STUDIES ON LYSOSOMES

V. The Effects of Streptolysins and Other Hemolytic Agents on Isolated Leucocyte Granules

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ABSTRACT

Granules from rabbit peritoneal leucocytes were prepared in 0.3 M sucrose as an optically homogeneous suspension with the aid of heparin. Lysis of the granules *in vitro* was followed by measurement of decreases in the apparent absorbance of the suspensions at 520 mμ and was accompanied by solubilization of beta-glucuronidase from the particles. Streptolysins O and S from hemolytic streptococci lysed the granules at 20°C; the initial rate of lysis by streptolysin O was greater than that by streptolysin S. Cysteine activated, and specific antibody inhibited, streptolysin O; antimycin and bovine serum albumin inhibited streptolysin S. The granules were not lysed by any other streptococcal exotoxins. Lysis was irreversible and depended neither upon oxidative phosphorylation, nor upon intact respiration. The granules were also lysed by lysolecithin, at concentrations from 2×10^{-6} M to 1×10^{-4} M; bovine serum albumin and antimycin also inhibited this lytic agent. Such other hemolytic agents and procedures as vitamin A, non-ionic detergents, and ultraviolet irradiation also disrupted leucocyte granules. In susceptibility to lysis and other properties, the granules of white cells resembled erythrocytes. Leucocyte granules differed from mitochondria in that they did not appear to take up or extrude water reversibly; they were unaffected by thyroxine, phosphate, or metabolic substrate. The studies are compatible with the hypotheses that white cell granules are similar to lysosomes isolated from other tissues, and that they share common surface properties with erythrocytes.

Streptolysins O and S from hemolytic streptococci injure not only the membranes of red cells, but disrupt hepatic lysosomes (1) and cause the swelling of mitochondria with solubilization of mitochondrial enzymes (2). They are also lethal for intact leucocytes, the death of which has been attributed to an action of the lysins on the specific granules of white cells (3). Leucocyte granules, when isolated, have been shown to possess many of the properties of lysosomes from other tissues: a number of hydrolases with an optimal pH in the acid range such as cathepsins, DNase, RNase, acid phosphatase, and beta-glucuronidase, etc. can be released from the granules by such methods as freezing and thawing, incubation at acid pH, or exposure to detergents (4).

Studies of the actions of streptolysins on lysosomes have left several unresolved problems (1). Whereas streptolysin S was at least ten times more active than streptolysin O in causing the release of enzymes from mitochondria and lysosomes (1, 2), it was *less* active than streptolysin O in causing damage to the specific granules of intact leucocytes and acted on these only after a considerable lag period (3). Lysis of leucocyte granules followed shortly after the addition of strepto-

lysins to white cells; however, it was impossible to determine whether the lysins acted directly on the membranes of the granules or exerted an indirect effect through mechanisms operative only in the living cell (3). Earlier studies on lysosome-rich fractions of rabbit liver were also complicated by the presence of numerous mitochondria (1, 2), which constituted the bulk of particles in such suspensions; it was not clear to what extent mitochondria influenced the uptake and distribution of streptolysins or other agents active on lysosomes *in vitro*. By means of electron microscopy, over 85 per cent of the particles in such suspensions could be identified as mitochondria.

To resolve these difficulties it became necessary to study the effects of such agents directly on isolated leucocyte granules. By an extension of methods described by Cohn and Hirsch (4), it has been possible to follow lysis of leucocyte granules in an optically homogeneous system, and to relate reductions in the apparent absorbance of a dilute granule suspension to release of enzyme from the particles. Since less than 10 per cent of the granules in these suspensions could be identified as mitochondria by electron microscopy (4), the results obtained should not reflect actions modified by the presence of mitochondria. The experiments to be described below have shown that not only streptolysins, but such other hemolytic agents as vitamin A, lysolecithin, non-ionic detergents, and ultraviolet radiation, cause lysis of the granules with release of beta-glucuronidase into the suspending medium. In these and in other respects, the isolated granules behaved somewhat like erythrocytes; the membranes bounding both leucocyte granules and red cells were susceptible to lysis by similar means and may indeed share other surface properties.

MATERIALS AND METHODS

Preparation of Leucocyte Granule Suspensions

The specific granules of leucocytes were prepared as described by Cohn and Hirsch (4). Briefly, leucocytes were harvested from the exudate formed in the peritoneal cavities of New Zealand or albino male rabbits 4 hours after 200 ml of 0.1 per cent (w/v) glycogen in saline had been introduced. Heparinized saline (100 ml) was used to wash the peritoneal cavity, and the leucocytes which were obtained in turbid suspension were separated by centrifugation for 5 minutes at 250 *g*. The cells were resuspended in 0.34 M ice-cold, ion-free sucrose, centrifuged again at 250 *g* for 5 minutes, and finally resuspended in 12 to 15 ml of 0.34 M sucrose. They were disrupted by repeated, vigorous pipetting in a Pasteur pipette and the specific granules obtained by differential centrifugation. The granules, which sedmented between 400 *g* (10 minutes) and 8,200 *g* (15 minutes), were resuspended in sucrose. At this point, a difficulty, already described by Cohn and Hirsch (4), arose: if the granules were examined before the 8,200 *g* centrifugation (either macroscopically or by phase microscopy), they appeared to be well dispersed and were optically uniform. However, after the 8,200 *g* centrifugation, the granules clumped quite markedly upon resuspension: aggregates were observed grossly and microscopically. Since it was intended to do optical studies, and since suspensions as homogeneous as those used in mitochondrial swelling experiments were sought (2, 5), a variety of agents were tested for their ability to prevent the aggregation or clumping of the resuspended granules.

