ANTISERUM TO LEUCOCYTE LYSOSOMES

Its Cytotoxic, Granulolytic, and Hemolytic Activities*

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Plates 14 to 18

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The cytoplasmic granules of rabbit polymorphonuclear leucocytes contain various hydrolytic enzymes and bactericidal substances (1). These organelles may be considered a specialized form of lysosome (2).

Several observations point to a similarity in behavior between the membranes surrounding leucocyte granules and erythrocytes, and a difference in behavior between these and the membranes about the white cell itself. For instance acid or streptolysins (3) lyse red cells and granules but do not disrupt the leucocyte, whereas ion-free sucrose solutions (1) or electric current (4) disrupt the polymorph membrane without evident lysis of granules or erythrocytes.

These observations suggest that other hemolytic agents or conditions, such as antibody plus complement, might also be granulolytic. This report describes observations on the cytotoxic, granulolytic, and hemolytic activities of guinea pig antisera to rabbit leucocyte granules and to rabbit erythrocytes.

Methods

Polymorphonuclear leucocytes were harvested from rabbit peritoneal exudates induced by glycogen as described previously (5). Only those exudates free of detectable red cell contamination were used. The exudates each contained 5×10^8 to 1×10^9 leucocytes, more than 95 per cent of which were polymorphs. The cells were washed by suspension in saline and centrifugation at 250 *G*. These operations were performed at room temperature.

Leucocyte granules were recovered from the rabbit polymorphs by the sucrose lysis procedure (1). Granules were washed three times by suspending in sucrose or in saline and spinning at 5000 *G* at 0–4°C.

Rabbit erythrocytes were collected from heparinized blood. After centrifugation at 800 G for 20 minutes, a portion of red cells from the lowermost portion of the tube was removed and washed 6 times by suspension in phosphate-buffered saline and centrifugation.

Antisera against leucocyte granules or against red cells were prepared by injecting guinea pigs subcutaneously with 0.5 ml washed granule suspension (containing granules from approximately 4×10^8 polymorphs) or with 0.5 ml washed erythrocytes (containing 5×10^8

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red cells). Three such injections were made at 7-day intervals, and the guinea pigs were bled from the heart 10 days after the last injection. The clotted blood was held at room temperature for 1 hour, and then serum was separated by centrifugation. Serum was used promptly or was stored at −20°C and used within 1 month.

Cytotoxicity and degranulating effects of these antisera were observed and recorded on motion picture film using equipment and procedures described previously (3).

For *electron microscopy*, cells were deposited by centrifuging lightly. The pellet was chilled on ice for 2 minutes and then suspended and fixed for 10 minutes in cold phosphate-buffered 1 per cent OsO_4. Standard procedures were employed for dehydration and embedding in epon (6). Thin sections were double stained with uranyl and lead ions and examined in a Siemens elmiskop I.

Granule lysis on exposure to antisera was also detected by following liberation into the medium of certain hydrolytic enzymes normally contained in or associated with the granules. Lysozyme, aryl sulfatase, and cathepsin were measured by methods employed previously (1, 7). Intact polymorphs or isolated granules were suspended in saline and divided into several equal aliquots. One portion was frozen in dry ice–alcohol and thawed at room temperature 6 times to liberate the lysosomal enzymes and serve as a measure of total enzyme available in the suspension. Other aliquots were incubated for 1 hour at 37°C with 30 per cent antigranule serum, antierythrocyte serum, or normal guinea pig serum. Following incubation these suspensions were centrifuged at 800 *G* (to deposit leucocytes) or 5000 *G* (to deposit granules or granule debris) and enzyme analyses were done on the supernates. The other aliquots were frozen and thawed 6 times and then incubated with normal guinea pig serum or the antisera; assays on supernates from these samples permitted measurement of enzyme inhibition, when present, by normal serum factors or by antibodies.

Appropriate dilutions of immune and normal guinea pig sera were also assayed directly to allow correction for enzyme activity in the serum when present.

Titrations of hemolytic activity on rabbit erythrocytes were done in barbital-buffered saline containing divalent cations and 10 per cent normal guinea pig serum to provide excess complement. Serial twofold dilutions of the guinea pig antisera were made in a final volume of 0.5 ml, and 0.1 ml of a 4 per cent suspension of washed rabbit red cells was added to each sample. After 1 hour at 38°C and 4 hours at room temperature, the highest dilution of antiserum giving complete hemolysis was recorded as the end-point.

Titration of granulolytic activity on intact leucocytes was performed in a similar barbital buffer system containing normal guinea pig serum. Serial dilutions of the antisera were made. To each sample was added 0.1 ml of a saline suspension of washed rabbit peritoneal exudate polymorphs containing 1×10^8 cells per ml. After 1 hour at 38°C, the samples were mixed thoroughly and a loopful of each was made into a thin coverslip preparation and examined by high-dry phase contrast microscopy. The end-point was taken as the highest dilution of antiserum which caused degranulation of more than 50 per cent of the leucocytes. Though somewhat cumbersome, this technique allowed quite precise titrations, as substantiated by reproducible results when the microscopic readings were made in control fashion on coded slides.

Absorption of antisera with rabbit red cells or with rabbit polymorph granules was accomplished as follows. Antiserum was held at 56°C 30 minutes to inactivate complement. Washed packed rabbit red cells were suspended in an equal volume of antiserum, or washed packed granules from approximately 10^9 polymorphs were suspended in 1 ml of antiserum. After 1 hour at 0–4°C, the specimens were centrifuged at 1000 *G* to deposit red cells or at 5000 *G* to deposit granules. Absorption was repeated on the supernatant serum in some instances. These absorptions were not quantitatively comparable, as the red cell/serum ratio was at least 10 times greater than the granule/serum ratio. For control purposes certain immune guinea pig sera were absorbed with human and horse erythrocytes.

