THE MECHANISM OF THE INHIBITION OF HEMOLYSIS

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This paper is concerned with the general nature of the reactions involved in the phenomenon of the inhibition of hemolysis by a variety of substances (serum, hemoglobin, glucose, etc.). Since the investigations of Bayer (1907), who showed that the inhibition produced by serum on bile salt hemolysis is largely accounted for by its protein content, the literature has been dominated by the idea that inhibitors such as the serum proteins produce their effect by reacting with the lysin in the same sort of way as when reactant A precipitates reactant B from solution. More specifically, the inhibition produced by serum, plasma, and hemoglobin has been described by supposing that a quantity Q of the inhibitor "removes" (in the same sense as cholesterol, an inhibitor of digitonin hemolysis, precipitates digitonin quantitatively) a quantity Δ of the lysin, so that the original concentration c_1 of the lysin falls to a concentration c_2, and where $c_1 - c_2 = \Delta$. This treatment of the problem is unsatisfactory for three reasons. (1) The relations between Q and Δ turn out to be very complex. (2) The inhibitory effects of serum, plasma, and hemoglobin have to be treated in a manner entirely different from that used to treat the inhibitory effect of substances such as the sugars and certain electrolytes. (3) This treatment of inhibitory phenomena is quite different from that used to treat acceleratory phenomena. There is, of course, no *a priori* reason why the inhibition produced by serum and plasma, the inhibition produced by substances such as glucose, and the acceleration produced by substances such as benzene and indol, should all be brought about in the same way; the possibility of a single underlying mechanism, however, ought to be more thoroughly investigated than it has been.

I. The Inhibitory Effect of Serum and Its Protein Content

The inhibitory effect of serum on saponin (or digitonin) hemolysis is not entirely paralleled by its protein content. Two kinds of experiment show this.

1. One takes a number (six or so) of specimens of human plasma with varying protein content, and finds the asymptotes of the time-dilution curves for saponin in the absence and in the presence of the inhibitor plasma diluted 1 in 1000. This is done by preparing a series of saponin concentrations in phosphate buffer (pH = 7.3) varying by steps of 5 γ/cc. upwards from 50 γ/cc. to about 200 γ/cc. One series is put up without the addition of inhibitor, the

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systems consisting of 0.8 cc. of lysin, 0.8 cc. of buffer, with 0.4 cc. of red cell suspension added, and the asymptote of the time-dilution curve is found by observing the concentration c_2 which produces complete hemolysis after 300 minutes (for technique, see Ponder, 1934). A second series is set up with 0.8 cc. of plasma in a dilution of 1 in 1000 replacing the 0.8 cc. of buffer, and the asymptotic concentration c_1 for this series is found in a similar way. Table I

TABLE I

Plasma	System	Protein	Asymptotic concentrations c_1 and c_2	Δ	*R*
		gm. per cent			
1	Standard + plasma 1:1000	— 7.6	36 52	16	1.44
2	Standard + plasma 1:1000	— 7.3	35 52	17	1.48
3	Standard + plasma 1:1000	— 6.6	38 52	14	1.37
4	Standard + plasma 1:1000	— 6.2	35 50	15	1.43
5	Standard + plasma 1:1000	— 5.9	38 50	12	1.32
6	Standard + plasma 1:1000	— 5.8	36 46	10	1.28

shows how the difference $\Delta = c_1 - c_2$ and the ratio $R = c_1/c_2$ vary with the protein content of the plasma. Both the difference Δ and the ratio *R* are given, because it will be shown directly that these two measures of the extent of the inhibition imply the existence of quite different mechanisms.

