ON THE RELATIVE HAEMOGLOBIN-VALUE OF THE
RESISTANT ERYTHROCYTES DURING THE HAE-
MOLYSIS OF BLOOD WITH HYPOSMOTIC SODIUM
CHLORIDE SOLUTION, AND ON THE PERMEABILITY
OF THE ERYTHROCYTES TO WATER AS A FACTOR
IN THE PRODUCTION OF HAEMOLYSIS

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(Received May 10th, 1909)

In a previous paper¹ I have pointed out that the dark coloration described by Wright, and obtained by mixing one part of blood with two parts of a progressive dilution of saline does not represent the point of complete haemolysis. This point is obtained in the observations of McCay² and my own observations by mixing one part of blood with two parts of $\frac{N}{40}$ to $\frac{N}{50}$ saline solution. It may, for the sake of convenience, be called *Wright's haemolytic point*.

This point probably represents the stage at which a large number of the erythrocytes undergo haemolysis as the result of osmosis and rupture. The corpuscles that do not haemolyse at Wright's haemolytic point will be termed in this paper the *resistant corpuscles*.

By quantitatively estimating the amount of dissolved haemoglobin in 20 cb.mm. of the supernatant fluid obtained after centrifugalisation of a mixture of blood and two volumes of $\frac{N}{x}$ saline solution, where x is any number from 20 upwards, I have made out the *curve of haemolysis with hyposmotic saline solutions* (see fig. 1).

From the curve below it will be seen that the very beginning of haemolysis starts with $\frac{N}{20}$ saline solution. Then the degree of haemolysis suddenly increases from $\frac{N}{20}$ to $\frac{N}{30}$ saline. From $\frac{N}{30}$ to $\frac{N}{45}$ or $\frac{N}{50}$ it is somewhat gradual, while from $\frac{N}{50}$ upwards it increases very slightly with the higher dilutions.

1. *Bio-Chemical Journal*, Vol. IV, p. 59, 1909.

2. *Ibid.*, Vol. III, p. 97, 1907.

FIG. 1.—The curve of haemolysis of human blood with hypotonic NaCl solution, the curve being drawn by estimating the amount of haemoglobin in 20 cb. mm. of the supernatant fluid from the centrifuged mixture.

The fact that some of the erythrocytes haemolyse with higher dilutions of saline than others leads to the conclusion that either they are less permeable to water or they can bear the tension of distension from osmosis better than others, and therefore do not rupture so readily. I have, however, already pointed out that osmosis and rupture alone cannot explain the whole phenomenon of haemolysis with hypotonic saline solutions, and that one has to take the question of mass action into consideration in explaining it¹. The presence of erythrocytes containing partially discharged haemoglobin among the sediment corpuscles goes against the theory of rupture.

The relation of the amount of haemoglobin in the resistant corpuscles to the total amount in the sample of blood under examination appears to me from observations in health and disease to have an important bearing, and I would suggest that this be called the *relative haemoglobin-value of the resistant erythrocytes*. It may be expressed as the quotient obtained by dividing the amount of haemoglobin in the resistant corpuscles by that of the total blood.

The method by which I estimated the amount of haemoglobin in the resistant corpuscles is described as follows:—In all cases the blood was haemolysed with two parts of $\frac{N}{50}$ saline solution, with which in the case of healthy individuals Wright's haemolytic point is with certainty obtained. After thoroughly mixing 5 cb.mm. of the blood with 10 cb.mm. of $\frac{N}{50}$ saline, the mixture is centrifugalised as thoroughly as possible, and then the sediment is washed several times with $\frac{N}{10}$ saline till the supernatant fluid at the top is perfectly colourless. The sediment is now dissolved in a small quantity of distilled water with the addition of a drop or two of chloroform, and then the amount of haemoglobin is estimated by a Haldane's haemoglobinometer. In those cases in which the amount of haemoglobin in the resistant corpuscles is less than 10 per cent., 10 or 20 cb.mm. of blood is taken and then treated with 20 or 40 cb.mm. of $\frac{N}{50}$ saline respectively, and the amount of haemoglobin in the resistant corpuscles is then estimated. This number divided by two or four, as the case may be, gives the amount of haemoglobin in the resistant corpuscles of 5 cb.mm. of blood.

