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Scope and Method of Study: This study consists of a collection and organization of much of the published research done on the Escherichia coli, T2 bacteriophage system. An attempt was made to trace the elucidation of the complexities of this system from as early as 1948 to the present, by reading many of the sources and utilizing those which most clearly traced the evolution of the knowledge of this system.

Findings and Conclusions: The T2 phage, in order to reproduce itself in its host E. coli, must stimulate the formation of several enzymes, or enzyme complexes, which in turn catalyze the reactions which produce phage precursor molecules.

The phage must first halt all macromolecule synthesis within the host cell by a DNase, then produce a phage specific messenger RNA. With this RNA carrying the code for phage specific protein, the bacterial ribosomes and the transfer RNA of the host cell are borrowed, resulting in protein which functions as enzymes, or is phage precursor in nature.

The majority of the phage induced enzymes are concerned with the formation or replication of DNA, which is unlike that of the host cell in that it contains glucosylated deoxyhydroxymethyl cytosine derivatives. These enzymes lead directly to the synthesis of deoxyhydroxymethylcytidylate, its subsequent polymerization into phage DNA where it is glucosylated by further phage induced enzymes. Also deoxythymidylate is produced in the infected cell from deoxycytidylate by deamination and addition of a mole of formaldehyde.

At this point, the phage specific DNA and precursor protein pools in the host cell are "full". The DNA is crosslinked, the polyhedral head and tail are added, and the resultant phage are mature. The host cell is then lysed from within by a lysozyme whose synthesis was also induced by the attacking phage, and the phage progeny are released into the medium.

ADVISER'S APPROVAL L. Herbert Bruneau

# RESULTANT CHANGES IN THE METABOLIC PATHWAYS OF *ESCHERICHIA COLI* AFTER ATTACK BY T2 BACTERIOPHAGE

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RESULTANT CHANGES IN THE METABOLIC PATHWAYS

OF ESCHERICHIA COLI AFTER ATTACK

BY T2 BACTERIOPHAGE

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# PREFACE

At first thought, the value of extensive research into a seemingly inconsequential virus-host system such as the T2 bacteriophage and Escherichia coli may seem nil. However, if it is considered in the sense in which it is employed, as a model system, it becomes invaluable. Many characteristics of the system lend to its value as a model system; the availibility of both the host cell and the virus, the ease with which the bacteriophage can be assayed, the hydroxymethyl cytosine common to T-even phages being but a few.

Though researchers are well aware that all virus-host relationships are not as in the T2, E. coli system, they see the value in their investigations because of the similarity to other systems. Total enlightenment of the complexities of this system will surely add to the pool of general knowledge of viruses.

It is in this vein that I have researched and written this paper; the specific knowledge of this system being of little importance, rather the insight I have gained into the effects resultant in the host cell from viral infection.

Indebtedness is acknowledged to Dr. L.H. Bruneau, and Dr. E.T. Gaudy for their assistance.

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# CHAPTER I

## INTRODUCTION

In order for a given virus to reproduce itself after attacking any living system, be it a somatic cell of a vertebrate, a plant cell, or a bacterium; it must be capable of converting the "metabolic machinery" of that cell to such an extent that any resultant molecules produced are of a viral species. The attacking virus may stimulate the formation of entirely new metabolic pathways as well as "borrow" some of those which are active in the uninfected cell, but the end result must be those processes which ultimately result in the formation of new virus particles.

One may immediately think that such metabolic conversion would be quite simple in the T2, Escherichia coli system, due to the simple structure of the T2 bacteriophage. However, some insight into the structure of this phage at the molecular level, indicates that several differences exist which will make it necessary for the phage to create totally new enzyme series in order to produce the substances necessary for phage maturation.

In this system, the resultant metabolic pathways are clearly categorized relative to the time when they first appear after infection of the cell, and the duration of the

alteration. All these changes are such that they are totally necessary for the production of new phage particles, and the sequence in which they appear is perfectly adapted to the development of the mature phage particle.

## CHAPTER II

## METABOLIC CONVERSION

In order for virus induced enzymes to exert their total influence upon the metabolic machinery of the cell, the chemical synthesis in the infected cell must be brought to a halt. Cohen and Anderson (1946) have shown that immediately upon infection by T2, the formation of further respiratory enzymes cease, and the energy flow of the cell proceeds at a constant rate all during the latent period. This is to say that anabolic activities concerned with macromolecule synthesis cease, but catabolic, or energy producing reactions continue.

