ACCELERATION OF HAEMOLYSIS IN RELATION TO CHEMICAL STRUCTURE

I. BENZENE DERIVATIVES

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(With Four Text-figures)

Since methods for measuring acceleration and inhibition of haemolysis were first described (Ponder, 1926), very little attention has been paid to the way in which accelerators and inhibitors act. The effects of only a few chemical substances have been studied, and detailed investigations on the accelerating and inhibiting effects of various ions, etc., have certainly not tended to simplify the situation (Yeager, 1929, 1931; Gordon, 1933 a, b); recently, however, the problem has become of interest from quite a different point of view.

A study of the haemolytic effects of the triphenylmethane dyes by Blum (1935), both in the dark and in the light, has shown that the order of activity of these substances is fluorescein < eosin < erythrosin < rose bengal, and the same result has been obtained in an investigation of the concentration of the dyes necessary to produce prolytic spheres (Ponder, 1936). Since fluorescein contains no halogens, while eosin has two Br, erythrosin two I, and rose bengal either two I and two Cl or four I and two Cl, increasing the number and reactivity (Cl < Br < I) of the halogens attached to the rings increases the lytic activity of the molecule as a whole, and Astbury has suggested to me that the addition of more and more polar groups confers on the molecule the ability to react with the side chains of the proteins of the red cell surface, thus exerting a solvating effect. This suggestion is so interesting that one would like to find other lysins in the case of which one could study the relation of lytic activity to the addition of reactive groups, but a difficulty immediately presents itself in that most active lysins (e.g. saponin, the bile salts, and even the soaps) are structurally too complex for the desired substitutions to be easily made.

Many accelerators, on the other hand, are simple substances, and so, instead of trying to relate lytic activity to the molecular structure of the lysin, one can try to relate accelerating power to the structure of the accelerator. The two problems are somewhat similar, for while lysin breaks down the red cell membrane, the accelerator modifies the membrane so as to make it more easily disintegrated by the lysin, added subsequently.

As a starting point I chose benzene and some of its derivatives.
METHODS

To measure the extent to which a substance is an accelerator or inhibitor of haemolysis, one plots two time-dilution curves at the same temperature, the first curve being a “standard” curve for a system containing lysin, saline, and cells, and the second being a time-dilution curve for a system containing lysin, a solution in saline of the substance to be tested, and cells. In practice, the standard system A contains 0·8 c.c. of the lysin (e.g. saponin) in various dilutions in isotonic NaCl or NaCl-buffer (saline), 0·8 c.c. of saline, and 0·4 c.c. of a red cell suspension made by suspending the thrice-washed cells of 1 c.c. of rabbit blood in 20 c.c. of saline. The second system B contains 0·8 c.c. of the lysin in various dilutions, 0·8 c.c. of a solution in saline of the accelerator or inhibitor, and 0·4 c.c. of the red cell suspension, as before.

If the substance is an accelerator, lysis will be faster in system B than in system A, and complete lysis in any time will be brought about by a smaller concentration of lysin in system B than in system A. If the former concentration is \( c_1 \) and the latter \( c_2 \), it will be found that plotting \( c_1 \) against \( c_2 \) usually gives quite a good straight line (but see below, § IV), so that we can use the slope of this line \( R = \frac{c_1}{c_2} \) as a measure of acceleration when \( R < 1 \cdot 0 \), or as a measure of inhibition when \( R > 1 \cdot 0 \). This value of \( R \) corresponds to one particular concentration of the accelerator or inhibitor, but we can get \( R \) values corresponding to other concentrations by plotting other time-dilution curves, C, D, etc., for systems containing the substance in different amounts. If this is done, and the values of \( R \) are plotted against the concentration of the accelerator or inhibitor, it will be found that the points usually lie on a good straight line (but see § IV), necessarily passing through \( R = 1 \cdot 0 \) at zero concentration (see Fig. 1). The slope of such a line \( (R - 1) / c \) is a complete expression of the accelerating or inhibiting power of a substance, \( c \) being the concentration in millimols/litre. Expressed in this way, values for acceleration are negative, and decrease numerically to zero as the accelerating power becomes less; values for inhibition are positive, and become greater as the inhibitory power increases.

