

THE ELECTRIC IMPEDANCE OF HEMOLYZED SUSPENSIONS OF MAMMALIAN ERYTHROCYTES

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Determinations of the complex impedance (measured in terms of resistance and capacitance) have already provided a means of characterizing the surface of the red corpuscle (1). This paper is concerned with an extension of this method to a study of hemolysis.

EXPERIMENTAL PROCEDURE

The impedance of a suspension of cells may be represented as a resistance and a parallel capacitance which, per centimeter cube of suspension, are referred to as the resistivity R (ohms-cm.) and the capacity C (micromicrofarads-cm.⁻¹) of the suspension.

The values of R and C at frequencies between 1/4 and 2000 kilocycles per second were measured with a Wheatstone bridge, using a substitution method (1). The electrolytic cell containing the corpuscles is replaced by a similar cell filled with a salt solution of the same resistance and connected in parallel to a condenser (of negligible inductance). The function of the bridge is solely to indicate the existence of electrical equivalence in the two cases. The capacitance of the suspension is obtained by adding the static capacitance of the comparison cell to the capacitance of the parallel condenser. The static capacitance of the comparison cell equals

$$\frac{D}{4\pi K} \cdot \frac{9}{10} = \frac{7.1}{K} \mu\mu F$$

where D is the dielectric constant of water (79 at the experimental temperature of 21.4°C.), and K is the cell constant.

The same procedure of substitution was used from 2000 to 16,000 kilocycles/sec., but a resonance method was used instead of the bridge to indicate the existence of equivalence.

The electrolytic cells are cylindrical with one electrode mounted on a micrometer screw which allows a fine adjustment of the resistance of the comparison cell to be made. The electrodes are of platinum and coated with platinum black to

decrease the polarization. At the lowest frequencies, this polarization at the electrodes may be appreciable. Its influence is eliminated by making measurements at two different distances of the electrodes. The influence of the polarization is the smaller the further the electrodes are apart. In certain cases, an electrolytic cell was used where the maximum electrode separation was 20 cm. The area was 10 sq. cm. and the volume about 300 cc.

Normal Corpuscles

At low frequencies the resistivity of the red corpuscle is very high compared to that of the serum,¹ and measurements of the complex impedance indicate that this high resistivity is derived from the surface of the corpuscle, while its interior is composed of a fluid having a resistivity and a dielectric constant not greatly different from that of normal serum.² The surface of the corpuscle acts as an electric condenser with a rather small power loss.

The values of C and R , for blood of the rabbit, are shown in Fig. 1 as functions of the frequency. At the lower frequencies C and R are also shown in a magnified scale. Our earlier measurements (4) did not go below 4 kilocycles and from these it appeared that C and R were constant at the lower frequencies. The present extension of the frequency range down to $\frac{1}{4}$ kilocycle shows, however, that this is not the case, but that a rise in C and R occurs at the lowest frequencies.

A unit surface of the corpuscle may be represented as a complex impedance, the parallel components of which are the surface capacity C_m (micromicrofarads/cm.²) and the surface resistivity R_m (ohms/cm²).

The phase angle θ , which is always small, is given by $\tan \theta = \frac{1}{2\pi n C_m R_m}$

where n is the frequency of the alternating current.

¹ This may be concluded from the excellent agreement between the observed value of the resistance of a suspension of red corpuscles, and that calculated theoretically (2) assuming infinite resistivity of the corpuscles. More direct evidence is obtained by measuring the resistance of a dense mass of cells, packed by centrifugation. Values of the resistance from 30 to 40 times the resistance of the serum are then obtained. The temperature coefficient of this resistance (measured between 0 and 37°C.) is found to be the same as that of the serum, which suggests that to a great extent this resistance is due to the serum still left between the corpuscles.

² The resistivity of the interior fluid is about twice that of the serum (3) (rabbit, sheep, and chicken).

From the observed values of C and R , C_m and R_m may theoretically be derived if we know the resistivities and dielectric constants of the inter- and intracellular fluids, and the form and volume concentration of the corpuscles. A discussion of this problem will be given at another place. For the spherical form, a theory has been worked out by