Morphological changes (to be discussed later in this paper) that occur in the host cell immediately after infection, make it evident that something is happening to the bacterial deoxyribonucleic acid (DNA). Pardee and Williams (1952) found an increase in deoxyribonuclease (DNase) activity in the infected cell and the same was found by Kunkee and Pardee in 1956. The results of their work led them to believe it was definate increase in the activity of DNase and they could not create the same effect by a variety of environmental changes. They also showed that the phage DNA must enter the cell to stimulate this

effect, as a phage "ghost" caused no increase in the activity of this enzyme. Both workers felt the observed effect resulted from bacterial DNase whose activation resulted from the destruction of a DNase inhibitor by the attacking phage.

Later work by Stone and Burton (1962) disproves the earlier theory of the destruction of an inhibitor, in that they were able to isolate a DNase whose highest specific activity is during that time of greatest protein synthesis in the host cell, and whose sensitivity to p-chloromercuribenzoate (PCMB) is a great deal different. This certainly tends to support the concept of a totally new phage induced enzyme. With the synthesis of this DNase, the "code center" for macromolecule synthesis is destroyed, and the invading virus is now in a position to rebuild a portion of the cell's metabolic machinery.

Almost immediately after infection by T2 (i.e. the injection into the host cell of the viral DNA), a change is detectable in the RNA produced by the infected cell. Cohen (1948), in his early experiments observed a cessation of RNA synthesis immediately after infection, and could not positively establish if RNA synthesis was then reestablished, or if so, the degree to which it was carried on. But he positively proved that protein synthesis continued in an infected cell at the same rate as that found in the cell prior to infection.

Several years later however, it was shown by Hershey

(1951) that there was active RNA synthesis in the cell after phage infection, and he felt it was most certainly coded from the viral DNA. This discovery, when considered in the light of Cohen's constant level of protein synthesis in the post infection period, was inevitable. The RNA synthesis in the infected cell was maintained at such a low level, however, that it did not seem capable of producing the amount of protein necessary for the production of mature phage.

Volkin and Astrachan (1956), when considering uridylic (dUMP) and cytidylic acid (dCMP) as equivalents of their natural analogues, hydroxymethylcytidylic acid (dHMCMP) and thymidylic acid (dTMP) of phage DNA, found that the post infection RNA is characteristic of the phage DNA rather than the host cell. That is, the base sequence (or their analogues) conforms to the sequence of bases found in the phage DNA. They also explained the extremely low level of RNA present at any given time in the cell by showing this to be highly unstable nucleic acid, i.e. its turnover was rapid.

RNA, which was obviously coded from the phage DNA, was further observed by Nomura et al (1960), and they felt the majority of this RNA probably always exists in a bound form, i.e. as a ribonucleoprotein particle. However, later work by Brenner et al (1961) found that stable RNA was not made after T2 infection, and existed only in the unstable form as formerly postulated. This unstable form served as a "messenger" RNA which carries the code

from the phage DNA to the ribosomes, and that the ribosomes themselves are an inert mechanism which cannot function without information from the DNA. This unstable messenger RNA (M-RNA) was found to possess molecular weights from 250 to 500,000.

Osawa et al (1962) added that a phage specific M-RNA is formed from the phage DNA, is liberated from it, and then passes to the ribosomes. This RNA was definitely a virus species. About 90% of this nucleic acid in the cytoplasm could be demonstrated in the ribosomal pellet, and the remaining 10% was in a soluble form in the cytoplasm. Also, at any given time, a percentage of the total cell RNA could be found in close proximity with the phage DNA, as it was being templated from it.

Stent (1963) strengthens the concept that a highly unstable M-RNA is formed, by stating that the rate of protein synthesis in the infected cell can be controlled by the number of operable ribosomes in the bacterium prior to infection. This can them be interpreted as meaning the phage "borrows" the bacterial ribosomes and produces its own protein by the M-RNA carrying messages coded from viral DNA. Other investigators such as Gros et al (1961) demonstrated an RNA in both the infected and uninfected cell and felt it definitely had a messenger function. This M-RNA was found in each case to have a base sequence which was characteristic of the DNA in the respective cell in which it was found.

