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LYSOSOMAL DISRUPTION BY BACTERIAL TOXINS

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ABSTRACT

BERNHEIMER, ALAN W. (New York University School of Medicine, New York), AND LOIS L. SCHWARTZ. Lysosomal disruption by bacterial toxins. J. Bacteriol. **87**:1100-1104. 1964.—Seventeen bacterial toxins were examined for capacity (i) to disrupt rabbit leukocyte lysosomes as indicated by decrease in turbidity of lysosomal suspensions, and (ii) to alter rabbit liver lysosomes as measured by release of β-glucuronidase and acid phosphatase. Staphylococcal α-toxin, *Clostridium perfringens* α-toxin, and streptolysins O and S affected lysosomes in both systems. Staphylococcal β-toxin, leucocidin and enterotoxin, Shiga neurotoxin, *Serratia* endotoxin, diphtheria toxin, tetanus neurotoxin, *C. botulinum* type A toxin, and *C. perfringens* ε-toxin were not active in either system. Staphylococcal δ-toxin, *C. histolyticum* collagenase, crude *C. perfringens* β-toxin, and crude anthrax toxin caused lysosomal damage in only one of the test systems. There is a substantial correlation between the hemolytic property of a toxin and its capacity to disrupt lysosomes, lending support to the concept that erythrocytes and lysosomes are bounded by similar membranes.

The presence in mammalian cells of subcellular particles containing a variety of hydrolytic enzymes has been established by the work of several investigators, among whom the most notable is de Duve (1959). He termed the particles lysosomes. It seemed possible that some of the effects of bacterial toxins might be mediated through release into cytoplasm of hydrolases normally confined to lysosomes, and evidence supporting this hypothesis has been presented in several reports (Weissmann, Keiser, and Bernheimer, 1963; Hirsch, Bernheimer, and Weissmann, 1963; Weissmann, Becher, and Thomas, J. Cell Biol. *in press*) which demonstrate disruption of lysosomes by the cytotoxic products of streptococcal growth, streptolysins O and S. An extension of this work, designed to explore the generality of lysosome-destroying capacity among diverse bacterial toxins was undertaken, and the results are presented herewith.

MATERIALS AND METHODS

Two methods were used to estimate disruptive activity of toxins on lysosomes: (i) capacity of toxin to decrease the optical density of a suspension of cytoplasmic granules prepared from leukocytes, and (ii) capacity to release into suspending medium the lysosomal enzymes, β-glucuronidase and acid phosphatase, from granules derived from liver cells.

Optical density method. The specific cytoplasmic granules of leukocytes were prepared from rabbit peritoneal exudate according to the method of Cohn and Hirsch (1960) with slight modification (Weissmann et al., *in press*). The concentration of the stock suspension of granules was such as to give an OD_{520} (optical density at 520 mμ with 10-mm light path) of 0.25 to 0.45 when diluted 30-fold in buffered sucrose solution. To cuvettes of 1.8-ml capacity were added 25 μliters of granule suspension, a measured, small volume of diluted toxin, and sufficient diluent to bring the volume to 750 μliters. The diluent was 0.34 M sucrose buffered at pH 7.6 with 0.05 M tris(hydroxymethyl)aminomethane (tris) and containing 0.05 M sodium malate. After mixing, the OD_{520} of the suspension was read in a Beckman DU spectrophotometer every few minutes for 0.5 hr or longer. The reaction was allowed to proceed at the temperature of the cell compartment, which was usually about 25 C. Decrease in OD attributable to settling of particles was ordinarily negligible, but was always controlled by use of a cuvette containing granules without added toxin, and also by comparing the OD of test mixtures at the end of the experiment, before and after mixing the contents of the cuvette.

Enzymatic method. Suspensions of the large-granule fraction derived from rabbit liver were prepared according to methods previously described (Weissmann et al., 1963; Weissmann and Thomas, 1962, 1963). To 1.0 ml of liver granule suspension was added 0.5 ml of toxin diluted in 0.125 M sucrose solution. After 60 min at 37 C, the mixture was centrifuged at 20,000 × *g* for 20 min,

and the supernatant fluid was assayed for β-glucuronidase and acid phosphatase with 0.5 and 0.25 ml, respectively. β-Glucuronidase was assayed according to Talalay, Fishman, and Huggins (1946) with a 30-min incubation period at 37 C. Acid phosphatase was assayed according to Huggins and Talalay (1945). Tests with control solutions containing no liver granules indicated that none of the toxins, in the concentrations used, contained measurable amounts of either enzyme.

