CHEMICAL STUDIES IN HOST-VIRUS INTERACTIONS*

VII. A Comparison of Some Properties of Three Mutant Pairs of Bacterial Viruses, T2r$^+$ and T2r, T4r$^+$ and T4r, T6r$^+$ and T6r

By SEYMOUR S. COHEN, Ph.D., and RACHEL ARBOGAST

(From The Children's Hospital of Philadelphia (Department of Pediatrics) and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia)

(Received for publication, January 23, 1950)

Some metabolic properties of virus-infected bacteria have been described in previous papers from this laboratory and have been summarized elsewhere (1, 2). It has been shown that many aspects of the metabolism of the bacteria seem unaltered, *e.g.* respiration and the assimilation of constituents from the media, while virus and virus constituents are synthesized. It would appear that the host's enzymes still maintain vigorous activity.

However, the qualitative course of synthesis is changed. The infected bacteria are unable to multiply or synthesize many substances and enzymes which the uninfected cells are fully capable of making. The bulk of the products of synthesis are virus materials, in large measure isolable in virus. These phenomena are particularly striking in the case of nucleic acid synthesis. The host cell formerly capable of synthesizing both ribose nucleic acid (RNA) and desoxyribose nucleic acid (DNA) is capable of synthesizing only DNA, the sole nucleic acid shown to be a virus constituent.

It has also been shown that the kinetics of the synthesis of virus constituents of virus are unlike the multiplication of normal cells, being independent of the number of intracellular virus particles. And it has been found that virus synthesis occurs in stages, protein being formed first, followed by purine and pyrimidine synthesis (3), succeeded by the formation of protein-bound DNA, and finally intact virus particles. It has been considered desirable to extend much of the data, obtained for the most part on T2r$^+$-infected bacteria, to some other viruses.

The accumulation of these data was also considered important for a problem with which we are concerned, namely the specific nature of inheritable differences among the bacterial viruses. Although some data are available comparing the chemical properties of some related plant viruses (4) it should be noted that the genetics and physiology of the bacterial viruses are more readily susceptible of analysis. Therefore the bacterial virus systems provide for the

* This research was conducted at The Children's Hospital of the University of Pennsylvania under Office of Naval Research Contract N6ori-188, Task Order 1, NR 136-055.

time being a much better system for the correlation of genetic, chemical, and physiological similarities and differences.

In this paper data will be presented constituting a comparison of some properties of three mutant pairs of bacterial viruses, T2r$^+$ and T2r, T4r$^+$ and T4r, T6r$^+$ and T6r, their one-step growth curves, adsorption to their host bacteria, and protein and DNA synthesis within the infected cells.

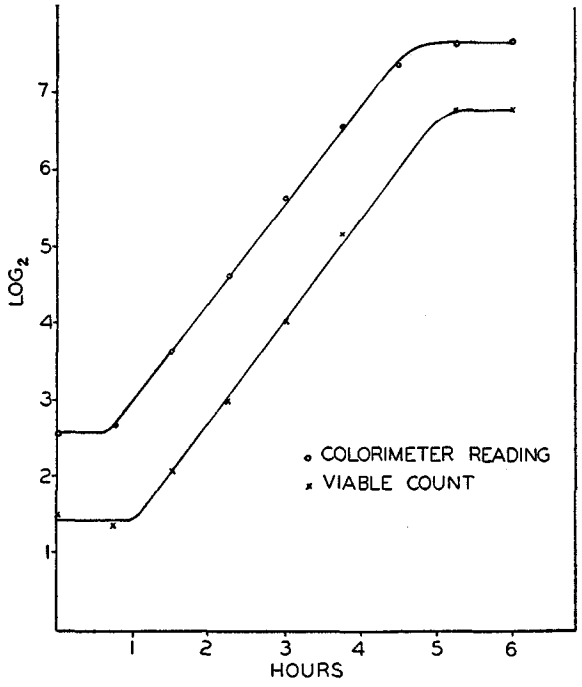

FIG. 1. A comparison of the viable count and turbidity of a culture of *E. coli* strain B during active growth at 37° in an aerated glucose medium. Viable count = colony count $\times 10^7$ per cc.

Methods and Materials

Bacterial Growth.—Escherichia coli strain B was subcultured monthly to Difco agar nutrient broth slants. From these, weekly subcultures were made to a chemically defined liquid medium containing (in per cent): glucose 0.1, Na_2HPO_4 1.64, KH_2PO_4 0.15, $(NH_4)_2SO_4$ 0.2, $MgSO_4 \cdot 7\ H_2O$ 0.02, $CaCl_2$ 0.001, and $FeSO_4 \cdot 7\ H_2O$ 0.00005. These cultures were aerated at 37° overnight; they grew to a concentration of about 1.2×10^9 bacteria per cc., as determined by a viable count. The limiting factor in this medium, as described elsewhere (5), was glucose. The organisms stopped multiplying while still in their exponential phase. On daily subculture from the exhausted medium to fresh medium containing glucose, the organisms had a lag phase of about 45 minutes, unlike strain K-12 which had no lag phase on subculture under these conditions. It can be seen in Fig. 1 that the turbidity and viable count curves as a function of time were exponential and parallel throughout the entire period of multiplica-

tion, unlike these functions for this strain in the lactate medium or in broth used in earlier studies (6). For most studies a culture freshly grown to 2×10^8 bacteria per cc. was used, as determined in the Klett-Summerson photoelectric colorimeter fitted with a 420 filter. The colorimeter at this bacterial concentration gave a reading equivalent to that of the same concentration of broth-grown organisms, indicating that the sizes of the two were similar. On the other hand, lactate-grown organisms were much smaller when cultured to this concentration and gave a lower colorimeter reading.

Virus Strains.—The bacteriophages T2r$^+$, T2r, T4r$^+$, T4r, T6r$^+$, and T6r were obtained from Dr. A. H. Doermann, then of the Carnegie Institution. The r strains were obtained by picking an r plaque which appeared as a spontaneous mutation in the plating of the r$^+$ strain. These r plaques were subcultured on *E. coli* and the lysates so obtained were reanalyzed by plating on various resistant and non-resistant *E. coli* strains. It was found that the T2, 4, or 6 phages were free of the other types. The r or r$^+$ cultures contained less than 0.1 per cent of r$^+$ or r phages respectively. All the r phages gave clear-edged, haloed plaques. However, the plaque sizes of the viruses were in the order T4r > T6r > T2r. The r$^+$ phages uniformly gave small fuzzy-edged plaques due to the phenomenon of r$^+$ lysis inhibition (7, 8). This phenomenon explains respiration and turbidimetric experiments reported in Paper I in this series, in which periods of measurement were used far greater than the normal latent period observed in singly infected cells.