The principal difficulty in this investigation has been that of bringing the benzene derivatives into solution in saline. Very few data as to their solubilities are to be found in the literature, and the amount dissolved at saturation is so dependent on the way in which saturation is brought about (shaking, temperature, etc.) that I have not been able to use even the few figures which are given. The procedure, accordingly, has been to dissolve weighed quantities of the various substances in 1 l. of saline contained in a 2 l. glass-stoppered bottle. This bottle with its contents is vigorously shaken at room temperature for several hours, and is then warmed to 60° C. in an oven with further shaking; if the contents remain clear and free of undissolved material after cooling to room temperature, the solution is tested for its accelerating effect. If the solution is cloudy or if there is undissolved material, a smaller quantity of the substance is weighed out, and the whole procedure repeated. In the case of some of the very insoluble substances, weighed quantities were dissolved in absolute alcohol, and 2 c.c. of the alcoholic solution added to 1 l. of saline.¹ The essential point is to be sure that a known amount of the substance is actually dissolved, so that its concentration is not in doubt. In the tables which follow, the greatest concentration of each substance tested is shown, so as to give the reader an idea of the amounts which can be dissolved.

Except for the estrogens, which were kindly given to me by Dr Marrian, the substances used were from the Eastman Kodak Company. All the time dilution curves were plotted at 25° C., and in all cases the red cells, the lysins, and the solutions of the added substances were made up in an isotonic NaCl-phosphate buffer mixture at pH 7·2.

¹ The resulting concentration of ethyl alcohol, 0·2 %, has no effect on the velocity of haemolysis.
I. EFFECT OF HALOGENATION

Benzene itself is an accelerator of lysis by saponin, sodium taurocholate, and sodium glycocholate, but its effect is not very great, the introduction of 0.8 c.c. of 10.2 mM./l. into systems containing saponin giving an $R$ value of 0.74, so that the value of $(R - 1)/c$ is only $-0.025$. The substitution of halogen atoms for one or more of the hydrogens, however, increases the accelerating power, as can be seen from the results in Fig. 1 and Table I. The first column of the table shows $c_{\text{max}}$, the greatest concentration of each substance tested, the second column gives $(R - 1)/c$ when the lysin is saponin, and the third the number of times which the substance
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exceeds benzene as an accelerator. Columns 4 and 5 give similar information when the lysin is sodium taurocholate. In all cases the results are given in terms of the concentration of the substance which is added in an amount of 0.8 c.c. to a system containing 0.8 c.c. of lysin and 0.4 c.c. of cells; the absolute concentration of the substance, when the lytic system is complete, is thus 0.4 time that shown in the tables. This must be borne in mind in carrying out certain types of computations (see § III).

Table I

<table>
<thead>
<tr>
<th>Substance tested</th>
<th>(c_{\text{max}}), mM./l.</th>
<th>Saponin ((R - 1)/c) × benzene</th>
<th>Taurocholate ((R - 1)/c) × benzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{C}_6\text{H}_4\text{Cl})</td>
<td>1.0</td>
<td>-0.025</td>
<td>1</td>
</tr>
<tr>
<td>(\text{C}_6\text{H}_5\text{Cl})</td>
<td>0.5</td>
<td>-0.00</td>
<td>7</td>
</tr>
<tr>
<td>(\text{C}_6\text{H}_5\text{Br})</td>
<td>0.0</td>
<td>-0.00</td>
<td>8</td>
</tr>
<tr>
<td>(\text{C}_6\text{H}_5\text{I})</td>
<td>0.0</td>
<td>-0.00</td>
<td>25</td>
</tr>
<tr>
<td>(\text{o-C}_6\text{H}_4\text{Cl})</td>
<td>0.0</td>
<td>-0.00</td>
<td>21</td>
</tr>
<tr>
<td>(\text{p-C}_6\text{H}_4\text{Cl})</td>
<td>0.0</td>
<td>-0.00</td>
<td>28</td>
</tr>
<tr>
<td>(\text{m-C}_6\text{H}_4\text{Cl})</td>
<td>0.0</td>
<td>-0.00</td>
<td>26</td>
</tr>
<tr>
<td>(\text{C}_6\text{H}_5\text{Br})</td>
<td>0.0</td>
<td>-0.00</td>
<td>88</td>
</tr>
<tr>
<td>(\text{p-C}_6\text{H}_5\text{Br})</td>
<td>0.0</td>
<td>-0.00</td>
<td>155</td>
</tr>
</tbody>
</table>

