LXVI. STUDIES ON THE KINETICS OF HAEMOLYTIC SYSTEMS, I.

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By a method fully described elsewhere [1926] it is possible to express the resistance of many haemolytic systems, as compared with many others, in terms of an easily determined constant R , together with a small additional constant k . This method has already been shown to be applicable to haemolytic systems containing acids, certain bases, a large number of the sugars, and many neutral salts—all substances which either accelerate or inhibit saponin or bile salt haemolysis [Ponder and Kennedy, 1926; Kennedy, 1926]. The method is very general, and in this paper we shall apply it to three problems in the kinetics of haemolytic systems: (1) to the measurement of the resistance of the cells of different individuals to lysis, (2) to the measurement of the resistance of the cells of different animals to lysis, and (3) to the effect of varying the strength of the cell suspension in haemolytic experiments. The first two problems are of frequent occurrence and often referred to in the literature; the third is of first importance in connection with the theory of the kinetics of these simple haemolytic systems.

1. THE RESISTANCE OF THE CELLS OF DIFFERENT INDIVIDUALS.

In order to find the variation in the resistance to saponin lysis of the cells of normal persons, the cells of fifty different persons were examined, the procedure being in every case as follows. From an arm vein about 2 cc. of blood is withdrawn into sufficient oxalate to prevent coagulation. Of this, 1 cc. is carefully measured into four centrifuge tubes, each containing about 10 cc. of 1.5 % sodium citrate. The tubes are centrifuged, the supernatant fluid pipetted off, and the cells washed twice with 0.85 % NaCl. After the last washing, the fluid is removed, and the cells suspended in 20 cc. of 0.85 % NaCl. This constitutes the suspension for the experiments. If one can work rapidly, it is possible to withdraw the blood without the use of oxalate and then to distribute the 1 cc. into the four tubes. This alternative procedure gives the same result as that involving the use of an anti-coagulant, and is in some respects preferable. An arbitrary standard suspension is also prepared in exactly the same way. In this series of experiments the standard was made from the blood of the investigator. The time-dilution curves for saponin

acting at 25° on the suspension to be examined, and also on the standard suspension, are then plotted in the usual way. To these curves is then applied the method of analysis previously described [Ponder, 1926], and c_1 plotted against c_2 . The result is always a straight line passing, as nearly as can be judged, through the origin. Its equation is $c_1 = Rc_2$, where R measures the resistance of the suspension examined in terms of that of the standard. The method is very simple in practice, although perhaps a little difficult to grasp at first, and has the advantage that it gives the relative resistances of the two suspensions for all dilutions of lysin, and not for one arbitrarily selected dilution, as do other methods.

In the following table is shown the number of cases in which values of R , ranging from 0.8 to 1.15, were found. It may be noted that if R is less than 1, the resistance of the suspension to which it refers is less than that of the standard, while if R is greater than 1 the resistance is greater in that proportion; when $R = 1$, the suspension is identical with the standard.

Value of R	No. of cases	Value of R	No. of cases
0.80	1	1.00	13
0.85	3	1.05	9
0.90	7	1.10	2
0.95	14	1.15	1

Fig. 1.

When plotted, these results give a frequency polygon as in Fig. 1. The polygon is roughly symmetrical, with a mean in the groups $R = 0.95$, $R = 1.0$. From this may be deduced that the arbitrary standard is one of approximately average resistance, and that the cells of normal persons vary little in resistance from this standard. Pickering and Taylor [1923], it should be noted,

have observed certain variations in the resistance of normal cells to saponin; these variations, according to them, appear to be small, although it is difficult to interpret their results in strictly quantitative terms.

The possibility of the variations being dependent on variations in the number of cells in the suspensions will at once suggest itself. In order to deal with this point, we have made red cell counts in a number of samples of blood, for the cells of which the resistance constant R was known. Below are reproduced ten of the results as examples.

Value of R	Cell count per mm. ³	Value of R	Cell count per mm. ³
1.0	4,880,000	0.95	5,200,000
1.0	5,330,000	0.95	4,750,000
1.15	5,200,000	0.9	5,220,000
1.05	4,900,000	0.85	5,150,000
1.1	5,000,000	0.85	5,800,000

The explanation of the different resistances of the suspensions clearly does not lie in the mere cell content.