The purified phage preparations were of two types. T2r$^+$ and T2r preparations had been grown by the method of confluent lysis in Hershey agar and purified as described (9). T4r$^+$ T4r, T6r$^+$ and T6r were grown in the glucose medium by multiple infection of bacteria at 2×10^8 per cc., and harvested at an approximately maximal titer. This titer was reached in 1 hour with the r strains, as determined from the one-step growth curves and turbidimetric studies, while direct assay established the appropriate harvest time of 3 hours for T6r$^+$ and 5 hours for T4r$^+$. Thus r$^+$ lysates were initially obtained at titers of 5 to 7×10^{10} per cc., while r lysates had 3 to 4×10^{10} virus particles per cc. In this medium a spontaneous inactivation of 10 to 20 per cent per hour took place at 37° and rapid chilling and isolation of virus at the correct harvest time were important. The relationship of these points to the purity of the isolated virus product has been discussed at some length elsewhere (9).

The virus concentrates, prepared by differential centrifugation, were stored in 0.85 per cent NaCl at 4°. Periodic examination and assay over the course of a year revealed a slow inactivation which was far less than that of virus similarly prepared in lactate medium (10). The viscosity of the glucose-grown virus preparations was comparable to broth-grown virus, a relationship in accord with the high depolymerase activity of the host cells, revealed in lysis.

Analyses.—One-step growth curves were determined by the technique of Delbrück and Luria (11). Virus assay employed the spreading and layering modification of Hershey *et al.* (12). The ultraviolet absorption spectra of virus concentrates were determined in a Beckman spectrophotometer in 0.0335 M phosphate buffer at pH 7.0. The estimation of DNA and protein in infected cells has been described (8).

The adsorption of the virus strains to B was studied by two techniques. The bacteria were grown to 1.3×10^9 per cc. overnight, sedimented, washed with the defined medium free of glucose, and resuspended in this medium at about 1 to 2×10^8 per cc. The bacteria were assayed by colony count. Virus was added to give a final concentration of 1 to 2×10^7 per cc. at 38°. Aliquots were removed periodically and centrifuged at 2500 R.P.M. for 4 minutes. The supernatant was assayed for free virus.

The screening of the virus for adsorption cofactor requirements (13) was done by plating a given virus strain by the Hershey technique on broth agar and on agar containing the glucose medium described above. It was anticipated that viruses requiring an adsorption cofactor would give rise to plaques in broth agar but not in glucose agar.

RESULTS

Ultraviolet Absorption Spectra.—No significant differences between r and r^+ strains of the T pairs have been observed. In Fig. 2 is presented a comparison of spectra for $T6r^+$ and T6r determined at 0.0303 mg. virus DNA per cc. The analyses were run in 0.0335 M phosphate buffer at pH 7.0. The K values at 2600 Å (K = density per mg. P per cc., as defined by Putnam *et al.* (14)) for these T6 preparations and DNA were 363 and 278 respectively.[1]

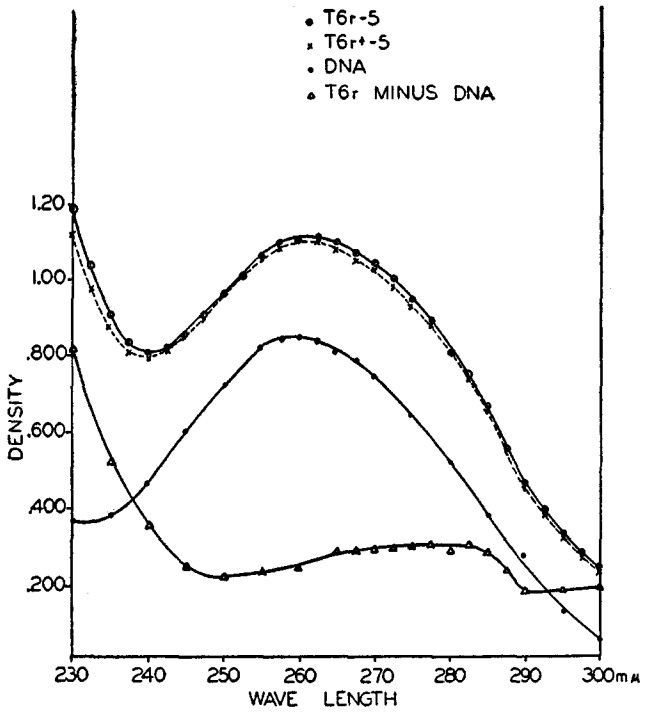

FIG. 2. A comparison of the ultraviolet absorption spectra of $T6r^+$, T6r, and desoxyribose nucleic acid (DNA) at the same DNA concentrations.

The densities of the various T virus preparations at 2600 Å were quite similar. No effort will be made to consider the significance of slight quantitative differences in the K values at various wave lengths. These were of the order of 10 per cent and less among the T group. However, when the spectra for T2, T4, and T6 viruses were compared by adjustment to the same density at 2600 Å, it could be seen clearly that the three virus groups had differences in absorption spectra which have been found to be reproducible for our strains.

[1] The K values for $T6r^+$ given by Putnam *et al.* indicated densities about 2.2 times those which we have found. We have recently been informed by Dr. Putnam that his values are in error and have been redetermined.

In Fig. 3 are presented such a comparison of a T2r, T4r, and T6r strain in phosphate buffer. Any one of these r strains had been shown to give essentially the same absorption spectrum as the homologous r^+ strains. It can be seen that there are significant differences between T2, T4, and T6, T4 and T6

