THE MECHANISM OF COMPLEMENT ACTION.

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It has been shown in the preceding paper that the hemolytic action of serum is to be attributed primarily to a single substance which is destroyed under the influence of ultra-violet light by a monomolecular reaction. This fact immediately raises the question of the nature of the photosensitive substance, and leads the writer to make some suggestions as to what such a substance might be. 1

I.

Certain experiments of the writer seem definitely to exclude the possibility that the serum proteins are primarily responsible for the power of serum to act as complement in specific serum hemolysis, for this power can be abolished by the action of light without producing the sensitization to heat which is characteristic of the effect of light on serum proteins. These experiments, which are summarized in Table I, show that radiated complement is not thereby made more susceptible to injury by heating. 2

If the rates of heat inactivation of any individual serum before and after radiation are compared, it will be noted that the differences of efficiency are so small as to be attributable to errors in titration;

* The experiments upon which this paper is based were done by the writer as Research Fellow in the Harvard School of Tropical Medicine.

1 In this paper the word "complement" will be used to denote any solution of serum used as one component in specific serum hemolysis (the other component being amboceptor); while that particular ingredient of serum primarily responsible for its hemolytic power is designated as the "lytic substance" or "lytic principle."

2 All three experiments were done before the development of dependable methods of titrating complement, and a probable error of about *3 per cent is to be expected.
and furthermore that the average value of the rate before radiation is almost exactly the same as the average value after radiation. This shows that radiation does not sensitize complement to heat. Experiments conducted for another purpose incidentally supply further proof that at temperatures up to 37°C, radiated complement deteriorates at the same rate as it did prior to radiation. ⑧

Now Bovie ④ and Chalupecky ⑤ independently pointed out that ultra-violet radiation sensitizes such proteins as egg white and lens protein to subsequent heating, so that they coagulate much more rapidly or at lower temperatures. In Bovie's experiments radiated egg white coagulated even at room temperature. Schanz ⑥ showed that ultra-violet light has a similar effect on serum proteins; he also confirmed and extended the findings of Chalupecky on egg white and lens proteins.

If the hemolytic power of the serum were due to its protein content, radiation sufficient to destroy a large part of that power should be

④ Bovie, W. T., Science, 1913, xxxvii, 373.
accompanied by changes which are at least qualitatively like those displayed by the pure proteins themselves under radiation. Since the lytic principle can be nearly destroyed without any perceptible alteration of the serum proteins, we are forced to the conclusion that complement is not one of the serum proteins, and is, to a certain extent at least, independent of them.

II.

This must not be thought to mean that the hemolytic power of serum is wholly independent of proteins under all conditions. The experiments of Jacoby and Schütze 7 on the inactivation of complement by shaking, those of Michaelis and Skwirsky 8 on the effect of proteases on complement, and many others indicate the contrary.

To obviate confusion it may be well to point out that while the hemolytic substance is probably not a protein, as explained in the preceding section, this is not incompatible with the fact that complement may be profoundly affected by changes in the serum proteins; inactivation may be the direct effect of some agent, e.g. light, or it may be indirect and due to the effect of a change produced by the action upon serum proteins of such an agent as a protease. The serum proteins thus altered may then act upon the lytic substance.

The following experiments bear out this idea, since they suggest that acid may inactivate complement not by means of its effect upon the lytic substance itself, but by altering the state of the serum proteins.

It has long been known that complement could be made inactive by the addition of acid and alkali, and that if more than a small amount of acid or alkali was added the inactivation was not reversible by subsequent neutralization of the added reagent. The precise limits of pH value between which inactivation is still reversible have not heretofore been determined.

Fresh guinea pig serum was diluted to 20 volumes with 0.85 per cent NaCl solution 9 and kept in containers immersed in ice water

9 The balanced solution employed for dilution throughout all other parts of this work would have resisted changes in reaction by reason of its bicarbonate content, and hence could not be used in this particular operation.
at all times except for one period of about $\frac{1}{2}$ hour while the acid was acting; during this period a temperature of 10°C. was maintained.

Three solutions were prepared for admixture with the complement: 0.04 N HCl, 0.04 N NaOH (adjusted by comparison with HCl), and a 0.02 N NaCl solution made by mixing equal volumes of these two solutions. All these solutions were cooled to the temperature of the diluted complement before mixing.