It was soon found that cationic agents such as lysozyme, cetylpyridinium chloride, or hexammine-cobaltic chloride (at concentrations from 10^{-3} M to 10^{-4} M) accentuated aggregation. It therefore appeared likely that the net surface charge of the granules was anionic. Consequently, a number of anionic polymers such as chondroitin sulfate, hyaluronate, and heparin were tested. It became possible to obtain optically uniform suspensions (Fig. 1) of granules of 0.34 M sucrose containing 30 to 40 units (U.S.P.) of heparin/ ml, provided final resuspension was done with vigorous pipetting and with dense suspensions. In the presence of polyanion, granule suspensions remained relatively uniform for periods up to 6 days at 4°C; only gentle pipetting was needed to achieve the same degree of suspension as upon first preparation. The exudate from the peritoneum of a well primed rabbit yielded a final volume of 3 to 4 ml of final granule suspension.

Measurement of Granule Lysis and Enzyme Release

Aliquots of granule suspension (0.1 ml) and test reagents (0.1 ml) were added to 2.8 ml of 0.3 M sucrose buffered at pH 7.4 with 0.02 M tris,[1] the suspensions having been previously adjusted to yield an apparent absorbance of 0.5 at 520 mμ. This system was chosen since it duplicated one employed in studies of mitochondrial swelling induced by the identical agents (2). The resultant change in absorbance at 520 mμ (A_{520}) was recorded at 5-minute intervals in a Beckman DB spectrophotometer. After

[1] The following abbreviations will be used: BSA, bovine serum albumin; DNP, 2,4, dinitrophenol; SLO, streptolysin O; SLS, streptolysin S; tris, tris (hydroxymethyl) aminomethane.

30 minutes, the 3.0-ml samples were centrifuged for 20 minutes at 20,000 *g* and the clear supernatant assayed for beta-glucuronidase activity by the method of Fishman *et al.* (6); activity is expressed as the absorbance at 550 mμ following 18 hours' incubation of tubes containing 0.5 ml of granule supernatant, 0.1 ml of phenolphthalein glucuronidate, and 1.5 ml of 0.1 M acetate buffer (pH 4.5); thereafter, 5 ml of glycine buffer, 0.02 M (pH 10.5), were added to stop the reaction and develop the chromogen. The granules proved to have a relatively uniform content of enzyme from day to day: solubilization of total enzyme activity was achieved by incubation of intact granules with Triton X-100 0.1 per cent v/v.

Optical lysis was followed at temperatures indicated below; these were kept constant by means of a water bath in which the cuvettes were maintained, between readings, and by means of a water-jacket of constant temperature housing the cuvettes while in the spectrophotometer. *Ultraviolet irradiation* was by means of a Hanovia 100-watt high-pressure mercury arc lamp under conditions previously described (7, 8). Samples were prepared for radiation on a tenfold larger scale (30 ml) than for other studies. Since exposure of the granules to radiation in dilute suspension for periods beyond 10 minutes resulted in the inactivation of beta-glucuronidase, aliquots removed at 10 minutes were used to measure beta-glucuronidase activity; optical measurements were carried out on 3-ml samples throughout the 30-minute experimental period.

Streptolysins and other Streptococcal Products

These were obtained through the generosity of Dr. Alan W. Bernheimer and have been described in previous reports from this laboratory (1–3). Antistreptolysin globulins, also previously described, were obtained from the Serum Institute, Carshalton, England.