2. THE RESISTANCE OF THE CELLS OF DIFFERENT ANIMALS.

The cell suspensions were prepared as in the previous section, each suspension containing the cells, thrice washed, for 1 cc. of blood of the particular animal concerned, and suspended in 20 cc. of 0.85 % NaCl. The standard with which they were compared was a similarly prepared suspension of human cells. In each case a red cell count was carried out on the whole blood. Time-dilution curves were constructed for each suspension in the usual way, and thereafter analysed. The plotting of c_1 against c_2 in these cases gives a straight line which does not pass through the origin, but which makes an intercept on one or the other axis; the expression for this line is $c_1 = R(c_2 - k)$, where R is the resistance constant, and k a small empirical constant.

The significant figures for several animals are given below.

Cat cells		Rabbit cells		Ox cells		Pig cells		Sheep cells	
c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2
0.33	0.083	0.040	0.10	0.5	0.066	0.066	0.055	0.5	0.057
0.20	0.055	0.025	0.056	0.4	0.053	0.05	0.042	0.4	0.047
0.13	0.042	0.020	0.042	0.33	0.043	0.04	0.034	0.33	0.039
0.10	0.034	0.016	0.032						
$R=4.5$		$R=0.35$		$R=8.0$		$R=1.2$		$R=9.0$	
$k=0.012$		$k=-0.013$		$k=-0.003$		$k=0.0$		$k=0.002$	

In order of resistance, as compared with human cells, the cells of these animals, therefore, are: rabbit, 0.35; man, 1.0; pig, 1.2; cat, 4.5; ox, 8.0; and sheep, 9.0. This is the order in which they are placed by Ryvosh [1907], whose results are thus confirmed and amplified by the relative resistances being expressed as figures.

In the case of the suspension of each animal, red cell counts were carried out. The following table expresses the results, together with a calculated figure showing the relative surface of the suspension in each case.

Animal	Cells per mm. ³	Relative surface
Rabbit	5,780,000	5.37
Man	5,100,000	6.06
Pig	5,460,000	3.49
Cat	6,076,000	3.39
Ox	5,990,000	3.95
Sheep	9,041,000	3.79

It is plain that the resistance is not entirely, or even greatly, dependent on the red cell count, or on what is proportional, the number of cells in the suspension. The relative surface in the case of man and the rabbit is somewhat larger than that in the cases of the other animals; this fact will be commented upon later, and is noted here as being of importance.

It must be borne in mind that the accuracy with which the necessary curves can be obtained in an experiment such as this is considerably less than in the experiments of Section 1. This is principally due to the fact that much greater concentrations of lysin have to be used for such cells as those of the ox and sheep, and that the errors, which appear in milligrams, are correspondingly greater, but it is also due to some extent to the end-points for certain of the suspensions being peculiarly bad.

3. THE EFFECT OF VARYING THE STRENGTH OF CELL SUSPENSION.

It might be, and indeed generally is, assumed that if the strength of a cell suspension be doubled, the effect of a haemolysin upon it is halved. Such an assumption is perfectly reasonable, and is merely an expression of the elementary facts of velocity equations; in the nomenclature of this paper, it would lead to the result that an increase in the strength of a cell suspension would be accompanied by a proportional increase in R , the resistance constant. If, for example, the strength of a suspension giving $R = 1$ were doubled, the suspension of double strength would give $R = 2$. This may be put to the test of experiment.

From an arm vein 4 cc. of blood is withdrawn and distributed into four centrifuge tubes containing citrate; the cells are washed thrice with 0.85 % NaCl. They are then washed with a small quantity of saline into a 10 cc. flask, and saline added to the 10 cc. mark. This suspension, diluted eight times, is approximately of the same strength as the standard usually employed, and is used as a standard for the experiment; by suitable dilution other suspensions can be made, four times as strong as the standard, twice as strong, one-half as strong, or one-quarter as strong, as well as the standard itself. Time-dilution curves for saponin acting on each of these suspensions at 25° are plotted in the usual way. The only point necessary to note is that the end-points become somewhat difficult to determine for suspensions stronger than four times the standard, and weaker than one-third the standard.

The results are most easily summarised in Fig. 2, in which the time-dilution curves for various strengths of suspension are shown. It will be noticed at once that the strength of suspension makes comparatively little difference

to the time taken by any dilution of lysin to complete haemolysis; taking, for example, the dilution 1 in 50,000, the standard suspension is haemolysed in 3.2 minutes, a suspension one-quarter the strength of the standard in 2.2 minutes, and a suspension four times as strong as the standard in 8.5 minutes. These differences are surprisingly small.

Fig. 2.

