LXXXIII. THE RELATIONSHIP BETWEEN HAE-
MOLYTIC COMPLEMENT OF GUINEA-PIG SERUM
AND LIPASE.

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The system in serum which is responsible for the haemolysis of sensitised red
cells appears to have many of the properties of an enzymic system, and it
has been suggested by several authors that this haemolysis is due to the
action of an enzyme on integral constituents of the red cell, leading to a
rupture of the membrane [cf. Landsteiner, 1929]. Complement and enzymes
have many properties in common, but certain facts have been adduced to
disprove the view that complement is enzymic in nature. Complement,
unlike the enzymes, appears to take part in specific haemolysis according to
the law of definite proportions [Liebermann, 1906] and is used up as the
reaction proceeds; these facts can probably be explained, however, by a
destruction of complement by secondary processes [cf. Liefmann and
Cohn, 1911] and, indeed, a similar inactivation occurs in many enzymic reactions.

The investigations described here were concerned with (a) the general
relationship between the complement power and the esterase activity
of guinea-pig serum and heated guinea-pig serum (56° for 30 mins.); (b) the
lipase and esterase activity of guinea-pig serum which has been inactivated
for haemolytic power by treatment with zymín, which inactivates the third
component, or with ammonia which inactivates the fourth component;
(c) the influence of the amboceptor on the lipase and esterase activities
of guinea-pig serum; and (d) the esterase activity of a special protein prepara-
tion from guinea-pig serum, a preparation which has no complete complement
power but retains part of the complement system.

If the action of haemolytic complement can be regarded, according to the
lipolytic theory, as the action of a serum-lipase on the fatty constituents of
the envelope of the sensitised red cells, then it might be expected that the
haemolytic activity would run parallel with the power to hydrolyse fats.
The lipase of guinea-pig serum has, however, little or no action on such fats
as olive oil, and therefore the lipase (or esterase) activity has to be determined
by the use, as substrate, of some ester such as ethyl butyrate. Our experi-
ments, in which the sera of four or five guinea-pigs were compared at the
same time, have shown that the two activities, haemolytic power and esterase
power, are not always parallel, although a general parallelism has been observed. In addition, it has been found that guinea-pig serum heated at 56° for 30 mins. has no demonstrable haemolytic action on sensitised red cells, but has a significant esterase action.

Complement can be "split" by dilute acids into two separately inactive fractions, a globulin and an albumin fraction, each of which contains a heat-labile component (possibly of protein nature) and a relatively heat-stable component. It is possible, however, to destroy either of these two relatively heat-stable factors with no significant loss of the other components of the system, and, if the lipase and complement systems are identical, these methods of inactivation—the removal of the third component of complement by treatment of the serum with yeast (or zymin) or the inactivation of the fourth component by ammonia—should produce a diminution or destruction of the lipolytic power. It has been found, however, that zymin-inactivated serum and ammonia-inactivated serum, which have no haemolytic complement action, have lipase and esterase activities equal to those of the original serum. The third and fourth components of haemolytic complement, therefore, are not essential for serum-lipase action.

In view of the suggestions made by other authors that the immune body functions either as a specially active lipase or as a co-enzyme for the serum-lipase, we have carried out investigations to determine the lipase and esterase activities of guinea-pig serum before and after the addition of sufficient immune body to render the serum haemolytic for unsensitised red cells. These experiments have shown that the immune serum used has, in the concentrations necessary for promoting haemolysis, practically no lipase or esterase action, nor does it activate the lipase or esterase of normal guinea-pig serum.

Other investigations which we have been carrying out have been concerned with the rôle played by the proteins of serum in complement action, and it was thought of interest to compare the lipolytic activities of the protein preparations used with those of the original sera. Similar preparations from pig's pancreas, obtained by the method of Hardy and Gardner [1910] and Hartley [1925] with the modification introduced by Hewitt [1927] have proved very useful in lipase and esterase investigations [Dawson and Platt, 1928] (Phillos and Platt, unpublished investigations), and from the results of the last-named authors we expected to find a marked esterase power with our protein preparations from guinea-pig serum. This proved to be the case, and these preparations possess a high percentage of the lipolytic power of the original guinea-pig serum. The preparations have, however, no haemolytic action on sensitised red cells, and thus support is furnished for the view that the action of complement is not due to the action of a simple lipase or esterase system.

