

THE EFFECTS OF POSTURE ON THE CIRCULATING BLOOD VOLUME.

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THOMPSON, Thompson, and Dailey [1928] made the discovery that if an individual remained stationary in the erect posture his total plasma volume gradually diminished for about half an hour when it reached a constant level approximately 12 p.c. less than in the recumbent posture. Further the hæmatocrit reading rose in such a way as to indicate that the total cell volume remained unaffected. Closely parallel with these changes was an increase in the red cell count, the specific gravity of the blood and the concentration of the plasma protein, together with a diminution in the percentage of plasma water. Their figures indicated that in the erect posture there was an absolute diminution in the amount of water and diffusible substances in the circulation; and they suggested that the loss was the result of a filtration of these substances through the capillary walls of the lower extremities owing to an increase in the erect posture of the hydrostatic pressure within them.

The blood volumes in Thompson's experiments were determined by the dye-method of Keith, Rowntree and Geraghty. The present paper gives results in a similar investigation, in which, however, the carbon-monoxide method was used for blood-volume determinations.

METHOD.

The modification of Haldane's carbon-monoxide method due to Chang and Harrop [1928] was employed and their technique followed closely.

After a light lunch¹ the subject lay on a couch for forty-five minutes.

¹ The blood volume was determined immediately before lunch on a subject who had starved since the previous night, and again two hours later, one hour following a heavy lunch. The close agreement between the two figures indicates that even a large meal has no appreciable effect on the blood volume.

	Whole blood	Cells	Plasma
Fasting before lunch	5260 c.c.	2230 c.c.	3030 c.c.
One hour after heavy lunch	5300 c.c.	2250 c.c.	3050 c.c.

Blood was then taken from a vein, under oil into a bottle containing sodium oxalate, for hæmatocrit, plasma protein, and carbon-monoxide estimations. Still lying down the subject rebreathed a measured quantity of CO for twenty minutes when a second sample of blood was taken for CO determination. He then stood stationary for forty minutes, partially supported by arm rests and with his legs as far as possible relaxed. Blood was again taken for hæmatocrit, protein and CO determinations. Finally another measured quantity of CO was inhaled and, after twenty minutes rebreathing, the fourth sample was taken for CO determination.

RESULTS.

The results in general confirmed those of Thompson and his associates, though certain differences were found which may be due to the difference in the method used. While Thompson with the dye

TABLE I. Changes depending on posture in whole blood, plasma, and cell volume for eight normal subjects.

Case No.	Age Year	Height cm.	Weight kg.	Ideal weight kg.	Whole blood volume				Plasma volume					
					Horizontal posture c.c.	Vertical posture c.c.	Change c.c.	Change p.c.	Horizontal posture c.c.	Vertical posture c.c.	Change c.c.	Change p.c.		
1	39	169	74	70	4330	4110	220	5.1	2490	2300	190	7.6		
2	38	167	75	70	4850	4040	810	16.7	2760	2140	620	22.4		
3	28	181	61.8	78	5280	4660	620	11.7	2980	2420	560	18.8		
4	27	160	—	61	4850	4470	380	7.8	2630	2280	350	13.3		
5	35	171	—	72	4980	4620	360	7.2	2780	2490	290	10.4		
6	23	189	74.1	82.5	5750	5080	670	11.6	3390	2900	490	14.5		
7	29	184	72.3	81	5780	5080	700	13.8	3290	2720	570	17.3		
8*	29	178	70	74.5	6130	5800	330	5.4	3520	3190	330	9.4		
Averages					5244	4732	512	9.9	2980	2555	425	14.3		
Case No.	Age Year	Height cm.	Weight kg.	Ideal weight kg.	Cell volume				Volume c.c. per kg. ideal weight					
					Horizontal posture c.c.	Vertical posture c.c.	Change c.c.	Change p.c.	Blood volume		Plasma volume		Cell volume	
1	39	169	74	70	1840	1810	30	1.6	61.8	58.7	35.6	32.9	26.3	25.9
2	38	167	75	70	2090	1900	190	9.1	69.4	57.7	39.4	30.6	29.9	27.2
3	28	181	61.8	78	2300	2240	60	2.6	67.6	59.7	38.2	31.0	29.5	28.7
4	27	160	—	61	2220	2190	30	1.4	79.5	73.3	43.1	37.4	36.4	35.9
5	35	171	—	72	2200	2130	70	3.2	69.2	64.2	38.6	34.6	30.8	29.6
6	23	189	74.1	82.5	2360	2190	170	7.2	69.9	61.6	41.1	35.1	28.6	26.5
7	29	184	72.3	81	2490	2360	130	5.2	71.4	62.7	40.6	33.6	30.7	29.1
8*	29	178	70	74.5	2610	2610	0	0	82.3	77.8	47.2	42.8	34.9	34.9
Averages					2264	2179	85	3.8	71.4	64.5	40.5	34.7	30.9	29.7

