XC. THE EFFECTS OF CONGESTION AND HEAT ON THE COMPOSITION OF VENOUS BLOOD SAMPLES TOGETHER WITH SOME OBSERVATIONS ON THE INDIRECT DETERMINATION OF IRON AND CHLORINE IN BLOOD-CORPUSCLES.

BY FREDERICK HORACE SMIRK.

*From the Laboratory of Clinical Investigations and Research,
Manchester Royal Infirmary.*

(Received April 6th, 1928.)

IN certain investigations on the blood composition in nephritis it became obvious that the determination of the iron and chlorine content of red blood-corpuscles was beset with errors, many of which would ordinarily pass unnoticed. The effect of congesting and heating the arm on the composition of a venous blood sample taken from the median basilic vein also appears to have been inadequately studied.

The corpuscular iron or chloride may be estimated directly or by calculation from other results.

Now let % Cs = percentage volume of red blood-corpuscles,

W.B.Fe. = whole blood-iron in mg. per 100 cc.

C.B.Fe. = corpuscular blood-iron in mg. per 100 cc.

P.B.C. = plasma blood-chloride in g. per 100 cc.

W.B.C. = whole blood-chloride in g. per 100 cc.

C.B.C. = corpuscular blood-chloride in g. per 100 cc.

The iron is almost entirely contained in the corpuscles and therefore in the absence of haemolysis the following relations hold within experimental error:

$$W.B.Fe. \times 100 = C.B.Fe. \times \% Cs,$$

$$\% Cs = 100 \times \frac{W.B.Fe.}{C.B.Fe.}.$$

The % Cs in a sample of blood may therefore be calculated if the value of the W.B.Fe. and C.B.Fe. are known.

Now the W.B.C. is the sum of the chloride contained in the corpuscles and in the plasma, therefore

$$100 W.B.C. = (100 - \% Cs) P.B.C. + \% Cs \times C.B.C.,$$

$$\text{and} \quad C.B.C. = \frac{100 W.B.C. - (100 - \% Cs) P.B.C.}{\% Cs}.$$

The values of W.B.C. and P.B.C. are readily estimated without appreciable error, so that the value of the C.B.C. obtained by this equation depends on the accuracy of the estimation of % Cs.

The C.B.C. may be obtained in several ways and apart from the chemical analysis its accuracy will depend on the purity of the corpuscular sample.

Separation of corpuscles and plasma is most completely effected by the haemocrit; the reading of % Cs being less than is obtained in a macro-centrifuge even when the haemocrit and centrifuge are spun at the same speed for the same time [Smirk, 1928]. The most accurate estimate of C.B.C. should therefore be obtained by calculation from P.B.C., W.B.C., and the haemocrit reading.

If the reading on a graduated centrifuge tube is used as a measure of the correct % Cs the value of C.B.C. will be too high because the plasma has not been completely removed. If, however, P.B.C. is known, a haemocrit determination on the corpuscular sample may be used to correct C.B.C. obtained by analysis.

In this investigation the blood-iron and blood-chlorides have been estimated by the methods described by Smirk [1927, 1, 2]. The following experiments illustrate the reliability of the chemical methods employed.

Exp. 1. 2 cc. of well-mixed oxygenated blood were delivered to a centrifuge tube and spun for 5 minutes. Exactly 0.5 cc. of plasma was removed in a standardised pipette and used for analysis, the remaining blood being mixed carefully and analysed.

A = original sample of blood.

B = sample of 2 cc. of whole blood from which 0.5 cc. of plasma has been removed.

