MECHANISMS INVOLVED IN THE DEPOSITION OF IMMUNE COMPLEXES IN TISSUES*

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The observations that immune complexes circulate in the bloodstream in experimental and human diseases stimulated considerable research into their role in immunologic injury. Studies of experimental models in the late 1950s demonstrated that the presence of complexes in the circulation coincides temporally with the development of lesions in glomeruli, arteries, endocardium, spleen, and elsewhere. As complexes were demonstrable in the lesions using fluorescent antibody techniques, it was reasonable to assume that they reached their target sites after passage through the bloodstream. The immune complex in the circulation, in fact, provided the most plausible explanation of the question of how antibody molecules could be transported from the lymphoid organs, where they were fabricated, to the target tissue.

One of the major areas to be investigated at that point was the mechanism whereby the circulating complexes left the bloodstream to be deposited in tissues. Were the complexes phagocytized by endothelial cells or did they have a particular affinity for cells or structures in the vessel walls? Did the complexes possess an electrostatic charge that allowed binding to occur in vessels? Was there an active mechanism that induced changes in the blood vessels leading to deposition of the complexes?

Work being conducted in several laboratories was directed at these questions. Immune complexes infused intravenously in animals could be shown to induce injury histologically, but interestingly, the results were variable and the lesions were not always the same as those seen in the primary model, immune complex disease (serum sickness) in rabbits. Also, in our hands and others, fluorescent antibodies, when employed, often revealed a pattern of immune complexes in glomeruli different from that seen in experimental serum sickness. Germuth and Pollack (1) infused antibody into rabbits having antigen already present in the circulation. The antigen was eliminated at much the same rate as in serum sickness and the rabbits developed mild swelling of glomerular endothelial cells and occasional foci of arterial inflammation in vessels of the stomach,

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duodenum, or peripancreatic tissues. Functional evidence of glomerular injury, i.e. proteinuria, was not tested. The coronary arteries, most frequent targets in serum sickness, were not involved. About half the rabbits showed one or a combination of these lesions. McCluskey and coworkers (2–4) injected immune complexes into mice and rats which developed glomerular, endocardial, and, to a lesser extent, arterial lesions within 36 hr of the first injection. The glomerular lesions contained large numbers of neutrophils and thus differed from the acute lesions in serum sickness of rabbits. In our laboratory, the results obtained from injecting preformed immune complexes in over 80 rabbits and 200 mice were inconsistent and variable. In mice, lesions were found in the glomeruli on occasions, but these appeared to be caused by immune precipitates in glomerular capillary lumina and mesangial cells as seen by immune fluorescence. A granular pattern of fluorescence distributed along the glomerular basement membrane, the hallmark of serum sickness, was not seen. In rabbits, mild swelling of glomerular endothelial cells was not accompanied by deposited immune complexes along the basement membrane and thus the mild lesions could not be attributed positively to immune complexes. In addition, Michael et al (5) infused aggregated gamma globulin in mice leading to uptake of aggregates by mesangial cells, i.e., in a location not associated with typical glomerular injury. The difficulty in obtaining histologic changes and significant fluorescent patterns consistently with passively transferred, preformed immune complexes suggested that some essential factor was missing in the experimental protocol. This prompted a series of investigations dealing with the mechanisms responsible for deposition.

Evidence of an active process required for deposition of circulating colloidal particles was obtained by Benacerraf et al. (6). Mice injected intravenously with colloidal carbon were then given histamine, 5-hydroxytryptamine (serotonin), epinephrine, or preformed immune complexes. In each case, carbon deposited in the intimal layer of large arteries, endocardium of the heart, and in the walls of venules in various sites. The immune complexes were thought to liberate vasoactive amines in vivo, leading to deposition of the carbon. In our laboratory, immune complexes themselves (7, 8) could be made to deposit in the walls of venules in guinea pigs by simultaneous infusion of agents that increased vascular permeability or liberated vasoactive amines from the mast cells. The immune complexes could be detected in the vessel walls in a fine granular pattern with the fluorescent antibody technique, almost identical to the pattern seen in vessels in serum sickness (Fig. 1). Anaphylatoxin, passive anaphylaxis, and the known histamine liberator octylamine all produced release of histamine from mast cells in vivo and the deposition of circulating immune complexes. Pretreatment with antihistamines prevented increased vascular permeability and deposition of circulating complexes.