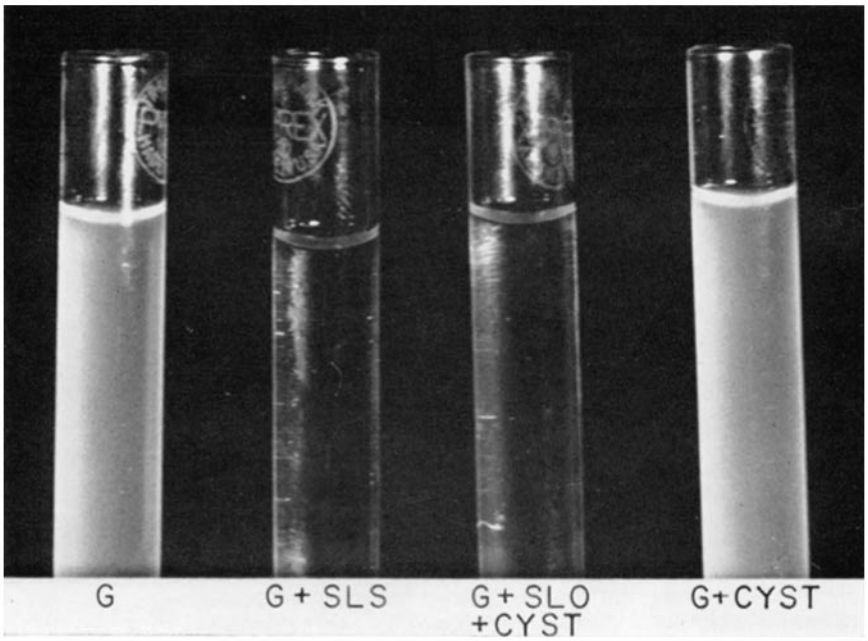

FIGURE 1 Suspensions of isolated leucocyte granules in 0.3 M sucrose, buffered with 0.02 M tris at pH 7.4 and dispersed with the aid of heparin. All tubes incubated for 30 minutes at 20°C. *G*, granules alone. *G + SLS*, granules after incubation with 333 hemolytic units of streptolysin *S*/ml. The tube has become clear after 30 minutes at 20°C. *G + SLO + cysteine*, granules incubated 30 minutes at 20°C with 333 hemolytic units of streptolysin O/ml activated with 0.1 mM cysteine. *G + cysteine*, granules with cysteine alone.

Reagents and Chemicals

Vitamin A alcohol (Nutritional Biochemicals, Inc., Cleveland) was dissolved in anhydrous ethanol before use. Lysolecithin was a gift from Dr. Peter Elsbach and was dissolved in 50 per cent ethanol. Thyroxine was dissolved in a minimal volume of 0.1 N NaOH and further diluted in aqueous sucrose before use; this and other reagents were obtained from Cali-

fornia Biochemicals, Los Angeles. BSA was obtained from Armour & Co., Chicago. Endotoxin, (preparation LPS, Pfizer, Brooklyn, New York) was prepared from *A. aerogenes*, or from *E. coli* (Difco Labs., Detroit).

RESULTS

Lysis of Leucocyte Granules by Streptolysin S

Upon the addition of streptolysin S to isolated granules, the A_{520} of the suspensions decreased rapidly; the previously opaque tubes became clear (Fig. 1). Decreases in A_{520} were directly related to the amount of streptolysin added; simultaneously beta-glucuronidase was released from the granules (Fig. 2, Table I). There also appeared to be a direct relationship between the activity of beta-glucuronidase rendered unsedimentable at 20,000 *g* and decreases in turbidity which resulted from streptolysin. These effects were specific for streptolysin S in the preparations studied: neither a product prepared in the same manner as SLS from a mutant strain which lacked the ability to produce SLS, nor the RNA core used in the production of SLS (9), was active on leucocyte granules (Table I).

After exposure to SLS, the granules appeared fragmented: only debris admixed with a few intact granules was seen by phase or darkfield microscopy; Wright's stain showed few granules left intact.

Solubilization of enzymes and lysis of the granules were temperature dependent; although some activity was noted at 4°C, lysis was as rapid at 20°C as at 37°C (Fig. 3).

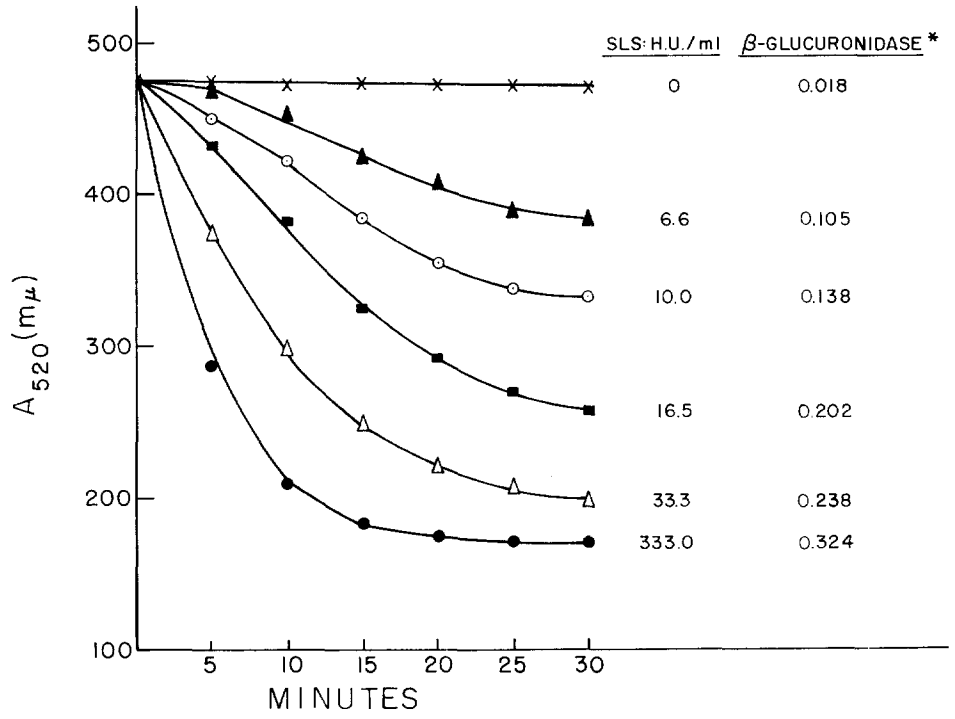

FIGURE 2 Lysis of leucocyte granules by streptolysin S. Reductions in turbidity (A_{520}) induced by increasing concentrations of SLS (expressed as hemolytic units/ml) Beta-glucuronidase activity rendered unsedimentable is listed to the right of each curve; units as in text.

