INTERACTION OF CELLS WITH IMMUNE COMPLEXES: ADHERENCE, RELEASE OF CONSTITUENTS, AND TISSUE INJURY*

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The pathogenic effects of immune complexes generally follow their deposition in the walls of blood vessels in different parts of the body. A mechanism for this deposition in experimental immune complex of the rabbit has been described previously.[1] It involves a role for IgE antibody, basophils, and platelets in the induction of increased vascular permeability, which then leads to trapping of large immune complexes along filtering surfaces. This process is independent of the complement system beyond the activation of C2 (1).

The next step to consider in the pathogenesis of immune complex diseases is the mechanism whereby the deposited complexes produce damage to the tissues. The role of neutrophils in the injury will first be discussed. A consideration of two in vitro mechanisms by which neutrophils release injurious constituents to the extracellular medium will follow. Finally, the ability of different immune reactants to stimulate these release processes will be described.

(A) THE ROLE OF NEUTROPHILS IN THE TISSUE INJURY PRODUCED BY IMMUNE COMPLEXES

There are at least two broad categories of immune complex-induced tissue injury, those dependent upon neutrophils and those which do not involve action of this type of cell. These categories can be illustrated by consideration of acute immune complex disease in rabbits.

(*1*) *The Arteritis of Acute Immune Complex Disease.*—This arteritis (Fig. 1) is a necrotizing vasculitis and is characterized by massive neutrophil accumulation with consequent damage to the internal elastic lamina, penetration of neutrophils into the media, and adventitia and subsequent necrosis of these

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[1] Cochrane, Charles G. 1971. Mechanisms involved in the deposition of immune complexes in tissues. *J. Exp. Med.* **134**(3, Pt. 2):75 s.

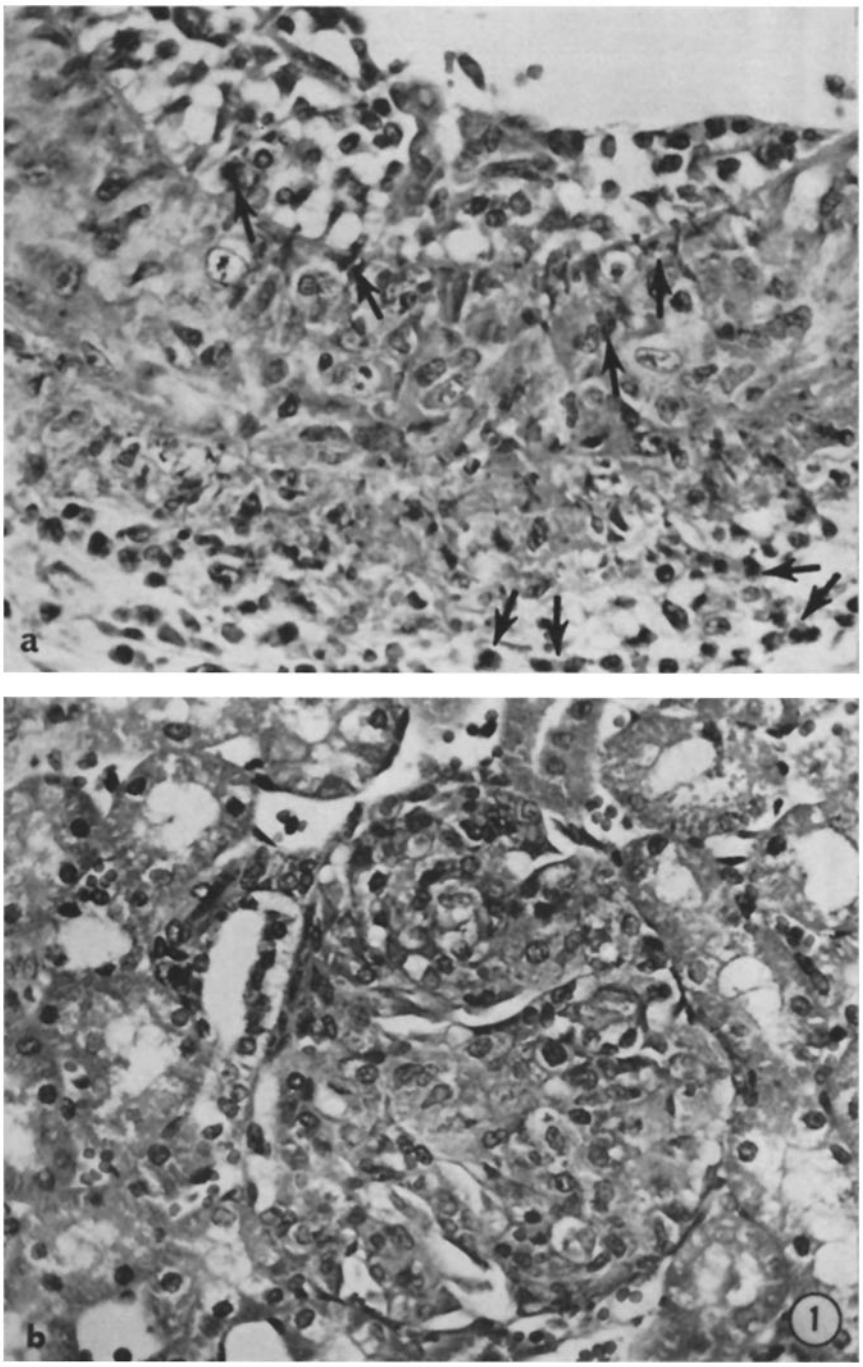


FIG. 1. Acute immune complex disease in the rabbit. (*a*) Arteritis: the characteristic neutrophil infiltration is apparent. These cells (arrows) have penetrated to all layers of the vessel wall. The lumen is at the top. (*b*) Glomerulonephritis: neutrophils cannot be seen in this esion which is characterized by swelling and proliferation of endothelial cells. × 450.

