CLIII. STUDIES ON THE KINETICS OF HAEMOLYTIC SYSTEMS.

VII. THE DISAPPEARANCE OF LYSIN DURING STROMATOLYSIS.

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THE observations to be described in this paper are related to others which have been recently published on the disappearance of lysin during the process of haemolysis. Since lysin disappears during the latter process, presumably as a result of its combining with some component of the cell envelope [Ponder, 1934, 1], it is not surprising to find that the disappearance continues after haemolysis is complete, and during that period in which the cell envelopes are being further destroyed (stromatolysis). A study of the later stages of the process, however, reveals certain unexpected relations.

METHODS.

These are an extension of methods already described [Ponder, 1934, 1] and can be summarised as follows.

A suspension of thrice washed red cells is prepared, the cells from 1 ml. of blood being finally suspended in 20 ml. of 1 % NaCl. A suspension of stromata is also prepared from the blood of the same animal, the stromata being obtained by freezing and thawing, with subsequent washing until all haemoglobin is removed. This suspension is diluted so that 0.4 ml. of it is exactly equivalent, so far as its power to combine with lysin is concerned, to 0.4 ml. of the red cell suspension.

To find that quantity of lysin, *e.g.* saponin, which has disappeared from a system at the end of any time t_1 , 0.4 ml. of the equivalent stroma suspension is allowed to react with 1.6 ml. of a given dilution of the lysin for this time at a constant temperature (30° in the following experiments, except where otherwise stated). At the end of the time t_1 , 0.4 ml. of the red cell suspension is added to the system and the time t_2 required for its complete haemolysis determined. From a suitable time-dilution curve, the quantity of lysin which corresponds to t_2 is found, and this is the amount of lysin left free in the system at time t_1 . Subtraction gives x , the quantity which has disappeared. This quantity can be found by this method for any initial concentration of lysin which is not so great as to give exceedingly rapid lysis or so small as to give values of t_2 in excess of about 60 minutes, and also for any value of t_1 , from values much less than that which would correspond to complete lysis of 0.4 ml. of red cell suspension by the lysin initially present, up to values very much greater.

A detailed description of the procedure will be found in the paper referred to above, and it is enough to say that the results to be described here were obtained

with cell and stroma suspensions made from the blood of both the rabbit and of man. The lysin used was saponin throughout, principally because its disappearance follows a regular course as haemolysis and subsequent stromatolysis proceed. Digitonin, the bile salts and the soaps, all show a rapid "initial absorption" at the cell surfaces, and only when this is complete do these lysins disappear in a regular manner; digitonin, moreover, is difficult to work with because of the high order of its time-dilution curves, and the bile salts and the soaps tend to be unstable.

EXPERIMENTAL RESULTS.

The course of the disappearance.

It can readily be shown that the disappearance of free saponin from a system containing stromata does not cease at a time corresponding to the complete lysis of an equivalent number of red cells but continues at a slower rate for a long time thereafter. This is shown in Table I and in Fig. 1.

Table I.

Initial lysin, γ	"Complete lysis"	γ lysin disappeared after				
		30 mins.	90 mins.	180 mins.	300 mins.	900 mins.
400	(30)	80	100	130	—	170
200	30	60	70	82	85	90
100	30	46	50	51	53	54
67	31	34	36	36	37	37

Fig. 1. Ordinate, γ of lysin used up; abscissa, time in mins. The scale on the abscissa has been broken up in order to show the general course of the curves. Squares, initial lysin concentration of 400 γ ; crosses 200 γ , circles 100 γ , dots 67 γ .

Table I and Fig. 1 show (a) that after the lysin and stromata have reacted for a time which would just be necessary for haemolysis of 0.4 ml. of the equivalent red cell suspension ("complete lysis"), a constant amount of lysin has disappeared from the system, in this case 30 γ [*cf.* Ponder, 1934, 1], and (b) that afterwards there is a continuous disappearance of lysin, the quantity which disappears tending to reach a final value asymptotically. The time it takes

for the final value to be approached varies with the initial concentration of the lysin, for when the latter is small the final value is reached quite rapidly, whereas when it is great the concentration of lysin can be shown to be still falling even after many hours. Further, the amount of lysin which ultimately disappears is a function of the initial concentration and an almost linear function moreover, at least within the comparatively small range in which reliable experimental values can be obtained¹. For very large values of initial lysin concentration the quantity finally removed appears to be less than would be obtained by extrapolating linearly from the values shown in Table I, but with very high lysin concentrations the errors of observation are enormous.

