THE STREPTOCOCCUS LACTIS HOST-VIRUS SYSTEM[1]

II. Characteristics of Virus Growth and the Effect of Electrolytes on Virus Adsorption

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The development of a technique for isolating one step in the growth of a virus for *Escherichia coli* was described by Ellis and Delbrück (1939). This method permits the determination of the latent period for virus growth and the yield of virus per bacterium under defined physiological conditions. If the composition of the medium is varied, the resulting effect on virus growth will be reflected in changes in the characteristics of the one-step growth curves. It seemed desirable to apply the one-step growth technique to a study of the characteristics of virus growth in the *Streptococcus lactis* host-virus system.

In attempting to study virus production by this system in a synthetic medium, difficulty was experienced in infecting the cells with virus. This was discovered to result from the presence of buffer salts in the medium. Therefore, a study of the effect of electrolytes on virus adsorption was undertaken.

There are many reports of the effect of electrolytes on virus multiplication. Scribner and Krueger (1937) have reviewed the work reported up to 1937. Relatively few workers have analyzed the influence of salts on adsorption of virus by the host cells. Krueger and Strietmann (1938) observed that in the presence of M/8 sodium sulfate the adsorption of virus by a culture of *Staphylococcus aureus* was reduced by a factor of 2. Scribner and Krueger (1937) reported that at 0 C there was no significant difference in the quantity of virus adsorbed by *S. aureus* cells between those suspended in 0.25 M sodium chloride and those suspended in a medium without salt. Gest (1943) concluded that 0.01 M magnesium chloride had no observable effect on the adsorption of virus by *E. coli* cells. Hershey *et al.* (1943) noted that a relatively high concentration of sodium and a low concentration of calcium ions were essential for plaque formation for T_2 virus. Part of the present report is concerned with the determination of optimal salt concentrations for adsorption of virus on *S. lactis* host cells.

MATERIALS AND METHODS

The host-virus system used consisted of a strain of *S. lactis* 122-4 and its homologous virus. Both virus lysates and cell suspensions were prepared as de-

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scribed in a previous paper (Cherry and Watson, 1949). The bacteria for all experiments were grown in an unbuffered medium consisting of 1 per cent tryptone (Difco), 0.3 per cent yeast extract (Difco), and 0.2 per cent glucose. This medium will be referred to as "complete," and a similar medium from which tryptone was omitted will be called "deficient." The initial pH of the media for all experiments was 7.0. Adsorption mixtures of cells and virus were incubated in the water bath at 30 C for 10 minutes. Aliquots of 1 ml of the mixtures were removed and centrifuged for 6 minutes at 2,400 rpm in the angle centrifuge to sediment the cells. Free virus in the supernatants was assayed by the plaque count method previously described (Cherry and Watson, 1949). Adsorbed virus was given by the difference between the initial concentration and the free virus at the end of the adsorption period. Salt solutions were prepared in either 0.1 M or 0.2 M concentrations in sterile distilled water and stored at 1 C. The pH was adjusted to 7.0 with 0.5 N sodium hydroxide when necessary. The salts were added to the medium immediately before beginning an experiment. The effect of electrolytes on lysis of cells by virus was determined by making turbidity readings after 2 to 4 hours of incubation at 30 C. The virus to cell ratio in all experiments was about 4, and the cell concentration was about 5×10^7 per ml.

One-step growth experiments were conducted according to the technique of Delbrück and Luria (1942). After an adsorption period of 10 minutes at 30 C, free virus was determined as described above. At the same time adsorption was arrested by diluting the cell-virus mixture to 10^{-5} or 10^{-6} and continuing the incubation. Aliquots were removed periodically and after suitable dilution were assayed for total virus by the plaque-counting method. All dilutions were made in broth of the same composition as that used in the adsorption tube. The average burst size was calculated from the formula given by Delbrück and Luria (1942). Bacterial cell counts were computed by arbitrarily multiplying the plate count by 2, since it was found that this figure expressed the ratio of the count obtained by the microscopic method to that given by the plate count.