* These values are only approximate, because of the extreme insolubility of this substance. 1

The results illustrate several points.

1) The introduction of one Cl enhances the accelerating power of benzene 7 times; one Br, however, increases it 8 times, and one I 25 times. This order, \(\text{Cl} < \text{Br} < \text{I}\), occurs repeatedly in experiments on the acceleration of lysis, and is the order of reactivity of the halogens.

2) The introduction of two Cl (para) has a greater effect than has one Cl, and increased the accelerating power to 26 times that of benzene, but two Br (para) are more effective (88 times benzene), while two I (para) enhance the accelerating power of benzene 155 times.

3) The effect, however, also depends on the position of the halogens on the ring. Thus two Cl in the ortho position are more effective than two Cl in the meta position, and these more effective than two Cl in the para position. The effect of the position on the ring is more pronounced when taurocholate is the lysin.

4) As might be expected 1, 2, 4, tri-chlor-benzene exceeds the di-chlor-benzenes in accelerating power, and exceeds benzene 62 times in activity. 2

These conclusions bear out the general hypothesis that the accelerating effect is related to chemical configuration, and that the introduction of the halogens enhances the accelerating power of the benzene nucleus in the order of their own characteristic reactivities. The number of the halogen atoms, and also their position, determine the extent of the acceleration produced. It will be observed that the

1 See footnote 2 on p. 42.

2 The relation between configuration and accelerating power could be further tested if substances such as 1, 2, 3, tri-chlor-benzene, 1, 3, 5, tri-chlor-benzene, etc., could be obtained in a sufficiently pure state. Unfortunately, I have not been able to obtain many substances which I should have liked to have tested, and this accounts for many obvious lacunae in this investigation.
results obtained when taurocholate is the lysin are substantially the same as when the lysin is saponin, although there are numerical differences. We have examined a number of the substances with respect to their accelerating effect on sodium glycocholate lysis at pH 6·2, and on lysis by digitonin: again there are numerical differences, but the order of effectiveness is substantially the same.  

II. EFFECT OF ADDING RINGS

The detailed results are contained in Table II, drawn up in the same way as Table I.