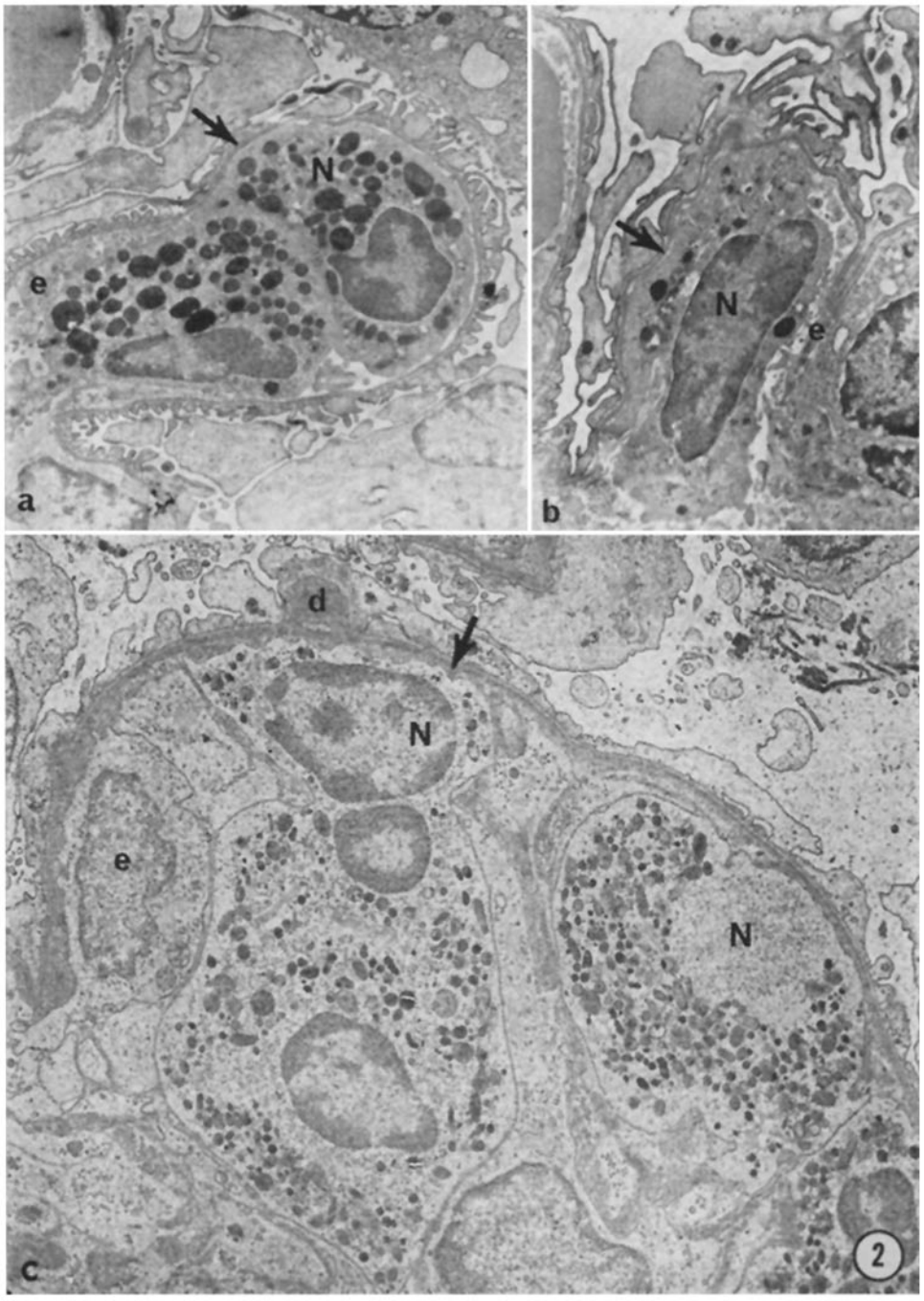
structures. The neutrophil accumulation and tissue damage, but not the deposition of complexes, can be prevented by depletion of circulating complement (C3 and later-acting components) with the anti-complementary factor from cobra venom (1) or by depletion of circulating neutrophils (2). It is presumed that the neutrophils accumulate by complement-mediated processes (chemotaxis and perhaps especially immune adherence), phagocytose the immune complexes, and release injurious constituents capable of digesting structures within the vessel wall.

(*2*) *The Glomerulonephritis of Immune Complex Disease.*—In contrast, the glomerulonephritis of the acute disease model (Fig. 1) is not characterized by neutrophil infiltration and is unaffected by depletion of either complement (1, 3) or neutrophils (2). Neutrophil-independent pathogenic mechanisms, whose nature is at present unknown, are therefore involved in the development of this glomerulonephritis. On the other hand, the glomerular lesions of chronic immune complex disease of the rabbit or the diseases thought to be associated with immune complexes in man frequently contain neutrophils (Fig. 2 *c*). In this situation, therefore, where deposition of large quantities of complexes occurs,[2] neutrophils and their products may contribute to the tissue damage.

(*3*) *Glomerulonephritis Produced by Anti-Glomerular Basement Membrane Antibodies.*— The situation in which complexes of antigen and antibody are dispersed along a surface is even more clearly represented by the glomerulonephritis produced by antibody directed against the glomerular basement membrane. This occurs experimentally in nephrotoxic nephritis in the rabbit or naturally in Goodpasture's disease in man (4). Antibody binds along the membrane and in the experimental system produces injury by both neutrophil-dependent and independent processes (5). When the former predominates, as depicted in Fig. 2 *a*, neutrophils fill the glomerular capillary lumen, having pushed aside the endothelial lining and become closely adherent to the antibody and complement bound to the basement membrane. As in the arteritis of acute immune complex disease, depletion of circulating complement or

[2] Wilson, Curtis B., and Frank J. Dixon. 1971. Quantitation of acute and chronic serum sickness in the rabbit. *J. Exp. Med.* **134**(3, Pt. 2):7 s.

FIG. 2. Neutrophils adherent to immune reactants along glomerular basement membranes. (*a*) Nephrotoxic nephritis in the rabbit. A glomerular capillary loop is depicted, 5 hr after injection of sheep anti-glomerular basement membrane antiserum. Neutrophils (*N*) have filled the capillary lumen, pushed aside the endothelial cell (*e*), and have become closely adherent to the basement membrane (arrow). × 6300. (*b*) A neutrophil in this glomerular capillary from the same rabbit has lost most of its granules. The foot processes of the epithelial cell have fused, indicating early damage to the vessel wall. × 8300. (*c*) Neutrophils in a human glomerulus from a case of poststreptococcal glomerulonephritis. The neutrophils are again in close contact with the basement membrane which in this case shows evidence of immune complex deposits (*d*). × 12,000. Photograph courtesy of Dr. J. D. Feldman.



neutrophils prevents the neutrophil accumulation and the subsequent tissue injury (6). However, in this situation, the neutrophils are adherent to immune reactants along a surface which they cannot phagocytose. Nevertheless, release of constituent enzymes is postulated to lead to digestion of basement membrane and, moreover, a neutrophil-derived proteolytic enzyme and fragments of the basement membrane have been detected in the urine (7).

TABLE I

Release of Enzymes from Human Neutrophils after Adherence to Immune Complexes

Stimulus*	Per cent release of enzymes	
	β-glucuronidase	LDH‡
Phagocytosable		
Ag + Ab	10.2	2.6
Ab	2.5	2.4
ZC	11.1	2.2
Z EDTA C	2.8	2.0
—	2.2	2.7
Nonphagocytosable		
Filter + Ag + Ab	13.3	2.7
Filter + Ag	3.1	3.1
Filter + normal human IgG	4.1	2.0

* Ag, tetanus toxoid; Ab, human IgG anti-tetanus. Ag + Ab, 30 μg of precipitates at equivalence. ZC, zymosan incubated with human serum as complement source and then washed (2.0 mg). Z EDTA C, zymosan incubated with serum and 0.01 M EDTA. Filters were incubated with tetanus, washed, incubated with antibody, and again washed, and 5×10^6 neutrophils were drawn gently down onto them. Incubated for 60 min at 37°C.

‡ LDH, lactic dehydrogenase.

(B) RELEASE OF CONSTITUENTS FROM NEUTROPHILS

In the continuing study of the pathogenesis of immune complex–induced tissue injury, it therefore became important to examine the mechanisms of release of constituents from neutrophils. Extracellular release of lysosomal materials from neutrophils after phagocytosis of immune complexes (8–12), bacteria (10, 13, 14), starch (15), or zymosan particles (16) has been described in a number of laboratories. In our experiments, two model systems have been employed, simulating the two in vivo situations described above (Table I). In one, neutrophils were allowed to phagocytose immune complexes or particles with antibody or complement fixed to them, and the release of enzymes to the outside of the cell was examined. In the other, the immune complexes were bound along a nonphagocytosable surface, in this case a micropore filter, and the reaction and release processes of adherent neutrophils were observed.[3]

[3] Henson, P. M. Manuscript submitted for publication.