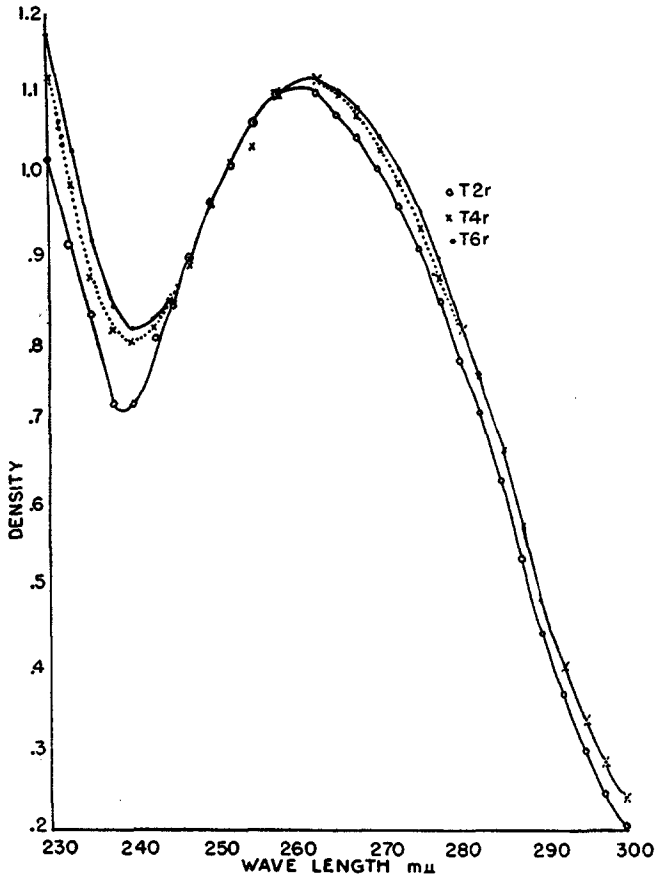

FIG. 3. Comparison of the ultraviolet absorption spectra of T2r, T4r, and T6r bacterial viruses.

containing relatively more ultraviolet-absorbing protein constituents. It can be seen in Fig. 2 that these protein constituents in T6 absorbed in the region 2700 to 2850 Å. For T2 these protein constituents were shown by a similar technique to absorb predominantly about 2800 Å suggesting relatively more tyrosine in T6 than in T2.

Adsorption.—In the screening tests for adsorption cofactors it was noted that the plating efficiencies of all the phages were markedly decreased on glucose agar. This, of course, would tend to obscure the results. In Table I

are presented data on such a test. It can be seen that on glucose agar the assays of T2r$^+$, T2r, T4r$^+$, and T4r were quite low but by roughly the same amount. The apparent titer of T6r however had fallen to one-fiftieth of that on broth, that of T6r$^+$ being about one-third to one-fifth of the titer in broth. These results were reproducible with other preparations of the same strains.

Although this crude test suggested that only T6r and possibly T6r$^+$ required a cofactor for the development of a reproductive cycle, the more exact adsorption tests revealed that only our T2r$^+$ and T2r strains lacked adsorption

TABLE I

The Assay of Bacterial Viruses on Nutrient Broth Agar and on Glucose Agar

Virus	Nutrient agar titer per cc.	Glucose agar titer per cc.
T2r$^+$	5.16×10^{11}	3.29×10^{11}
T2r	3.79×10^{11}	2.37×10^{11}
T4r$^+$	1.86×10^{11}	1.31×10^{11}
T4r	2.19×10^{11}	1.15×10^{11}
T6r$^+$	1.48×10^{11}	4.4×10^{10}
T6r	1.89×10^{11}	3.5×10^{9}

TABLE II

Adsorption Rate Constants K Cm.^{3}/Min.

Virus	0 tryptophane	50 γ tryptophane per cc.	Limiting tryptophane concentration*
T2r$^+$	1.63×10^{-9}	—	—
T2r	1.65×10^{-9}	—	—
T4r$^+$	0	9.0×10^{-10}	0
T4r	0	7.6×10^{-10}	6.0×10^{-10}
T6r$^+$	0	8.9×10^{-10}	2.1×10^{-10}
T6r	0	8.8×10^{-10}	0

$$K = \log_e \frac{\text{Initial virus}}{\text{Free virus}} \times \frac{1}{\text{Time}} \times \frac{1}{\text{Concentration B}}$$

B, 1.6 to 2 $\times 10^8$ per cc.; temperature, 38°C.; initial virus, 2×10^7 per cc.

* T4, 0.2γ tryptophane per cc.; T6, 0.05γ per cc.

cofactor requirements. As shown in Table II, T2r$^+$ and T2r had the same adsorption rate constants in the absence of added cofactor.

The T4 and T6 strains were then tested in the presence of added *l*-tryptophane and as shown in Table II, it was found that this amino acid acted as an adsorption cofactor. In excess tryptophane, the adsorption rate constants for T4r$^+$, T4r, T6r$^+$, and T6r were quite similar. Typical data are given in Fig. 4 for T4r$^+$ and T4r. In view of these results, the screening experiments indicated that the bacteria used in the assay secreted enough adsorption cofactor

for T4r$^+$, T4r, T6r$^+$, but not T6r. This suggested that tests at limiting tryptophane concentrations might reveal differences in the adsorption cofactor requirements especially between the T6 viruses.

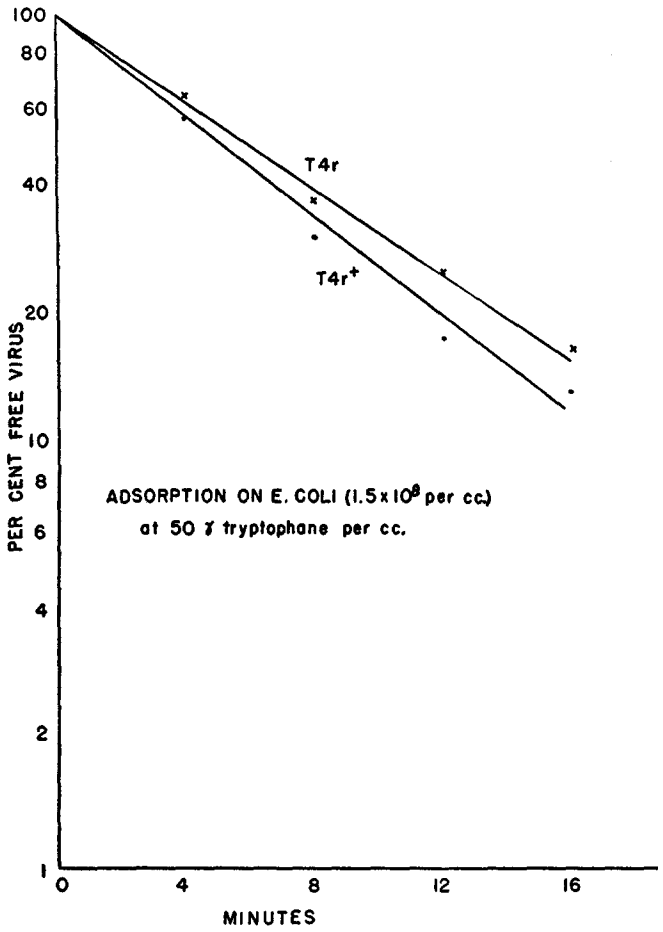

FIG. 4. The adsorption curves of T4r$^+$ and T4r on *E. coli* in a mineral medium containing an excess of tryptophane.