2. These determinations of the position of the time-dilution curve asymptotes in the absence and in the presence of the inhibitor are troublesome, partly because of the long times involved, and partly because of the difficulty in reading the 300 minute end-points. One can arrive at the same result by plotting a time-dilution curve for saponin, selecting a concentration c_1 (say 1 in 33,000 in phosphate buffer) which gives complete lysis in a relatively short time (about

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5 minutes), and then finding the concentration c_2 which appears to be present when sera in dilution of 1 in 5000 are added to the system. Either $\Delta = c_1 - c_2$, or $R = c_1/c_2$, would be a measure of the inhibition, and what we find is that neither of these functions is wholly determined by the protein content of the serum. This is shown in Table II.

The conclusion to be drawn is that the inhibitory effect on saponin hemolysis is not entirely dependent on the protein content of the plasma. Farley (1942), indeed, has suggested that plasma contains powerful inhibitors other than proteins, and the poorness of correspondence between inhibitory effect and plasma protein content shows that this possibility has to be recognized. This idea is not altogether new, for it has already been suggested that the effect of plasma on hemolysis, while in general inhibitory, is a statistical effect of a mixture of many inhibitors and accelerators (Ponder and Abels, 1936; Ponder, 1937; Collier and Allen, 1942). The question then arises as to whether we ought not to treat it in the same way as we treat acceleratory phenomena and as taking place at the cell surface rather than in the lysin-inhibitor phase.

TABLE II

	Plasma			
	1	2	3	4
Protein, *gm. per cent*	5.92	6.56	6.80	7.21
Globulin, *gm. per cent*	2.46	2.70	2.90	2.90
Albumin, *gm. per cent*	3.46	3.86	3.90	4.31
A/G ratio	1.4	1.4	1.3	1.5
Δ	14	22	16	20
R	1.26	1.46	1.30	1.40

When we write $\Delta = c_1 - c_2$, we imply that the inhibitor reacts with the lysin c_1, and renders inert a quantity Δ of it; the amount of lysin which remains is then c_2. When we write $R = c_1/c_2$, quite a different mechanism is implied. In a system containing sucrose (an inhibitor), for example, a concentration of lysin c_1 produces lysis as if it were of the smaller concentration c_1/R ($R > 1.0$) acting on the cells in the absence of sucrose. This, of course, is the same thing as saying that the resistance of the cells is increased R times by the sucrose, R times more lysin being needed to combine with the membrane component in the presence of the sucrose to produce the same effect as in its absence. The reaction implied by expressing the inhibition as a value of Δ, a function of c_1, or of c_2, is one in which the inhibitor reacts with the lysin, but in which the locality of the reaction is not specified (although it has been usually considered as being in the bulk of the fluid of the hemolytic system); when the inhibition is expressed as a value of R, however, the site of the reaction is specified

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as being at the cell surfaces, and the effect of the inhibitor has to be regarded as in general similar to the effect of an accelerator (for which see Ponder, 1941). The description of inhibition in terms of R values has, indeed, been used in the case of inhibitors such as certain sugars and salts ever since the R notation was introduced (Ponder, 1926; and see Kennedy, 1926; Bodansky, 1928; Yeager, 1929; and Gordon, 1933 *a* and *b*), while, except in one instance (Kennedy, 1925), Δ has been used to describe the inhibition only in the cases of the inhibitors plasma, serum, and hemoglobin (Ponder, 1924, 1925, 1932; Ponder and Gordon, 1934).

From a mathematical point of view, the expressions $\Delta = c_1 - c_2$ and $R = c_1/c_2$ transform into one another, and are two different ways of expressing the same experimental results. The relation between the two expressions has been fully discussed already (Ponder, 1934, pp. 200–205). In considering the inhibition produced by plasma and serum in terms of R instead of Δ, we can accordingly retain all the existing experimental results, although we may change our interpretation of them. It is first necessary, however, to show that a change in interpretation is necessary, and so I shall next consider some hitherto undescribed properties of saponin-plasma-cell systems.