For these studies, pure populations of cells (90–98% neutrophils) were obtained from the peripheral blood of human beings or rabbits and the reactions were carried out in a Tyrode's solution containing added albumin. Control preparations of rabbit neutrophils incubated for 1 hr at 37°C had the

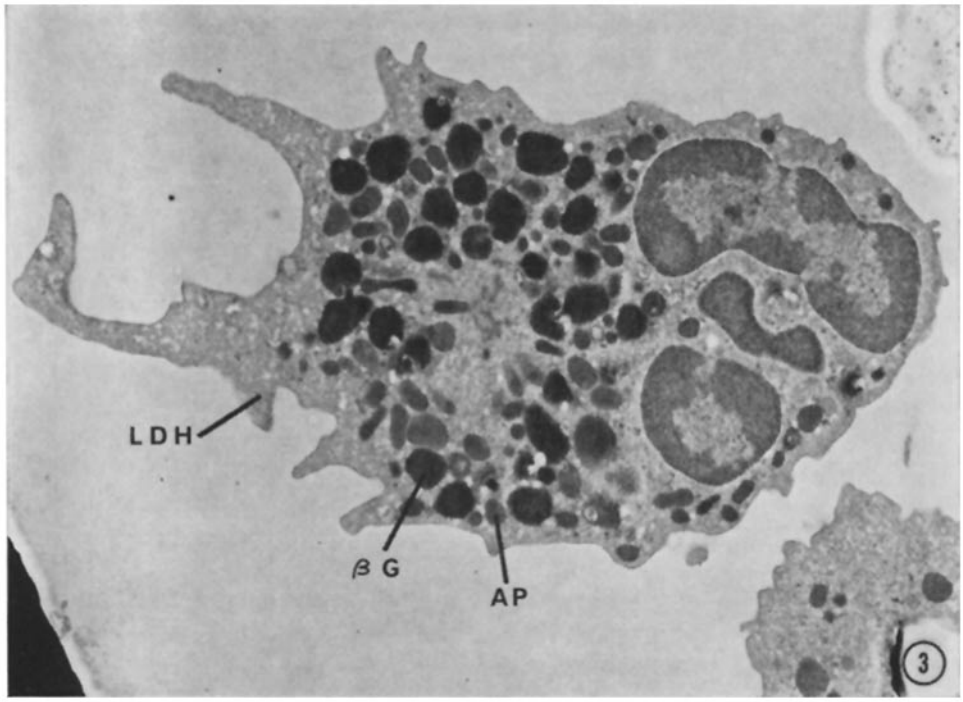


FIG. 3. Rabbit neutrophil from the blood, washed and incubated for 1 hr at 37°C. This control cell has not lost its complement of granules. The origin of the enzymes which were assayed (see text) are indicated. Lactic dehydrogenase (LDH) from the cytoplasm, β-glucuronidase (βG) from the primary granule, and alkaline phosphatase (AP) from the secondary granule. Fixed with glutaraldehyde and osmic acid, embedded in Vestopal W, and stained with uranyl acetate and lead citrate. × 12,500.

appearance shown in Fig. 3. The two major types of granules are clearly seen in this photograph. Release of enzymes from each type has been examined. β-glucuronidase was chosen as an enzyme which resides in the primary or "azurophil" granule and alkaline phosphatase as an enzyme of the secondary or "specific" granule (17, 18). In addition, to indicate cell death and release of cytoplasmic constituents, liberation of lactic dehydrogenase was also measured and was in most cases low, indicating that little cell lysis was occurring.

(*1*) *Release of Enzymes from Neutrophils on Nonphagocytosable Surfaces.*—

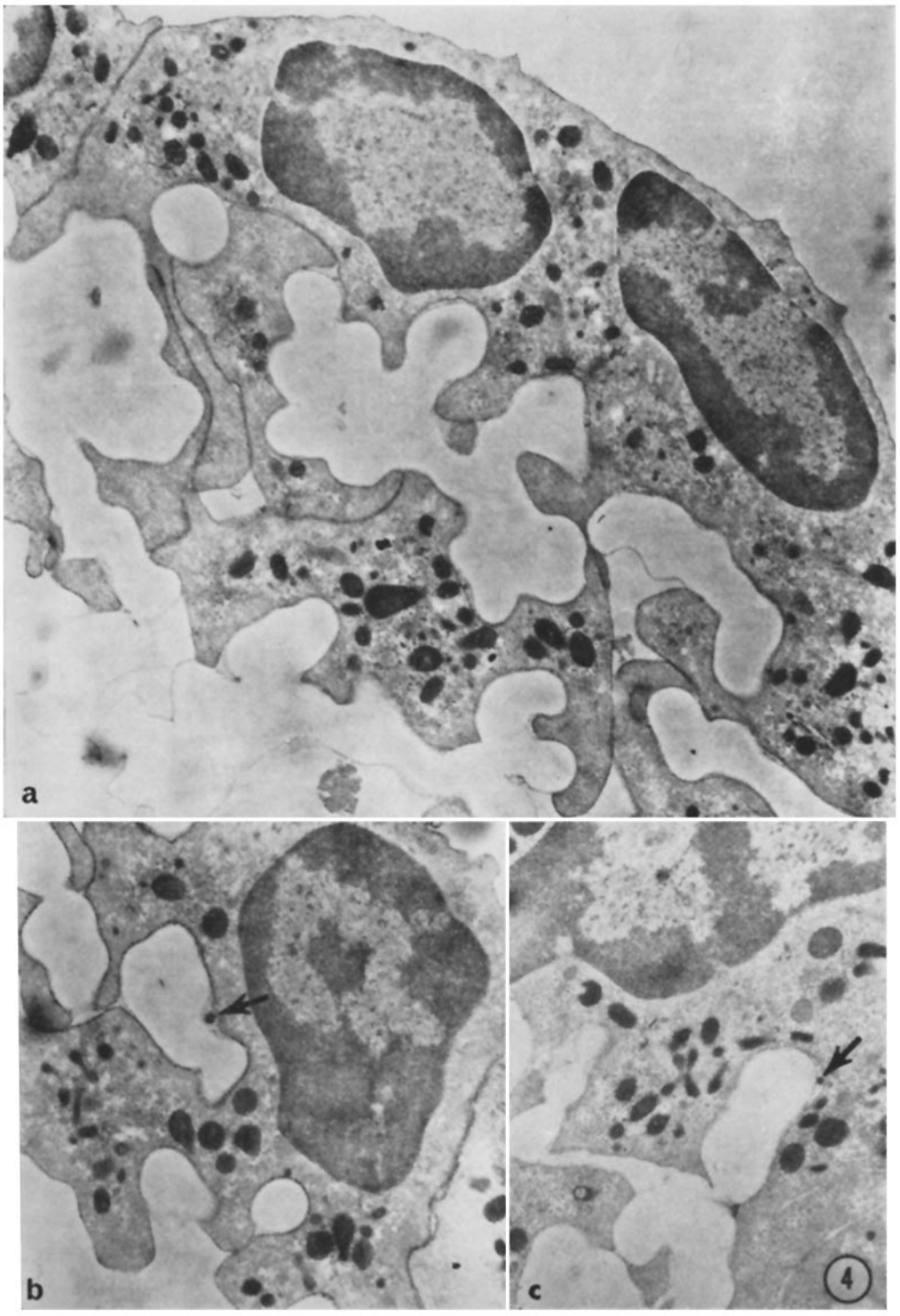
Fig. 4 *a* depicts a human neutrophil adherent to the surface of a micropore filter coated with antigen and antibody. The filter had been incubated first in a solution of antigen (tetanus toxoid), washed, and then reacted with human IgG anti-tetanus antibody. After further washing, 5×10^6 neutrophils were gently drawn down onto the surface and the filter with adherent neutrophils was incubated at 37°C. The neutrophil may be seen to be very closely adherent to the surface in a similar manner to the previously described adherence along the basement membrane.

