ACUTE IMMUNE COMPLEX DISEASE IN RABBITS*

THE ROLE OF COMPLEMENT AND OF A LEUKOCYTE-DEPENDENT RELEASE OF VASOACTIVE AMINES FROM PLATELETS

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Acute immune complex disease (serum sickness) in rabbits may be induced by a single large, intravenous injection of an antigen, such as bovine serum albumin (BSA). When the animal mounts an immune response against the antigen and synthesizes antibodies, immune complexes between antibody and antigen form and circulate in the blood. Deposition of these complexes in blood vessel walls initiates the characteristic glomerulonephritis and vasculitis. The deposition is associated with increased vascular permeability (6, 7) and can be prevented by administration of antagonists of histamine and serotonin or by depletion of the circulating platelets (22). These observations led to the suggestion that release of vasoactive materials from platelets might cause the increased vascular permeability and that immune complexes above a certain minimum size (19S) would then become trapped along the filtering basement membranes (8, 9).

In order to examine further this hypothesis, immunological mechanisms of release of vasoactive amines from platelets were sought in vitro which might describe the events taking place in vivo leading to deposition of the circulating complexes. To date, four immunologic mechanisms have been found which result in release of histamine and serotonin from rabbit platelets (15, 18, 20). Three of these mechanisms apparently require activation of the complement sequence by immune complexes, i.e., at least through the third component. Immune adherence of the platelets to a complex consisting of antibody and particulate antigens resulted in release of vasoactive amines by a process requiring active participation and energy metabolism on the part of the platelet. Not all of the complement components were involved, and lysis of the platelets did not occur. Immune adherence to immune complexes (in antibody excess) with soluble antigens in the presence of serum resulted in lysis of the platelets and release of their constituents. This probably resulted from the lytic action of terminal comple-

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Abbreviations used in this paper: BSA, bovine serum albumin; 1*BSA, BSA labeled with 131I or 125I; LDR, leukocyte-dependent reaction; PRP, platelet-rich plasma.
A fourth immunological mechanism resulting in release of histamine and serotonin from rabbit platelets involved the reaction of antigen with sensitized blood leukocytes, which then subsequently acted on the platelets to induce release of their vasoactive amines (17). The leukocyte responsible has not, as yet, been unequivocally identified. Since this process did not require complement (17), an attempt could be made to determine whether it or the complement-dependent reactions of platelets was involved in the in vivo deposition of immune complexes. This has been done by determining whether the deposition of immune complexes and the induction of lesions of immune complex disease occur in rabbits depleted of their complement with the anticomplementary factor from cobra venom (1, 5, 11, 21, 25). Reduction in levels of circulating complement by administration of additional antigen–antibody complexes to rabbits undergoing immune complex disease was reported to prevent the necrotizing arteritis lesions but not to affect the glomerulonephritis (27). The experiments described herein attempt to ascertain the role of complement and of the different mechanisms of vasoactive amine release from platelets in acute immune complex disease.

**Materials and Methods**

*Induction of Acute Immune Complex Disease.*—New Zealand rabbits weighing 1.5-2.0 kg were used. The immune complex disease was induced by a single intravenous injection of 250 mg/kg of BSA labeled with $^{131}$I or $^{125}$I ($I^*_{BSA}$). To enhance the incidence of disease, rabbit hyperimmune anti-BSA antiserum (5 mg of antibody N/kg) was administered 24 hr before the antigen injection (22). The course of the disease was followed by frequent determinations of the $I^*_{BSA}$ remaining in a sample of plasma counted in a well-type scintillation counter.

*Study of the Circulating Immune Complexes.*—The amounts of immune complexes circulating in the blood were measured by a determination of the $I^*_{BSA}$ precipitated from the plasma by 50% ammonium sulphate and, therefore, bound to globulin (14). The size of the circulating complexes was estimated by ultracentrifugation in 10–37% sucrose gradients as described previously (8). Fractions of 15 drops were counted for their content of $^{131}$I BSA.