II. Certain Properties of Saponin-Plasma-Cell Systems

1. The Temperature Coefficient.—In 1925 I was unable to find any clear cut temperature dependence for the inhibition of serum on saponin hemolysis (Ponder, 1925) although there were indications that the inhibition becomes less as the temperature increases. The failure of these experiments to show a definite effect of temperature was probably due to the method used for analyzing the curves (in terms of the slope of log c plotted against log Δ). Much more satisfactory results are obtained when time-dilution curves for standard systems and for saponin-inhibitor-cell systems are plotted and analyzed in terms of R values. Table III shows the average values of R obtained at two different temperatures for systems containing saponin, human red cells, and (*a*) plasma with a protein content of 7.0 gm. per cent, and (*b*) M/3.5 sucrose.[1]

In each case the values of R show that the temperature coefficient is small but negative, and therefore the same sign as the temperature coefficient for accelerators (Ponder, 1941). This result can be interpreted as pointing to surface phenomena being involved in the reaction which brings about inhibition, just as it has been shown to be in the case of acceleration (Ponder, 1939). In the case of inhibition by plasma, the surface might be either the extended

[1] Since R is a function of c_1, the temperatures chosen should be such that the same range of lysin concentration is covered by the time-dilution curves for the standard systems. This requirement considerably limits the range of temperatures over which R values can be properly compared. The standard curves can be plotted over the same concentration range at 22°C. and at 37°C., for example, but not at 22° and at 10°.

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surface of the colloidal inhibitor or that of the red cell; in the case of inhibition by sucrose, on the other hand, the latter is almost certainly involved.[2]

2. The Inhibition in Systems Containing Different Kinds of Cell.—The extent of the inhibition of saponin or digitonin hemolysis produced by plasma or serum varies according to the type of red cell used in the hemolytic system. Table IV shows the values of R obtained in systems containing 0.8 cc. of saponin in dilutions from 1 in 10,000 to 1 in 35,000 (22°C.), 0.8 cc. of plasma diluted 1 in 500, and 0.4 cc. of a standard suspension of the cells of the sheep, man, rat, and guinea pig.

For any quantity of lysin c_1, the R values are in the order sheep > man > rat > guinea pig, so the extent of the inhibition depends on the type of cell used

TABLE III

Inhibitor	Quantity	R, 22°C.	R, 37°C.
Plasma............	0.8 cc. of 1 in 250	1.23	1.14
Sucrose............	0.8 cc. of 0.286 M	1.21	1.12

TABLE IV

1 in	Sheep	Man	Rat	Guinea pig
10,000	1.80	1.23	1.20	1.13
15,000	1.67	1.19	1.14	1.11
20,000	1.60	1.15	1.11	1.10
25,000	1.50	1.15	1.10	1.08
30,000	1.40	1.13	1.08	1.07
35,000	1.31	1.10	1.06	1.06

in the hemolytic system. It is virtually impossible to reconcile this result with the idea that the inhibitor reacts solely with the lysin in the bulk of the system, removing an amount Δ by forming an inactive compound with it. The inhibition is clearly the result of some reaction which involves the cell surface, and which varies with its nature.

3. R As a Function of c_1.—Inspection of Tables IV and V will show that the value of R is not constant in systems containing either plasma or sucrose as inhibitors, and that R falls steadily as c_1 decreases, so that the curves relating c_1 and R are convex to the R axis (Fig. 1).

In the case of the inhibition produced by most sugars and certain electrolytes, it has been recognized at least since 1928 that R falls with c_1, Ponder and Yeager

[2] Even in the case of a substance such as sucrose, the possibility of a surface reaction in the bulk of the system has to be considered, for Kleinberg (1933) has shown that saponin may exist in a micellar state.