The accompanying table gives the relative haemoglobin-value of the resistant corpuscles in the blood of some of my students as well as in some cases of anaemia in my wards:—

1. *Loc. cit.*

TABLE I.—HEALTH

Haemoglobin in 5 cb.mm. of blood		Haemoglobin in the Resistant Corpuscles in 5 cb.mm. of blood		Relative Haemo- globin-value of the Resistant Corpuscles
95	...	32	...	0.336
96	...	40	...	0.416
96	...	40	...	0.416
92	...	36	...	0.391
98	...	35	...	0.357
110	...	48	...	0.436
96	...	42	...	0.437
92	...	34	...	0.369

ANAEMIA

TABLE II.

Haemoglobin in 5 cb.mm. of blood		Haemoglobin in the Resistant Corpuscles in 5 cb.mm. of blood		Relative Haemo- globin-value of the Resistant Corpuscles
40	...	8	...	0.200
30	...	4	...	0.133
35	...	10	...	0.285
36	...	10	...	0.277

TABLE III

Haemoglobin in 5 cb.mm. of blood		Haemoglobin in the Resistant Corpuscles in 5 cb.mm. of blood		Relative Haemo- globin-value of the Resistant erythrocytes
60	...	21	...	0.350
46	...	19	...	0.413
30	...	14	...	0.467
36	...	15	...	0.417

TABLE IV

Haemoglobin in 5 cb.mm. of blood		Haemoglobin in the Resistant Corpuscles in 5 cb.mm. of blood		Relative Haemo- globin-value of the Resistant erythrocytes
35	...	20	...	0.571
42	...	23	...	0.547

It will be seen from the above tables that while in health the relative haemoglobin-value of the erythrocytes varies within small limits; in anaemia it varies within much wider limits. Thus, in some cases, it is

much below the normal, in others it is almost the same as normal, while in others again it is above the normal. In kala-azar it is generally below the normal, while in ankylostomiasis it is above the normal. The forms of anaemia in which this value is increased or diminished and its clinical significance can only be determined by further investigation.

PERMEABILITY OF THE ERYTHROCYTES TO WATER AS A FACTOR IN THE PRODUCTION OF HAEMOLYSIS

An explanation may here be offered as to the cause of the differences of the haemoglobin-value of the resistant corpuscles in health and disease. It is possible that the resistant corpuscles are less permeable to water or can bear the tension of distension better than those that haemolyse. This permeability, or the power of resisting rupture, is probably altered in anaemia, being increased in some and diminished in others, while in others again it remains normal.

The entrance of water into the erythrocytes may, therefore, to some extent, be dependent upon their specific permeability, and this may be independent of the force of osmosis. So, also, their power of resisting rupture from distension after the entrance of water into their substance may vary in the different erythrocytes. That these are important factors in the phenomenon of haemolysis is borne out by the following facts:— If one part of human blood is mixed with one part of $\frac{N}{10}$ saline solution and then treated with two parts of $\frac{N}{80}$ saline, we find that the amount of haemolysis is much greater than when the $\frac{N}{10}$ saline contains 1 per cent. formol. The presence of formol cannot in any way change the concentration of the salts in the corpuscles, and its action must result either in increasing the resistance of the erythrocytes to rupture from osmotic distension or diminishing the permeability of water. Similarly, again, when blood is allowed to crenate between the slides for twenty-four hours or more, and then treated with $\frac{N}{10}$ saline solution, we find that some of them still remain crenated. Now, if crenation were simply due to osmosis, then the corpuscles would swell up and lose their crenation by re-absorption of water when treated with $\frac{N}{10}$ saline solution. The fact that some of them do not lose their crenation shows that they have become less permeable to water. In other words, along with crenation the outer portion of the erythrocytes undergo some changes, as a result

of which they do not allow the free passage of water into their structure by the process of osmosis. The same is also borne out by the fact that crenated corpuscles sometimes occur in the blood in some forms of anaemia. This cannot be due to any stronger concentration of saline in the serum, as in anaemia the chlorides are not much appreciably increased in the serum (see Tables IV and V).

TABLE V

	1	2	3	4	5	6	7	8	Average
Salinity of) Normal Serum)	0.6435%	0.5850%	0.6435%	0.7020%	0.7020%	0.6435%	0.7020%	0.7020%	= 0.6654%

TABLE VI

	1	2	3	4	5	6	Average
Salinity of) Serum in Anaemia)	0.7605 %	0.5850 %	0.6727 %	0.6727 %	0.6435 %	0.8772 %	= 0.7019 %