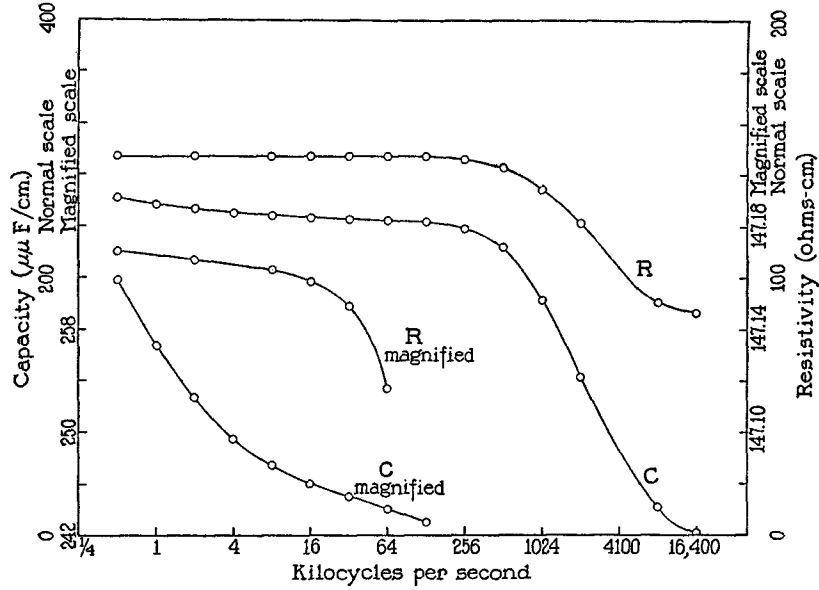


FIG. 1. Resistivity (R) and capacity (C) for a suspension of normal rabbit corpuscles.

Cole(5). At sufficiently low frequencies the following formulae are valid:

$$C_m = \frac{2/3 (C - C_s) (1 + \frac{1}{2} \rho)^2}{\alpha a \rho} \tag{1}$$

$$\frac{1}{R_m} = \frac{2/3 (1/R - 1/R_s) (1 + \frac{1}{2} \rho)^2}{\alpha a \rho} \tag{2}$$

where $2a$ is the major axis of the corpuscle, α a constant dependent on the form of the corpuscle, and ρ the volume concentration. The value of α is not known, but we have carried through the calculation by assuming the corpuscle to be equivalent to a sphere, in which case

$\alpha = 1.50$, taking as the diameter $2a$ of this sphere the average of the three major axes of the corpuscle. For the dimensions, data given by Ponder (6) have been used.

The terms C_s and R_s are respectively the capacity and the resistivity of the suspension which would be observed if the dielectric constant and conductance of the corpuscles were negligible. The dielectric constant and the resistivity of the serum were measured at frequencies from $\frac{1}{4}$ to 2000 kilocycles and found to be independent of the frequency. Therefore, C_s and R_s are also independent of the frequency, or, expressed in a different way, no part of the frequency variation of C and R for the blood is derived from the serum.

The value of C_s is given by:

$$C_s = \frac{D \cdot \frac{R_1}{R_o}}{4 \pi} \cdot \frac{9}{10} = 7.1 \frac{R_1}{R_o} \mu\mu F \quad (3)$$

where D is the dielectric constant of the serum, (which may with sufficient accuracy be taken as equal to that of water, $D = 79$), and where $\frac{R_1}{R_o}$ is the ratio of the resistivity of the intercellular fluid to the low frequency resistivity of the suspension.

The value of R_s probably would be the resistivity of the suspension at zero frequency. The value is not known so absolute values of $1/R_m$ cannot be calculated. Since R_s is independent of the frequency, we can, however, give a graphical representation of $1/R_m$ with an arbitrary zero point.

Graphical representations of C_m and $1/R_m$, (which are absolute values) as obtained from equations (1) and (2), are given in Fig. 2. The constant value of C_m , obtained at the higher frequencies, is 1.10 ± 10 per cent $\mu F/cm.^2$ and within the limits given, this value is reproducible and the same for corpuscles of rabbit and sheep, in serum, plasma, or saline. In order to obtain the absolute values of C_m and $1/R_m$ we need to know the size and form of the corpuscle. This constitutes a limitation to the use of these absolute values as characteristics of the cell surface. It is therefore fortunate that at low frequencies relative values which express the way in which C_m and $1/R_m$ vary with the frequency can be obtained independently of the form and

size of the corpuscles, since by (1) and (2) such values are given by $(C - C_s)$ and $(1/R - 1/R_s)$, respectively. Curves in which these values are plotted accordingly afford the most practicable way in which the present method can be used to characterize the corpuscle surface. Since C_s is small and constant, these relative values of C_m are practically given by the values of C . At the higher frequencies the calculation of even relative values involves the form of the corpuscle. Owing to the peculiarity of the form, an exact theory would be difficult, but