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(1928) giving values of R which decrease from 1.50 to 1.25 for taurocholate-sucrose-human cell systems. In some systems, however, the curve relating

TABLE V

1 in	c_1	R human cells and sucrose	R sheep cells and plasma
10,000	200	1.40	1.80
15,000	133	1.31	1.67
20,000	100	1.25	1.60
25,000	80	1.20	1.50
30,000	67	1.13	1.40
35,000	57	1.09	1.31
40,000	50	1.07	1.25

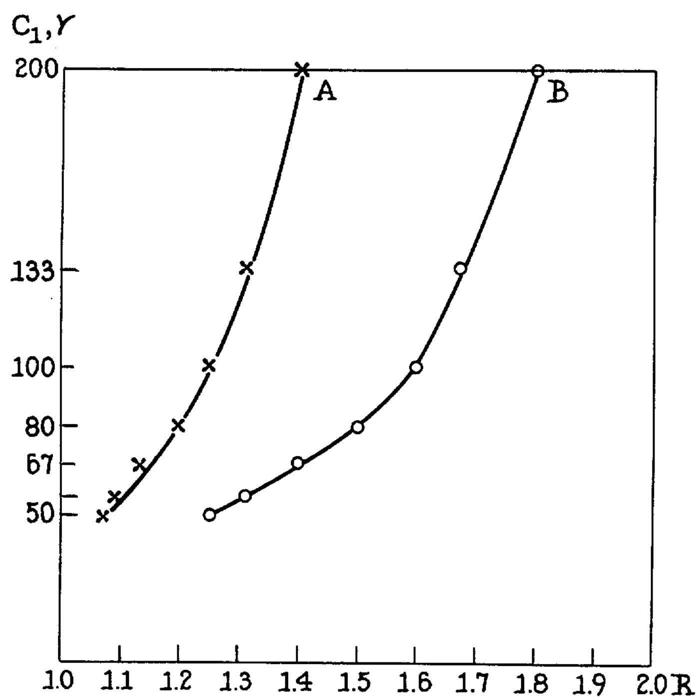

FIG. 1. R as a function of c_1. Curve A, inhibition of saponin hemolysis (human cells) by M/3.5 sucrose. Curve B, inhibition of saponin hemolysis (sheep cells) by plasma.

R and c_1 can be approximately represented by a straight line within a limited range of lysin concentration. Because it is only an approximation to the curve, this line usually makes a small intercept on one of the axes. In such cases, R can be treated as substantially constant, as in Yeager's investigation of the in-

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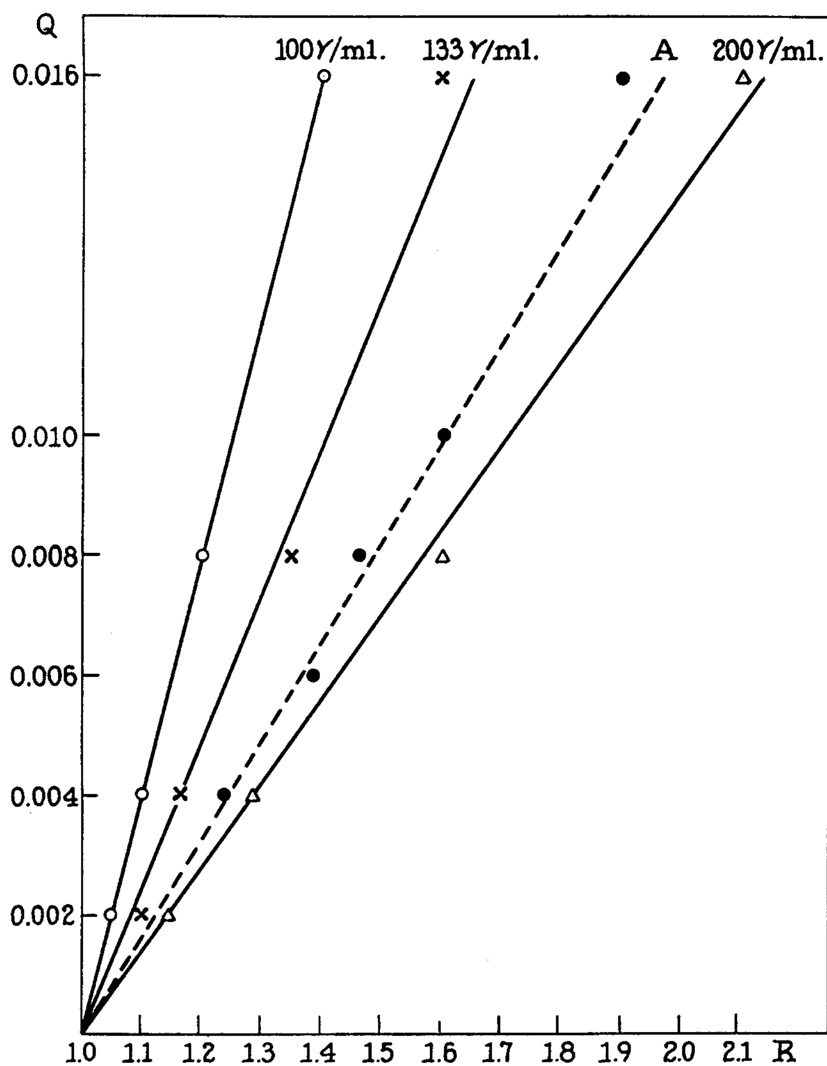