After the wide acceptance that M-RNA coded from viral DNA had the ability to control protein synthesis in the bacterial ribosome, workers turned toward the sequence of information transfer rather than the mechanism. Green (1963) found a great diversity in the molecular weight of the M-RNA as a result of sucrose gradient centrifugation, and by hybridization of this RNA with phage and bacterial DNA found that the RNA varied greatly with the time in which it was made.

Further investigation by Spiegleman (1963) led to his total agreement of the messenger function of the phage induced RNA, and that specific RNA molecules are produced at a given time. He found that most molecules of RNA were between 16 and 30S, such size that they are coded for more than one protein. He felt this could arise from continuous transcriptions of contiguous cistrons found in the same operon. This would then provide a simple explanation for the physical basis of the turning off and on of such groups of cistrons.

By checking the structure of M-RNA, he found that transcription of the entire phage genome is not a random thing because RNA produced in the same period was always structurally alike, and different if from a different period.

Along with the work by Nomura et al (1960) on M-RNA, they also found an RNA subsequent to infection which was soluble in the cytoplasm of the host cell. This RNA had

about the same sedimentation coefficient as E. coli soluble RNA, and is probably in about the same size range, but possessed the same base ratios as the ribosomal fraction. Nomura et al felt that this molecule could be breakdown products of precursors of the ribosomal RNA, but probably functioned as an amino acid acceptor, an integral component in the present scheme of protein synthesis. Cohen (1948) had previously shown that the normally appearing soluble RNA (s-RNA) fraction in E. coli does not exhibit comparable metabolic actibity in protein synthesis subsequent to infection. Also Osawa et al (1962) demonstrated 10% of the phage induced RNA as being soluble in the cytoplasm.

These facts, though they prove the presence of a s-RNA or transfer RNA (as would certainly be expected), do not show in any manner that these molecules are phage specific. When considering the function of transfer RNA in protein synthesis, there is no need that it actually be phage specific.

With the virus induced formation of a M-RNA and a s-RNA which is known to have an amino acid acceptor function, the synthesis of phage specific protein may begin, and the first protein of this type to appear does so at about three minutes after infection. This molecule is given the name internal protein, and though its function is not definitely known, there is much conjecture on the subject.

## CHAPTER III

## INTERNAL PROTEIN SYNTHESIS

Internal protein was first found by Hershey (1955) and was given the name L-protein. It has specific antigenic properties and is closely associated with viral DNA. It accounts for 2% of the total phage induced protein and consists of two polyamines, spermadine and putrescine, and a polypeptide containing primarily aspartic and glutamic acid, and lysine.

This protein may function as the "condensation principle" (to be discussed later in this paper), which is recognized by many researchers. Stent (1963) feels that it probably functions in this capacity, as there is little doubt that the condensation principle is protein in nature, because a protein inhibitor administered at the time of infection will prevent cross linking of the phage DNA. The internal protein is found in the host cell in close proximity to the phage DNA and remains there when the DNA is enclosed in the phage head. This close association of internal protein with DNA, has led some workers to postulate that it may serve to carry some hereditary traits or play an integral role in the synthesis of DNA. (Murakami et al, 1959).

## CHAPTER IV

## INDUCED ENZYME SYNTHESIS

Further phage induced protein synthesis gives rise to a protein group commonly called early enzymes or early protein. This category would include all that protein produced after infection except phage precursor molecules and the lysozyme synthesized later in the eclipse period.

Innumerable works have been published to show this group of large molecules are phage induced and that their synthesis is completely necessary for the later replication of viral DNA. Watanabe (1957) was able to stimulate a normal rate of protein synthesis in an ultra violet inactivated E. coli cell by allowing the attack of T2. Cohen and Fowler as early as 1947 found that the early protein synthesis can be inhibited, thus inhibiting DNA synthesis, by the application of one of several common protein inhibitors. It has been further found that if these inhibitors are administered a few minutes after infection, DNA synthesis will follow, and that rate of synthesis is proportional to the time after infection of administration of the inhibitor. This early evidence pointed toward the fact this group of protein was enzymes which were directly involved in the formation of phage DNA precursors, and further work has

fully supported this concept.