Contrary to expectation it was found that such differences could be readily demonstrated with the T4 strains but only slightly, if at all, with the T6 viruses. In Fig. 5, it can be seen that at tryptophane concentrations of 0.2 γ per cc., T4r was scarcely adsorbed, if at all, whereas T4r was adsorbed at almost a normal rate. At this concentration of tryptophane the T6 viruses were both slowly adsorbed at similar rates. No tryptophane concentration was

found at which T6r was markedly less rapidly adsorbed than T6r$^+$ or the T4 strains.

The significance of the screening test for adsorption cofactors by plating on glucose agar is therefore somewhat obscure. However, in additional tests

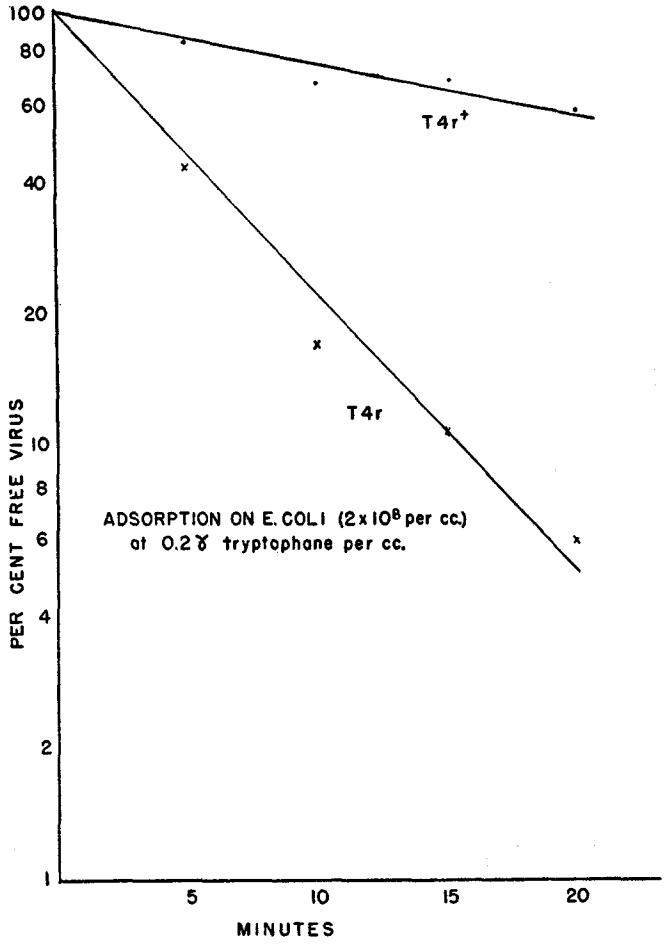

FIG. 5. The adsorption curves of T4r$^+$ and T4r on *E. coli* in a mineral medium containing limiting amounts of tryptophane.

it has been shown that supplementing the glucose agar with tryptophane did increase the plating efficiency of T6r.

One-Step Growth Curves.—

In these studies, bacteria were grown to the same concentration as was used in subsequent chemical studies; *i.e.*, 2×10^8 per cc. Experiments were run under conditions of single and

multiple infection, adsorption being carried out at relatively high concentrations of both reactants; *i.e.*, 2×10^8 bacteria per cc. or 2×10^7 2×10^9 virus particles per cc. The tubes were diluted during the latent periods to eliminate readsorption of virus liberated from infected cells. Free virus was estimated in supernatant fluids after centrifugation of infected cells.

In Table III are presented data summarizing the latent periods, rise periods, and burst sizes for the 6 viruses under conditions of single infection. It can be seen that the T2 viruses have indistinguishable latent periods and burst sizes, with a slightly shorter rise period for the r strain. The consistently low burst sizes suggest an inability of a large proportion of infected cells to lyse. This hypothesis was supported by turbidimetric and other studies. It has been shown (3) that cells infected with this strain of T2r$^+$ under conditions of mutual

TABLE III

*One-Step Growth Experiments for Various Bacterial Viruses at Single Infection**

Virus	Latent period	Rise periods, approx. time	Apparent burst size
	min.	*min.*	
T2r$^+$	27–28	20	50–60
T2r	27–28	11	35–80
T4r$^+$	33–36	20	70–115
T4r	27	12	110–180
T6r$^+$	30	15	75–105
T6r	35	20	75–120

* Adsorption period—5 minutes. Several experiments were made for each virus.

reactivation did not lyse even though intracellular virus was demonstrable by a cyanide-lysis technique (15).

The T4r$^+$ strain was shown to have a consistently longer latent period and rise period than the T4r strain. On the other hand, it was found that T6r had significantly longer latent and rise periods than the homologous T6r$^+$ virus. Under conditions of single and multiple infection an increase in infectious centers of about 50 per cent appeared at about 25 minutes in the T6r systems and remained level until the major burst occurred at 35 minutes. One-step growth experiments on multiple infection were determined in systems in which other functions were simultaneously studied and will be discussed below.

Virus Production and DNA Synthesis.—Studies of DNA synthesis were undertaken under conditions of infection, with about 5 virus particles per cell; *i.e.*, 2×10^8 bacteria and 10^9 virus per cc. The adsorption rates of the phages in these media indicated that each bacterium would successively adsorb virus particles throughout the latent period. Under the conditions of our experiments with our virus strains, lysis inhibition was not only a reinfection phenomenon by virus liberated at the beginning of the burst in the culture but

also due in part to repetitive infection during adsorption even at these low multiplicities. When one-step growth of r^+ strains was determined in dilution after a 20 minute adsorption period, marked increases of latent periods and rise periods were found. Data are presented in Table IV summarizing one-step growth experiments under these conditions for the various mutant pairs which were examined simultaneously.