The general plan of the experiments involved the addition of the desired amount of acid, and after a certain length of time its neutralization by the equivalent amount of alkali. In each case enough 0.02 N NaCl was added to make the final volume and concentration the same in all the tubes. The effect of the change in NaCl concentration (from 0.85 to 0.73 per cent) was negligible in view of the subsequent dilution with much larger amounts of balanced solution. The dilution of the serum was taken into account in the calculations.

Sets of clean sterile test-tubes were set in an ice water bath, each set consisting of a tube in which was placed 10 cc. of 5 per cent complement, and tubes for the acid, alkali, and NaCl solutions. At the beginning of the experiment the samples of complement were in turn poured into the tubes containing acid and quickly mixed by pouring back and forth twice. After the desired interval these mixed samples were poured in like manner into the tubes containing alkali, and then into the final tube containing NaCl to equalize the volumes. The same operations were performed even when no acid or alkali was to be added, to obviate any possible effect due to the slight foaming involved in mixing the samples even though this caused no demonstrable inactivation.

After this treatment the complement was further diluted to the desired extent and titrated as described in a previous paper, by a method in which the probable error of determining the relative efficiency of samples of complement lies between 1 and 2 per cent.

The action of the acid apparently takes place very quickly; certainly in less than 15 minutes at 0°C. A preliminary experiment will serve to demonstrate this point. The efficiencies of two samples of complement left in the acid for 15 and for 66 minutes respectively.

were 88.6 and 89.7 per cent respectively. These figures differ by considerably less than the probable error and are therefore to be considered identical. In the remaining experiments the acid was allowed to act for about 30 minutes (the time varying by not more than ½ minute in any one experiment) so that there could be no doubt that it would have its full effect.

![Graph](image)

**Fig. 1.** The effect of temporary acidulation upon the subsequent efficiency of radiated and normal complement. The ordinates represent efficiency in per cent of that of the corresponding unacidulated complement, and the abscissae represent the pH to which the complement was exposed during the period of acidulation. Open circles, normal complement; solid circles, radiated complement; where these coincide the circles are shaded.

The relation between the true reaction during acid treatment and the hemolytic activity after the restoration of the normal reaction of serum (pH = 7.6) will be most easily grasped by a study of Fig. 1 in which relative efficiencies are plotted as ordinates against hydrogen ion concentration in terms of pH. These values of pH are accurate.
only within 0.2 pH units, because they were judged only on the basis of the approximate colors, in samples of the acidulated complement, of two or more of the following indicators; phenol red, brom cresol purple, methyl red, and brom phenol blue. No standard comparison solutions were used. Bearing these facts in mind it will be seen that the experiments agree in showing very little change in the efficiency of complement (perhaps even a slight increase in some cases) as long as the hydrogen ion concentration is less than pH = 6.0; when the

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Sample.</th>
<th>Exposure to light.</th>
<th>Efficiency at indicated pH; referred to corresponding unacidulated sample as 100 per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min.</td>
<td>pH</td>
</tr>
<tr>
<td>90</td>
<td>Normal.</td>
<td>0</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Radiated.</td>
<td>4</td>
<td>6.5</td>
</tr>
<tr>
<td>96</td>
<td>Normal.</td>
<td>0</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>Radiated.</td>
<td>5</td>
<td>5.4</td>
</tr>
<tr>
<td>97</td>
<td>Normal.</td>
<td>0</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Radiated.</td>
<td>5</td>
<td>6.4</td>
</tr>
<tr>
<td>98</td>
<td>Normal.</td>
<td>0</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>Radiated.</td>
<td>6</td>
<td>6.2</td>
</tr>
</tbody>
</table>

pH = 5.5, there is some decrease in the hemolytic power, and as it exceeds 5.0 there is a rapid drop in efficiency, complete inactivation being the result at pH = 4.3. The data for these experiments are given in detail in Table II.

There is a remarkable parallelism between this behavior and the known characteristics of the serum proteins. The serum proteins are usually classified as eu- and pseudoglobulins and albumins according to their solubility in water and in salt solutions; but there are no sharp divisions between these groups. The isoelectric point of euglobulins as prepared by Rona and Michaelis is given by them as 3.6

\( \times 10^{-4} \), which is equivalent to a pH of about 5.4; that is, the point at which complement begins to be affected by acid. The hydrogen ion concentration at which the inactivation of complement becomes complete is about pH = 5.0; this is just on the acid side of the isoelectric point of the serum albumin, which is at pH 4.7.\(^{12}\)