FIG. 2. R as a function of Q. Inhibition of saponin hemolysis (human cells) by plasma in various amounts Q; data for three initial concentrations of lysin, from 100γ/ml. to 200γ/ml. Curve marked A: data from Ponder and Gordon (1934), included in this figure only for the purpose of illustrating linearity. The absolute values of Q shown on the ordinate do not apply to Curve A.

hibitory effect of sucrose (1929) but where the relation is too curvilinear for the fall of R with c_1 to be ignored, the expedient has been resorted to of measuring the extent of the inhibition by the ratio of the asymptotic values of c_∞ for the

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standard system and for the inhibited system respectively; *i.e.*, $R = c_{1_\infty}/c_{2_\infty}$. Yeager (1928), in his paper on the Rywosch series with taurocholate, and Gordon (1933 *b*), in his investigation of the effects of electrolytes, have used this method of expressing inhibitory effects, and so have avoided the difficulties presented by the R, c_1 relation being curvilinear; evidence of its being so, however, appears in all the papers on the subject of inhibition of hemolysis up to the present time.

In the case of the inhibition produced by plasma, serum, and hemoglobin, a relation between Δ and c_2 (Ponder, 1924, 1925), or between Δ and c_1 (Ponder and Gordon, 1934) has been used instead of a relation between R and c_1 principally because the R, c_1 relation is so obviously curvilinear as to apparently separate it from the nearly linear relation met with in the case of the inhibition produced by many sugars, electrolytes, etc. Looking back on the development of the subject, it is now clear that the R, c_1 relation is *always* curvilinear if the experimental range is sufficiently extended, and that the Δ, c_1 relation, which first attracted attention because of the idea that the inhibitor forms an inactive compound with the lysin in the bulk of the fluid in the system, represents an attempt to distinguish between two kinds of hemolytic system (sugar-lysin-cell systems and plasma-lysin-cell systems) between which there is no real difference.

4. R As a Function of Q.—It has been known since 1924 that the inhibition produced by a quantity of serum Q increases as Q increases. Although a relation between Δ and Q, which turns out to be curvilinear and complex, has been obtained (Ponder, 1924, 1925; Ponder and Gordon, 1934), no one seems to have considered R as a function of Q. The best existing values are those of Ponder and Gordon (1934, their Table II), and plotting R against Q gives the line marked A in Fig. 2. The relation is a linear one over a very extended experimental range, and the slope of the line depends on the concentration of lysin in the system, since R is also a function of c_1. The other lines in Fig. 2 illustrate this.[3]

DISCUSSION

The first point which emerges from these experiments is the similarity between inhibitory and acceleratory phenomena. Both appear to involve the region of the surface of the red cell (*cf.* Ponder, 1939, 1941), both have a negative temperature coefficient, in both cases R, the measure of the amount of

[3] In the case of inhibition of sodium taurocholate hemolysis by plasma, the R, Q relation is better represented by a curve convex to the R axis than by a straight line (Ponder and Gordon, 1935). It is possible, indeed, that the relation would be curvilinear even in the case of saponin if the experimental range were to be sufficiently extended.