*Sacrifice of Animals and Examination of Tissues.*—Histologic studies of the kidneys and coronary arteries were performed following sacrifice of 16 rabbits at the time when 99% of the $^{131}$I BSA had been eliminated. Frozen sections of the kidneys were examined by fluorescent antisera to BSA, rabbit IgG, and rabbit C3 using the same techniques and reagents as described previously (8).

*Detection of the Leukocyte-Dependent Mechanism of Release of Histamine from Platelets.*—Since more than 80% of the histamine in rabbit blood is present in the platelets, release of this amine from whole blood cells was taken to represent, at least in greatest part, release from the platelets. Blood was obtained at intervals before, during, and after the occurrence of acute immune complex disease and the presence of the leukocyte-dependent reaction, and thus the presence of the requisite sensitized cells was determined. The simple procedure for detecting the reaction has been described previously (17). 2 ml of blood was removed from the ear artery
and the formed elements, including platelets, were washed. Antigen (in this case, 60 μg BSA) was added to a portion of the washed cells and platelet suspension and was incubated in 2.5 ml of Tyrode's solution containing 0.25% gelatin for 30 min at 37°C. A control tube contained cells but no antigen. After incubation, the cells were removed by centrifugation and the supernatant fluid tested for released histamine on the atropinized guinea pig ileum. The percentage of histamine released was determined from the total amount liberated by boiling.

**Examination of the Complement-Dependent Mechanisms of Histamine Release from Platelets:**

The details of the preparation of the reagents and performance of the reactions have been described previously (18). Platelet-rich plasma (PRP) was prepared by centrifugation (400 g for 20 min) of blood taken from the ear artery directly into ethylenediaminetetraacetate (EDTA), a mixture of the disodium and tetrasodium salts, to yield a cell of 7.2 to a final concentration of 0.005 M. Before the test, 1/50 volume of a mixture of 2% CaCl₂ and 2% MgCl₂ was added and 10 units/ml heparin was included to prevent clotting. The PRP (0.5 ml) was incubated with or without antigen (0.1 μg N-BSA) or antibody (10 μg N-IgG rabbit anti-BSA) in a total volume of 1 ml for 30 min at 37°C. The diluent was Tyrode's solution with 0.25% gelatin. The platelets were then sedimented and the percentage of histamine released into the supernatant fluid was determined. Platelet-rich plasma (0.5 ml) was also incubated at 37°C for 30 min with 3 mg zymosan (Nutritional Biochemical Corp., Cleveland, Ohio) and the release of histamine was again assayed.

**Preparation of Cobra Venom Factor:**

The anticomplementary factor in the venom of the cobra *Naja naja* (Ross Allen Reptile Institute, Silver Springs, Fla.) was prepared according to previously described methods (11) by diethylaminoethyl (DEAE) cellulose chromatography and G200 Sephadex (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) filtration. The material was assayed for anticomplementary activity on normal human serum. One unit of cobra venom factor was defined (11) as the quantity of cobra venom factor in 0.1 ml that reduces the hemolytic capacity of a 1/50 dilution of normal human serum by 50%. In general, this amounted to 4-5 μg of the purified material.

**Injection of Cobra Venom Factor and Assay of Depletion of Complement:**

The cobra venom factor was administered to rabbits essentially according to the regimen outlined previously (11). A total of 800 units of cobra venom factor were injected intraperitoneally into each rabbit being divided into two injections per day from the 6th to the 11th day after administration of antigen. On the 8th day, just before the peak incidence of the disease, the cobra venom factor was injected intravenously. Alternatively, cobra venom factor was injected intravenously (200 units/kg) on the 5th, 6th, 7th, and 8th day or on the 6th and 8th day after antigen injection. Both regimens were equally effective in reducing complement levels but the intravenous injections resulted in some intravascular hemolysis (1) and so the intraperitoneal route was preferred.