Flaks and Cohen (1959) were able to demonstrate the presence of the enzyme responsible for the synthesis of dHMC in cells infected with T even phages. It was found to catalyze the addition of formaldehyde, in the presence of tetrahydrofolic acid, to the 5-position of dCMP to yield 5-dHMCMP. This enzyme could not be demonstrated in uninfected cells, and first appears three minutes after infection in the infected cell, with synthesis continuing up to fifteen minutes after infection. If the formation of this enzyme is blocked with a protein inhibitor, no phage specific DNA is synthesized. The origin of the substrate of deoxycytidylate hydroxymethylase (dHMCase) was at that time unknown, but it was felt that it probably was the nucleotide that resulted from host DNA destruction and that the presence of the nucleotide triggered the formation of the enzyme. (Flaks et al, 1959).

Wyatt and Cohen (1953) demonstrated the efficiency of this enzyme in their comparisons of the amount of phage and cellular DNA found within the infected cell at given times after infection. Cytosine containing DNA (bacterial DNA) decreases to about one third at twenty minutes after infection, and the infected cell contains virtually no cytosine DNA thirty minutes after infection if lysis is inhibited. At ten minutes after infection, phage and bacterial DNA are found in equal amounts in the infected cell.

Workers involved with the discovery of the new pyrim-

idine base and the enzyme responsible for this substance were puzzled as to how dHMC was phosphorylated and incorporated into phage DNA. Kornberg et al (1959) found several new enzymes in the infected cell which appeared about four minutes after attack by phage and like all other early protein was inhibited by the common protein inhibitors.

Among these enzymes was a kinase which phosphorylated dHMCMP to the triphosphate form, and it was given the name dHMCMP kinase. This enzyme was undetectable in uninfected cells and in those cells infected with bacteriophage T5 (a phage which contains cytosine rather than its hydroxymethyl derivatives in its DNA). Somerville et al (1959) demonstrated this enzyme by providing dHMCMP to both infected and uninfected cells, and this monophosphate was converted to the di- and triphosphate only in the infected cells. Somerville (1962) also showed this enzyme to be present as early as one minute after infection, and at this time both the enzyme and the product could be demonstrated in the infected cell.

While these workers were concerned with this kinase, they found that the T2 infected cell loses its ability to phosphorylate dCTP at the same time the new kinase appears. It was found that this phenomenon resulted from the action of another phage induced enzyme, deoxycytosine triphosphatase (dCTPase). This enzyme was aptly demonstrated by Kornberg et al (1959) by mixing extracts of T2 infected cells with uninfected cells and an inhibition of the forma-

tion of dCTP, which is normally formed in uninfected cells, was seen. This enzyme does not directly inhibit the action of the normally occurring dCMP kinase, because the dCMP kinase was shown to be produced at preinfection levels at this time; rather it was found to cleave the terminal pyrophosphate from this compound as rapidly as it was formed. Uninfected cells were found to have less than 1% of the dCTPase as were cells infected with phage T5.

Koerner et al (1960) in further investigations found that dCTPase is a very specific enzyme, i.e. it will not react if the nature of the pyrimidine base has been altered in any way, if the substrate is not in the triphosphate form, or if ribose is substituted for deoxyribose. It will not react with dCDP, which shows the cleavage must be of the pyrophosphate. This was shown to be true by virtue of the discovery of one molecule of pyrophosphate formed for each mole of dCTP used. It was also found that the enzyme rapidly increases from two to fifteen minutes after infection, and at the time DNA replication begins (6-8 mins.), the enzyme has attained nearly 50% final activity, and shows 10% final activity at three minutes after infection.

Bessman (1959) demonstrated the ten to twenty fold increase not only in dHMC kinase, but also in a dGMP and a dTMP kinase as well. This increase, of course, was not apparent in the dCMP kinase, due to the phosphatase discussed earlier in this chapter.

It was not positively known during this period whether these kinases which phosphorylated dTMP and dGMP were virus specific enzymes, or merely increased activity of a naturally occurring bacterial enzyme present prior to infection.

Bessman and Van Bibber (1959) later showed that dGMP kinase was probably a phage induced enzyme by virtue of its reaction KCl. This salt stimulated the formation of the kinase in an uninfected cell and inhibited its formation in an infected cell.