* In this case vertical determination done on a different day from that of horizontal determination. It is the only one showing no change in cell volume. The very high volume figures may be associated with a considerable degree of varicose veins.

method found a loss of plasma volume amounting to 12 p.c., I obtained a loss of nearly 15 p.c. with the gas method. While they found that the cell volume underwent no change, I found a diminution of about 4 p.c.

in the cell volume. Finally, whereas Thompson obtained figures indicating that none of the plasma protein disappeared from the circulation in the erect posture, my results require that over half of the protein is lost with the rest of the plasma. [Compare Tables II and IV, and III and IV.]

A possible explanation of this diminution in cell volume in the erect posture is as follows. The increase in plasma protein percentage (which we both find) results in an increase in the plasma-osmotic pressure and therefore in a withdrawal of water from the cells with their consequent shrinkage.

TABLE II. Changes in *percentage* cell volume indicating a diminution in *total* cell volume in the erect posture.

Case No.	Haematocrit p.c. cell volume			Obs. loss cell vol.	Obs. p.c. loss plasma vol.	Calc.† p.c. loss plasma vol.	Obs. p.c. loss plasma vol.
	Horiz.	Vert.	Calc.* vert.				
1	42.5	44.0	44.4	.992	1.6	6.0	7.6
2	43.0	47.2	49.1	.962	9.1	15.6	22.4
3	43.5	48.0	48.7	.986	2.6	16.5	18.8
4	45.8	49.0	49.4	.992	1.4	12.1	13.3
5	44.2	46.0	46.9	.980	3.2	7.1	10.4
6	41.0	43.0	44.8	.960	7.2	7.9	14.5
7	43.0	46.5	47.1	.988	5.2	13.0	17.3
Averages	43.2	46.1	47.2	.980	4.3	11.2	14.9

* Calculated, on assumption that total cell volume remains constant, from observed change in plasma volume.

† Calculated, on assumption that total cell volume remains constant, from observed change in percentage cell volume.

As regards the fact that over half the protein was found to filter through the capillary wall with the rest of the plasma, the following observations are of interest. Lymph from the thoracic duct of anæsthetized dogs [Smith, 1925], and of normal human subjects obtained under certain unusual circumstances [Hammarssten, 1900] has been found to contain protein in about 50 or 60 p.c. of its concentration in the plasma. Further I determined the albumin-globulin ratio in the recumbent and erect postures in two cases of nephrosis and in one normal subject. In all three the increase in the erect posture in the albumin percentage was negligible, though in the globulin percentage very considerable. These observations suggest that the capillary wall is only partially impermeable to plasma protein, and further that its impermeability is more or less limited to the globulin fraction.

In Table III, therefore, the loss of plasma fluid on two different hypotheses has been calculated from the observed changes in protein concentration. In one, following Thompson, I have assumed the capillaries to be completely impermeable to protein; in the other, taking

TABLE III.* Showing that the observed change in plasma volume is more closely parallel to that calculated on the assumption that albumin only is diffusible than to that calculated on the assumption that none of the protein is diffusible.