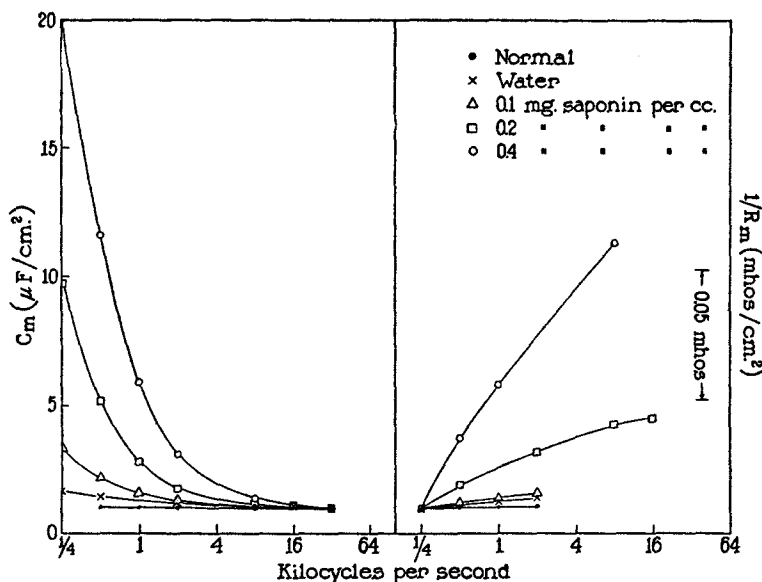


FIG. 2. Surface conductivity ($1/R_m$) and surface capacity (C_m) for normal and hemolyzed rabbit corpuscles.

calculations have been carried out under simplifying assumptions as to the form. These show that C_m and R_m remain constant or very nearly so at high frequencies. The value of θ is small at all frequencies.

For a tentative interpretation of the surface impedance of the red corpuscle, it may be assumed that the latter is surrounded by a membrane which has a small selective ionic permeability. When placed in an electric field, it therefore shows a small conductivity, due to the small ionic transport across it, and a polarization due to the difference in permeability to anions and cations. At high frequencies, the in-

fluence of this polarization is negligible,³ and the value of C_m is then the static capacitance of the membrane, and $1/R_m$ represents the conductivity due to the ionic movement of the ions in the membrane, as well as that due to any dielectric loss present. Since dielectric constants generally depend on the frequency, and since a dielectric loss is probably present, neither C_m nor R_m could be expected to remain constant at high frequencies. However, a more exact method of calculating C_m and R_m is required before the expected decrease can be established. At low frequencies the influence of the polarization becomes appreciable, leading to an increase of C_m and R_m , as observed.⁴

Hemolysis

General.—In applying these methods to a study of hemolysis, we have used suspensions of red corpuscles hemolyzed with water, with various chemical lysins (including saponin, complement and amboceptor, sodium taurocholate, and digitonin) and by freezing and thawing. Rabbit corpuscles were used throughout except in the experiments with complement and amboceptor, which were carried out with sheep corpuscles. Measurements have been made on completely hemolyzed suspensions only. It has not been found possible to obtain significant results for partially hemolyzed suspensions, owing to a marked dependence of the values of C and R on stirring.

In Fig. 3 are shown the curves for C and R obtained for a suspension of rabbit corpuscles hemolyzed by adding three parts of water to one

³ Compare with observations on polarization at metal electrodes (7).

⁴ (Added February, 1935.) Recent work has shown that there may be another explanation of the rise of C and R at low frequencies. Since the red corpuscle migrates in an electric field, it must be charged and therefore must have a diffuse ionic double layer at its surface. Some small part of the conductance of a cell suspension must be derived from this double layer, this being what is generally referred to as surface conductance. On theoretical grounds we should expect the electric current passing through the double layer to become polarized and this polarization would result in an increase of C and R as the frequency decreases. Such increases have actually been observed in suspensions of non-conducting particles such as rouge, sulfur, and paraffin oil. While the increase in C and R , at low frequencies, may be of this origin in the case of normal corpuscles, in the meantime it still seems to us reasonable to suppose that the still greater increase of C and R observed in the case of hemolyzed corpuscles, is due to an increased permeability of the cell membrane, as stated in the text.

part of packed corpuscles. The large value of C and the general similarity of these curves to those for normal corpuscles give evidence of the presence of "cells" in the hemolyzed suspension. A difference from normal corpuscles is shown by the more pronounced rise of R and C at the lower frequencies.

