood volume studies. VII. Comparative values of Welcker, carbon monoxide and dye methods for blood volume determinations. Accurate estimation of absolute blood volume. *Amer. J. Physiol.* **56**, 336–360.

STEAD, E. A. & EBERT, R. V. (1941). Relationship of the plasma volume and the cell plasma ratio to the total red cell volume. *Amer. J. Physiol.* **132**, 411–417.

VAN SLYKE, D. D., WU, H. & MCLEAN, F. C. (1923). Studies of gas and electrolyte equilibria in the blood. V. Factors controlling the electrolyte and water distribution in the blood. *J. biol. Chem.* **56**, 765–849.

WARBURG, E. J. (1922). Studies on carbonic acid compounds and hydrogen-ion activities in blood and salt solutions; a contribution to the theory of the equation of Lawrence J. Henderson and K. A. Hasselbalch. *Biochem. J.* **16**, 153–340.