Bessman, now working with Belle (1963), continued his investigation of these enzymes, and in this later work has grouped what he considered three kinases previously, into one enzyme, a deoxynucleotide kinase. This enzyme is active in phosphorylating dTMP, dGMP, and dHMCMP, but is ineffective on dCMP and dAMP. Bessman and Belle feel this enzyme has three active sites, one specific for each of its respective substrates. When any one of the three substrates attack an active site on the enzyme, it so modifies the enzyme that it alters the remaining active sites and they can no longer function. This opinion is strengthened by the fact that each of the substrates are competitive inhibitors of the other two. Surprisingly enough, this enzyme was shown to be capable of adding only one phosphate, i.e. bringing the nucleoside to the nucleotide form. On the strength of this discovery, one might predict the isolation of further kinases in this system, though there is nothing more at the time of writing of this paper.

It is probably apparent at this point that no mention has been made of any enzyme which is capable of phosphorylating dAMP. This function is not necessary, even under the accelerated metabolic rates after T2 infection, due to the normally occurring high level of adenosine triphosphate (ATP) relative to its function as a high energy compound.

After the triphosphates of each of the four nucleotides are formed, the polymerization of these substrates into DNA then follows. Lehman et al (1958) demonstrated the presence of a DNA polymerase, but could not state definitely whether this was an accelerated bacterial enzyme or another which is phage induced. Aposhian et al (1962) then reported that the DNA polymerase present subsequent to infection is distinctly different from that produced by the host cell on the strength of immunological specificity, primer preference, etc. They also showed a ten fold increase in the activity of this enzyme as would be expected when one considers the increased rate of activity of the respective kinases.

Early workers were aware that upon analysis of T-even phage DNA, glucose derivatives of dHMC were present, and were even found to be present in definite percentages in each T-even phage as will be discussed later in this paper.

Zimmerman et al (1959) first found an enzyme which was capable of catalyzing the transfer of glucose from uridyl diphosphoglucose (UDPG) to dHMCTP and gave it the name glucosyl transferase. It was very obvious that was a

phage induced enzyme, as the glucosylated purine is common only to the DNA of the phage. At this time there was no distinction made as to whether this was a single enzyme or a complex of enzymes which resulted in the various glucosyl derivatives common to the T-even phage.

Lehman et al (1960) published their results showing that the proportion of glucose to phosphate in the T-even phage is a heritable property of each phage type. This proportion of glucosylated derivatives is so inflexible, they feel it may in some manner carry genetic information. Further proof of the inheritable characteristic of glucosylated dHMC was published by Streisinger et al (1959) when they found that crosses between T2 and $T^4$ phages resulted in intermediate structure of the glucose derivatives in the DNA of the resulting progeny.

Lehman et al (1960) further showed the extent of glucosylation of the dHMC in T2; 25% is not glucosylated, 70% is a monoglucose derivative, and 5% is a diglucose derivative. They also found the specificity of the glycosidic bonds to be as follows: those molecules of glucose which are bonded to the hydroxyl group on dHMC are always joined through an alpha linkage, and the second mole, which is of course bonded to the first, is always in beta linkage. At this point, these workers felt probably two enzymes existed, an alpha and beta glucosyl transferase.

Further work in this area by Zimmerman et al (1962) proved the glucosyl derivatives of dHMC must be polymerized

into DNA before they can act as a substrate for the glucosyl transferases. They found that if only dHMCTP in the glucosylated form was present, no DNA synthesis would take place, but dHMCTP was readily polymerized into phage specific DNA. The DNA polymer then became the substrate for the enzyme, resulting in the glucosylated DNA.

At that point when the triphosphates of the four nucleotides are polymerized into phage specific DNA, and dHMC is glucosylated, the phage DNA molecule is complete and will be found in progeny in this form. At the time this pathway from dCMP to DNA-glucose was traced, another acquired metabolic pathway was found to arise also from dCMP.

Flaks and Cohen (1959), while investigating the reason that phage could mature within, and burst a thymineless mutant of E. coli which was growing in a medium containing no thymine, found a new enzyme capable of converting dUMP to dTMP. This reaction required formaldehyde from which the one carbon fragment is obtained, and tetrahydrofolate, which they felt probably functions as the carbon carrier and a reducing agent as well. They postulated that this enzyme, which they gave the name thymidylate synthetase, transfers the formaldehyde to dTMP by way of a methylene bridged complex between dUMP and the intermediate, tetrahydrofolate. The enzyme then reduces the formaldehyde to the methyl group found on dTMP. Thymidylate synthetase was found to be present in the uninfected cell, but increases seven fold in the infected cell, exactly that rate

which would make the specific activity of this enzyme comparable to that of the phosphorylation of dTMP and DNA polymerization.