DNP and cyanide did not inhibit SLS-induced lysis of leucocyte granules, suggesting that neither tightly coupled oxidative phosphorylation nor intact respiration (carried out by the few contaminating mitochondria) was necessary for the lysis of granules by SLS. It had been found (2) that antimycin and BSA, as well as Mg^{++}, could prevent mitochondrial swelling and the solubilization of enzymes by SLS. These agents were added to leucocyte granules; data in Fig. 3 indicate that

TABLE I

Lysis of Leucocyte Granules by Streptolysin S (SLS)

Agent	Concentration	ΔA_{620}*	Beta-glucuron-idase‡
Control	—	0.008	0.072
SLS	333 h.u./ml§	0.352	0.194
SLS-less mutant	50 μg/ml	0.010	0.074
RNA core	"	0.012	0.070
SLS and Mg^{++}	333 h.u./ml, 1×10^{-3} M	0.350	0.186
SLS and Mg^{++} after 10″	333 h.u./ml, 1×10^{-3} M	0.346	0.179
SLS and BSA	333 h.u./ml, 8×10^{-5} M	0.020	0.102
SLS and cortisol	333 h.u./ml, 1.5×10^{-4} M	0.316	0.180

* Reduction in apparent absorbance of a dilute granule suspension after 30 minutes' incubation in 0.3 M sucrose. Results are means of three experiments. Temperature: 20°C.

‡ Beta-glucuronidase activity rendered unsedimentable after 30 minutes' incubation; units as in text.

§ Hemolytic units/ml = 43 μg of SLS.

TABLE II

Lysis of Leucocyte Granules by Streptolysin O (SLO) and other Streptococcal Exotoxins

Agent	Concentration	ΔA_{620}*	Beta-glucuronidase§
Control	—	0.004	0.062
SLO	333 h.u./ml‡	0.068	0.098
SLO, cysteine‖	333 h.u./ml	0.364	0.305
Cysteine	0.1 mM	0.010	0.049
DPNase	50 μg/ml	0.002	0.058
DNase A	50 μg/ml	0.060	0.063
Streptokinase	50 μg/ml	0.010	0.059
Erythrogenic toxin	50 μg/ml	0.008	0.067
Proteinase	50 μg/ml	0.042	0.078

* Reduction in apparent absorbance of a dilute granule suspension after 30 minutes' incubation in 0.3 M sucrose. Results are means of three experiments. Temperature: 20°C.

‡ Hemolytic units per ml = 21 μg of SLO.

§ beta-glucuronidase activity rendered unsedimentable after 30 minutes' incubation; units as in text.

‖ Cysteine: 0.1 mM.