The experiments described here indicate, therefore, that there is no simple relationship between haemolytic complement and serum-lipase or -esterase, but there is still the possibility to be considered that this enzyme is one of
the components of the complement system. The experiments with ammonia-
treated serum and zymin-treated serum show, however, that no relationship
exists between the serum-esterase or -lipase and the relatively heat-stable
components of complement, whilst the retention of a significant part of the
esterase power, when the serum is heated at 56° for 30 mins., appears to
indicate that the lipolytic function cannot be attributed to the heat-labile
components since the latter have no appreciable haemolytic activity after
this heating.

Experimental.

Guinea-pig serum was used as the source of complement and for the
haemolytic tests the serum was diluted accurately with 0·9 % NaCl or, in
certain experiments, as described later, with a phosphate-NaCl mixture. To
varying amounts of the diluted serum, 1 cc. of a 4 % suspension of sensitised
red-cells (ox) and sufficient 0·9 % NaCl to make the total volume 2 cc. were
added, and the mixtures were incubated at 37°. The degree of haemolysis
after 0·25, 0·5 and 1 hour was recorded as follows:

\[+ + + + \text{ Complete haemolysis} \quad + + + \text{ Intermediate grades of haemolysis}\]

\[+ + + \text{ Complete haemolysis} \quad + + \text{ No haemolysis}\]

The esterase activity of a serum was determined by allowing the serum to
act on ethyl butyrate in the presence of a phosphate buffer of \(p_H\) 7·6. The
mixtures were placed in small stoppered bottles and were shaken for 2–4 hours
at 37°. The contents of the bottles were then titrated with 0·1 \(N\) sodium
hydroxide to a standard colour with phenolphthalein or thymol blue. Control
experiments with serum plus buffer (no ester) were made in every case and
toluene was added to each bottle to inhibit changes due to bacterial action.
The figures given in the tables below represent the mean of two duplicate
determinations.

The “true” lipase activity of guinea-pig serum, or the power to hydrolyse
olive oil, is very slight, and the amount of hydrolysis noted is very little
greater than the experimental error of the method, but it was thought de-
sirable to make determinations of this nature, especially in those experiments
(e.g. those with the immune serum) where an increase in this power would
be of some significance. The technique was similar to that for the esterase
determinations. A neutralised emulsion of olive oil was used as substrate
and the mixtures were titrated at the end of the experiment with 0·05 \(N\)
alcoholic KOH after the addition of 5 to 6 volumes of an alcohol-ether
mixture (4 volumes of absolute alcohol and 1 volume of ether), thymol blue
or thymolphthalein being used as indicator. The amount of hydrolysis was
very small even after incubation for 3 days. It was also thought desirable
to determine whether any relationship exists between the lecithin-splitting
power of the serum and complement. It has been shown, however [Schumoff-
Simanowski and Sieber, 1906], that the sera of various animals have no appreci-
ciable hydrolytic action on lecithin, and we are indebted to Dr E. R. Dawson
for confirming this with the guinea-pig sera which we have used for our tests.
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(a) Comparison of the complement and esterase activities of normal serum and those of heated serum.

Serum was collected from several guinea-pigs and the complement and esterase powers of each serum were determined. A result which is typical of many experiments is recorded in Table I.

Esterase activity.

(a) 10 cc. phosphate buffer pH 7·6 (or NaCl-phosphate solution) plus 1 cc. serum plus 0·5 cc. ethyl butyrate plus 1 cc. toluene.

(b) Control—as in (a) but no ethyl butyrate.