The essential point is that the disappearance of lysin apparently ceases while there is yet a large quantity of free lysin present in the system, and not, as has been supposed hitherto, when the concentration of free lysin has fallen to zero. It is unsafe, however, to attempt to determine the exact form of the curves in Fig. 1, for at least two reasons. (1) There is doubt regarding the reference time-dilution curve which should be used in finding how much lysin is left free in the system. During the early stages of the reaction, when the lysin is being used up rapidly, one must use a reference curve obtained by adding 0.8 ml. of cell suspension to 1.6 ml. of different dilutions of the lysin, for a system in which free lysin is energetically reacting with 0.4 ml. of stroma suspension *plus* 0.4 ml. of cell suspension is more like one in which the lysin is reacting with 0.8 ml. of cell suspension than it is like any other. When the reaction between lysin and stromata has reached equilibrium, on the other hand, the free lysin left reacts only with the added 0.4 ml. of cell suspension, and the proper reference time-dilution curve is one for 0.4 ml. of cell suspension added to 1.6 ml. of lysin *plus* 0.4 ml. of saline. The fact that one has to use one reference curve at the beginning of the reaction and another at the end introduces uncertainty into the units in which the disappearance of lysin is measured. The uncertainty may not be great, for there is not much difference between the two reference curves, but it is great enough to make a detailed analysis of the curves in Fig. 1 almost futile. (2) The results in Table I under the head "complete lysis" are those obtained at times which would be required for complete haemolysis of 0.4 ml. of the red cell suspension by the particular lysin dilution concerned. Now when lysin reacts with a red cell suspension, haemolysing it in time t , lysin is used up in reacting both with the cell envelopes (as in the case in which it reacts with a stroma suspension) and also in reacting with liberated haemoglobin; when the lysin reacts with a stroma suspension, on the other hand, there is no pigment and no inhibition, and so more lysin will be used up as a result of a combination with the cell envelopes in the same time t . It will be apparent that the quantity which has to unite with the envelopes in order to produce complete lysis cannot be found exactly unless the inhibitory effect of the liberated pigment is allowed for, and this cannot be done quantitatively in the meantime.

Although these difficulties prevent one from analysing the situation fully, there is no doubt as to the departure of the curves from the course which would be expected on the basis of existing theory, and at the same time the experimental results are sufficiently definite to allow the course of the disappearance

¹ For very large initial amounts of lysin, the times for complete haemolysis are too short to be measured exactly, and even such measurements as can be made are complicated by the mixing of the cell suspension and the lysin requiring appreciable time [v. Ponder, 1932]. For amounts less than about 67%, the times for complete lysis may be so great that no measurements can be obtained at all. The experimental range is therefore severely limited and the difficulty in obtaining results correspondingly increased.

of lysin to be discussed in a general sort of way. More specifically, it will be observed that the experimental curves are not described by the equations¹

$$dx/dt = k(c-x)^n \quad \text{.....(1),}$$

$$\text{or} \quad kt = \frac{p}{p-1} \left\{ c^{\frac{p-1}{p}} - (c-x)^{\frac{p-1}{p}} \right\} \quad \text{.....(2),}$$

where

$$p = \frac{1}{n},$$

even if the values assigned to c are those which correspond to the equilibria finally reached (the values in the last column of Table I). Fig. 2 shows an experimental and a calculated curve side by side, and it will be seen that the former

Fig. 2. An experimental curve and a calculated curve obtained from expression (2) with $n=2.0$, both approaching the same asymptote. Ordinate, x in γ ; abscissa, time in mins.

falls off much too quickly. The only simple way of accounting for its course is to suppose that the velocity constant of the reaction is continually diminishing, the reaction itself being generally described by expressions (1) and (2). The matter will be discussed more fully below.

The irreversible nature of the combination.

Since the equilibrium conditions are so different from those expected, it is important to test the assumption that the combination between lysin and cell component is irreversible. This is done in the following way.