Now amphoteric substances, among which we must number the serum proteins, behave as anions on the alkaline side of their isoelectric points, and as cations on the acid side of their isoelectric points; while at this point the sign of their charge changes from negative to positive, and there are great changes in their physical properties corresponding to slight changes of hydrogen ion concentration.\(^{13}\)

### Table III.
The pH of Samples of the Same Lot of 5 Per Cent Complement Radiated for Different Lengths of Time.

<table>
<thead>
<tr>
<th>Exposure (min.)</th>
<th>Efficiency (per cent)</th>
<th>Hydrogen ion concentration (pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>7.55</td>
</tr>
<tr>
<td>1/2</td>
<td>95.7</td>
<td>7.52</td>
</tr>
<tr>
<td>1</td>
<td>90.9</td>
<td>7.52</td>
</tr>
<tr>
<td>2</td>
<td>85.6</td>
<td>7.52</td>
</tr>
<tr>
<td>4</td>
<td>74.0</td>
<td>7.55</td>
</tr>
<tr>
<td>7</td>
<td>57.6</td>
<td>7.50</td>
</tr>
<tr>
<td>10</td>
<td>43.6</td>
<td>7.55</td>
</tr>
<tr>
<td>18</td>
<td>23.6</td>
<td>7.55</td>
</tr>
</tbody>
</table>

Since the hydrogen ion concentration at which these changes occur is coincident with that at which complement loses its hemolytic power, it is not improbable that there is a connection between the phenomena. We might suppose that the protein cation takes part in an irreversible reaction with the lytic substance or some one of its constituent groups. Other possibilities might be suggested, but the evidence is not adequate for distinguishing between one possibility and another, and the essential point remains the same; that there is some close connection between the state of the serum proteins and the effectiveness of the lytic substance.


\(^{13}\) Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 237.
If this is true then the lytic substance may be inactivated in either of two ways: either directly, as by ultra-violet light; or indirectly by means of agents affecting other serum constituents; e.g., the proteins (Table III).

These same experiments show also that radiation sufficient to destroy the hemolytic power of serum may fail to have any appreciable effect on the serum proteins; otherwise there should be a difference between radiated and normal complement in their susceptibility to inactivation by acids (Fig. 1 and Table II). Differences between radiated and normal complement do occur occasionally, but they are so irregular as to have no obvious general significance. Radiation fails to sensitize complement to either heating (Section I) or acidulation, and the two sets of experiments substantiate each other in this respect.

III.

In view of the fact that surface tension of complement has been thought by some to be connected directly with its hemolytic power it is interesting to note the change in surface tension taking place when complement is inactivated by ultra-violet light.

The surface tension of 5 per cent complement solution was measured by means of a Traube stalagmometer and was found to decrease slightly (Table IV and Fig. 2). Measurements of the time of outflow made at the same time are probably not reliable because of the presence of occasional wisps of cotton in the solutions, but since they seem to indicate a change in viscosity they are also given: if there is any definite change of viscosity it is a decrease.

In this connection it should be pointed out that changes in hydrogen ion concentration play no part in the ordinary course of photoinactivation. Samples of 5 per cent complement were taken immediately after radiation for various lengths of exposure and their hydrogen ion concentration determined by the addition of an appropriate amount of phenol red and comparison of the resulting color with that produced in solutions of known hydrogen ion concentration. The least active sample had been reduced to a relative efficiency of 23.6 per cent. There was an irregular variation of between pH = 7.55 and 7.50, which is so small as to be utterly negligible (Table III). The complement is at all times exposed to a hydrogen ion concentration the same as that prevailing in the blood plasma.

Traube, J., Biochem. Z., 1908, x, 371.

Traube, J., Biochem. Z., 1908, x, 380.
TABLE IV.
The Surface Tension and Viscosity of 5 Per Cent Complement Solutions Radiated for Different Lengths of Time.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Exposure</th>
<th>Efficiency</th>
<th>Outflow from Stalagmometer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>per cent</td>
<td>No. of drops</td>
</tr>
<tr>
<td>71</td>
<td>0</td>
<td>100.0</td>
<td>56.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>91.0</td>
<td>56.4</td>
</tr>
<tr>
<td></td>
<td>3½</td>
<td>82.4</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>52.4</td>
<td>57.8</td>
</tr>
<tr>
<td>Diluent alone</td>
<td></td>
<td></td>
<td>56.0</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td>100.0</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>½</td>
<td>95.7</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>85.6</td>
<td>57.2</td>
</tr>
<tr>
<td></td>
<td>8½</td>
<td>50.0</td>
<td>57.25</td>
</tr>
<tr>
<td>Diluent alone</td>
<td></td>
<td></td>
<td>56.1</td>
</tr>
</tbody>
</table>