For the theoretical interpretation, the determination of the resistivity of the intercellular fluid of the hemolyzed suspension is required. The intercellular fluid cannot be separated by centrifugation,

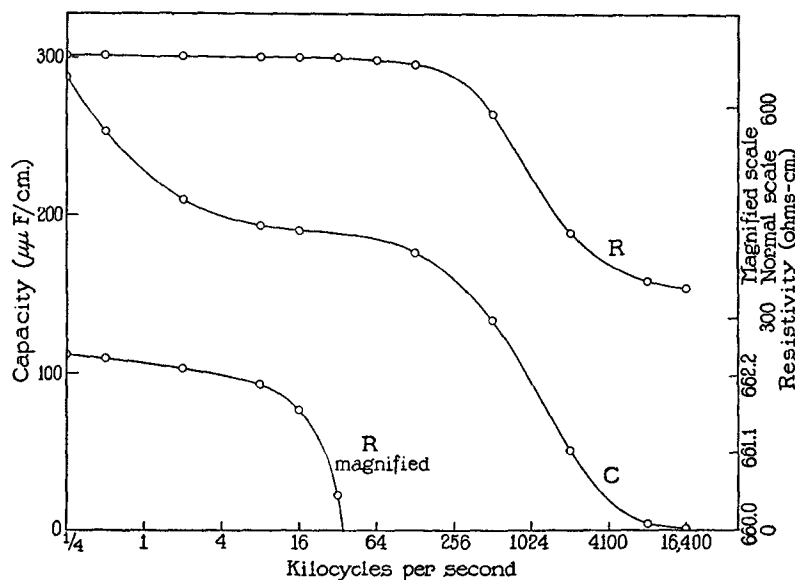


FIG. 3. Resistivity (R) and capacity (C) for a suspension of rabbit corpuscles hemolyzed by adding three parts of water to one part of packed corpuscles.

but the following two indirect methods have been used for the determination:

1. At the frequency of 16,000 kilocycles the resistivity of the hemolyzed suspension becomes nearly constant (Fig. 3). By an extrapolation the resistivity at "infinite frequency" is determined. At infinite frequency, the impedance of the cell membranes is negligible and therefore this extrapolated value, R_{∞} , is the resistivity which would be obtained at low frequency if all the cell membranes could be removed without any other change taking place. If we make the reasonable

assumption that after hemolysis the intercellular and intracellular fluids are identical, it follows that R_{∞} gives their resistivity.

2. The addition of sufficient saponin to the suspension completely destroys the membranes of the cells, leaving a homogeneous fluid, as will be shown later. With a suitable correction for the influence of the added saponin, the resistivity, R_{sap} , of this fluid should equal that of the intercellular and intracellular fluids.

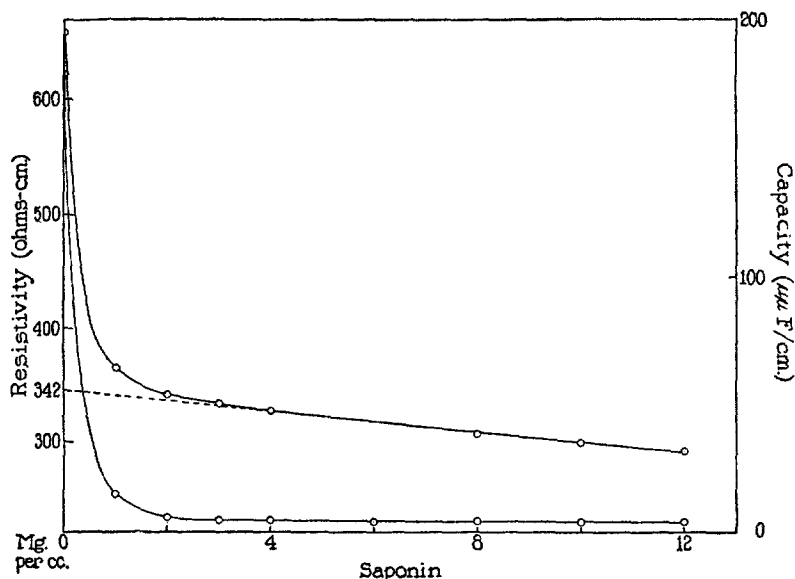


FIG. 4. Stromatolysis of the hemolyzed suspension of Fig. 3 with saponin, in order to obtain the resistivity of its suspending fluid. Resistivity (R) and capacity (C) as functions of saponin concentration.

The values obtained by these two methods are found to be in satisfactory agreement. The average value is used as the resistivity of the intercellular fluid.

