Therefore it seems that the observation here reported may aid in furnishing at least part of the mechanism for the high sedimentation rate seen in pneumonia.

9214

Effect of Phage on Electrokinetic Potential of Susceptible Cells.*

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Earlier work[1, 2] has served to establish the following essential facts concerning the phage-bacterium reaction:

A. Phage-production is conditioned by bacterial growth.

B. There is at all times a normal distribution of phage between susceptible cells and the surrounding medium providing the cells are alive, *i. e.*,

$$\frac{\text{intracellular phage per cell}}{\text{free phage}} = K$$

C. Lysis of bacteria depends upon the attainment of a critical ratio of phage to bacteria. In our experiments this threshold is approximately 100 activity units[3] per bacterium.

While the above relationships are significant for the development of a rational mechanism of phage action on bacteria[4] and have been proved to apply to more than one organism and the corresponding phage,[5] they give no evidence as to how phage induces cellular dissolution. Apparently phage does not measurably alter the normal bacterial growth-rate or the rate of cellular metabolism;[4, 6, 7] it may or may not bring about swelling of susceptible bacteria just before lysis begins. Bronfenbrenner[8] has found that phage produces hydrolytic cleavage of bacterial proteins although

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1 Krueger, A. P., and Northrop, J. H., *J. Gen. Physiol.*, 1930, **14**, 223.

2 Krueger, A. P., *J. Gen. Physiol.*, 1931, **14**, 493.

3 Krueger, A. P., *J. Gen. Physiol.*, 1930, **13**, 557.

4 Krueger, A. P., *Physiol. Reviews*, 1936, **16**, 129.

5 Clifton, C. E., and Morrow, G., *J. Bact.*, 1936, **31**, 441.

6 Eaton, M. D., *J. Bact.*, 1931, **21**, 143.

7 Hallauer, C., *Centralbl. f. Bakt., I. Orig.*, 1933, **130**, 194.

8 Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, **3**, 562.

this does not seem to be a constant concomitant of phagic action.[9] Since the site of action has never been clearly demonstrated and it is not even known whether phage taken up by a bacterium is inside the cell or merely held on the cell's surface, we felt that measurements of the electrokinetic potential of susceptible staphylococci which had been exposed to anti-staphylococcal phage would be of interest.

I. *Measurement of the rate of electrophoresis.* To estimate the electrokinetic potential the rate of electrophoresis of untreated and phage-treated staphylococci was determined. Two types of suspensions were employed:

A. Live Cells. A suspension of living staphylococci was made from a 16-hour culture of organisms which had been grown on agar, thoroughly washed, and resuspended in sterile water. From this dense suspension the following mixtures were prepared: $12x10^7$ staphylococci per ml. in undiluted phage ($1x10^{10}$ activity units/ml.) and $12x10^7$ staphylococci per ml. in broth. Both mixtures were kept in ice-water for 2 hours to allow equilibrium between intracellular and extracellular phage to become established. They were then centrifuged, the supernatants decanted, and the discarded solution replaced with an equal volume of secondary sodium phosphate and primary potassium phosphate buffer-mixture of pH 7.0.

B. Dead Cells. A 16-hour culture of staphylococci grown on agar was washed twice with sterile distilled water. The washed cells were made into a dense suspension in sterile distilled water and were heated at 80°C. for one hour. Aliquots were added to broth and to undiluted phage so that the final concentrations in both preparations were $1x10^8$ bacteria per ml. The rest of the treatment was identical with the procedure outlined under A.

The rates of migration in the electric field were measured in the Northop-Kunitz microcataphoretic cell[10] and the figures were substituted in the Helmholtz-Lamb equation for calculating the zeta potential. The average electrokinetic potential for living untreated cells was −31 millivolts, while the corresponding figure for phage-treated cells was −59 millivolts. Dead untreated cells had an average electrokinetic potential of −23 millivolts, and dead phage-treated cells an average zeta potential of −46 millivolts.

II. *Test of colloidal stability.* As a further test of the differences in electrokinetic potential engendered by phage, the stability of untreated and phage-treated staphylococcal suspensions to the

[9] Bayne-Jones, S., and Sandholzer, L. A., *J. Exp. Med.*, 1933, **57**, 279.