Sample <i>A</i>	Sample <i>B</i>
W.B.Fe. 56.0 mg. per 100 cc.	75.0 mg. per 100 cc.
W.B.C. 0.487 g. per 100 cc.	0.430 g. per 100 cc.
P.B.C. on both samples: 0.641 g. per 100 cc.	
Calculated value of W.B.Fe. in <i>B</i> = $\frac{56 \times 2}{1.5} = 74.7$ mg. per 100 cc.	
Actual value of W.B.Fe. in <i>B</i> by direct analysis = 75.0 mg. per 100 cc.	
Calculated value of W.B.C. in <i>B</i> = $\frac{0.487 \times 2 - 0.641 \times 0.5}{1.5} = 0.435$ g. per 100 cc.	
Actual value of W.B.C. in <i>B</i> by direct analysis = 0.430 g. per 100 cc.	

Exp. 2. 3 cc. of well-mixed oxygenated blood were delivered to a centrifuge tube and spun for 5 minutes. Exactly 1 cc. of plasma was removed in a standardised pipette and used for analysis, the remaining blood being mixed and carefully analysed.

C = original sample of blood.

D = 3 cc. of original blood - 1 cc. of plasma.

Sample <i>C</i>	Sample <i>D</i>
W.B.Fe. 54.9 mg. per 100 cc.	81.8 mg. per 100 cc.
W.B.C. 0.480 g. per 100 cc.	0.402 g. per 100 cc.
P.B.C. on both samples: 0.626 g. per 100 cc.	
Calculated value of W.B.Fe. in <i>D</i> = $\frac{54.9 \times 3}{2} = 82.4$ mg. per 100 cc.	
Actual value of W.B.Fe. in <i>D</i> by direct analysis = 81.8 mg. per 100 cc.	
Calculated value of W.B.C. in <i>D</i> = $\frac{0.480 \times 3 - 0.626}{2} = 0.407$ g. per 100 cc.	
Actual value of W.B.C. in <i>D</i> by direct analysis = 0.402 g. per 100 cc.	

It will be seen that there is a close approximation of results by calculation and analysis in both the above experiments.

The following series of observations on a single sample of venous blood illustrates the relationship between the values of C.B.Fe. and C.B.C. and % Cs as determined experimentally and by calculation from the formulae:

$$\% \text{ Cs} = \frac{100 \text{ W.B.Fe.}}{\text{C.B.Fe.}},$$

$$\text{C.B.C.} = \frac{100 \text{ W.B.C.} - (100 - \% \text{ Cs}) \text{ P.B.C.}}{\% \text{ Cs}}.$$

1. W.B.Fe. by analysis	= 62.6 mg. per 100 cc.
2. C.B.Fe. by analysis of sample obtained by macro-centrifuging ...	= 121.0 mg. "
3. C.B.Fe. by calculation from (2) and (5)	= 129.5 mg. "
4. C.B.Fe. by calculation from (1) and (6)	= 134.0 mg. "
5. Purity of corpuscular sample obtained by macro-centrifuging as determined by haemocrit	= 93.2 %
6. % Cs by haemocrit on whole blood	= 46.7 %
7. % Cs calculated from (1) and (3)	= 48.3 %
8. P.B.C. by analysis	= 0.647 g. per 100 cc.
9. W.B.C. by analysis	= 0.459 g. "
10. C.B.C. by analysis of sample obtained by macro-centrifuging ...	= 0.274 g. "
11. C.B.C. by calculation from (7), (8) and (9)	= 0.258 g. "
12. C.B.C. by calculation from (10) and (5)	= 0.247 g. "
13. C.B.C. by calculation from (6), (8) and (9)	= 0.244 g. "

It will be observed that the two lowest values of C.B.C. obtained by methods (12) and (13) agree within experimental error. Both depend for their accuracy on haemocrit determinations. The methods (10) and (11) give high results because of admixture of the sample with serum. The % Cs in (7) is too high for a similar reason and the C.B.Fe. in (2) too low. The separation of serum from the corpuscular mass by the haemocrit has not been as complete as the separation in the case of the whole blood sample. In consequence of this, the C.B.Fe. is slightly less in method (3) than in method (4) and the C.B.C. slightly greater in method (12) than in method (13). From these results it follows that the most accurate method is to obtain the corpuscular blood-iron and -chloride by haemocrit observations on whole blood and subsequent calculation from the plasma- and whole blood-chlorides.