The NaCl-phosphate solution, which was used in certain experiments in the esterase determinations and used also for diluting the complement for the haemolytic test, consisted of a mixture of equal volumes of 1·35 % NaCl and a phosphate buffer of pH 7·6 [see Cole, 1926, p. 29]. This mixture was used in many experiments since it rendered possible a comparative measurement of lipolysis and haemolysis with a constant salt and phosphate concentration. The results were always identical with those in which the simple phosphate buffer was used for the esterase (or lipase) determinations and 0·9 % NaCl for the haemolytic tests.

Table I. Complement activity and esterase activity of different guinea-pig sera.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Complement activity</th>
<th>Haemolysis of sensitised cells after 1 hr. by</th>
<th>Esterase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0·010–0·03 cc. of serum</td>
<td>0·025 cc. of serum</td>
<td>0·018 cc. of serum</td>
</tr>
<tr>
<td>1</td>
<td>++ + + +</td>
<td>++ + + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>2</td>
<td>++ + + +</td>
<td>+ + + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>3</td>
<td>++ + + +</td>
<td>++ + + +</td>
<td>++ + + +</td>
</tr>
<tr>
<td>4</td>
<td>++ + + +</td>
<td>++ + +</td>
<td>++ + + +</td>
</tr>
</tbody>
</table>

From the results given in Table I and from those of a large number of experiments in which the conditions of the determinations have been varied, the conclusion is reached that the two functions, the haemolytic complement activity and the esterase activity of guinea-pig serum, do not show that relationship which would be expected if the haemolytic action of complement were due to the action of the serum-esterase on the fats of the red cell.

Since haemolytic complement is readily inactivated when guinea-pig serum is heated at 56° for 0·5 hour, it was thought of interest to determine the influence of this treatment on the lipase and esterase of the serum. Jobling and Bull [1913] were unable to obtain a lipase-free complement by heating the serum and found that the lipase was somewhat more resistant to heat than complement.

The results of typical experiments are given in Table II, and they show that, whereas the heating process destroys all the measurable complement activity of the serum, a significant part of the esterase activity is retained;
in addition, no detectable diminution of the slight "true" lipase activity of the serum occurs as a result of the heating process.

**Esterase activity.**

(a) 2.0 cc. serum plus 10 cc. phosphate buffer $p_H 7.6$ (or NaCl-phosphate solution) plus 0.5 cc. ethyl butyrate plus 0.5 cc. toluene.

(b) Control (no ethyl butyrate).

**Lipase activity.**

(a) 4.0 cc. serum plus 4 cc. phosphate buffer $p_H 7.6$ (or NaCl-phosphate solution) plus 1 cc. neut. emulsified olive oil plus 1 cc. toluene.

(b) Control—no olive oil.

(c) Control—olive oil plus phosphate buffer plus toluene.

Control (c) showed that a very slight hydrolysis of the oil occurred even in the absence of serum, and a correction for this hydrolysis has been made in all the experiments with olive oil.

**Table II.**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Esterase activity (cc. 0.1 N NaOH)</th>
<th>Lipase activity (cc. 0.05 N alc. KOH)</th>
<th>Complement activity (amounts of serum as in Table I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.56</td>
<td>0.25</td>
<td>Good (as in Table I)</td>
</tr>
<tr>
<td>Heated (56° for 30 mins.)</td>
<td>0.85</td>
<td>0.27</td>
<td>None</td>
</tr>
<tr>
<td>Incubation period (in hrs.) for lipase determinations</td>
<td>2 1/2</td>
<td>44</td>
<td>70 72</td>
</tr>
</tbody>
</table>

(b) The esterase and lipase activities of NH$_3$-inactivated serum and zymin-inactivated serum.

