CHEMICAL STUDIES ON HOST-VIRUS INTERACTIONS

I. The Effect of Bacteriophage Adsorption on the Multiplication of Its Host, Escherichia coli B

With an Appendix Giving Some Data on the Composition of the Bacteriophage, T2

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INTRODUCTION

The past decade of chemical virology has been concerned in the main with the characterization of the virus particle. This approach to the chemical problems of viral infection has systematically relegated to the background the rôle of the host and the specific nature of its response to the virus. However, investigators have noted that variations in the nutritional state of the host markedly change the course of a specific infection (1–6). Chemical alteration of tobacco mosaic virus has produced a greater change of infective competence of the virus with respect to one host than another (7, 8). More recently attempts have been made to study directly the metabolic behavior of virus-infected tissue (9) and of virus-resistant tissue (10–13). The more detailed metabolic studies on tumors are pertinent to this line of attack if it can be demonstrated that these tumors are associated with a viral infection (14).

The characterization of the virus particle has contributed a great deal to our knowledge. Much information has been obtained concerning (1) the specific nature of the virus active in a specific infection, and concerning (2) the dissimilarities and similarities of virus particles, information essential to the understanding of viral parasitism in general. This type of information has not yet permitted extrapolation to the chemotherapy of virus diseases, a few of which have responded only to an empirical approach (15, 16). Nor have any specific clues been gleaned in recent years as to the mechanism of autocatalytic duplication although frequent analogy has been drawn to normal nucleoprotein synthesis and function within the cell.

Nevertheless, the numerous analytical studies on different virus preparations lead to a general type of approach, away from the previous main line of study. Thus, the enzymatic equipment of the viruses which have been studied to date is so limited as to strongly suggest that they are incapable of carrying out the metabolic reactions essential for independent energy production and utilization.

This biochemical limitation has been defined by the very organization of the viruses, their inadequate content of internal metabolites, coenzymes and enzymes, and the presence of unusual chemical groupings which may reflect an incomplete metabolic processing (17). One can conclude from this generalization that the host must provide the major part of substance and energy essential for virus synthesis, in a manner directed by the presence of the mediating virus particle. It is clear then that the crux of the problem of viral infection in general lies within the sphere of the host-virus interaction.

With a theory of this sort, some disquieting data in the realm of the animal viruses become not only reasonable but also expected. It has been reported, for instance, that in preparations of Rous sarcoma virus (18), fowl leucosis virus (19), influenza virus (20, 21), and mouse pneumonitis virus (22) antigens and structures characteristic of the normal host tissues are to be found in a manner suggestive of organic union. This situation obviously makes difficult the interpretation of numerous belabored chemical analyses on the aforementioned viruses, the equine encephalomeylitis group, rabbit papilloma virus, etc. These situations must be expected at some phase of virus synthesis, since it is highly improbable that metabolism of the host modified by the virus can occur without a close relationship of the virus particle and the organized metabolizing structures of the host.

An approach to this problem is not a simple matter. It is difficult enough to analyze the metabolic behavior of normal tissues such as liver, muscle, nerve, etc.; this analysis becomes much more complicated when the metabolism is modified by viral infection. The metabolism of a complex tissue such as lung or liver during infection raises problems concerning which of the various types of cells present in the tissue have been infected, what proportion of the whole they comprise, etc. The importance of this point has been attested to recently in the study of poliomyelitis-infected nervous tissue (23). In addition, alterations of the metabolism of non-infected cells may also be produced as a result of the products of metabolism of infected cells.

Of considerable importance to the biochemist is the additional requirement that the metabolic process which he has chosen to follow is of such a qualitative and quantitative nature that variations may indeed be observed with available techniques. Thus variations in the metabolism of ribose nucleic acid of tobacco cells infected with tobacco ring spot virus (24) would be difficult to study since the quantity of this substance which appears in the small yield of virus is very low indeed and the nature of metabolism of this substance in normal tissue is almost totally unknown. Despite the interest of this problem, the choice of the ring spot virus-tobacco system would not facilitate the production of significant results concerning the aberrant metabolism of this substance.