Mathews and Cohen (1963) were able to substantiate the earlier theory that tetrahydrofolate functioned as a reducing agent as well as a carbon carrier, by showing that it was stochiometrically oxidized to dihydrofolate, and that the rate of reduction of this compound was approximately seven fold as in other steps in the synthesis of dTMP. They found that a new enzyme, dihydrofolate reductase, was responsible for reducing the dihydrofolate with the cofactor $TPNH_2$ to tetrahydrofolate and TPN plus. This enzyme was present in the uninfected cell also, but there appeared to be some difference in properties when compared to that enzyme which is probably phage induced, but one cannot positively make this conclusion.

Keck et al (1960) found the link between dCMP and dUMP by isolating an enzyme which cleaves the amino group from dCMP. This enzyme, named dCMP deaminase, appears about three to five minutes after infection. Unlike other enzymes in this group, it could not be demonstrated in the uninfected cell.

With the isolation of dCMP deaminase, a totally new pathway of dTMP synthesis opens, and as did the pathway to glucosylated DNA, arises from dCMP. However, the products resulting from a single substrate are quite different, yet complement one another to produce a glucosylated

DNA which will become phage precursor molecules.

## CHAPTER V

## PHAGE PRECURSOR SYNTHESIS AND MATURATION

With the synthesis of all enzymes necessary for the polymerization of phage DNA, this process starts about six to eight minutes after infection. There are certain morphological changes in the host cell prior to the advent of DNA synthesis which are worthy of some discussion in light of past remarks made in this paper, and these changes are also directly related to the maturation process of the phage.

Kellenberger *et al* (1959) by viewing with the electron microscope ultra thin slices of *E. coli* at various times after infection by T2, was able to learn much about DNA synthesis and phage maturation.

They saw that immediately following infection, a rapid distortion of the bacterial nucleoids occurs within two minutes, followed by a migration of this nuclear material to the protoplast membrance of the host cell. At six to eight minutes after infection, large vacuoles containing a fibrillar material are evident, and these enlarge until they are clearly evident within the cell.

Kellenberger *et al* spoke of these large fibril-containing vacuoles as the "morphological pool", and felt they were composed largely of phage DNA. These conclu-

sions were drawn from their demonstrating DNA, which was known to have been synthesized at different times during the latent period within the same phage offspring. If this theory is not true, there would have to be much recombination of homologous subunits during their sojourn to the DNA pool. However, if DNA subunits are synthesized, one must accept the concept of a new system which will dictate the ordering of these subunits when they leave the pool. In no other manner could phage DNA exhibit that sequence of bases which make it phage specific DNA.

Kellenberger (1961) showed that DNA passes from his "morphological pool" to the maturing phage, as he found that the pool does not increase in size from the end of the eclipse period even though DNA synthesis positively continues until burst. He explained this phenomenon by showing DNA was being condensed and incorporated into the phage head as rapidly as it was being synthesized.

At about the same time after infection (seven minutes), Koch et al (1959) readily identified a phage precursor protein by immunological techniques, and found it to be those protein subunits which go into the head of the mature phage. These protein subunits have a molecular weight of 80,000 and probably exist in a pool as is characteristic of DNA.

At ten minutes after infection, Kellenberger et al (1959) viewed electron dense bodies with the polyhedral shape characteristic of the T2 head. These polyhedrals were shown to display various stages in the ontogeny of the phage head, as the number of such polyhedral bodies

per cell visible at various times during the latent period, always exceeded the number of empty phage heads and intact phage particles per cell an any premature lysates of the same population of infected cells. Some of these polyhedrons must then reflect an earlier stage in the development of the phage head, and this structure corresponds nicely with the cross linked DNA resulting from action by the condensation principle discussed earlier in this paper.

At this point the protein coat forms about the crosslinked DNA. Koch et al (1959) state that an unstable protein coat is first formed and maintains a membrane-characteristic for approximately five minutes before becoming stable in the sixth minute. The tail of the phage is then added and the maturation process is complete, thus ending the eclipse period at approximately fifteen minutes after infection.