The "third component" of serum, a relatively heat-stable component, is inactivated when the serum is treated with yeast [Coca, 1914; von Dungern, 1900; Ehrlich and Sachs, 1902] or zymin [Whitehead, Gordon and Wormald, 1925]. Another component is destroyed or inactivated by small amounts of ammonia [Gordon, Whitehead and Wormald, 1926] and zymin-inactivated serum and NH$_3$-inactivated serum are each devoid of complement activity. Experiments have been made to determine the effect on the esterase and lipase of the serum of destruction of these two components, and it has been found (cf. Table III) that the three sera concerned—untreated guinea-pig

**Table III.** Lipase and esterase activities.

Cf. details for Table II. The period of incubation for these determinations was 2–4 hrs. for the esterase and 48–72 hrs. for the lipase determinations.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Esterase activity</th>
<th>Lipase activity</th>
<th>Complement power (amounts as in Table I)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc. of serum used</td>
<td>cc. 0.1 N NaOH</td>
<td>cc. of serum used</td>
</tr>
<tr>
<td>Normal</td>
<td>0-4</td>
<td>2.40</td>
<td>4-0</td>
</tr>
<tr>
<td></td>
<td>2-0</td>
<td>3.40</td>
<td>4-0</td>
</tr>
<tr>
<td>Zymin-inactivated</td>
<td>0-4</td>
<td>2.40</td>
<td>4-0</td>
</tr>
<tr>
<td>NH$_3$-inactivated</td>
<td>2-0</td>
<td>3.70</td>
<td>3-90</td>
</tr>
</tbody>
</table>
serum, zymin-treated serum and NH₃-treated serum—have approximately the same esterase power. The three sera have also the same "true" lipase activity, although this activity, as mentioned above, is extremely weak compared with the esterase activity. These results indicate, therefore, that the two relatively heat-stable components are essential for haemolytic complement action but not for the action of the esterase or lipase of guinea-pig serum.

(c) *The influence of the immune serum on the esterase and lipase activities of guinea-pig serum.*

Sufficient immune serum (anti-ox-erythrocyte serum) was added to guinea-pig serum to render the mixture haemolytic for unsensitised ox red-cells and the lipase and esterase powers of this mixture were compared with those of the guinea-pig serum. The results of these experiments (Table IV) indicate that the immune serum (previously heated at 56° for 30 mins. to destroy the complement present) has no appreciable lipolytic activity in the amounts used, nor has it any activating action on the esterase or lipase of guinea-pig serum.

**Esterase activity.**

Series (a). A. 0·4 cc. guinea-pig serum plus 10 cc. NaCl-phosphate buffer pH 7·6 plus 0·5 cc. ethyl butyrate.

B. 0·4 cc. guinea-pig serum plus 10 cc. NaCl-phosphate buffer pH 7·6 plus 0·5 cc. ethyl butyrate plus 0·025 cc. inactivated immune serum.

C. 0·4 cc. 0·9% NaCl plus 10 cc. NaCl-phosphate buffer pH 7·6 plus 0·5 cc. ethyl butyrate plus 0·025 cc. inactivated immune serum.

Series (b). The same as series (a) with 5 times the amount of guinea-pig and immune sera. Controls, with no ester, were made for all these determinations, and 0·5 cc. of toluene was added to each solution. The mixtures were shaken at 37° for 2–4 hours and were then titrated with 0·1 N NaOH, phenolphthalein being used as indicator.

**Lipase activity.**

Amounts of serum and buffer as for Table II—incubation period, 72 hours.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Esterase activity (cc. 0·1 N NaOH required)</th>
<th>Lipase activity (cc. 0·05 N alc. KOH)</th>
<th>Haemolysis of unsensitised ox-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal guinea-pig serum (A)</td>
<td>(a) 3·22 2·26 2·60</td>
<td>0·10 0·10</td>
<td>None</td>
</tr>
<tr>
<td>Guinea-pig serum + immune serum (B)</td>
<td>(a) 3·36 2·28 2·70</td>
<td>0·13 0·09</td>
<td>Good</td>
</tr>
<tr>
<td>Immune serum (C)</td>
<td>(a) 0·06 0·06 0·05</td>
<td>0·0 0·03</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>(b) 0·14 0·08 0·05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(d) Esterase activity of a protein preparation from guinea-pig serum.