(*a*) *Release of lysosomal constituents*: Neutrophils adherent to antigen and antibody released β-glucuronidase into the external medium (Table I) but not the cytoplasmic enzyme lactic dehydrogenase. In contrast, cells on filters with antigen only did not liberate their lysosomal enzymes. In similar experiments performed with rabbit neutrophils, a wide variety of lysosomal constituents, including cathepsins and permeability factors, were released when neutrophils reacted with immune complexes on these filters. In similar experiments with neutrophils upon a collagen membrane, D. Hawkins (personal communication) has also found that neutrophils release lysosomal enzymes to the external environment. It may be noted that a greater percentage of enzymes was consistently released by all types of stimuli from rabbit neutrophils than from human cells. Nevertheless, the processes of release appeared to be identical in each species.

(*b*) *Release of alkaline phosphatase*: Alkaline phosphatase, an enzyme from the secondary granule, was not detected in the supernatant fluid after the reaction. Nevertheless, release of the enzyme to the exterior of the cells did occur. This enzyme appears to be insoluble or to have the property of binding to membranes (19) and it remains adherent to the outside of the neutrophil cell membrane, where it could be detected histochemically (Fig. 4).

(*c*) *Mechanism of release*: Release of enzymes from neutrophils adherent to antigen and antibody on a nonphagocytosable surface appears to result from a direct degranulation to the outside of the cell. This degranulation has been observed for both human (Fig. 4) and rabbit (Fig. 5) neutrophils. It is postulated that the external cell membrane is stimulated by adherence to the fixed antibody such that granules fuse with it and discharge to the outside as

FIG. 4. Human neutrophils adherent to antigen and antibody on a nonphagocytosable surface (micropore filter). (*a*) The close adherence of the neutrophil to the convoluted surface of the filter is apparent. The free surface of the cell is to the top right. × 15,300. (*b*) A similar neutrophil after 5 min incubation showing the presence of alkaline phosphatase activity (18). The black precipitate of lead phosphate can be seen outside the cell, adherent to the neutrophil membrane where it is in contact with the antigen and antibody on the filter. No activity is present on the free surface of the cell (top right). The arrow indicates discharge of a granule. × 15,600. (*c*) A control preparation in which the reaction for alkaline phosphatase was performed in the absence of enzyme substrate. No precipitate has occurred. × 15,600.



if to a phagocytic vacuole. The process may thus be considered one in which the complete phagocytic process is "frustrated" by the large size of the surface.

(*d*) *Sequential discharge of secondary and primary granules*: Examination of

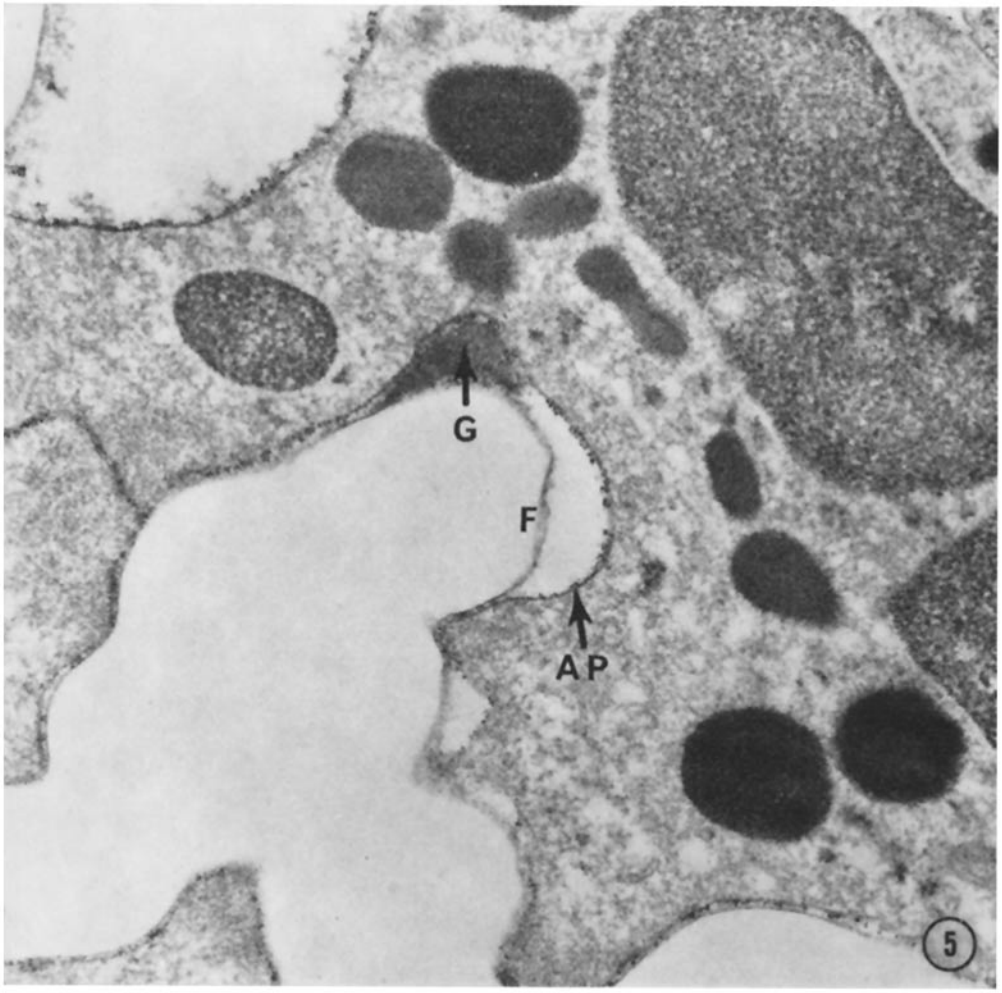


FIG. 5. Discharge of a secondary granule (G) to the outside of a rabbit neutrophil adherent of antigen and antibody on a micropore filter (F) after 5 min incubation. Alkaline phosphatase reaction product (AP) is visible along the cell membrane. × 39,700.

the time at which the different enzymes were released suggested that secondary granules (alkaline phosphatase) were discharged at an earlier time than the primary granules (β-glucuronidase) (Fig. 6). After 5 min of incubation,

secondary granules but not primary granules have been seen degranulating to the outside of the cell (Fig. 5). These experiments confirm the work of Bainton (20) who clearly demonstrated an earlier release of alkaline phosphatase than

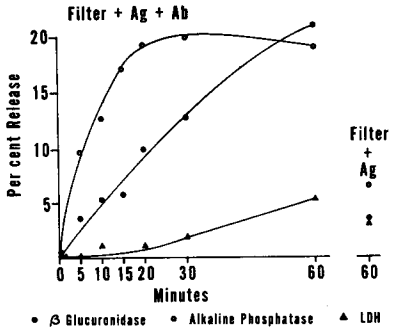


FIG. 6. Release of enzymes from rabbit neutrophils on nonphagocytosable surfaces with increasing time of incubation. Ag, bovine serum albumin (BSA); Ab, rabbit IgG anti-BSA; LDH, lactic dehydrogenase. The alkaline phosphatase was released more rapidly than the β-glucuronidase.