Sera obtained at intervals during the period of complement depletion were assayed for total

<p>| TABLE I |
|------------------|------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>CH₅₀ Units in Sera from Rabbits with Immune Complex Disease Given Cobra Factor Venom</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>With cobra factor</td>
<td>61*</td>
<td>52</td>
<td>&lt;6</td>
<td>&lt;6</td>
</tr>
<tr>
<td>Without cobra factor</td>
<td>52*</td>
<td>48</td>
<td>22</td>
<td>25</td>
</tr>
</tbody>
</table>

* Mean of 8 rabbits.
hemolytic complement (CH50) by the method of Osier et al. (26) and for C3 protein by immunodiffusion against guinea pig anti-rabbit C3. Table I and Fig. 1 show representative assays which indicate that the complement levels are reduced to below 15% and the C3 protein has almost entirely disappeared during the time when immune complexes are circulating.

![Fig. 1. Immunodiffusion analysis of C3 depletion of rabbits by cobra venom factor. Center well, guinea pig anti-rabbit C3. Wells 1–4, sera from rabbits treated with cobra venom factor. Wells 5 and 6, sera from control rabbits. The antiserum recognizes a serum protein in addition to C3, but shows clearly the almost complete loss of C3 protein in the cobra factor-treated sera on both the 8th and 11th days after antigen administration.](image)

**TABLE II**

<table>
<thead>
<tr>
<th>Proteinuria during Immune Complex Disease in C3-Depleted Rabbits</th>
<th>Normal C3 levels</th>
<th>C3 depleted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number rabbits positive/total</td>
<td>20/21</td>
<td>19/20</td>
</tr>
<tr>
<td>Proteinuria (mean total mg)</td>
<td>945</td>
<td>1145</td>
</tr>
<tr>
<td>Range</td>
<td>(58–2785)</td>
<td>(65–3193)</td>
</tr>
</tbody>
</table>

**RESULTS**

*Induction of Immune Complex Disease in Rabbits Depleted of C3 with Cobra Venom Factor: Proteinuria.*—In these experiments, immune complex disease was induced in two groups of rabbits totalling 23 animals each. C3 levels in one group were depleted by the administration of cobra venom factor from the 6th day after the injection of antigen. Animals in the other group served as controls with normal complement levels. Table II shows the incidence and extent of proteinuria in these two groups of rabbits. In each case, animals which did not develop an antibody response, i.e. did not exhibit immune elimination of the 125I BSA, have been excluded from the results shown. As noted, depletion
Fig. 2: Glomerular lesions in a rabbit with normal C3 levels. Endothelial proliferation is marked but there is little neutrophil accumulation. × 450.

Fig. 3. Glomerular lesions in a rabbit depleted of C3 with cobra factor. The lesion is identical with that in Fig. 2. × 450.
FIG. 4. Arterial lesions (coronary artery) in a rabbit depleted of C3 with cobra factor. There is lifting of the endothelium and some proliferation and/or round cell accumulation but neutrophils are absent. X 300.

FIG. 5. Arterial lesion (coronary artery) in a rabbit with normal levels of C3. Endothelial proliferation, neutrophil accumulation, and breaks in the internal elastic lamina are apparent. X 300.
of C3 had no effect on the development of proteinuria in acute immune complex disease.

**Histology and Immune Complex Deposition.**—Rabbits in one experiment were sacrificed at the time when 99% of the antigen had been eliminated and tissues were taken for immunofluorescent and histologic examination. The typical granular pattern of IgG localized in the glomeruli was observed in rabbits from both groups. However, C3 could only be found in the kidneys of those animals with normal complement levels. In vivo depletion of C3 with cobra venom factor had thus prevented the fixation of C3 to the immune complexes depositing in the kidneys.

Histologically, glomeruli from rabbits with normal or depleted C3 levels appeared identical (Figs. 2 and 3), showing typical proliferative glomerulonephritis. The incidence of these glomerular lesions is shown in Table III and was similar in both groups. Neutrophil accumulation was not seen in glomeruli of either group of animals.

