GENETIC RESTRICTION IN ESCHERICHIA COLI STRAIN W

A thesis

presented in partial fulfilment

of the requirements for the degree of

Master of Science

in Microbiology

at

Massey University

A.F. JAMIESON

1971

ABSTRACT

The ability of various phages to propagate on Escherichia coli strain W was investigated. Phages P1, T2, T3, T4, T5, T6 and T7 could not be shown to form plaques on this strain. Phages T2, T3, T5 and T6 prevented the development of a bacterial lawn when added to a plate at an input ratio of about three phage per bacterium; it appears these phages exerted a killing effect on strain W. Phage P1 and phage T4 did not exhibit this killing effect. Phage T1 formed atypical plaques on strain W with an efficiency of plating of 10^{-4}; it appears these plaques are due to mutants occuring in the T1 population able to propagate on strain W.

All of the above phages adsorbed efficiently to strain W with the exception of T4, explaining its inability to either propagate on or kill strain W. The infection of strain W by P1 was similar in most respects to that of λ but in order to establish the occurence of conventional restriction, DNA degradation would need to be demonstrated.

Phages were isolated which propagate on strain W; they are similar in morphology to phages T5 and λ and do not readily adsorb to E coli strains B, C or K.

The supernatant from broth cultures of strain W was shown to contain two closely related phages, one plating on E coli C, and the other on E coli K. Each possesses a characteristic pattern of plating efficiencies on strains C and K when propagated alternately in these two hosts but the two phages were shown to be co-immune and identical with respect to heat sensitivity, morphology and serology.

Both tended to lose the ability to exclude phage P1 on lysogenising strain C once having mutated to plate on strain K. This may be due to the integration of the mutated phage at alternate "non-restricting" sites on the E coli C chromosome.

A series of conjugal crosses was employed to determine the sites of integration of the phages on the chromosomes of E coli strains W and C. The phage present in the W supernatant which plated on K was found to integrate close to the proline loci on the chromosome of E coli W but the phage plating on C appeared to have more than one locus, one of which may map close to the 85 minute mark on the linkage map of E coli W (36, Figure 22). No information has so far been obtained concerning the sites of integration of the w phages in restrictive and non-restrictive lysogens of E coli C. The failure to obtain a 'cured' strain of E coli W by elimination of the prophage integrated at the two mapped sites leaves open the possibility of the existence of more than one integration site of phage w.C on the E coli W chromosome.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Professor D.F. Bacon for his invaluable guidance throughout the course of this work and also for the supply of many of the bacterial and bacteriophage strains.

Thanks are also due to the staff of the Palmerston North D.S.I.R. electron microscope unit for their assistance in the preparation of the electron micrographs, to Miss J. Quigan for her expert typing and to all staff in the Microbiology and Genetics Department who have helped in work leading to the presentation of this thesis.

A.F. JAMIESON

TABLE OF CONTENTS

****

LIST OF TABLES

****

LIST OF FIGURES

****

LIST OF PLATES

****

INTRODUCTION

Several different mechanisms may prevent a viable phage from multiplying in a host cell after the phage has adsorbed to the cell surface and injected its DNA. This abortive infection may take one of several forms:

I Superinfection immunity

II Superinfection exclusion

III Inhibition by F^+

IV Restriction

I Superinfection immunity

This mechanism occurs in lysogenic cells where superinfecting phage particles of the same, or similar type to the integrated prophage are unable to initiate a round of vegetative propagation. Replication of the superinfecting genome is prevented by the action upon it of a cytoplasmic repressor protein, which is synthesised under prophage direction and which prevents expression of genes responsible for initiation of a lytic cycle (1,2,3,4,5).

II Superinfection exclusion

When bacteria lysogenised by temperate phages such as λ or P2 are superinfected by distinguishable mutants of their prophage, the superinfecting phage appears in the progeny released after induction of vegetative phage growth.

Work by Rao, Walsh and Meynell has shown this is not the case in P22 lysogens of Salmonella typhimurium (6,7). No super-

infecting genetic markers like C_2 (clear plague) or h (host range) appear in the phage obtained after induction, although superinfecting phage are adsorbed normally. The wild type prophage is therefore termed excluding or χ^+. Complementation of ts P22 prophage mutants by superinfecting ts phage is also substantially decreased, indicating the exclusion mechanism operates to block gene expression as well as replication. It is most likely that DNA penetration of superinfecting phage occurs as lysogens can be induced by superinfection at high multiplicity, suggesting the superinfecting genomes are available for interaction with the phage immunity repressor protein.

DNA degradation was not observed and hence this may rule out the possibility that exclusion is a restriction phenomena. This leaves open the possibility that exclusion is mediated by any one of the control mechanisms operating at the series of steps required for informational transfer from the genome.

Mutants of P22 which are non-excluding (χ) on lysogeny of Salmonella typhimurium have been isolated suggesting the exclusion is prophage controlled. These mutants are indistinguishable from P22 (χ^+) in morphology, neutralisation kinetics with anti-P22 antisera, heat sensitivity and inability to lyse P22-lysogenic bacteria.

III Inhibition by F^+

The efficiency of plating of certain phages, notably T3 (8), T7 (9), ϕI (10), ϕII (11) and W31 (12) is reduced on infection of cells carrying F^+. The inhibition of T7 has recently been shown by Malamy and Morrison to be due to a control mechanism operating at the level of translation which affects production of certain classes of T7 protein (13)

IV Restriction

(i) The restriction and modification processes.

The term restriction is employed to describe a particular type of abortive infection involving the endonucleolytic scisson of DNA molecules foreign to the invaded cell at specific sites on these DNA molecules. It is carried out by specific nucleases whose synthesis is mediated by the host genome or by a plasmid carried by the host. Different hosts have different specificities.

Restriction is almost always associated with modification which may involve either methylation or glucosylation, mediated by host cell or plasmid enzymes at the specific DNA cleavage sites. This modification protects host DNA and DNA of infecting phage which escape the restricting system against nucleolytic attack.

Modification is not inheritable as a genetic marker and although retained as long as phage particles are propagated in a modifying host, is lost on replication in a cell population lacking restriction and modifying activities except for the two parental phage DNA strands which still retain their modification. (Reviews: 14,15,16, 17,18,19). An example of a typical system is the infection of E coli K and E coli C with phage λ (Fig.1). See section (ii) for explanation of the notations used in the figure. (eop = efficiency of plating)

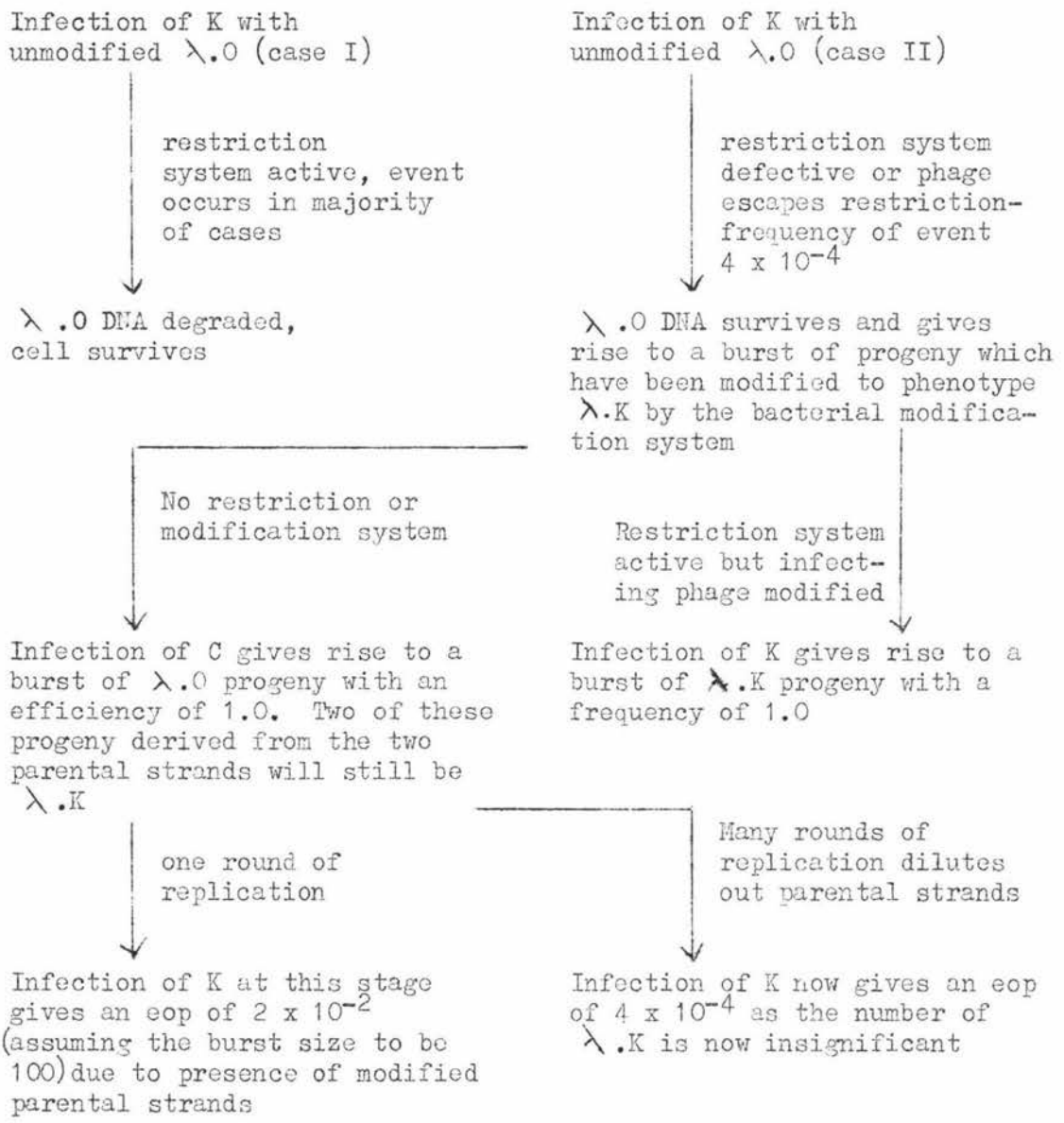


Figure 1: Restriction and modification of phage λ in *Escherichia coli* strains K and C.

(ii) Definitions and notations (from Arber 14,16)

(a) Host specificity types - these are noted by their bacterial or plasmid origin. Hence K, B and A signify host specificity controlled by E coli strains K12, B and A respectively, W signifies host specificity controlled by the genome of phage W. 0 indicates the absence of any detectible host specificity, i.e. a strain exerting neither restriction nor modification ($r^- m^-$), such as E coli C.

(b) Modification type carried by phage particles or DNA molecules - these types are marked with the notation for the particular host specificity type preceded by a dot. For example λ.K indicates phage λ carrying K- specific modification. Notations such as λ.K.0.K are used to express the history of a phage population obtained by successive infection of K, 0 and K bacteria.

(iii) Breakdown of DNA.