THE EFFECTS OF CONGESTION AND HEAT ON THE COMPOSITION OF VENOUS BLOOD SAMPLES.

It is well known, and is illustrated later in this paper, that variations in the CO₂ content of the blood cause movements of chlorine ions between corpuscles and plasma, the total or whole blood-chloride remaining constant.

Any acceleration or retardation of the blood flow may therefore be of importance in investigations of the distribution of chlorine in the blood.

The effect of congestion.

Where veins are normally inconspicuous the tendency when removing blood from them is to leave the tourniquet in position for a longer time until they are more easily visible.

Experiments have been performed on human subjects revealing considerable differences in the composition of venous blood from congested and uncongested veins. The blood in four of these cases was collected in an air-tight syringe, the last bubble of air being replaced by sterile paraffin. The sample was delivered under paraffin to the bottom of a tube containing a small amount of potassium oxalate. The paraffin prevents loss of CO_2 and, by exclusion of air, oxygenation of the sample is avoided. The following results were obtained.

Test	Treatment	W.B.Fe. mg. per 100 cc.	Change in W.B.Fe.	P.B.C. g. per 100 cc.	Change in P.B.C.	W.B.C. g. per 100 cc.	Change in W.B.C.	C.B.C. g. per 100 cc.	Change in C.B.C.
1	None	60.3	+ 10.7	0.568	- 0.006	0.450	- 0.010	0.304	+ 0.035
	Congestion	71.0		0.562		0.440		0.339	
2	None	66.5	+ 12.3	0.596	- 0.005	0.474	- 0.014	0.312	+ 0.005
	Congestion	78.8		0.591		0.460		0.317	
3 A	None	64.0	+ 6.7	0.587	- 0.008	0.456	- 0.008	0.300	+ 0.017
	Congestion	70.7		0.579		0.448		0.317	
3 B	Oxygenation of untreated sample	64.0	+ 6.7	0.612	+ 0.007	0.456	- 0.008	0.253	+ 0.011
	Oxygenation of congested sample	70.7		0.619		0.448		0.264	
4	None	47.3	+ 0.1	0.568	- 0.001	0.472	- 0.002	0.301	- 0.003
	Slight temporary congestion	47.4		0.567		0.470		0.298	
5 A	None	62.6	+ 2.9	0.596	- 0.012	0.459	- 0.005	0.306	- 0.009
	Congestion	65.5		0.584		0.454		0.297	
5 B	Oxygenation of untreated sample	62.6	+ 2.9	0.647	- 0.010	0.459	- 0.005	0.274	- 0.005
	Oxygenation of congested sample	65.5		0.637		0.454		0.269	

It will be observed that the whole blood-iron was greater where veins were congested than in uncongested vessels, but unchanged in test 4 where congestion was slight and temporary. The plasma-chloride was diminished and the corpuscular chloride increased wherever the change was definitely outside the range of experimental error. These chloride transferences are due to the greater CO_2 tension of venous blood.

The increase in blood-iron denotes an increase in the proportion of corpuscles and, in consequence of this, there is a fall in the whole blood-chloride, because corpuscles contain less chloride than plasma.

In two experiments, 3 B and 5 B, the blood was shaken thoroughly with air so that the effect of the increased CO_2 was removed. The considerable increase in the plasma-chlorides and the diminution in the corpuscular chlorides as compared with experiments 3 A and 5 A are worthy of note.

It is clear, therefore, that when venous samples are used as an index of the chlorine and iron content of the circulating blood it is necessary that the arm should be only temporarily congested and that the tourniquet should be preferably released for several seconds after the needle is in the vein before the blood sample is actually taken. In addition, the venous blood obtained should be thoroughly oxygenated to remove CO_2 and obtain a value of the

plasma-chloride independent of variations in CO_2 produced by local metabolic changes in the arm.