Case no.	Total protein			Obs. calc.†	Obs. calc.‡	Calc.§ p.c. loss plasma	Calc. p.c. loss plasma	Obs. p.c. loss plasma
	Horiz.	Vert.	Calc.† vert.					
1	730	743	790	.94	.97	1.8	4.6	7.6
3	577	616	711	.87	.95	6.3	14.5	18.8
4	524	579	604	.96	1.09	9.5	20.7	13.3
5	682	714	761	.94	1.00	4.5	10.5	10.4
6	656	727	766	.95	1.09	9.7	21	14.4
7	668	720	807	.89	.99	7.4	16.3	17.3
3b*	656	674	706	.95	.99	2.7	6.5	7.1
Averages	642	682	735	.93	1.01	6.0	13.4	12.7

* 3b same subject as 3: the observations 3b were made after standing erect only for ten minutes.

† Calculated, on assumption that all the plasma protein is indiffusible, from observed plasma loss.

‡ Calculated, on assumption that globulin alone is indiffusible, from observed plasma loss.

§ Calculated, on assumption that all the plasma protein is indiffusible, from observed change in protein concentration.

|| Calculated, on assumption that globulin alone is indiffusible, from observed change in protein concentration.

an average albumin-globulin ratio of 60:40, I have assumed that the globulin alone was held back by the capillary wall. It is seen that the figures calculated on the second hypothesis are in better agreement with the *observed* loss of plasma fluid.

TABLE IV. Thompson's figures for hæmatocrit readings and total plasma protein correlated with his diminution in plasma volume.

Case no.	Hæmatocrit p.c. cell volume			Vert. obs. calc.	Total protein				
	Horiz.	Vert.	Calc.* vert.		Horiz.	Vert.	Calc.† vert.	Obs. calc.	Obs. p.c. loss plasma
1	44.6	49.6	48.8	1.02					15.5
2	45.9	48.8	49.0	1.00					11.9
3	40.1	44.2	43.0	1.03	7.2	8.3	8.1	1.02	11.2
4	35.6	38.1	38.6	0.99	6.9	8.2	7.9	1.04	12.0
5	48.0	50.9	51.7	0.98	7.0	7.8	8.1	0.96	13.6
6	28.1	30.2	30.2	1.00	6.5	7.9	7.2	1.10	9.8
Averages	40.4	43.6	43.6	1.00	6.9	8.1	7.8	1.03	12.3

* Calculated on assumption that total cell volume is unchanged, from observed plasma loss.

† Calculated on assumption that all the plasma protein is indiffusible, from observed plasma loss.

In Table IV are reproduced for comparison the hæmatocrit and protein

figures obtained in Thompson's experiments. It must be emphasized that the reason these conclusions differ in regard to the postural effect on the total cell volume and on the total amount of circulating protein, has nothing to do with the respective hæmatocrit and protein estimations. These, taken by themselves, are in agreement. The divergence only arises when we calculate *absolute* changes from the observed *percentage* changes, and so is entirely due to our getting divergent values for the postural change in plasma volume.

Before discussing this difference in the postural change in plasma volume as determined by the dye and gas methods, I must refer to an investigation published elsewhere in the current number of this *Journal* [Waterfield, 1931]. In their paper Thompson and his associates suggested that the postural changes in the volume and composition of the blood, which they observed, were due in the erect posture to a filtration of plasma fluid into the lymph-spaces of the lower extremities, and in the recumbent posture to a diffusion of it back again into the capillaries. In order to test this experimentally an apparatus was constructed for measuring changes in the volume of the leg. In the second paper is given the evidence for believing that there is a swelling of the leg in the erect posture due to a "physiological œdema," the quantity of which is of the same order of magnitude as the quantity of plasma fluid lost from the circulation. The truth of Thompson's theory will therefore be assumed in what follows.