To 1.6 ml. of a series of saponin dilutions, from about 1 in 5000 to about 1 in 20,000, is added 0.4 ml. of a stroma suspension about five times as concentrated as that used in the above experiments. The tubes are allowed to stand for several hours, at the end of which time the amount of lysin left free is determined. An exactly similar series of tubes is allowed to stand for the same length of time, at the end of which 2 ml. of saline are added to each; 2 ml. of the mixture are removed into a series of clean tubes, and the quantity of lysin left free is found by adding 0.4 ml. of cell suspension, as before. If the amount of lysin left free is exactly half that found in the case of the corresponding lysin dilution in the first series, addition of an equal volume of saline to the systems has done nothing more than dilute the free lysin, and has not caused any of the combined lysin to be liberated again.

The results given by this procedure are shown in Table II. In this experiment the stromata and lysin were allowed to react at 30° for 8 hours. All values are in γ .

¹ These equations ought to contain terms for the inhibitory effect of liberated haemoglobin on the course of the lytic reaction, but, as the introduction of these terms does not affect the general form of the expressions, the terms have been omitted. See Ponder [1932], and Ponder and Gordon [1934].

Table II.

Initial lysin concentration	Free lysin before dilution	Free lysin after dilution	Ratio
400	160.0	78.5	0.49
200	51.5	25.0	0.49
133	42.0	21.0	0.50
100	37.5	18.0	0.48

The fact that the ratio of free lysin found before and after dilution is so nearly 0.5 shows that the dilution does not cause lysin to be liberated from combination, *i.e.*, that the reaction between the cell component and the lysin is not reversible under these conditions. This is what has always been believed, and it is scarcely necessary to point out that had any other result been obtained the methods used in this and many earlier investigations would have been open to serious objection.

Temperature effects.

The failure of expressions such as equations (1) and (2) to describe the experimental results obtained during the whole course of haemolysis and subsequent stromatolysis is not altogether unexpected, for the expressions themselves have always been unsatisfactory in that they contain an arbitrary constant $n=1/p$. The values assumed by this constant under various conditions have always been puzzling, and it has already been recognised that the introduction of the constant may have no better justification than that it reconciles experimental results with a theory possibly faulty in itself [*v. Ponder*, 1934, 2]. It will suggest itself that the departure of the experimental curves (Fig. 1) from the course expected on the basis of the existing equations may throw light on the meaning of the constant n and its variations, and so I have carried out experiments which attempt to relate the behaviour of the constant to the course of the curves for the disappearance of lysin during haemolysis and stromatolysis. It happens that the easiest way in which n can be varied is by varying the temperature of the haemolytic systems [*Ponder and Yeager*, 1930].

1. The first step in such an investigation consists in showing that the concentration of the stroma suspension which is equivalent, so far as its power in reacting with lysin is concerned, to a given quantity of red cell suspension, does not itself vary with temperature. The procedure referred to under Methods, above, and already fully described [*Ponder*, 1934, 1], is repeated at 38, 22 and 7°, three temperatures associated with very different values of n for saponin systems. In each case one begins with a stroma suspension of unknown concentration (because of the variable yield in preparing it), and finds the extent to which it must be diluted so that 0.4 ml. of it is equivalent to 0.4 ml. of red cell suspension. It is necessary to select a dilution of lysin for which results can be obtained at all three temperatures, and so the lysin concentration with respect to which the determinations can be made is exceedingly limited; if the concentration is too great, lysis is so rapid at 38° that the time for complete lysis cannot be observed, and if the concentration is too small, complete lysis never occurs at 7°. This, however, is of little consequence, for the extent to which the stroma suspension has to be diluted seems to be virtually independent of the lysin concentration used to determine it. Table III shows typical results for an experiment in which the dilution of saponin was 1 in 30,000, and the cells and stromata were derived from the rabbit.

Table III.

Required dilution of stroma suspension	Temperature (° C.)
1 in 3.0	38
1 in 3.0	22
1 in 2.8	7

Allowing for experimental error, which in determinations such as these is considerable, these results show that the "equivalence" of the stroma suspension is independent of temperature.

2. The next step is to see whether the amounts of lysin which finally disappear from systems containing stromata and different initial concentrations of lysin are themselves affected by variations in temperature. This is done by repeating experiments such as shown in Table I at different temperatures, 0.4 ml. of equivalent stroma suspension being added to various dilutions of lysin, and the quantity of the latter left free after about 420 minutes being found as already described. For all practical purposes, equilibrium is reached within this time, even at 6°. Table IV shows results obtained for saponin and a rabbit stroma suspension at 38, 20 and 6°.

Table IV.