From these points of view, it has appeared potentially fruitful to study the metabolic phenomena associated with the bacteriophage—*E. coli* system. The

uniform preparation of host and virus in suitable amounts is relatively simple. Each component of the system may be assayed with a relatively high degree of accuracy and ease. Known numbers of host cells can be infected with known numbers of virus particles. Production of the host and infection and multiplication of virus can occur, in the same synthetic medium, under relatively controlled conditions. Many biological aspects of infection in this system are known (25–29), and the genetics and physiology of this and related systems are being actively studied. Some of the biological attributes of this system are markedly similar to the behavior of other virus systems, for instance, the interference phenomenon, etc. Hence data obtained in studies on the bacterial virus systems are at least potentially pertinent to other virus systems.

As described in the appendix to the present paper, chemical data have been obtained which describe the T2 bacteriophage as a form possessed of the limited equipment of the viruses in general and an unusually high desoxyribose nucleic acid content which would facilitate studies of this component in viral synthesis.

In this paper are presented simple respiration studies of two bacterial viruses acting on their host in a synthetic ammonium lactate medium. These orienting examinations were undertaken to enable us to see whether the utilization of this medium is affected during infection.

The observation was made that a primary effect of viral adsorption in this system is the loss of the host's ability to multiply without any gross change in the rate of O_2 consumption or the respiratory quotient.

Material and Methods

Nomenclature.—E. coli B (28) is identical with strain PC of Kalmanson and Bronfenbrenner (30). Virus T2 has been called PC phage by the latter authors (30), and virus gamma by Luria and Delbrück (29). The relationships of T4 to T2 and the other viruses in the "T" set have been described by Anderson, Delbrück, and Demerec (31).

Preparation of Bacteria.—Escherichia coli B was used as the host for the bacterial viruses T2 and T4 and was subcultured daily. The organisms were grown in an aerated synthetic medium (F) containing 10 gm. sodium lactate, 1 gm. NH_4Cl, 0.7 gm. K_2HPO_4, 0.3 gm. KH_2PO_4, 0.1 gm. Na_2SO_4, 0.01 gm. $MgSO_4$ per liter of distilled water. After suitable periods of growth at 37°, they were sedimented and washed in 0.85 per cent NaCl or in F medium. Plating on solid nutrient agar or on the synthetic medium containing 1 per cent agar revealed a maximum growth of 5×10^9 cells per cc. after 24 hours of aeration in F medium at 37°. The turbidity of this suspension measured on a Klett-Summerson photoelectric colorimeter with a 540 filter yielded a reading of 385–415. This instrument was used in following gross turbidity changes in several of the experiments.

Preparation of Purified Bacteriophage.—The purification procedure employed and the chemical properties of T2 are described in the appendix to this paper. Virus assay followed the procedure of counting plaques described by Delbrück and Luria (28). The ultraviolet irradiation and inactivation of phage have been described previously (32).

Fragmentation of Bacteria.—The bacteria were disrupted with a modified Peirce magnetostriction oscillator with a frequency of about 8900 cycles per second, as described by Chambers and Flosdorf (33). The vibrated suspensions were fractionated into low speed sedimentable

debris, a fraction sedimented at 30,000 R.P.M. for 1 hour, and a high speed non-sedimentable supernatant fluid.

Estimation of Respiration.—The consumption of oxygen was determined by the usual methods in Warburg manometers at 38°C. Respiratory quotients were determined by measuring the CO_2 liberated on tipping in acid at the start and conclusion of an oxygen consumption determination. We are indebted to Dr. W. C. Stadie of the Department of Research Medicine of the University of Pennsylvania for the use of his Barcroft-Warburg apparatus in some of the experiments reported in this paper.