Examination of the affected blood vessels histologically indicated that the

immune complexes were trapped along a limiting membrane. Using particles visible with the electron microscope, the membrane was identified as the vascular basement membrane (7). This suggested that a process of filtration existed in which the basement membrane prevented passage of the immune complexes through the vessel wall during a state of increased permeability.

In keeping with the notion that immune complexes deposit by a process of filtration was the finding that only large immune complexes became entrapped

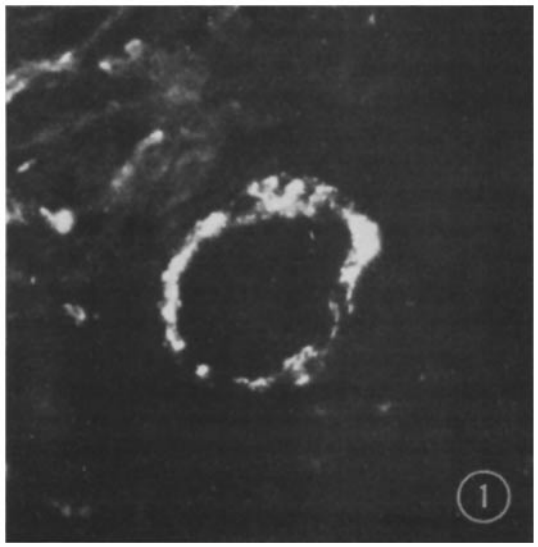

FIG. 1. Fluorescent photomicrograph of a small vessel in the lung of a guinea pig showing the deposition of bovine serum albumin (BSA)–anti-BSA complexes in the vessel wall. This section was treated with fluorescent anti–rabbit gamma globulin. Treatment with fluorescent anti-BSA or anti–guinea pig C'3 gave a similar fluorescent pattern. Electron photomicrographs in previous studies showed the complexes and other markers to lie between and beneath endothelial cells, up against the limiting basement membranes. × 350.

in vessel walls (9). Complexes of various sizes, i.e. prepared by varying the quantities of antigen, were assayed for their ability to deposit in vessel walls. Only large immune complexes were found in vessel walls. When immune complexes were sedimented in a sucrose gradient and the various fractions assayed, only those complexes greater in size than 19S were deposited in vessel walls. The data are shown in Fig. 2. Individual protein molecules were also able to become lodged in vessel walls provided they were of adequate size. Hemocyanin from the keyhole limpet in its associated form (7×10^6 mol wt) was capable of depositing, while in its dissociated form (0.8×10^6 mol wt) it would not deposit (9). Native IgG would not deposit, while if aggregated by heat it would. The

critical size was greater than that of molecules of 19S, as IgM and thyroglobulin would not deposit.

A series of experiments was then performed to find if a release of vasoactive agents increased vascular permeability, and if filtration of large immune complexes occurred in acute immune complex disease (serum sickness) of rabbits. Evidence was obtained indicating immune complexes were depositing from the circulation. Colloidal carbon, injected intravenously, became localized along

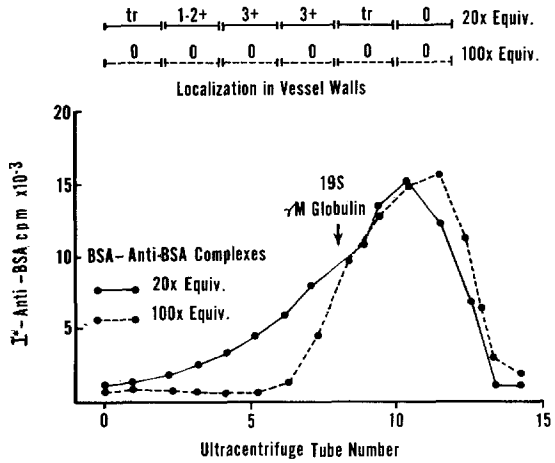

FIG. 2. Patterns of sedimentation of anti-I*BSA complexes with BSA using two quantities of BSA. Sedimentation carried out in a linear sucrose gradient, 10–37%, 4 hr, at 50,000 rpm. Purified human γM served as marker. Those made using a large excess of BSA (100 × equivalence) yielded slowly sedimenting complexes, while those prepared in moderate antigen excess (20 × equivalence) demonstrated both slowly sedimenting and more rapidly sedimenting components. Fractions collected after centrifugation and injected into guinea pigs showed localizing complexes only in tubes taken from complexes prepared at 20 × equivalence and greater than 19S in size (from reference 9).