Initial lysin, γ	γ lysin disappeared after 420 mins. at		
	38°	20°	6°
670	—	420	370
500	—	300	280
400	220	230	210
333	170	190	180
250	130	140	130
200	120	120	120
100	70	70	—
67	51	49	—

Reading across the rows it will be seen that for each initial quantity of lysin the amount which finally disappears is substantially the same irrespective of the temperature, *i.e.*, the equilibrium conditions in these systems are not appreciably affected by temperature. This is interesting in view of the many theories which associate haemolysis *etc.* with an adsorption of lysin by the material of the cell envelope [Clark, 1933; Reiner, 1934], for in adsorption reactions larger amounts are usually adsorbed at lower temperatures. It may also be observed that the equilibrium conditions for the process of haemolysis, which is dependent on the value of x in expressions (1) and (2), are also scarcely affected by temperature. A study of the equilibrium conditions accordingly throws no light on the arbitrary constant n .

3. We can, however, extend the investigation by plotting the course of the curve for lysin utilisation at different temperatures, *e.g.*, at 38° and at 6°. Consider one concentration of lysis, such as 200 γ , reacting with stromata at the two temperatures, and imagine the amount of lysin x which disappears plotted on the ordinate against time t on the abscissa (as in Fig. 3). When $t=0$, $x=0$, and when $t=\infty$ both curves pass to the same final value of x (2, above). They may nevertheless pursue entirely different courses between these two points, and on each there is a point of particular interest, *viz.*, the point which corresponds to the time required for the complete lysis of a quantity of red cell suspension equivalent to the amount of stroma suspension with which the lysin is

reacting. The peculiarity of this point lies in the fact that it has already been shown that, at any one temperature, the quantity of lysin used up at the time needed to produce complete haemolysis is constant, irrespective of the initial lysin concentration [Ponder, 1934, 1]. It is therefore important to establish whether or not the amount of lysin used up at the time required for complete lysis of a red cell suspension is constant irrespective of temperature, and at first sight it would appear that this could be done by plotting the curves for the utilisation of lysin in their entirety at the two different temperatures and seeing if both curves pass through points characterised by the same value of x on the ordinate and by values of t corresponding to the times for complete lysis of 0.4 ml. of red cell suspension at the two different temperatures on the abscissa. This, however, is impracticable, for if we select a concentration of lysin which produces complete haemolysis of 0.4 ml. of red cell suspension in a reasonable time at 6°, the time for complete lysis at 38° is so short that small variations from it cannot be measured with certainty. Conversely, if we select a concentration which gives complete lysis at 38° in a time sufficiently long to allow of small variations to be satisfactorily measured, lysis at 6° is not complete even in six or eight hours. We have accordingly to change the lysin concentration when we change the temperature, and to rely on the fact that the amount of lysin utilised at the moment of complete haemolysis is constant at any one temperature, irrespective of the initial lysin concentration. The procedure is best illustrated by a typical experiment.

Taking 1.6 ml. of 1 in 100,000 saponin at 38° and adding 0.4 ml. of red cell suspension, one gets complete lysis in 11 seconds. Allowing the same amount of lysin to react with 0.4 ml. of equivalent stroma suspension for 11 seconds and then adding 0.4 ml. of cell suspension, one gets complete lysis in 42 seconds. The time required for complete lysis when 1.6 ml. of 1 in 120,000 saponin reacts with 0.8 ml. of cell suspension is 32 seconds, and that required when 1 in 160,000 saponin reacts under the same conditions is 90 seconds. Interpolating, the time of 42 seconds corresponds to a dilution of 1 in 134,000. Converting to the number of γ of lysin in the system of volume 2.4 ml., the initial amount of lysin is 40 γ , and the amount left free at the end of 11 seconds is 30 γ . The difference, x , is 10 γ .

Repeating the same experiment at 6° with a lysin dilution of 1 in 1000, complete lysis of the 0.4 ml. of cell suspension occurs in 1.0 minute. Allowing the lysin to react with 0.4 ml. of equivalent stroma suspension for 1.0 minute, and then adding 0.4 ml. of cell suspension, we get complete lysis in 1.35 minutes. This corresponds to a lysin dilution of 1 in 10,300 reacting with 0.8 ml. of red cell suspension. Converting to γ , the initial amount of lysin is 400 γ , and the amount left free after 1.0 minute is 389 γ . The difference is 11 γ , which is substantially the same as the difference obtained at 38°.

