ELECTRIC IMPEDANCE OF INJURED AND SENSITIZED RED BLOOD CORPUSCLES

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INTRODUCTION

In a recent paper on the electric impedance of hemolyzed suspensions of mammalian erythrocytes (Fricke and Curtis, 1935a), it was shown that even after a red cell is hemolyzed it still offers a high resistance to the passage of an electric current, or, in other words, that the membrane is still relatively impermeable. It was further shown that for normal cells the membrane capacity and resistivity at low frequency increase only slightly with decreasing frequency, while they change quite rapidly with the frequency in the hemolyzed suspensions. Moreover, in the case of a chemical lysin such as saponin the increase becomes more marked as stromatolysis proceeds.

It was pointed out that a polarization capacity due to selective ionic permeability could account for this increase and the more marked increase in the case of the hemolyzed cells could be explained as due to an increased permeability. However, another explanation was advanced, namely, that the increase might be due to a polarization of the current passing through the double layer, such as has been observed in aqueous suspensions of non-conducting particles like powdered glass or paraffin oil (Fricke and Curtis, 1935b). On this hypothesis, the change on hemolysis would be due to some sort of a change in the double layer. The former of these two possibilities seemed the most likely.

Thus it appeared as though the electrical method presented a new way to measure changes in the cell membrane. In order to test this, it was decided to make a series of measurements on red cell suspensions which had been treated in various ways to produce changes in the...
membrane, to see if the low frequency variation of capacity is affected by any of these changes.

EXPERIMENTAL PROCEDURE

The method of measurement was the same as that previously described (Fricke and Curtis, 1935a) except that the measuring cells were of a different size. The ones used were 20 cm. long and had a cross-sectional area of 1.3 cm.² The method consists essentially in using a Wheatstone bridge to indicate the electrical equivalence of a measuring cell filled with the suspension and an exactly similar cell filled with a salt solution with a variable air condenser in parallel to it. The resistance of the suspension is then the resistance of the salt solution and its capacity is the capacity of the condenser plus the static capacity of the comparison cell. The greatest single error in such measurements at low frequencies is due to electrode polarization. Because of this and other errors which will be discussed in a subsequent paper, it has not been found possible to make these measurements with sufficient accuracy for the present purpose at frequencies below 2 kilocycles per second.

Rabbit blood or washed rabbit cells was used in all the experiments. To obtain lecithinated cells, just enough lecithin was added to the plasma to make the cells completely spherical. The complete change of shape presumably corresponds to some important change at the cell surface (Ponder, 1936). In the case of systems containing glucose, the cells were suspended in 5.6 per cent glucose; their permeability is presumably modified in such a medium, for they lose salts (Joel, 1915, Kerr, 1929), and they may eventually hemolyze (Yeager, 1929). In the case of saponin, half as much saponin was added as would cause hemolysis in 30 minutes. In the colloidal silicic acid, amboceptor, and tannic acid systems, enough sensitizing agent was added in each case to sensitize the cells completely to guinea pig complement, but not enough to cause agglutination. It is not known whether permeability changes are associated with sensitization or not, but the nature of the cell surface must be considerably modified. In no case was there any hemolysis at the end of the experiment.

RESULTS

The results are shown in Fig. 1, the membrane capacity in \( \mu \text{f. per cm.}^2 \) being plotted against the frequency. Since the absolute value of the membrane capacity cannot be measured with as great an accuracy as the variation of this capacity with the frequency, and since we are interested primarily in this variation, the capacity values have arbitrarily been made to agree at 32 kilocycles. There is no discernible systematic variation in the absolute values.

It will be seen that the largest variation is exhibited by the leci-
thinned corpuscles, but even here it is somewhat doubtful if the experimental accuracy is good enough to enable us to say definitely that the increase is greater than that for normal cells. The dotted curve is a reproduction of a typical curve obtained for a suspension of hemolyzed red cells, and is included to give an idea of the relatively enormous increases encountered in these cases, and to show that the present increases do not in any way approach them.

It was not possible in all cases to measure the variation of resistivity with frequency, but where such measurements were made it was found that, within the experimental error, the variation was the same as for normal cells.

**Figure 1.** Membrane capacity vs. log frequency for normal, injured, and sensitized red cells. The dotted curve represents hemolyzed red cells.
The results show conclusively that there is no marked increase in the frequency variation of the membrane capacity of rabbit red cells when they are "injured" or sensitized up to the point of hemolysis or agglutination. While it is not possible to state exactly what sort of changes in the membrane have taken place in each case, it can certainly be said that marked changes in permeability have taken place at least in the cases of glucose and saponin, and in all cases the membrane is far from being unchanged. This, therefore, is not a sensitive method for measuring changes in membrane properties, although a prolonged and careful investigation might show a statistical difference between normal and injured cells.

The result may mean that the form of the frequency variation is an extremely insensitive measure of permeability and other membrane changes, and capable only of disclosing the very great changes associated with hemolysis, or it may mean that the change in the frequency variation at low frequencies has nothing to do with permeability, as has been already pointed out.

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SUMMARY

On the basis of previous work on the electrical properties of hemolyzed red cells, it might be supposed that the variation of the capacity with frequency at low frequencies is an indication of membrane permeability. To test this, rabbit red cells were subjected to treatment with lecithin, tannic acid, glucose, saponin, amboceptor, and colloidal silicic acid, each in sub-lytic doses. No change in any of the electrical properties of any of the suspensions could be detected. The result may mean that the form of the frequency variation is an extremely insensitive measure of permeability and other membrane changes, and capable only of disclosing the very great changes associated with hemolysis, or it may mean that the change in the frequency variation at low frequencies has nothing to do with permeability.
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