A suspension of 1.1×10^{10} active T2 particles in 0.5 cc. of F medium consumed no measurable quantity of oxygen in 60 minutes after the addition of 1.25 cc. of F medium. A culture of bacteria at maximal growth, 6.25×10^9 bacteria in 1.25 cc. F, consumed 8.45 μ M O_2 in 30 minutes. The rate of O_2 consumption was constant for this period.

The Effect of the Addition of T2 or T4 to E. coli B.—A fresh bacterial suspension at a concentration of about 10^9 organisms per cc. was placed in Warburg vessels and its O_2 consumption rate determined for 20 minutes. The basal rate at such high bacterial concentrations changed very slowly or was constant in this interval. With the basal rate established, F medium or phage particles in F medium were tipped from a sidearm into the vessel and the continuing O_2 consumption measured. The addition of fresh F medium alone produced an increase in the rate of respiration which roughly paralleled bacterial multiplication and the turbidity of the bacterial suspensions in separate simultaneously shaken flasks. In one of these experiments in which the turbidity-time curve paralleled the O_2 consumption curve, an increase in turbidity of 189 per cent was matched with an increase in bacterial count of 183 per cent.

The addition of T2 resulted for the most part in an inhibition of the increase of respiration, with a maintenance of the basal respiratory rate for 1 to 3 hours depending on the conditions of the experiment. There would then occur a slow decrease of the rate of O_2 consumption. This effect occurred with bacteria in their lag phase or with actively multiplying organisms.

The adsorption of T4 on B has been demonstrated to require a cofactor such as *l*-tryptophane (34). Therefore, in experiments in F medium, it was necessary to add this substance to permit cellular infection by T4. In this system, essentially the same phenomenon was observed as in T2 infections: cells infected with T4 in the presence of tryptophane failed to increase their rate of O_2 consumption but maintained the basal rate of respiration characteristic of the bacteria prior to infection. In Table I are presented the contents of the Warburg vessels corresponding to the O_2 consumption curves presented in Fig. 1. Bacteria plus T4 without cofactor apparently multiplied normally.

The Respiration of Bacteria Treated with Partially Inactivated T2.—T2 can be partially inactivated by ultraviolet light (28); *i. e.*, rendered incapable of multiplying within its host. T2 inactivated in this way, however, is still capable of being adsorbed by the bacteria, of inhibiting bacterial multiplication,

TABLE I

The Oxygen Consumption of E. coli Infected with Bacteriophage T4 in F Medium

Vessel	Center compartment	Side arm	Center well
1	1 cc. B* + 0.1 cc. T4 + 0.4 cc. F	0.4 cc. Try + 0.1 cc. F	0.2 cc. 2 N NaOH
2	1 cc. B + 0.5 cc. F	0.4 cc. Try + 0.1 cc. T4	
3	1 cc. B + 0.1 cc. T4 + 0.4 cc. F	0.5 cc. F	
4	1 cc. B + 0.5 cc. F	0.4 cc. Try + 0.1 cc. F	

* B = bacteria in F medium (5×10^8/cc.); T4 = bacteriophage in F medium (7×10^{10}/cc.); Try = *dl*-tryptophane in F medium (200 γ per cc.). The virus to cell ratio of this experiment was $\frac{0.1 \times 7 \times 10^{10}}{1 \times 5 \times 10^8} = 1.4$.

Results presented in Fig. 1.