In contrast to the lack of effect on the glomerulonephritis, depletion of C3 did change the quality of the arterial lesions. Figs. 4 and 5 show sections of the coronary artery from a rabbit treated with cobra venom factor and from a control animal with normal complement levels. The characteristic neutrophil infiltration, the subsequent damage to the internal elastic lamina, and necrosis of media and adventitia were absent in the cobra factor-treated rabbits. However, of interest was the elevation of intima away from the underlying internal elastic lamina with slight endothelial proliferation and/or round cell accumulation in the coronary arteries in these latter animals. This is illustrated in Fig. 4. The incidence of these lesions and the site (around the heart valves, aorta, and at the coronary outflow) were the same as the more complete, necrotic lesions in normal rabbits (Table III).

**The Effect of Cobra Factor on the Presence of Circulating Immune Complexes.**—In order to determine whether treatment with cobra venom factor was affecting the immune response of the rabbits or the quality of the immune complexes, certain parameters of the immune response were examined. These are shown

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**TABLE III**

| Incidence of Lesions of Immune Complex Disease in C3-Depleted Rabbits |
|-----------------------------|-----------------------------|
| Number of animals showing lesions | Normal C3 levels | C3 depleted |
| Glomerular lesions          | 16/17         | 13/13        |
| Cardiovascular lesions      |               |              |
| Intimal changes             | 5/5           | 6/6          |
| Neutrophil infiltration and necrosis | 5/5       | 0/6          |
for two representative rabbits (one depleted of C3 and one with normal C3 levels) in Fig. 6. It was found that the pattern of antigen clearance was identical in the two groups of animals. Although individual variation in rates of antigen elimination did occur, rabbits from either group exhibited the same variation and individuals could be paired as shown in Fig. 6. The percentage of $^{125}${I} BSA which was bound to globulin (as determined by ammonium sulphate precipitation of the plasma) again showed individual variation but was similar in the two groups. This indicated the presence of comparable amounts of circulating immune complexes. Fig. 6 also demonstrates that the presence of proteinuria in these two rabbits occurred over the same time period and to approximately the same degree.

At the time when the largest amount of circulating immune complexes could be detected, plasma was taken from the rabbits in each group and immediately sedimented in sucrose-density gradients to determine the size of the immune complexes. Again, no difference could be detected between those rabbits receiving cobra venom factor and those which did not, although individual variation was noted. Rabbits producing proteinuria had complexes extending up to and beyond 19S in size, as described previously (8).
Effect of Cobra Factor on the Release of Histamine from Platelets.—Since the release of vasoactive amines from platelets has been implicated in the deposition of immune complexes (23), the ability of immunological reactions to induce this release of amines from the blood of the C3-depleted rabbits undergoing immune complex disease was examined. Table IV depicts the results. Three reactions were performed. (a) A test for the presence of complement-dependent (nonlytic) release of histamine from platelets (18). Zymosan particles were added to platelet-rich plasma of rabbits with normal or depleted C3 levels. The zymosan fixes complement through C3 and induces adherence of the platelets and release of their vasoactive amines. As shown in Table IV, the release of histamine, which was observed in PRP from animals with normal C3, was inhibited in PRP from C3-depleted rabbits, confirming the complement-dependence of this reaction. (b) A test for the presence of complement-dependent (lytic) release of histamine from platelets (18). Antigen-antibody complexes formed in antibody excess with protein antigens also induce release of histamine from platelets by a complement-dependent mechanism. The ineffectiveness of this process in PRP from C3-depleted animals is again shown in Table IV. In addition, the release which did occur with PRP from rabbits with normal complement levels was inhibited if cobra factor was added in vitro. Antigen alone incubated with PRP at the time of antigen elimination did not induce histamine release. However, if incubated with PRP