Table II

<table>
<thead>
<tr>
<th>Substance tested</th>
<th>$c_m$</th>
<th>Saponin $(R - 1)/c$</th>
<th>Taurocholate $(R - 1)/c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM./l.</td>
<td>x benzene</td>
<td></td>
</tr>
<tr>
<td>C$_2$H$_6$</td>
<td>0.02</td>
<td>-0.025</td>
<td>1</td>
</tr>
<tr>
<td>C$_6$H$_5$</td>
<td>0.02</td>
<td>-1.2</td>
<td>48</td>
</tr>
<tr>
<td>aC$_{10}$H$_7$Cl</td>
<td>0.1</td>
<td>-1.7</td>
<td>68</td>
</tr>
<tr>
<td>$\beta$C$_{10}$H$_7$Cl</td>
<td>0.1</td>
<td>2.0</td>
<td>80</td>
</tr>
<tr>
<td>aC$_{10}$H$_7$Br</td>
<td>0.08</td>
<td>-2.5</td>
<td>100</td>
</tr>
<tr>
<td>$\beta$C$_{10}$H$_7$Br</td>
<td>0.05</td>
<td>-3.0</td>
<td>120</td>
</tr>
<tr>
<td>aC$_{10}$H$_7$I</td>
<td>0.04</td>
<td>-4.3</td>
<td>172</td>
</tr>
<tr>
<td>$\beta$C$_{10}$H$_7$I</td>
<td>0.04</td>
<td>-4.7</td>
<td>187</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.014</td>
<td>$&lt;\pm 0.71$</td>
<td>$&lt;28$</td>
</tr>
<tr>
<td>*Phenanthrene</td>
<td>0.025</td>
<td>$&lt;\pm 1.3$</td>
<td>52</td>
</tr>
<tr>
<td>*Benzpyrene</td>
<td>0.010</td>
<td>$&lt;\pm 1.0$</td>
<td>40</td>
</tr>
<tr>
<td>1, 2, 5, 6, Di- benzanthracene</td>
<td>0.010</td>
<td>$&lt;\pm 1.0$</td>
<td>40</td>
</tr>
</tbody>
</table>

* The values obtained appear to depend on the freshness of the solutions, and on the colloidal state in general.

Several points emerge from the data.

(1) Naphthalene is a much better accelerator than benzene (48 times better), and its halogenated derivatives are very powerful accelerators; again the order is Cl < Br < I, and the position of the halogen on the ring seems to have a slight effect, the $\beta$ position being the better. It is interesting that the introduction of one Cl, one Br, and one I increases the effectiveness of naphthalene 1-4, 21, and 3·6 times respectively, while one Cl, one Br, and one I increase the accelerating power of benzene 7·2, 8·0, and 25 times respectively. The enhancing effect of the halogens is accordingly less for naphthalene than for benzene.

(2) When we pass to anthracene, we find that the accelerating power, if still present, is less than 28 times that of benzene, and certainly less than half of that of anthracene.  

1 There are cases, however, in which a substance may be an accelerator or an inhibitor for one lysin, and inert with respect to another. For example, estriol inhibits saponin lysis, but has no effect in a taurocholate system; phenanthraquinone (0.02 mM./l.) is a powerful accelerator in a taurocholate system, but has little or no effect in a saponin system; aniline is a good accelerator for the bile salts, but affects saponin very little. These instances, however, are more the exception than the rule.

A technical difficulty appears here, for the greatest concentration of anthracene which can be made is about 0.014 mM./l. Suppose the smallest acceleration which could just be observed were $R = 0.99$; then $(R - 1)/c$ would be $-0.71$, an acceleration 28 times that given by benzene. In fact, it would scarcely be possible to distinguish between the small acceleration $R = 0.99$ and the small inhibition $R = 1.01$, so, in the case of a substance such as anthracene, all we can do is to set an upper
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naphthalene; adding the third ring, therefore causes a relative depression of the accelerating power. Phenanthrene, the isomer of anthracene, has a greater accelerating power, about 50 times that of benzene, and about the same as that of naphthalene. The position with respect to which the rings are connected together is therefore apparently of importance.

(3) The only 4-ring compounds which have been tested are estrin and estriol, both of which are inhibitors; these substances, however, scarcely fall into the same category as the other benzene derivatives which have been considered, the latter containing only C, H, and halogens.

Benzpyrene and dibenzanthracene, 5-ring compounds (C_{20}H_{14}) are so insoluble that they appear to have no effect on the velocity of haemolysis.

III. THE CONCENTRATION OF BENZENE AT THE RED CELL SURFACE

(1) It is obviously of considerable importance to determine the extent to which an accelerator such as benzene is concentrated at the cell surface, where its action is presumably exerted. This can be done in the following way.