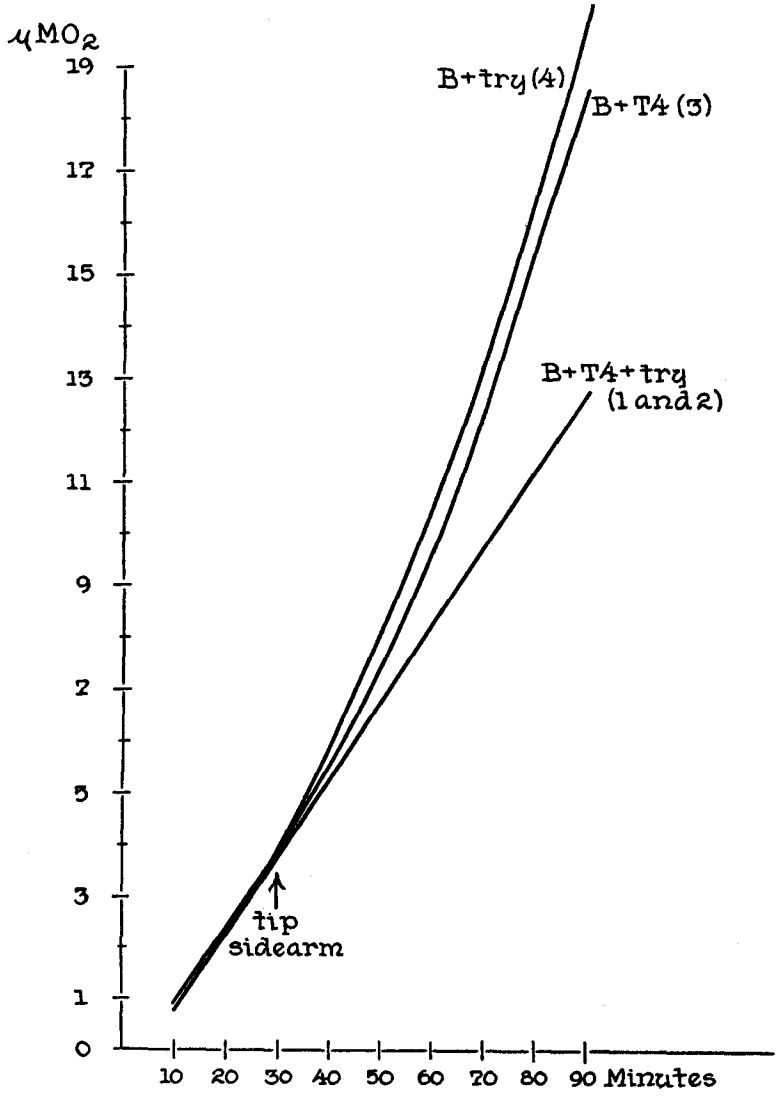

FIG. 1. The oxygen consumption of *E. coli* infected with bacteriophage T4 in synthetic medium. See Table I.

of interfering with the growth of other viruses on the bacteria, and of partially inhibiting the multiplication of fully active T2 on the infected cell (29). The ultraviolet irradiation of purified solutions of active T2 of a titer of 10^{10} was carried out for periods necessary to reduce the titer of active T2 by a factor of 10^6.

The addition of irradiated partially inactivated T2 to bacteria in Warburg vessels resulted in the maintenance of the linear basal respiratory rate as did the fully active bacteriophages. The turbidities of such cultures remained approximately constant in contrast to those of multiplying cultures.

The Respiratory Quotient of Infected and Normal Bacteria.—The ratios of CO_2 production to O_2 consumption were determined for bacteria infected with active T2, for bacteria treated with ultraviolet-inactivated T2, and for normal bacteria. In the experiment presented in Fig. 2, the reaction vessels contained 1 cc. of bacteria in F, with a titer of 1.5×10^9/cc., and 0.3 cc. F, to which were added 0.5 cc. F, or 0.5 cc. purified T2 in F (titer 1.7×10^{10}/cc.), or 0.5 cc. irradiated purified T2 in F (original titer 10^{10}; titer after irradiation, 3×10^4 cc.). Normally multiplying organisms had an R.Q. of 1.06 ± 0.04. Cells infected with active T2 had an R.Q. of 1.07; those treated with partially inactivated T2 had an R.Q. of 1.02. It is considered that these differences are not significant. Thus, active or partially inactive T2 appeared to stop bacterial multiplication without reducing respiratory rate or inducing any gross change in the respiratory quotient in F medium.