These results are in accord with the observations of Peters, Bulger, Eisenman and Lee [1926], who found an increase in the plasma-proteins and a diminution in plasma-chloride on congesting the source of a venous sample.

The effect of heat.

When the hands and arms are heated by immersion in hot water for several minutes, changes occur in the venous blood composition which, however, are less definite than after congestion. The blood, when withdrawn from the heated arm, appears distinctly arterial. The following results were obtained:

Test	Treatment	W.B.Fe. mg. per 100 cc.	Change in W.B.Fe.	P.B.C. g. per 100 cc.	Changes in P.B.C.	W.B.C. g. per 100 cc.	Change W.B.C.	C.B.C. g. per 100 cc.	Change in C.B.C.
1	None	51.5	+0.7	0.652	+0.009	0.486	-0.001	0.285	-0.008
	Immersion in hot water	52.2		0.661		0.485		0.277	
2	None	60.3	-2.6	0.568	+0.010	0.450	-0.001	0.304	-0.031
	Immersion in hot water	57.7		0.578		0.449		0.273	
3	None	66.5	+8.2	0.596	+0.013	0.474	+0.005	0.312	+0.005
	Immersion in hot water	74.7		0.609		0.479		0.317	
4 A	None	64.0	+6.7	0.587	-0.001	0.456	+0.001	0.300	0.000
	Immersion in hot water	70.7		0.586		0.457		0.300	
4 B	Oxygenation of untreated sample	64.0	+6.7	0.612	+0.004	0.456	+0.001	0.253	+0.003
	Immersion in hot water and oxygenation	70.7		0.616		0.457		0.256	
5	None	47.3	-3.2	0.568	+0.008	0.472	-0.001	0.301	-0.003
	Immersion in hot water	44.1		0.576		0.471		0.298	

In all cases where any chloride transference takes place owing to the diminished CO_2 content of the arterialised venous blood the plasma-chloride is raised.

The corpuscular chloride falls in all cases except 3 and 4 A. In the latter no fall in corpuscular chloride would be expected since the plasma-chloride was not increased.

The iron and whole blood-chloride are not uniform in their deviation; the tendency however is for a rise in iron content. The total iron increase in the five tests amounts to 15.6 mg. and the total fall to 5.8 mg. The fact that the whole blood-chloride and iron values do not maintain the theoretical inverse relationship may suggest that the plasma-chloride can interchange with the tissue chlorides in much the same way as with the chloride of the corpuscles. There is, however, no direct evidence of this and changes are of too small an order to allow safe deduction.

SUMMARY.

1. The corpuscular blood-chloride and corpuscular blood-iron have been estimated by direct and several indirect methods. The errors in these determinations have been shown to be due to incomplete separation of plasma from the corpuscles, and not to the chemical analysis.

2. Congestion of the arm causes a concentration of iron and increased venosity in samples from the median basilic vein. This is associated with a reduced percentage of chlorine in the plasma and an increased percentage in the corpuscles, and is of practical importance in blood sampling.

3. Heating the arm renders the venous sample more arterial in appearance and this is associated with a transference of chlorine ions from corpuscles to plasma. The iron content of the blood may be raised.

4. Venous samples should be taken with a minimum of congestion and should be thoroughly shaken with atmospheric or alveolar air if they are to form a reliable index of changes in the plasma-chlorides of the general circulation.

I wish to acknowledge my indebtedness to Prof. H. S. Raper and Dr Craven Moore for their kind interest, criticism and advice, which have alike proved invaluable, and to the Medical Research Council for a grant.

REFERENCES.

- Peters, Bulger, Eisenman and Lee (1926). *J. Biol. Chem.* **67**, 175.
Smirk (1927, 1). *Biochem. J.* **21**, 36.
—— (1927, 2). *Biochem. J.* **21**, 31.
—— (1928). *J. Exp. Path.* (in the Press).