The unexpected nature of the result becomes even more apparent when we inspect the position of the asymptotes to which the various curves approach. These positions can be determined with a considerable degree of accuracy by mere inspection, especially if care be taken to make observations on those parts of the curves which are rapidly receding to the right. The positions of the various asymptotes for the different suspensions of this experiment are shown in the following table; ∞ is the concentration of lysin at which the time for complete haemolysis becomes infinite.

Suspension compared to standard	Position of asymptote as value of ∞
4	0.0350
2	0.0285
1 (standard)	0.0255
$\frac{1}{2}$	0.0237
$\frac{1}{4}$	0.0222

If we were to apply to these curves the equation given by Arrhenius for haemolysis—an equation which is the well-known monomolecular formula for chemical reactions—we should have the positions of the asymptotes as values of x in the expression

$$kt = \log \frac{c}{c-x},$$

in which k is a velocity constant, t the time for complete lysis, and c the initial concentration of lysin. In such an expression, x , given by the position

of the asymptote, would indicate the amount of lysin used up by the cells in their haemolysis. We should then be confronted with the experimental finding that twice the number of cells use up, not twice the amount of lysin, but a very much smaller quantity; taking, for example, the suspensions one-quarter and one-half, the ratio is 1.07, and not 2.0. In view of this finding, we are entitled to conclude that the description of haemolysis by simple monomolecular formulae is an erroneous proceeding, for it leads to a result which, in terms of simple reactions, is inexplicable and at variance with experience. So far as we can find, any theory of the action of the simple lysins hitherto put forward breaks down on this point.

Fig. 3.

The result of plotting c_1 against c_2 in this experiment is interesting. Taking the suspension of quarter-strength as the new standard, we proceed as previously described [1926]. The result is shown in Fig. 3, in which the points for the suspension of half the strength of the experimental standard are omitted, as the figure otherwise becomes too crowded. The plotting results in what appear to be very good straight lines, each of which, if produced, cuts the c_1 -axis; probably these lines are really flat curves, but the experimental range prevents an investigation of their upper extremities. Each line comes to a stop at a point the coordinates of which represent the positions of the asymptotes, the c_2 coordinate giving the position of the asymptote of the standard, and the c_1 coordinate giving the position of the asymptote of

the suspension which is being compared with the standard. The lines extend upwards to infinity.

These lines obtained by an analysis of the experimental findings may be compared with the three lines (dotted) on the left of the figure, which are those which would result from this analysis if the simple monomolecular equation applied to the haemolytic phenomena. The divergence of the dotted lines from those obtained by experiment is very striking, and indicates the complete failure of the monomolecular formula to describe the results.

One additional point may be added. If suspensions stronger than four times the experimental standard be used, the end-points become very indistinct, but nevertheless it may be observed that the delay in haemolysis becomes more noticeable, and may, in the case of suspensions about ten times the strength of the standard, become very great indeed in the dilutions of lysin which lie within the ordinary experimental range. If, on the other hand, suspensions less than one-quarter of the standard are used, it will be observed that lysis takes much longer to occur than might be expected, and that, if the suspension be diluted until its addition gives but a trace of cloudiness in the tubes containing the lysin, the haemolysis of these few cells takes almost as long as does the haemolysis of a suspension of one-quarter the strength of the standard. It appears, indeed, that such a suspension provides a kind of limiting case, and that suspensions more dilute are haemolysed in no shorter a time. We state these points somewhat vaguely, because exact measurement is impossible.

It will, of course, be understood that this experiment is typical of a large number carried out; there is no difficulty in obtaining the general result, and nothing but the lack of suitable quantitative methods can have prevented it having been described before. Nor is the general result obtained with saponin alone, for it can be obtained either with the soaps, with alkalis or with sodium taurocholate as a lysin—indeed, with any simple lysin for which it is possible to construct accurate time-dilution curves. In its general nature it is not dependent on the species of cell used in the suspensions, nor would one expect it to be.

These results are obviously of first importance in connection with the theory of the action of the simple haemolysins, and may be used as a crucial test of any hypothesis put forward to explain this action. Failure of a theory to account for them in a rational way we should take to dismiss that theory from further consideration; any hypothesis which would lead to their theoretical establishment would, on the other hand, derive a very strong support from the fact.

SUMMARY.

1. The difference of resistance of the cells of normal persons to saponin haemolysis is studied, and it is shown, by quantitative methods, that the differences are small.

2. A method is described for studying the relative resistances of the cells of different animals. Quantitative results are obtained, confirming the findings of Ryvosh.

3. The effect of varying the strength of cell suspension in haemolytic experiments is studied in detail. The results show that the monomolecular formula is inapplicable to haemolysis by the simple haemolysins, for very unexpected relations are found.

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