Gel diffusion precipitin reactions were observed by the Ouchterlony method using 1 per cent agar in a veronal-buffered medium at pH 8.6. Granule suspension was placed in the center well and various sera were introduced in peripheral wells. Diffusion was for 48 hours at room temperature.

RESULTS

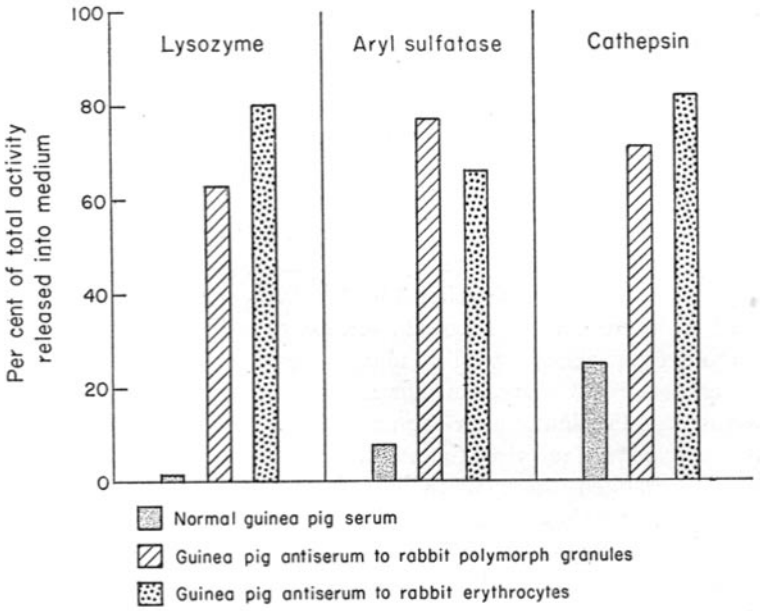
Cytotoxicity on Polymorphs of Antigranule and Antierythrocyte Sera.—Normal guinea pig serum and serum from guinea pigs immunized with ovalbumin did not exert any detectable damaging effects on rabbit polymorphonuclear leucocytes in the coverslip preparations during a 30 minute period of observation. In contrast, addition of serum from guinea pigs immunized with rabbit polymorph granules resulted in dramatic cytoxicity. Immediately following addition of such antiserum the cytoplasmic granules began to burst in rapid succession and the cells became immobilized and swollen. In most instances granules appeared to discharge their contents directly into the cytoplasm, giving it a moth-eaten appearance. The rapidity of individual granule lysis is illustrated in Fig. 1; phase-dense granules circled in the photographs on the left have disappeared in pictures taken 0.1 second later, shown at right. Following degranulation the nuclear lobes rounded up and frequently fused, as is illustrated by the sequences in Fig. 2. The cell membrane remained smooth and apparently intact for at least 15 minutes after degranulation and nuclear fusion.

We were somewhat surprised to find that guinea pig antisera to rabbit erythrocytes also exhibited toxicity on rabbit polymorphs. Furthermore the rapidity and detailed morphology of the cytoxicity of antigranule (Fig. 2) and antierythrocyte (Fig. 3) sera were indistinguishable.

The cytotoxic effects of antigranule sera were studied in more detail by electron microscopy. Fig. 4 *a* shows a rabbit polymorph fixed after incubation with normal guinea pig serum for 2 minutes. The appearance is typical of the undamaged rabbit cell with the fixation and staining procedures employed. These procedures were selected to preserve cell and granule membrane structure; although the cytoplasmic granules varied greatly in size and internal density, they appeared to be surrounded by an intact membrane in most instances. A rabbit polymorph damaged by a 2 minute exposure to antigranule serum is shown in Fig. 4 *b*. Nuclear lobes were swollen, rounded, and appeared to be fusing. A few granules and membrane residues were seen at the upper left portion of the cell. Despite the evidence of severe internal damage, the cell membrane remained intact.

Fig. 5 *a* shows a typical cell fixed after exposure to antigranule serum for only 30 seconds. Nucleus, cytoplasm, most of the granules, and cell membrane showed little or no evidence of injury. However some of the granules, indicated by arrows, appeared to be fixed in the act of disrupting. Their membranes showed wide openings and there was diminished density within these granules

and in the cytoplasm adjacent to the openings. Fig. 5 *b* shows one of these granules at higher magnification. The arrows point to infolded portions of the broken membrane, thus establishing that the discontinuity was real, and not due to technical artifacts such as grazing sectioning. It should be pointed out, however, that broken granules with similar appearance were also seen on occasions in sections of normal polymorphs.



TEXT-FIG. 1. Release of three lysosomal enzymes into the medium from rabbit polymorphs exposed to normal guinea pig serum, guinea pig antiserum to rabbit polymorph granules, and guinea pig antiserum to rabbit erythrocytes. The 100 per cent value is that amount of enzyme released in a similar cell suspension repeatedly frozen and thawed.

Release of Hydrolases from Intact Polymorphs and from Isolated Granules by Antigranule and by Antierythrocyte Sera.—It was desirable to confirm and extend the morphologic evidence for granule rupture by biochemical studies demonstrating release of the granule-associated hydrolytic enzymes. The results of such studies on intact rabbit polymorphs are presented in Table I and Text-fig 1. As is seen, aryl sulfatase, lysozyme, and cathepsin were released in soluble form after exposure of the cells to antigranule or to antierythrocyte serum, but not after exposure to normal guinea pig serum.

The granulolytic effect of the immune sera on intact leucocytes might, of course, have been an indirect action secondary to some other site of cell injury. Studies were therefore made on interactions between isolated granules and the

antisera. Direct microscopic observation demonstrated that isolated granules were, in fact, lysed in the presence of either antigranule or antierythrocyte serum.