[10] Northrop, J. H., *J. Gen. Physiol.*, 1921-22, **4**, 629.

addition of thorium nitrate was tested. The bacterial suspensions used were those described above and were prepared both with and without exposure to phage.

In the case of living cells it was found that agglutination was very hard to induce even with high concentrations of thorium nitrate. There was no clear-cut endpoint which would serve to differentiate the untreated and the phage-treated suspensions. Apparently concentrations of thorium nitrate sufficient to reduce the electrokinetic potential below the critical level for agglutination also alter the cohesive properties of the cells.

In carrying out tests with dead bacteria, the cells were prepared as described under I-B with the exception that the final concentrations of bacteria in broth and in phage were 6×10^8 cells per ml. After exposure to phage and to broth for 3 hours the suspensions were centrifuged, the supernatants decanted, and the discarded fluid replaced with equivalent volumes of distilled water. The packed cells were thoroughly shaken up and 0.5 ml. aliquots of each cell suspension were added to 0.5 ml. portions of thorium nitrate dilutions. The suspensions were shaken in the waterbath at 36°C. and were read for agglutination at 0.1-hour intervals. Table I summarizes the general results observed in a series of such tests.

TABLE I.

Effect of phage on stability of heat-killed staphylococcal suspensions exposed to action of thorium nitrate. [Bacteria] = 3×10^8/ml. Agglutination expressed as 1+, 2+, 3+, 4+. Reaction carried out at 36° C. with shaking. Higher dilutions were ineffective.

Time (Hrs.)	Broth-Treated Staphylococci				Phage-Treated Staphylococci	
	Normality of Thorium Nitrate					
	1/10000	1/12000	1/16000	1/20000	1/10000	1/12000
0.1	4+	4+	0	0	0	0
0.2	4+	4+	2+	2+	1+	0
0.3	4+	4+	4+	2+	1+	0
0.4	4+	4+	4+	2+	1+	0
0.5	4+	4+	4+	2+	1+	0
0.75	4+	4+	4+	2+	1+	0
1.0	4+	4+	4+	2+	1+	0

Apparently when dead staphylococci take up phage they become quite resistant to the precipitating action of thorium nitrate. It should be noted that the sorption of phage by living staphylococci differs from that observed with dead cells.[2] When living cells are placed in phage-solutions equilibrium between intracellular and extracellular phage fractions is quickly established and maintained in accordance with a partition-coefficient; this attachment of phage to the cell is readily reversible. With dead cells phage distribution is

expressible as an adsorption-isotherm equation and the fraction attached to the bacteria is irreversibly bound.

The attachment of phage to either living or dead susceptible staphylococci increases the negative electrokinetic potential as measured directly by the rate of cataphoretic migration and indirectly by the increased stability of suspensions to the action of quadrivalent cations. This is not proof that phage action is limited to the cell surface but it does furnish evidence that certain of the bacterium's surface-properties are altered either directly as a result of phage-attachment or indirectly as a reflection of intracellular reactions in which cellular constituents and phage participate.

9215 P

Liver Proteins. II. Liver Albumin.

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In the course of investigations on the chemistry of the liver proteins we have had occasion to determine the liver-albumin content under a variety of conditions. The fundamental problem which we desired to elucidate was whether liver albumin exists as a preformed protein or whether it arises as an artefact during fractionation. It occurred to us that a partial answer would be obtained by studying the ratio, albumin/total salt-soluble protein, at different hydrogen ion concentrations maintained during the preliminary sodium chloride extractions. It is known[1] that the total salt-soluble protein extractable from liver increases greatly with increase of pH over the range pH 4 to 8. If the liver albumin were largely an artefact, arising as a dissociation product from the salt-soluble protein fraction, one might reasonably expect that increases in the latter would be reflected by equi-proportional increases in the former; the ratio, albumin/total salt-soluble protein, would remain constant. Although the present work does not give a decisive answer to the question it yields certain information about the liver-albumin content which is pertinent and significant.

For the purposes of this investigation we used dog liver, rapidly perfused *in situ* to remove blood, then excised, frozen with liquid air, powdered, and preserved at −10°C.

[1] Luck, J. M., and Nimmo, C. C., *Proc. Am. Soc. Biol. Chem.*, in press.