RESULTS

The effects of electrolytes on lysis of cells by virus. The influence of several salts on the lysis of *S. lactis* 122-4 by virus was tested. It was found that the omission of tryptone from the medium reduced virus adsorption from about 80 per cent to 20 to 30 per cent of the initial virus. Therefore, a medium free of tryptone was considered "deficient" for virus adsorption. The stimulating effect of electrolytes could be measured by noting the increase in adsorption resulting from the addition of salts. "Deficient" medium supplemented with salts never gave better adsorption than could be obtained in the same medium to which 1 per cent of tryptone was added ("complete"). Therefore, virus adsorption in the "complete" medium was determined in each experiment and served as a marker of adsorption efficiency. The salt concentrations employed ranged from 0.001 M to 0.1 M. The results of these experiments are presented in figures 1, 2, and 3, in which the percentage of light transmittance after a 2- to 4-hour incubation

period is plotted against molar concentrations of the salts added. The peaks of the curves represent lysis of cells at the corresponding salt concentrations. Lysis usually began at the end of the latent period of virus growth (32 minutes). Sodium and potassium phosphate and magnesium sulfate were most effective at 0.005 to 0.01 M concentrations, whereas calcium chloride was effective over a larger range (0.005 to 0.05 M). Sodium and potassium sulfate had their greatest activity at 0.01 M concentrations, sodium chloride at 0.02 to 0.05 M levels, and potassium chloride at the 0.02 M level. Lithium chloride (figure 3) was somewhat effective at 0.05 to 0.1 M concentrations and sodium acetate at 0.02 to 0.05 M

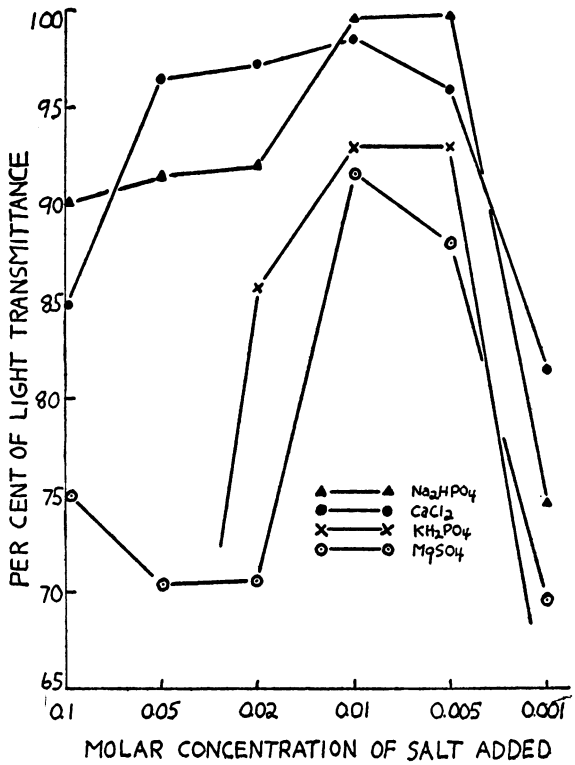

Figure 1. Effect of salts on lysis of *S. lactis* 122–4 by virus at pH 7.0 and 30 C.

salt levels. Manganous sulfate was toxic to the growth of bacteria at all levels, and cells were not lysed by virus in the presence of the salt. Potassium oxalate in a 0.1 M concentration inhibited growth of the cells and allowed no appreciable lysis even at lower salt levels. Sodium citrate was toxic at the higher concentrations and appeared to be somewhat inhibitory to the bacteria at all salt levels.

These data indicate that many electrolytes are active in promoting lysis of *S. lactis* cells by virus. The effective range of salt concentrations is usually rather small and in some cases very sharp.