Evidence in support of a direct granulolytic action of the antisera was obtained by measuring release into the medium from isolated granules of bound hydrolases, as shown in Table II and Text-fig. 2. Either type of antiserum brought about release in a soluble, active form of 40 to 70 per cent of granule-associated enzymes. These hydrolases were also liberated to a considerable but lesser degree on incubation with normal guinea pig serum or, not shown in the

TABLE I

Release of Granule-Bound Hydrolases from Intact Rabbit Polymorphs on Exposure to Various Guinea Pig Sera

Cells exposed to:	Enzyme activity released into medium		
	Lysozyme*	Aryl sulfatase‡	Cathepsin§
Normal guinea pig serum	<1.0	27	58
Guinea pig antiserum to rabbit polymorph granules	6.0	360	161
Guinea pig antiserum to rabbit erythrocytes	7.8	312	190
Repeated freezing and thawing (total enzyme available for release)	9.5	470	228

* Micrograms egg white lysozyme equivalents per 10^8 cells per hour.

‡ Micrograms 4-nitrocatechol per 10^8 cells per hour.

§ Micrograms trichloracetic acid–soluble protein per 10^8 cells per hour.

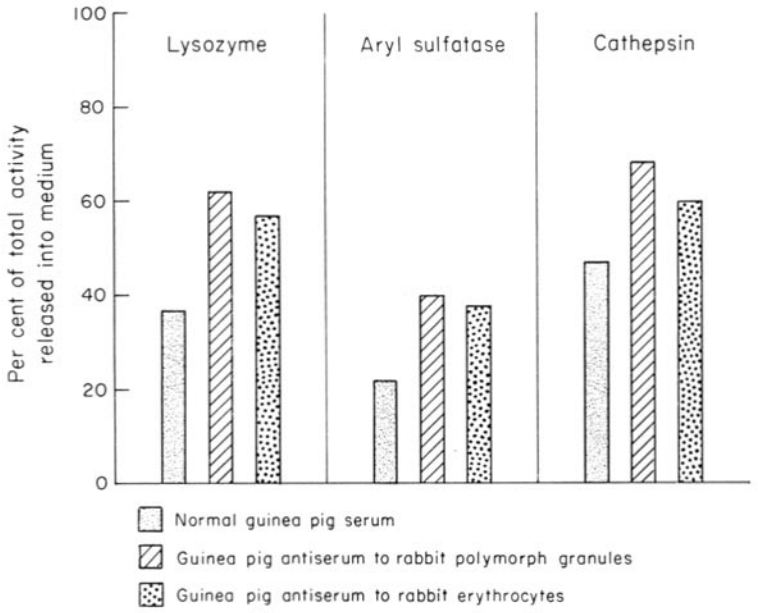
table, with saline or normal rabbit serum. In all of these situations severe clumping of the isolated granules occurred.

Many guinea pig sera inhibited nearly completely the activity of rabbit polymorph beta glucuronidase or acid phosphatase, and significantly depressed catheptic action. The inhibitory activity of immune sera was not appreciably greater than that of normal sera, indicating that the inactivation was not primarily due to antibodies to the individual enzymes.

Conditions Required for Granule Lysis by the Immune Sera.—Heating the guinea pig antisera at 56°C for 30 minutes destroyed completely their capacity to damage and degranulate rabbit polymorphs or to release hydrolases from these cells. Granulolytic activity of the heat-inactivated antisera could be restored by addition to the test system of normal guinea pig or of normal rabbit serum. Chelation of divalent cations in the test system by addition of versene led to loss of granulolytic action; this action could be restored by removal of the versene by dialysis and addition of calcium to the medium. Gamma globulin

fractions of the immune sera exhibited granulolytic activity, provided normal serum was added to the test system.

Studies on Quantitative Aspects and on Specificity of the Antigranule and the Antierythrocyte Sera.—Specificity of the antisera was studied from two points of view: (*a*) the apparent cross-reactivity of antigranule and antierythrocyte sera on both granules and red cells, suggesting the possibility of a common



TEXT-FIG. 2. Release of three lysosomal enzymes into the medium from isolated rabbit polymorph granules on exposure to normal guinea pig serum, guinea pig antiserum to rabbit polymorph granules, and guinea pig antiserum to rabbit erythrocytes. The 100 per cent value is that amount of enzyme released in a similar granule suspension repeatedly frozen and thawed.

antigen in granule and red cell membranes, and (*b*) the action of the antisera on rabbit cells other than polymorphs and erythrocytes, and the action on leucocytes of other mammalian species.

Normal guinea pig serum occasionally exhibited slight hemolytic action on rabbit red cells, but never in a dilution greater than $\frac{1}{2}$. The titers of granulolytic and of hemolytic activity in antisera prepared against granules or red cells are shown in Table III. As is seen, antigranule and antierythrocyte sera were approximately equally active in lysing granules. The antierythrocyte serum was, however, considerably more active than the antigranule serum in lysing red cells. It seemed unlikely that the cross-reactivity of the antisera was due to contamination of the immunizing materials; *i.e.*, contamination of the washed

red cells with polymorph granules or their membranes, or contamination of the washed granule suspension with erythrocyte membranes.

Absorption studies were done to obtain more definitive evidence on the suggested similarities of the antibodies in antigranule and antierythrocyte sera. As is seen from the results in Table IV, the granulolytic and hemolytic activities of an antigranule serum were removed equally effectively by absorption with either granules or red cells. The granulolytic activity of an antierythrocyte

TABLE II

Release of Granule-Bound Hydrolases from Rabbit Polymorph Granules on Exposure to Various Guinea Pig Sera

Granules exposed to:	Enzyme activity released into medium		
	Lysozyme*	Aryl sulfatase‡	Cathepsin§
Normal guinea pig serum	2.1	42.3	90
Guinea pig antiserum to rabbit polymorph granules	3.5	81	130
Guinea pig antiserum to rabbit erythrocytes	3.2	74	115
Repeated freezing and thawing (total enzyme available for release)	5.6	194	190

* Micrograms egg white lysozyme equivalents per mg granule protein per hour.