To explain the fact that with the carbon-monoxide method I obtain a greater postural change in plasma volume than Thompson does with the dye method, it is only necessary to assume the validity of an old and oft-made criticism of the dye method: that there is a partial diffusion of the dye into the lymph-spaces, that consequently the dye method includes in "the blood volume" a certain percentage of the lymph volume. Smith [1925], working at this Hospital, confirmed the work of others by showing that the loss of dye from the circulation, after the lapse of four minutes following its injection, took place so slowly that no appreciable error was introduced thereby into the blood-volume determination. He then investigated the behaviour of the dye *during* those first four minutes after its injection. In anæsthetized dogs he found the lymph coming from the thoracic duct to be stained with dye less than three minutes after its injection into a vein. Within fifteen minutes the concentration of the dye in the thoracic duct lymph was 60 p.c. of its concentration in the plasma. He pointed out that, since a long column of unstained lymph had to be displaced before the dye could appear at the

mouth of the cannula in the thoracic duct, the dye must have passed almost immediately upon its injection into the lymph-spaces. The dye method would therefore have included as a part of the "blood volume" of these dogs, 60 p.c. of their lymph volume, exclusive of that part of their lymph volume represented by the contents of the thoracic duct. Smith concluded that, if there was as much as 13 c.c. of lymph per kilogram of body weight, this diffusion of the dye into the lymph would explain the much discussed excess of the dye-determined over the gas-determined blood volumes in normal individuals. Since, however, there was no evidence that the lymph was present in such a large amount as 13 c.c. per kg. body weight, he contented himself with the view that his observations explained the *greater part* of the discrepancy between the two methods.

In my opinion this qualification of Smith's conclusions may now be removed. The present experiments and those of Thompson indicate that between 12 and 15 p.c. of the total plasma volume is lost in the erect posture, while my measurements of the swelling of the leg suggests that this fluid passes into the lymph spaces. The average gas-determined plasma volume is about 40 c.c. per kg. of body weight, while 15 p.c. of this volume—or 6 c.c. per kg. body weight—passes into the tissues of the lower extremities in the erect posture. But that is merely the *increment* in the tissue fluid, and moreover in that of the lower extremities only. It therefore seems probable that the total lymph volume might be expected to reach at least the figure of 13 c.c. per kg. of body weight that Smith required to explain the whole of the difference between the results of the two methods.

If that is true the explanation straightway follows of the cause of the difference in the results given by the two methods for the *postural* changes in plasma volume. If the dye diffuses into the tissues in such a way that (as Smith found) the concentration of it in the lymph is less than in the plasma, the dye method must indicate a smaller postural change in plasma volume than the gas method. If the diffusion of the dye was so rapid that the concentration of it in the lymph was equal to the concentration of it in the plasma, then the dye method would obviously be incapable of demonstrating any postural change in plasma volume. On the other hand, if no diffusion of dye occurred into the lymph, the postural change recorded by the dye method would be equal to that recorded by the gas method. In fact the difference between the postural changes in plasma volume given by the two methods should theoretically enable one to determine the relative concentrations of the

dye in plasma and lymph, and moreover the actual volume of the lymph¹.

One qualification, however, must be made. It is not known precisely how much of the muscle hæmoglobin is included in the carbon-monoxide method as part of the blood volume. Strictly speaking, therefore, the figure obtained in this way will give a minimal value for the lymph volume².

It is very uncertain in the first place how much muscle hæmoglobin is contained in the body; and in the second place whether all or only part of the total muscle hæmoglobin is reckoned in the gas-determined blood volume. Gescheidtlen [1878] considered the muscle hæmoglobin to be under 5 p.c. of the total blood hæmoglobin, while Whipple [1926], more recently in dogs, found it to vary between 10 and 80 p.c. of the total blood hæmoglobin. In a paper [Chang and Harrop, 1928] already referred to, some experiments were described in which the effect of exercise was studied on the concentration of CO in the blood. During exercise the capillary bed of the muscle is considerably increased and the blood flow through it correspondingly augmented; it would be expected

¹ The following considerations will make this clear. Let Dh , Dv , Gh , Gv , be the dye-determined and gas-determined "plasma volumes" in the horizontal and vertical positions. Let P and L be the true plasma and lymph volumes respectively, and ΔP the amount of plasma fluid which in the erect posture passes out from the plasma into the lymph. Then

$$Dh = P + fL,$$

and

$$Dv = P - \Delta P + f(L + \Delta P),$$

where f is the ratio of the concentration of dye in the lymph to its concentration in the plasma.