with the complexes in the developing intimal lesions (10). The carbon filtered between endothelial cells and lined up along the internal elastic lamina of the coronary arteries. This elastic lamina (or a membrane closely associated with it) apparently acted as the filtration barrier, and lost its integrity only when neutrophils entered the reaction (11).

Evidence of an active mechanism of inducing vascular permeability was also observed. Antagonists of vasoactive amines given during the time immune complexes appeared in the circulation prevented in great part the deposition of immune complexes in arteries and glomeruli (Table I). In addition, when the most important reservoirs of vasoactive amines in the circulation of rabbits, i.e. platelets (12, 13), were depleted in rabbits subjected to serum sickness,

deposition of immune complexes and the development of arterial and glomerular lesions were markedly suppressed (Table I).

The size of the immune complex is also important in determining whether deposition and development of lesions occur. In a correlative study, the deposition of circulating complexes and the development of glomerular and arterial lesions in acute immune complex disease of rabbits was found almost exclusively in animals forming large complexes, i.e., greater than 19S in size (9) (Table II). The sedimentation patterns of complexes in serum of rabbits that were sufficiently large to deposit in vessels, as opposed to those that were too small, are

TABLE I

*Incidence and Severity of Serum Sickness Lesions in Treated and Control Rabbits**

	Treated rabbits (% positive)		Control rabbits (% positive)
	Antihistamine anti-serotonin 11 rabbits	Platelet depletion 16 rabbits	10 rabbits
Coronary artery			
Endothelial proliferation	9	44	90
Medial necrosis	9	19	80
Glomeruli			
Immunofluorescent deposits‡	0 to + (fine)	0 to + (fine)	+ to ++ (coarse)
Endothelial swelling and proliferation§	1.0+	1.7+	2.2+

Values tabulated are averages for each group.

* Data from Kniker and Cochrane (10).

‡ Grading of immunofluorescent deposits of IgG, antigen (BSA): 0 to +++.

§ Grading of glomerular endothelial swelling and proliferation 0 to 3+.

shown in Fig. 3. In chronic immune complex disease in rabbits, a similar correlation has been observed (14).

The data suggest that a complex series of events takes place leading to deposition of circulating complexes. Increased vascular permeability occurs, brought about most probably by the release of vasoactive agents from their reservoirs in the circulation or the tissues. In the rabbit, the circulating reservoirs, i.e., platelets, are probably most important for disease in glomeruli and arterial intima where mast cells do not exist. Then in the presence of increased permeability, the large macromolecular immune complexes deposit along a filtering membrane. Inflammation ensues.

Hydrodynamic forces also play a role in the deposition of circulating complexes. Arterial lesions in acute immune complex disease of rabbits occur most commonly along heart values, at the entrance of the coronary arteries, and at

branches and bifurcations of the aorta. In addition, if a coarctation is induced artificially in the lower aorta, lesions develop around the constricted zone (10). This is of great interest since it has been shown that platelets clump and adhere with leukocytes to endothelium around these areas of turbulence (15).

TABLE II

*Relationship of the Sedimentation Characteristics of Circulating Immune Complexes to the Development of Lesions in Serum Sickness**

Rabbits	Avg. amt. BSA-^{131}I bound to globulin at maximum‡	Pattern of BSA-^{131}I sedimentation§	Glomerular lesions	Total amt. proteinuria	Maximal complement depletion
	%			*mg*	
9	43.3	Heavy	2.3+	567	78
5	41.6	Light	0 to ±	0	67

* Data taken from Cochrane and Hawkins (9).

‡ Per cent (average) of BSA-^{131}I bound to globulins determined by precipitation of the globulins with ammonium sulfate at 50% saturation.

§ Pattern of sedimentation of BSA-^{131}I complexed to globulin as determined in sucrose gradient. Heavy = BSA-^{131}I sedimentation pattern extends below 19S marker. Light = BSA-^{131}I sedimentation pattern does not reach 19S marker (see Fig. 2).