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inhibition or acceleration, is an approximately linear function of the quantity of inhibitor or accelerator Q, and in both cases R is a function of c_1.[4]

It should be observed that these relations apply to all inhibitors yet studied, just as they apply to all accelerators, and since it has been shown (Ponder, 1926; Ponder and Yeager, 1928) that inhibitors such as sucrose react more or less irreversibly with components of the cell membrane just as accelerators do, we have to entertain the hypothesis that the inhibitors contained in plasma react with the components of the red cell membrane so as to increase their resistance to the action of hemolysins.

This idea is not so strange as might appear at first sight, quite apart from the fact that plasma apparently contains inhibitors other than the plasma proteins (Section I, above), and that some of these inhibitors may be substances of low molecular weight which can be imagined to react with membrane components. When considering the inhibitory effect of the plasma proteins, it should be remembered that some of the protein components of the membrane can leave the cell surface to appear in the surrounding fluid, while some of the protein components of plasma can leave the fluid to take up positions in the membrane and apparently to become part of its structure. This transferability is shown in the clearest way in the experiments of Furchgott (1940) and of Furchgott and Ponder (1940) on the disk-sphere transformation between glass slide and cover glass. The "anti-sphering substance," identified as a crystalbumin, leaves the cell surfaces to be adsorbed on the glass, and at the same time the cell becomes spherical. The lost anti-sphering substance can be replaced in the cell surface by adding plasma, which reconverts the spheres into disks. A 30 per cent change in surface area is involved in these transformations, and the amount of crystalbumin which leaves and returns is sufficient to form about one-third of the estimated mass of the membrane, although it would form a layer only a few molecules thick if spread over the cell surface. A protein can accordingly be thought of as reacting with components of the membrane ultrastructure.

The process underlying the phenomena of lysis and its acceleration or inhibition accordingly seems to be one in which the lysin reacts with a component

[4] The R, c_1 curve in the case of an accelerator can be analyzed (Ponder, 1941), a sharp curvature occurring at a point corresponding to the asymptote of the standard curve. R is virtually constant below this point and linear with c_1 below it. The form of the R, c_1 curve in the case of the inhibitors is as yet unaccounted for. Since the asymptote for the inhibited system corresponds to a greater value of c_1 than that for the standard system, an analysis parallel to that for accelerated systems is not possible, but the underlying explanation is probably fundamentally similar, *viz.* the inhibitor is present at the cell surface in excess, its effect increasing as the concentration of lysin present at the surface, and therefore capable of being inhibited, increases.

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or components of the cell membrane in such a way as to break down its semipermeability to hemoglobin, and in which the accelerator or inhibitor also reacts with the component in such a way as to increase or decrease the effectiveness of the lysin in producing breakdown. By breakdown we mean the local or general dissolution of the continuity of the membrane components upon which the properties of semipermeability depend, and we express the change in effectiveness by saying that R times as much lysin is required in the presence of the accelerator or inhibitor in order to produce the same destructive effect, or that the accelerator or inhibitor changes the resistance of the membrane to the lysin by a factor R. This statement is sufficient so far as an investigation into the kinetics of lysis and its acceleration or inhibition is concerned, but before it can have any real meaning in terms of mechanism it is necessary both to identify the components which enter into the reactions and to show how the reactions result in hemolysis, accelerated or inhibited. The relations between quantities, expressed by the equations for the kinetics, must correspond to actual occurrences on the molecular scale, and if the reactions give a few hints as to the nature of the occurrences, we cannot omit following them up. There are several points which are of particular interest in this connection.

