CHEMICAL STUDIES IN HOST-VIRUS INTERACTIONS*

V. Some Additional Methods of Determining Nutritional Requirements for Virus Multiplication

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It has been demonstrated in the previous paper (1) that the composition of the medium of the host cell affects the rate and amount of T2 bacteriophage synthesized in and liberated from infected *Escherichia coli*, strain B. The effects of numerous compounds on virus multiplication in a minimal medium (F) have been described; stimulatory effects by single supplementary compounds have been interpreted to signify that the compound plays a rôle in virus synthesis. Thus, a number of amino acids and nucleic acid derivatives were shown to be of interest as a result of this test. It was found that methionine sulfoxide, an antagonist of the stimulatory glutamic acid, interfered with virus reproduction.

A complex defined medium has been described consisting of the F medium supplemented with amino acids, purines, and pyrimidines, which almost duplicated the nutritive qualities of the broth medium for virus multiplication. The necessity for these amino acids, purines, or pyrimidines may be tested by studying the course of virus multiplication in the complex defined medium, in which single constituents are omitted. In this type of experiment, the course of virus multiplication has been followed by two methods: (1) the one-step growth technique previously described (1), and (2) the estimation of the synthesis of desoxyribose nucleic acid (DNA) in multiply infected cells in the appropriate media.

The use of DNA synthesis as a measure of virus multiplication in the T2 system follows from studies on synthesis in infected cells in F media. Thus it has been shown elsewhere (2, 3) that the onset and amount of DNA synthesis, which occurs at a constant rate, can be correlated with (1) the time of appearance of more than one virus particle within an infected cell, (2) the amount of virus liberated at 25 to 30 minutes, and (3) the amount of virus liberated by lysis-inhibited cells. The method has already been used to demonstrate the greater stimulatory value of supplementing the F medium with

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a mixture of amino acids as compared to the most stimulatory single amino acid, glutamic acid (3).

Materials and Methods

Preparation of Bacteria.—*E. coli* B were grown in nutrient broth (N) as described previously (1). The bacteria, B_N, were grown to about 5×10^7 per cc. for one-step growth studies, and to 2×10^8 per cc. for studies of DNA synthesis in infected cells. The bacteria continued in a logarithmic phase with respect to division until about $1\text{-}2 \times 10^9$ per cc. However, the amount of protoplasm as followed turbidimetrically in a Klett-Summerson colorimeter with a 420 filter increased at the same logarithmic rate only until $2\text{-}4 \times 10^8$ per cc. and then increased at a much slower rate. Thus B_N studied in the one-step growth experiments were in their logarithmic phase with respect to division and growth, while B_N used in studies of DNA synthesis were approaching a change in synthesis affecting growth but not division. Nevertheless, B_N concentrations of 2×10^8 per cc. were chosen to avoid the use of large volumes of media, suspensions, and aliquots otherwise required for the DNA estimations.

The bacteria were washed twice in F medium and resuspended in the medium of desired composition at the concentration to which the bacteria had been grown. In almost all of the experiments, B_N from the same culture were used for the two different tests involving the same media.

Virus.—Purified concentrates of $T2r^+ - F$ and $T2r^+ - N$ in 0.85 per cent NaCl were used (4).

Media.—The amino acids used were all of the *l*-configuration. The complete defined medium has been described in the preceding paper (1). The relative proportions of the amino acids were arbitrarily chosen to approximate a casein hydrolysate in the minimal F medium (4). To this mixture were added adenine, guanine, cytosine, and thymine at 10 γ per cc. In the following experiments, virus synthesis in B_N was tested in the complete defined medium, the F medium, and the complete medium minus a single constituent

Analyses.—Virus production in singly infected cells in the various media was determined by the one-step growth technique of Delbrück and Luria (5). The course of DNA synthesis in the various media was determined by the application of the diphenylamine reaction to the trichloroacetic acid precipitates of infected cells (3).