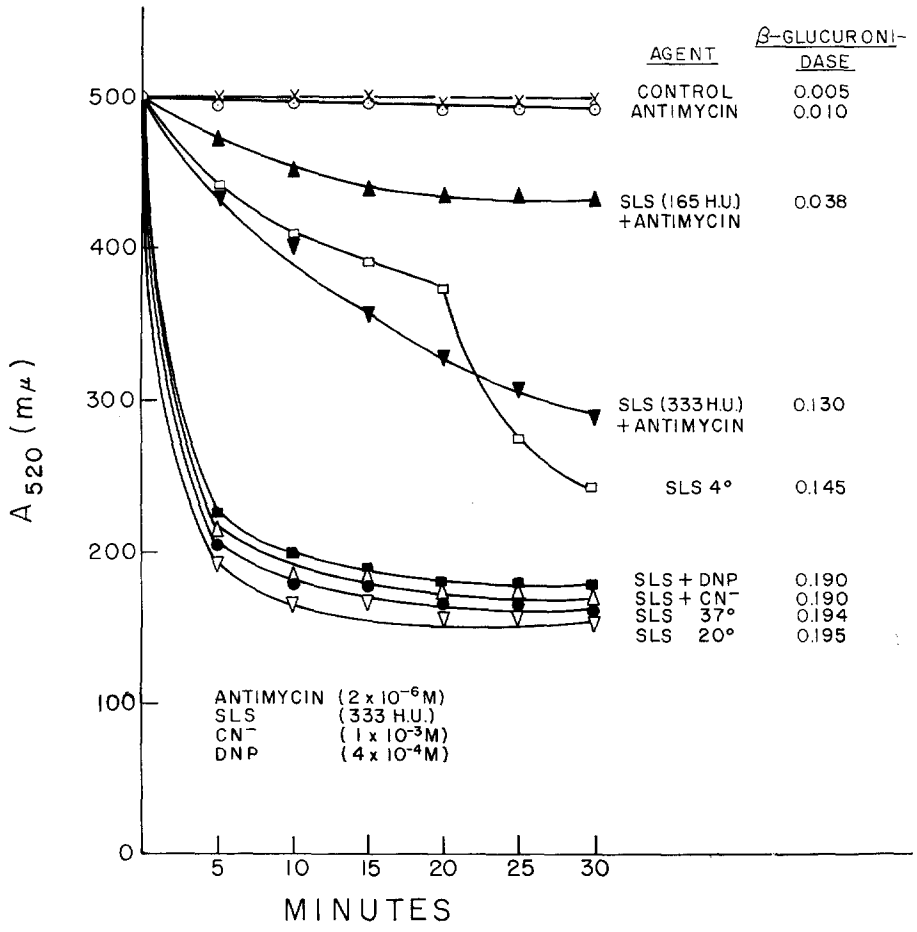

FIGURE 3 Lysis of leucocyte granules by streptolysin S and inhibition by antimycin. Reductions in turbidity (A_{520}) induced by the lysin are plotted against time. Beta-glucuronidase activity rendered unsedimentable is listed to the right of each curve; units as in text.

antimycin significantly inhibited granule lysis by SLS. BSA also retarded lysis and release of enzymes, an effect similar to this agent's protection of mitochondria. In contrast, Mg^{++} did not inhibit lysis of granules by SLS, either when present at the outset or when added 10 minutes after lysis was initiated. At this concentration (10^{-3} M) Mg^{++} inhibited or reversed mitochondrial swelling induced by SLS (2). Cortisol as the hemisuccinate did not protect the granules (Table I).

Lysis of Leucocyte Granules by Streptolysin O and Other Streptococcal Exotoxins

Lysis of leucocyte granules occurred promptly after the addition of streptolysin O, provided the lysin was first activated with cysteine. At the concentrations present, cysteine had no effect on the granules (Figs. 1 and 4, Table II). As with lysis by SLS, lysis by SLO could be related to the concentration of the lysin; solubilization of enzyme correlated well with decreases in A_{520}. In contrast to SLS, however, SLO appeared to have a greater initial rate of action: compare the lytic curve for 16.5 hemolytic units/ml of SLO (Fig. 4) with the curve for the equivalent concentration of SLS (Fig. 2). The slower initial rate of action seen with lower concentrations of SLS, but not with SLO, is characteristic of the action of each lysin on red cells (10). The presence of antibody sufficient to neutralize the hemolytic activity of SLO inhibited completely any effect on granules (Fig. 4); antibody preparations did not them-

TABLE III

Lysis of Leucocyte Granules by Several Hemolytic Agents

Agent	Temperature	Concentration	ΔA_{520}*	Beta-glucuronidase‡
Control	20°	—	0.020	0.068
Control	37°	—	0.048	0.076
Lysolecithin	20°	3×10^{-5} M	0.296	0.235
Vitamin A	20°	1×10^{-5} M	turbid	0.130
	20°	1×10^{-4} M	turbid	0.140
	37°	1×10^{-4} M	turbid	0.206
Ultraviolet radiation (UV)	20°	—	0.164	0.168
UV + BSA	20°	8×10^{-5} M	0.160	0.165
UV + antimycin	20°	2×10^{-6} M	0.130	0.154
UV + cortisol	20°	1.5×10^{-4} M	0.142	0.164
Triton X-100	4°	0.1% v/v	0.402	0.392
	4°	0.01% v/v	0.338	0.354
	20°	0.1% v/v	0.410	0.398
	20°	0.01% v/v	0.357	0.384
Thyroxine	20°	3×10^{-5} M	0.032	0.064
Phosphate	20°	3×10^{-4} M	0.028	0.058
Succinate	20°	3×10^{-3} M	0.030	0.070
Endotoxin	20°	1, 10, 100 μg/ml	0.048	0.080
	37°	1, 10, 100 μg/ml	0.060	0.089

* Reduction in apparent absorbance of a dilute granule suspension after 30 minutes' incubation in 0.3 M sucrose. Results are means of three experiments.

‡ Beta-glucuronidase activity rendered unsedimentable after 30 minutes' incubation; units as in text.

selves affect granules. Cortisol as the hemisuccinate did not protect leucocyte granules from lysis by SLO; this is in contrast to the protection by cortisol of lysosomes in liver homogenates.

Streptolysin O was far less active in the absence of cysteine (Table II); no other streptococcal exotoxins affected leucocyte granules. These findings agree with earlier observations on liver granules (1).

Lysis of Leucocyte Granules by Lysolecithin

Since streptolysins appeared to attack leucocyte granules in the same manner that they affected red cells, other hemolytic agents were investigated. An acid phospholipase has been identified as one of the enzymes contained within leucocyte granules (11). Were a phospholipase to be released from the granules, *e.g.* during phagocytosis, the formation of lysolecithin, a potent hemolytic agent (12), might be expected to facilitate membrane rupture. Therefore, lysolecithin was added to the granules: prompt falls in A_{520} were observed; these were related to the concentrations of lysolecithin present and were seen with levels as low as 2×10^{-6} M. Concurrently, there was release of beta-glucuronidase from the disrupted granules (Fig. 5). Microscopic changes were indistinguishable from those described for SLS.