The effect of electrolytes on virus adsorption. Experiments were performed in order to test whether or not the effects noted above resulted from stimulation of

virus adsorption. The salt concentration giving the greatest activity for cellular lysis was found to be the one giving the greatest adsorption of virus to the cell as measured by the plaque count assay. Table 1 shows the results obtained with potassium phosphate. As predicted from figure 1, maximum stimulation was obtained in the presence of 0.01 M phosphate. Lower or higher salt concentrations depressed the adsorption of virus below that obtained in the "deficient" medium without added phosphate. The effect of calcium chloride is evident in the data of table 2, in which the greatest activity was obtained in the presence of 0.01 and 0.02 M salt concentrations. Cellular lysis was noted only at these salt levels. Similar results were obtained with sodium citrate (table 3), with the ex-

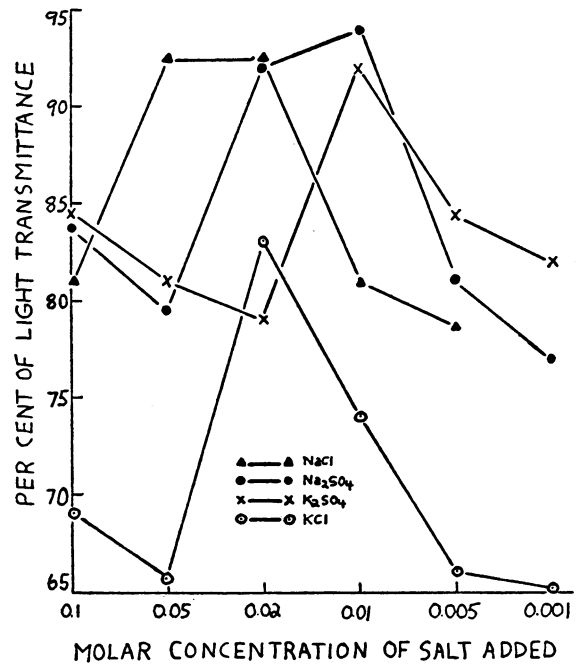

Figure 2. Effect of salts on lysis of *S. lactis* 122-4 by virus at pH 7.0 and 30 C.

ception that cellular lysis was not observed at any level of salt concentration used. Potassium and sodium chloride, magnesium sulfate, and sodium acetate were tested in the same way and found to stimulate or depress virus adsorption in accordance with the previously noted effect on cellular lysis.

An experiment was performed to show what part of the total adsorbing ability of the "complete" medium was contributed by yeast extract and what by tryptone. In the medium composed of yeast extract and glucose, 21 per cent of the initial virus was adsorbed in 10 minutes at 30 C. The tryptone plus glucose medium supported 59 per cent adsorption under the same conditions. The sum of these (80 per cent) equals the adsorption given by the "complete" medium (82 per cent). If the concentration of yeast extract in the "deficient" medium was increased from 0.3 per cent to 1.0 per cent, virus adsorption was approxi-

mately as good as in the "complete" medium. This fact indicates that the inability of the "deficient" medium to support virus adsorption is the result of quantitative rather than qualitative factors.

One-step growth experiments in "complete" medium. Typical one-step growth curves of virus on *S. lactis* 122-4 in "complete" medium at 30 C and an initial pH of 7.0 are shown in figure 4. In table 4, experiments 1 and 3, these data are summarized. The latent period of virus growth is in the range of 31 to 33 minutes, and the rise period is 15 to 18 minutes when the multiplicity of infection is about 3. With virus to cell ratios of less than 1, similar results were obtained.