The procedure may be illustrated by the results on the suspension to which Fig. 3 refers. For R_{∞} , the value $R = 340$ ohms is obtained. In Fig. 4 are shown C and R , at 128 kilocycles, as functions of the amount of saponin added (given in milligrams per cubic centimeter of suspension). The destruction of the cell membranes is indicated by the decrease of C and R . When more than 4 mg. of saponin have been added, R changes slowly and at a constant rate, and C is very

nearly zero. At the same time, it is found that R and C become independent of the frequency. (The small final value of C of about $7 \mu\mu F$ corresponds to a dielectric constant nearly equal to that for water.) These facts indicate that complete destruction of the membranes has taken place. Extrapolating the straight part of the curve for R back to zero concentration of saponin, the value $R_{sap} = 342$ ohms is obtained. This value is in good agreement with the value for $R_\infty = 340$ ohms. The average $R_1 = 341$ ohms is taken as the value for the resistivity of the intercellular fluid.

We now make use of the following formula (2),

$$\frac{\frac{R_1}{R} - 1}{\frac{R_1}{R} + X} = \rho \frac{\frac{R_1}{R_2} - 1}{\frac{R_1}{R_2} + X} \quad (4)$$

where R , R_1 , and R_2 are the resistivities of the suspension, suspending fluid, and suspended cells respectively, where X depends on R_2/R_1 and on the form of the cells, and where ρ is the volume concentration. The form of the hemolyzed cells is unknown, but there is evidence that they change to the spherical form just before hemolysis occurs (6), and although we do not know that they retain this form, we have assumed them to do so, giving $X = 2$. The error introduced, even with a considerable deviation from the spherical form, is comparatively small.

We introduce now

$$\rho_o = 2\rho \frac{1 - \frac{R_1}{R_2}}{\frac{R_1}{R_2} + 2} \quad (5)$$

where R_2 is the resistivity of the hemolyzed cell at low frequencies. We term ρ_o the equivalent non-conducting volume concentration. It is the true value of the volume concentration only if $R_2 = \infty$, that is, if the hemolyzed corpuscles are non-conducting at low frequencies. The value of ρ_o is determined from

$$\rho_o = 2 \frac{1 - \frac{R_1}{R_o}}{\frac{R_1}{R_o} + 2} \quad (6)$$

which is obtained by substituting (5) in (4) with $X = 2$ and where R_o is the low frequency and practically constant resistivity of the suspension.

The value of ρ_o is most conveniently expressed as a fractional part of ρ_1 , where ρ_1 is the volume concentration of the original suspension. As an example of the calculation of ρ_o/ρ_1 , consider again the suspension of Fig. 3. The low frequency resistivity of the suspension is $R_o = 662$ ohms. The resistivity of the suspending fluid was determined above to be $R_1 = 341$ ohms. Therefore, from (6), $\rho_o = 38.5$ per cent. The packed suspension, from which the hemolyzed suspension was prepared, was found, from its electrical resistance, to have a volume concentration of 95 per cent. The addition of water in the relation 1:3 reduces this volume concentration to $\rho_1 = 23.8$ per cent, giving $\rho_o/\rho_1 = 1.61$.

Water.—Several experiments were carried out in which the volumes of water added to produce lysis were varied from three to nineteen parts of water for each part of packed corpuscles. The values of C and R showed no change with time, during observations extending over several hours. No measurements could be made, however, until a few minutes after hemolysis was complete. Values of ρ_o/ρ_1 between 1.45 and 1.65 were obtained, with no apparent dependence on the volume of water used. The average was $\rho_o/\rho_1 = 1.54$. This factor gives a reasonable value for the swelling which the corpuscle would undergo before hemolysis takes place. This agreement shows that the resistivity (at low frequencies) of the hemolyzed corpuscles is high as compared with that of the suspending fluid. Since we do not know either the exact volume of the hemolyzed corpuscles or their form, an accurate determination of the resistivity of the hemolyzed corpuscles is not possible by this method.

The calculation of the surface resistivity R_m and the surface capacity C_m of the hemolyzed corpuscles is carried out by the same method as used for normal corpuscles. For this purpose we assume that the hemolyzed corpuscle is spherical, giving $\alpha = 1.50$, and that its volume is ρ_o/ρ_1 times that of the normal corpuscle, which for rabbit is taken to be $57 \mu^3$ (6). From the volume, the diameter is determined. The dielectric constant of the inter- and intracellular fluids is taken as equal to that of water, $D = 79$.