Rabbit red cells are thoroughly washed, and made up to about the original volume concentration with 1% NaCl (saline). The volume concentration is found by haematocrite. Five c.c. of the suspension are then added to 5 c.c. of benzene in various concentrations in saline, and after about a minute, the cells are thrown down, and the quantity of benzene left in the supernatant fluid determined by the method of Schrenk et al. (1935: aeration and nitration).

Table III shows a set of typical results, obtained with a cell suspension of volume concentration 0.395. The first column shows C, the concentration to which the cells were exposed, and the second column the loss X, i.e. the quantity taken up by the cells. All quantities are in mg./c.c.

<table>
<thead>
<tr>
<th>C</th>
<th>X</th>
<th>X/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.250</td>
<td>0.178</td>
<td>0.71</td>
</tr>
<tr>
<td>0.125</td>
<td>0.096</td>
<td>0.76</td>
</tr>
<tr>
<td>0.063</td>
<td>0.050</td>
<td>0.79</td>
</tr>
<tr>
<td>0.032</td>
<td>0.025</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The relative constancy of the ratio X/C means that the amount of benzene taken up by the cells is roughly linear with the concentration to which they are exposed, although there is a little more taken up, proportionately, at the lower concentrations. Since the acceleration \((R - 1)\) is also roughly linear with the concentration, the accelerating effect tends to be proportional to the amount of the benzene taken up by the cells, which in itself is quite probable.

The accelerating power and say that it is less than 28 times that of benzene. Generally speaking, values are apt to be only very approximate in the case of very insoluble substances, because the acceleration produced is usually small and difficult to measure, yet \((R - 1)/c\) is relatively great because c is so small.
Assuming all the benzene which is taken up to be at the red cell surfaces, it is interesting to calculate how many monolayers could be formed. The number of rabbit cells contained in 1·98 c.c. of packed cells is about $10^{10}$, and their total surface would be about $10^{20} \text{A}^2$. A solution of benzene containing 0·25 mg./c.c. is 0·32 (10$^{-4}$) $M$, and so 5 c.c. of it contains $9·5(10^{18})$ molecules; of these, $0·71 \times 9·5(10^{18})$, or $6·8(10^{18})$ molecules are taken up by the cells (Table III, row 1). Each benzene molecule covers about $30 \text{A}^2$, so if all the molecules taken up from a 0·25 mg./c.c. solution were arranged at the cell surfaces, we would have about

$$2 \left(\frac{10^{20}}{10^{20}}\right) = 2 \text{ monolayers.}$$

By a similar calculation based on Table III, the amount of benzene taken up from a 0·125 mg./c.c. solution would make a single monolayer, that from a 0·063 mg./c.c. solution 0·5 of a monolayer, and that from a 0·032 mg./c.c. 0·25 of a monolayer. When the accelerating effect of benzene is observed in a system containing about $\frac{1}{40}$th of this number of cells, an $R$ value of about 0·97 is observed in a system containing 0·032 mg./c.c. of benzene in the completed system of 2 c.c. If the taking up of benzene is proportional to the cell concentration, this acceleration would result from the formation of about one-quarter of a monolayer of benzene at the cell surfaces.

It is possible, of course, that the amount of benzene taken up is not proportional to the cell surface, but a similar result may be arrived at in another way. Consider $p$-di-brom-benzene, one of the most active accelerators, which in a concentration of 0·1 mM./l. is 88 times as potent as benzene. In the volume of 0·8 c.c. added to the haemolytic system, there are $4·8(10^{16})$ molecules, each covering about $50 \text{A}^2$. The cell surface of the system is about $4·8(10^{17}) \text{A}^2$, so that there would be about five monolayers if all the accelerator were taken up. The concentration 0·1 mM./l. gives an $R$ value of 0·78, and an acceleration of about one-tenth of this, $R = 0·98$, can be observed with 0·01 mM./l.; this latter acceleration must therefore correspond to the formation of less than a monolayer at the cell surfaces, even if all the di-brom-benzene is taken up, which it probably is not.

