THE LAW REGULATING HAEMOLYSIS OF ERYTHROCYTES IN HYPOSMOTIC SALINE SOLUTION OR DISTILLED WATER

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In studying the haemoglobin-value of the resistant erythrocytes¹ when blood is treated with hyposmotic saline or distilled water, I have obtained results which lead to the conclusion that the amount of haemoglobin in a suspension of erythrocytes that can be dissolved in a definite volume of hyposmotic saline solution or distilled water is proportional to the total amount of haemoglobin present in the suspended corpuscles. The suspension of the erythrocytes was obtained by taking a volume of blood, which was allowed to clot after being quickly centrifuged. The clot was separated and the erythrocytes washed three or four times with 0.85 per cent. solution of sodium chloride, and a suspension of the erythrocytes obtained. One volume of this was treated with two volumes of distilled water, or N/50 sodium chloride solution. The amount of haemoglobin dissolved was then estimated, and compared with the total amount of haemoglobin present in the suspended corpuscles. The blood of various animals was thus tested and the results compared.

In the following tables, t denotes the total amount of haemoglobin in a suspension of erythrocytes, d the amount of dissolved haemoglobin in any particular suspension of erythrocytes, and $p = \frac{d}{t}$

TABLE I (a)

Normal Human Blood (1 part of erythrocytes suspended in 0.85 % NaCl solution + 2 parts of N/50 NaCl solution)—

1. $t = 110$; $d = 62$. $p \times 10^2 = 56.3$
2. $t = 100$; $d = 58$. $p \times 10^2 = 58.00$
3. $t = 90$; $d = 50$. $p \times 10^2 = 55.55$
4. $t = 77$; $d = 44$. $p \times 10^2 = 57.14$

TABLE I (b)

Normal Human Blood (1 part of erythrocytes suspended in 0.85 % NaCl solution + 2 parts of distilled water)—

1. $t = 150$; $d = 106$. $p \times 10^2 = 70.66$
2. $t = 118$; $d = 90$. $p \times 10^2 = 76.27$
3. $t = 50$; $d = 35$. $p \times 10^2 = 70.00$

TABLE II

Fowl's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 105$; $d = 88$. $p \times 10^2 = 83\cdot80$
2. $t = 96$; $d = 78$. $p \times 10^2 = 81\cdot25$
3. $t = 90$; $d = 80$. $p \times 10^2 = 88\cdot88$
4. $t = 84$; $d = 68$. $p \times 10^2 = 80\cdot95$
5. $t = 42$; $d = 34$. $p \times 10^2 = 80\cdot95$

TABLE III (a)

Sheep's Blood (1 part of erythrocytes suspended in the serum of the sheep + 2 parts of distilled water)—

1. $t = 120$; $d = 120$. $p \times 10^2 = 100\cdot00$
2. $t = 70$; $d = 70$. $p \times 10^2 = 100\cdot00$

TABLE III (b)

Sheep's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 150$; $d = 150$. $p \times 10^2 = 100\cdot00$
2. $t = 70$; $d = 70$. $p \times 10^2 = 100\cdot00$

TABLE IV (a)

Frog's Blood (1 part of erythrocytes suspended in the serum of the frog + 2 parts of N/50 NaCl)—

1. $t = 46$; $d = 10$. $p \times 10^2 = 20\cdot87$
2. $t = 28$; $d = 8$. $p \times 10^2 = 28\cdot57$

TABLE IV (b)

Frog's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 90$; $d = 10$. $p \times 10^2 = 11\cdot11$
2. $t = 70$; $d = 8$. $p \times 10^2 = 11\cdot42$

TABLE V

Cat's Blood (1 part of erythrocytes suspended in 0·85 % NaCl solution + 2 parts of distilled water)—

1. $t = 122$; $d = 106$. $p \times 10^2 = 86\cdot88$
2. $t = 62$; $d = 55$. $p \times 10^2 = 88\cdot71$

TABLE VI

Value of $p \times 10^2$ in the blood of a number of healthy students, the blood being treated with 2 parts of N/50 NaCl (deduced from the author's paper in the *Bio-Chemical Journal*, Vol. IV, Nos. 5, 6, and 7)—

1	2	3	4	5	6
58·4	58·4	60·9	64·3	56·4	56·3

From the above observations the following laws can be deduced:—

1. The amount of haemoglobin dissolved in a given volume of hyposmotic sodium chloride solution or distilled water is proportional to

the amount of haemoglobin in the erythrocytes presented for solution in the form of a suspension.

2. The ratio between these above two factors varies in different animals, being the smallest in our table in the case of the frog, and greatest in the case of the sheep. (In the case of the rabbit it is also very high.)

3. It is fairly constant in healthy individuals, but varies widely in disease.¹

From the above it will be seen that the same law that regulates the solution of globulin in solution of neutral salts also regulates the solution of haemoglobin contained within erythrocytes. In other words, the latter follows the law enunciated by Mellanby and Hardy.² This fact probably throws some light as to how haemoglobin exists inside erythrocytes. Each individual erythrocyte allows a certain quantity of water to enter into its substance by process of osmosis when blood is treated with a hyposmotic sodium chloride solution or distilled water. If, now, we consider that the haemoglobin exists in the form of a suspension inside erythrocytes, it follows, from the law of Mellanby and Hardy, that the amount of haemoglobin that will be dissolved by water entering into the substance of the erythrocytes is proportional to the amount of haemoglobin in each individual corpuscle. Accordingly, the amount of dissolved haemoglobin that will pass out of the corpuscles is proportional to the amount of haemoglobin inside them. It follows, therefore, that the amount of haemoglobin dissolved in a suspension of erythrocytes when the latter is treated with distilled water or hyposmotic saline, is proportional to the total amount of haemoglobin present in them. We can, therefore, conclude that haemoglobin as it exists inside erythrocytes is in a state of suspension.

REFERENCES

1. *Bio-Chemical Journal*, Vol. IV, p. 280, 1909.
2. *Journal of Physiology*, Vol. XXXIII, 1905; also referred to in the *Proceedings of the Royal Society*, Series B, Vol. LXXIX, 1907.