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>C3 depleted (6 rabbits) (%)</th>
<th>Normal C3 levels (6 rabbits) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP + zymosan</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>PRP + Ag</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>PRP + Ag + Ab</td>
<td>1</td>
<td>30*</td>
</tr>
<tr>
<td>Washed blood cells</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Washed blood cells + Ag</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2–3 days after Ag elimination</td>
<td>Washed blood cells</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Washed blood Cells + Ag</td>
<td>75</td>
</tr>
</tbody>
</table>

Zymosan = 3 μg; Ag = 0.1 μg BSA; Ab = 10 μg rabbit IgG antiBSA; PRP = platelet-rich plasma (see Materials and Methods section). Washed blood cells include the platelets. The figures represent mean percentages of histamine released.

* Addition of cobra factor in vitro to these PRP reduced the released histamine due to Ag + Ab from 30 to 4%.
taken 2 days after elimination (not shown), i.e. when free antibody was circu-
lating, 20% histamine was released in the case of animals with normal C3
levels. (c) A test for the presence of the leukocyte-dependent release of hist-
amine from platelets (17). The complement-independent release of histamine
from platelets requires blood leukocytes but not plasma. Accordingly, rabbits
from both groups were examined at the time of antigen elimination by in-
cubating washed blood cells (including platelets) with antigen. At this time,
blood from neither group exhibited release of histamine (Table IV). However,
this reaction has previously been reported (17) to appear after the antigen
has been eliminated, not while it is still circulating in the blood. For this
reason, some rabbits in each group were not sacrificed at the time of antigen
elimination but were allowed to proceed for 2–3 days while their blood was
being examined for the presence of this leukocyte-dependent release of hista-
mine from platelets.

Table IV shows that 2 days after the immune elimination of antigen, the
leukocyte-dependent reaction (LDR) appeared in the blood of rabbits from
either group. Fig. 6 includes measurements of this reaction in a rabbit with
normal C3 level or a rabbit depleted of C3 during the complete course of the
immune complex disease. The results of each examination of the washed blood
cells are expressed as the percentage of histamine released by 62 μg of BSA.
It should be noted that the test is only roughly quantitative since the platelets
within the blood sample are being used as a source of the release of histamine
and these may vary in number. Nevertheless, it does give a guide to the strength
of the reaction, i.e., the number of reactive leukocytes (17). In every case,
tubes without antigen were included as controls. It was found that identical
responses occurred, whether or not the rabbits were depleted of C3. This was
equally true in the first few days after antigen elimination, when the LDR was
becoming detectable and while the C3 levels were still depressed.

Correlation of Leukocyte-Dependent Release of Vasoactive Amines from Platelets
and the Deposition of Immune Complexes in Vessels and Glomerular Injury.—

The ability of rabbits to succumb to the glomerulonephritis of immune complex
disease, even though the C3 had been depleted with cobra factor, suggests that
the mechanism of release of histamine from platelets which did not require
complement might be involved in the deposition of the complexes. Unfor-
nately, the presence of the reaction in the blood cannot be detected in
the presence of circulating antigen. However, since the LDR can be observed
after antigen elimination, immune complex disease was induced in 30 rabbits
whose blood was examined during and after the clearance of radiolabeled BSA.
The presence of the disease was determined by daily examination of the urine
for protein. 17 out of 30 rabbits produced antibody, formed complexes in
the circulation, and developed proteinuria; 8 others with complexes did not
develop proteinuria, while 5 rabbits failed to produce antibody and show
immune elimination or the presence of complexes. The poorer responsiveness of these rabbits permitted an interesting correlation between the presence of proteinuria and the LDR. Of the 17 rabbits with proteinuria, i.e. having immune complex deposition in the glomeruli and glomerulitis, 16 developed a detectable LDR within 2 days after antigen elimination (Table V). Of the 8 rabbits which formed complexes in the circulation and eliminated the antigen but did not develop proteinuria, 7 did not exhibit an LDR in the blood at any time. The 8th animal was of particular interest since it eliminated the antigen, exhibited an LDR, but did not develop proteinuria. Examination of the sedimentation characteristics of the circulating immune complexes in this rabbit (see below) revealed that they were less than 19S in size, i.e., too small to become deposited in glomeruli and induce nephritis (8).