The Effect of Initial Turbidity Change on the Rate of O_2 Consumption.—The frequent occurrence in these systems of constant respiratory rates without apparent lysis of the bacteria as reflected in absence of turbidity decrease (32) is most puzzling. The one-step growth curves described by Delbrück and Luria (28) which demonstrate a relatively short period of virus multiplication within the bacteria, constant for single or multiple infections, followed by a period of rapid rise of virus release concomitant with lysis in the *E. coli*—T2 system were not observed in the systems just described. The one-step growth curves have been worked out for much more dilute bacterial suspensions than those employed for measurements of O_2 consumption and apparently do not apply in concentrated systems.[1]

An initial turbidity decrease is to be seen occasionally, accompanied by a roughly proportional decrease in the basal rate of O_2 consumption. In one instance, the addition of active purified T2 to B in the ratio of 3.3 virus particles to 1 cell produced a slow turbidity decrease of 23 per cent over 40 minutes. The linear rate of O_2 consumption for 50 minutes was 30 per cent less than the basal rate. The addition of partially inactivated T2 to B in the same ratio of original active particles to B of 3.3:1 produced a rapid turbidity decrease of 30 per cent for 10 minutes, and a continuing 13 per cent decrease for 60 minutes.

[1] Delbrück, M., personal communication.

The linear rate of O_2 consumption for this entire period was 34 per cent less than the basal rate.

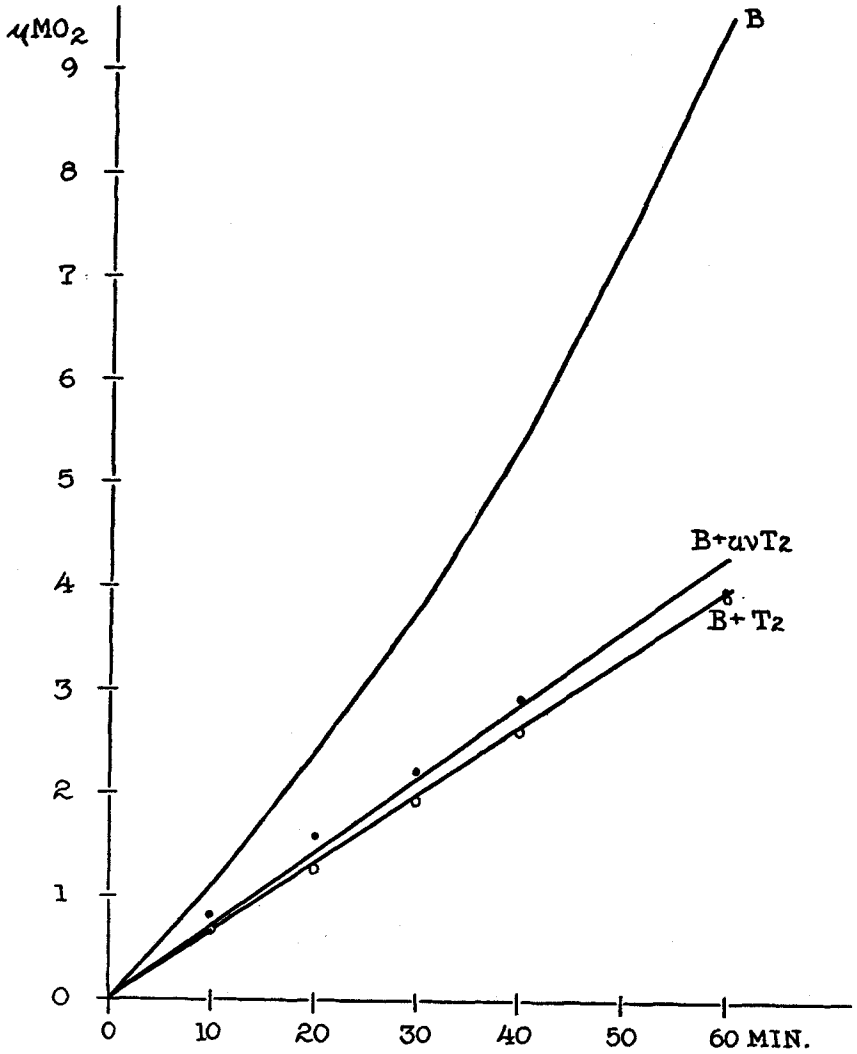

FIG. 2. The oxygen consumption of *E. coli* B, *E. coli* infected with active bacteriophage T2 (B + T2), or *E. coli* treated with irradiated partially inactivated T2 (B + UVT2).