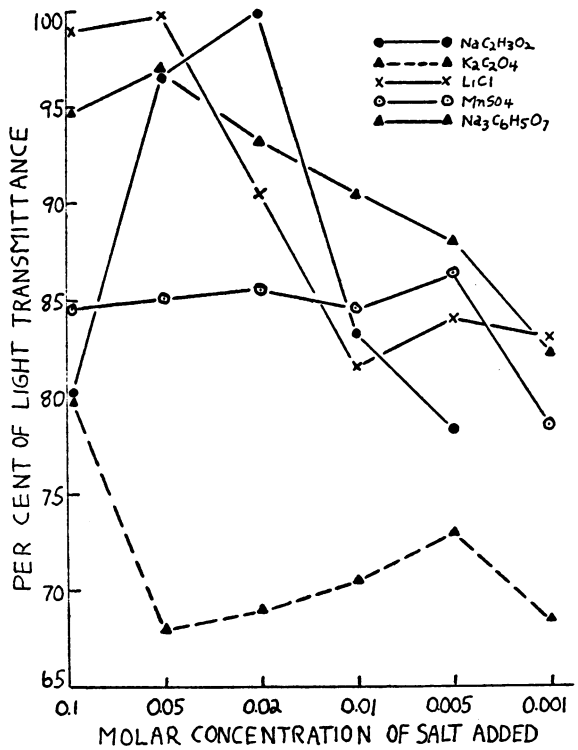

Figure 3. Effect of salts on lysis of *S. lactis* 122–4 by virus at pH 7.0 and 30 C.

The average yield per infected bacterial cell was about 70 plaque-forming particles of virus. Final concentrations of virus were about 20 times the initial input.

One-step growth experiments in "deficient" medium. Experiment 4 of figure 5 illustrates the effect of removing tryptone from the "complete" medium. The medium was not "deficient" for virus adsorption since it was supplemented by the addition of monobasic potassium phosphate to give a 0.01 M concentration. This results in adsorption of virus equivalent to that obtained in "complete" medium. However, the latent period of virus growth is increased by about 20 minutes and the average burst size is reduced to 24 (table 4). The final to initial

TABLE 1

The effect of potassium phosphate on the adsorption of virus by S. lactis 122-4

MEDIUM	PLAQUE COUNTS OF FREE VIRUS DUPLICATE PLATES	VIRUS ADSORBED PER ML × 10^7	PERCENTAGE OF INITIAL VIRUS* ADSORBED	EXPECTED PERCENTAGE OF ADSORPTION†
"Complete"‡	174–175	14.5	80	75–85
"Deficient"§	57–66	6.0	33	20–30
"Deficient" + 0.001 M KH_2PO_4	71–79	3.0	17	20–30
"Deficient" + 0.01 M KH_2PO_4	156–167	14.8	82	75–85
"Deficient" + 0.05 M KH_2PO_4	76–89	1.5	8	20–30

* 1.8×10^7 per ml.

† Estimated from figure 1.

‡ Tryptone, 1 per cent; yeast extract, 0.3 per cent; glucose, 0.2 per cent.

§ Same as "complete" medium minus tryptone.

TABLE 2

The influence of calcium chloride on the adsorption of virus by S. lactis 122-4

MEDIUM	PLAQUE COUNT OF FREE VIRUS DUPLICATE PLATES	VIRUS ADSORBED PER ML × 10^7	PERCENTAGE OF INITIAL VIRUS* ADSORBED	LYSIS OF CELLS
"Complete"	87–76	9.8	86	+
"Deficient"	43–60	1.1	10	−
"Deficient" + 0.001 M $CaCl_2$	32–53	2.9	25	−
"Deficient" + 0.01 M $CaCl_2$	149–163	8.3	73	+
"Deficient" + 0.02 M $CaCl_2$	76–93	9.7	85	+
"Deficient" + 0.1 M $CaCl_2$	46–58	1.0	9	−

* 11.4×10^7.