Restriction as defined above always involves breakdown of invading DNA. Infection of a restricting host with ^{32}P labelled DNA has been shown by Dussoix and Arber to give rise to breakdown products ranging from acid insoluble fragments to oligonucleotides and inorganic phosphorus (20,21). This process probably occurs in two steps - a few endonucleolytic scissons by the restricting enzyme, followed by slower non-specific digestion by other, non-specific DNAses. Evidence supporting this view includes observation of a slight functioning of restricted phage genes in a restricting host (22) and rescue of unmodified phage genetic markers from restrictive hosts on super-infection with modified phage (20).

(iv) Factors governing the level of restriction.

Restriction results in a reduced plating efficiency of phage on infection of a restricting host. In a specific system each phage has a characteristic probability of escape which is governed by several major factors. The smaller the number of specificity sites carried by the phage, the more likely it is to escape restriction. Wild type phage fd has two B-specificity sites and has a plating efficiency of 7×10^{-4} on E coli B. Arber has shown that on the loss of one of the two sites its plating efficiency on B is increased to 3×10^{-2}(14). This implies competition between modification and restriction activities for the sites. However, in cases where the infecting phage has three or more sites it is unlikely that it would survive this competition and hence, in this case, the plating efficiency is probably a reflection of the proportion of cells in a physiological condition which causes them to be weak or lacking in ability to restrict. These 'weak' or restrictionless infected cells will still retain their modifiing activity and hence their progeny phage will be modified and will initiate a spreading infection. Restrictionless mutants do exist but their levels are negligible in normal populations and hence centers of infection arise mainly from events initiated by the 'weak' cells.

Various external factors such as culture age, media composition, and temperature influence the functioning of the restriction enzymes (14). When these factors are constant, the plating efficiency of a given unmodified phage on a specific bacterial strain is generally characteristic of that system.

(v) Restriction in other systems.

Restriction is not only confined to infecting phage DNA. In the case of bacterial conjugation and of phage-mediated transduction, a restrictive recipient strain gives rise to fewer genetic recombinants or transductants than a homologous nonrestrictive recipient strain. Hence restriction nucleases also act against bacterial DNA (23). Wood has shown that these results are due to restriction and not wholly to incomplete pairing between the DNA of the donor and the recipient (24).

An interesting phenomena was observed in bacterial conjugation by Glover, Copeland and other workers involving a breakdown in the restrictive mechanism of the zygote about twenty minutes after the commencement of mating. This may be due to saturation of the restricting nucleases with incoming unmodified donor DNA (25,26).

(vi) Isolation of restricting nucleases and determination of the nature of the specificity sites.

The restricting nucleases for Haemophilus influenzae (27), E coli K12 (28) and E coli B (29) have been isolated. The nuclease from Haemophilus influenzae isolated by Smith and Wilcox was active against DNA from a wide variety of sources including that of T7, P22, S typhimurium, B subtilus and salmon sperm. It was not active against Haemophilus influenzae DNA. With T7 DNA it produced forty double-stranded 5'-phosphoryl, 3'-hydroxyl molecules, each about 1000 base pairs long. It appears likely that the ability of the endonuclease to recognise only a few specific sites resides in the site base sequence. On the assumption of a random sequence any unique run of six nucleotides will occur once in 1024 base pairs, therefore six base pairs seems to

be the likely length for the recognition site of T7.

The substrate site sequence was determined by sequencing the DNA on either side of the cleavage point. The sequence from the 3' end was found to be unique for a distance of three base pairs and complementary to the sequence from the 5' end for a distance of three base pairs, hence the enzyme appears to recognise a six nucleotide sequence in the middle of which is situated the "chopping" site (Fig.2).

5'..... Gp Tp Py ↓ p Pu p Ap C ..... 3'

3'..... Cp Ap Pu p ↑ Py p Tp G ..... 5'

Figure 2: The specificity site nucleotide sequence of Haemophilus influenzae.

It is likely that the enzyme is made up of subunits - one a recognition subunit, the other a nuclease subunit. It seems very likely on the basis of economy of genetic information and the symmetry of the site that there are in fact four subunits - one recognition subunit recognising perhaps GTPy on one strand on one side of the chopping site and another identical subunit recognising the same sequence on the complementary strand on the other side of the chopping site. A nuclease would be associated with each recognition subunit, severing a single strand in each case.

Present evidence tends to suggest the nucleases are located in the periplasm between the cell wall and the cell membrane. This evidence includes treatments which are known to remove surface localised enzymes such as EDTA treatment (30), conversion to spheroplasts (31) and an experiment in which Schell and Glover

infected restricting cells with unmodified phage at a temperature which decreased restriction. When the system was resuspended in a hypertonic medium at 37°C restoring restriction activity as evidenced by the fact that in a control, phage which infected cells after this step were restricted, a significant number of phage escaped restriction, indicating that once penetration had occured the phage was no longer susceptible (32,33). In the case of the E coli W system where prophage w - mediated restriction but no modification occurs, the bacterial genome cannot be modified and hence must be protected, possibly by compartmentalisation of the nucleases in the periplasm. There is also, however, the possibility that strain W lacks the sequence recognised by the prophage w-mediated restricting function.

(vii) Function of restriction.

The function of restriction seems to be a bacterial defense mechanism against viral attack. However, this system would also seem to inhibit diversification of genetic material by conjugation and transduction and thus retard bacterial evolution although one of its functions may indeed be that of genetic isolation. To quote Arber (14) ".....modification and restriction provides a unique defense mechanism for the bacterial cell, DNA foreign to the strain is specifically identified and inactivated. This system is particularly effective towards a virus which has been recently introduced from a foreign environment, a virus to which the bacterial population has not yet built up an immunity. Although this, or an analogous restriction process has not as yet been identified in cells of higher organisms, the existence of such a mechanism remains an intriguing possibility."

(viii) The nature of modification.

DNA which overcomes a restriction activity and is produced in a particular host strain is not restricted when it again enters cells of that strain. This DNA is said to be modified. Modification frequently consists of a specific methylation of either adenine or cytosine at the specificity sites, rendering the DNA insensitive to the specific restriction activities. The possibility that methionine is involved was suggested by inhibition of modification in experiments involving methionine starvation (15) and pre infection with U.V.-irradiated phage T3 which causes cleavage of s-adenosyl methionine (34). These methods are limited in that physiological conditions which affect modification may also inhibit phage growth.

Another line of evidence is the determination of the relative amounts of methylation of modified and unmodified DNA. A major problem is that most bacterial and phage DNA is extensively methylated for unknown reasons and the few extra methylations due to modification are not readily detected by present analytical methods (35). Arber, however, was able to demonstrate a correlation between B-specific modification and the presence of 6-methyl amino purine (6-MAP) in phage fd, which is not extensively methylated. In labelling experiments fd.0 was shown to have about one 6-MAP per 4,000 nucleotides while phage fd.B was shown to have two 6-MAP per 4,000 nucleotides. A single-stranded fd molecule contains 6,000 nucleotides hence the difference between fd.0 and fd.B is about two methylation events. This correlates with genetic evidence which suggests wild type fd carries two B-specific sites on its DNA (16).

The second type of modification involves the T-even phages (17). These phage possess hydroxy-methyl deoxy cytidylate (dHMP)

instead of deoxycytidylate (dCMP) in their DNA. The hydroxy-methyl group is normally glucosylated. Glucosylation is mediated by phage-induced glucosyl transferases utilising uridine diphospho glucose (UDPG). Lack of either glucosyl transferases or UDPG in an infected host prevents modification of infecting phage DNA, thus exposing it to restriction by various hosts. In contrast to infection by λ however, restricting cells are frequently killed (17).

A significant operational difference between the two systems of methylation and glucosylation is that the bacterial methylation system includes both a restricting and a modifying component which are specifically related to one another and in which the two genetic loci are closely linked. The modifying component acts on internal endogenous DNA, as may the restricting component. (This is presumably the function of modification - to prevent breakdown of host bacterial DNA which is carrying a restriction activity, although there is some evidence for compartment alisation of the restriction activity). Modification of T-even phage DNA (glucosylation) is brought about by a mechanism completely separate from that of restriction - that of phage-induced glucosyl transferases utilising UDPG provided by the host cells.

(ix) The genetic determinants of the restriction and modifying activities.

Genes containing structural and regulatory information for the production of restriction and modifying enzymes are carried on bacterial chromosomes and on the chromosomes of many plasmids.

Restriction-deficient bacterial and prophage mutants (r^- phenotypes) have been isolated (14). About one half of the r^- mutants

were found to be of the m^- phenotype, unable to carry out strain-specific modification. No strains isolated were r^+m^- - the only known occurrence of this phenotype is in E coli W. This may reflect the method by which the mutants were isolated, i.e. by selecting cells affected in restriction - no direct selection technique is available for selection of m^- phenotypes - but it is more likely to reflect the possibility that r m^- cells are normally lethal.

E coli K, B, 15 and A have been shown to lose their restriction ability completely in one step mutations indicating that each strain possesses the information for only one system of host specificity. This was confirmed by mapping the restriction and modification (or host specificity, hs) characters by conjugation and transduction. The hs markers for K-, B-, 15- and A-specific modification and restriction form a closely linked cluster of genes with a location about one minute to the left of threonine on the linkage map of E coli K12 (14,36). The hs genes of these strains have been shown to be allelic as concluded from their cotransducibility with ser B and thyR. This allelism might suggest that the hs genes of the E coli strains are related, having evolved from a common ancestor.

It has been postulated that the hs site consists of three genes - the hsr and hsm genes which code for the restricting nuclease and the modifying enzyme respectively, and the hss gene which codes for a product which carries out site recognition for the restriction and modifying activities. Neither the hsm nor hsr gene product is postulated to be functional unless it is associated with that of the hss gene. This is supported by complementation studies where an F-prime factor carrying hs genes of known phenotype is introduced into various bacterial strains carrying hs genes of known phenotype, such as the diploid (F' - r^- m^- / r^- m^-) in which complementation

occurs, enabling restriction and modification to take place. This can best be explained if the system is in fact (F' - *hsr*$^-$ *hsm*$^+$ *hss*$^+$ / *hss*$^-$ *hsr*$^+$ *hsm*$^+$) (14). The *hss* gene product seems to be equivalent to the postulated subunit which recognises the DNA cleavage site in *Hemophilus influenzae* although no concrete evidence for this has yet been presented.

(x) Restriction in *E coli* W.

Supernatants from log phase broth cultures of *E coli* W contain a phage, w, which forms λ-like plaques about 2-3mm in diameter on *E coli* C (Plate 13) at an efficiency arbitrarily called 1.0 (37). These cultures contain about 10^6 plaque forming units per cm^3. The phage is non inducible by UV radiation.

P1 and P2, but not λ, have been shown by Glover to be antigenically related to w, P2 more so than P1. The bouyant densities of w and P2 are very similar, as well as their morphology. Electron micrographs show a tadpole-like phage very similar to P2 and T1. It has a head approximately 65 x 65nm and a tail 140nm long with a contractile sheath (Plates 14,15,16,13). In spite of these similarities between w and P2 they are not co-immune as P2 plates on C(w) and w plates on C(P2). Phage w also plates on C(P1) and hence P1 and w are not co-immune.