(2) It is equally important to ascertain whether the accelerating effect of a substance such as benzene is wholly or partially reversible or irreversible, i.e. whether it exerts its effect by permanently altering the cell membrane as a result of a reaction with its components. This can be investigated in the following manner.

To 10 c.c. of benzene solutions of known concentration are added 5 c.c. of the standard red cell suspension. After standing for varying lengths of time, the mixture is divided between two 15 c.c. centrifuge tubes, and saline is added; the cells are then washed once or more often, and are finally made up to 5 c.c. with saline. Time-dilution curves are plotted for (a) a system containing this suspension, (b) a system containing a suspension of untreated cells, and (c) a system containing the particular concentration of benzene as an accelerator. The more closely the time-dilution curve (a) resembles the time-dilution curve (c), the more irreversible (by washing) is the effect of the accelerator; if curve (a) were identical with curve (b), the effect of the accelerator would be wholly reversible. To express the results quantitatively, $R$ values may be calculated in the usual way.
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Experiments of this kind with benzene show that its irreversible effect on the resistance of red cells depends on the length of time during which the accelerator and the cells are in contact. This is shown in Fig. 2, in which the abscissa shows \( t \), the time in minutes during which the cells were left in contact with 5·1 mM. /l. benzene before the washing was begun, and in which the ordinate shows \( R \), the resistance of the cells, as tested by comparing a time-dilution of the type (a), above, with one of the type (b). In this particular case, a curve of the type (c), with the accelerator present in the system during the lytic process, gave an \( R \) value of 0·73. It will be seen that the longer the period of contact, the smaller the value of \( R \), the latter constant falling off in a roughly exponential fashion towards the value 0·73; this means that the accelerator produces irreversible changes in the red cell resistance, these becoming greater as the period of contact becomes greater. I have no suggestion to make as to the nature of these changes, but presumably they are of the same nature as those changes in the cell surface which finally result in lysis in systems containing benzene in sufficient concentration. Similar results have been obtained with mono-chlor-benzene and with indol.

IV. OBSERVATIONS ON THE KINETICS OF THE ACCELERATION

Since the study of acceleration and inhibition of haemolysis is becoming as important as the study of lysis itself, it is necessary to add a few remarks regarding the kinetics of the acceleration produced by the benzene derivatives.

\( c_1/c_2 = \text{constant} \). Plotting \( c_1 \) against \( c_2 \) gives quite a good straight line in most cases, and this relation might arise in at least two ways: (a) We might imagine that the accelerator molecules, inserting themselves between the protein chains and lipoid layers which make up the membrane ultra-structure (Schmitt et al. 1936, 1938), break down or weaken

1 There seems to be a general belief that organic solvents denature proteins by forming interfacial films, but there does not appear to be any literature on the subject. The problem of the adsorption of protein by oils has been investigated in a semi-quantitative manner by St von Przylecki.
some of the intermolecular forces upon which the integrity of the membrane depends. The subsequently added lysin then finds some of its work done for it, as it were, and a smaller concentration \( c_2 \) is sufficient to complete the destruction which a larger concentration \( c_1 \) would effect in absence of the accelerator. Under such circumstances, the ratio \( c_1/c_2 \) would tend to be constant, for any particular concentration of accelerator would tend to break down the intermolecular forces in the membrane to a constant extent. (b) A layer, or layers, of the accelerator, situated at the red cell surface, might constitute a phase in which the lysin was \( 1/R \) times as soluble as it would be in the surface layers in the absence of the accelerator. A concentration of lysin \( c_2 \) would then be concentrated \( 1/R \) times at the surface, so as to become a greater concentration \( c_1 = 1/Rc_2 \). Either of these mechanisms, and probably a number of others, would lead to the same result, and the results of § III, above, suggest that both may be operative.
It should be emphasized, however, that the constancy of \( R \) is only approximate, for it is a familiar experimental fact that the values of \( R \) frequently show a definite tendency to approach unity as the asymptotes of the curves which are being compared are approached, i.e. whether an acceleration or an inhibition is being measured, the greater acceleration or inhibition is found corresponding to the greater lysin concentrations. This has been known to be the case for a long time, and most workers in the field have observed it, but it has always been put down as an "experimental inconsistency", about which the less said the better; if accelerations and inhibitions are to be closely studied, however, the inconsistency has to be taken into account. It is best observed with a very powerful accelerator; the effects of which can be accurately observed over a long range, and Fig. 3, which shows the \( R \) values obtained for systems in which \( 1 \) mM./l. indol is added to various concentrations of saponin in the usual proportions, illustrates the phenomenon under discussion.