TABLE 3

The influence of sodium citrate on the adsorption of virus by S. lactis 122-4

MEDIUM	PLAQUE COUNTS OF FREE VIRUS DUPLICATE PLATES	VIRUS ADSORBED PER ML × 10^6	PERCENTAGE OF INITIAL VIRUS* ADSORBED	LYSIS OF CELLS
"Complete"	138–113	62	71	+
"Deficient"	348–300	22	25	−
"Deficient" + 0.005 M sodium citrate	134–132	60	69	−
"Deficient" + 0.01 M sodium citrate	225–221	42	49	−
"Deficient" + 0.02 M sodium citrate	292–315	26	30	−
"Deficient" + 0.05 M sodium citrate	312–322	24	27	−

* 87×10^6 per ml.

virus ratio is low (6). If yeast extract (Difco) is added to the "deficient" medium to give a total of 1 per cent, virus production approaches that obtained in the "complete" medium. This is shown in experiment 5 of figure 2 and in table 4.

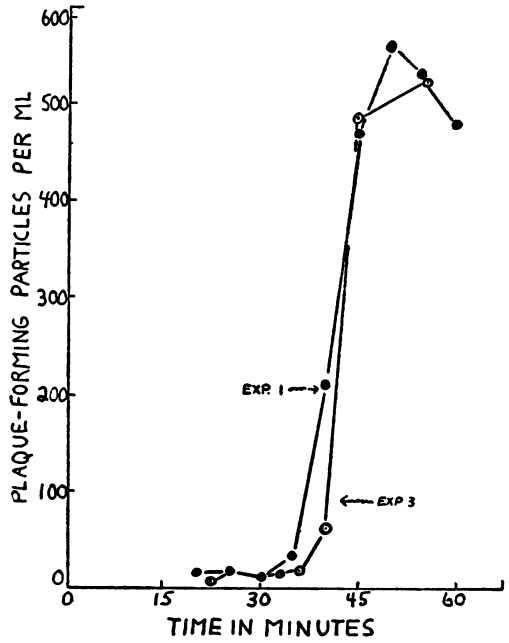

Figure 4. One-step growth curves of virus on *S. lactis* 122–4. Experiments 1 and 3 in "complete" medium at pH 7.0 and 30 C.

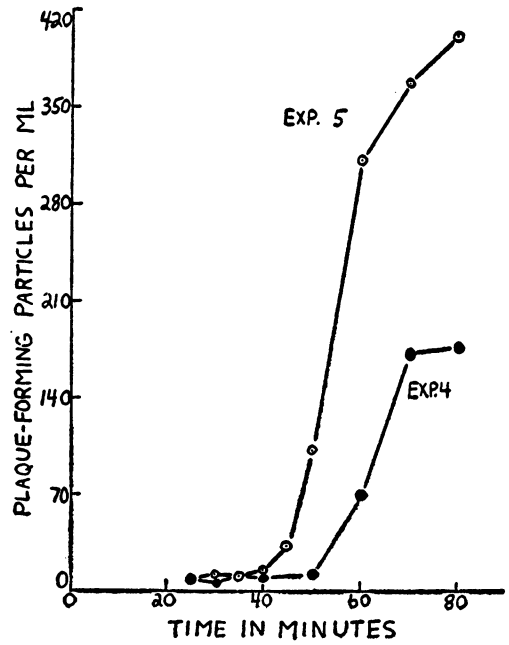

Figure 5. One-step growth curves of virus on *S. lactis* 122–4. Experiment 4 in "deficient" medium at pH 7.0 and 30 C. Experiment 5 in "deficient" medium plus 0.7 per cent yeast extract at pH 7.0 and 30 C.

However, the latent period of virus growth was about 10 minutes longer than it was in the tryptone medium. Thus it seems that on a weight basis yeast extract does not completely replace tryptone for virus production.

The effect of sodium citrate on virus production. As shown in figure 3, sodium citrate did not allow lysis of cells by virus. However, bacterial growth was inhibited to some extent even at the lower salt concentrations. Table 3 indicates that virus is readily adsorbed in the presence of 0.005 M sodium citrate. To determine whether virus was produced in the absence of bacterial lysis, one-step growth experiments were made in both "complete" and "deficient" media containing 0.005 M sodium citrate. In "deficient" medium no increase in virus was noted over a period of 93 minutes (table 4, expt. 6). After a latent period of 60 minutes, there was a small increase in virus in the "complete" medium (expt. 8). The final to initial virus ratio was about 4, and the average burst size was 5.