Glover (37,38) was unable to demonstrate plating of the *E coli* W supernatant (w.W) on strain K. Phage w propagated on C, however, would infect K at a frequency of 10^{-6}. A suspension of phage prepared from a single plaque on K plated with an efficiency of 1.0 on both K and C. This phenomena is not due to host endowed modification as after several cycles of growth in C the phage still

retains its ability to plate on K. It was postulated that a w phage particle able to plate on K is in fact a host range mutant wk.

Phage	Plating Bacteria	
	K	C
w.W	$<10^{-6}$	1.0
w.C	10^{-6}	1.0
wk.K (or w.C.K.)	1.0	1.0
wk.C (or w.C.K.C.)	1.0	1.0

Table 1. Efficiency of plating of phage w on *Escherichia coli* strains K and C (37).

Notations such as wk.K should not be confused with the notations defining host specificity and merely indicate the series of hosts in which the phage was grown.

In contrast to these results, Pizer et al (42) reported that supernatant from the *E coli* W strain with which they were working plated with an efficiency of 1.0 on both strains K and C.

Phage	Plating Bacteria	
	C	K
w.W	1.0	1.0
w.C	1.0	10^{-5}
w.K	10^{-3}	1.0

Table 2. Efficiency of plating of phage w on *Escherichia coli* strains K and C (42).

Phage w and its supposed mutant w.k were used by Glover to prepare the lysogenic strains C(w), K(wk) and C(wk). Phage λ is restricted in W to a level of 10^{-10}(39), hence it was of interest to determine if the w lysogens were also restrictive. E coli C(w) was restrictive for λ(eop $<10^{-8}$) but K(wk) and C(wk) were not. DNA was found to be degraded in W(w) and C(w) on infection with λ(38). Therefore wk might be regarded as a mutant which has lost its ability to direct the degradation of λ DNA and that this mutation is somehow linked with the change in host range.

Damaging to this hypothesis was the discovery by the same workers that not all C(w) isolates behave in the same way; different strains of C lysogenised with w did not restrict λ and again some lysogens gave intermediate levels of restriction. The frequency of these three different classes among C(w) lysogens varied according to the strains of C made lysogenic. The reason for these differences is not known. However, w is very much like P2, and like P2, may occupy several alternate locations on the chromosome of E coli C. Furthermore, some of these P2 integration sites seem to be located in different positions in K12 (40,41). If the expression of restriction is dependent on chromosome location it should be possible to demonstrate differences between the location of w in C(w) strains which restrict λ and those which do not.

Glover was unable to obtain a cured strain of W by normal screening methods (37), although he did obtain strains no longer producing w but which nevertheless failed to propagate the phage. These bacterial isolates were presumably still lysogenic for a defective form of w. Lederberg (42) claimed to have obtained a cured strain of W called WS by selecting for sensitivity to infection

by phage T2. (T2 infection of W is abortive). Again this isolate produced phage in its supernatant but would not readily propagate phage w.W. It would, however, plate w at a very low frequency when it had previously been grown in strain K. It is possible that this low plating efficiency may be attributable to a W genome restriction activity but seems excessively low compared with the W genome restriction exhibited on infection with λ w (38, see later). Strain WS has so far been used exclusively in experiments involving abortive infection of W by the T-even phages and T5. The mechanism of abortive infection of E coli W by T5 and the T-even phages seems to be somewhat less straight forward and will be dealt with more fully in the following section.

Glover therefore used lysogens of strain C to demonstrate the role of prophage w in restriction of λ(38). Phage λ was demonstrated to have a low plating efficiency on W and on C(w). Adsorption occurs normally in both cases hence labelled ^{32}P. λ DNA was used to infect C(w). It was found that greater than 20 percent of the ^{32}P became acid soluble shortly after infection, a figure comparible to infection of strain K by phage λC. Hence in the lysogen C(w), the w prophage controls a restriction process which operates against λ like that controlled by P1. It was found, however, that no w-specific modification was carried by the small number of λ particles which escaped this restriction process. When strain W is infected with λ, a small fraction (10^{-4}) of infected cells give rise to small bursts of phage progeny, unable to form plaques on W. Hence phage w controls a host specificity type which fulfils all the requirements of a r^+m^- system. In such a phenotype it might be expected that the restriction enzymes specified by the prophage would indulge in a suicidal attack on the host DNA. A mechanism must however, be

operating to prevent this from happening. This may take the form of nuclease compartmentalisation which is in agreement with the likely site of the nucleases in the periplasm, or alternatively there may be a lack of specificity sites which are recognised by the w-specified nucleases on the C, K and W genomes.

A mutant of phage λ, λw, was isolated which forms plaques on strain W and on C(w) lysogens with an efficiency of 1.0 (38,39). This mutant occurs in the λ population at a frequency of about 10^{-10}. It was concluded that it carried a mutation at the specific nucleotide sequence recognised by the w restriction nuclease. Phage λw grown in strain W plates on W with an efficiency of 1.0. Grown in strain C or C(w.C), it plates on W with an efficiency of 10^{-3}. It was concluded that there is a W-specific host modification and restriction mechanism controlled by the W genome, and the actual frequency of λw mutants in the λ population is probably therefore 10^{-7}, not 10^{-10}, as the mutants are able to avoid the prophage w-specified restriction but not that of the W genome-specified type.

ABORTIVE INFECTION BY T5 AND THE T-EVEN PHAGES

Infection of K12 (λ) by an r_{II} mutant of T4 is abortive (43). In addition, abortive infection occurs following adsorption of T5 and the T-even phages to Shigella dysenteriae lysogenic for phage P2, to Escherichia coli W and to Escherichia coli Co 270. (42,44,45,46,47). Abortive infection of Escherichia coli B by non-glucosylated T-even phages has been covered (p.18).

With reference to the case of abortive infection of K12 (λ) with T4 r_{II}, Krylov found that the surface charge of E coli B cells infected by r_{II} mutants differed from that of r^+ infected cells (48). He suggested that the r_{II} protein participates in the sealing of the cell envelope following infection of bacteria with the T-even phages and that a non-functional r_{II} gene results in leakage of cell constituents leading to death of the cell.

A similar mechanism has been suggested by Pizer, Fields and others to be responsible for the abortive infections observed in the remaining cases (42,45). A comparison of events occuring after T2 infection of SH(P2) with those occuring after T2 infection of E coli W is relevant as strain W carries a prophage which is morphologically and antigenically related to P2 (37) and which is responsible in part for restriction of T2 in E coli W.(42) The abortive infection of T2 in both SH(P2) and W is characterised by an abrupt, early cessation of all macromolecular syntheses. In the infection of strain W by T2, degradation of 20-50% of T2 DNA has been reported by Smith and Pizer (42,46), but they have suggested that DNA degradation may not be the cause of abortive infection as intact T2 DNA was detected by sucrose gradient centrifugation analyses long after phage infection had

occured (46). In addition, these authors and others could detect no degradation of T2 DNA on infection of SH(P2)(47).

To determine whether the presence of the temperate phage w is responsible for the inability of E coli W cells to act as hosts for T2 and T4, a cured strain of W, WS, was isolated by Pizer. This strain allowed adsorbtion and propagation of both T2 and T4. Phage T2 adsorbed to and killed the lysogen WS (w); but phage were produced in less than 1 percent of infected cells. Phage T4 did not readily adsorb to WS (w), suggesting a change in the cell envelope which is mediated by the w prophage. No phage progeny resulted from those adsorption events which did occur.

In further support of the view that prophage w is responsible for the abortive infection of strain W by the T-even phages, phage w lysogens of strain C plated T2 with an efficiency of less than 10^{-2}, while T4 failed to produce phage on either C or C(w). Lysogens of strain K, however, could be successfully infected with both T2 and T4 and therefore the presence of the w prophage does not seem to be the only factor involved in limiting infection. In a parallel situation, lysogen B (P2) acts as a normal host for the T-even phages (44).

Three separate lysogens isolated following infection of WS by w were challenged with T2. Two of these degraded the T2 DNA but no DNA degradation was detected on infection of the third lysogen. None produced phage progeny. This seemed to support the view that degradation of T2 DNA may not be an essential feature of abortive infection in E coli W or SH(P2)

There is evidence (49) that virulent phage may damage the cytoplasmic membrane of the host cell during the infection process

but possess the capacity to repair this damage. Pizer et al have suggested (42,45) that the presence of either P2 or w as prophages may result in an altered membrane which the infecting phage more readily damages or is unable to repair. As a consequence of a damaged membrane, the cell may leak metabolites and be unable to concentrate nutrients from the medium; Fields has demonstrated leakage of ATP and an inability to accumulate galactosides in T2 - infected SH(P2) cells (45). In either case, as a consequence of depletion of substrates, macromolecular synthesis would soon cease. The difference in behavior after infection shown by different bacterial strains lysogenic for the same prophage may result from differences in cell envelopes, and hence the degree of alteration produced by the establishment of lysogeny.

However, any hypothesis involving the repair of the host cell membrane by phage induced enzymes has been rendered less credible by evidence presented in a recent paper by Duckworth (50). This worker studied the metabolism of E coli B cells infected with phage T4 and T4 ghosts. It was shown that adsorption of the phage protein coat can inhibit all bacterial macromolecular syntheses within two minutes and can also inhibit the transport of many small molecules into the cell, but that the intact phage can very rapidly reverse or prevent these latter effects even in the presence of inhibitors of protein synthesis. Cells prelabelled with nucleosides and then infected with T4 ghosts almost immediately released labelled material into the surrounding medium; phage-infected cells had the ability to maintain their soluble pools at their preinfection levels: nucleoside retention occured even in the presence of chloramphenicol and puromycin, suggesting that production of phage-mediated repair

enzymes may not be necessary for this retention.

The following hypothesis was put forward to explain these results. Attachment of the phage protein coat causes allosteric changes in certain membrane proteins leading to functional (but not necessarily physical) detachment of the host chromosome and loss of other membrane associated functions; the injection of the phage DNA and/or internal protein and its attachment to the cell membrane allows the membrane to retain its function, but in a slightly altered state that allows phage macromolecular biosyntheses in lieu of those of the host cell.

It is tempting to employ this hypothesis to explain the abortive infection observed in T5 and T-even phage infection of E coli W and SH(P2). Adsorption of phage may lead to loss of membrane function; the invading phage genome may fail to attach to the cell membrane due to a mechanism mediated by the prophage carried by the host cell and lead to death of the infected cell on the one hand and failure of the phage to propagate on the other. A mechanism other than DNA degradation may prevent attachment of the phage genome to the cell membrane as no DNA degradation has been detected following infection of SH(P2) with T2. The hypothesis also leaves unexplained the inability of T4 to adsorb to strain WS after it has been lysogenised by phage w.

An additional anomaly was reported by Pizer et al (42a, 47) who observed that only 50 percent of T2-cellular interactions resulted in death of the cell on infection of strain W. This survival seemed to be linked with the ability of some of the cells to degrade T2 DNA; all T2-cellular interactions on infection of SH(P2), which apparently

does not degrade T2 DNA, resulted in death of the infected cell. Hence it seems that some cells can survive after infection in spite of the presence of an adsorbed phage coat.