Experiments of this kind leave no doubt as to the constancy of the quantity of lysin which disappears in the time required for complete haemolysis, and that the amount appears to be independent of both temperature and initial lysin

Fig. 3. Curves for the using up of lysin from an initial lysin concentration of 200 γ at 38 and 7° respectively. Ordinate, x in γ ; abscissa, time in mins. The equilibrium values, reached at about 500 mins, are substantially the same, and are shown by horizontal lines.

concentration. The determinations, of course, have a considerable experimental error attached, but the consistency with which the same result is obtained is, all things considered, remarkable.

4. Table V shows the result of an experiment in which the curves for the disappearance of lysin reacting with stromata are obtained in their entirety at 38° and at 7°. The lysin concentration used was 200 γ , and the times are in minutes.

38°		7°	
<i>t</i>	<i>x</i>	<i>t</i>	<i>x</i>
0.0	0	0.0	0
0.017	(15)	2.2	15
2.2	33	5.0	23
10.0	52	10.0	34
500.0	65	500.0	67

Assuming equilibrium to have been reached in 500 minutes, both curves pass to the same equilibrium value (*cf.* 2, above). The point (0.017, 15) has been inserted in order to make the amount of lysin which disappears in 1 second at 38° and the time required for complete lysis of 0.4 ml. of red cell suspension at that temperature, correspond to the 15 γ of lysin used up in 2.2 minutes at 7°, this being the time required for complete lysis at 7°. The results are plotted in Fig. 3, and it will be seen that the two curves approach the same equilibrium value in quite different ways. They are like the curves of Fig. 1 in that neither can be described by expressions (1) and (2) with a constant value of *k*, but their course throws some light on the value of *n* in these equations, for the curve at 38°, with its sharp curvature, bears a general resemblance to a curve described by expression (2) with a value of *n* of about 3.0, while the flatter curve at 7° is not unlike that for a first order reaction, *i.e.*, a curve described by expression (2) with *n* = 1.0. The variations of the value of *n* which have already been described as a result of changes in temperature are accordingly reflected in the form of the curve for the utilisation of lysin at various temperatures; at high temperatures, the value of *n* in the existing equations is 2.5 or greater, and the curve for the utilisation has a high degree of curvature, while at low temperatures the value of *n* approaches 1.0, and the curve for the utilisation of lysin is relatively flat.

DISCUSSION

The experimental curves for the process of haemolysis followed by stromatolysis differ from those expected on the basis of existing theory in two ways. (1) The equilibrium conditions are different, the using up of lysin ceasing while considerable quantities of lysin are still free in the system, and not when the free lysin concentration has fallen to zero. (2) The course of the curves for the utilisation of lysin cannot be described by the existing equations unless the value of the velocity constant *k* is imagined to vary. In considering the effect of these two observations on existing theory, it is convenient to treat them separately.

1. As shown in Tables I and IV, the amount of lysin which finally disappears as a result of reacting with the stromata is very nearly a linear function of the initial lysin present. Let the slope of the line be *a*. Rewriting (1) to express the idea that the velocity of the reaction is dependent on the quantity of lysin in excess of the equilibrium value, we have

$$\text{which gives on integration} \quad \frac{dx}{dt} = k(ac - x)^n \quad \dots\dots(3),$$

$$kt/a \frac{1-p}{p} = \frac{p}{p-1} \left\{ c \frac{p-1}{p} - (c-x/a) \frac{p-1}{p} \right\} \quad \dots\dots(4).$$

Comparing this expression with expression (2), it will be clear that the two equations can be made to give the same values of t by multiplying every value of c in expression (4) by $1/a$, and changing the value of the velocity constant. Since the only way of measuring x is in terms of c and in units which are really arbitrary, and since there is no independent way of finding the value of the velocity constant of the reaction, the two equations are identical in so far as numerical results are concerned. The fact that the equilibrium conditions are other than those postulated in the existing theory accordingly makes no difference; this, of course, is wholly due to the relation between the values of x at equilibrium being linear with the initial values of c .