Size of Immune Complexes, the LDR, and Glomerulonephritis.—Plasma was taken from many of the rabbits in the experiment described above for sucrose density gradient analysis of their circulating immune complexes. Control

<table>
<thead>
<tr>
<th>TABLE V</th>
<th>Correlation of Leukocyte-Dependent Release from Platelets and Glomerular Injury</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number of animals with leukocyte-dependent release of histamine</td>
</tr>
<tr>
<td></td>
<td>Rabbits with proteinuria</td>
</tr>
<tr>
<td></td>
<td>Rabbits without proteinuria</td>
</tr>
</tbody>
</table>

* This rabbit had light complexes, see Fig. 3.

samples analyzed 2 days after antigen administration revealed only a homogeneous 4.5S peak of radiolabeled BSA. Plasmas were then examined at a time when at least 25% of the circulating antigen was bound to globulin (determined by precipitation with 50% saturated ammonium sulphate). The samples were immediately placed on a 10–37% sucrose density gradient and centrifuged at 50,000 rpm for 5 hr. 15 drop samples were removed and counted for 125I BSA. IgM, IgG, and albumin were used as 20S, 7S, and 4.5S markers, respectively. Figure 7 shows plasma from three representative animals analyzed in this way.

In the plasma from rabbit 90, antigen was found extending into the gradient, i.e. complexed to globulin, as far as the 19S marker. The animal developed an LDR after antigen elimination and exhibited proteinuria. In contrast, rabbit 88 did not develop an LDR and did not excrete protein in the urine even though the circulating immune complexes were as large in size as those of rabbit 90. Rabbit 93 is the one referred to above whose immune complexes were small, only extending slightly beyond the 7S marker. In this case, despite the presence of an LDR, no proteinuria occurred.
DISCUSSION

Three major observations have arisen from the experiments described herein. (a) Complement components beyond C2 did not appear to be involved in the deposition of immune complexes, either in the arteries or in the glomerulus of rabbits with acute immune complex disease. (b) A strong correlation existed between the presence of a mechanism of release of vasoactive constituents from platelets that requires leukocytes and the deposition of immune complexes in blood vessels with the subsequent development of injury to the glomeruli. Complement did not appear to be required for this process. (c) The glomerular injury which accompanies the deposition of complexes in the kidney still occurred in the absence of C3. In complete contrast, however, the necrotizing arteritis lesions which follow the deposition of immune complexes in the arteries did require the action of complement components beyond C2 in addition to the presence of circulating neutrophils (22).

Plasma Complement and the Deposition of Immune Complexes.—The results
indicated that deposition of complexes in the glomeruli was completely unaffected by depletion of C3 with cobra factor since the extent and the severity of glomerulonephritis was unaltered (Table II). In addition, the complexes could be detected in the glomeruli by immunofluorescence, although as expected and in confirmation of the effectiveness of the cobra factor treatment, C3 was not seen with the immune complexes in these rabbits.

Evidence of deposition of immune complexes in the arteries was also obtained in that distinct changes in the intima were observed in the C3-depleted rabbits. These lesions consisted of raising of the intimal layer, endothelial cell proliferation, and/or mononuclear cell infiltration. These changes have been described as the earliest manifestations of the arteritis of immune complex disease occurring before accumulation of neutrophils (23), and were felt to indicate the immune complexes had accumulated at these sites. In addition, the incidence of these alterations in the rabbits treated with cobra factor was the same as that of the more severe necrotic lesions in the animals with normal levels of complement (see discussion below).