It appears that one of two events may occur on infection of strain W by T2. Firstly, DNA degradation may occur resulting in survival of the cell; secondly, in those cells which fail to degrade the infecting phage DNA a mechanism may operate resulting in death of the cell, possibly associated with loss of membrane function.

AIMS OF THE INVESTIGATION

1. To determine the presence or absence of abortive infection of *E coli* W with respect to various strains of laboratory phage by measuring their plating efficiencies and adsorption kinetics on strain W;

2. To isolate phage from various sources which would propagate readily on *E coli* W;

3. To clarify the confusion surrounding the plating efficiencies of phage from the supernatant of *E coli* W on strains C and K and the subsequent plating efficiencies of the phage particles on C and K after passage through these strains;

4. To attempt to gain an insight into the basic mechanisms of abortive infection and restriction operating in *E coli* W.

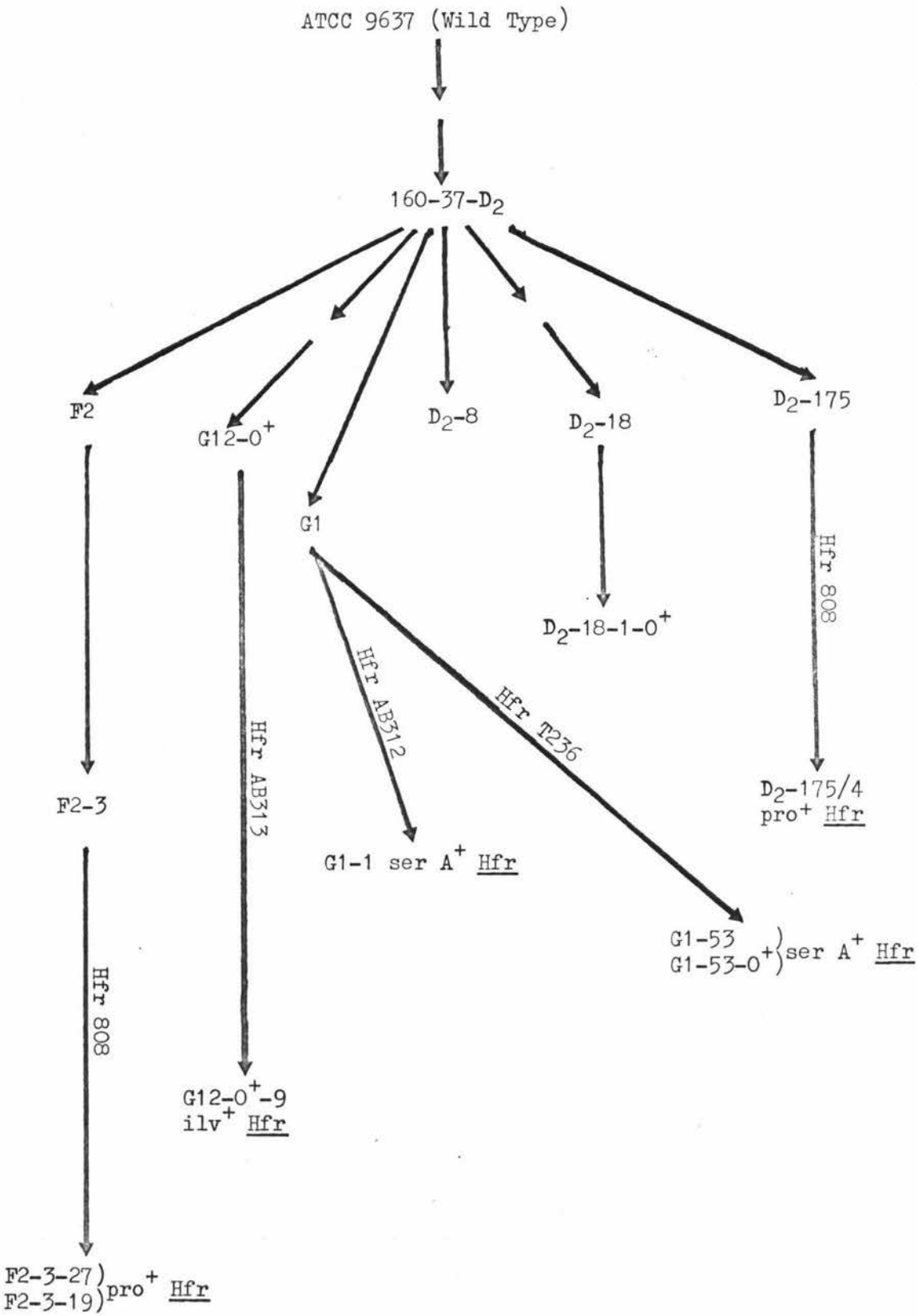
BACTERIA

Strain	Substrain	Phenotype	Source
Escherichia coli W	ATCC 9637	Wild-type (WT)	D.F. Bacon
	160-37-P2	pro^-orn^-, F^-	D.F. Bacon
	D2-8	$pro^-orn^-cit^-, F^-$	D.F. Bacon
	D2-18-1-0$^+$	pro^-leu^-, F^-, Sm^r	D.F. Bacon
	D2 175/4	orn^-met^-, Hfr_{808}	D.F. Bacon
	F2	$pro^-orn^-met^-his^-, F^-$	D.F. Bacon
	F2-3	$pro^-his^-met^-, F^-$	D.F. Bacon
	F2-3-27	met^-his^-, Hfr_{808}	D.F. Bacon
	F2-3-19	met^-his^-, Hfr_{808}	D.F. Bacon
	G1-1	$pro^-orn^-tryp^-, Hfr_{312}$	D.F. Bacon
	G1-53	$pro^-orn^-tryp^-, Hfr_{236}$	D.F. Bacon
	G1-53 0$^+$	pro^-tryp^-, Hfr_{236}	D.F. Bacon
	G-12-0$^+$-9	pro^-tryp^-, Hfr_{313}	D.F. Bacon
Escherichia coli C	C1-a	WT, non lysogenic, F^-	S. Howarth-Thompson
	C1-a/4	thr^-	from C1-a
	C1-a/4/50	thr^-pro^-	from C1-a/4
Escherichia coli B		WT, F^-	D.F. Bacon
Escherichia coli K12	T186	WT, λ_s	D.F. Bacon
	AB 266	$thr^-leu^-B1^-pro^-gal^-$ lac^-Sm^r, F^-	J.S. Loutit
	PB 240	WT λ^+	P. Bergquist
	AB 259	WT, Sm^s, Hfr_H	J.S. Loutit
	Hfr808	WT, Sm^s	D.F. Bacon

Table 3. Bacterial strains.

The derivation of the substrains of *E coli* W is described in Figure 3. Strain W Hfrs were originally obtained by D.F. Bacon who employed K12(Hfr) x W(F^-) conjugal crosses involving selection of late donor markers (51). The Hfr type is denoted by the figure following the mating type description. The origins and direction of transfer of markers of these Hfr strains are depicted in Figure 4.

Figure 3. Derivation of Substrains of Escherichia coli Strain W (D.F. Bacon)



Markers involved in selection of Hfr strains only are noted. Other markers are detailed fully in Table 3.

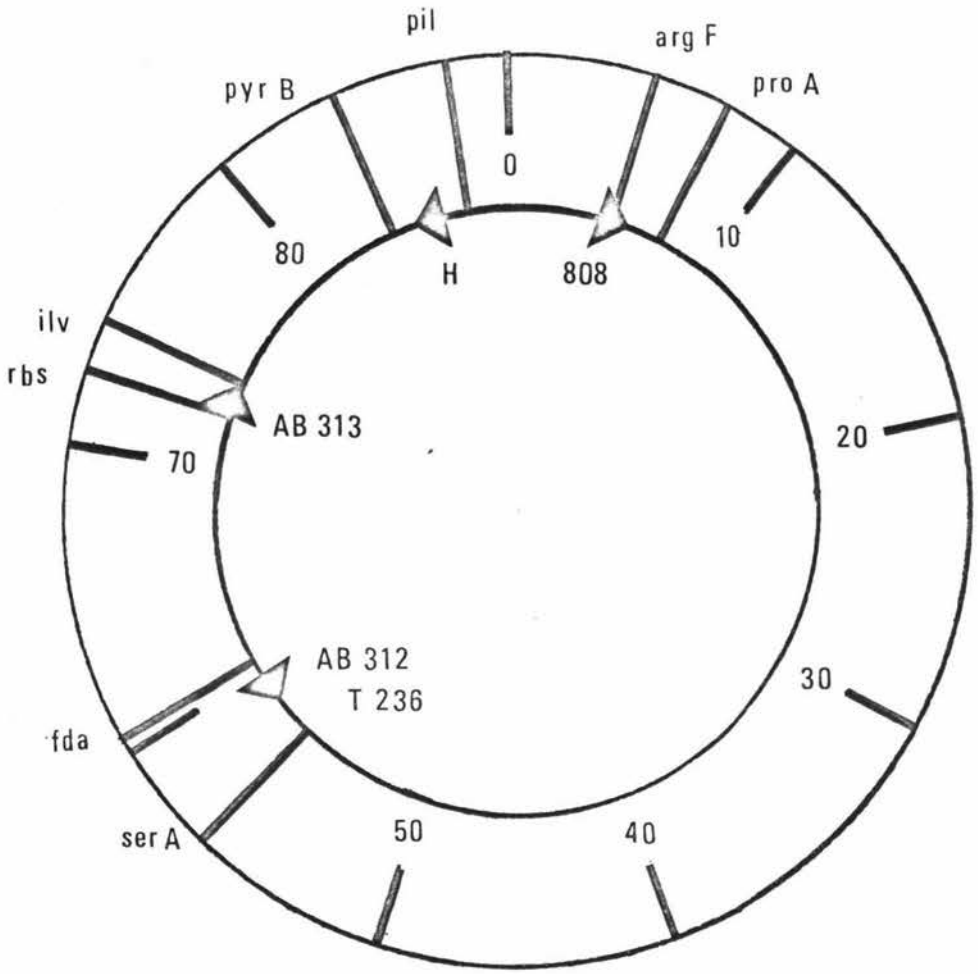

pil
arg F
pro A
pyr B
0
10
80
H
808
ilv
rbs
AB 313
70
20
AB 312
T 236
30
fda
ser A
50
40

Figure 4

Figure 4. Linkage map showing the point of origin of chromosome transfer for several Hfr strains of Escherichia coli. Arrowheads on the inner circle indicate direction of transfer. The first and last markers known to be transferred by each Hfr are displayed on the outer circle (36,52).