RESULTS

One-Step Growth Experiments

In Table I is presented a summary of results obtained by this technique. It may be seen that there was very little change in the latent period on omission of a single constituent, despite the fact that with many amino acids the burst size or average number of virus particles per infected bacterium was significantly reduced. Although tests have not been carried out to determine whether all the infectious centers liberate virus on the depleted medium, it appears probable that they do. It is considered that a decreased burst size means a decreased number of particles synthesized per infected bacterium. The lack of correlation of latent period to burst size is striking; lysis appears to be almost independent of the number of virus particles in the cell.

In Fig. 1 are presented some experiments of this type. The effect of omission of leucine and tryptophane is readily apparent. Although tryptophane has been found in T2 (4), and its importance for multiplication affirmed by the

antimetabolite technique (6), tryptophane was not stimulatory in the single supplement study (1). It can be seen, however, that its omission from the complete medium markedly reduced burst size.

By the single supplement technique, leucine was found to be inhibitory although the inhibition was readily overcome by the action of isoleucine, norleucine, or valine (1). Despite its inhibitory action in one type of test,

TABLE I

Effect of Omission of Single Constituents

Compound omitted	Increase in latent period	Decrease in burst size	Approximate delay in DNA synthesis
	min.	*per cent*	*min.*
Alanine	0	0	0
Arginine	0	0	0
Aspartic acid	0	0	0
Cystine	0	0	0
Glutamic acid	0.5	38	3
Glycine	0	0	0
Histidine	1	45	6
Hydroxyproline	0	0	0
Isoleucine	2	47	0
Leucine	3	57	11
Lysine	0	0	0
Methionine	1.5	0	25–45
Phenylalanine	0.5	32	7
Proline	0	0	0
Serine	0	0	0
Threonine	0	0	0
Tryptophane	1	50	11
Tyrosine	0	0	7
Valine	1	66	6
Adenine	0.5	0	Variable
Cytosine	0	0	0
Guanine	0	0	0
Thymine	0	0	0

leucine was, nevertheless, important in virus synthesis, as detected by the omission technique.

Some compounds such as glycine or threonine did not show an effect when omitted from the complete medium. This need not mean necessarily these compounds are not incorporated into virus or are not involved in some intermediary rôle in virus synthesis. Since many compounds, such as desoxyribose phosphate, must be synthesized from lactate and inorganic phosphate to form virus, these results may merely signify that compounds such as glycine and threonine are synthesized at a faster rate than other rate-determining compounds, such as desoxyribose phosphate.

Desoxyribose Nucleic Acid (DNA) Synthesis

Eight amino acids have been found important by the one-step growth test described in the previous section: they include isoleucine, phenylalanine, tryptophane, leucine, valine, glutamic acid, methionine, and histidine. By following the course of DNA synthesis in infected cells, effects have been noted with all of these but isoleucine. In addition, omission of tyrosine and adenine produced effects on the course of DNA synthesis but not on the one-step growth curves.

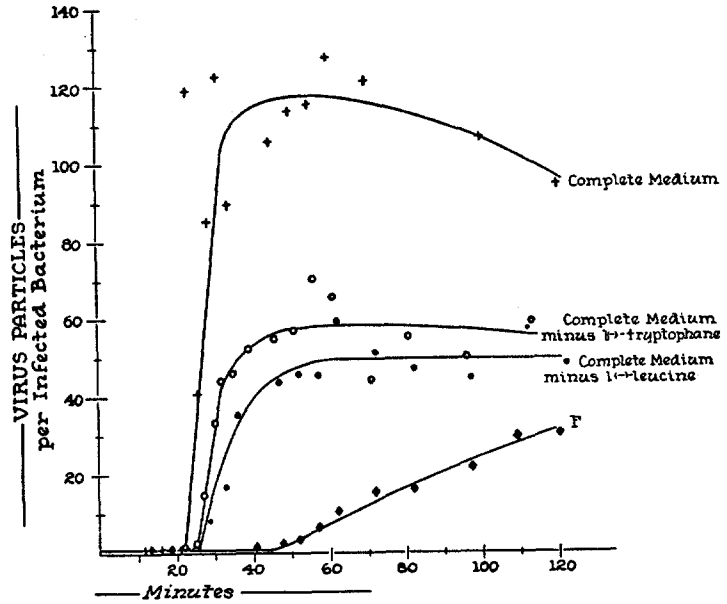

FIG. 1. The one-step growth curves of the T2r$^+$-*E. coli* system in media of different compositions.