In Figs. 6 to 8 are presented the DNA syntheses simultaneously determined in these same systems of undiluted suspensions of infected bacteria. It is seen in these studies that no significant difference in the rate of DNA synthesis was observed between r^+ and r strains. Each pair had a comparable short delay before DNA synthesis began, the delays with T2 and T6 being about 6 minutes, that with T4 10 to 12 minutes. The rates of increment were identical and constant through most of their course. The sole detectable

TABLE IV

*One-Step Growth Experiments for Various Bacterial Viruses under Multiple Infection**

Virus	Latent period	Rise periods, approx. time	Apparent burst size
	min.	*min.*	
$T2r^+$	30	40	7
T2r	27	13	15–37
$T4r^+$	33	40	60
T4r	27	13	200–230
$T6r^+$	45	15	35
T6r	35–38	15	120–160

* Adsorption period—20 minutes. The virus to cell ratio was 5.0.

difference was the time at which r-infected bacteria stopped synthesizing DNA. This corresponded to some point within the rise period, at which point lysis was considerably advanced.

It was possible to estimate from the T4r and T6r experiments the amount of DNA synthesized per particle. In the T4r experiment presented, a burst size of 230 had been obtained in a one-step growth experiment from a culture of 1.54×10^8 infected bacteria per cc. which had synthesized 9.5 γ DNA per cc. The DNA synthesized per liberated particle was therefore 2.6×10^{-16} gm.

In the T6 experiment in Fig. 8 a burst size of 120 was obtained in a system containing 2.65×10^8 bacteria per cc. which had synthesized 10.4 γ DNA per cc. The DNA synthesized per liberated particle was therefore 3.3×10^{-16} gm.

Protein Synthesis in T4r$^+$- and T4r-Infected Cells.—In Fig. 9 is presented a comparison of protein and DNA syntheses in bacteria simultaneously infected with a 5-fold multiplicity of $T4r^+$ or T4r. It can be seen that no significant difference is to be observed between these systems over the period before

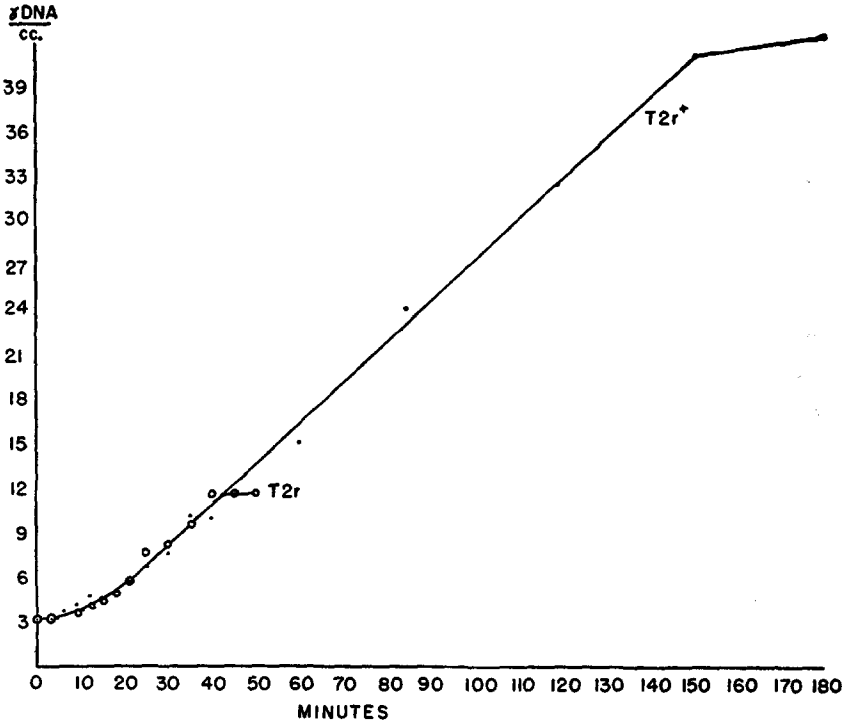

FIG. 6. A comparison of synthesis of DNA in cells multiply infected with T2r+ or T2r virus.

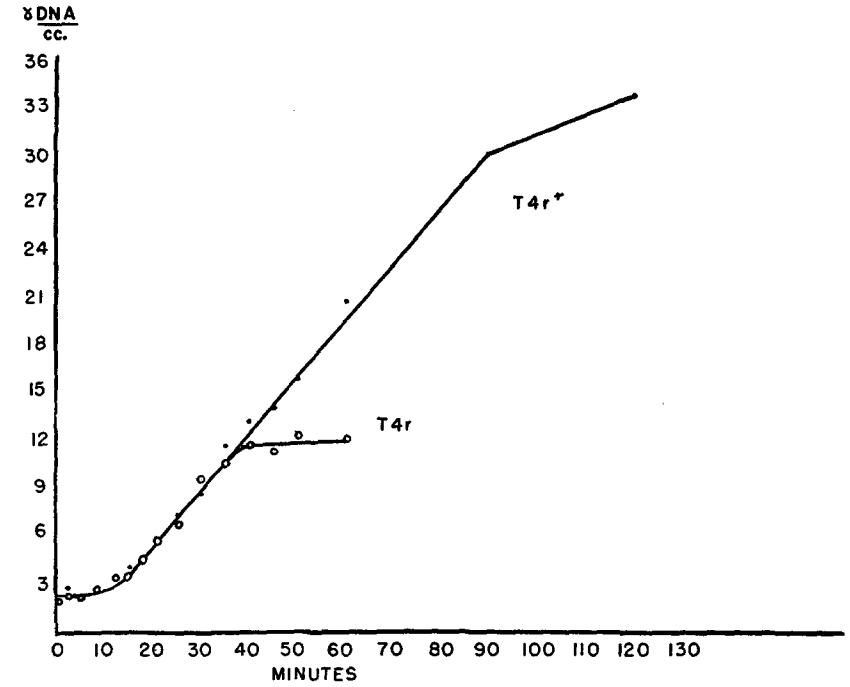

FIG. 7. A comparison of synthesis of DNA in cells multiply infected with T4r+ or T4r virus.