Gene symbol	Map position (min)	Phenotypic trait affected
arg F	5	Ornithine transcarbamylase
fda	60	Fructose 1,6 diphosphate aldolase
ilV	74	Isoleucine-valine
met C	59	Cystathionase
pil	88	Presence or absence of pili
pro A	7	Proline synthesis
pyr B	84	Aspartate transcarbamylase

BACTERIOPHAGES

Phage	Source
T1	S. Howarth-Thompson
T2 - T7	D.F. Bacon
P1	J. Pittard
λ	from culture fluids of PB 240
V7	from culture fluids of an *E coli* strain isolated from cat rib
V10	from culture fluids of an *E coli* strain isolated from sheep intestine
V11	from culture fluids of an *E coli* strain isolated from fowl liver

Table 4. Bacteriophages.

MEDIA

Modified Lennox broth (ML broth)

Bacto -tryptone	15 g
Yeast extract	4.5 g
NaCl	7.5 g
Tris (hydroxymethyl) methylamine buffer	1.2 g
Glucose	4.0 cm^3 of 50% w/v solution added after autoclaving
$CaCl_2.6H_2O$; 0.05M	2.0 cm^3 added after autoclaving
Distilled water	1 liter

The medium was adjusted to pH 7.2 with 2N HCl before autoclaving.

Modified Lennox plate agar (ML agar)

ML broth	1 liter*
$CaCl_2.6H_2O$; 0.5M	5.0 cm^3 added after autoclaving
Agar (NZ Davis)	10 g

Thirty cm^3 of ML agar was dispensed per plate to form the basal layer.

* The 0.05M $CaCl_2$ was omitted and the glucose was added after autoclaving.

Modified Lennox soft agar (ML soft agar)

Prepared as for ML agar with the exception of the agar which is added to give a concentration of 0.6%. Stored in 50 cm^3 quantities.

Minimal Medium

K_2HPO_4	7.0 g
KH_2PO_4	2.0 g
Sodium citrate.$2H_2O$	0.5 g
$MgSO_4.7H_2O$	0.1 g
$(NH_4)_2\ SO_4$	1.0 g
Distilled water	1 liter
Glucose	4.0 cm^3 of 50% w/v solution added after autoclaving

Minimal Agar

Minimal medium	1 liter*
Agar (NZ Davis)	10 g

Twenty cm^3 was dispensed per plate.

* Glucose was added after autoclaving, medium and agar were autoclaved separately.

Tetrazolium Solution

Triphenyl tetrazolium chloride	0.3 g
NaCl	0.8 g
KCl	0.04 g
Distilled water	100 cm^3

Plate count agar

Dehydrated Difco Brain -Heart Infusion Broth (BHI)	37 g
Agar (NZ Davis)	10 g
Distilled water	1 liter

Slant culture agar

Dehydrated Difco Nutrient Broth Agar	23 g
NaCl	5 g
Distilled water	1 liter

METHODS

Several of the methods below have been adopted from Adams (53). Unless otherwise stated, all temperature controlled experiments were held at 37°C; incubation was also at 37°C.

(i) Tetrazolium agar overlay technique for phage titrations

A soft agar overlay consisting of 1 cm^3 of tetrazolium solution, 2 cm^3 of ML soft agar, 0.1 cm^3 of overnight bacteria and 0.1 cm^3 of an appropriately diluted phage lysate was poured onto a plate of ML agar. The tetrazolium in the overlay is initially colourless but is reduced to the red formazan by growth of the seed bacteria in the overlay. Very small plaques can be resolved with this method which either would not be seen or would be seen with great difficulty on the more traditional overlay plates. Tetrazolium overlay plates also permit easier counting of lysogenic phage; the plaque areas appearing a lighter red.

(ii) Preparation of high titer phage stocks

(a) Tube method (for lytic phage)

0.1 cm^3 of a phage lysate containing 10^{10} pfu per cm^3 was added to a 10 cm^3 culture of ML broth containing approximately 5×10^8 sensitive log phase bacterial cells per cm^3. The mixture was aerated and frequently cleared after several generations giving rise to a lysate containing $10^{10} - 10^{11}$ pfu per cm^3. These lysates were centrifuged for 10 minutes at 10,000 x g to remove bacterial debris and stored over chloroform at 4°C.

(b) Plate method (for lytic or temperate phage)

A dilution of phage was added to 3 cm^3 of soft agar and poured onto a plate of ML agar. The dilution was such that the

plaques were almost confluent when fully developed. Three cm^3 of ML broth was added, the soft agar overlay broken up, the resultant suspension shaken vigourously, allowed to stant 30 minutes, centrifuged at 10,000 x g for 10 minutes and the supernatant (or lysate) stored over chloroform at 4^oC. These lysates usually contained 10^{10} - 10^{11} pfu per cm^3.

(iii) Isolation of phage which propagate on Escherichia coli strain W (ATCC 9637)

(a) Temperate phage.

E coli strains isolated during post-mortems on various species of animal were spotted onto ML agar plates which were incubated overnight; the resultant colonial growth on the plates was exposed to chloroform, killing the bacteria present. ML soft agar overlays containing E coli strain W were now poured onto the plates which were then given an additional overnight incubation. A zone of clearing around a colony is indicative of either a temperate phage or a colicin active on the seed bacterium (E coli W) present in the overlay (plate 5). Colicins tend to give larger clear zones. Bacterial strains which produced zones by this test were grown overnight, centrifuged, and the supernatants titrated against E coli W. Only phage would be expected to give rise to plaques.

(b) Phage from sewage.

A sewage sample was centrifuged at low speed to remove debris, 1 cm^3 of supernatant was added to 30 cm^3 of ML broth containing 10^7 cells of E coli W per cm^3 and incubated overnight. The resulting growth was centrifuged at 10,000 x g for 10 minutes and the supernatant titrated against E coli W.

(iv) Isolation of T-phage-resistant mutants of E coli B.

Enough phage was added to a young culture of strain B at 5×10^8 cells per cm^3 to give an input ratio of 30 to 60 phage per cell. This mixture was allowed to adsorb for 30 minutes after which 0.1 cm^3 was spread directly onto ML agar plates. The colonies obtained were subcultured to remove residual phage then spread as lawns onto ML agar plates onto which were spotted T-phage (at approx. 10^3 phage per spot) in order to check for cross resistance patterns.

(v) Preparation of samples for the electron microscope

(a) For observation of lysates.

Ten cm^3 of a lysate containing at least 10^{10} pfu per cm^3 was spun in a preparative ultracentrifuge at 100,000 x g for 2 hours, the supernatant decanted and the pellet resuspended in 1 cm^3 of distilled water. In some cases, in order to obtain 'clean' preparations, it was necessary to repeat the centrifugation to remove the residual broth. A sample was then mixed with phosphotungstic acid negative strain (PTA) and sprayed onto a grid.

(b) For observation of adsorption.

One cm^3 of log phase bacterial cells at 2×10^8 per cm^3 was added to 4 cm^3 of a phage lysate (10^{10} pfu per cm^3) in ML broth, 10 minutes allowed for adsorption, the mixture centrifuged at 10,000 x g for 2 minutes, the pellet resuspended in distilled water and fixed with 1% formaldehyde to prevent lysis. Samples were then mixed with PTA negative stain and sprayed onto a grid.

(vi) Measurement of adsorption coefficients

The bacterial strain was grown overnight in BHI broth, diluted one in ten, again in BHI broth, and grown 2 hours for strain W,

$2\frac{1}{2}$ hours for strains B and K. The cells were then washed once in saline and resuspended in BHI broth. 0.9 cm^3 of this bacterial cell suspension, now at approximately 10^8 cells per cm^3, was added to 0.9 cm^3 of a phage lysate with 10^6 - 10^7 pfu per cm^3, plus 0.2 ml of 5×10^{-3}M Ca^{++}. Aliquots of 0.1 cm^3 were removed at suitable intervals into 9.9 cm^3 of diluent containing chloroform in order to kill infected cells and to prevent further adsorption by dilution. Unadsorbed phage were then titrated.

(vii) Determination of phage heat sensitivity

(a) Phage at about 10^7 pfu per cm^3 were subjected to various temperatures for 10 minutes while suspended in 0.1M NaCl. One cm^3 samples were then diluted into 9.0 cm^3 of BHI broth at room temperature and assayed.

(b) A temperature was selected at which 90% inactivation of phage occurs after 10 minutes. Phage at 10^7 pfu per cm^3 were subjected to this temperature and one cm^3 samples were diluted at 2 minute intervals into BHI broth at room temperature. These tubes were then titrated at appropriate dilutions.

(viii) Preparation of antisera

Two cm^3 of phage lysate with a titer of at least 10^{10} pfu per cm^3 was mixed with 2 cm^3 of Freund's complete adjuvant, homogenised, and four 0.5 cm^3 aliquots injected subcutaneously into each of two rabbits. Prior to these injections, normal serum was obtained as a control from each rabbit. After four weeks the rabbits were bled from the ear veins to yield about 30 cm^3 of blood which was allowed to coagulate, and the sera harvested. Thiomersal was added as a preservative and the antisera stored frozen.

(ix) Determination of K for antisera

Sera were diluted in BHI broth to give between 90 and 99 percent neutralisation in 10 minutes. 0.1 cm^3 of phage at 10^7 pfu per cm^3 was added to 0.9 cm^3 of diluted serum and 0.1 cm^3 samples were removed at 2 minute intervals. These were immediately diluted into 9.9 cm^3 of 10 percent BHI broth to stop further neutralisation. These tubes were then titrated at appropriate dilutions.

(x) Lysogenisation of E coli K and C with phage w

Phage w was plated on either K or C to give well-spaced plaques which were stabbed with a sterile needle and innoculated into 2.5 cm^3 of BHI broth, grown up overnight and streaked onto BHI agar plates. The selected single colonies which developed were grown up in BHI broth, tested for insensitivity to the w phage and the supernatants titrated for phage w. Clones were designated lysogenic if they produced phage w and were immune to infection by phage w, carrying the appropriate host range specificity. To test for insensitivity of suspected lysogens to w, about 10^4 pfu of phage w were spread onto a ML agar plate onto which the suspected lysogens were spotted. Strains lysogenic for phage w did not give plaques within the spot (40) (Plates 17,18,19).

(xi) Isolation of mutants of E coli C (52)

E coli C was grown overnight in minimal medium, diluted 1:10, incubated 2 hours, diluted 1:2 and 8 ml of this bacterial cell suspension U.V. - irradiated for 60 sec., 60 cm from a Chromolux 24 U.V. lamp (catalog No.633000) to give 0.1% survival of the cells. This irradiated culture was pipetted into 100 cm^3 of minimal broth containing 50 ug cm^3 of each of the amino acid growth requirements for which mutants were to be selected, and incubated overnight. This culture

was diluted 1:2, grown one hour to bring it out of the lag phase, and a 10 cm^3 sample washed twice in saline and resuspended in 1 cm^3. Enough of the suspension to give an optical density of klett 10 was placed into 10 cm^3 of minimal medium in a side arm klett flask. These cells were incubated until the optical density doubled, when 0.1 cm^3 of penicillin at 2 x 10^4 ug per cm^3 was added and the flask incubated 90 min. The survivors were plated onto minimal agar supplemented with a mixture of the appropriate amino acid growth requirements at a level which would just sustain the growth of a mutant (about 1-2 ug per cm^3) and incubated 48 hours. The mutant colonies were much smaller than those of the wild type. Their phenotype was determined by spotting them onto minimal agar as a control, on which mutants did not grow, and onto minimal agar plates supplemented with individual amino acids at a concentration of 50 ug per cm^3.