Fig. 2. The progressive changes in surface tension and viscosity of complement during photoinactivation. The squares indicate the data for the diluent alone. The ordinates represent the time, or the number of drops required for the outflow of a constant amount of complement from a Traube stalagmometer. The abscissae represent in minutes the time of exposure of the samples to the light. Open circles and square, surface tension; solid circles and square, viscosity.
The change of surface tension is like that accompanying thermo-
inactivation, and contrary to the hypothesis advanced by Traube, is probably not the cause of the change in hemolytic power.

**IV.**

In the following paragraphs there is suggested an hypothesis which has the advantage of explaining many of the known properties of serum complement with more definiteness than current theories and without being in conflict with any well established fact. Definite substances are named only for the sake of making it easier to grasp certain essential ideas, and not with any pretense that complement must be supposed to consist of just these particular substances. The main ideas are these: that there is a hemolytic substance in serum which is constantly breaking down into non-lytic material, and constantly renewed from a store of some "precursor" substance; that this lytic substance is so related to the serum proteins that it is more or less permanently inactivated by certain changes in the serum proteins; and that the hemolytic substance and all its precursors contain a certain photosensitive molecular grouping whose alteration results in photoinactivation.

These essential points may be embodied in the following scheme

\[
A \rightarrow \text{changed by light to } a \\
\downarrow \\
B \rightarrow \text{" " " " } b \\
\downarrow \\
B' \rightarrow \text{" " " " } b' \\
\downarrow \\
C
\]

in which the different letters represent different chemical individuals; the changes \( A \rightarrow B \), \( a \rightarrow b \), \( B' \rightarrow C \), and \( b' \rightarrow C \) represent hydrolyses, and the changes \( B \rightarrow B' \) and \( b \rightarrow b' \) the passage of \( B \) or \( b \) from solution in fats to solution in water as a result of a change in relative solubility produced by the preceding hydrolysis. \( B \) (i.e. \( B' \)) is the principal lysin, and \( b' \), formed by radiation, is probably also hemolytic to a certain extent.

When complement is heated we need consider only the left-hand series, \( A \rightarrow B \rightarrow B' \rightarrow C \). \( A \) is a precursor present in large amount

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in serum, and undergoing transformation into $B$ at a rate which is not very greatly accelerated by an increase in temperature. The change $B \rightarrow B'$ is very rapid and is probably never a limiting factor. $B'$ is the lysin and undergoes hydrolysis at a rate markedly influenced by changes of temperature, and proportional to the amount of $B'$ present at the moment, as in any monomolecular reaction such as most hydrolyses.

Let us suppose serum to be collected and placed at $0^\circ C$. The breakdown of $B'$ may be retarded by the lowered temperature, and $B'$ will accumulate until there is so much that the amount broken down in a unit of time will equal the amount formed from $A$. (We may suppose the process $B \rightarrow B'$ to be so rapid as to have no perceptible influence on variations in the amount of $B'$.) Since the reaction $A \rightarrow B$ is also monomolecular, the amount of $B$ formed in any time interval will be less and less as $A$ is used up; and to keep pace with this change $B'$, the lysin, must also gradually decrease in amount. Thus the hemolytic power, which is proportional to the amount of $B'$, will gradually decrease. This is a well known phenomenon.

Let us suppose that serum which has reached this steady state of equal formation and destruction of $B'$ is suddenly raised to a high temperature, e.g. $56^\circ C$; the destruction of $B'$, which we have assumed to have a high temperature coefficient, will be enormously accelerated while its formation will still be relatively slow. As a result the hemolytic power of the serum will rapidly decrease, and, since its rate of formation is at first relatively negligible, the decrease will follow the course of a monomolecular reaction. This will continue until so little $B'$ is left that it decomposes at a rate comparable with that of the change $A \rightarrow B$. Madsen and Watabiki\(^{18}\) present data which not only show that thermoinactivation of complement follows the course of a monomolecular reaction (about as closely as photoinactivation) but that the temperature coefficient at $50$–$56^\circ C.$ is very high ($Q_{10}$ lying between $123$ and $366.8$), but between $3$ and $37^\circ C.$ is about that of typical dark reaction ($Q_{10}$ between $1.98$ and $2.94$).\(^{19}\)


