XXVII. THE RELATION BETWEEN THE NUMBER AND SIZE OF RED CORPUSCLES AND THE OPACITY OF THEIR SUSPENSIONS.

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IN a recent investigation on the standardisation of vaccines [1921], the author was led to observe that a constant could be obtained for each kind of organism, which was not only useful in determining the number of bacteria per cubic centimetre of a suspension, but whose reciprocal was also a measure of the average size of the organism. It seemed advisable therefore to study more fully the influence of number and size of particles on the opacity of their suspension. Bacteria however did not lend themselves to exact methods owing to their small and varying size and shape. Mammalian red corpuscles, on the other hand, being much larger and of much more constant shape lent themselves very well to the proposed work, especially as reliable data had already been published as to their size and shape. For the purpose of this paper then, the red corpuscles of the animals quoted below were used.

Animal		Diameter of red corpuscles	Authority
Sheep	...	5.0 μ	Vierordt [1888]
Rabbit	...	6.9 μ	Vierordt [1888]
Guinea-pig	...	7.2 μ	Gulliver [1875]
Man	...	7.5 μ	—

As the medium in which the corpuscles were suspended was a solution of sodium chloride, it was first sought to determine the effect of varying concentration of saline on the opacity of a suspension of red corpuscles of constant concentration. The red corpuscles used were those of the sheep and were "washed" by the method commonly adopted in preparing them for the Wassermann reaction. This was as follows. Freshly collected sheep's blood having been defibrinated by shaking with glass beads, the red corpuscles were then powerfully centrifuged for 10 minutes. The supernatant defibrinated plasma was then decanted, replaced by 0.85 % sodium chloride, and the resulting suspension again centrifuged for 10 minutes. Decantation and renewal of saline, followed by a third centrifuging lasting half an hour, completed the process. When a suspension of corpuscles was required for experimental purposes the supernatant saline was decanted from the centrifuge tube and replaced by physiologically normal saline.

A suspension of washed sheep's corpuscles having been prepared and diluted with normal saline until the opacimeter reading was a fairly low one of about 50, the correct reading of this was then accurately determined by means of the apparatus recently described by the author [1921, Part I]. With this stock suspension the effect of endosmosis and exosmosis was measured. For testing the effect of endosmosis the stock suspension was diluted in a volumetric flask with varying amounts of hypotonic saline, and the opacity of each dilution determined and expressed as a percentage of the opacity of the stock suspension.

The results are collected together in Table I.

Table I. *Effect of endosmosis on the opacity of a suspension of red corpuscles.*

Concentration of NaCl %	Opacity of suspension expressed as a percentage of the stock suspension
0.85	100
0.799	89.3
0.765	81.6
0.731	64.1

The effect of exosmosis was determined by adding varying amounts of hypertonic saline to the stock suspension, a volumetric flask being used as before. The opacities of the suspensions having been determined, the results were again expressed as a percentage of the opacity of the stock solution. The results are shown in Table II and the curve is shown in Fig. 1.

Table II. *Effect of exosmosis on the opacity of a suspension of red corpuscles.*

Concentration of NaCl %	Opacity of suspension expressed as a percentage of the stock suspension
0.85	100
0.956	99.4
1.488	95.5
2.550	87.2
3.613	79.3
4.675	71.2

On plotting the opacity of the suspension against the concentration of sodium chloride, the curves are seen to be straight lines but sloping in opposite directions and at different angles from a point which represents the isotonic strength of sodium chloride. The two lines represent separate and distinct processes, which may be explained as follows. As a result of endosmosis, some of the red corpuscles are broken up, with a corresponding reduction in opacity. The reduction in opacity is proportional to the reduction in strength of the sodium chloride. In the case of exosmosis the corpuscles shrink, and the shrinkage is proportional to the excess of sodium chloride used. This steady diminution in size of the red corpuscles with increasing strength of the saline above the isotonic point produces a corresponding diminution of the

opacity. The curve representing the action of varying concentration of sodium chloride on the opacity of a given suspension of red corpuscles consists then of two parts, one limb showing the change in opacity due to the change in the number of corpuscles, the other limb showing the change in opacity due to change in size of the corpuscles, and the junction of the two lines represents very sharply the point at which there is no change either in the number or size of the corpuscles, *i.e.* the isotonic point. The effect of endosmosis is much more marked than that of exosmosis.

Fig. 1.

In the second series of experiments the concentration of the saline was maintained constant at 0.85 %, while the size of the corpuscle was made to vary by using the blood of the animals quoted above, "washed" corpuscles being used in each case. It was found that for each type of red cell the curve obtained on plotting the concentration of the red cells against the opacity of their suspension was a straight line, and the equation was of the simple order $T = kC$, where T = opacity of the suspension, C = number of red cells per cubic millimetre as determined by the haemocytometer, and k = constant which represents the theoretical opacity produced by one red cell per cubic millimetre. To obtain as accurate a value of k as possible, a suspension of red

cells was diluted a known number of times so as to produce a field in the haemocytometer showing about five or six corpuscles per square, and the whole field of four hundred squares then counted. At the same time the original suspension was diluted a known number of times so as to produce a convenient reading of about one hundred in the opacimeter, and the opacity accurately determined. The opacity and the cell count of the original suspension were then calculated and the ratio determined. In this way the following constants were obtained.