(xii) The standard conjugal cross

The Hfr and F^- strains were grown overnight in 2.5 cm^3 of BHI broth to give a cell density of approximately 2 x 10^9 per cm^3. These cultures were diluted 1:2, incubated 1 hour to bring them out of the lag phase, centrifuged, and resuspended in 2.5 cm^3 of BHI broth. 0.1 cm^3 of the Hfr was then added to 0.9 cm^3 of the F^- and the mixture allowed to mate. After one hour samples were taken, diluted appropriately and plated on media which would select for recombinants. The parents were plated separately on the selective media at appropriate dilutions as controls.

In the case of the cross F2-3-27 x C1-a/50, the Hfr was washed twice to reduce the concentration of phage w and 0.1 cm^3 of a 1:10 dilution of the w antiserum (K = 230) was added to the mixture to suppress infection of the F^- from without by phage w. Additional controls

included titration of the cross supernatant after one hour for phage w and testing F^- cells from the cross for lysogenisation by spotting them on a 'lawn' of w. The frequency of lysogenised cells in the F^- population was too low to be detected by this method and had lysogens been detected these would probably have arisen by infection from without by phage w.

RESULTS AND DISCUSSION

I. INVESTIGATION OF THE ABORTIVE INFECTION OF ESCHERICHIA COLI BY:

(i) T5 and the T-even phages

Phages T2, T4, T5 and T6 grown on strain B could not be shown to form plaques on a lawn of strain W (Table 7) although, with the exception of phage T4, adsorption occured to these cells (Figures 6,8,10,11). In addition, phages T2, T5 and T6 appeared to exert a killing effect on strain W; an input of three phage particles per bacterial cell in a plated soft agar layer resulted in failure of the cells to develop a lawn. When phage were incorporated at successively lower input ratios no plaques were ever observed although the bacterial lawn developed fully. In the case of phage T4, however, an input ratio of 20:1 still permitted the development of a confluent bacterial lawn, consistant with the observation that T4 does not readily adsorb to strain W (Figure 8).

Pizer et al reported that T4 would not plate on strain C (42). This has been shown to be due to the inability of T4 to adsorb to this strain (Figure 9). A host range mutant of T4 occuring in the T4 population at a frequency of 10^{-6} (Table 7) was isolated which would plate on strains B and C (but not B/4) with an efficiency of approximately 1.0.

Table 5. Plating effects at different input levels of phage w on *E coli* strain W.

Phage	Number of phage particles plated	Input ratio phage/bacterium	Bacterial lawn development
T2.B	3×10^8	3	-
	3×10^6	3×10^{-2}	+
	3×10^4	3×10^{-4}	+
	3×10^2	3×10^{-6}	+
T3.B	6×10^8	6	-
	6×10^6	6×10^{-2}	+*
	6×10^4	6×10^{-4}	+*
	6×10^2	6×10^{-6}	+
T4.B	2×10^9	20	+
	2×10^8	2	+
	2×10^6	2×10^{-2}	+
	2×10^4	2×10^{-4}	+
	2×10^2	2×10^{-6}	+
T5.B	4×10^8	4	-
	4×10^6	4×10^{-2}	+
	4×10^4	4×10^{-4}	+
	4×10^2	4×10^{-6}	+
T6.B	4×10^8	4	-
	4×10^6	4×10^{-2}	+
	4×10^4	4×10^{-4}	+
	4×10^2	4×10^{-6}	+
T7.B	3×10^8	3	-
	3×10^6	3×10^{-2}	+*
	3×10^4	3×10^{-4}	+*
	3×10^2	3×10^{-6}	+
P1.K	6×10^8	6	+
	6×10^6	6×10^{-2}	+
	6×10^4	6×10^{-4}	+
	6×10^2	6×10^{-6}	+

continued.....

Table 5 continued.....

In each case a soft agar layer was innoculated with 10^8 *E coli* W cells per plate.

* mottling observed on these plates - see text P.51.

These results are generally in agreement with those of Pizer et al (42,46) who reported killing of strain W by T2, and inability of T2, T4, T5 and T6 to plate on strain W. Their system, however, did not enable them to measure a plating efficiency of less than 2×10^{-6} for T2, 6×10^{-6} for T6 and 7×10^{-4} for T5 as these were the lowest dilutions of phage allowing a confluent bacterial lawn. This may have been due to the possible use of a low concentration of bacterial cells in the lawn. They suggested that failure of the lawn to develop on addition of more phage may have been caused by lysis from without but it has been shown (Table 5) that low mutiplicities in the vicinity of three will cause death of a cell. This confirms the later observation by Pizer et al that infection by one phage particle is frequently lethal (42a).

(ii) T3 and T7

Phages T3 and T7 grown on strain B also failed to produce plaques on infection of strain W (Table 7) although normal adsorption occured (Figures 7,12). However a mottling of the bacterial lawn was observed on these plates at an input ratio of 10^{-2} to 10^{-3} phage per bacterial cell. It seems unlikely that these areas were plaques as it was demonstrated for T7 that although bursts of phage do occur at a low frequency, the phage produced are not modified and are still unable to infect strain W (Table 6). In addition it is probable that, a small number of T3 and T7 phage particles per E coli W cell is sufficient to produce cell death (Table 5).

Table 6. Determination of the frequency of productive infection of E coli W cells by phage T7.B.

Host cell concentration	Phage input ratio	Unadsorbed phage at 5 min.	Free phage after antiserum	Infective centers on		*Yielder frequency
				B	W	
$1.25 \times 10^8/cm^3$	1	$8 \times 10^6/cm^3$	$45/cm^3$	$9.5 \times 10^3/cm^3$	$<10/cm^3$	7.5×10^{-5}

Phage T7.B was adsorbed to young growing cells of strain W for 5 minutes. Unadsorbed phage were assayed after killing the infected bacteria with chloroform both before and after the addition of antiserum. Assays of infective centers on B and W were completed within 9 minutes of mixing phage T7.B and the E coli W cells to ensure titration of infective centers only.

* Yielder frequency - the proportion of infected bacteria which produced infective centers on B.

(iii) Phage T1

Phage T1.B plated on strain W with an efficiency of 10^{-4} producing sharply defined plaques of irregular size and shape which were always much smaller than those of T1.B plated on strain B, (Plates 1,2). Phage from these small plaques plated on strain W with an efficiency of 10^{-1} to 2×10^{-2} compared with their plating efficiency on strain C. On this latter host they produced typical T1 plaques. After several rounds of replication in strain C the resultant progeny (T1.W.C) still retained their ability to plate on strain W with an efficiency of 10^{-1} to 2×10^{-2}, (Table 7) suggesting that the ability of T1,W to plate on strain W is not due to host controlled modification but is genetically inherited.

Lederberg (44) reported that T1.B plated on the lysogen B(P1) with an efficiency of 10^{-4}. Some of the resultant progeny were then able to plate on B(P1) with an efficiency of 1.0; the yielder cells giving rise to a mixture of restricted and unrestricted phage. The ability of the unrestricted phage to plate on B(P1) was lost after several cycles of growth in strain B. He concluded that T1.B was restricted in B(P1) and that some of those phage which were able to escape this restriction and propagate in the B(P1) cell became modified thus enabling them to infect B(P1) with an efficiency of plating of 1.0.

In contrast, phage T1.B was not modified after propagation in E coli W and it seems likely that its ability to propagate on strain W is due to the presence of a mutant occuring at a frequency of 10^{-4} in the T1.B population. The mutation may involve a change in the nucleotide sequence recognised by either the w prophage specified restriction system or a change in the nucleotide sequence

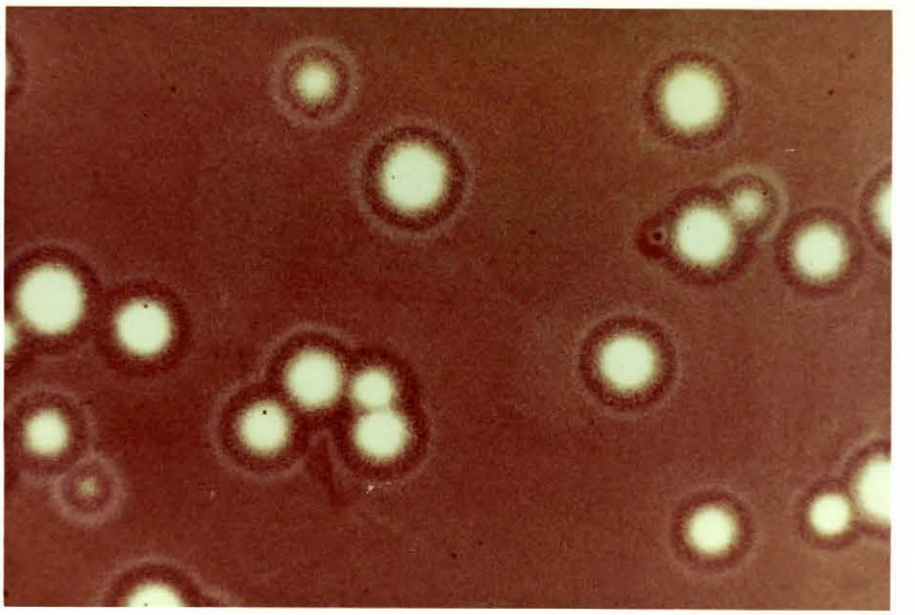


Plate 1. Plaques formed by phage T1.B on E coli B (x3)

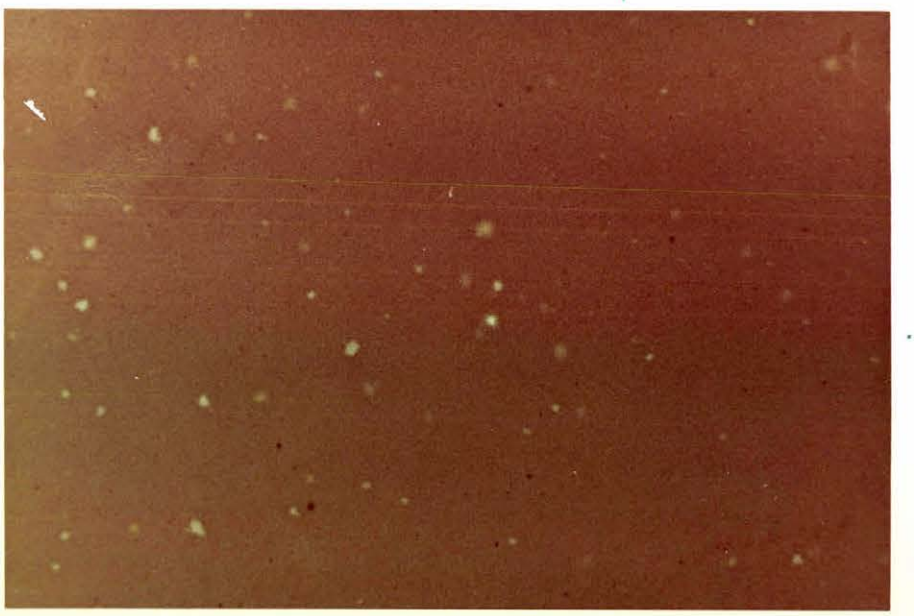


Plate 2. Plaques formed by phage T1.B on E coli W (x3)

recognised by that of the W genome but only if one of these systems was not active against T1.B DNA. The alternative explanation that a mutation occured in the nucleotide sequences recognised by both types of restriction seems unlikely because of the high frequency of the mutational event.