In Fig. 2 C_m and $1/R_m$ are given as functions of the frequency. The values show no systematic dependence on the amount of water used to produce lysis, and the curves represent averages of all experiments. Since the values of $1/R_m$ can be expressed only as differences from an undetermined constant, the curves for $1/R_m$, for water, as well as for the other lysins used, have arbitrarily been made to agree with the one for normal corpuscles, at 250 cycles/sec. The constant value of C_m , obtained at high frequencies, is the same as that found for normal corpuscles, which shows that lysis produces no change in the static capacitance of the membrane. The injury to the corpuscles due to the lysis is shown by the changes in C_m and $1/R_m$, at low frequencies. These changes are presumably due to an increased ionic permeability of the membrane, and it may be worth noting that since the lysis produces a change in the frequency dependence of C_m and $1/R_m$, this increased permeability cannot be due to a rupture of the membrane, as has sometimes been proposed, but must be due to an actual change in the membrane itself.

Saponin.—An important difference between osmotic lysis and chemical lysis is shown by the fact that in the latter case, C and R do not become constant until a considerable time after hemolysis is complete, during which period both decrease. This may be seen from Fig. 5 which shows C and R , measured at 128 kilocycles, plotted against the time, after the addition of 0.1 mg./cc. of saponin to a 39 per cent suspension of rabbit corpuscles in saline. The initial increase of R is due to the release of the hemoglobin and gives a graphical representation of the course of the lysis. This is complete in 8 minutes. C and R do not become constant until 30 minutes later. As the concentration of saponin is increased, these final values of C and R decrease until, with the addition of sufficient saponin (compare Fig. 4), R reaches a final low value, while C reaches the low value (about $7 \mu\mu F$) characteristic of a homogeneous aqueous solution. At the same time R (as well as C) becomes independent of the frequency. The curves in Fig. 6 represent these final values of C and R , as functions of the frequency, for suspensions of rabbit corpuscles in saline, hemolyzed with three different quantities of saponin. The original volume concentration of the suspension was in each case 39 per cent, and in a suspension of this concentration 0.02 mg. of saponin

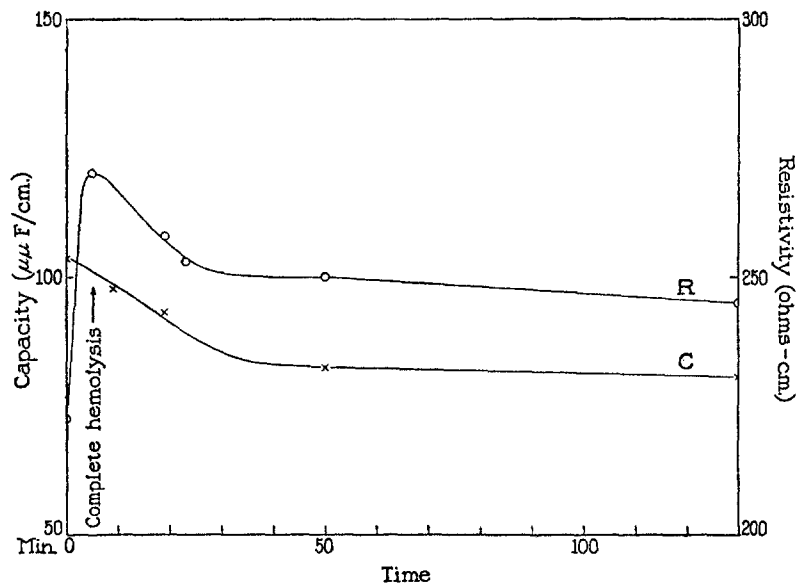


FIG. 5. Resistivity (R) and capacity (C) as functions of time after the addition of 0.1 mg./cc. of saponin to a 39 per cent suspension of normal rabbit corpuscles in saline.