TABLE 4

Characteristics of the growth of virus on S. lactis 122-4

EXPERIMENT NO.	MEDIUM	MULTIPLICITY OF INFECTION	MEAN LATENT PERIOD IN MINUTES	MEAN RISE PERIOD IN MINUTES	AVERAGE BURST SIZE PER INFECTED CELL	RATIO OF FINAL TO INITIAL VIRUS
1	"Complete"	3	32	17	66	18
3	"Complete"	3	31	15	77	21
4	"Deficient"	4	55	10	24	6
5	"Deficient" + 0.7% yeast extract	3	42	15	47	13
6	"Deficient" + 0.005 M sodium citrate	1	—	—	<1	0.5
7	"Deficient" + 0.005 M sodium citrate + 0.02 M $CaCl_2$	1	—	—	<1	0.5
8	"Complete" + 0.005 M sodium citrate	1	60	10	5	3.5
9	"Complete" + 0.005 M sodium citrate + 0.02 M $CaCl_2$	1	48	8	6	4.7

An attempt to relieve the inhibition of virus production by the addition of calcium chloride to give a 0.02 M solution was unsuccessful. Sodium citrate and calcium chloride were added to both "complete" and "deficient" media immediately before the experiments were begun. Calcium had no effect on increasing virus production in "deficient" medium (table 4, expt. 7), and the only observable effect in "complete" medium (table 4, expt. 9) was a reduction in the latent period of virus growth. Assays for virus were made up to 80 minutes. However, it was noted that after a long incubation period the cells in the tubes to which calcium chloride was added were completely lysed. The multiplicity of infection in the experiments conducted in media containing sodium citrate was about 1. Under these conditions many cells are not infected. Poisson's formula for calculating the fraction of uninfected bacteria is not applicable since the efficiency of plating is rather low (Cherry and Watson, 1949). However, even if only 50 per cent of the bacteria were infected the results would not be changed significantly. It appears that sodium citrate greatly depresses virus production

and that this effect can be reversed by the addition of calcium chloride only after a very long lag period, if at all. Virus assays throughout the course of the experiments gave no indication that citrate, at the levels used, had any appreciable effect in accelerating virus inactivation.

DISCUSSION

The average yield of virus obtained per infected cell of *S. lactis* 122-4 under the experimental conditions used was lower than the burst sizes that have been determined for the T series of viruses for *E. coli*. Delbrück (1946) reported burst sizes ranging from 120 to 300 for the seven viruses of the coli group. It is quite possible that other media may increase the yield of viruses for *S. lactis* above the burst size of 70 determined in tryptone yeast extract broth. The tendency of *S. lactis* cells to grow in short chains makes it difficult to determine absolute burst size values.

Tryptone contributed approximately a 3-fold stimulation to both adsorption and synthesis of virus for *S. lactis* 122-4. On a weight basis, yeast extract completely replaced tryptone for virus adsorption but could not be entirely substituted for tryptone for virus production. In view of the studies (Cohen, 1949) that have been reported on the growth requirements of the T series of viruses for *E. coli*, it is probable that this is an expression of similar requirements for the *S. lactis* host-virus system. Further studies along these lines are in progress. The stimulatory effects on virus adsorption of both 1 per cent yeast extract and 1 per cent tryptone are believed to be the result of the salts that they contain. The increased adsorption of virus occurring when salts are added to media deficient for virus adsorption is the evidence for this belief. The mechanism by which electrolytes produce these effects are obscure. The studies of Greenstein *et al.* (1947) on the importance of salts in the enzymatic degradation of nucleic acids by extracts of animal tissues suggest the possibility that adsorption of virus to the cell surface could be mediated by salt-activated enzymes. Anderson (1948) showed that the characteristics of activation of T_4 virus for adsorption on its host were not incompatible with an enzymatically controlled mechanism. It seems unlikely that the effect of electrolytes on adsorption of virus by *S. lactis* 122-4 can be entirely explained by purely physical phenomena involving the electrokinetic potential of cell and virus.