Toxins. Staphylococcal α-toxin was prepared as described by Bernheimer and Schwartz (1963) and was estimated to be 70% pure. Highly purified staphylococcal β-toxin, supplied by William R. Chesbro (Heydrick and Chesbro, 1962), had a hemolysin titer of 1:64,000 at a concentration of 1 mg of protein per ml. It was tested in the presence of 0.1% $MgSO_4$. Staphylococcal δ-toxin was crystallized (2×) material prepared by Yoshida (1963). Highly purified S and F components of staphylococcal leucocidin were those prepared by Woodin (1960). Pure lyophilized staphylococcal enterotoxin, type B, and crystalline *Clostridium botulinum* toxin, type A, were supplied by Edward J. Schantz. Streptolysin S was a lyophilized product containing 7,500 hemolytic units per mg prepared as described elsewhere (Bernheimer, 1949). Streptolysin O was a partially purified product described elsewhere (Weissmann et al., 1963*b*) and designated preparation A. It was activated with 0.1% cysteine before use. Shiga neurotoxin and tetanus neurotoxin were provided by W. E. van Heyningen. The former was a crude preparation; the latter was highly purified and contained, per mg of protein, 25 to 50 million mouse LD_{50} and 375 L_+ units. A sample of highly purified endotoxin derived from *Serratia marcescens* was supplied by Arthur G. Johnson. Purified diptheria toxin containing, per ml, 2.8 mg of protein, 1,100 L_f and 75,000 MLD was made available by A. M. Pappenheimer, Jr., α- and β-toxin and ε-prototoxin of *Clostridium perfringens* were provided by R. O. Thomson. The α-toxin was a relatively crude preparation containing 26 minimal indicating lecithovitellin units per mg; it was contaminated with collagenase but contained no detectable λ- or θ-toxin. Tests of it were carried out in presence of 0.01 M Ca^{++}. The β-toxin was also relatively crude, containing 240 L_f per mg, and some α- and θ-toxins but no λ-toxin. The ε-prototoxin was crystalline (Thomson, 1963) and contained 2,000 L_f per mg. Before use, it was activated by incubation with trypsin (4 μg of crystalline trypsin per 100 μg of prototoxin) for 30 min at 37 C. *C. histolyticum* collagenase was purchased from Worthington Biochemical Corp., Freehold, N.J. Anthrax toxin was a crude preparation supplied by Dorothy M. Molnar.

Antitoxin. A commercial product containing approximately 1,000 International Units of staphylococcal (α) antitoxin per ml was used.

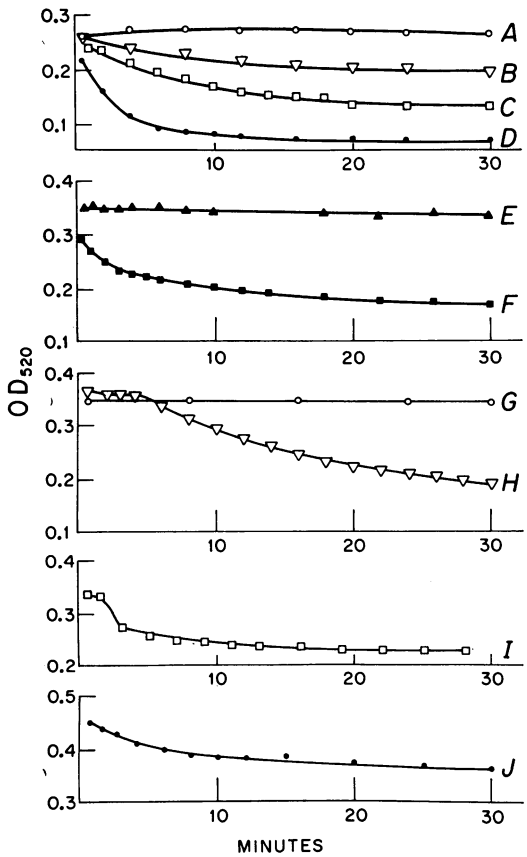

FIG. 1. *Optical density of suspensions of leukocyte lysosomes as a function of time in the presence and absence of various toxins. Quantities are per ml of reaction mixture. (A) 33 μg of staphylococcal α-toxin plus 50 units of antitoxin; (B) 2 μg of staphylococcal α-toxin; (C) 6.6 μg of staphylococcal α-toxin; (D) 33 μg of staphylococcal α-toxin; (E) no additions; (F) 10 μg of staphylococcal δ-toxin; (G) no additions; (H) 24 μg of streptolysin S; (I) 6.7 μg of streptolysin O; (J) 10 μg of Clostridium perfringens α-toxin.*

RESULTS

In addition to streptolysins, several other toxins were found to bring about decreases in turbidity of suspensions of specific granules derived from leukocytes. The effects are illustrated in Fig. 1, which demonstrates also the capacity of antitoxin to prevent the optical change caused by staphylococcal α-toxin. Evidence that the decrease in turbidity produced by staphylococcal α-toxin involved actual lysis of granules was provided by phase-contrast microscopy. Faint structures, which appeared to represent lysosome ghosts, were observed. The presence of intact granules as well, however, indicated that not all of the granules had undergone lysis.

