CXXIX. THE DISPERSED PHASE OF THE BLOOD CORPUSCLES.

By RICHARD EGE.

From the Physiological Institute at the University of Copenhagen.

(*Received November 3rd, 1926.*)

A. Gough in an extremely interesting paper [1924] on "The nature of the red blood corpuscle" also treats the question of the dispersed phase of the blood corpuscle. He determines the dispersed phase by computing the changes of volume of the blood corpuscles in fluids of different osmotic pressure by Hamburger's principle [1898], and he comes to the conclusion that the dispersed phase constitutes two-thirds of the volume of the blood corpuscle, while the watery phase, in which the electrolytes, etc., are found to be dissolved, only forms one-third of the blood corpuscle.

The value which Gough indicates for the dispersed phase of the blood corpuscle is even greater than that which Hamburger has found, but which, according to my investigations, must be said to be too high.

It need not be specially emphasised that the knowledge of the volume of the dispersed phase is of great interest, for not till the magnitude of the dispersed phase in the plasma and blood corpuscles be known will it be possible to discuss and explain the empirically found relations of partition which have been determined for non-electrolytes capable of permeating the membrane of the blood corpuscle. Thus, if one finds that the partition of urea and acetone between the blood corpuscles and the plasma corresponds to the proportion between the magnitude of the dispersion medium in the two systems, the most reasonable explanation will be that the two substances are only found dissolved in the dispersion medium of the two systems. On finding, however, that

$$\frac{\text{Concentration in the blood corpuscles}}{\text{Concentration in the plasma}} \gtrless \frac{\text{Dispersion medium in the blood corpuscles}}{\text{Dispersion medium in the plasma}}$$

after equilibrium has been established, it is seen that the partition cannot simply be interpreted by referring to the difference of volume of the dispersion medium. The substance in question must also be soluble in the dispersed phase of the one system, being adsorbed to the same or complexly bound.

It is therefore of great importance for the comprehension of these relations to ascertain whether the value for the dispersed phase found by Gough [1924], or that found by Ege [1920], is correct.

Gough's findings are:

Dispersion medium 30–35 % Dispersed phase 70–65 %.

Ege finds:

Dispersion medium about 60 % Dispersed phase 40 %.

By determinations performed by Hamburger's principle, I came to the result that the changes of volume of the blood corpuscles in a cane-sugar solution varied in the manner which would be expected in accordance with osmotic theories, if the dispersed phase of the blood corpuscles was 35–45 % [Ege, 1922].

When Gough, using the same method, finds a value about twice as great, this may be partly due to the circumstance that the volume of the blood corpuscles does not keep constant in such NaCl solutions as he used, but particularly to an incomplete sedimentation of the column of blood corpuscles in those experiments where the blood corpuscles are suspended in a hypertonic salt solution, which renders sedimentation much more difficult than iso- and hypo-tonic solutions [Ege, 1923]. Gough only says that all the samples were spun in a water-centrifuge for 3 hours.

Gough does not think that this has led to a complete sedimentation of the blood corpuscles, but he considers the error which will arise from this as negligible. However, he has furnished no proof of this being actually the case, and, as will easily be seen, an incomplete sedimentation may bring about a very considerable error in his determination and computation of the volume of the dispersed phase.

If the centrifuging of the blood corpuscles is continued until the whole of the column of blood corpuscles acquires the appearance of laked blood, that is to say, until an absolute sedimentation is attained, one will find that different periods of centrifuging will be required, according as the blood corpuscles are suspended in hypo- and iso-tonic solutions in which the absolute sedimentation takes place relatively rapidly, or in hypertonic solutions, with which a prolonged centrifuging is required if the same quantitative sedimentation is to be obtained. If all the samples are centrifuged for an equally long time, the values found for the volume of the blood corpuscles may become too high, particularly in the hypertonic solutions.

Even fairly small errors may lead to very great errors on computation of the volume of the dispersed phase. Thus, supposing that a complete sedimentation is attained in experiments with an isotonic fluid as well as with a fluid whose osmotic concentration is 50 % higher, the dispersed volume will be found to be = 40 %, if the first volume be = 100 and the other = 80, as becomes evident from the following formula:

$$1{\cdot}00\,(100 - x) = 1{\cdot}50\,(80 - x),$$
$$x = 40.$$

If, however, on account of imperfect centrifugalisation, the complete sedimentation were attained in the isotonic solution only, but not in the hypertonic solution, so that there still remained, for instance, 12–13 % of fluid among the blood corpuscles, that is to say, that the volume of blood corpuscles amounted to 90 instead of the true volume of 80; the dispersed volume would then be found to be 70 %:

$$1{\cdot}00\,(100 - x) = 1{\cdot}50\,(90 - x),$$
$$x = 70.$$

The method of determining the dispersed phase introduced by Hamburger—employed by Gough—will thus easily lead to excessively high values for the dispersed phase unless the centrifuging is guaranteed to be absolutely quantitative in all the samples.

If the centrifuging is continued until the column of blood corpuscles has acquired its true volume without interstitial plasma, this manifesting itself by the column of blood taking the appearance of laked blood [Köppe, 1905] in its whole extent, a diminution of volume in the hypertonic solutions approximately corresponding to a dispersed phase of 35–45 % will be found, as has previously been mentioned.

As it might be presumed that the envelope of the blood corpuscles will resist the changes of volume, the method mentioned may, however, give rise to some objections. Another determination of the volume of the dispersed phase is derived from making solutions of non-electrolyte in equal volumes of water and blood corpuscle press-juice, and afterwards, determining the change in the freezing-point of the fluid.

8 g. of cane-sugar in water, $\Delta = 1{\cdot}22°$.

8 g. of cane-sugar in the same volume of blood corpuscle press-juice[1], $\Delta = 1{\cdot}81°$.

The volume x of the dispersed phase can then be calculated by the following formula:

$$(100 - x) \times 1{\cdot}81 = 100 \times 1{\cdot}22,$$
$$x = 33\ \%.$$

For the sake of comparison the following determination of dry matter in the blood corpuscle is mentioned:

	Dry matter	Water
Percentage of weight	34	66
Percentage of volume	28	72
	Dispersed phase	Dispersion medium
	33	67

The volume of the dispersed phase is thus 10–15 % greater than the volume found by the determination of dry matter (corresponding however rather closely to the percentage of weight).

Summary

By means of various methods of determination of the volume of the dispersed phase of the blood corpuscle, this volume is found to approach very nearly to the percentage of dry matter of the corpuscle.

[1] From this value the depression of freezing point of the ordinary corpuscle press-juice has been subtracted.

The figures given by Gough, according to which the volume of the dispersed phase is double the content of dry matter, must be due to a systematic error.

REFERENCES.

Ege (1920). Studies upon partition of glucose between the plasma and the red blood corpuscles. (Monograph. Copenhagen, 1919–1920.) Translated into German: *Biochem. Z.* (1920), **107**, 109; (1921), **114**, 115.

—— (1922). *Biochem. Z.* **130**, 99.

—— (1923). *Biochem. Z.* **134**, 234.

Gough (1924). *Biochem. J.* **18**, 202.

Hamburger (1898). *Arch. Anat. Physiol.* 317.

Köppe (1905). *Pflüger's Arch.* **107**, 86.