Then

$$Dh - Dv = \Delta P (1 - f).$$

Again

$$Gh = P,$$

and

$$Gv = P - \Delta P.$$

Therefore

$$Gh - Gv = \Delta P$$

.....(1).

Also

$$(Gh - Gv) - (Dh - Dv) = f(\Delta P)$$

.....(2).

Finally

$$Dh - Gh = fL$$

.....(3).

From this it is obvious that, if the plasma volume is determined in the recumbent and vertical position both by the dye and gas method, we can evaluate P , $f(\Delta P)$, and fL , and hence can determine both f , the distribution factor of the dye between lymph and plasma, and L the actual lymph volume.

² If we put M for the spurious component of the gas-determined plasma volume due to the absorption of CO by muscle hæmoglobin we have

$$Gh = P + M$$

and

$$Gv = P - \Delta P + M.$$

Equation (1) and (2) remain unchanged, but equation (3) becomes

$$Dh - Gh = f(L) - M;$$

and

$$L = 1/f(Dh - Gh + M).$$

that the muscle hæmoglobin might thus become more completely saturated with CO during exercise than during rest. Actually, Chang and Harrop found a very small change in CO concentration—in fact it represented an increase in blood volume of between 1.3 and 7.3 p.c. of the total blood volume. Moreover, part of this change was probably the result of other factors, such as the throwing into the circulation of blood from the spleen. These figures, of course, give no definite information as to the amount of muscle hæmoglobin included in the blood-volume estimation during rest; but they suggest that the amount included is nearer to the lower than the upper limit which Whipple gives for the total muscle hæmoglobin in the dog.

Theoretically, therefore, a minimal value for the lymph volume could be obtained by making gas and dye determinations of "blood volume" in both the erect and recumbent postures on a given subject. The figures thus obtained would require the addition of an unknown but probably small factor. Thus although the quadruple experiment would undoubtedly be tedious both for the subject and the observer, the interest of the result would probably repay the effort¹.

CONTRACTION OF THE SPLEEN.

On two subjects, while the double blood volume for the effect of posture was being determined, there occurred in the second determination (*i.e.* in the erect posture) an increase instead of a diminution in blood volume. Now in this second determination, which followed soon after the first, the blood CO necessarily rose to a considerably higher level. De Boer and Carroll [1924], and later Barcroft and his associates [Barcroft, Murray, Orahovats, Sands and Weiss, 1925] have shown that in certain animals the inhalation of CO produces a splenic contraction. On one of the two subjects (No. 4), who had twice given an abnormal response, the double experiment was repeated with smaller quantities of CO and the normal postural change already given for this subject in the above tables was obtained. There was no opportunity for repeating the experiment on the other subject (R.B.). In these three anomalous experiments the changes in percentage cell volume and plasma-protein concentration were also abnormal—the usual increase did not take place in the erect posture.

¹ A second correction E , probably too small to be more than of academic interest, should theoretically be applied for the spurious component of the dye method due to the adsorption on to the surface of the cell of a monomolecular layer of dye. The final expression then assumes the form:

$$L = 1/f(Dh - Gh + M - E).$$

It is possible that these two subjects were abnormally sensitive to CO, with the result that their spleens contracted and their blood volumes increased (Table V).

TABLE V. Anomalous results in two subjects presumably sensitive to CO, giving an *increase* in blood-volume figures in the erect posture after a previous determination in the recumbent posture.