As is rather obvious to the reader, little information has been given in this paper concerning the assembly process of the bacteriophage, i.e. the specific nature of addition of the protein coat and tail to the crosslinked DNA. Of all phases in production of mature phage, this seems to be the least understood and the least explained in literature.

Though the presence of mature phage within the host cell indicates the end of the eclipse period, these mature particles are still within the bounds of the cell and must be released into the medium to complete the cycle.

This is accomplished by a lysozyme, the final phage specific protein synthesized within the host cell. Though this enzyme lyses the cell at twenty five minutes after infection, it appears as early as seven to eight minutes after infection.

When this enzyme was first isolated, it was thought that its only capabilities were protein degradation. Then it was shown to lyse E. coli when in purified form and as a result considered as a true lysozyme. (Koch and Dreyer, 1958). With the lysozyme actively bringing about a lysis from within, the host cell is ruptured, the phage progeny released, and the cycle is complete.

## CHAPTER VI

## SUMMARY AND CONCLUSIONS

Certain metabolic changes are apparent in the bacterium Escherichia coli after infection with T2 bacteriophage. A phage stimulated DNase appears immediately after the injection of viral DNA, resulting in the destruction of bacterial DNA. Thus, all anabolic activities resulting in the formation of large molecules are brought to a halt; however the energy producing reactions common to the preinfection host cell continue at their normal rate.

At about the same time, the synthesis of phage specific RNA is detectable, thus establishing a phage oriented metabolism which will readily produce protein of a phage variety. This phage oriented protein producing mechanism consists of a M-RNA to carry the "code" of proteins to be synthesized from the viral DNA to the "borrowed" bacterial ribosomes. Probably a s-RNA has also been detected which has an amino acid acceptor function.

After establishing the necessary metabolic pathways, a number of phage specific early enzymes are produced which will in turn catalyze reactions that result in the formation of phage precursor molecules.

The majority of these early enzymes probably are con-

cerned with the production of glucosylated DNA which is common to the T2 bacteriophage. Two general enzyme groups exist, and both have as their ultimate precursor, dCMP.

A dHMCase catalyzes the addition of formaldehyde to dCMP to form dHMCMP, which is then phosphorylated by its respective kinase. The other three nucleotides found in phage DNA, dAMP, dTMP, and dGMP, are also found in high levels in the host cell at this time, the latter two due to the action of their respective kinases, and the former by virtue of its function as a high energy compound.

At this point, the triphosphates of the nucleotides are polymerized into an intact DNA by a phage specific DNA polymerase. This then results in DNA containing dHMC derivatives common to the T2. Also found in phage T2, are glucosylated derivatives of dHMC in the intact DNA as a result of two phage specific enzymes, alpha and beta glucosyl transferase, which transfer glucose from UDPG to dHMC.

Another enzyme system which functions simultaneously, catalyzes a series of reactions which change dCMP to dTMP by removal of the amino group from dCMP to form dUMP, then the addition of a formaldehyde to the 5' position on dUMP. Deoxycytidylic deaminase and thymidylate synthetase are the enzymes involved in these reactions. One other enzyme is also functional in this series. Dihydrofolate reductase is found to reduce dihydrofolate to tetrahydrofolate with $TPNH_2$ as the cofactor. Tetrahydrofolate is itself oxidized

in reducing formaldehyde to the methyl group found on the 5' carbon of dTMP.

With the appearance of all necessary enzymes, the phage precursor molecules are then synthesized. DNA replication is followed by its condensation into polyhedral shapes slightly smaller than the intact phage head. Protein subunits are then drawn from the protein pool to form a coat over the condensed DNA. The assembly of the phage is completed by the appearance and attachment of the tail.

During the maturation process just described, the final phage induced protein is synthesized in the form of a lysozyme, which lyses the cell from within, releasing the mature phage.

Further research into the E. coli, T2 system will probably be directed toward the assembly of precursor substances rather than their synthesis. Phage specific DNA synthesis is relatively well outlined and seemingly requires little more study; however, further insight into phage precursor protein will probably be attained. There will then only remain an elucidation of the actual mechanism of assembly to complete the total picture of this system.

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VITA

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