SUMMARY

The adsorption of virus by *Streptococcus lactis* 122-4 was about 3 times as great in a medium consisting of 1 per cent tryptone, 0.3 per cent yeast extract, and 0.2 per cent glucose as in the same medium when tryptone was omitted.

On a weight basis, yeast extract replaced tryptone for virus adsorption.

Salt stimulation of cellular lysis by virus can be used as a method of screening electrolytes for effects on virus adsorption. Electrolytes were most stimulating for both cellular lysis and virus adsorption at concentrations ranging from 0.005 M to 0.05 M. Potassium phosphate, potassium, sodium and calcium chloride, magnesium sulfate, and sodium acetate promoted lysis of the host cells

according to their efficiency in promoting virus adsorption, as measured by plaque count assays.

Sodium citrate did not permit lysis of host cells by virus although it allowed maximum adsorption and did not inactivate the virus. Virus synthesis was greatly reduced in the presence of 0.005 M sodium citrate. This level of salt is somewhat inhibitory to multiplication of the bacteria. Addition of 0.02 M calcium chloride did not release the citrate inhibition except to permit cellular lysis after a very long lag period.

The average yield of virus per infected cell of *S. lactis* 122-4 in tryptone yeast extract medium at pH 7.0 and 30 C was about 70 plaque-forming particles. The burst size was reduced by a factor of three when tryptone was not present in the medium even though adsorption of virus was maintained at a high level by the addition of potassium phosphate to give a 0.01 M concentration. On a weight basis, yeast extract only partly replaced tryptone for virus synthesis. This suggests that this host-virus system requires growth factors that are not entirely supplied by yeast extract.

REFERENCES

ANDERSON, T. F. 1948 The activation of the bacterial virus T_4 by L-tryptophan. J. Bact., **55**, 637–649.

CHERRY, W. B., AND WATSON, D. W. 1949 The *Streptococcus lactis* host-virus system. I. Factors influencing quantitative measurement of the virus. J. Bact., **58**, 601–610.

COHEN, S. S. 1949 Growth requirements of bacterial viruses. Bact. Revs., **13**, 1–24.

DELBRÜCK, M. 1946 Bacterial viruses or bacteriophages. Biol. Rev. Cambridge Phil. Soc., **21**, 30–40.

DELBRÜCK, M., AND LURIA, S. E. 1942 Interference between bacterial viruses. I. Interference between two bacterial viruses acting upon the same host, and the mechanism of virus growth. Arch. Biochem., **1**, 111–141.

ELLIS, E. L., AND DELBRÜCK, M. 1939 The growth of bacteriophage. J. Gen. Physiol., **22**, 365–384.

GEST, H. 1943 The effects of inorganic salts on the multiplication of bacterial viruses. J. Infectious Diseases, **73**, 158–166.

GREENSTEIN, J. P., CARTER, C. E., AND CHALKLEY, H. W. 1947 Enzymatic degradation of ribosenucleic and desoxyribosenucleic acids. Cold Spring Harbor Symposia Quant. Biol., **12**, 64–94.

HERSHEY, A. D., KALMANSON, G., AND BRONFENBRENNER, J. 1943 Quantitative methods in the study of the phage-antiphage reaction. J. Immunol., **46**, 267–279.

KRUEGER, A. P., AND STRIETMANN, W. L. 1938 Effect of sodium sulfate on the phage-bacterium reaction. J. Gen. Physiol., **22**, 131–138.

SCRIBNER, E. JANE, AND KRUEGER, A. P. 1937 The effect of NaCl on the phage-bacterium reaction. J. Gen. Physiol., **21**, 1–16.