Depletion of C3 (and later components) also prevented release of histamine from platelets by the complement-dependent reactions. Neither a particulate antigen (zymosan) nor antigen-antibody complexes formed with BSA as antigen were capable of inducing release of histamine from platelets in platelet-rich plasma from the rabbits treated with cobra venom factor. This was contrasted with the release observed in the case of the control animals not receiving the anticomplementary material. The results suggested that these complement-dependent mechanisms of release of platelet constituents were not of prime importance in the generation of increased vascular permeability and deposition of complexes in acute immune complex disease.

Correlation of the Leukocyte-Dependent Mechanism of Histamine Release from Platelets with the Deposition of Immune Complexes.—The leukocyte-dependent mechanism of immunological release of histamine from platelets, however, did not require plasma as a source of complement (17) and was observed in rabbits depleted of C3 with cobra venom factor. When attempts were made to determine the incidence of the LDR mechanism in rabbits developing glomerulonephritis, a striking correlation was observed. Of 17 rabbits with circulating complexes and evidence of glomerular injury, 16 possessed the LDR mechanism. By contrast, of 8 rabbits with circulating complexes but without glomerulonephritis, 7 did not exhibit the LDR mechanism. The sole exception in this latter correlation was found to have immune complexes in the circulation that were too small, i.e. less than 19S in size, so that deposition could not be expected in any event. Since the blood was examined for LDR after the elimination of antigen, the rabbits could not be sacrificed at the time of maximum severity of arteritis. Accordingly, no direct correlation with this type of lesion was possible.
The leukocyte-dependent reaction has been described in a number of laboratories (2, 17, 28, 29, 31) and involves the reaction of specific antigen with a circulating sensitized leukocyte. This interaction subsequently causes platelets to release their content of vasoactive amines. The nature of the leukocyte has not yet been unequivocally determined. There is some evidence that a soluble factor is released from these cells after reaction with antigen and that this then acts on the platelets to induce release of at least some of their histamine and serotonin (17). Recently, experiments in this laboratory and in others (13) have shown that the LDR can be transferred to normal recipient rabbits with serum from immunized animals. Washed leukocytes from the recipients then react with antigen, and in the presence of platelets release of histamine ensues. These experiments have raised the possibility that a form of cytophilic (4) or cytotropic antibody is responsible for the LDR. An LDR was not detected during the time that antigen was still circulating. In possible explanation of this, when leukocytes were subjected to incubation with antigen in vitro in previous studies (17), they were no longer capable of causing release of histamine from platelets. This desensitization may account for the inability to detect the reaction in the blood before antigen elimination. Alternatively, when sensitized leukocytes react with antigen in vivo, they may be removed from the circulation, obviating detection. On the other hand, after the antigen has gone, the LDR could be detected, increasing in strength for a few days, and then waning slowly. The presence of sensitized leukocytes in the blood indicated by this reaction, therefore, follows a similar time course to the presence of circulating antibody.

The Mediation of Glomerular and Arterial Injury.—Consequent to the deposition of immune complexes, injury to the tissues occurs. The studies reported herein indicate that injury to the glomerulus does not follow the same mediation pathway as damage to the arteries.

Arteritis.—Depletion of C3 (and C5 and C6) had a marked effect on the arteritis in this disease since it prevented the characteristic neutrophil accumulation. There are two likely ways in which fixation of complement might contribute to the accumulation of neutrophils. The chemotactic activity for neutrophils of a number of complement components (or fragments thereof) are well-known (3, 30, 32-35). However, it is difficult to imagine how chemotactic factors released from complexes deposited in the walls of large arteries with high flow rates could set up suitable concentration gradients for this process to be important in neutrophil accumulation. Instead the process of immune adherence seems a more likely possibility to account for the complement requirement. Neutrophils show strong adherence reactions to complexes which have bound C3 (16, 24), (and to a lesser extent to the fixed immunoglobulin itself) and might thus remain adherent to antigen—antibody—complement complexes which they have contacted in their passage through
the arteries. This may be augmented by the occurrence of the lesions at sites of more turbulent flow where greater contact between neutrophils and bound C3 would occur.