\(^{19}\) Madsen and Watabiki express their results in terms of the temperature coefficient of the van't Hoff-Arrhenius formula. The values of $Q_{10}$ given above are calculated directly from their data.
This agrees exactly with our hypothesis, both as to the order of the reaction of thermoinactivation, and in that the destruction of $B'$ with its high temperature coefficient is predominant at temperatures above 50°, while the limiting reaction at temperatures below 37° is the breakdown of $A$ which has a relatively low temperature coefficient. The gradual decrease of $B'$ then keeps pace with the gradual decrease of $A$. Whenever, as after brief heating to 56° for example, the concentration of $B'$ is disproportionately reduced and a large amount of $A$ is still left, the latter will act as a reservoir from which $B'$ will be restored to the concentration appropriate to the temperature and the remaining amount of $A$. The hemolytic power being proportional to $B'$ will be low immediately after heating and will then be regenerated as Gramenitzki\textsuperscript{20} and the writer\textsuperscript{1} have shown to be the case.

Turning now to the question of what occurs during photoinactivation we find that if the time curves of several experiments are averaged the process follows the course of a monomolecular reaction.\textsuperscript{21} Now if only $B'$ were hemolytic and the members of the left-hand branch of our hypothetical series (i.e. $A$, $B$, and $B'$) contained the same photosensitive group, the proportion of each substance destroyed would be the same; that is, one-tenth or one-half or nine-tenths of each of them would be destroyed, but not one-half of $A$ and nine-tenths of $B$ and $B'$. Under certain conditions which were defined in a previous paper\textsuperscript{21} the destruction of a substance by light will follow the course of a monomolecular reaction, and if $B'$ is so destroyed, and no disproportionate amount of $A$ and $B$ is left (the latter case occurs when complement is briefly heated), then there can be no regeneration. The theory then accounts for the observed course of photoinactivation and for the fact that no regeneration follows photoinactivation.

The reader should now note carefully the curves shown in Fig. 2, of the preceding paper. They are all characterized by the fact that at some time during the process the hemolytic activity exceeds that to be expected if the process followed exactly the course of a monomolecular reaction; there is a "wave" of excess activity. If we suppose that $b'$ is hemolytic and that it forms and decomposes at a relatively

\textsuperscript{20} Gramenitzki, M., Biochem. Z., 1912, xxxviii, 501.
rapid rate it is easy to see that as A is changed to a the concentration of a will increase; later as A is used up a will decrease again. Similarly b and b' will appear, increase, and disappear. If b' is hemolytic there will be a "wave" of hemolytic power in addition to that due to B' and not only the average time curve of photoinactivation, but also the divergence of individual curves from this average is satisfactorily accounted for. The time at which this wave occurs in the course of the process will depend on the rate of the slower of the processes, a to b and b to b'; presumably this is a to b which may well be dependent on an enzyme whose concentration is different in different samples of serum. If the conditions are favorable we may expect the wave of excess hemolytic power to be so large and so early that the complement becomes more efficient when radiated, as during the early part of Experiment 69 of the preceding paper.22

In order to render this hypothesis more tangible it is desirable to suggest the nature of the substances A, B, a, b, and so on, even if there is no direct evidence for their exact composition. There is considerable evidence that fatty acids are important in immune reactions: Warden23 has synthesized from fatty acids antigens which produce specific antibodies against blood cells and gonococcus; Jobling and Bull24 have shown that the lipase content and hemolytic power of human sera vary together. Noguchi,25 among others, has shown striking analogies between complement and solutions of fatty acid compounds dissolved in serum albumin solutions. All these facts suggest the presence of a fatty acid compound acting as hemolysin in complement.

The most active hemolysin of which the writer has found any record is the "lysocithin" produced by the action of cobra venom upon crude lecithin and studied by Fourneau and his students. It appears to be at least 50 times as active as saponin and perhaps 100 times as active as the most hemolytic soaps. It is a fatty acid compound: choline monopalmitoglycerophosphatate.26 It should be noted that

22 Brooks, S. C., J. Gen. Physiol., 1920–21, iii, 180, Table IV; and 181, upper curve of Fig. 2.
25 Noguchi, H., Biochem. Z., 1907, vi, 327.
this substance is soluble in warm water, and hardly soluble in benzene, while complement is an aqueous solution, and is not easily injured by extraction with benzene.\textsuperscript{27} Lysocithin also forms an irreversible compound with emulsified cholesterol, thus reminding one of the fact that emulsions of cholesterol "fix" complement. Furthermore, lysocithin is formed by the action of venom lipase on lecithin but is destroyed by the further action of the same agent; removal of the second fatty acid leaves the lecithin complex inactive.\textsuperscript{28} These two steps might be compared with the process which results in spontaneous deterioration of complement.