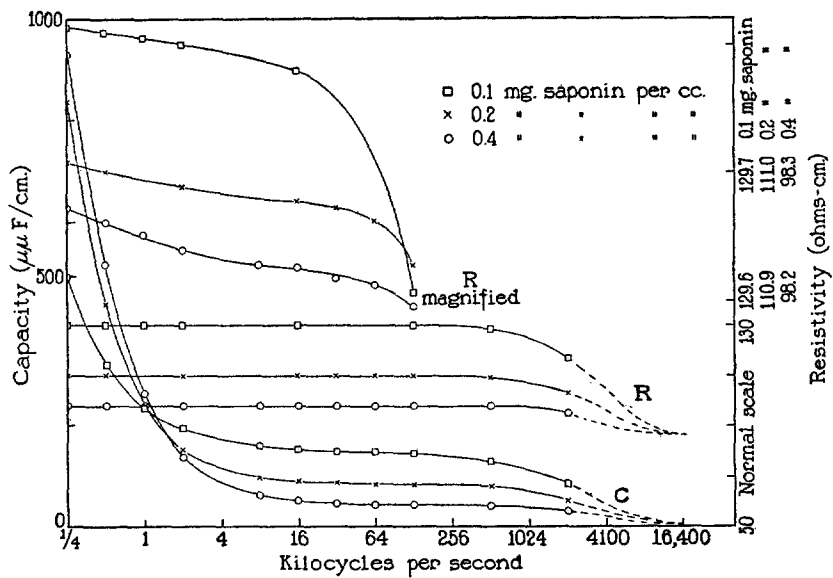


FIG. 6. Resistivity (R) and capacity (C) for suspensions of rabbit corpuscles hemolyzed by the addition of saponin.

per cc. of suspension is required to produce 50 per cent hemolysis. The form of the curves, at low frequencies, shows evidence of injury to the membranes of the corpuscles of the same type as that found after osmotic lysis, and to an increasing extent as the concentration of the saponin increases.

From the facts given it follows that with a large amount of saponin the membranes of all the corpuscles become completely permeable, the reasonable inference being that disintegration has taken place. For the case of the suspensions to which saponin has been added in more moderate concentrations, it is found most satisfactory to assume that the decrease of C and R , which takes place after lysis, is due to the disintegration of the membranes of a certain number of the corpuscles while the remainder are injured to a slight extent only, retaining in particular a high resistance, as compared with that of the intercellular fluid. These remaining cells, as regards our measurements, are the only "effective" cells of the suspension. As the concentration of saponin is increased, the number of disintegrated corpuscles increases, until with sufficient saponin, all corpuscles are so affected. The essential point which is brought out by this evidence is that as the injury proceeds, the permeability of the membrane of the corpuscle does not increase steadily, but the injury ends with a kind of all-or-none process, during which the membrane passes from a state of high to one of negligible resistance.

Since it is assumed that the resistivity of the effective cells is high as compared with that of the intercellular fluid, the values of C_m and $1/R_m$ can be calculated by the method used for normal corpuscles. For this purpose we assume the corpuscles to be spherical, giving $\alpha = 1.50$, and we assume furthermore that their volume is the same as it was originally. By these assumptions, the diameter $2a$ is determined. The graphical representations of C_m and $1/R_m$, given in Fig. 2, are for the suspensions, for which data are given in Fig. 6. The curves confirm the statement made as to the general similarity of the injury produced by saponin and by water. The fact that the constant value of C_m , at high frequencies, is the same as that for the normal corpuscle, lends justification to the theoretical treatment used.

It may be assumed that the disintegration of the membranes of the corpuscles is related to the gradual disappearance of ghosts noted

when increasing concentrations of saponin are added to a corpuscle suspension, and usually referred to as stromatolysis. Although the two phenomena may not be identical, we may for the present designate them by the same term.

The volume of the stromatolyzed corpuscles, as a percentage of the original volume of the corpuscles, is given by $100(1 - \rho_0/\rho_1)$. We refer to this term as the "percentage stromatolysis." Fig. 7 shows

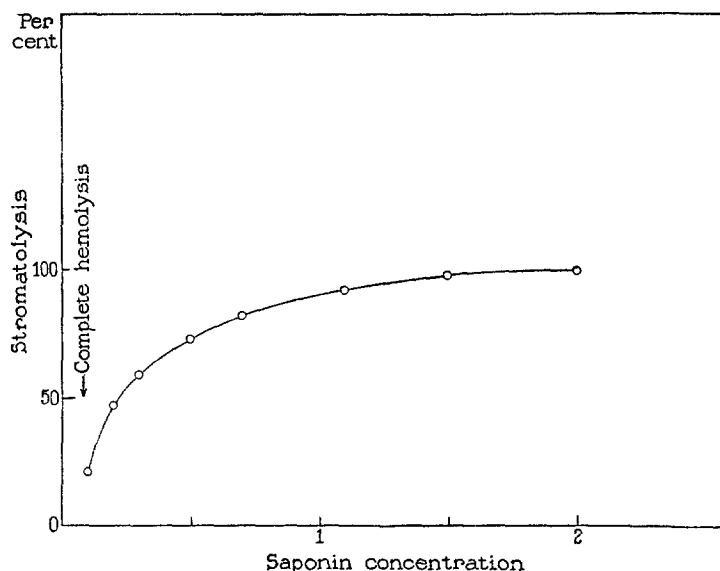


FIG. 7. Percentage stromatolysis as a function of saponin concentration for a 40 per cent suspension of rabbit corpuscles in saline.

the percentage stromatolysis as a function of the concentration of saponin, for a 40 per cent suspension of rabbit corpuscles in saline.