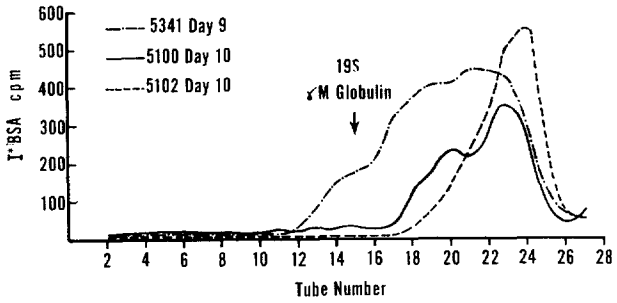

FIG. 3. Sedimentation patterns of I*BSA in sera obtained from three rabbits at the times shown after injection of I*BSA. Centrifugation carried out in linear sucrose density gradient 10–37% at 50,000 rpm for 4 hr. No. 5100 demonstrates a typical "light" pattern and No. 5341 a "heavy" pattern. No. 5102 failed to develop antibodies and showed a sedimentation pattern of free BSA in normal serum (from reference 9).

Release of Vasoactive Amines from Platelets.—Clearly a subject of pressing interest has been the immunologic mechanisms responsible for the release of histamine from platelets. To date, four immunologic reactions have been observed that lead to release of vasoactive amines:

(*a*) Immune complexes have been found by many investigators to bring about the release of amines from rabbit platelets in the presence of plasma (16–23). The reaction is augmented greatly in antibody excess (17–19, 22). The require-

ment of fresh plasma has been shown in several laboratories (16, 20, 22). Strong evidence favoring the participation of complement components emerged from the use of plasma from genetically C6-deficient rabbits. Immune complexes failed to induce release of histamine and serotonin from rabbit platelets in the presence of C6-deficient plasma, and the ability was restored by addition of semipurified rabbit C6 (22). The requirement of complement has been questioned (23) in view of an apparent requirement of Mg^{++} but not Ca^{++}. The observation may be explained by the activation of C3 proactivator by the immune complexes (see reference 24) which would allow participation of the terminal components. During the reaction of platelets and immune complexes in plasma, clumping of platelets to the complexes occurred by C3 immune adherence, and, through the action of terminal components, lysis of the platelets followed. The lysis was evident with the electron microscope and by measuring the release of several enzymes from cytoplasmic and granular compartments of the platelets. Acid phosphatase, β-glucuronidase (α-granules), and lactic dehydrogenase (cytoplasmic enzyme) were released. ^{86}Rb, previously incorporated into the cytoplasm of the platelets, was also released. The reaction was one of passive lysis, not requiring active participation of the platelet (22, 25).

(*b*) A second mechanism of histamine release from platelets was observed. When C6-deficient plasma was mixed with immune complexes and platelets, release of histamine and serotonin could be induced by adding a few neutrophils (26). Immune adherence but not lysis of platelets resulted, and the reaction required active participation of the platelet. Inhibition of the glycolytic pathway of the platelet prevented release of vasoactive amines. Release of granular and cytoplasmic enzymes did not occur.

(*c*) If a particulate antigen such as zymosan was used together with antibody to its surface determinants, complement components through C3 were required to induce immune adherence of platelets and the particles, and the release of histamine and serotonin occurred (25, 27, 28). Complement components beyond C3 were not required. The release by this mechanism also required the active participation of the platelet, and lysis was not observed (27, 28). Presumably, the surface of the particle together with antibody and C3 offer an adequate stimulus to initiate release of vasoactive amines from the platelet.