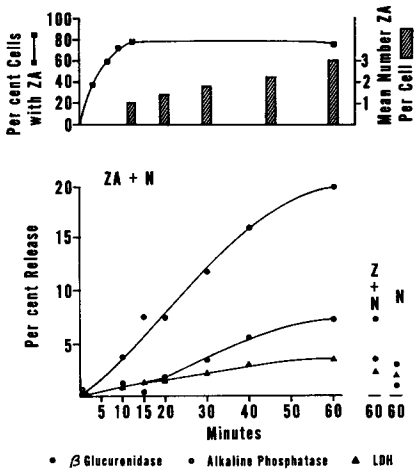


FIG. 7. Release of enzymes from rabbit neutrophils phagocytosing zymosan particles. ZA, 2 mg zymosan incubated with rabbit IgG anti-zymosan antibody and then washed; N, 10^7 neutrophils; LDH, lactic dehydrogenase. The alkaline phosphatase was only poorly released.

myeloperoxidase (which comes from the primary granule) into phagocytic vacuoles of neutrophils. The mechanisms whereby granules may be discharged at different rates is at present unknown but from a functional standpoint it is of interest that, as suggested by Bainton (20), secondary granules may release

enzymes with pH optima near neutrality before the pH within a vacuole (or inflammatory lesion) is reduced.

(*e*) *Degranulation in vivo*: Neutrophils adherent to antibody and complement along basement membranes in vivo have not yet been observed in the process of discharging granules. Nevertheless, cells may be seen in lesions of nephrotoxic nephritis which have almost completely lost their complement of granules (Fig. 2 *b*). The process described in vitro may then also contribute in vivo to the release of injurious consitituents from neutrophils.

(*2*) *Release of Enzymes from Neutrophils during Phagocytosis.*—Phagocytosis of either immune complexes or antibody-coated particles induces release of enzymes from the neutrophil primary granules (Table I) (12).[1] As depicted in Fig. 7, phagocytosis of zymosan–antibody complexes was a very effective stimulus for the release of β-glucuronidase from the primary granule to the exterior of the cells. The minimal release of lactic dehydrogenase and the exclusion of Trypan blue by the cells indicates that this release did not result from lysis of the cells.

(*a*) *Release of alkaline phosphatase*: When alkaline phosphatase was examined, however, a striking difference was noted between the release stimulated by a nonphagocytosable surface and by a phagocytosed particle. As shown in Fig. 7, phagocytosis of zymosan antibody induced little extracellular release of this enzyme (whether in the medium or bound to the external cell membrane) and, in addition, it was liberated over the same time-course as β-glucuronidase.

(*b*) *Mechanism of extracellular release*: Morphologic and histochemical studies in the electron microscope helped explain this difference (Fig. 8). In confirmation of Bainton's experiments (20), alkaline phosphatase was liberated into the phagocytic vacuoles after only a short period (2–5 min) of incubation, even though it was not released to the outside. It could be detected there, bound to the surface membrane of the vacuole. It is postulated that the release of enzymes during phagocytosis is a two-stage phenomenon, first a degranulation into the phagocytic vacuole and then an opening of the vacuole, probably transiently, to the outside of the cell. The soluble enzymes, such as β-glucuronidase, escape, but the alkaline phosphatase remains in the vacuole bound to its surface membrane. Direct discharge of granules to the cell surface has not been observed.

One situation in which phagocytic vacuoles may open to the extracellular medium is during the process of phagocytosis of an additional particle, which may be taken into an already existing vacuole before the neutrophil pseudopodia have closed behind it. Evidence for this process has been obtained (Fig. 8). Moreover, the release of enzymes correlates well with the uptake of additional zymosan particles (Fig. 7), not with phagocytosis of the first particle.

A corollary of this hypothesis would suggest that phagocytosis of large particles would induce greater release than that of small particles, since the latter

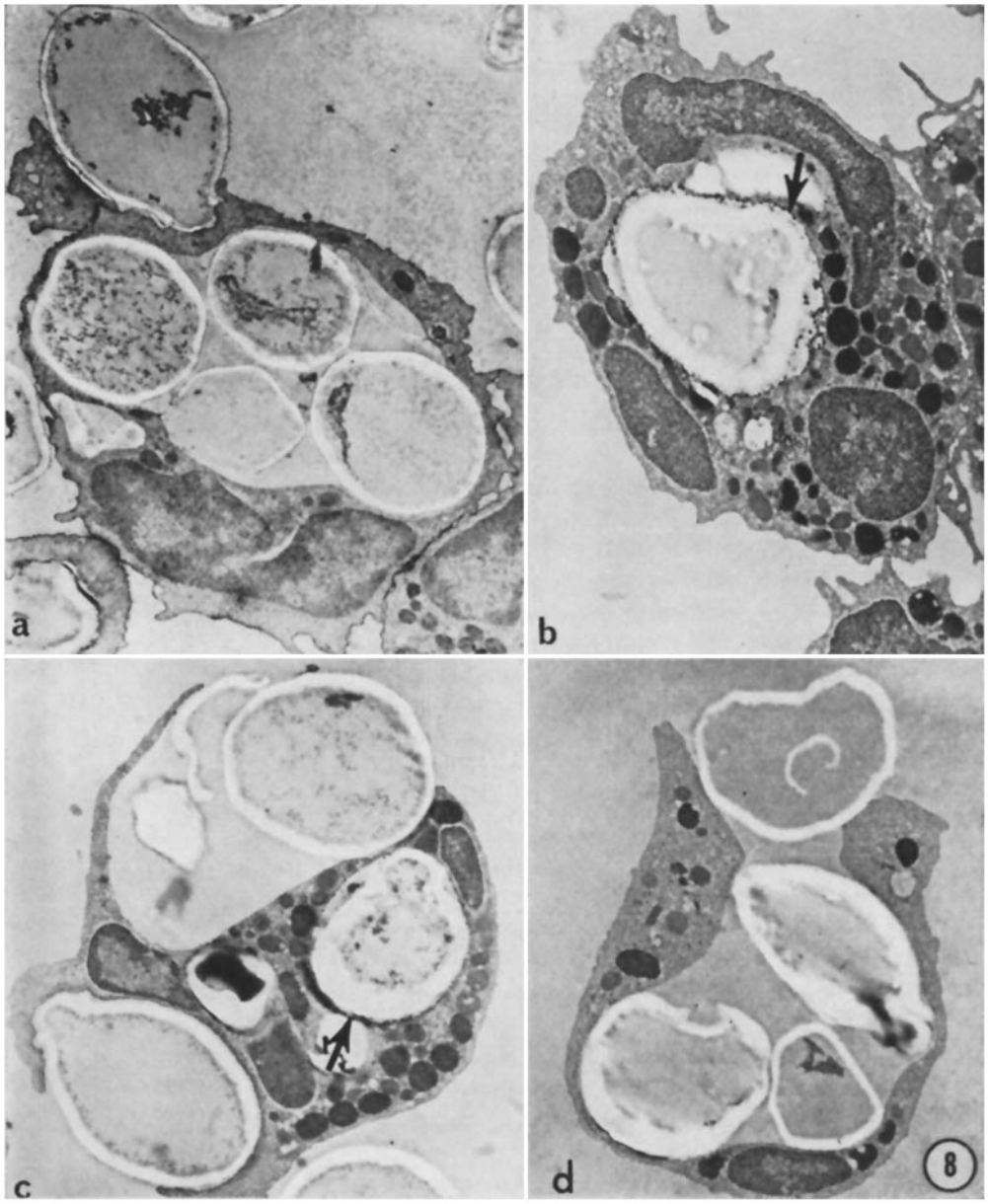


FIG. 8. Rabbit neutrophils phagocytosing zymosan particles. (*a*) Neutrophil containing six particles of zymosan complement (see text) and adherent to a seventh. × 7300. (*b*) Neutrophil after 10 min incubation with zymosan antibody showing alkaline phosphatase activity. The reaction product (arrow) may be seen along the surface of the phagocytic vacuole which contains one zymosan particle. × 5100. (*c* and *d*) Neutrophils after 30 min incubation with ZA. Stages in the uptake of an additional particle into an already formed phagocytic vacuole are shown. A number of granules remain in *c* but have mostly disappeared in *d*. The latter cell shows an opening of the vacuole, presumably containing lysosomal enzymes, to the outside. Many instances of this phenomenon have been observed. Arrow in *c* same as in *b*. × 8200 and 7600, respectively.