(iv) Phage P1

Phage P1.K did not produce plaques on strain W or prevent development of a bacterial lawn of W at an input ratio of 6 phage per cell. P1 was shown to adsorb to strain W (Figure 13) and hence this infection appears to be very similar to that of λ which plates on strain W with an efficiency of 10^{-10} and which does not kill the cell unless phage progeny are produced. It remains to be shown, however, that degradation of P1 DNA occurs in strain W. It is possible, therefore, that mutants of P1 could be obtained at a low frequency which would then plate on W with an efficiency of 1.0. P1.K is restricted by some strains of C lysogenic for prophage w and this fact might be employed to facilitate isolation of a P1 mutant able to propagate in W as the W genome restriction activity would not be present in the C (w) lysogen.

Table 7. Efficiencies of plating of phages on Escherichia coli strains W, B and C.

Phage	eop on B	eop on C	eop on W
T1.B	1.0	1.0	10^{-4}
T1.C	1.0	1.0	10^{-4}
T1.W	1.0	1.0	10^{-1}-2 x 10^{-2}
T1.W.C	-	1.0	2 x 10^{-2}
T2.B	1.0	0.5*	$<3.3 \times 10^{-7}$
T2.C	1.0	0.5*	$<5.0 \times 10^{-7}$
T2.B	1.0	1.0	$<1.7 \times 10^{-5}$
T3.C	1.0	1.0	$<1.3 \times 10^{-5}$
T4.B	1.0	10^{-6}	$<5.0 \times 10^{-10}$
T4.C	1.0	0.5*	$<2.0 \times 10^{-9}$
T5.B	1.0	1.0	$<1.5 \times 10^{-7}$
T5.C	1.0	1.0	$<4.0 \times 10^{-7}$
T6.B	1.0	0.5*	$<1.5 \times 10^{-7}$
T6.C	1.0	0.5*	$<3.3 \times 10^{-7}$
T7.B	1.0	1.0	$<3.3 \times 10^{-5}$
T7.C	1.0	1.0	$<1.6 \times 10^{-5}$
P1.K	10^{-2}	1.0	$<1.7 \times 10^{-9}$

eop = efficiency of plating

* Phages T2 and T6 always plated on C with an efficiency of 0.5 compared to that on B irrespective of whether they had been grown on B or on C. The plaque size of T2 on C was always less than that on B and the 'halo' was absent (Plates 3 and 4). This effect was also noted on infection of C by the host range mutant of T4 able to place on strain C.

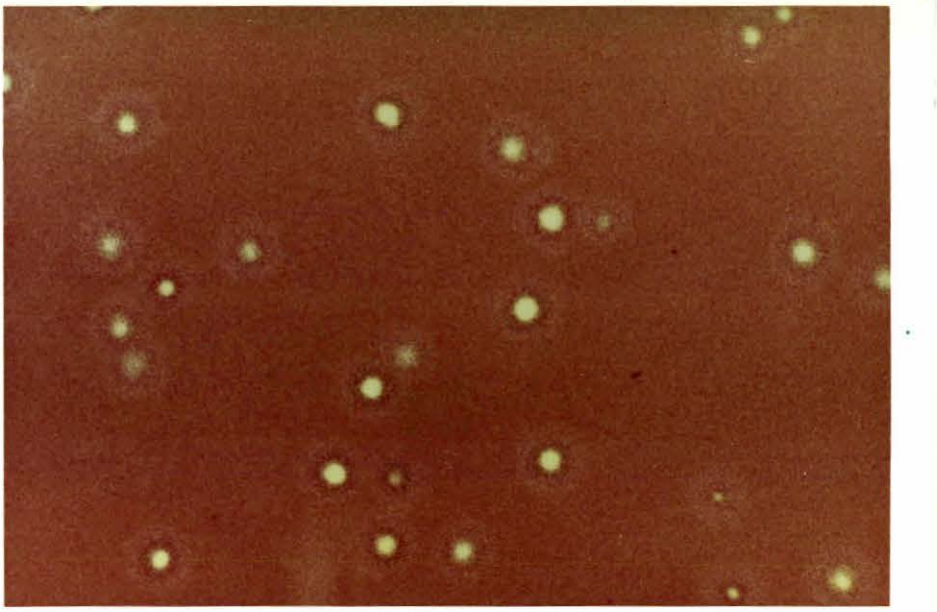


Plate 3. Plaques formed by phage T2.B on E coli B (x3)

Plate 4. Plaques formed by phage T2.B on E coli C (x3)

Table 8. Adsorption coefficients of the T phages and P1 on adsorption to *E coli* strains W, B and K12.

Phage	K_{ADS} on W	K_{ADS} on B	K_{ADS} on K12
T1.B	0.7×10^{-9}	1.9×10^{-9}	-
T2.B	1.2×10^{-9}	2.9×10^{-9}	-
T3.B	3.0×10^{-9}	2.6×10^{-9}	-
T4.B	0*	2.8×10^{-9}	-
T5.B	0.4×10^{-9}	0.5×10^{-9}	-
T6.B	1.9×10^{-9}	3.2×10^{-9}	-
T7.B	4.7×10^{-9}	4.6×10^{-9}	-
P1.K	0.7×10^{-9}	-	0.4×10^{-9}

* no measurable adsorption

$K_{ADS} = 2.3/(B)t \times \log Po/P$

Where Po = phage assay at time 0

P = phage not adsorbed at time t min

B = number of bacterial cells/cm^3

K_{ADS} = velocity constant with dimensions cm^3/min

The figures obtained for adsorption to B are in general agreement with those of other workers (53,55,56). It is notable the K_{ADS} values for the T-even phages are always less on strain W than on strain B and in fact approaches 0 in the case of adsorption of T4 to W.

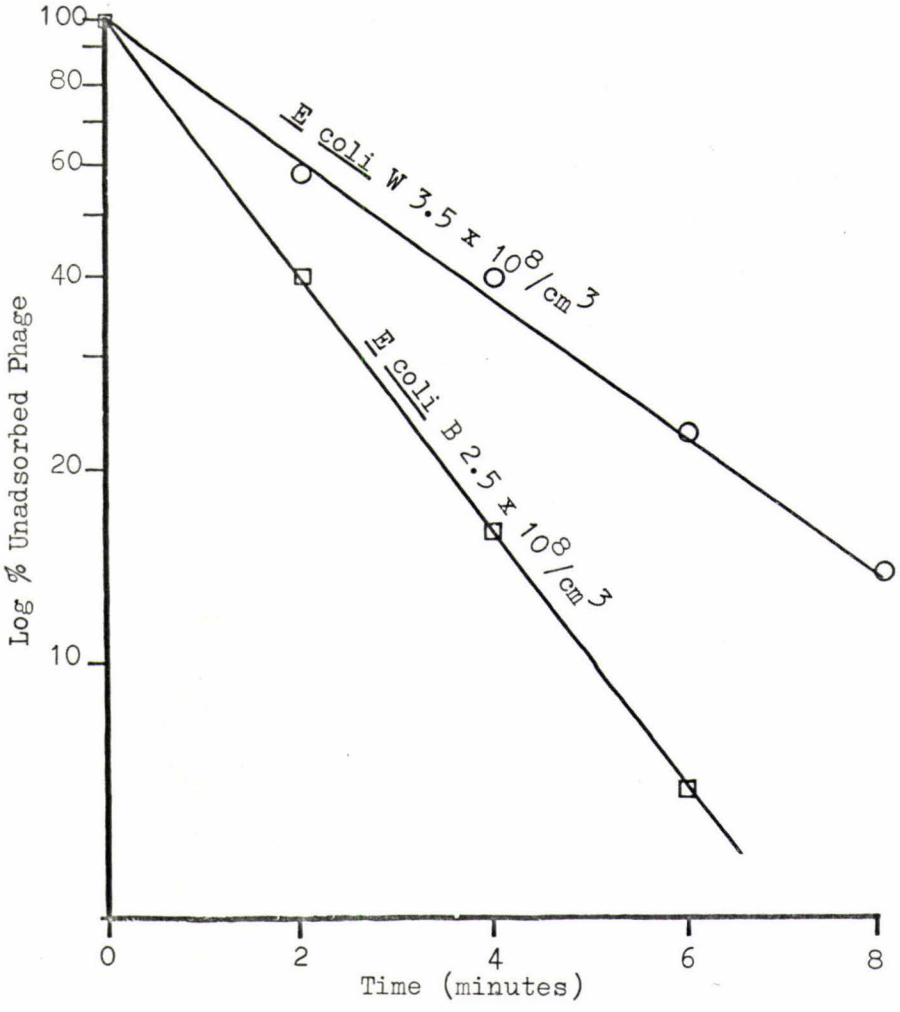


Figure 5. Adsorption Kinetics of Phage T1 to Escherichia coli strains B and W.

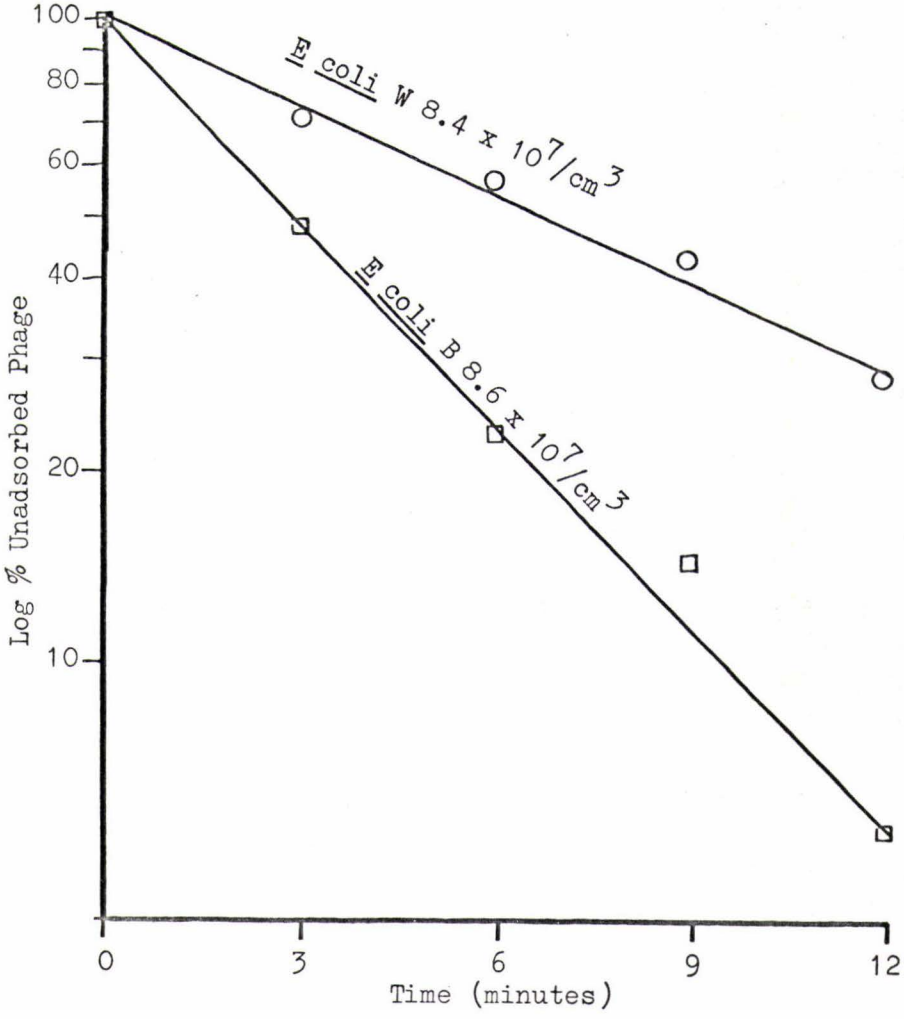


Figure 6. Adsorption Kinetics of Phage T2 to Escherichia coli strains B and W.