1. The conclusion has already been reached that lysis occurs through a breakdown of spots or patches in the cell membrane, although the lysin acts over the surface of the membrane as a whole, these spots ("key spots") being regions at which the resistance to the lysin is particularly low (Ponder, 1941). The combined area of the spots can be inferred from experiments on the "fading time" or length of time taken for the hemoglobin to diffuse out of a single cell during its hemolysis (Ponder and Marsland, 1935). In systems in which approximately asymptotic concentrations of lysin are present, the fading time is about 15 seconds, and this corresponds to a value of Nd of 0.25, where N is the number of holes or permeable patches, and d their mean diameter in μ. Considering that hemoglobin diffuses through these holes, and that the diameter of the hemoglobin molecule is in the neighborhood of 50 Å, the minimum mean diameter of the holes would be 50 Å, and in an approximately asymptotic concentration of lysin for which $Nd = 0.25$, the maximum number N would be 50. These observations show that the structure of the membrane is discontinuous, certain spatially separated areas breaking down more readily than others as a result of the reaction with the lysin.

2. If the resistance of the least resistant spots is x_0, there must also be present a number of spots of greater resistance, x_1, x_2, and so on. If N is the number of spots of any degree of resistance as measured in terms of x, the plotting of N as a function of x will give rise to some kind of frequency distribution, the integral of which is the experimental curve obtained by plotting the average fading time of the cells against the concentration of lysin in the system (Ponder and Marsland, 1935; Ponder, 1941). The distribution is very skew, which

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means that a small part of the surface is made up of areas which are relatively readily broken down as lysin is transformed, while the greater part is made up of areas which have a higher and much less uniform resistance.

3. The breakdown of the spots of resistance x_0, x_1, etc., is not the first change which occurs in the red cell membrane under the action of the hemolysin, for lysis is always preceded by a loss of the discoidal form. The membrane ultrastructure must therefore (*a*) preserve a surface greater than the minimum for the enclosed volume, thus maintaining the discoidal form, (*b*) undergo some modification as a result of the action of the lysin, so that the rigidity of form is lost, and the expanded surface cannot be maintained, and (*c*) break down at spots as a result of the further action of the lysin. Like the change which results in loss of semipermeability, the form change is a process which has a measurable velocity. It does not occur immediately on the addition of the lysin, but only after a "latent period;" the first signs of it then appear at localized areas on the surface, so that the discoidal form develops crenations in increasing number as the disk passes into the sphere. Just as the membrane ultrastructure has spots of varying resistance to permeability breakdown, so it seems that it has spots of varying rigidity of form; in the case of any one cell, however, the shape change is always complete before the least resistant key spot breaks down.

4. The spots of resistance x_0 can be thought of as being distributed through the membrane ultrastructure either at random or in some kind of pattern, but since the number of these spots may be as small as 50, the unit pattern must be large enough to cover some 10^8 Å^2 before repeating itself. This is a very large unit pattern. The same alternative applies to the spots of greater resistance and to the spots of varying rigidity of form. While there is not conclusive evidence in favour of it, the idea of a pattern is the more attractive of the two, if for no other reason than that the cell has a regular geometrical form.[5]

[5] Wrinch (1942) has put forward an attractive hypothesis regarding the general nature of the membrane ultrastructure. In her model the skeleton of the membrane is composed of multiply connected proteins arranged in a definite pattern. Because of differences in their side chains, these protein molecules and their linkages are not all the same, and this would permit of local differences in reactivity with lysin molecules. The size of a protein unit in the model is from 50 to 100 Å, which roughly corresponds to the size of a key spot, and the model would not exclude a sufficiently infrequent repetition of the unit pattern to allow for the number of spots of resistance x_0 being comparatively few. Cephalin molecules are conceived of as being attached to the protein network with a more or less normal orientation to it, and the network may be not single, but multiple; in this way a laminated structure may be built up, and the polarization optics of such a structure would be something of the kind already shown to exist for the membrane of the red cell ghost (Schmitt, Bear, and Ponder, 1936, 1938). From the standpoint of the red cell, Wrinch's model is interesting not only because it pictures the membrane as having a molecular pattern, but because it