would be more likely to be sequestered in individual vacuoles and the neutrophil would more readily enclose the particle before incorporation into already existing vacuoles. Studies with IgG globulin bound to latex particles of different sizes have revealed that this is indeed the case. Particles of 2, 1, 0.5, and 0.1 μm in diameter induced release of 22, 19, 16, and 9% β-glucuronidase, respectively, where equal surface area (and, therefore, equal amounts of IgG) were presented to the neutrophils. This finding, therefore, confirms the original observations of Cohn and Hirsch (21) who found less than 10% release of lysosomal enzymes to the exterior of neutrophils (heterophils) phagocytosing small particles (bacteria).

Two other related mechanisms of release may also contribute to the reaction and have occasionally been observed. Phagocytosis of one particle by two neutrophils leads to degranulation into a vacuole which is essentially open to the exterior[1] (22). In addition, during phagocytosis, degranulation into a developing vacuole sometimes occurs before the vacuole is completely closed. However, these are likely to be secondary granules (see above), and yet alkaline phosphatase was found in only low levels outside the cell and not generally along the surface of developing vacuoles before they were closed off. Moreover, the release of enzymes correlated with the uptake of not the first, but of additional zymosan particles. These observations do not suggest a major role for these two mechanisms although they probably add to the total release.[1]

(*c*) *Release in vivo:* Although it has not yet been clearly demonstrated that uptake of particles into preexisting phagocytic vacuoles containing lysosomal enzymes does occur in vivo, Fig. 9 shows that it may be possible. This depicts an Arthus reaction produced with ferritin antigen and shows that precipitates of large size may be formed which may lead to suitably large vacuoles for the postulated release process.

(*3*) *Comparison of Release of Constituents from Neutrophils Adhering to Phagocytosable Particles or Nonphagocytosable Surfaces.*—Fig. 10 summarizes our concept of the predominant mechanisms of release in these two circumstances. On the surface, there is a degranulation to the external membrane of the cells which is in contact with the immune complexes. After phagocytosis, there is a degranulation into the phagocytic vacuole which later opens to the outside during ingestion of additional particles, a process which is more likely to occur with larger than with smaller particles.

The relative sensitivities of these processes in vitro are shown in Table II To achieve 13% release of β-glucuronidase, 31 μg of antibody in immune complexes in suspension was required. However, on the nonphagocytosable surface, less than 2 μg of antibody was effective. (In fact, more antibody is present on the filter but neutrophils are drawn down onto only a small portion of the total area available.) Immune complexes along surfaces within the body may,

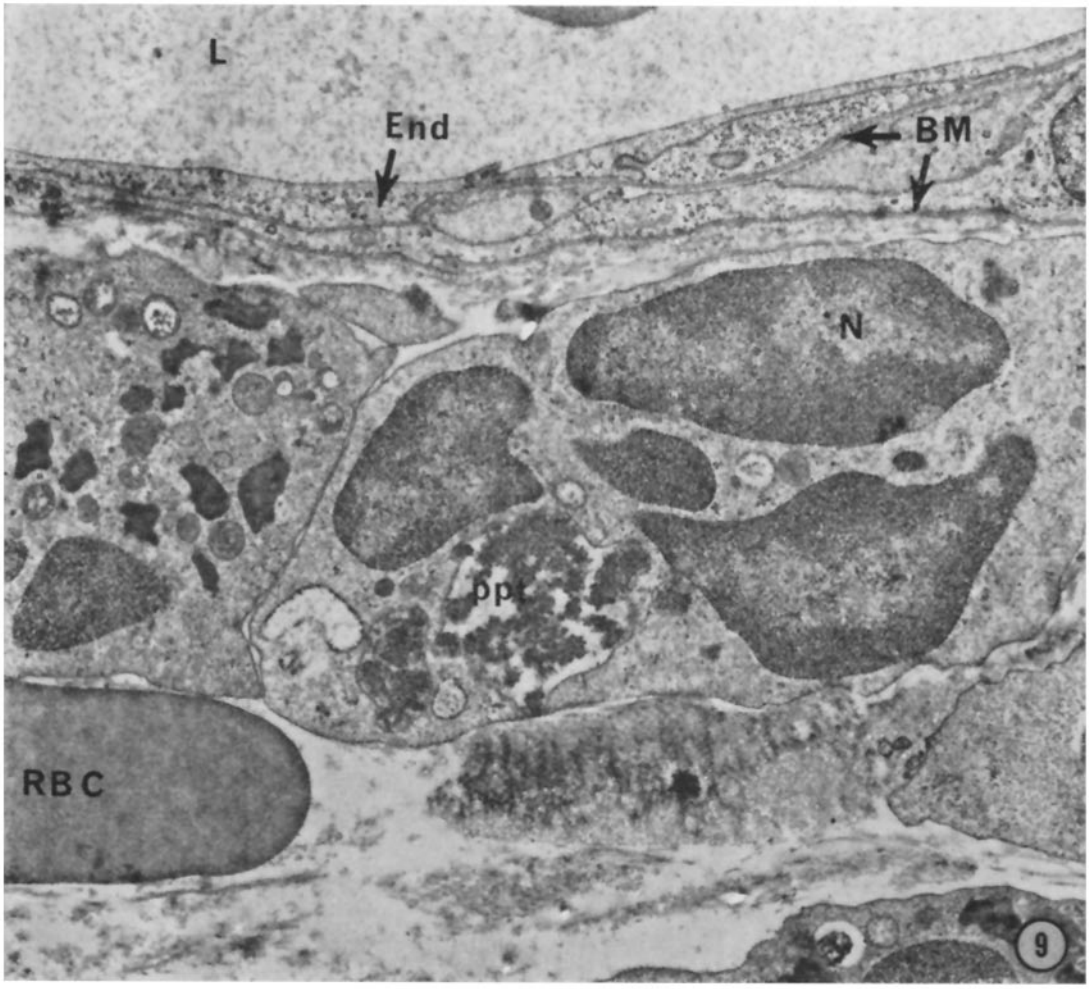


FIG. 9. A reversed passive Arthus reaction in the rabbit bladder wall. Neutrophils (N) are visible in the vessel wall and have phagocytosed immune complexes (ferritin–anti-ferritin). One large vacuole containing such a precipitate (ppt) can be seen and is almost open to the outside of the cell. RBC, red blood cell; *BM*, basement membrane; End, endothelial cell; L, lumen. × 14,500.

therefore, be more effective stimuli to tissue injury than when free in the blood stream.