Lysis of granules by lysolecithin was dependent upon temperature. Considerable lysis was noted at 4°C, but at 20°C, and especially at 37°C, more complete lysis and release of beta-glucuronidase was achieved. Both BSA and antimycin inhibited the granule lysis induced by lysolecithin (Fig. 6).

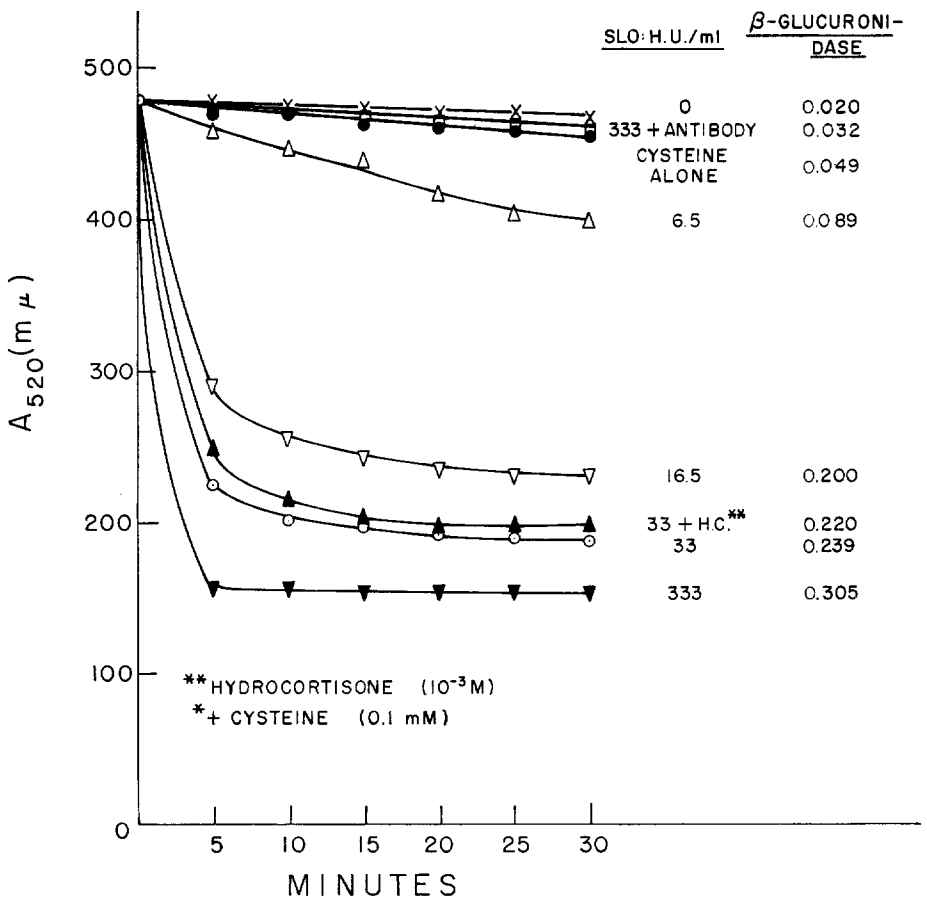

FIGURE 4 Lysis of leucocyte granules by streptolysin O. Reductions in turbidity (A_{520}) induced by increasing concentrations of the lysin are plotted against time. Antibody sufficient to neutralize hemolytic activity was present in one sample. Beta-glucuronidase activity rendered unsedimentable is listed to the right of each curve; units as in text. Hydrocortisone present as the hemisuccinate.

Indeed, the inhibition of lysolecithin lysis by BSA was more complete than the inhibition by BSA of SLS-induced lysis. Antimycin at 2×10^{-6} M was incapable of preventing lysis by 3×10^{-5} M lysolecithin, although the same concentration of antimycin sufficed to inhibit substantially the effects of 1.5×10^{-5} M lysolecithin.

Lysis of Leucocyte Granules by Vitamin A and Ultraviolet Irradiation

Vitamin A has been shown to damage lysosomes of rat and rabbit liver (8, 13), and to release lysosomal enzymes from chick and rabbit cartilage (2, 14, 15); the vitamin is also hemolytic (12). The sparing solubility of vitamin A made optical measurements in the usual system impossible; when, however, vitamin A alcohol was incubated with leucocyte granules, a temperature-dependent release of enzyme was observed. This effect was similar to the action of vitamin A on suspensions of lysosomes from other tissues (8, 13, 14).