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5. The fact that the same value of R is obtained by comparing a system containing an accelerator or an inhibitor with a standard system, irrespective of the percentage of hemolysis used as an end-point (Ponder, 1934, p. 190), shows that the effect of the accelerator or inhibitor is to change the resistance of every reactive spot in the membrane ultrastructure by a factor R. The resistance of each area is accordingly changed by a constant factor and not by a constant amount. This result suggests that the accelerator or inhibitor has some over-all effect, the simplest example of which would be that of changing the extent to which the lysin is concentrated at the surface, or is partitioned between the material of the membrane and the surrounding fluid. Some kind of combination between the accelerator or inhibitor and the membrane ultrastructure is presumably involved. Part of the effect of most accelerators and inhibitors is reversible by such a simple procedure as washing (Ponder, 1926, 1939), and this probably corresponds to a loose adsorption-like combination involving large parts or the whole of the surface. Some of the loose links are later replaced by more permanent combinations involving the same types of bond as are broken down by lysins; the accelerator or inhibitor so combined cannot be reversed by washing, and many accelerators *and inhibitors* (*e.g.* sucrose) themselves produce lysis if present in sufficient concentration.

SUMMARY

The principal conclusion of this investigation is that the inhibitory effect of plasma or serum on hemolysis by saponin and lysins of the same type is similar in nature to the inhibitory effects of certain sugars and electrolytes, which again are similar to the acceleratory effects produced by indol, benzene, and other substances already studied. All these effects, both inhibitory and acceleratory, are the result of reactions between the inhibitors or accelerators and those components of the red cell membrane which are broken down by lysins.

The inhibitory effect of plasma on saponin hemolysis has a number of properties in common with the inhibition produced by sugars and electrolytes and with accelerations in general. (*a*) The temperature coefficient is small and negative. (*b*) The extent of the inhibition depends on the type of red cell used in the hemolytic system. (*c*) The most satisfactory measure of the extent of the inhibition, the constant R, is a function of the concentration of lysin in the system, and (*d*) R is a linear function of the quantity of inhibitor present.

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supposes two sets of structures, the first of which tends to form the smallest surface for the enclosed volume and the second of which may, under the proper circumstances, tend to form an expanded surface. This is reminiscent of Norris' theory (Norris, 1882; Ponder, 1933), with the distinction that the expansive component in Wrinch's model would be the protein network, while in Norris' theory it is the layer of lipoid molecules.

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It is also shown that the inhibitory effect of plasma and serum is not entirely dependent on its protein content.

The process underlying the phenomenon of lysis and its acceleration or inhibition seems to be one in which the lysin reacts with a component or components of the cell membrane in such a way as to break down its semipermeability to hemoglobin, and in which the accelerator or inhibitor also reacts with the same component in such a way as to increase or decrease the effectiveness of the lysin in producing breakdown. The membrane is considered as being an ultrastructure made up of small areas or spots of varying degrees of resistance to breakdown, the resistances being distributed according to a negatively skew type of frequency curve, and the process of lysis seems to begin with the least resistant spots breaking down first. These spots may be arranged in some regular spatial pattern, and the membrane has also to be regarded as possessing spots of varying rigidity of form. The accelerator or inhibitor changes the resistance of every reactive spot in the ultrastructure by a factor R, which suggests that acceleration and inhibition are results of some over-all effect, such as that of changing the extent to which lysin is concentrated at the surface or partitioned between the material of the membrane and the surrounding fluid. Some kind of combination between the accelerator or inhibitor and the material of the ultrastructure is presumably involved; at first the combination seems to be a loose one and partly reversible, but later some of the loose links are replaced by more permanent combinations involving the same types of bond as are broken down by the lysins themselves.

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