It is to be noted, however, that frequently neither of these types of changes occurred although high ratios of virus to host cells tended to produce turbidity decreases. Delbrück (27) has distinguished between lysis from within and lysis from without due to a high ratio of externally adsorbed virus to cell. It is likely that these turbidity decreases were of the latter type.

The Inhibition of Cell Multiplication by a Lysate after Removal of Phage.—It was observed that, after removal of 97 per cent of the phage from a bacterial lysate by sedimentation at 10,000 R.P.M. for 2 hours, the supernatant fluid on addition to bacteria at an active virus to cell ratio of 1:6 produced complete inhibition of multiplication for 1 hour, as indicated by the constancy of the respiration rate. The R.Q. of the inhibited organisms over a period of an hour was 1.08. When the supernatant fluid from the lysate, diluted with two parts of F, was added to *E. coli*, a short temporary inhibition and a rapid recovery to the normal multiplication rate were observed. This effect was not produced by the supernatant fluid of a 24 hour culture of *E. coli* B at maximal growth. These observations led us to examine the bacteriostatic action of fractions of artificially disrupted *E. coli*.

An Inhibitor of Multiplication Released from Sonically Vibrated Organisms.—A culture (250 cc.) of 24 hour aerated organisms in F was centrifuged. The bacteria were then suspended in 18 cc. of F, and vibrated for 1 hour. A portion of the disintegrated material was centrifuged at 4000 R.P.M. for 30 minutes and the supernatant fluid was sedimented at 30,000 R.P.M. for 40 minutes. The resulting pellet was resuspended in the same volume of F. The cellular particles appearing in this high speed sedimentable fraction seem to be comparable to cytoplasmic particles in their structural and functional properties. Thus this fraction contains a complete lactic acid dehydrogenase system, comprised of cyanide-sensitive and cyanide-insensitive portions.[2] In the F medium this would be the first carbon-utilizing system of *E. coli*. The addition, therefore, of the high speed sediment to fresh F medium containing lactate results in the rapid linear consumption of O_2 with respect to time, according to mechanisms which have been described (35–37). A much smaller fraction of lactic acid dehydrogenase was found in the high speed non-sedimentable fraction.

It might be expected that the addition of either of these fractions to a multiplying bacterial culture should appear in a respiration experiment as the superposition of the linear O_2 consumption of the sedimentable enzyme system on the increasing respiration of multiplying organisms. The inhibition of bacterial multiplication should appear as a constant rate of respiration, although at a higher rate than the basal rate of the bacteria alone. This inhibition was produced by the high speed sedimentable fraction. On dilution of this fraction the inhibitory effect disappeared only when the increment in basal rate approached zero.

The high speed non-sedimentable fluid contained far less inhibitory substances. The multiplication rate of *E. coli* was considerably increased in the presence of this high speed non-sedimentable fluid at a dilution beyond which the inhibition of multiplication occurred.

It appears, therefore, that an inhibitor of *E. coli* multiplication is present

[2] Haugaard, N., personal communication.

within the organism. Sedimented preparations of the inhibitor contained particles bearing lactic acid dehydrogenase activity. It is not unlikely that this inhibitor is similar to that present in an *E. coli* lysate.