Ultraviolet irradiation also causes injury to the membranes of red cells, lysosomes, and mitochondria (16–18). The deleterious effects of radiation are believed to result, at least in part, from the formation of lipid peroxides during the process of irradiation (19). Formation of lipid peroxide in dilute suspensions of mitochondria and lysosomes from rabbit liver has recently been described (20). When leucocyte granules were exposed to radiation from a mercury arc, substantial reductions in A_{520} were observed; these never approached the

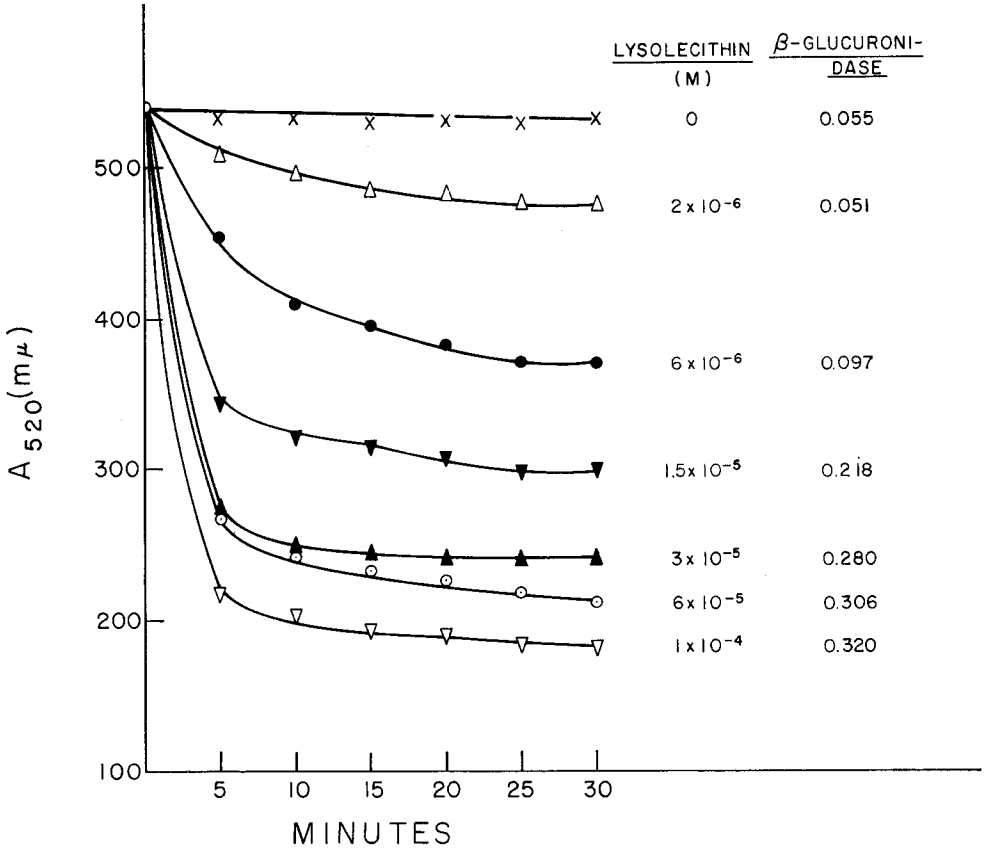

FIGURE 5 Lysis of leucocyte granules by lysolecithin. Reductions in turbidity (A_{520}) induced by increasing concentrations of the lysin are plotted against time. Beta-glucuronidase activity rendered unsedimentable is listed to the right of each curve; units as in text.

drops seen with other hemolytic agents. Neither antimycin nor BSA inhibited lysis of granules by ultraviolet irradiation.

The Effects of Other Agents upon Leucocyte Granules

Triton X-100 at concentration of 0.1 per cent v/v to 0.01 per cent caused rapid and complete lysis of the granules, independent of temperature. At lower concentrations, temperature-dependence was, however, observed. This finding is in contrast to those of Cohn and Hirsch (4), who found Triton relatively ineffective in lysing less well dispersed granules. Conceivably, this difference may be due to the presence in our suspensions of heparin, or due to the removal, by washing, of inhibitors of Triton.

Thyroxine, phosphate, or succinate, induce falls in the A_{520} of mitochondrial suspensions from rabbit liver prepared in 0.3 M sucrose (2). In the case of mitochondria, such reductions represent mitochondrial swelling dependent upon electron transport and are not accompanied by solubilization of mitochondrial or lysosomal enzymes (2, 5). These agents did not decrease the A_{520} of leucocyte granule suspensions (Table III).

Endotoxin, injected during life, causes the membranes of liver lysosomes to become more fragile to *in vitro* procedures; lysozyme is released into the circulation following the injection of lipopolysaccharide (21). However, as shown in Table III, concentrations of *A. aerogenes* endotoxin up to 100 μg/ml were without effect upon isolated granules. Nor was an endotoxin from *E. coli* (Difco) active at these concentrations. This finding would support those of Hirsch and Cohn (22) who noted that exposure of intact leucocytes to endotoxin failed to induce degranulation.