‡ Micrograms 4-nitrocatechol per mg granule protein per hour.

§ Micrograms trichloracetic acid soluble protein per mg granule protein per hour.

TABLE III

Hemolytic and Granulolytic Titers of Antisera to Red Cells and to Granules

Antiserum prepared against	Hemolytic titer*	Granulolytic titer*
Granules	64	16
Red cells	512	16

* Reciprocal of highest twofold dilution giving lysis.

serum could be also absorbed by either granules or red cells, whereas its hemolytic activity was removed by absorption with red cells but not by repeated absorption with granules. The granulolytic and hemolytic titers of these sera were not decreased by absorption with either human or horse erythrocytes.

Gel diffusion studies, shown in Text-fig. 3, revealed 4 precipitin bands between rabbit polymorph granules and the guinea pig antigranule serum. Antigranule serum absorbed with rabbit erythrocytes and thus rendered nongranulolytic give the same 4 precipitin bands. The strongly granulolytic antierythrocyte serum gave no precipitin bands with polymorph granules.

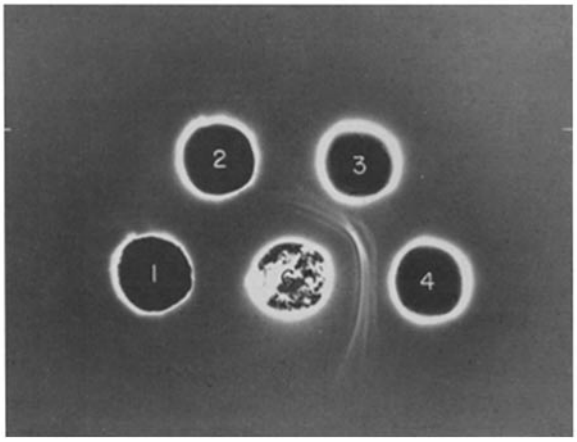
Although not yet studied thoroughly, rabbit macrophages, and perhaps other

types of rabbit cells, were apparently damaged by the antiserum against rabbit polymorph granules. The antigranule serum exerted cytotoxicity on rabbit alveolar macrophages in thin coverslip preparations and released hydrolytic enzymes from lysosomes isolated from these cells.

TABLE IV

Absorption of Guinea Pig Antisera with Rabbit Polymorph Granules or Erythrocytes

Antiserum	Absorption	Granulolytic titer	Hemolytic titer
Antigranule	Nil	16	32
	Granules	<2	<2
	Red cells	<2	<2
Antierythrocyte	Nil	64	512
	Granules	<2	256
	Red cells	<2	<2



TEXT-FIG. 3. Results of a gel diffusion study of the interaction between rabbit polymorph granules (center well) and various guinea pig sera (normal guinea pig serum in well *1*; antierythrocyte serum in well *2*; antigranule serum in well *3*; and antigranule serum absorbed with rabbit erythrocytes in well *4*).

The antigranule and antierythrocyte sera exhibited no toxicity on polymorphs of humans or horses, in contrast to their striking effect on rabbit cells.

A good antibody response was obtained in guinea pigs given the insoluble residue from granules disrupted by repeated freezing and thawing. For example one animal given a single injection of such a granule residue thoroughly washed by centrifugation (total of approximately 50 mg protein given) exhibited a

granulolytic and hemolytic titer of 1/16 10 days later, and serum from this animal remained cytotoxic for rabbit polymorphs for more than 6 weeks.

Attempts to induce homologous granule antibody were unsuccessful. Dutch-belted rabbits were injected up to 17 times with granules prepared from polymorphs of New Zealand rabbits without demonstrable adverse reaction in the recipient animals, and no demonstrable cytotoxic or granulolytic activity for the donor polymorphs appeared in their serum.

Guinea pigs immunized with rabbit polymorph granules or with rabbit erythrocytes showed no signs of illness. The total white cell count remained normal, and no morphologic or functional changes were evident in their polymorphs.

DISCUSSION

Antisera to rabbit polymorph granules or to rabbit erythrocytes both produce lysis of rabbit red cells, severe cytotoxicity on rabbit leucocytes apparently initiated by granule disruption, and liberation in soluble form of hydrolases normally bound to the granules. Release of lysosomal enzymes into the medium is nearly complete following exposure of intact cells to granule antiserum, whereas release from isolated granules is less impressive. Technical factors probably play a large role in rendering difficult the demonstration of release from granules. Granules in these preparations regularly clump severely; this clumping may well shield a significant portion of them from interacting with the antiserum. In addition, the clumping, along with the high centrifugal fields necessary for sedimentation of the granules or their residues, probably lead to non-specific damage which may have accounted for the release of enzymes observed in control preparations exposed to normal serum or saline.

Evidence available thus far indicates that the granulolytic antibody is directed against a constituent of the granule membrane. The residue from granules disrupted by freezing and thawing remains fully active as an antigen for induction of granulolytic antibody, even after repeated washing to remove soluble enzymes and other internal constituents. The precipitin studies indicate furthermore that diffusible granule constituents do not account for the granulolytic antibody. Absorption of granule antiserum with red cells results in complete removal of granulolytic activity, without detectably changing its content of antibody against diffusible granule components as indicated by the precipitin lines. Furthermore antierythrocyte serum is granulolytic, but has no precipitating antibodies directed against soluble granule components.

The cross-reactivity of the antisera on granules and red cells, and the results of absorption studies suggest that rabbit polymorph granules and rabbit red cells have in their membrane a common antigen or antigens, antibodies to which result, in the presence of complement, in membrane disruption. The failure of

polymorph granules to absorb all hemolytic activity from the antierythrocyte sera might be explained by the presence on the red cell membrane of other antigens, not present in granules, which also lead to hemolysis in the presence of antibody and complement.