With these facts in mind we may proceed to picture the nature of the hemolysin system in complement as follows:


In this scheme R represents choline glycerophosphoric acid or some similar substance, and, since compounds of lower fatty acids are markedly lacking in hemolytic power, the two fatty acids may each be supposed to contain at least ten carbon atoms. One of the acids is for two reasons supposed to be unsaturated: because compounds of unsaturated fatty acids are in general more hemolytic than those of the corresponding saturated acids, as shown by Lamar; and because this furnishes a molecular grouping known to be attacked by light with a consequent break in the fatty acid chain which might be expected to bring about a great decrease in hemolytic power.

Many phenomena displayed by complement are undoubtedly dependent in some way upon the physical or chemical state of the serum colloids, as is shown for example in the previously noted inactivation by acid. Sachs and Stilling have shown that inulin suspended in cold water affects complement, while if first dissolved by warming the water it no longer has any effect. It is not surprising then that complement is inactivated by shaking, since shaking, or rather the attendant foaming, causes irreversible coagulation of proteins, nor that processes which precipitate serum globulins should, if the process is reversible, produce the so called “fractions” which upon being recombined regain their hemolytic power. The conflicting nature of the evidence about these so called fractions, as well as the writer’s own experience with several methods of obtaining the fractions, leads him to doubt the uniformity of the preparations secured by different investigators. Evidently the lytic substance is usually held inactive in one of the fractions, since exposure of sensitized red blood cells to the globulin or “mid-piece” fraction results in a change which makes them susceptible of lysis by the albumin fraction, which is therefore supposed to combine with some element in the red blood cells indirectly through the “mid-piece.” It is for this reason that the albumin fraction has received the name “end-piece.”

33 Ramsden, W., *Arch. Physiol.*, 1894, 517.
When cobra venom is allowed to act upon complement under certain conditions there ensues a change in the serum such that when this cobra serum, as it is called, is combined with either mid- or end-piece of normal complement, or when either fraction of the cobra serum is combined with the supplementary fraction of normal serum the mixture is hemolytic. This is supposed to be due to the action of cobra venom upon a "third component" of complement. Hemolysis by complement would then be considered to result from the combined action of mid-piece, end-piece, and third component, all of which must be present.

The writer is inclined to regard the cobra serum as serum from which all the lysin and its precursors have been removed. The third component, which is destroyed by cobra venom, is the lysin and its related substances. This is in accord with the work of Delezenne and Fourneau and with the observed characteristics of cobra serum.

Phenomena susceptible of explanation in terms of the scheme here proposed abound in the literature, and their descriptions might be multiplied indefinitely if anything were to be gained by so doing. It is of greater significance that search of the voluminous literature on complement has so far failed to discover any well established fact which is incompatible with this hypothesis. If such facts are found, the hypothesis must of course be replaced by some better one; but the writer believes that the main ideas underlying it, namely successive transformations of precursor into lysin and then into inactive products, and the dependence of this lysin on the serum proteins, will prove to be valid. At any rate they seem preferable to certain prevailing explanations based upon the rather indefinite concept of a hemolytic power resulting from the colloidal properties or "lability" of serum proteins.

SUMMARY.

It has been shown:
1. That complement exposed to ultra-violet light is not thereby sensitized to the action of heat (which indicates that it is not protein).


Such ideas have been expressed by many investigators and have recently been developed and emphasized by Sachs in a review of the work of his laboratory (Sachs, H., Koll. Z., 1919, xxiv, 113).
2. That inactivation of complement by ultra-violet light is accompanied by a decrease in its surface tension.

3. That photoinactivation of complement is not a result of any changes in hydrogen ion concentration since these are less than 0.05 pH.

4. That hydrogen ion concentrations high enough to transform serum proteins from the cation to the anion condition (i.e. past the isoelectric point) permanently inactivate complement.

These facts together with those given in previous papers lead to the following hypotheses.

1. That there is present in serum a hemolytic substance which is formed from a precursor (which may resemble lecithin) and is constantly being formed and simultaneously being broken down into inactive products.

2. That both precursor and lysin contain the same photosensitive molecular group.

3. That the lytic substance is dependent for its activity upon the state of the serum proteins.