In Fig. 2 are presented the curves for the synthesis of DNA in infected B_N suspended in the complete medium, the F medium, and the complete medium with tryptophane or leucine omitted. To B_N at 2×10^8 per cc. was added T2r$^+$ virus to give a final concentration of virus of 1×10^9 per cc. Two types of effect may be noted in this experiment. First, the omission of leucine or tryptophane resulted in significant delays in the onset of synthesis. Nevertheless, when synthesis began, it occurred at the same rate as in the complete medium. This type of effect has been noted consistently although the length of delay, as presented in Table I, depends on the amino acid. Secondly, the rate of synthesis fell off earlier in the case of the omitted amino acids. This was not noted frequently in 2 hour experiments. It was quite variable with the same amino acid and was only observed for tryptophane, leucine, and adenine.

Systems omitting adenine showed great variability. In several one-step growth experiments, no differences were noted from the complete medium. However, in four DNA synthesis experiments in media without adenine a marked delay of onset was observed in two, once the rate decreased sharply after 60 minutes, and in one experiment no difference was noted during the 2 hour interval.

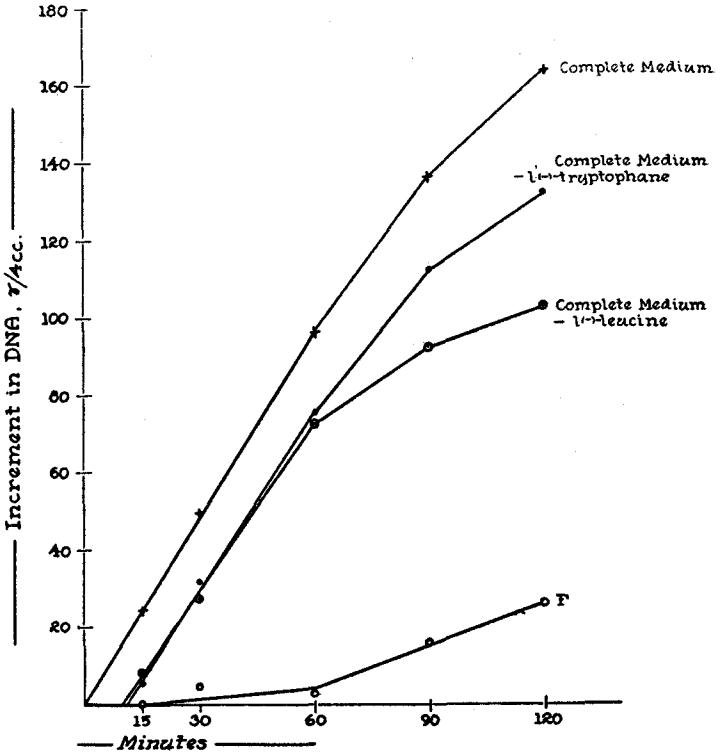

FIG. 2. The course of desoxyribose nucleic acid synthesis in the T2r$^+$-*E. coli* system in media of different compositions.

It was observed that the beginning of DNA synthesis in the complete medium varied from one culture of B_N to the next. This point is obtained by extrapolation of the linear increment of DNA to 0. In the experiment presented in Fig. 2, the curve was extrapolated very close to the origin. This occurred in a few experiments; in most cases, however, a latent period of up to 10 minutes was found.

In several experiments it was noted that the latent period of synthesis of DNA in F medium alone was exceedingly long; *i.e.*, about 80 to 90 minutes. The DNA formed after infection at this time in B_N at 2×10^8 per cc. could not

account for the virus liberated in comparable periods in this medium in the one-step growth test with B_N at 5×10^7 per cc. This effect is tentatively attributed to the decreased synthetic powers of B_N to the higher concentrations.

