XXXI. THE INFLUENCE OF NEUTRAL SALTS ON HAEMOLYSIS.

BY WALTER PHILLIPS KENNEDY (*Carnegie Research Fellow*).

From the Department of Physiology, University of Edinburgh.

(Received February 19th, 1926.)

IN a recent paper it was pointed out that in experiments on the kinetics of haemolysis it is of prime importance to have the tonicity of the system adjusted with strict accuracy. It has been thought that experimental evidence on this point would be desirable, and that at the same time the effects of various kations on saponin haemolysis should be investigated, since variations in the suspension medium also affect the rate of haemolysis and therefore frequently invalidate the comparison of different sets of results.

METHOD.

The method falls into two parts, the purely manipulative and the mathematical analysis. Detailed descriptions of these will be found in papers by Ponder [1926], and by Ponder and Kennedy [1926]. It is only necessary to add that the chemicals used were B.D.H. analytical reagents and that in the experiments with various ions the system consisted of 0.4 cc. blood suspension in 0.85 NaCl + 0.8 cc. of a $M/6.88$ solution of the salt to be examined + 0.8 cc. saponin in varying dilution in 0.85 % NaCl. The principal point of the analysis is that if a first time-dilution curve for a saponin-cell-NaCl system be drawn together with a second curve for a system in which another salt replaces the NaCl, then a straight line may be obtained by plotting the ordinate c_1 of the second curve against the ordinate c_2 of the first curve; and this line is represented by the equation $c_1 = R(c_2 - k)$, where R is the slope of the line and k an empirical constant, usually small. In the case of accelerations R is less than 1 and k is negative; in the case of inhibitions R is greater than 1 and k is positive. R affords a measure of the amount of acceleration or inhibition, and the values of R for the various ions can be compared legitimately as they stand, although for strictly quantitative considerations Ponder [1926] should be consulted.

It may be mentioned that there is no difficulty in obtaining these lines from the experimental results, which show negligible deviations as can be seen from the figures.

RESULTS.

Table I. *Effect of varying tonicity on saponin haemolysis.*

	Tonicity of system				
	0.85 %	0.76 %	0.69 %	0.595 %	0.51 %
<i>R</i>	1.0	1.2	1.27	1.29	1.3
<i>k</i>	0.0	0.006	0.006	0.006	0.006

Table II. *Relative effects of kations on saponin haemolysis.*

Salt added to system	p_H of salt solution	Sheep		Cat		Human		Rabbit	
		<i>R</i>	<i>k</i>	<i>R</i>	<i>k</i>	<i>R</i>	<i>k</i>	<i>R</i>	<i>k</i>
LiCl	6.3	0.9	-0.01	1.4	0.04	1.2	0.003	1.0	0.0
MgCl ₂	6.5	0.77	-0.035	0.6	-0.02	0.74	-0.0045	0.9	-0.001
NaCl	6.2	1.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0
KCl	5.58	0.85	-0.02	0.7	-0.05	0.9	-0.0015	1.0	0.0
CaCl ₂	6.34	0.77	-0.035	0.35	-0.06	0.74	-0.0035	1.4	0.004
RbCl	5.28	0.85	-0.02	0.69	-0.04	0.82	-0.001	1.0	0.0
SrCl ₂	6.24	0.85	-0.02	0.50	-0.02	0.74	-0.0035	0.9	-0.001
BaCl ₂	6.24	0.93	-0.05	0.60	-0.02	0.9	-0.0015	1.0	0.0

It will be noted from the above that *k* in the case of the sheep and cat is in general some ten times greater than in the human and rabbit. This may be connected with the greater resistance of the former to the haemolytic action of saponin. To minimise experimental error it is expedient to choose a series of dilutions of saponin such as will give end points in a time range of half to 6 or 7 minutes: in the sheep this is from 1 in 2000–10,000, in the cat 1 in 6000–25,000, in the human 1 in 20,000–60,000 and in the rabbit 1 in 50,000–120,000. Of these four animals the cat has the most satisfactory cells for haemolytic experiments, as the suspension, in this dilution, is of a beautiful rich colour and the end points are sharp; the rabbit is the least satisfactory on this score, and, owing to the greater liability of clotting, the blood is not so easy to obtain. As the p_H has a definite effect on haemolysis the solutions were tested with regard to this, but as will be seen from Table II the variations were small and quite unconnected with the results. It is obvious too that the effect on haemolysis in these systems is not connected with the molecular weight, and indeed it is not justifiable in the present state of knowledge to seek correlations between the action of the ions and their other physical properties. It is interesting to note, although no reason is assignable at the moment, that lithium is acceleratory in the sheep and inhibitory in the other cases, whilst calcium is inhibitory in the rabbit and acceleratory in the others. Two figures are given for the cat and the human showing c_1 and c_2 plotted against each other for the various salt systems. It will be seen at a glance that the action of the kations is very different. Similar plottings of the sheep and rabbit merely emphasise this still further.

These results are so much at variance with those of Höber [1917], and Höber and Nast [1914], that some discussion of these papers is requisite.

Fig. 1.

Fig. 2.

Note: the line of Mg is very near to, but not actually coincident with, that of Ca and Sr.

In the first case it may be pointed out that their methods contain certain fallacies which obscure the results. It can be readily calculated from Höber's own figures that during his experiments with saponin the cells were suspended in a medium containing serum in a concentration of not less than 1 in 600, and such a concentration in itself will produce a very considerable inhibition of haemolysis, as may be seen from the tables of Ponder [1925]. Thus it follows

that the kinetics of the haemolytic systems used were such as to be incapable of simple solution even if the exact concentration of serum were known, which was not the case. The experiments were conducted in an ice-chest, and whilst this is quite a permissible method it has the objection that it places inconvenience and experimental error at a maximum. Especially, it so lengthens the end point that no accurate time relation can be ascertained. This, however, was not attempted as the amount of haemolysis is throughout expressed in such vague terms as "weak," or "strong haemolysis," "colourless" and the like. These pseudo-quantitative measurements are of such a nature that the conclusions drawn from them are quite unreliable: even then the results are tabulated in a way which permits of considerable variety of interpretation.

Among their findings they state that variations of the saponin concentration result in a reversal of the order of ionic activity. This would imply that the time dilution curves crossed, a result which has not been met with throughout the long series of different experiments conducted by Ponder's method.

The results recorded in this paper are quite opposed to Höber's, and indeed often diametrically opposite, but they are given additional weight by the mathematical analysis which results in all cases in a straight line relationship between the curves, in which incidentally, each point is checked by that above and below it. These straight line relationships are found in the cases of many other systems, such as those containing sugars [Ponder and Kennedy, 1926], certain acids and alkalis, and other acceleratory and inhibitory substances.

SUMMARY.

The effect of hypotonicity on a cell-saponin-NaCl haemolytic system is shown to be inhibitory.

The effects of various kations in cell-saponin-salt systems are compared with a standard cell-saponin-NaCl system, and with each other. The method employed measures the resistances of the systems in terms of a constant which can readily be evaluated.

The equations applicable to these systems are identical with the general equations for inhibition and acceleration. Höber and Nast's results are not confirmed by these methods.

REFERENCES.

- Höber (1917). *Pflüger's Arch.* **166**, 531.
Höber and Nast (1914). *Biochem. Z.* **60**, 131.
Ponder (1925). *Proc. Roy. Soc. Lond. B.* **98**, 484.
— (1926). *Proc. Roy. Soc. Lond. B.* **99**, No. 699.
Ponder and Kennedy (1926). *Biochem. J.* **20**, 237.