Previous studies of others on the effects of immune serum on ascites tumor cells (8), led to the conclusion that the primary toxic action was on the cell membrane. Other observations (9) however, have suggested that antibody and complement reach and damage intracellular organelles, including lysosomes, in the ascites tumor cell system. Since in our studies an early sign of toxicity is cell swelling, perhaps the antisera also damage, but do not grossly disrupt, the cell membrane so as to permit passage of protein molecules. Cell swelling *per se* appears not to play a role in degranulation and nuclear changes, since in hyperosmolar media the swelling can be prevented but granule lysis and nuclear fusion still take place.

Very recently Weiss and Dingle (10) reported the cytotoxic action on rat liver cells and fibroblasts of rabbit antiserum to rat liver lysosomes. They demonstrated histochemically the disappearance of acid phosphatase granules on exposure of cells to the antiserum, but did not find hydrolases released into the medium from cells or from lysosome suspensions.

In earlier studies on the toxicity of streptolysins for leucocytes (3), the initial toxic event appeared to be granule rupture, soon followed by cytoplasmic liquifaction and signs of nuclear digestion. The present observations on cytotoxic action of antigranule antibodies and complement also show degranulation and ensuing cytoplasmic and nuclear degeneration. This similar course of events following granule disruption, whether the disruption is caused by streptolysin or by antibody and complement, suggests an autolytic effect of released granule enzymes, since it is unlikely that these two different agents would produce essentially identical changes in cytoplasmic and nuclear structures by a series of independent direct toxic events. The electron microscopic studies also point to granule disruption early in the course of the toxic action.

Polymorphonuclear leucocytes play a role in the inflammation accompanying Shwartzman (11) or Arthus (12) reactions. In the neutropenic animal these reactions are blocked or markedly reduced in severity. Speculation has grown that neutrophils accumulating at these and other allergic or infectious sites may release their granule enzymes, and that irritating or autolytic effects of these enzymes may lead to severe inflammation or necrosis. Electron microscopic studies of Arthus reactions have, in fact, shown degranulation of neutrophils in the lesion (13). However only limited direct evidence is now available to incriminate lysosomal hydrolases from polymorphs as instigating agents of inflammation and necrosis. Polymorph granules injected directly into skin do apparently cause some vascular changes and swelling (14), and such an injection followed in 4 to 12 hours by systemic endotoxin leads to extensive hemorrhagic necrosis at the injection site (15).

The present studies indicate that antigen-antibody reactions, in the presence of complement, cause polymorph degranulation and release of autolytic enzymes. Antigranule antibodies of the type used by us would not likely be present in allergic reactions, but it is reasonable to speculate that antigens (or antibodies) might be taken up by the cells and become associated with membranes of intact granules or of phagocytic vacuoles, perhaps rendering these structures susceptible in some situations to lysis on exposure to antibody (or antigen) and complement.

SUMMARY

Antisera to rabbit polymorph granules and to rabbit erythrocytes have been prepared in guinea pigs.

Both antigranule and antierythrocyte sera are hemolytic and both exhibit striking cytotoxicity on leucocytes. The sequence of toxic events, as observed by phase contrast cinemicrophotography and electron microscopy, consists of explosive granule lysis, cell swelling, cytoplasmic liquifaction, and nuclear fusion. Other rabbit cells are also susceptible to these cytotoxic effects, but cells, including polymorphs, of other mammals are not.

Cytotoxic action of the antisera requires, in addition to the antibody, heat-labile serum factors and divalent cations, suggesting that the action is a combined one of antibody and complement.

The morphologic observations have been supported by biochemical studies demonstrating release into the medium of granule-bound hydrolases following exposure of polymorphs or of isolated granules to the antigranule or antierythrocyte sera.

Granulolytic activity of the antisera can be reduced or removed by absorption with either rabbit leucocyte granules or with erythrocytes, indicating that leucocyte granules and erythrocytes have an identical or similar membrane constituent.

The observations lend support to the notion that lysosomal hydrolases may exert autolytic effects in some situations.

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EXPLANATION OF PLATES

PLATE 14

FIG. 1. Lysis of individual cytoplasmic granules in rabbit polymorphonuclear leucocytes. The upper row shows a cell soon after exposure to antigranule serum. On the left a dense granule is seen in the circled area; 0.1 second later, at right, this granule has disappeared from view.

The cell below is in a late stage of degranulation and cytotoxicity following exposure to antierythrocyte serum. The rapidity of granule lysis is illustrated in the circled area. Approximately × 2000, phase contrast.