Under the conditions of this test, the effect of omission of methionine was the most marked of any of the amino acids, resulting in delays of synthesis of

TABLE II

Comparison of Results of Single Supplement and Single Omission Techniques

Compound	Effect of supplement	Effect of omission	
		One-step growth	Synthesis of DNA
Alanine	0	0	0
Arginine	+	0	0
Aspartic acid	+	0	0
Cystine	Inhibitory	0	0
Glutamic acid	+	+	+
Glycine	0	0	0
Histidine	Variable	+	+
Isoleucine	+	+	0
Leucine	Inhibitory	+	+
Lysine	+	0	0
Methionine	0	+	+
Phenylalanine	+	+	+
Proline	+	0	0
Serine	Inhibitory	0	0
Threonine	0	0	0
Tryptophane	0	+	+
Tyrosine	Variable	0	+
Valine	Variable	+	+
Adenine	0	0	+
Cytosine	0	0	0
Guanine	0	0	0
Thymine	0	0	0

The symbols + and 0 mean positive effect and no effect, respectively.

over 25 minutes. In contrast to this, one-step growth curves in media without methionine showed relatively small differences from the complete medium. This situation appears analogous to that described for F alone.

Simplification of the Complete Medium

As summarized in Table II, the single supplement technique indicated the need for seven amino acids, the omission techniques indicating a total of nine amino acids and one purine. Combination of these to include valine, isoleucine, leucine, phenylalanine, histidine, arginine, lysine, aspartic acid, glutamic acid,

methionine, tryptophane, tyrosine, and adenine as supplements to the F medium supported one-step growth curves comparable to that in the complete medium. The burst size was within 10 per cent of that in broth, the latent period being 1 to 2 minutes longer.

DISCUSSION

It is apparent that the techniques described in this paper permit the detection of compounds important for virus synthesis in addition to those revealed by the method previously described (1). By the three techniques as presented in Table II, thirteen amino acids and a purine have been shown to markedly affect the course of synthetic mechanisms tied to virus synthesis. However, the development of a medium for maximal virus synthesis in which the synthetic needs of the organism have been reduced to a minimum has not been completed. Compounds such as thymine or guanine are probably synthesized at a rate that precludes the detection of an effect on omitting them from the medium. The detection of a rôle for these compounds in virus synthesis might be accomplished by the use of competing structural analogues or more demanding host cells, such as mutant strains incapable of these syntheses.

In a host cell such as *E. coli* strain B capable of such varied synthesis, the interpretation of all the results is difficult. The more rigorously defined requirements of isolated animal cells, or of many bacteria may be expected to reveal many all-or-none effects in virus synthesis, which might be more easily interpreted.

In most of the experiments described in this paper the rate of DNA synthesis accounted very well for the amount of virus produced. For instance, the most active T2 preparation isolated in this laboratory contained 1.6×10^9 active particles per γ DNA. It may be calculated from the data in Fig. 2 that the amount of DNA synthesized at 30 minutes in the complete, tryptophaneless, and leucineless media accounted for burst sizes at that time of 99, 63, and 55, respectively. The observed burst sizes as presented in Fig. 1 were 118, 58, and 50, respectively. However, some conflicting results have been recorded. DNA synthesis did not correspond to virus production in the methionine-, tyrosine-, and isoleucine-deficient media. At present, these differences are attributed to differences in the age of the bacteria, and the difference in the ratio of virus to host cell in the two experimental systems.

SUMMARY

Omission of a single constituent from a chemically defined medium approximating the virus growth-promoting properties of broth affects virus production in infected bacteria. This may be estimated by the one-step growth technique and the course of desoxyribose nucleic acid synthesis. Nine amino acids and one purine have been shown to be important by these tests. A combination

of all constituents observed to be important by the single supplement and single omission techniques has approximated the virus growth-promoting properties of broth. Certain anomalous results have been commented upon.

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