Complement and Amboceptor; Sodium Taurocholate; Digitonin.—The results obtained are in general the same as those for saponin. Expressing the quantity of a lysin in terms of that required to produce 50 per cent lysis, it is found that the stromatolysis produced with complement and amboceptor is considerably less than that produced with the corresponding quantity of saponin. Stewart (8) noted that the electric resistance of a suspension of red corpuscles

hemolyzed with saponin was less than when lysis was produced with complement and amboceptor, and drew from this observation the conclusion that the pigment and the electrolytes were liberated each independently of the other. This conclusion is not justified since it does not consider the difference in stromatolysis, and we have found no indication of any such differential liberation of pigments and electrolytes.

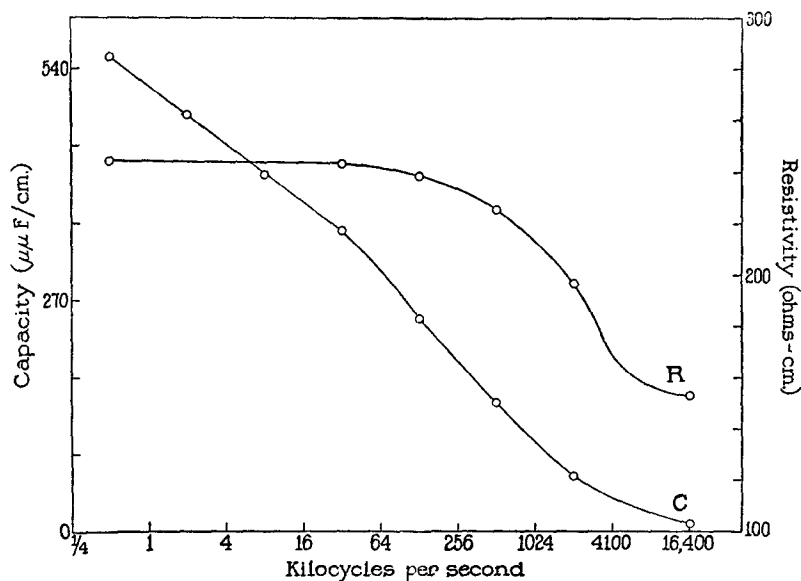


FIG. 8. Resistivity (R) and capacity (C) for a suspension of rabbit corpuscles hemolyzed by freezing and thawing four times with carbon dioxide snow.

Freezing and Thawing.—In Fig. 8 are given C and R , as functions of the frequency, for a 74 per cent suspension of rabbit corpuscles, frozen and thawed four times with carbon dioxide snow. The value of $\rho_o/\rho_1 = 0.43$. Repetition of the freezing leaves the general form of the curves for C and R unchanged, but the value of ρ_o/ρ_1 decreases. We are doubtful about the interpretation of these curves, and have not attempted to derive the values of C_m and $1/R_m$. The form of the curve for C appears to indicate a distinct difference between lysis produced by freezing and thawing and by the other lysins used.

SUMMARY

This paper is concerned with the changes in the electric surface capacity and surface resistivity of the membrane surrounding the mammalian red corpuscle, as a result of various types of hemolysis. In the case of hemolysis with water, the cells swell with no apparent change in the electric properties of the membrane. They then hemolyze, but the membrane persists, although showing evidence of injury, as indicated by a change in the frequency dependence of its capacity and resistivity at low frequencies. The fact that a change of the frequency dependence takes place shows that the injury cannot be due merely to a rupture in the membrane, but must be due to changes in the properties (increased permeability) of the membrane as a whole.

With chemical lysins (saponin, complement and amboceptor, digitonin, sodium taurocholate) a similar type of injury to the membranes of a certain number of the corpuscles takes place, to an increasing extent as the concentration of lysin is increased. The rest of the corpuscles become completely permeable to the electric current, and as the amount of lysin is increased, this number of completely permeable corpuscles increases until all are affected. This change, presumably associated with a disintegration of the corpuscle membrane, is referred to as stromatolysis, and the method gives a quantitative means of determining percentage stromatolysis.

For lysis by freezing and thawing, the results obtained indicate this type of lysis to be different from that of the others studied.

In its earlier stages, this work was carried out in collaboration with Dr. H. Goldblatt of Western Reserve University. For the greater part, we have had the benefit of the active interest of Dr. E. Ponder of this laboratory.

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