(*d*) A fourth mechanism of immunologic release of constituents from rabbit platelets was described independently by Siraganian et al (29) and Schoenbechler and Sadun (30). This mechanism involves a synergy between sensitized leukocytes and platelets. If leukocytes from recently sensitized rabbits are washed and then mixed with platelets in the presence of antigen, histamine and serotonin are released from the platelets. Plasma is not required. While washed leukocytes and platelets were originally employed from rabbits infected with *Schistosoma mansoni*, protein antigens have also been used to elicit this reaction (20, 21, 25). The leukocyte involved was thought at first to be mononuclear

in type although direct evidence of its participation was uncertain (31, 32). More recently, the basophil has been implicated as the leukocyte most likely involved in the synergistic action. This has been demonstrated in several ways. Leukocytes from sensitized rabbits were fractionated on a column of glass beads or on a gradient of Ficoll by sedimentation (33). The fractionated cells were then assayed for total content of histamine, morphologic characteristics, and ability to stimulate release of histamine from platelets on exposure to antigen. The ability to release histamine from platelets corresponded to the leukocytes that contained histamine, i.e., the basophils (34). Further studies have correlated the presence of basophils from sensitized rabbits with the presence of the platelet-activating capacity (35). Basophils in these studies have been visualized directly. In experiments in our laboratory, the serum of rabbits having strong reactivity was transferred to normal recipient rabbits, and the recipients were then endowed with reactivity in a manner similar to that reported by Colwell et al. (36). The serum also was rich in homocytotropic antibody as determined by its capacity to transfer passive cutaneous anaphylaxis to normal recipients. Fractionation of antiserum by anion exchange chromatography and gel filtration indicated that the antibody responsible was a fast gamma and larger in size than IgG (i.e., in the range of 200,000 Daltons). In addition, the leukocyte-sensitizing antibody eluted together with homocytotropic antibody activity from the columns. In other experiments, when leukocytes from a sensitized rabbit were treated with anti-IgE (anti-homocytotropic antibody[1]) and platelets added, histamine was released from the platelets. Prior treatment of the leukocytes with this antibody led to desensitization: when the leukocytes from sensitized rabbits were first exposed to anti-IgE, followed by washing, antigen could no longer stimulate them to induce the release of histamine of platelets.

A soluble factor released from the leukocytes that induces clumping of platelets and release of histamine has been described by Henson (28, 32). The factor was labile and readily inactivated by platelets contaminating the leukocyte suspension. This may explain the difficulty noted by others (37) in its detection. It release from sensitized leukocytes with antigen was inhibited when glucose in the medium was replaced by 2-deoxyglucose or when Mg ethylenediaminetetraacetate (EDTA) or acetyl salicylate were added (32). Pretreatment of the cells with antigen induced a state of desensitization. The release of histamine from the platelets by the soluble factor was also inhibited by replacement of glucose by 2-deoxyglucose (10^{-3} M) diisopropyl fluorophosphate (2×10^{-3} M), Mg ethylene glycol Bis(β-aminoethyl ether) N,N,N',N' tetraacetic acid (EGTA) (5×10^{-3} M), adenosine (10^{-3} M), and acetyl salicylate (10^{-3} M). The soluble factor induced clumping of the platelets and release of histamine and serotonin but not enzymes associated with platelet granules

[1] Antibody to homocytotropic antibody was generously given by Dr. Nathan Zvaifler.

(acid phosphatase and β-glucuronidase) or cytoplasm (lactic dehydrogenase). By electron microscopy, the platelets were not lysed in confirmation of the observed specific release. The presence of a soluble intermediate has recently been confirmed (38), although lysis of platelets was reported as evidence by release of ^{86}Rb.

A scheme of this mechanism of release of vasoactive amines from platelets is shown in Fig. 4.