The importance of neutrophils in the production of injury to blood vessel walls in acute immune complex disease has been previously reported (22). Depletion of the neutrophils by anti-neutrophil antibody was effective in preventing the necrotizing arteritis while not affecting the deposition of the immune complexes. Adherence of neutrophils to immune complexes and the subsequent phagocytosis of them are both stimuli which lead to release of enzymes and tissue-damaging agents from the cells (Henson, P. M., manuscript in preparation). It is therefore suggested that the pathogenesis of the arteritis comprises (a) increased vascular permeability following platelet impingement on vessel walls and release of vasoactive amines by the leukocyte-dependent process; (b) deposition of immune complexes greater than 19S in size at these sites; (c) fixation of complement by the complexes (which probably occurs while they are still circulating); (d) complement-dependent accumulation of neutrophils; (e) adherence to, and phagocytosis of, the complexes by neutrophils; (f) release of neutrophil constituents; (g) digestion of materials in the vessel walls and penetration of neutrophils, resulting in medial and adventitial necrosis.

**Glomerulitis.**—In contrast to its effect on the arteritis, depletion of the C3 did not alter in any measurable way the incidence, character, or severity of the glomerulonephritis. It had previously been shown that depletion of neutrophils had little effect on the kidney lesion in this acute immune complex disease (22). Indeed, few neutrophils are seen in acute immune complex nephritis of rabbits. This should be contrasted with the importance of these cells and complement in nephrotoxic nephritis produced by antibodies to glomerular basement membrane (12). It appears, therefore, that deposition of immune complexes along the filtering basement membrane in the glomerulus results in damage (exemplified by proteinuria and proliferative glomerulonephritis) by an as yet undefined process. It may be that mere deposition of material along the membrane itself alters its permeability properties.

**Effects of Cobra Venom Factor.**—In the course of these studies, conclusions have been drawn from the effect of cobra venom factor in depleting C3 and later complement components from the blood of rabbits. Consideration must be given to the possibility that cobra venom factor is interfering with the disease process in a manner unrelated to its direct effect (in combination with the proinactivator in plasma) on C3, and thus on the later components of complement. However, in a study of many physiological parameters (11), little or no effect of cobra venom factor was found on numbers or performance of neutrophils and platelets or on the kinin or coagulation systems.

The action of cobra venom factor on the complement system results in
the generation of anaphylatoxins, which in their turn induce release of histamine from mast cells (10). This raises the possibility that the cobra venom factor itself, by acting on the C3 which is synthesized during the course of the depletion process, can cause an increase in vascular permeability by liberating anaphylatoxins and thus permit complex deposition. However, it has been clearly demonstrated that platelets are required for the deposition (23) and anaphylatoxins do not affect this reservoir of vasoactive amines (20). Moreover, the quantities of C3 produced are not very great and the rate of anaphylatoxin inactivation in rabbit plasma is extremely rapid. It is felt, therefore, that a role for cobra factor in initiating the disease is unlikely and that the deposition of the immune complexes is independent of the later complement components.

SUMMARY

By depletion of C3 from rabbits undergoing acute experimental immune complex disease with an anticomplementary factor in cobra venom, it has been possible to demonstrate that deposition of the complexes in arteries and glomeruli does not require the complement components reacting after C2.

Immunological reactions, in which platelets release their vasoactive amines, have been examined in rabbits undergoing immune complex disease. A correlation was obtained between the presence of a complement-independent reaction which required blood leukocytes, antigen and platelets, the deposition of immune complexes, and the induction of glomerulonephritis.

C3 depletion did, however, have a marked alleviating effect on the severity of the arterial lesions. Neutrophil accumulation and the subsequent necrotizing arteritis were prevented. In contrast, the character and severity of the glomerulonephritis was not altered by depletion of later-acting complement components.

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BIBLIOGRAPHY