On the ordinate are shown the quantities of saponin, in micrograms, while the \( R \) values are shown on the abscissa. The arrows mark the approximate position of the asymptotes of the standard curve and of the curve for the system containing indol respectively.

It will be apparent that the acceleration is virtually constant as long as the quantity of lysin is great \(( R = 0.35)\), but that when the amount of lysin in the standard system is less than the asymptotic concentration, the value of \( R \) increases, i.e. the acceleration becomes less. A dubious extrapolation beyond the asymptote of the time-dilution curve for the accelerated system suggests that the curve passes through \( R = 1.0 \) when \( c_1 = 0 \). This means that the accelerating effect is constant for concentrations greater than the asymptotic concentration \( c_{1,0} \), but that for smaller concentrations \(( R - 1)\) is proportional to \( c_1 \). What this relation means I cannot at present say, but it is clear that the acceleration of sublytic quantities of lysin is very different from that of quantities which can produce lysis per se.

\[ (R - 1)/c = \text{constant}. \]

Because of their insolubility, the accelerating effects of most of the benzene derivatives can be examined over a comparatively small concentration range only, and within this range it seems that the slope of the line \(( R - 1)/c \) is sufficiently constant. By using a powerful accelerator such as indol, however, it is easy to show that this relation is only an approximation, and Fig. 4 shows the \( R \) values obtained with indol.

![Graph showing acceleration of haemolysis](image)
concentrations from 1 mM/l. downwards. The value corresponding to the smaller indol concentrations lie on a very good straight line, but when the concentration of the accelerator becomes great, the curve turns upwards, and the linear relation no longer holds. It ought to be pointed out, nevertheless, that the results of §§ I and II of this paper are not affected by this lack of perfect linearity between \((R-1)\) and \(c\), or between \(c_1\) and \(c_2\), for the absence of perfect linearity is not seen in the limited experimental range over which most of the benzene derivatives can be studied.

**SUMMARY**

1. Benzene and its halogenated derivatives are accelerators of saponin and bile salt haemolysis. The order of effectiveness is benzene < chlor-benzene < brom-benzene < iodo-benzene. The addition of two halogens is more effective than the addition of one, and again the order is Cl < Br < I. In the case of the di-chlor-benzenes, the order of effectiveness is ortho > meta > para, and the addition of three Cl increases the accelerating power more than does the addition of two Cl.

2. Naphthalene and its halogenated derivatives are also accelerators, and again the order of effectiveness is Cl < Br < I. The addition of the halogen in the \(\beta\) position is more effective than the addition in the \(\alpha\) position.

3. Anthracene does not produce appreciable acceleration, perhaps because of its insolubility, but acceleration is produced by its isomer, phenanthrene. The 5-ring compounds which have been examined are too insoluble to produce any measurable effect; of the 4-ring compounds, only estrin and estriol have been examined, and these are inhibitors.

4. Benzene is concentrated at the red cell surface, the amount taken up being roughly linear with the concentration to which the cells are exposed. Acceleration can be observed when there are too few molecules in the system to form even a monolayer.

5. If an accelerator such as benzene is washed off the cells immediately, no accelerating effect remains, but if some time is allowed to elapse before the washing, effects irreversible by washing can be detected.

6. The investigation has brought to light certain hitherto undescribed peculiarities in the kinetics of acceleration, and these are discussed in detail.

**REFERENCES**