The results, obtained by both optical and enzymatic methods, of all toxins examined are shown in Table 1. As an index of capacity of toxin to promote release of β-glucuronidase and acid phosphatase, activity is expressed as the ratio of enzyme liberated in the presence of toxin to that liberated in absence of toxin, enzyme activity being assumed to be directly proportional to color developed. Because of the small amounts of enzyme measured, significance is attached only to ratios greater than 2.

DISCUSSION

The results summarized in the table show that the toxins fall into four groups according to whether they affected lysosomes (i) in both optical and enzymatic systems, (ii) in neither system, (iii) in the optical system only, or (iv) in the enzymatic system only. Of the 17 toxins examined, 4 fall into the first and 9 into the second group. It it notable that the four toxins which caused significant effects in both systems were active in concentrations $\frac{1}{10}$ to $\frac{1}{100}$ those of the 9 toxins which clearly did not. The third group contains only staphylococcal δ-toxin which, although unequivocally destructive to leukocyte lysosomes, apparently does not act on liver lysosomes. Two (*C. perfringens* β-toxin and anthrax toxin) of the three preparations which liberated one or both

TABLE 1. *Capacity of toxins to reduce turbidity of leukocyte lysosome suspensions, and to render nonsedimentable the lysosomal enzymes, β-glucuronidase and acid phosphatase, in the large-granule fraction of liver*

Toxin	Nature of prepn	Concn in test	Reduction in turbidity of leukocyte granule suspension	Release of lysosomal enzymes from large-granule fraction of liver	
				β-Glucuronidase ratio*	Acid phosphatase ratio*
		μg/ml	%		
Staphylococcal α-toxin	Highly purified	7	50	2.7	2.3
Staphylococcal β-toxin	Highly purified	100	0	1.1	1.0
Staphylococcal δ-toxin	Crystalline	10	39	1.2	0.9
Staphylococcal leucocidin (S + F)	Highly purified	200	−2	1.0	1.1
Staphylococcal enterotoxin	Highly purified	100	4	1.5	0.9
Streptolysin S	Partially purified	2	47	2.5	6.6
Streptolysin O	Partially purified	7	34	1.5	2.9
Shiga neurotoxin	Crude	100	−4	0.9	0.9
Serratia endotoxin	Highly purified	100	6	1.0	1.0
Diphtheria toxin	Highly purified	100	1	1.8	1.7
Tetanus neurotoxin	Highly purified	100	2	1.1	1.0
Clostridium perfringens α-toxin	Crude	10	19	7.4	8.0
C. perfringens β-toxin	Crude	100	−2	6.2	3.9
C. perfringens ε-toxin	Prototoxin crystalline	100	0	1.6	1.8
C. histolyticum collagenase	Partially purified	100	−3	2.6	3.1
C. botulinum toxin, type A	Crystalline	100	5	1.9	1.5
Bacillus anthracis toxin	Crude	?†	3	8.9	1.7

* Ratio of enzyme activity, as color intensity, in presence of toxin to that in absence of toxin. None of the toxins yielded enzyme activity in the absence of granules.

† A 0.2-ml amount of solution of "standard anthrax toxin" containing 8 agar units per ml.

liver lysosomal enzymes but which did not affect leukocyte lysosomes were very crude, and the significance of the results obtained with them cannot be evaluated until purified preparations are examined.

Besides providing new information on a variety of bacterial toxins, the results serve also to confirm earlier findings that streptolysins (Weissmann et al., 1963, *in press*) are active on isolated lysosomes and that endotoxin (Weissmann et al., *in press*) and staphylococcal leucocidin (Woodin, *personal communication*) are not. The results with *C. perfringens* α-toxin confirm the observation of MacFarlane and Datta (1954) that this agent liberates acid phosphatase from a mitochondrion-containing particulate fraction of liver—a finding interpreted by Beaufay (1957) to signify lysosomal action.

A clear-cut qualitative correlation is evident between leukocyte lysosome-destroying activity and erythrocyte-destroying capacity: staphylococcal α-toxin, *C. perfringens* α-toxin, and the streptolysins are potent hemolytic, as well as lysosome-destructive agents, whereas, with one exception, none of the 12 leukocyte lysosome-negative agents is hemolytic. The exceptional agent, staphylococcal β-toxin, is known to exhibit a marked species preference according to whether rabbit erythrocytes (relatively resistant) or sheep erythrocytes (highly sensitive) are subjected to its action. Had lysosomes from sheep rather than rabbit cells been used, the results might have been different. Several lines of evidence suggest that the membranes bounding leukocyte lysosomes and those of erythrocytes are very similar, the most recent being a demonstration of immunological relatedness between the two (Quie, *personal communication*). The results of the present study provide additional information supporting this concept.

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