2. The fact that the course of the curves for the utilisation of lysin cannot be described by expression (2) with a constant value of the velocity constant, and therefore cannot be described by expression (4) either, leads to the unescapable conclusion that the existing theory is wrong in some fundamental respect. There are, of course, many ways in which it may be at fault, but the most obvious of these is that it supposes the velocity constant of the reaction to have the same value at all stages of the reaction, whereas in fact it may be steadily decreasing. On the basis that it is undergoing a continuous decrease it would obviously be possible to fit the experimental curves of Figs. 1 and 3 with a first order equation, or with an equation of any order less than that required to fit the time-dilution curve for haemolysis under the same conditions and by the existing equations, provided the velocity constant were allowed to decrease appropriately. If the possibility of such a decrease in k is once admitted, however, there is no justification for supposing that the decrease occurs only during stromatolysis and after haemolysis is complete; one might as well suppose it to begin to decrease from the very beginning of the process, and describe the entire course of the disappearance of lysin during both haemolysis and subsequent stromatolysis by a first order equation, together with an appropriate decrease in k as the reaction proceeds. Under such circumstances the meaning of the constant n in equations (2) and (4) would be clear, and this constant would merely express, in an arbitrary sort of way, the extent of the fall in the value of k during the course of haemolysis. For example, if the reaction were really of the first order, and n were 1.0, the value of k would not decrease at all, but if n were 2.0 and the reaction of the first order, k would have to decrease as the reaction proceeded, while if n were 3.0 the decrease would have to be greater. Such a hypothesis would, of course, leave us with no difficulty in accounting for the non-integral values which the constant n usually assumes.

Reaching a conclusion like this, of course, is tantamount to admitting that the equations hitherto used for describing the course of haemolysis are merely formal ones. In fact, it raises the disturbing doubt as to whether correct equations for the kinetics of any biological system can ever be formulated except in cases in which the behaviour of the velocity constant is first defined exactly, and such cases appear to be rare at the present time. The possibility of a mathematical description of the kinetics of haemolysis and stromatolysis, other than a purely formal one, at all events becomes rather remote¹.

¹ The reader will observe that, although the equations hitherto used to describe time-dilution and percentage haemolysis curves are probably only formal ones, the system of analysis used by myself and my collaborators in previous papers requires no essential modification. This is due to the analysis being based on the comparison of time-dilution and percentage haemolysis curves, obtained under one set of conditions, with similar "standard" curves obtained under simpler conditions. So far as the method of analysis is concerned, the experimental curves need never have been described by equations at all.

In conclusion, it ought to be remarked that the type of reaction which now appears to be responsible for haemolysis and stromatolysis is not a unique one. A reaction characterised by virtual irreversibility, a steadily decreasing velocity constant and an equilibrium which is reached when there is still free reactant in the system is met with in a number of processes, such as the dyeing of wool *etc.*, and is known as "pseudo-adsorption" or "anomalous adsorption", terms which mean little except that the reaction is not a stoichiometric one. In all such cases, as in the case of the reaction which results in haemolysis *etc.*, the decrease in the velocity constant is probably associated with the fact that the proper form of expression (1) is

$$dx/dt = k' \cdot F(c-x) \cdot F(S-x) \quad \dots\dots(5),$$

where S is the reacting surface of the substance which is acted on, and that the expression has to be simplified by writing $k = k' \cdot F(S-x)$. Obviously, were $F(S-x)$ to equal $F(c-x)$, we would get expression (1) with $n=2$, but since we know nothing as to how the reacting surface decreases as the reaction proceeds, the whole problem is indeterminate.

SUMMARY.

This paper is concerned with the rate at which a lysin such as saponin is used up during the process of stromatolysis, or breaking down of the red cell envelopes, which follows haemolysis. It is shown that the equations which have hitherto been used to describe the using up of lysin during haemolysis are not applicable to the subsequent process. The contents of the paper do not lend themselves to detailed abstraction, but the principal conclusions are (a) that the reaction between cells and lysin is characterised by peculiar equilibrium conditions, in which using up of lysin ceases while there is still free in the system a quantity of lysin which is nearly linear with the amount initially present, (b) that the course of the disappearance of lysin is probably described by an equation, the exact form of which cannot be defined, in which there is a velocity constant which undergoes continual decrease as the reaction proceeds, (c) that such a decrease in the velocity constant is probably responsible for our having to introduce an arbitrary constant n into the existing equations for the kinetics of haemolysis, and (d) that although these existing equations are probably only formal ones, the conclusions based on the existing analysis of time-dilution and percentage haemolysis curves are themselves essentially unchanged.

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