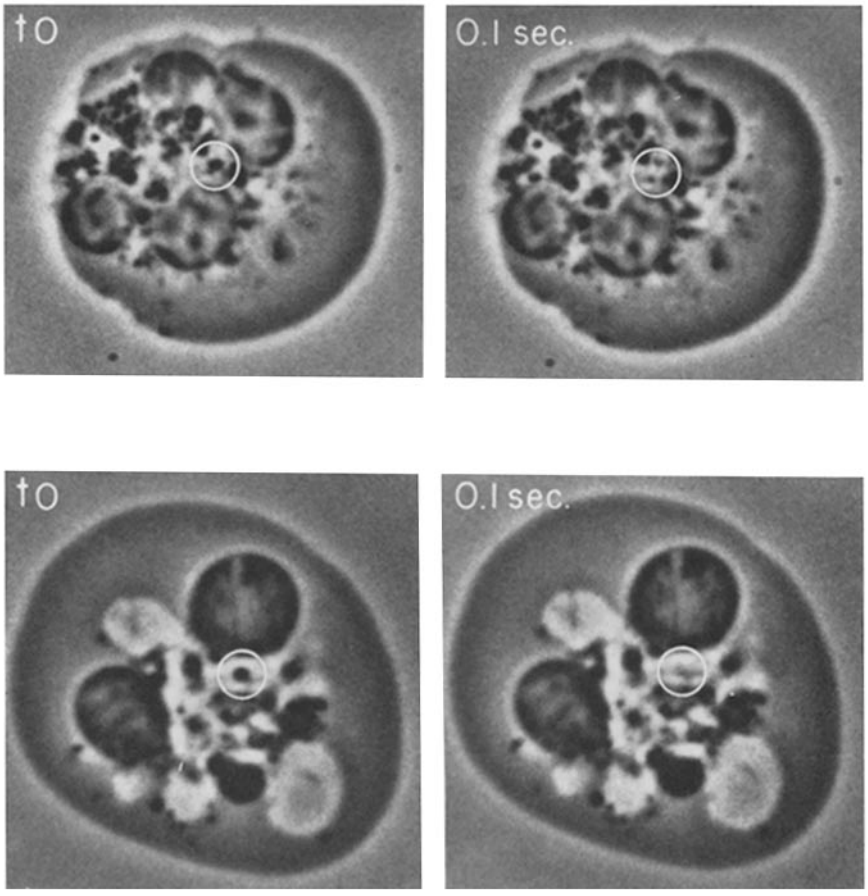


FIG. 1

(Quie and Hirsch: Antiserum to leucocyte lysosomes)

PLATE 15

FIG. 2. A rabbit polymorph at intervals after addition of antigranule serum. At 15 seconds the cell is swollen, most of the granules have lysed, and nuclear lobes are rounding up. The 30 and 45 second prints illustrate progressive degranulation and fusion of the two nuclear lobes on the right. The cell membrane appears to be intact. Approximately × 2000, phase contrast.

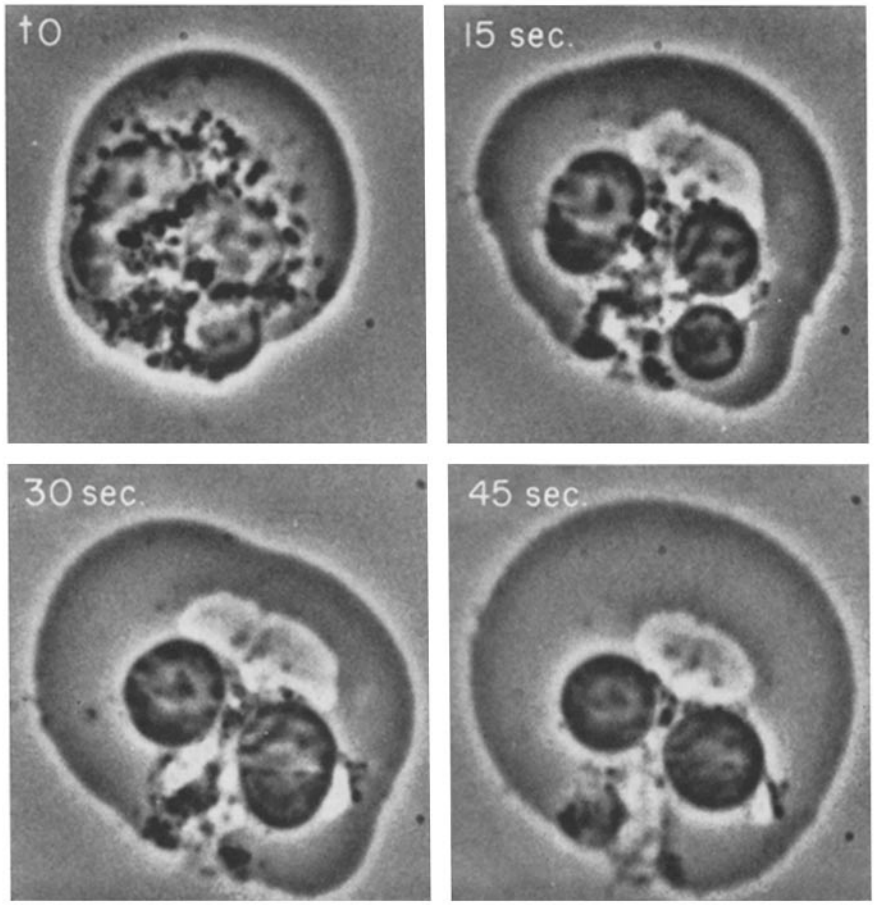


FIG. 2

(Quie and Hirsch: Antiserum to leucocyte lysosomes)

PLATE 16

FIG. 3. The cytotoxic action of antierythrocyte serum on two rabbit polymorphonuclear leucocytes. Nuclear lobes and cytoplasmic granules are well defined in the initial print. Photographs made from the motion picture film at 15 second intervals show extensive degranulation, swelling of the cells, and fusion of the nuclear lobes. Approximately × 2000, phase contrast.