Animal	Constant k
Sheep ...	4.29×10^{-6}
Rabbit ...	8.21×10^{-6}
Guinea-pig ...	8.86×10^{-6}
Man ...	9.50×10^{-6}

Once the constant k has been determined for each animal, the number of red cells per cubic millimetre is readily and accurately determined from the opacity by the equation $C = \frac{T}{k}$. Evidently the constant k is related to the size of the red corpuscles. So far as could be ascertained by watching with the microscope when the corpuscles fall under gravity in a specially made capillary tube whose walls were flat, the path taken was in general not vertical but sloping away from the vertical. The amount of light scattered by each corpuscle therefore may be taken as proportional to the incident light coming through the horizontal plane of projection of the area of an inclined circle whose diameter is equal to that of the cell, and therefore proportional to the area of the circle itself. The areas corresponding to the corpuscles examined were as follows:

Animal	Proportional area of red corpuscles
Sheep ...	$19.64\mu^2$
Rabbit ...	$37.39\mu^2$
Guinea-pig ...	$40.72\mu^2$
Man ...	$44.17\mu^2$

Bearing in mind that the constant k represents the turbidity produced by one corpuscle per cubic millimetre, it was decided to determine the turbidity proportional to $1\mu^2$ (one millionth of a square millimetre), *i.e.* the following fraction was evaluated $\frac{k}{\pi r^2}$, where r = radius of red corpuscles. The results were as follows:

Animal	Opacity proportional to $1\mu^2$ of surface of red corpuscle per cm. of suspension = $k/\text{proportional area}$
Sheep ...	0.218×10^{-8}
Rabbit ...	0.220×10^{-8}
Guinea-pig ...	0.218×10^{-8}
Man ...	0.215×10^{-8}
Average ...	$0.218 \times 10^{-8} = a$

Obviously then the opacity produced by one millionth of a square millimetre of the surface of a red corpuscle per cubic millimetre of suspension was the

same for all the types of cell examined. This constant α appears to be a characteristic of the mammalian red corpuscle in general.

Since

$$\begin{aligned}\frac{k}{\pi r^2} &= \alpha, \\ \therefore r &= \sqrt{\frac{k}{\pi \alpha}} \\ &= \sqrt{\frac{T}{\pi \alpha C}} \\ &= \sqrt{\frac{1}{\pi \alpha CD}},\end{aligned}$$

where D = opacimeter reading.

$$\therefore \text{Diameter} = 2 \sqrt{\frac{1}{\pi \alpha CD}}.$$

$$\text{Evaluating} \quad 2 \sqrt{\frac{1}{\pi \alpha}} = 2.402 \times 10^3,$$

$$\therefore \text{Diameter} = \frac{2.402 \times 10^3}{\sqrt{CD}}.$$

To obtain the average diameter of a mammalian red corpuscle in suspension in physiologically normal saline it is sufficient to make a cell count and to determine the opacimeter reading of the suspension at the end-point.

Again, since

$$\begin{aligned}\frac{k}{\pi r^2} &= \alpha, \\ k &= \pi \alpha r^2 \\ &= \frac{1}{4} \pi \alpha d^2,\end{aligned}$$

where d = diameter of corpuscle in μ .

$$\text{Evaluating } \frac{1}{4} \pi \alpha = 0.171 \times 10^{-6},$$

$$k = 0.171 d^2 \times 10^{-6}.$$

Therefore the constant for other mammalian red corpuscles may readily be calculated from the published data for their diameters. Since the opacity produced by one millionth of a square millimetre ($1 \mu^2$) of the surface of a red corpuscle per cubic millimetre of suspension is constant for the mammalian cell, it follows that the opacity of a suspension of red corpuscles is a measure of the total surface of such cells and, by inference, that opacity in general of a liquid is a measure of the total surface of the particles therein scattering light. Opacity does not measure the total surface of all matter present, because ions and molecules which are very small relative to the wave length of light, do not scatter light. Hence this peculiar property of opacity measurements should be useful in studying the course of reactions in which solute becomes amicros, amicros become submicros, and submicros become micros, or *vice versa*. Hence also in those reactions in physiology and pathology in which surface phenomena play a leading part, such as enzyme reactions and the Wassermann reaction, measurements of opacity should be of more value in elucidating the underlying phenomena than measurements of the quantity of material.

SUMMARY.

1. Endosmosis and exosmosis may be measured by means of the opacimeter.
2. By plotting the results of endosmosis and exosmosis the isotonic point may be accurately determined.
3. The opacity-concentration curve of a suspension of red corpuscles is a straight line with the equation $T = kC$, where T = opacity, C = concentration of corpuscles per cmm., and k = constant, which varies with the animal, and is a measure of the size of the red corpuscles.
4. The number of red corpuscles in a suspension can be readily and accurately determined from the opacity.
5. The average size of mammalian red corpuscles can be determined from the equation, diameter = $\frac{2.402 \times 10^3}{\sqrt{CD}}$, where C = number of cells per cmm. and D = opacimeter reading. The diameter of the corpuscle is inversely proportional to the square root of the product of the cell count and opacimeter reading.
6. The opacity constant k for any given mammalian red corpuscle, that is, the theoretical turbidity produced by one such corpuscle per cubic millimetre of suspension, can be determined from the equation $k = 0.171d^2 \times 10^{-6}$, where d is the diameter of the corpuscle.
7. The opacity of a suspension of red corpuscles is a measure of the total surface of the suspended particles.
8. In those physiological and pathological reactions where surface action plays a predominating part, opacity determinations offer a more strict measure of the changes taking place than number of particles.

REFERENCES.

- Gulliver (1875). *Proc. Zoolog. Soc.* 474.
 Holker (1921). *J. Path. Bact.* (In the Press.)
 — (1921). *Biochem. J.* 15. 216.
 Vierordt (1888). *Anatomische, physiologische und physikalische Daten und Tabellen zum Gebrauche für Mediciner*, 99.