Subject	Increase in whole blood volume		Increase in cell volume		Increase in plasma volume		P.c. cell volume hæmatocrit		Protein gm. p.c.	
	c.c.	p.c.	c.c.	p.c.	c.c.	p.c.	Horizontal	Vertical	Horizontal	Vertical
4a	360	8	70	3	290	12	46	44	7.35	6.94
4b	230	5	110	5	120	4	44	44	—	—
R.B.	990	18	—	—	—	—	—	—	6.57	6.18

On two subjects, R.L.W. and R.McI., the blood volume was determined immediately after the removal of about half a litre of blood and the result compared with determinations made on the previous day at the same time.

In R.L.W., who got up and walked about between the venesection and the determination, a remarkable *increase* in blood volume was observed (Table VI). Allowing for the 550 c.c. removed the increase amounted to 1200 c.c. of whole blood, 730 c.c. of plasma and 490 c.c. of cells; simultaneously the red cell count *increased* by 1.7 millions.

In R.McI. on the other hand, who remained lying quietly on the couch from before venesection until the end of the determination, no such dramatic rise in blood volume occurred (lower part of Table VI).

TABLE VI. Figures showing effect in two subjects of rapid removal of 500 c.c. of blood.

Subject	Date	Time	Blood vol. c.c.	Plasma vol. c.c.	Cell vol.	O ₂ Cap vol. p.c.	R.B.C. millions	
R.L.W.	May 9	3.0 p.m.	5435	—	—	—	—	
	„ 26	3.30 p.m.	5280	3190	2090	19.4	—	
	„ 31	2.30 p.m.	5530	3320	2210	19.7*	4.7	
	June 1	2 p.m.	550 c.c. blood removed from arm					—
	„ 1	2.30 p.m.	6200	3720	2480	21.7*	6.4	
	„ 1	5.30 p.m.	—	—	—	—	5.2	
	„ 2	2.10 a.m.	—	—	—	—	4.1	
	Sept. 5	12 noon	5260	3030	2230	—	—	
	„ 5	2 p.m.	5300	3050	2250	—	—	
	Oct. 16†	2.30 p.m.	5280	2980	2300	—	—	
R.McI.	Jan. 7†	3 p.m.	5190	3090	2100	—	—	
	Apr. 23	3.0 p.m.	5090	—	—	—	—	
	Sept. 17†	2.30 p.m.	5210	3150	2060	—	—	
	„ 18	2.30-2.45 p.m.	540 c.c. blood removed from arm					—
„ 18†	2.45 p.m.	5000	2900	2100	—	—		

* This figure includes the hæmoglobin present as carboxyhæmoglobin.

† Determinations made in recumbent position: all others in this series were done sitting upright in a chair.

Barcroft and his associates [Barcroft and Barcroft, 1923] found that in rats the inhaled CO reached a very much lower concentration in the splenic blood than it did in the general circulation, and suggested that the spleen acted as a reservoir of red cells shunted off from the active circulation. Barcroft, Murray, Orahovats, Sands and Weiss [1925] showed that splenectomized guinea-pigs died more quickly of CO-poisoning than normal guinea-pigs; because, when the CO reached a poisonous concentration, the spleens of the normal pigs contracted and threw into the circulation their reserve supply of blood. That same year [Barcroft, Harris, Orahovats and Weiss, 1925] developed new methods for studying the spleen in living dogs and cats, and demonstrated that (i) the living spleen might have several times its volume *post mortem*; (ii) hæmorrhage and exercise produced splenic contraction so that between 6 and 12 p.c. of the total blood volume might be added to the active circulation; (iii) contraction occurred early in the hæmorrhage; (iv) the splenic blood was richer than the systemic blood in red cells.

The above animal experiments suggest reasonable explanations of the present human experiments. In addition the different reaction to hæmorrhage shown by R.L.W. and R.McI. may be due to the fact that the former walked about, while the latter lay still after venesection: that the second subject lacked the trigger effect of exercise to fire off the splenic contraction.