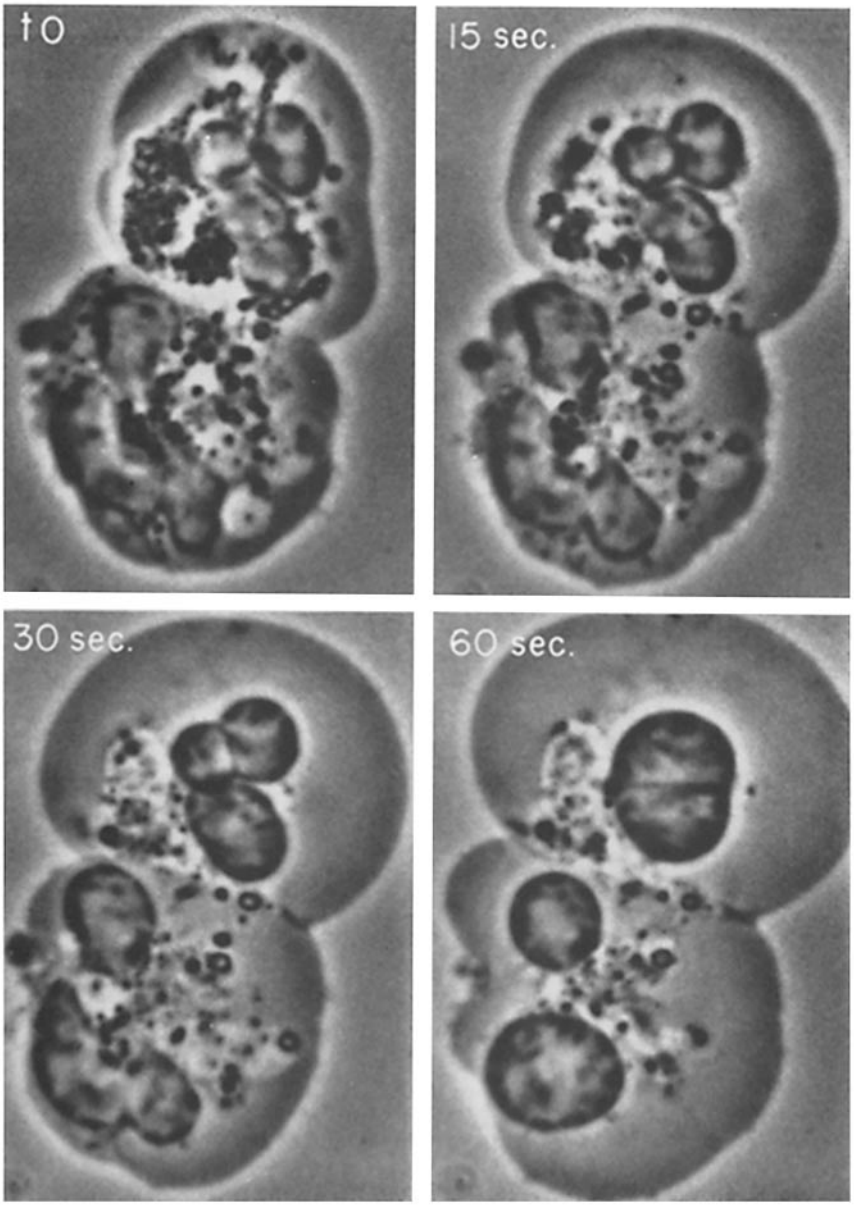


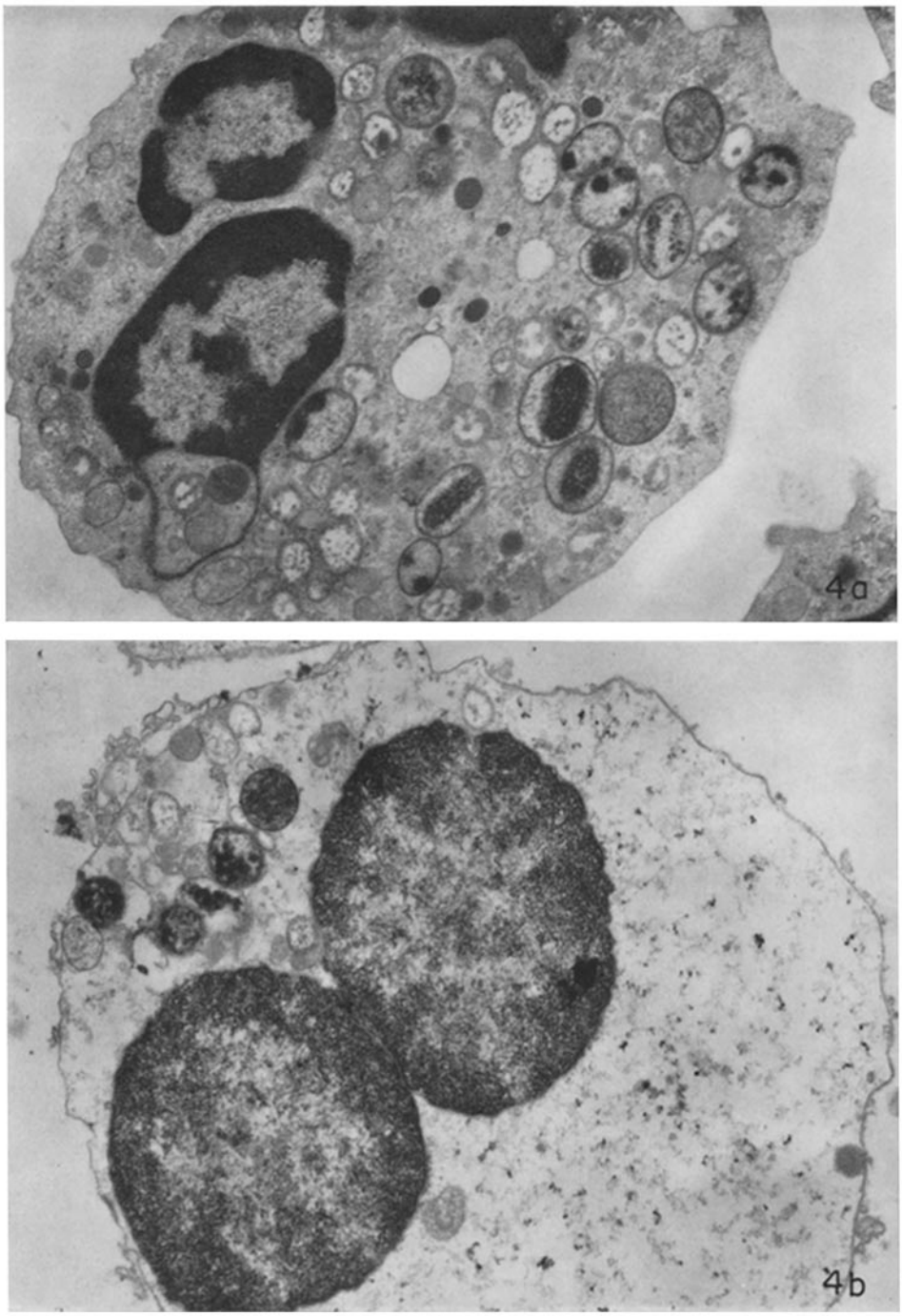
FIG. 3

(Quie and Hirsch: Antiserum to leucocyte lysosomes)

PLATE 17

FIG. 4 *a*. An electron micrograph of a rabbit polymorphonuclear leucocyte after incubation with 30 per cent normal guinea pig serum for 2 minutes. The appearance is identical with that of control cells fixed as they are collected from the peritoneal exudate. Nuclear lobes show condensation of densely staining material at the periphery. Cytoplasmic granules vary in both size and density, and many appear to be partially extracted under the conditions of fixation and processing used. Most of the granules have a well defined limiting membrane. × 17,500.