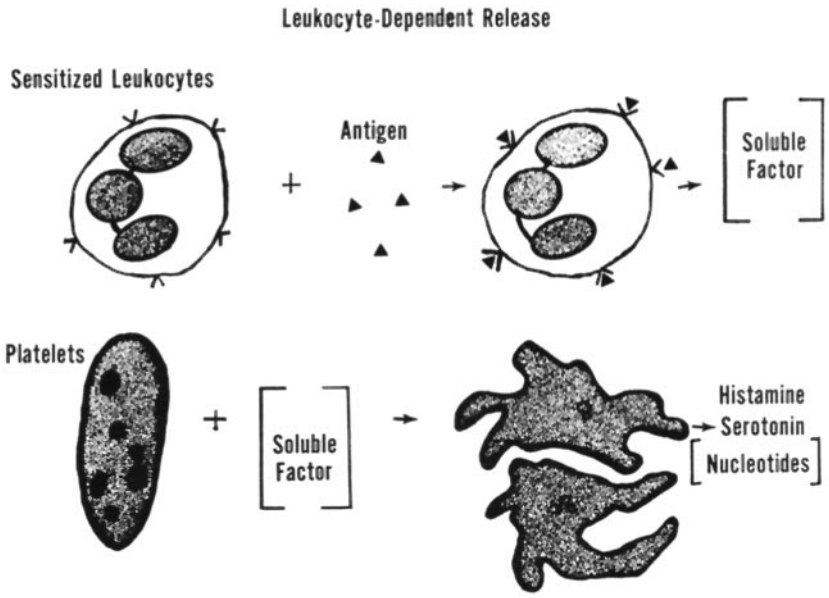

FIG. 4. Schematic presentation of the leukocyte-dependent mechanism of histamine release from platelets in rabbits. The sensitized basophils, having sensitizing antibody on the surface, are reacted with antigen. The basophils then give off a soluble factor that reacts with platelets to cause clumping and release of their vasoactive amines and, to a lesser degree, nucleotides.

Lack of Participation of Complement Components in the Release of Vasoactive Amines from Platelets in Acute Immune Complex Disease (Serum Sickness) in Rabbits.—Experiments have been conducted to determine if the complement-dependent mechanisms of histamine release play a role in the deposition of circulating immune complexes in rabbits. Rabbits were depleted of C3 and terminal components of complement by the injection of cobra venom factor (39). Immune complexes appeared in the circulation and, despite depletion of complement, they deposited in glomeruli to induce injury (Table III) (40). Arteries were also affected, but in the absence of sufficient C3, neutrophils did

not accumulate to produce necrotizing arteritis. The fact that the immune complexes are in antigen excess in acute immune complex disease may be important in this observation. The complement-dependent mechanisms of release of vasoactive amines from platelets are significantly augmented in antibody excess and diminished in antigen excess as noted above.

TABLE III

*Effect of C3 Depletion in Acute Immune Complex Disease (Serum Sickness) of Rabbits**

	Glomerulitis (No. positive total)	Avg. amt. proteinuria	Necrotizing arteritis (No. positive total)
		mg/day	
C3 depleted‡	13/13	664	0/6§
Control	43/45	456	20/34

* Data from Henson and Cochrane (40).

‡ C3 depleted by injections of cobra venom factor just before appearance of circulating immune complexes.

§ Arterial intimal proliferation and edema present in all six rabbits, but neutrophil accumulation did not occur in C3-depleted rabbits.

TABLE IV

Correlation of Leukocyte-Dependent Release of Histamine from Platelets and Glomerular Injury in Acute Immune Complex Disease (Serum Sickness) of Rabbits

No. of rabbits*	Glomerulonephritis	Rabbits with leukocyte-dependent release
17	Present	16
8	Absent	1‡

* All rabbits exhibited circulating immune complexes. Amount of complexes was comparable between the two groups.

‡ Complexes of "light" type in sedimentation pattern (see Fig. 2).

Correlation of the Leukocyte-Dependent Mechanism of Histamine Release from Platelets and the Deposition of Immune Complexes and Development of Injury.— A correlative study of the presence of the leukocyte-dependent (LDHR)[2] mechanism of histamine release from platelets and the deposition of immune complexes and injury is shown in Table IV. Only animals with circulating immune complexes are included. As noted, when circulating complexes deposited and injury resulted, the LDHR was detected in all but one instance (18). By contrast, rabbits with immune complexes but without deposition and the development of injury failed in all but one case to demonstrate the LDHR. It is of great interest that the single rabbit with circulating immune complexes and

[2] *Abbreviations used in this paper:* BSA, bovine serum albumin; LDHR, leukocyte-dependent mechanism of histamine release from platelets.

the LDHR was found to have complexes of the "light" type (see above, also Table II and Fig. 3) that were too small to be entrapped in vessel walls. This correlation implicated the LDHR as being the important immunologic method of releasing vasoactive amines thereby increasing vascular permeability and bringing about deposition of the circulating complexes. The LDHR may also be detected in rabbits with chronic immune complex disease.

