LXXXII. THE CHANGES IN CONDUCTIVITY OF RED CELL SUSPENSIONS DURING HAEMOLYSIS.

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(Received May 27th, 1925.)

SINCE Stewart [1899] first described the changes occurring in suspensions of cells subjected to the action of lysins, numerous investigators have applied his methods, with various modifications, to the same and similar subjects. With the application of the methods to the changes occurring in suspensions of erythrocytes we are particularly concerned, for some of the findings vary from each other, as well as from the results obtained by other methods.

The available methods for studying conductivity are three in number—the Kohlrausch method, the rotating commutator method of MacGregor, and the method of the ballistic galvanometer. The last is only of use as a final resort in certain special cases, since the effects of polarisation are not excluded.

The Kohlrausch method as used by Stewart in his original researches is open to grave objection. In the first place, the use of heavily platinised electrodes is a disadvantage as well as a necessity, for considerable error may be introduced when solutions containing substances such as the serum proteins or saponin are employed, since they may be deposited on the electrodes, thereby altering the resistance. This difficulty is overcome by Brooks [1923] who uses blank electrodes, and obtains the necessary improvement in the telephone minimum by the insertion of a variometer in the bridge circuit, as was also done by Osterhout and others. The method suffers from the disadvantage that it is dependent on the observing of a minimum of sound produced by the exact position of a sliding contact, interpolations between the effects produced by two different positions of the slider being impossible.

The remaining method avoids these faults. The electrodes do not need to be platinised, though platinised electrodes which have been heated have an advantage over polished electrodes; provided the commutator is run at a fair speed, polarisation is as completely eliminated as in the Kohlrausch method, galvanometer readings replace the subjective determination of a sound-minimum, and interpolation to any desired degree of accuracy is possible.

With the selection of a suitable electrical apparatus for the measurement of the conductivities the difficulties do not end, for it is necessary that the cell-containing fluids shall be such as will lend themselves to accurate measurement. Stewart used defibrinated blood as the basis of his experiments, the

cells being thus suspended in serum. This is not an ideal suspension medium for conductivity work; the cells are too numerous, rouleaux are present, sedimentation is rapid, the suspension is thick and difficult to remove from the electrodes, and the presence of the serum introduces fallacies, not only by the contained proteins inhibiting some forms of lysis, and by their tendency to be adsorbed on the electrodes, but because of their liability to spontaneous alterations of conductivity [Paoli, 1922]. Better results are obtained from suspensions of cells in saline, in which these fallacies are not encountered. The effect of any one of the above-mentioned factors in occasioning error may be small, but their added effects very great indeed.

Details of the apparatus employed, which was specially made to reduce these disturbing influences to a minimum, are given below.

The Rotating Commutator. One great advantage of the rotating commutator method is that, since the telephone is replaced by a galvanometer, the exact balancing resistance of the Wheatstone bridge can be interpolated from the deflections observed when resistances on either side of it are inserted. The galvanometer may be of any desired sensitiveness or the deflection for 1 ohm difference may be varied by alteration of the voltage. Moreover, the fact that a series of observations on both sides of the zero should give consistent results affords a valuable check.

The method was first described by MacGregor, was employed by Fitzgerald, and later by Whetham. The earlier forms of commutator were not very satisfactory in use, and several years ago one of us devised a pattern which obviated the defects. This model was shown at the Royal Society of Edinburgh, and at the International Congress of Physiology in Edinburgh, 1922. A brief description may be given. An ebonite cylinder of 10 cm. diameter and 3.5 cm. length is mounted on a ball-bearing axle. On the periphery are mounted a number of brass plates, moderately tapered, so that the gaps between them are wider at the one end of the cylinder. The drum now in use has 22 such plates, although for certain purposes the number should be a multiple of 4. After being fixed the plates are divided, giving in effect two coaxially mounted cylinders with the corresponding tapered plates all accurately oriented. Two concentric brass rings are let into each end of the cylinder, and the plates connected alternately to the outer and inner rings of the corresponding end. Contact with the plates and rings is made by brushes of fine brass wire or thin brass sheet. The brushes on the plates are placed so that when one is in contact with one plate, the other is in contact with a plate of the alternate series, so that, on rotation of the drum, a direct current applied to the rings is taken off by the plate brushes as an alternating current. This is led to the conductivity apparatus, from which it is taken back to the other end of the drum, where it is reconverted to a direct current and is led to the galvanometer. The end with the broader plates is used as the alternator, the object of the taper being to secure that the galvanometer contacts are made after, and broken before, the corresponding contacts on the alternator.