FIG. 4 *b*. A rabbit polymorph which was fixed 2 minutes after exposure to 30 per cent guinea pig antiserum to polymorph granules. Nuclear lobes are swollen, rounded, and appear to be fusing. The cytoplasm shows disappearance of most of the organized structures; a few intact granules and residual membranous forms are seen at the upper left. Despite the evidence of severe autolytic changes, the cell membrane remains intact. × 17,500.

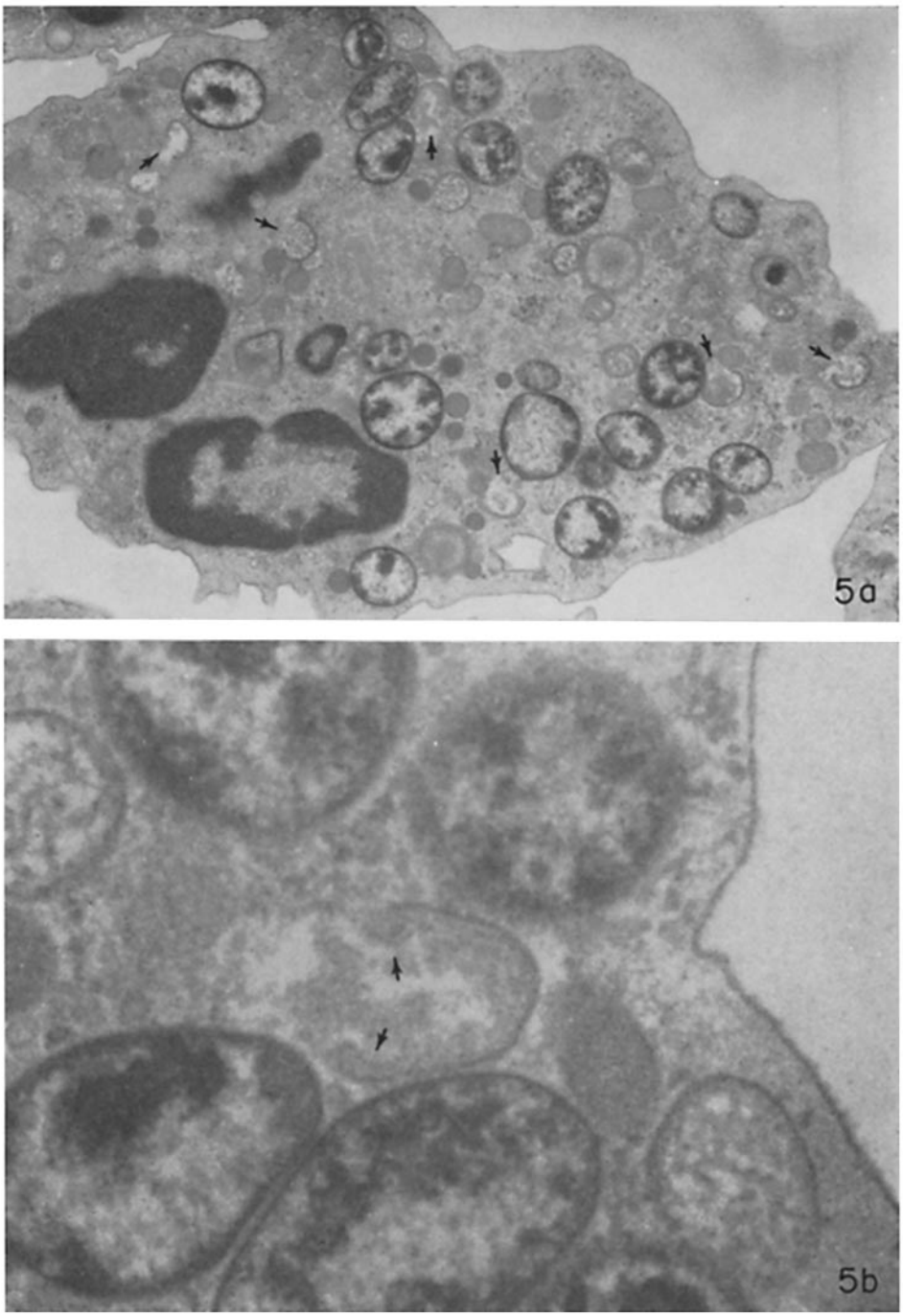


(Quie and Hirsch: Antiserum to leucocyte lysosomes)

PLATE 18

FIG. 5 *a*. A rabbit neutrophil fixed after exposure to antigranule serum for 30 seconds. From the overall aspect the cell appears undamaged (compare with Fig. 4 *a*). No nuclear or cell swelling is evident and the cell membrane appears intact. However, as indicated by arrows on the print, several of the cytoplasmic granules show disruptions in their membranes. The appearance of granule rupture is heightened by the empty or partially empty nature of these granules and by changes in the cytoplasm adjacent to their membrane discontinuity. × 17,500.

FIG. 5 *b*. One of these disrupting granules at higher power. Infoldings of the membranes (arrows) suggest that the appearance of membrane interruption is not simply a reflection of tangential sectioning. Similar ruptured granules are occasionally seen in sections of normal cells; it is thus possible that granule membrane damage arises, at least in part, during fixation and processing. × 100,000.



(Quie and Hirsch: Antiserum to leucocyte lysosomes)