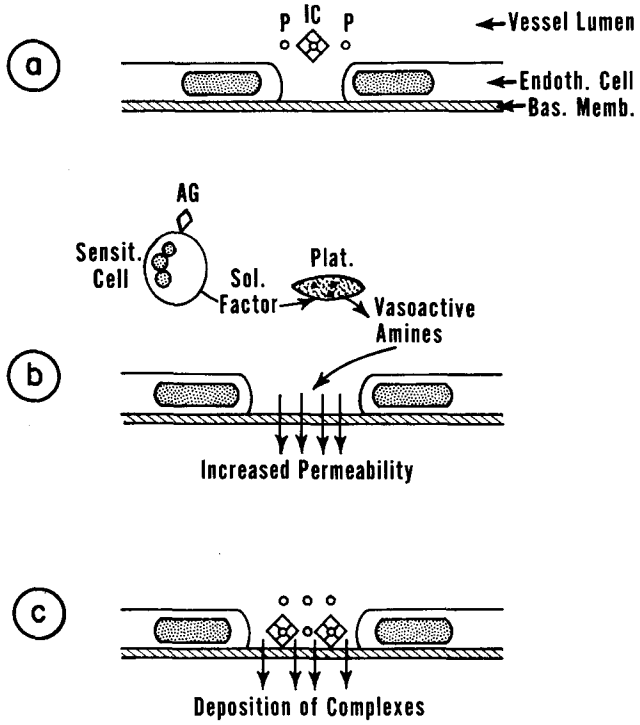

FIG. 5. Theoretical summary of the mechanism responsible for the deposition of immune complexes from the circulation in acute immune complex disease. Explanation given in text.

As discussed previously, Germuth and Pollack (1), by infusing antibody into rabbits with circulating antigen, were able to induce at least mild lesions in glomeruli and certain arteries in about half the recipients, while the injection of preformed complexes made with hyperimmune antibody was ineffectual in this regard.[3] An explanation may lie in the possibility that the infused antibody

[3] Cochrane, C. G., and W. O. Weigle. 1958. W. T. Kniker and C. G. Cochrane. 1964. Unpublished observations.

allowed a sensitization of leukocytes to take place for the LDHR reaction, while combining the hyperimmune antibody with an excess of antigen in vitro did not allow sensitization of leukocytes to take place. Also, since this leukocyte-sensitizing antibody disappears with repeated antigenic exposure, the hyperimmune antibody might not have contained sufficient sensitizing antibody.

Summary.—A summary of the mechanisms by which deposition of immune complexes may occur in acute immune complex disease (serum sickness) of rabbits is shown in Fig. 5. (*a*) Immune complexes and protein molecules circulate in a blood vessel. (*b*) In the presence of basophil leukocytes with adherent IgE antibody, the antigen induces release of the soluble intermediate. This intermediate activates platelets to clump and to release vasoactive amines. These amines then cause an increased permeability of blood vessels, especially in areas where the platelets clump and impinge upon blood vessels. (*c*) With the increased permeability, macromolecular (>19S) immune complexes become entrapped along filtering membranes in the vessel wall to induce injury.

SUMMARY

The mechanisms reponsible for the deposition of circulating immune complexes have been analyzed. An active process appears to be responsible in both a laboratory model in guinea pigs and in acute immune complex disease (serum sickness) in rabbits.

In rabbits, after the injection of antigen to induce serum sickness, immune complexes appear in the circulation. In addition, homocytotropic (IgE) antibody is formed which binds to the surface of basophils. Leukocyte suspensions containing these basophils, when combined with specific antigen, release a soluble factor that causes clumping of platelets and release of their vasoactive amines. An excellent correlation was found between the presence of this mechanism of release of vasoactive amine and the deposition of immune complexes in serum sickness of rabbits. Antagonists of vasoactive amines or depletion of platelets, the major circulating reservoir of these amines, suppressed the deposition of circulating immune complexes and inhibited glomerulitis and arteritis. Upon entering the walls of vessels, the complexes became lodged immune complexes, greater than 19S in size, were deposited along the membranes.

The data suggest that at a time when immune complexes appear in the circulation of an immunized rabbit, vasoactive amines are released from platelets in areas where turbulence of blood occurs. Sensitized basophils participate in the release of vasoactive amines from the platelets. The amines induced increased vascular permeability which leads to deposition of large complexes from the circulation in vessel walls by a process of filtration. The deposited complexes then induce inflammatory injury.

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