The Conductivity Cell. The cell used for these determinations was made for the special purpose in view. It consists of a glass tube of 6 mm. bore, the small platinum electrodes being placed vertically with their lower edges about 10 mm. from the bottom of the tube; with 1 cc. of liquid in the cell, the level of the liquid is 10 mm. above the tops of the electrodes. The part of the tube where the electrodes and their side tubes are situated is slightly blown out, so that a pipette can be passed to the bottom of the cell without contact with the electrodes. By these means, irregularities due to settling of suspensions on the electrodes and to sedimentation are reduced to a minimum. The electrodes should be grey, very slightly platinised.

Method. The conductivity cell is immersed in a thermostat, a large rectangular glass vessel electrically heated and controlled, the contents of which are stirred by a current of air. The temperature in these experiments was maintained at 25°.

In place of a bridge with a sliding contact, two resistance boxes are used. It is a convenience to keep the one box constant throughout and of the same resistance as the box in the cell circuit, so that the resistance of the cell, when balanced, is the same as that of the second box. The exact balancing resistance, to fractions of 1 ohm, is obtained by interpolations from the galvanometer deflections. The latter, of course, can be varied at will by altering the voltage applied.

The galvanometer employed is a Paul unipivot instrument of 732 ω , with a centre zero.

One very necessary precaution is to be mentioned. When the liquid in the cell is changed to another of different conductivity, the electrodes must be soaked repeatedly—some three or four times—with fresh portions of the fluid. If this is not done, errors of a magnitude greater than the differences one requires to measure may be introduced, and failure to carry out this soaking may completely invalidate results.

RESULTS.

We propose to present the results as a series of illustrative experiments, representative of a large number. It may be well to point out at the beginning the object of the experiments. As a result of some of the earlier work, it is generally held that conductivity measurements of cell suspensions at various stages of lysis by various haemolysins show that the haemoglobin and the electrolyte of the red cell may be liberated independently of each other, and that, in some cases, the pigment may escape while the electrolyte is retained. Such results are used to support the idea that the haemoglobin and electrolyte are combined to a cell-stroma, the existence of which, however, is made doubtful by other researches. The accuracy of this early work is scarcely sufficient for conclusions of such importance to be based upon it; we have therefore repeated the fundamental experiments with the superior methods at our disposal, in order to find to what extent the usually accepted facts are

correct, as well as to see if any alternative explanation of the observations can be offered.

1. *Effect of saponin on saline.*

	κ
Normal saline ...	0.01515
Plus saponin ...	0.01517

The amount of saponin added was about 5 mg.; the saponin used being Merck's purest. The effect on the conductivity of the saline is seen to be negligible.

2. *Effect of addition of water to cell suspension.*

The cell suspension used in this and other experiments was prepared by suspending the washed cells from 1 cc. of human blood in 5 cc. of normal saline (0.85 % NaCl). Such a suspension is of convenient strength, but it is to be noted that it contains fewer cells than the defibrinated blood used by Stewart in his work. Serum proteins are, of course, absent. The conductivity of this suspension was first taken alone, after which a known volume of water was added in order to produce laking of the cells. The conductivity was then again measured, and thereafter saponin (about 5 mg.) added to bring about complete lysis. Again the conductivity was ascertained:

	κ	
Normal saline	0.015180	
Plus 2 vols. H ₂ O	0.005572	
Suspension	0.013600	
Plus 2 vols. H ₂ O	0.005325	Lysis 51 %
Plus saponin	0.005648	

It is clear that as some of the cells haemolyse, electrolyte passes out of them along with the haemoglobin, for the diluted suspension, had no lysis occurred, should have given $\kappa = 0.004991$. All the electrolyte does not escape from the cells of the suspension, as shown by the further increase of conductivity on the addition of saponin, which produces complete lysis; this is simply because only about half the cells were laked by the two volumes of water. On the other hand, the lysis of the first 50 % of the cells by the water gives very nearly the same increase of conductivity as does the lysis of the second 50 % by the saponin, and there appears no evidence that electrolyte is retained in the haemolysed cell, and that it is not liberated as freely as is the pigment by the action of the water. Stewart does not seem to have taken into account the fact that, on his additions of various amounts of water to the cells, only a certain percentage haemolysis must have resulted, and when he says that it is possible, by the addition of a large quantity of water to bring out of the corpuscles as great a quantity of electrolyte as by any other method of laking employed, *e.g.* saponin, it would appear that the facts might be equally correctly accounted for by saying that a sufficiently large quantity of water will lake as many cells as will saponin, and therefore liberate as much electro-

Take this similar experiment:

3. *Saponin haemolysis.*

4. Complement-amboceptor haemolysis.

This is not dissimilar to the findings of Stewart. The lysis of cells by the complement-amboceptor system results in a very small change of conductivity—an increase of 0.00017—whilst the addition of saponin to the completely laked cells gives a further increase of 0.00087. On Stewart's hypothesis, this fact would be explained by a liberation of electrolyte from the ghosts by the

saponin, it being considered that the passage of haemoglobin from the cells is not accompanied by the passage of any large portion of the electrolyte.