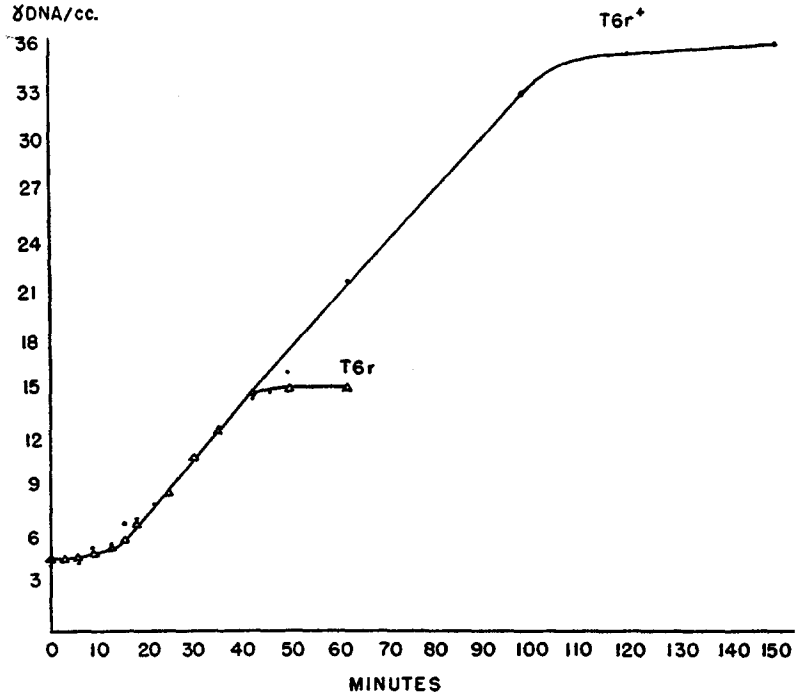

FIG. 8. A comparison of synthesis of DNA in cells multiply infected with T6r$^+$ or T6r virus.

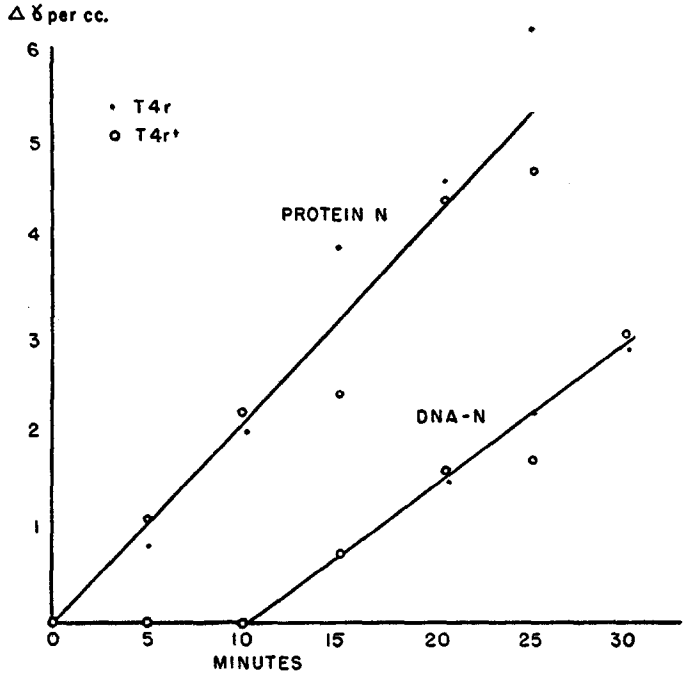

FIG. 9. A comparison of protein and DNA synthesis in cells multiply infected with T4r$^+$ or T4r virus.

reinfective lysis inhibition occurred. As described earlier, DNA synthesis stopped in the r system between 30 and 40 minutes as massive lysis occurred. Protein synthesis could not be studied in the r system further since lysis was accompanied by vigorous proteolysis.

In Fig. 10 is presented the complete course of a T4r$^+$ experiment. The slight but significant proteolysis, before reinfection inhibited further lysis, displaced

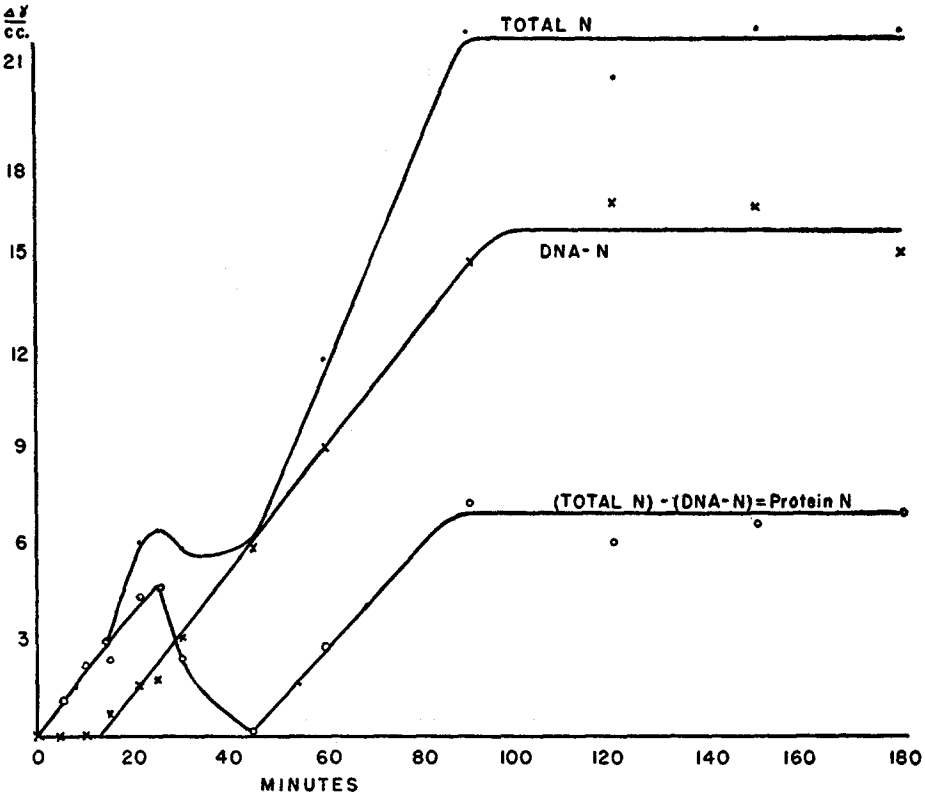

FIG. 10. The synthesis of protein and DNA in T4r$^+$-infected cells.

the protein increment curve, which then continued at approximately the initial rate. The DNA synthesis was not displaced indicating that the proteolysis was not associated with DNA-containing structures or did not affect precipitation of DNA. These results are in apparent contrast to earlier T2r$^+$ data in a lactate medium which suggested an inhibition of the rate of protein synthesis during lysis inhibition. The latter impression arose from the masking of proteolysis during primary lysis by the coincidental circumstances of especially poor lysis in T2r$^+$ systems and weak proteolysis during the lysis of lactate-grown bacteria.