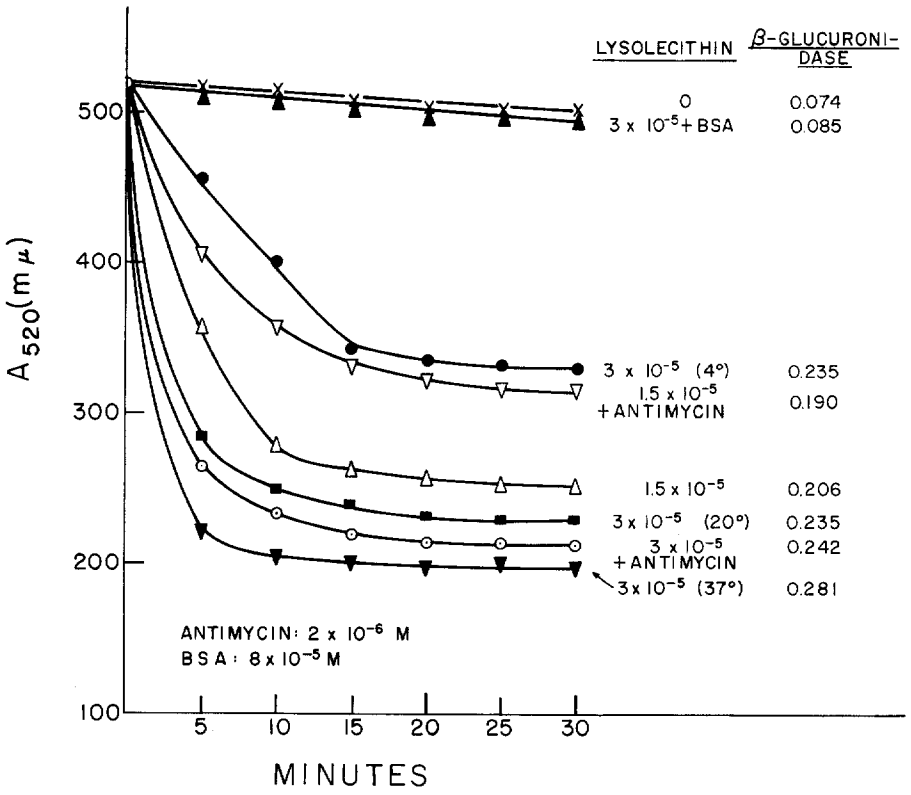

FIGURE 6 Lysis of leucocyte granules by lysolecithin and inhibition by antimycin. Reductions in turbidity (A_{520}) induced by the lysin are plotted against time. Beta-glucuronidase activity rendered unsedimentable is listed to the right of each curve; units as in text.

DISCUSSION

An optically uniform suspension of granules, prepared from the peritoneal leucocytes of rabbits, proved susceptible to lysis by several hemolytic agents and procedures: streptolysins O and S, lysolecithin, vitamin A, ultraviolet irradiation, and the non-ionic detergent, Triton X-100. Lysosomes from other tissues may also be disrupted by these means; the experiments, therefore, support the contention that leucocyte granules closely resemble the lysosomes of other tissues (4). In many ways, the isolated particles also resembled erythrocytes in behavior: they are relatively stable at 4°C for up to a week, they may be lysed by osmotic means or by freezing and thawing (4), they may be disrupted by dilute acids (4), and finally, the granules react in the same fashion as do red cells to streptolysins. Although each of the agents and procedures which proved lytic to the granules also damaged mitochondria (2), leucocyte granules differed from these particles in that falls of A_{520} of granular suspensions were irreversible, and that control suspensions of leucocyte granules failed to undergo "swelling" in a medium which regularly causes the swelling of mitochondria (5). Reductions in A_{520} were independent of respiration or coupled oxidative phosphorylation; and thyroxine, phosphate, or succinate failed to induce swelling or lysis.

The experiments described above demonstrate that streptolysins injure leucocyte granules directly. Earlier studies (3) have demonstrated that the lysins are leucotoxic; more detailed examination of their toxic effects revealed that degranulation of leucocytes was an early change following exposure of white cells to these agents. It is probable that direct lysis may suffice to explain the action of streptolysins on the granules of intact cells; no indirect effects mediated through mechanisms operative only in the living cell need be postulated.

When concentrations of the streptolysins were expressed as hemolytic units/ml SLS proved to be at least tenfold more active than SLO in releasing enzymes from liver lysosomes (1) and in inducing mitochondrial swelling (2). In contrast, SLO appeared to be slightly more active in inducing the degranulation of living white cells (3). Although this property of SLO may be a reflection of a more rapid rate of lysis of isolated granules, differences between the two lysins in their actions on intact cells may also reflect differences in their access to the cell interior. Similarly, the greater activity of SLS in preparations containing mitochondria may be due to the inactivation of SLO by its interaction with mitochondria or other contaminants.

Unlike changes in mitochondrial turbidity (5), changes in the absorbance of leucocyte granules appeared to result from physical lysis alone. Neither BSA nor Mg^{++} was able to reverse falls in A_{520} once they had been induced, nor could DNP or cyanide prevent granule lysis. Were antimycin to act exclusively upon the respiratory chain (5), it would be difficult to explain the inhibition of SLS- or lysolecithin-induced decreases of A_{520} by antimycin. However, other explanations for this effect may exist: antimycin may stabilize several cytoplasmic structures; the granules may possess structural groupings in common with those of the respiratory chain; or antimycin may bind the lytic agents themselves. Similarly, BSA may inhibit the actions of SLS and lysolecithin upon leucocyte granules either through a direct action on the particles or by binding the lysins. The present experiments do not permit any conclusions regarding the mode of action of either inhibitor.

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