One difficulty lies in the *size* of the increase in blood volume observed in R.L.W. and R.B. (Tables VI and V respectively), which amounted to about a litre. It cannot be supposed that all this came from the spleen. Hartwig [1929], from perfusion experiments on human spleens removed at autopsy, concluded that their volume *post mortem* is $\frac{1}{3}$ to $\frac{1}{2}$ their volume in life, and that they may contain up to 400 c.c. of cells. Binet and Fournier [1926] found that immediately following hæmorrhage in normal dogs the red count might increase by over 2 million cells, and that this did not occur in splenectomized dogs or in dogs in which the spleen's pedicle was lightly clamped.

Now following venesection the *cell volume* of R.L.W. increased by 490 c.c. while his red count rose by 1.7 million cells: the first figure is not much in excess of Hartwig's estimate of the capacity of the human spleen, and the second figure is similar to the figures of Binet and Fournier in dogs. It is possible, therefore, that the excess cells may come from the spleen, while the remainder of the increase in blood volume (the plasma increase) is secondarily derived from the tissues.

SUMMARY.

1. The blood volume as determined by the carbon-monoxide method is less in the erect than in the recumbent posture.
2. This loss of blood volume is mainly in respect of plasma, the loss of which averages 15 p.c. of the total plasma volume.
3. There is an accompanying loss of cell volume averaging 4 p.c. of the total cell volume.
4. There is an increase of the plasma-protein concentration in the erect posture such as would be expected if the globulin fraction alone did not diffuse through the capillary walls.
5. These results differ from those of observers using the dye method; the latter find a smaller diminution in plasma volume, no change in cell volume, and an increase in plasma-protein concentration such as would result from the albumin as well as the globulin being held back by the capillary walls.
6. All these differences between the gas and dye methods can be explained as the result of a diffusion of the dye into the lymph spaces.
7. A method is outlined whereby it should be possible to determine the total lymph volume of the body, or rather a minimal figure for it, from quadruple determinations of blood volume in both erect and recumbent postures by both gas and dye methods.
8. The "blood volume" does not include the blood in the spleen.
9. The spleen may contract and throw its red cells into the circulation either in subjects sensitive to CO as a result of inhaling that gas, or after the removal of about a pint of blood.
10. The increase in plasma volume following splenic contraction is probably derived secondarily from the tissue fluids.

REFERENCES.

- Barcroft, J. and Barcroft, H. (1923). *J. Physiol.* **58**, 138.
 Barcroft, J., Harris, H. A., Orahovats, D. and Weiss, R. (1925). *J. Physiol.* **60**, 443.
 Barcroft, J., Murray, C. D., Orahovats, D., Sands, J. and Weiss, R. (1925). *J. Physiol.* **60**, 79.
 Binet, L. et Fournier, B. (1926). *C. R. Soc. Biol. Paris*, **95**, 1141.
 Chang, H. C. and Harrop, G. A. (1928). *J. Clin. Inves.* **5**, 393.
 de Boer, S. and Carroll, D. C. (1924). *J. Physiol.* **59**, 312.
 Gescheidtlen, R. (1878). *Pfluegers Arch.* **7**, 530.
 Hammarsten, Olaf (1900). *Physiological Chemistry*, pp. 184, 185 (Chapman and Hall).
 Cited from Hoppe-Seyler, Owen Rees, Gubler, Quevenne and von Scherer.
 Hartwig, H. (1929). *Ziegler's Beiträge*, **83**, 431.
 Smith, H. P. (1925). *Bull. J. H. Hosp.* **36**, 325.
 Thompson, W. O., Thompson, P. K. and Dailey, M. E. (1928). *J. Clin. Inves.* **5**, 573.
 Waterfield, R. L. (1931). *J. Physiol.* **72**, 121.
 Whipple, G. H. (1926). *Amer. J. Physiol.* **76**, 685, 693, 708.