In inflammatory lesions in vivo, neutrophil degeneration and lysis may play an important role by contributing additional neutrophil enzymes and permeability factors. This cell death could occur for many reasons, but may in part

be caused by materials released by active (not degenerative) processes from neutrophils which arrived first at the site of injury and there interacted with the immune complexes by the mechanisms described.

(c) THE STIMULUS TO NEUTROPHILS FOR RELEASE OF CONSTITUENTS TO THE OUTSIDE

The initial reaction between neutrophils and immune complexes is one of adherence. This adherence is mediated by either antibody or complement

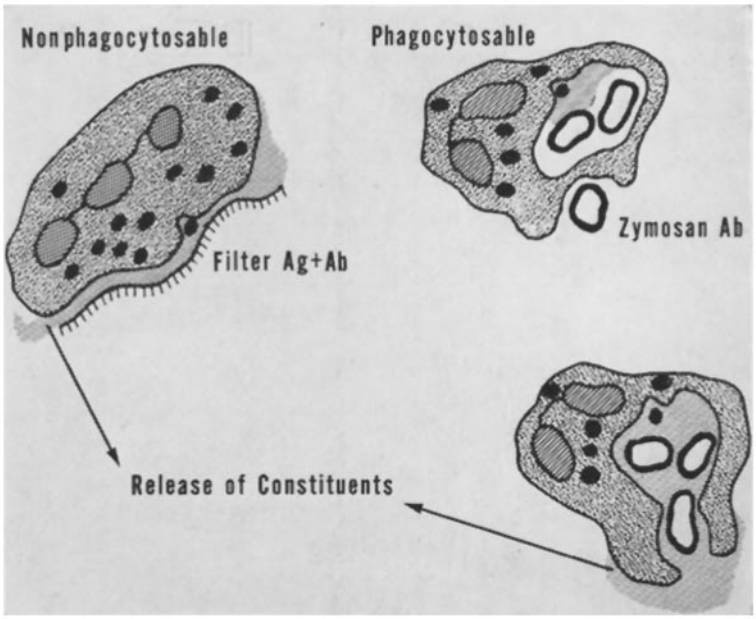


FIG. 10. Two mechanisms of release of granule constituents from neutrophils reacting with immune complexes which may be phagocytosed, or which are bound to nonphagocytosable surfaces.

(23–28) and may be clearly demonstrated by "rosette" formation if the particle is too large for easy phagocytosis or if the neutrophils are metabolically inhibited so that the subsequent phagocytosis is prevented (Fig. 11).

(*1*) *Complement.*—The fixation of complement components to erythrocytes or zymosan results in adherence of neutrophils and phagocytosis of the particles. Clear evidence from a number of laboratories (24–26, 28, 29) has implicated C3 as the complement component of major importance in these phenomena of adherence, phagocytosis, and release of constituents.

The ability of complement to induce release of enzymes from neutrophils on nonphagocytosable surfaces has also been examined. Incubation of antigen

and antibody bound to micropore filters in normal fresh serum allowed complement fixation. Neutrophils adherent to these complexes exhibited increased release of lysosomal constituents. By use of C6-deficient or C3-depleted sera, it was possible to implicate C3 in this reaction also.[1]

(*2*) *Immunoglobulins.*—The receptors on neutrophils (and also those on monocytes and macrophages) which allow adherence to C3 are different from those which permit adherence to IgG antibody (25, 26, 30). The former can be removed by trypsin or, in the case of rabbit neutrophils, prevented from reacting with C3 by the addition of chelating agents. In contrast, adherence of neutrophils to IgG antibody on a particle such as an erythrocyte is inhibited in the presence of excess free, monodisperse IgG, which has no effect on the adherence to complement.

TABLE II

Amounts of Antibody Required to Stimulate Release of Enzymes from Rabbit Neutrophils

	IgG antibody	Per cent release of enzymes	
		β-glucuronidase	LDH
	μg		
Ag Ab precipitates	31	13.2	6.1
Z Ab	5	13.7	5.5
Ag Ab on filter	<2	15.0	6.0

What are the classes of immunoglobulins which induce these reactions? To answer this question, the ability of neutrophils to release β-glucuronidase upon adherence to (or phagocytosis of) aggregated immunoglobulins was examined.[4]

(*3*) *Reaction of Neutrophils with Immunoglobulin Classes and Subclasses.*—Purified human myeloma proteins were aggregated with bisdiazo-benzidine (BDB) (31) or by heat. Table III shows the results of incubating aggregated immunoglobulins with human neutrophils. Each myeloma protein was tested in duplicate on a number of occasions with neutrophils from different normal subjects. It may be seen that with these insoluble aggregates (500 μg incubated with 5×10^6 neutrophils for 1 hr at 37°C), IgG_1, IgG_2, IgG_3, and IgG_4 all induced release of β-glucuronidase, and adherence of neutrophils to the microscopically visible precipitates was observed. There was also some suggestion that IgG_4 was less active but this was in part due to greater variation from myeloma to myeloma of this subclass.

Of great interest was the finding that neutrophils adhered to aggregates of all IgA examined and that these myeloma proteins proved very efficient in

[4] Henson, P. M., R. J. Bell, and H. L. Spiegelberg. Manuscript in preparation.

stimulating release of enzymes. IgM macroglobulins were unable to induce either adherence or release of β-glucuronidase and IgD was also inactive.