DISCUSSION

Luria and Delbrück have observed that virus-infected bacteria will not produce colonies and that irradiated virus, T2, does in fact prevent *E. coli* from dividing (28, 29). Furthermore, the ability of irradiated T2 to interfere with the multiplication of a dissimilar bacterial virus, α or T1, parallels the inhibition of bacterial multiplication. The development of a constant turbidity curve when fully active T2 was added to B had previously been noted (32). The observations of constant respiratory rate, reported in this paper, for cells infected with active or irradiated virus appear to agree with these previous observations and extend them more fully to active virus.

In studies to be published shortly, Dr. W. Henle and Dr. G. Henle have indicated that addition of irradiated influenza virus to the chorio-allantoic sac of the 8 day embryonated hen's egg inhibits the normal growth of the allantoic sac around the egg. In previous experiments by these authors (38) membranes treated with irradiated influenza virus did not support the growth of active influenza virus, or unrelated viruses. When these inhibited membranes were compared 8 days after inoculation with 16 day normal membranes which had markedly increased their weight in this period, the rates of O_2 consumed per mg. dry weights in glucose or allantoic fluid were found to be identical.[3] Thus an apparent parallelism has been noted between the *E. coli*-phage system and the chorio-allantoic membrane-influenza virus system with respect to (*a*) various aspects of the interference phenomenon, and (*b*) the inhibition of cellular multiplication, without gross change in oxygen utilization.

Cellular proliferation has been considered to be a characteristic feature of many virus diseases (39). Since the multiplication mechanisms of infected host cells are blocked in two diverse types of virus infection, the extension of this phenomenon to other systems is not unlikely. The problem is then posed whether the proliferation observed in many systems is present in infected cells or in normal cells stimulated to division by products other than virus from nearby infected cells. Although there would seem to be little doubt that the former is true for various tumor viruses, even here it has not yet been demonstrated rigorously on a cellular basis.

Inhibition of cellular division does not necessarily result in interference with virus multiplication. Spizizen (40) has reported phage multiplication in *E. coli* in glycine anhydride, a medium which did not support bacterial division. Anderson has shown that bacteria whose division has been inhibited by ultra-

[3] Cohen, S. S., Henle, W., and Henle, G., unpublished data.

violet irradiation still support phage multiplication.[4] Since cellular division probably requires the coordination of numerous enzyme systems, it is entirely possible that the stages affected by interference are more intimately associated with virus multiplication than are the stages affected by starvation with glycine anhydride or short periods of ultraviolet irradiation.

The relationships between inhibition of multiplication by phage and the inhibition produced by the intracellular substance liberated by lysis are not clear. One might speculate that the initial lysis of a few organisms in a concentrated suspension might liberate enough intracellular inhibitor to add an additional interference effect to that induced by the phage alone. This might then be responsible for the aberrancies in one-step growth curves determined in concentrated bacterial suspensions.

SUMMARY

The addition of active or irradiated T2 bacteriophage and T4 bacteriophage to *E. coli* B stops bacterial multiplication. The respiratory rate and respiratory quotient of the inhibited bacteria remained at the values observed just before infection. A respiratory rate decrease which occasionally appears can be roughly correlated with change of turbidity of the suspension. An intracellular inhibitor of multiplication appears to be liberated into lysates. A similar substance has been separated from normal *E. coli* B after sonic disintegration. These bacteriostatic preparations contain cytoplasmic granules with lactic acid dehydrogenase activity. The relationship of these phenomena to the interference effect in this system and others has been considered.

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APPENDIX

Some Data on the Composition of the Bacteriophage, T2

Electron micrographs of T2 bacteriophage (1) have revealed the presence of characteristic tadpole-shaped bodies consisting of a head containing regions of high and low density, and a tail. The dimensions of the head are 65 × 80 mμ, and the straight tail is 120 mμ long and 20 mμ thick. It has been suggested that the dense areas of the head, resembling similar differentiated structures in the elementary bodies of vaccinia virus (2) consist of nucleoprotein of characteristic high density. This hypothesis has conflicted with the analyses of purified preparations of this bacteriophage reported by Kalmanson and Bronfenbrenner (3, 4) who obtained a negligible phosphorus content. We have

found dialyzed dried preparations of T2 purified by differential centrifugation to contain 3.7 per cent phosphorus and 37 per cent desoxyribose nucleic acid as determined by two different methods. Schlesinger had reported a P content of 3.7 per cent and a positive Feulgen reaction for this, or what must have been a very similar bacteriophage (5, 6). The preparation of phage concentrates has been described by Anderson (7).