Turbidimetric Study of Infected Cultures.—In Figs. 11 and 12 are presented comparisons of the turbidities of cultures infected simultaneously with the r or r^+ strains respectively. In the comparison of the r strains an initial dip in the turbidities of the cultures was seen followed by a recovery of turbidity just prior to the burst to approximately the original level. The inception of the major dips in infected cultures occurred at times corresponding to the end of

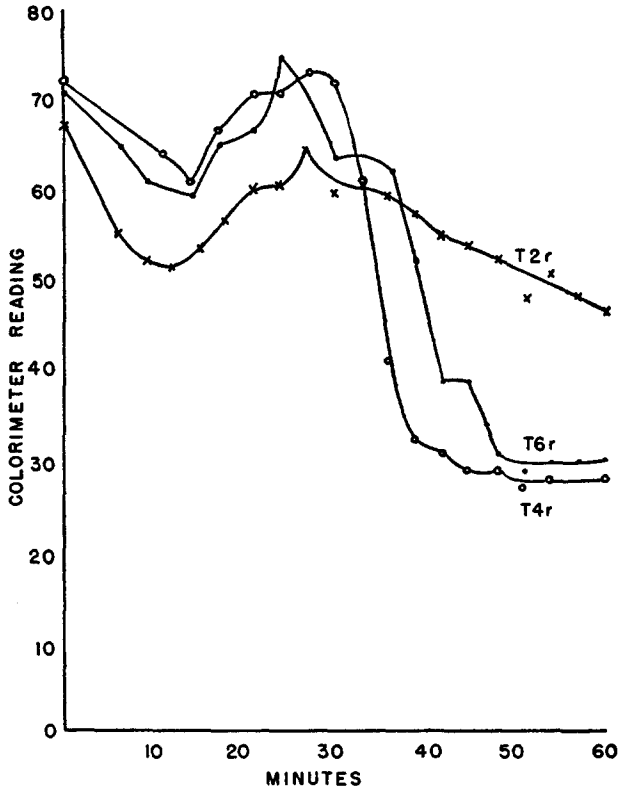

FIG. 11. A comparison of the turbidities of cultures containing *E. coli* multiply infected with T2r, T4r, or T6r virus.

latent periods, as determined in one-step growth experiments. A short preliminary dip in the T6r culture coincided very well in time with the small increment in infectious centers regularly seen at 25 minutes in one-step growth experiments. Characteristically the turbidity fall in the T2r culture was slow.

In the r^+ cultures it is seen that lysis inhibition was produced in the order $T2r^+ > T4r^+ > T6r^+$. Studies of virus liberation in these systems showed that the rise periods of the cultures ended at $T2r^+$–6 hours, $T4r^+$–5 hours, and $T6r^+$–4 hours. In these systems, however, virus appeared throughout the

period of turbidity increase to the extent of 10 to 20 per cent of the maximum titer obtained. This indicated a slow breakdown of some cells before the major lysis occurred.

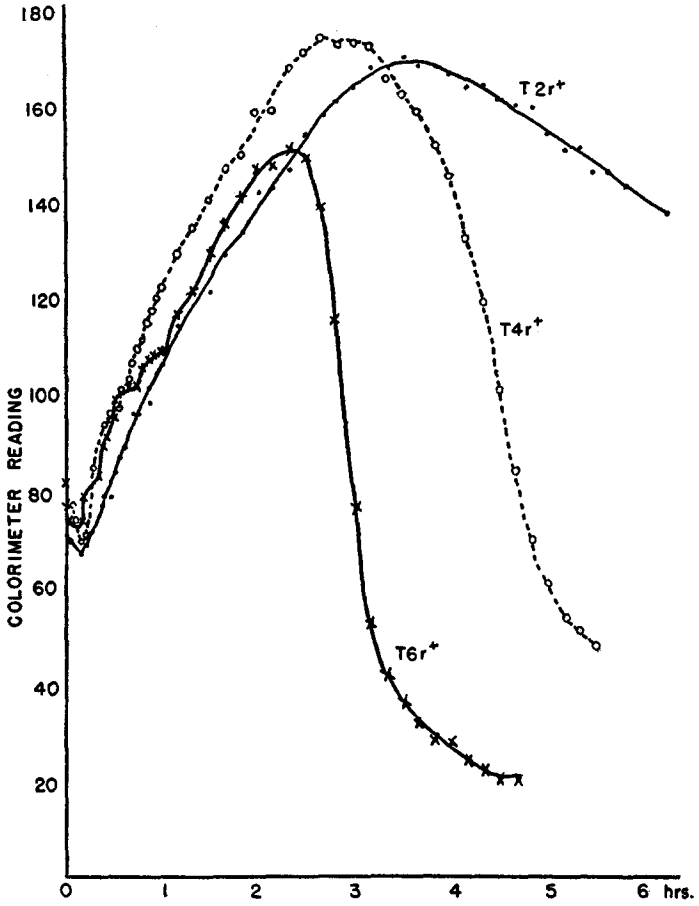

FIG. 12. A comparison of the turbidities of cultures containing *E. coli* multiply infected with T2r+, T4r+, or T6r+ virus.

Comparison of DNA Synthesis in r-Infected Bacteria.—The theory had been advanced that the quantitative aspects of the synthesis of virus constituents are predominantly a function of bacterial capabilities and enzymes. Therefore it might be anticipated that the rates of DNA synthesis, for example, would be the same in cells, aliquots of which were simultaneously infected with different viruses. As in Fig. 13 this was indeed found, thereby providing an additional partial confirmation of the theory. Although T4r-infected cells start somewhat

later, and lyse slightly earlier, the maximal rates of DNA synthesis for the three types of infection were independent of the virus strains used.

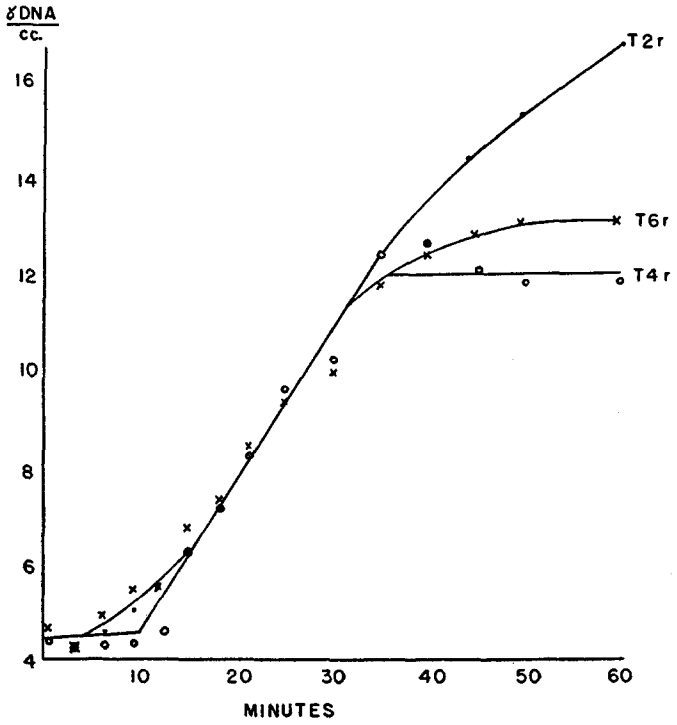

FIG. 13. A comparison of synthesis of DNA in cells multiply infected with T2r, T4r, or T6r virus.