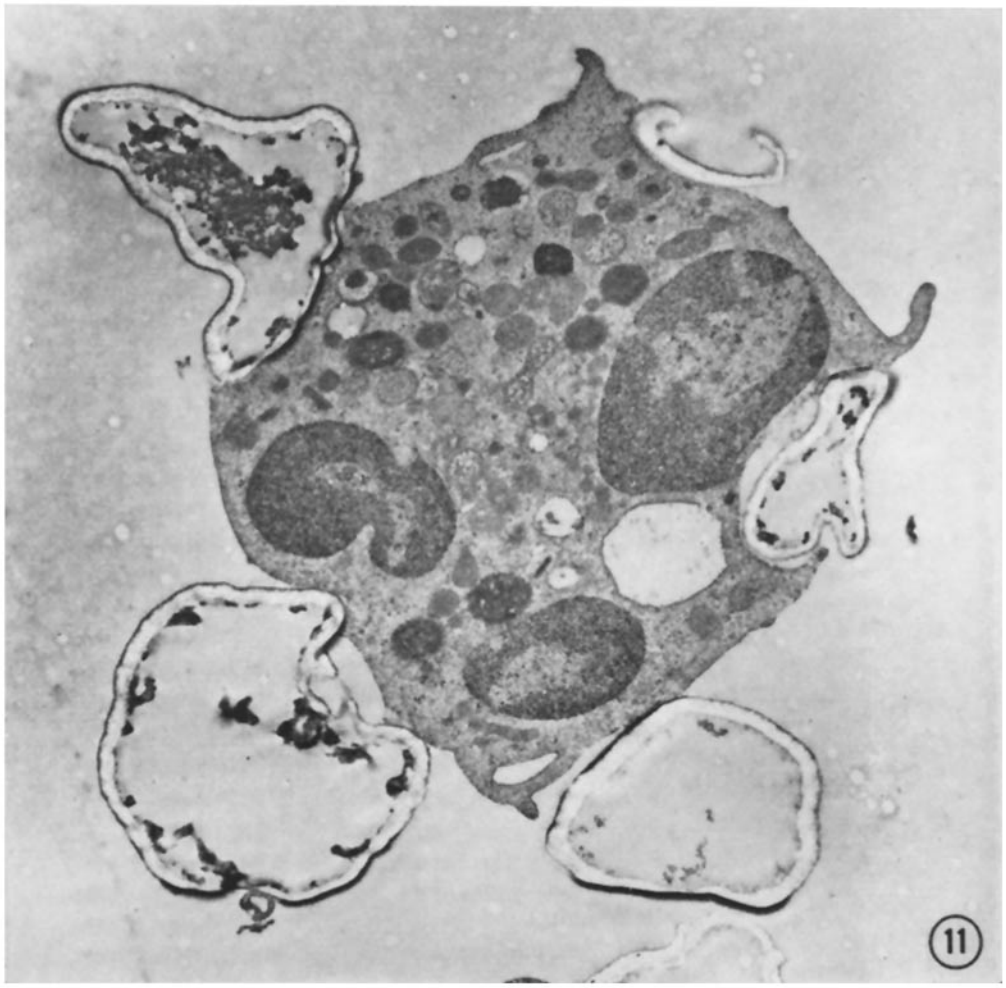


FIG. 11. Adherence of zymosan–anti-zymosan complexes to the surface of a rabbit neutrophil. The phagocytosis which would normally ensue has been inhibited with 10^{-3} M iodoacetate. × 13,900.

Table IV shows that for each myeloma, 500 μg was an effective stimulus and 50 μg was not. Since the maximum contamination of any of the myeloma proteins used was 5% and most contained much less, the possibility that the IgA was reacting with neutrophils by virtue of contamination with IgG was

excluded. Although the reaction of neutrophil suspensions with insoluble immunoglobulin aggregates resulted in release of enzymes, if soluble aggregates, prepared with less aggregating agent, were employed, no release oc-

TABLE III

Release of Enzymes from Washed Human Neutrophils by Aggregated Immunoglobulins in Suspension

Myeloma proteins	No.	Per cent release of enzymes*			Adherence to precipitates
		β-glucuronidase		LDH	
		Mean	Range		
IgG_1	(3)	14.6	(6.3–24.7)	2.3	+
IgG_2	(4)	9.4	(3.8–14.5)	2.2	+
IgG_3	(2)	12.1	(9.0–14.0)	2.5	+
IgG_4	(3)	8.3	(3.4–12.4)	2.1	+
IgA_1	(4)	10.3	(6.1–18.4)	2.3	+
IgA_2	(2)	10.7	(8.7–12.7)	3.0	+
IgD	(2)	1.2	(0.3–2.2)	1.7	
IgM	(4)	1.5	(0.6–2.0)	2.8	0
—		1.1	(0–3.1)	2.1	

* Aggregated immunoglobulins (500 μg aggregated with BDB) were incubated with 5×10^6 neutrophils for 60 min at 37°C, and the percentage of enzymes released into the supernatant fluid was measured.

TABLE IV

Percentage of β-Glucuronidase Released from Human Neutrophils by Aggregated Immunoglobulins

Immunoglobulin	Degree of aggregation	μg aggregated immunoglobulins			
		500	200	100	50
IgG_1	Soluble*	3.0	3.0	2.7	2.3
	Insoluble	20.3	9.1	5.2	3.4
IgG_2	Insoluble	13.8	8.3	6.1	3.7
IgG_3	Insoluble	13.3	10.0	6.6	4.1
IgG_4	Insoluble	12.4	7.7	6.3	5.4
IgA_1	Insoluble	14.6	10.4	8.0	3.5

* Soluble aggregates of immunoglobulins (2.5 mg/ml) were produced with 12 μg/ml BDB and insoluble aggregates with 50 μg/ml BDB.

curred (Table IV). This again demonstrates the requirement for large particles for this type of release reaction.

In contrast to the lack of release when neutrophils reacted with soluble immunoglobulins in suspension, when such soluble aggregated myeloma proteins were fixed to the surface of micropore filters, adherent neutrophils did release β-glucuronidase (Table V). This occurred if the neutrophils were in

contact with only 15 μg (or less) of protein. Once again, all four subclasses of IgG and both IgA_1 and IgA_2 were capable of inducing enzyme release while IgM was ineffective and IgD was only weakly active, if at all.

These results indicated that human neutrophils have receptors on their surface for, and are capable of being directly stimulated by, both IgG and IgA immunoglobulins. IgM does not react in this way and had previously been shown not to induce adherence by itself (26), but due to its complement-fixing activity would cause adherence secondarily by means of the C3 which is fixed. IgG_4 and IgA do not fix complement by the normal process but, nevertheless, as described above, do have the ability to react directly with the neu-

TABLE V

Release of Enzymes from Washed Human Neutrophils by Aggregated Immunoglobulins on Nonphagocytosable Surfaces

Myeloma proteins	No.	Per cent release of enzymes		
		β-glucuronidase		LDH
IgG_1	(3)	13.6	(12.1–16.4)	2.1
IgG_2	(2)	11.7	(9.9–13.2)	1.9
IgG_3	(2)	10.5	(8.3–12.7)	1.8
IgG_4	(3)	8.1	(4.5–12.0)	1.9
IgA	(3)	12.3	(7.2–16.4)	2.3
IgA_2	(1)	7.0	(5.3–8.6)	2.5
IgD	(2)	4.0	(0–5.7)	2.7
IgM	(3)	2.7	(0–5.5)	1.9
BSA		2.6	(1.8–3.9)	2.1

trophils. It has been reported (32) that binding of IgG_1 and IgG_3 by neutrophils as measured by inhibition studies was more efficient than that of IgG_2 and IgG_4. The studies described herein provided a semiquantitative assessment of the ability of neutrophils to react directly with the immunoglobulins and showed a reaction with all four subclasses of IgG, although IgG_1 and IgG_3 did appear to be more active.

The adherence of neutrophils to the immune complex (whether induced by complement or by immunoglobulins) is but the first step in the sequence of biochemical events leading to phagocytosis and/or release of enzymes. The release process is an active one and requires neutrophil energy metabolism, the action of serine esterases, and the presence of calcium ions. Moreover, its control may be mediated by cyclic-adenosine monophosphate.[5] However, much has still to be determined before the release process can be selectively

[5] Weissmann, Gerald, Robert B. Zurier, Paul J. Spieler, and Ira M. Goldstein. Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles. *J. Exp. Med.* **134**(3, Pt. 2):149 s.

inhibited in vivo in order to prevent the liberation of injurious materials into inflammatory lesions.

(D) SUMMARY AND CONCLUSIONS

Neutrophils are essential mediators of tissue damage in many forms of immune complex–induced injury. In vitro, they have been shown to release some of their content of injurious constituents upon reaction with immune complexes (Fig. 10). If the complexes are distributed along a nonphagocytosable surface, degranulation to the exterior of the cell is observed. When the complexes were phagocytized, however, degranulation into the phagocytic vacuole, and some loss of enzymes into the surrounding medium, occurred. This may have resulted from a momentary opening of the vacuole to allow ingestion of additional particles, as was demonstrated with the electron microscope. This phenomenon was particularly noticeable when the particles were relatively large. Far more immune complex is required to induce release when in a phagocytosable form than when on a nonphagocytosable membrane.

Neutrophils may be attracted to sites of immune complex deposition in many parts of the body (arteries, heart, skin, brain, kidney, joints) by complement-mediated processes. In some situations, e.g. in the joint fluid, they would encounter free immune complexes, phagocytose them, and release enzymes. In many others, in which immune complexes may be distributed along surfaces, such as in the glomerulus, adherence of neutrophils may also lead to release of injurious constituents (proteases, collagenase, elastase, permeability factors) capable of digesting and injuring the tissues.

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