The proteins of guinea-pig serum were precipitated with a mixture of alcohol and ether at low temperatures by the method of Hardy and Gardner [1910] as used by Hartley [1925] and stable, finely divided powders obtained. Similar preparations obtained by treating serum with alcohol at low temperatures were found by Dean [1913] to have no end-piece of complement and our preparations, in a similar manner, showed no complete complement power. These preparations retained, however, a large part of the esterase activity of the original serum and thus possession of esterase activity is not sufficient to endow the preparation with full complement power.

Experimental details.

A. 5 cc. of guinea-pig serum plus 36 cc. of cold alcohol-ether mixture [cf. Hartley, 1925]. The protein preparation was extracted continuously in a Soxhlet apparatus for 20 hours and then dried in vacuo. The powder was dissolved in 4·5 cc. of distilled water and made up to a volume of 5·0 cc. with sufficient NaCl to give 1 % NaCl in the final solution.

B. The same guinea-pig serum plus toluene, kept at room temperature.

C. The same guinea-pig serum, kept in the ice-box.

D. Fresh guinea-pig serum.

The esterase determinations were made as before, with phosphate buffer (pH 7·6) or, in some experiments, the NaCl-phosphate buffer mixture. Series (a) with 0·4 cc. serum and series (b) with 1·0 cc. serum.

Table V. The esterase activities of a protein preparation and of preserved and fresh sera.

<table>
<thead>
<tr>
<th>Serum</th>
<th>Esterase activity (cc. of 0·1 N NaOH)</th>
<th>Complement activity</th>
<th>Haemolysis of 1 cc. of sensitised cells after 1 hr. by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0·01 cc. of serum</td>
<td>0·05 cc. of serum</td>
</tr>
<tr>
<td>A. Protein preparation</td>
<td>1·66</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2·34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B. Preserved serum</td>
<td>2·82</td>
<td>++ + + +</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>3·54</td>
<td>+ + + + + +</td>
<td>+ +</td>
</tr>
<tr>
<td>C. Preserved serum</td>
<td>2·76</td>
<td>+ + + + + +</td>
<td>+ +</td>
</tr>
<tr>
<td></td>
<td>3·70</td>
<td>+ + + + + +</td>
<td>–</td>
</tr>
<tr>
<td>D. Fresh serum</td>
<td>2·96</td>
<td>+ + + + + +</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4·1</td>
<td>+ + + + + + +</td>
<td>–</td>
</tr>
</tbody>
</table>

Summary.

1. The complement activities of various guinea-pig sera tend to run parallel with the esterase activities of these sera, but discrepancies occur which do not support the view that the action of complement is a simple esterase function.

2. Destruction or inactivation of either of the two relatively heat-stable components of complement does not appreciably diminish the esterase activity, and, as far as it is significant, the lipase activity of guinea-pig serum.
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3. Guinea-pig serum when heated at 56° for 30 mins. loses all detectable complement action but still retains a distinct esterase power.

4. Protein preparations, obtained by the treatment of guinea-pig serum with alcohol and ether at low temperatures, possess no complete complement power but retain a large part of the original esterase of the serum.

5. The immune body essential for complement action has no influence on the esterase (or lipase) activity of guinea-pig serum when used in amounts sufficient to promote haemolysis of ox red-cells.

6. No evidence has been obtained that the esterase (or lipase) of guinea-pig serum takes part in specific haemolysis. The action of haemolytic complement cannot be ascribed to a hydrolysis of the fatty substances of the red-cell envelope by the powerful esterase or the very weak lipase of guinea-pig serum.

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REFERENCES.

Coca (1914). Z. Immunitätsforsch. 21, 604.
Liebermann (1906). Deutsch. med. Woch. 32, 249.
Liefmann and Cohn (1911). Z. Immunitätsforsch. 8, 58.
Schumoff-Simanowski and Sieber (1906). Z. physiol. Chem. 49, 50.