A suspension of *E. coli* B in aerated F medium was infected with T2 bacteriophage and incubated at 37° until lysis was considerably advanced. Debris and bacteria were removed by centrifugation at 5000 R.P.M. for 15 minutes. The supernatant fluid containing virus was centrifuged at 10,000 R.P.M. for 1 hour. The translucent pellet containing a large proportion of the activity of the fluid was resuspended and after a few days the fractionation was repeated. The purified T2 bacteriophage was resuspended in 1 per cent sodium chloride. When this preparation was examined in the electron microscope, its particulate elements appeared to consist of T2 phage particles. Examination of this material for purine desoxyriboside by the diphenylamine reaction (8) and for the total desoxyribose content by the perchloric acid-tryptophane reaction (9) in comparison with a standard of desoxyribose nucleic acid from thymus (10) revealed that the ratio of purine desoxyribose to total desoxyribose in T2 phage was 0.48. This ratio for the standard nucleic acid was 0.50, as determined in comparison with desoxyguanosine. This variation in T2 from the equivalence of purine and pyrimidine desoxyriboside found with the standard is probably within the experimental error inherent in the combined colorimetric methods used. T2 bacteriophage gave a color in the orcinol-sulfuric acid reaction which was not unlike that given by glucose.

The purified bacteriophage was dialyzed against running water. On dialysis the phage settled as a white gel which was difficult to disperse. It was dried *in vacuo* while frozen and finally over P_2O_5. The white solid had a nitrogen content by the Kjeldahl method of 11.84 per cent, a phosphorus content by the King method (11) of 3.66 per cent, and a desoxyribose nucleic acid content of 36.9 per cent (8). The orcinol-reactive carbohydrate content, after correction for the color produced by the desoxyribose nucleic acid present in this material was 5.40 per cent, calculated as glucose. The intensities of the Bial reaction for pentose and Seliwanoff reaction for ketose were equivalent to those of blanks containing the amount of desoxyribose nucleic acid present in the preparation (12). This indicated the absence of ribose and ketose.

Since the glyoxylic acid procedure for tryptophane is complicated by the presence of desoxyribose, the Lugg procedure (13) was employed on alkaline hydrolysates, yielding a tryptophane content of 1.45 per cent. The tyrosine content, as determined by the Bernhart method (14) on alkaline hydrolysates, was 2.77 per cent. The arginine content as determined by the method of Brand and Kassell (15) was 2.86 per cent. An inhibitor of arginine color formation

was not present in acid hydrolysates of T2, in contrast to hydrolysates of many proteins.

Crystalline ribonuclease, trypsin, and chymotrypsin, and Fairchild trypsin did not liberate soluble nitrogen from T2 nor inactivate the virus.

The molecular equivalence of desoxyribose to phosphorus, the former of which is distributed in two equivalent fractions, reactive as typical purine and pyrimidine desoxyribosides, indicates that all the phosphorus of the non-dialyzable constituents of T2 exists in a desoxyribose nucleic acid. This virus therefore is devoid of numerous phosphorylated compounds, such as ribose nucleic acid, phospholipid, etc. In this respect, namely, the exclusive presence of only one type of nucleic acid, the desoxyribose type, the T2 bacteriophage appears to be similar to two other cellularly organized viruses, the elementary bodies of vaccinia (16) and the rickettsiae of epidemic typhus (17). However, T2 contains much more of this substance than do vaccinia, rickettsiae, and rabbit papilloma virus (18).

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