Agglutination and Lysis of Erythrocytes by Lysozyme.* (23882)

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Utility of lysozyme as a bacteriolytic agent in the study of bacterial physiology and cell structure has been repeatedly demonstrated and recently reviewed(1). Though the specific substrate of lysozyme is unknown, this enzyme is classified as a mucopolysaccharidase. As such it should attack cells other than bacterial cells which contain superficially located mucoprotein. Erythrocytes are known to contain mucoprotein as blood group substances(2) and viral receptors(3). This article will report the hemolytic and hemagglutinating action of lysozyme upon these erythrocytes.

Materials and methods. Crystalline lysozyme was used throughout these studies and assayed 700,000 units/g(4). Dilutions of lysozyme were prepared in saline and assayed for their lytic activity by incubation for 2 hours at 37°C with an equal volume of a 0.25% suspension of the particular cells being studied. Hemagglutination assays were conducted in the same fashion but incubation was reduced to one hour. The red blood cell suspensions were pools of 3 or more donors except for sheep cells which were from individual donors or from a commercial source from an unknown number of donors. Pools were used to minimize difference between individuals, which, however, were later found to be minor. All titers represent the final concentration of lysozyme after addition of all reagents. Lytic titers do not represent complete lysis but are based upon the visual appearance of hemoglobin in the supernatant. Hemagglutination was determined by the cell settling pattern after refrigeration overnight and titers represent the greatest dilution of enzyme yet active. Kahn, Wassermann and 10 mm hemagglutination tubes were used without appreciable differences among the three.

Results. Lysis of erythrocytes by lysozyme was dependent upon the type of cell used (Table I). Human cells were consistently more sensitive than cells of other species yet apparently do not differ from one another. Pools of human AB blood were no different from other human types. Sheep and chicken cells were not as sensitive to lysis as human cells and rabbit cells were the most resistant of those studied. Lysis of chicken cells was frequently confirmed by microscopic examination since the hemoglobin is altered by lysozyme in such a way as to render it less visible. Assays of lysozyme with the Repaske technic(5) will increase the lytic titer against chicken cells approximately 2-fold. Similar experiments with other cell types have not been conducted.

TABLE I. Lysis of Erythrocytes by Lysozyme.

Cell type	Dilution of lysozyme 1:100	200	300	400	600	800	1200	1600
Human								
Group O	+	+	+	+	+	+	+	—
A	+	+	+	+	+	+	+	—
B	+	+	+	+	+	+	+	—
Sheep	+	+	+	+	+	±	—	—
Chicken	+	+	+	+	+	+	—	—
Rabbit	+	+	+	+	+	—	—	—

+ = lysis; — = no lysis; ± = lysis in some, but not all, repeats of the experiment.

When tubes of lysozyme solutions were placed in boiling water for a few minutes and then assayed the lytic ability of the enzyme for bacteria and erythrocytes was destroyed (Table II). During the heating process the solutions developed an hemagglutinating property. The destruction of the lytic ability and creation of the hemagglutinating activity were directly related to the duration of the heating and also to the decrease in the bacteriolytic titer. During the boiling process the solutions remain clear or become only slightly turbid.

Dilutions of lysozyme boiled for 20 minutes have the ability to agglutinate the cell types which were lysed by untreated lysozyme solutions (Table III). There was no direct re-

* Supported in part by Nat. Inst. of Health Research Grant.

TABLE II. Effect of Heating on Lysozyme Activity against Human O Erythrocytes.*

	Dilution of lysozyme in hundreds											Bacteriolytic units/g
	2	4	8	12	16	32	64	128	256	512	1024	
No treatment	L	L	L	L	L	—	—	—	—	—	—	700,000
Boiled 10 min.	L	L	L	H	H	H	H	H	—	—	—	20–40,000
" 20 "	L	H	H	H	H	H	H	H	H	H	H	5–10,000

* Individual, not pooled cells.
L = lysis; H = hemagglutination; — = no visible reaction.

TABLE III. Agglutination of Erythrocytes by Boiled Lysozyme.

Cell type	Dilution of lysozyme in thousands								
	13	26	51	102	205	410	819	1638	3277
Human Group O	+	+	+	+	+	+	—	—	—
" " A	+	+	+	+	+	+	—	—	—
" " B	+	+	+	+	+	+	—	—	—
Sheep	+	+	—	—	—	—	—	—	—
Chicken	+	+	+	+	—	—	—	—	—
Rabbit	+	+	+	+	+	+	+	+	—

+ = agglutination; — = no visible reaction.

lationship between lysis and agglutination since rabbit cells, the most resistant to lysis, were the most sensitive to agglutination. However, sheep cells were highly resistant to both of these properties of lysozyme solutions.

Discussion. Erythrocytes were seemingly more resistant to lysis by lysozyme than most Gram positive bacteria, requiring concentrations of 0.05 to 0.25 mg/ml as compared to μg/ml quantities for bacterial suspensions. It is doubtful that the 2 systems may be compared so directly, however; for the nature, amount and availability of the enzyme substrate in these systems is still unknown.

It is not yet clear if lysozyme is actually acting upon mucoprotein of the erythrocytes since, in data not presented, sublytic exposure of chicken cells to lysozyme with subsequent removal of the enzyme by repeating washing of the cells did not produce cells with diminished influenza virus A(PR-8) hemagglutinating titers. Likewise, human cells similarly exposed to lysozyme were agglutinated to the same titer as normal cells by specific immune serum.

Hemagglutination by lysozyme has not been described previously although agglutination of bacteria has been observed(6,7,8). These latter were largely random studies not given detailed study. It is possible that agglutination and hemagglutination occur by the same or similar mechanisms since weakly bacteriolytic preparations of lysozyme have been more reactive in bacterial agglutination. However, in one instance the ability of lysozyme to flocculate bacteria was destroyed by boiling(9).

The remarkable dilutions of denatured lysozyme which agglutinated red blood cells were unparalleled even by phytoagglutinins which are usually more active than immune sera.

Summary. Two new observations have been presented; lysis and agglutination of erythrocytes by lysozyme. The hemagglutinating reagent may be prepared by boiling the hemolytic enzyme solution.

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Received January 3, 1958. P.S.E.B.M., 1958, v97.