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Factors Influencing the Infectivity of Bacteriophage.

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When a mixture of virus and antiserum is titrated in animals it is the usual experience to find that the infectivity of the virus has been reduced but not abolished. By convention, one refers to such a mixture as representing so many units of neutralized virus, and so many units of active virus. In some instances this convention has been literally interpreted to reach conclusions probably not justified by the facts. In particular, decisions have been made regarding the reversibility of neutralization, and the heterogeneity of the viral population, on the insecure assumption that "neutral" and "active" viral particles in the mixture are different. It is not our purpose to question these conclusions, but to inquire into the validity of the underlying assumption.

The experiment described in Table I serves as an illustration of some of the difficulties inherent in an all-or-none hypothesis of neutralization. When the neutralization effected by a given exposure of bacteriophage to antiserum is evaluated by enumeration on three different media, 3 different results are obtained. With unsensitized phage, the media consistently fall into a definite order of effectiveness, but this order is profoundly changed after the phage has been exposed to antibody. Thus the medium representing the highest degree of "host-resistance"[1] to untreated phage, exhibits no unusual resistance after the phage has been sensitized. This experiment can be modified in many ways, but in each case it is evident that the active sensitized phage is qualitatively different from the unsensitized phage. Similar observations with the animal viruses[2] might be interpreted in the same way. The advantage of experiments with the simpler system lies in their susceptibility to further analysis, especially because the effects of uncombined antibody can be excluded.

The coliphage used (P9H) is weakly active in the absence of rather high concentrations of univalent cations. This is at least in part a result of poor adsorption to bacteria in the absence of salt. The effect of antibody is to increase the adsorbability in the presence or absence of salt. But antibody also inactivates phage, independently

[1] Hershey, A. D., and Bronfenbrenner, J., *J. Gen. Physiol.*, 1941, **24**, 703.

[2] Bronson, L. H., and Parker, R. F., *J. Immunol.*, 1941, **41**, 269.

TABLE I.
Phage-Neutralization Tests with Different Media

Medium	0.08 M NaCl	0.016 M NaCl	0.08 M NaCl + dead bacteria
Unsensitized phage	448	96	200
Sensitized phage	40	34	9

All counts shown express the phage-content of 10^{-7} ml of the same preparation of coliphage P9H. For sensitization, phage was exposed to the action of 1:5000 rabbit antiserum for 80 minutes at 37°C, and diluted beyond the range of activity of the serum. The counts were made simultaneously according to the method described in reference 1.

of the effect on adsorption. If the concentration of salt is low, antibody may either increase or decrease infectivity, depending on the balance between these two effects. If dead (or "resistant") cells are present, the effect of sensitization is to depress infectivity both by increasing adsorption to the dead cells, and by inactivating the phage.

We believe that these facts, as well as analogous results with animal viruses, can be better understood if it is assumed that the sensitized virus does not consist of a mixture of neutral and active particles, but that it represents an essentially uniform population of virus of altered infectivity. On this assumption, we have the general relation $P = (1-p_r-q)p_i$, where P is the infectivity expressed as the probability that any single specified viral particle will produce a lesion, p_r is the probability that the particle will be accepted by a resistant cell, q is the probability that it will escape fixation (or be lost into the circulation) and p_i is the probability that it will produce a lesion if fixed by a susceptible cell. If the susceptible cells are assumed to be uniform, p_i is a simple probability; otherwise it expresses the average susceptibility of the cells not perfectly resistant. It is evident in what way salt, dead bacteria, and antibody in our experiment, diversely affect P by influencing the various terms on the right. In the case of other viruses, the analogous factors are only now being elucidated.[3, 4]

The available experiments do not establish the truth of the assumption made above. It is suggested merely that this assumption is reasonable, and should be applied to the results of infectivity-measurements before far-reaching conclusions are drawn.

[3] Sprunt, D. H., *J. Exp. Med.*, 1941, **74**, 81.

[4] Friedewald, W. F., *J. Exp. Med.*, 1942, **75**, 197.