DISCUSSION

No chemical or physiological difference has as yet been found which clarifies the nature of the $r^+ \rightarrow r$ mutation. It has been shown that the occurrence of this mutation does not affect adsorption in T2 systems, DNA synthesis in T2, T4, and T6 systems, nor protein synthesis in T4 systems. Consistent differences between all r and r^+ strains have been revealed only in that functional character by which the r mutation is described, lysis and lysis inhibition.

Some other functional differences have been observed between some r and r^+ strains. For instance, significant differences in the adsorption cofactor requirements, and latent periods have been revealed between T4r^+ and T4r, T6r^+ and T6r. Their significance with respect to the $r^+ \rightarrow r$ mutation is obscure since the variations are in opposite directions in the T4 and T6 pairs and do not even appear in the T2 set.

The fact that inheritable differences can be found as a mutational step from one virus to another does cast light upon some current biochemical theories of gene action. One extreme view frequently expressed at the present time asserts that the loss of a gene or its change is invariably accompanied by a loss or change in a basic enzyme whose production the gene controls. No virus enzymes have as yet been implicated in virus synthesis; in fact the existing evidence strongly suggests the contrary view that the host cells effect the synthesis with host enzymes. Further, since a virus-infected cell has not yet been observed to synthesize enzymes of any type, it is difficult to see how genetic change in these viruses is associated with enzyme production. The existence of genetic change among the viruses would appear to be an outstanding exception to the one gene–one enzyme hypothesis. One alternative explanation could possibly point to structural alterations of the specific models which the virus presents to the synthetic mechanisms.

The figures presented above for the DNA synthesized per particle liberated in T4r and T6r infections show a fairly close agreement with the values presented elsewhere for the DNA per particle in the most active isolated virus preparations obtained. T4r and T6r had 2.6 and 3.3×10^{-16} gm. DNA synthesized per virus particle liberated respectively. Isolated T4r preparations had 2.4 and T6r 3.4×10^{-16} gm. DNA per active particle. In general, T4r was observed to have larger burst sizes than T6r although the nucleic acid synthesized by infected cells was of the same order. This may be ascribed to a lower DNA per particle of T4r or a lower efficiency of recovery of T6r from lysed cells, as does appear to be the case for T2r. However, from Fig. 11 T6r-infected bacteria appeared to lyse as thoroughly as in T4r systems.

The large turbidity increments observed with r^+-infected cells in this glucose medium were not previously observed in the lactate medium. They are ascribed to a swelling of the cells which has been observed microscopically by many workers immediately prior to lysis (see also section on bacterial growth). This swelling must have been due to osmotic effects as well as an increase of solids. The synthesis of both DNA and protein in the $T4r^+$ system for example, stopped at 90 minutes although the turbidity increased at about the same rate for 60 minutes more before lysis began. Therefore it cannot be concluded that lysis was a result of the depletion of structural elements for synthesis of various viral constituents. It is possible that the enzymatic mayhem observed in lysis is due to the structural disorganization of cellular enzymes provoked by osmotic distension. This, however, does not explain the fact that similarly swollen cells (infected by $T2r^+$ or $T4r^+$) lysed at very different rates.

Finally it is evident that these data provide an extension and confirmation of early data confined in the main to $T2r^+$ to r and r^+ strains of T2, T4, and T6. The picture elaborated then of the course of the phenomena in virus reproduction is in the main confirmed and extended.

SUMMARY

Various chemical and physiological aspects of the reproductive cycles of r^+ and r strains of T2, T4, and T6 viruses have been examined and compared. These include the ultraviolet absorption spectra in which differences between r and r^+ strains were not observed, though they were obtained in the case of T2, T4, and T6. Adsorption of T4 and T6 was found to require the adsorption cofactor *l*-tryptophane. Among the r and r^+ strains of these viruses limiting tryptophane requirements for adsorption were found to be different. Some differences were observed in the one-step growth curves of these viruses under conditions of single and multiple infection. The turbidity-time relations of infected cultures were characteristically different. The rates of DNA and protein synthesis in the infected cells were found to be independent of the viruses used. Certain implications of the data have been discussed.

BIBLIOGRAPHY

1. Cohen, S. S., *Cold Spring Harbor Symp. Quant. Biol.*, 1947, **12,** 35.
2. Cohen, S. S., *Bact. Rev.*, 1949, **13,** 1.
3. Cohen, S. S., and Arbogast, R., *J. Exp. Med.*, 1950, **91,** 637.
4. Knight, C. A., *J. Biol. Chem.*, 1947, **171,** 297.
5. Cohen, S. S., *J. Biol. Chem.*, 1949, **177,** 607.
6. Cohen, S. S., and Fowler, C. B., *J. Exp. Med.*, 1948, **87,** 275.
7. Hershey, A., *Genetics*, 1946, **31,** 620.
8. Cohen, S. S., *J. Biol. Chem.*, 1948, **174,** 281.
9. Cohen, S. S., and Arbogast, R., *J. Exp. Med.*, 1950, **91,** 607.
10. Cohen, S. S., *J. Biol. Chem.*, 1947, **168,** 51.
11. Delbrück, M., and Luria, S. E., *Arch. Biochem.*, 1942, **1,** 111.
12. Hershey, A. D., Kalmanson, G., and Bronfenbrenner, J., *J. Immunol.*, 1943, **46,** 267.
13. Anderson, T. F., *J. Cell. and Comp. Physiol.*, 1945, **25,** 17.
14. Putnam, F. W., Kozloff, L. M., and Neil, J. C., *J. Biol. Chem.*, 1949, **179,** 303.
15. Doermann, A. H., *Carnegie Institution of Washington Year Book No. 47*, 1948, 176.