It is, however, possible that the saponin has some effect on the liberated haemoglobin. Stewart was unable to find evidence for the existence of a compound, split by dilution, between haemoglobin and electrolyte, but there may be such a compound nevertheless, and it may be split by saponin. It has been shown that the addition of saponin to saline produces no appreciable change of conductivity; it now remains to be shown whether or not the addition of saponin to haemoglobin, as derived from laked cells, produces any alteration.

The cells of the suspension used were laked by the addition of an appropriate amount of complement and amboceptor, and the laked fluid passed through a Berkefeld filter. In the filtrate, it may be taken that no ghosts were present; apart from dilution, on the other hand, the condition of the haemoglobin and the electrolyte would be unaltered. To this filtrate saponin was added, with the following result:

Filtrate	...	...	...	κ	0.004864
Plus saponin	...	...	...		0.004961

This is an increase of about 2 %. It is to be noted that the concentration of this filtrate, both in electrolyte and in haemoglobin, is considerably less than that of the laked fluid resulting from the complement-amboceptor haemolysis, the concentration in the case of the filtrate being about one-third of that of the latter fluid; the 2 % alteration in conductivity on the addition of saponin is thus a very considerable one. Assuming, as appears necessary, that there is a compound between the haemoglobin and electrolyte, which is partly or completely broken down by the action of saponin, the change of conductivity in the fluid derived from complement-amboceptor haemolysis on the addition of saponin can be explained quite well without the necessity of postulating the liberation of electrolyte from haemolysed ghosts, for when we take into account the dilution of the filtrate, there is no difficulty in arriving at the 8 % alteration in the complement-amboceptor experiment.

This dilution, moreover, may explain why the alteration in the filtrate is as small as it is (2 %), for the dilution of the haemolysed fluid by water appears to be responsible for the splitting off of a certain amount of the electrolyte from the haemoglobin. In (2) above, the addition of saponin to cells partly laked by water does not appear to do more than lake the remaining cells—there is no necessity to call in an action on the haemoglobin to account for the final alteration in conductivity, presumably because the very addition of water has accomplished the greater part of the splitting. We do not wish, however, to lay stress on this point, for the differences are small; on the other hand, they are large enough to warrant attention being called to them, and to justify the suggestion that the true explanation of the results lies in there being a compound between haemoglobin and electrolyte, which compound is broken down, with the liberation of electrolyte, either by dilution with water, or by the addition of saponin.

5. *Heat haemolysis.*

We have been unable to confirm Stewart's results for the change of conductivity of heated suspensions, since we have found that heating suspensions or defibrinated blood to the temperature he mentions results in the formation of a thick fluid full of precipitate, and quite impossible to subject to accurate measurement. Even heating to much lower temperatures gives solutions which are very unsatisfactory for conductivity work. In this connection, however, one point may be recorded. The heating of a filtrate of laked cells is sufficient to change its conductivity appreciably, as the following figures show:

Filtrate	...	...	...	^K 0.004864
Heated to 60°	...	...	...	0.004941

This is nearly a 2 % difference, and as it occurs with a dilute solution of haemoglobin, the changes occurring in defibrinated blood or in a concentrated cell suspension are such as cannot be ignored.

In this paper we have purposely omitted to make freezing-point measurements, as these are generally admitted to be very unreliable in experiments of this sort, and as such measurements, owing to their inferior accuracy, are of little value in checking conductivity estimations. We have also purposely ignored the points made by Brooks [1925] in his theoretical paper on the subject; not that we do not agree entirely with his conclusions, but because they do not affect the essential points which we wish to emphasise.

In order to enable anyone to convert the specific conductivities in which these results are expressed into actual resistances, we give the cell constant as 0.6976.

CONCLUSIONS.

1. The liberation of electrolyte from red cells during haemolysis by water is such as may be explained by each cell giving up the electrolyte contained in it, liberation of electrolyte being proportional to the percentage number of cells haemolysed, and complete when haemolysis is complete.

2. In the case of complement-amboceptor haemolysis, the full amount of electrolyte does not appear to be liberated. This is due to some of the electrolyte passing out of the cells combined with haemoglobin, from which it can be split off by the addition of saponin, and also, to some extent, by heating.

3. There appears no necessity to postulate that electrolyte is retained in any quantity in the haemolysed cells, after the liberation of haemoglobin.

This research was carried out during the tenure of a Crighton Research Scholarship by one of us (E. P.). The expenses were defrayed by a grant from the Royal Society.

REFERENCES.

- Brooks (1923). *J. Gen. Physiol.* 5, 365.
 — (1925). *J. Gen. Physiol.* 8, 349.
 Paoli (1922). *Colloid Chemistry of the Proteins.*
 Stewart (1899). *J. Physiol.* 24, 211.