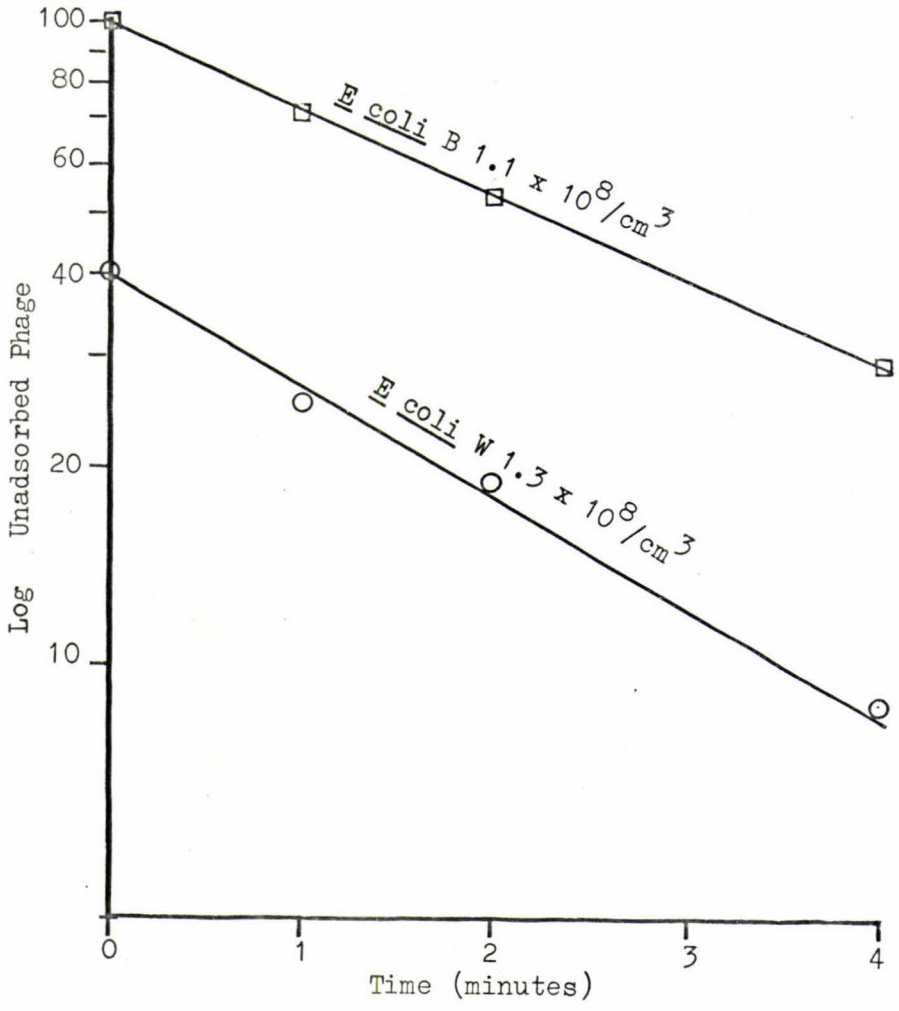


Figure 7. Adsorption Kinetics of Phage T3 to Escherichia coli strains B and W.

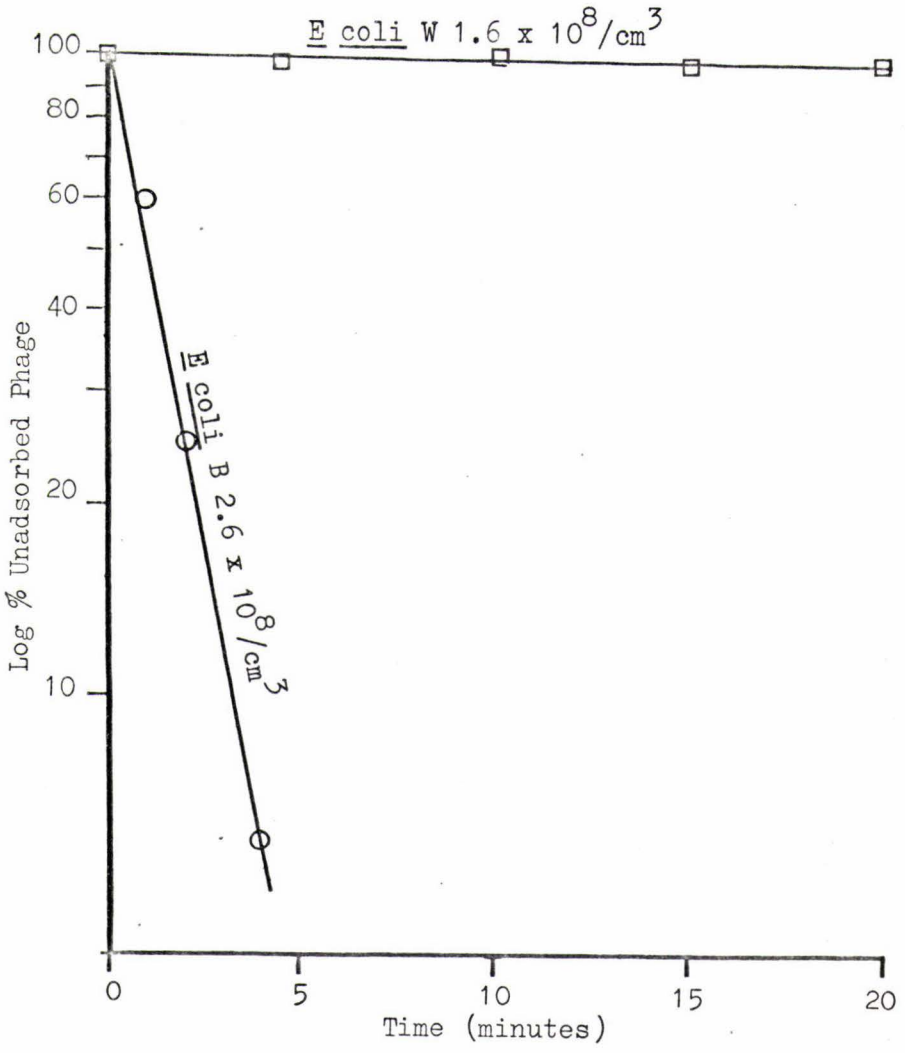


Figure 8. Adsorption Kinetics of Phage T4 to Escherichia coli strains B and W.

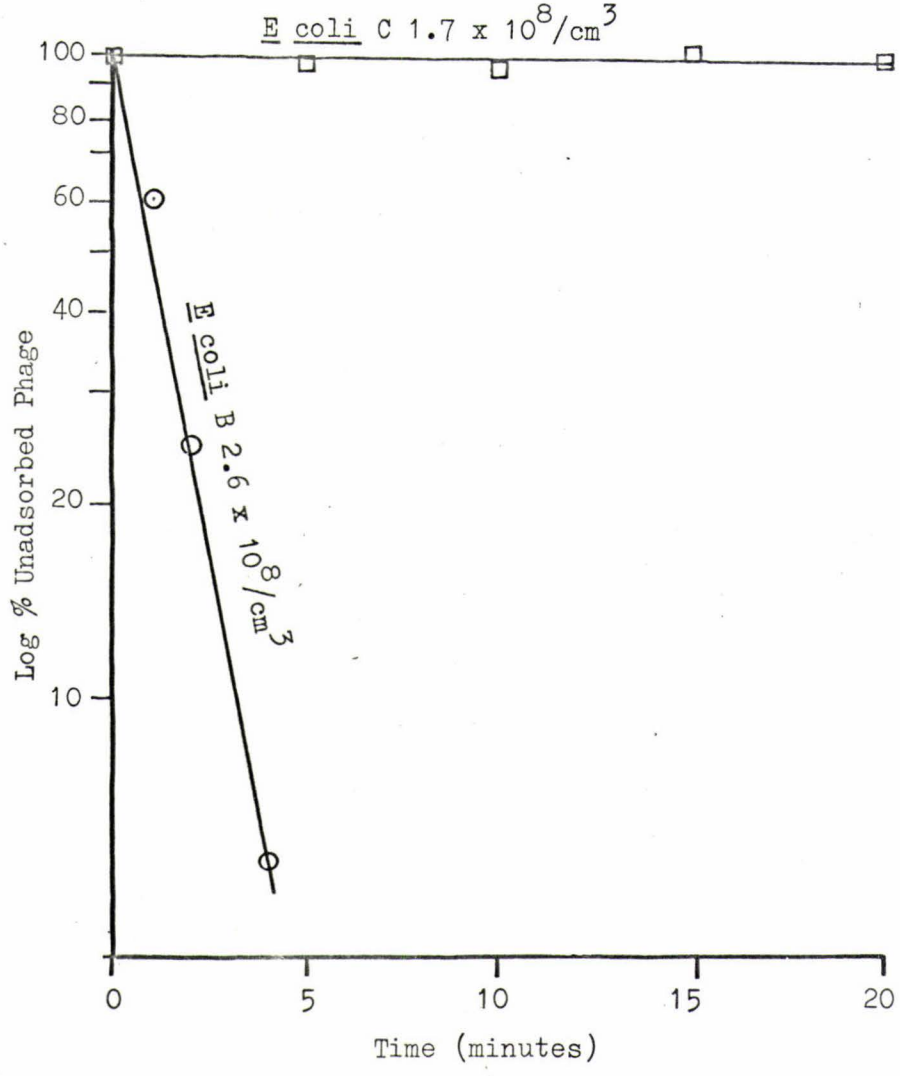


Figure 9. Adsorption Kinetics of Phage T4 to Escherichia coli strains B and C.

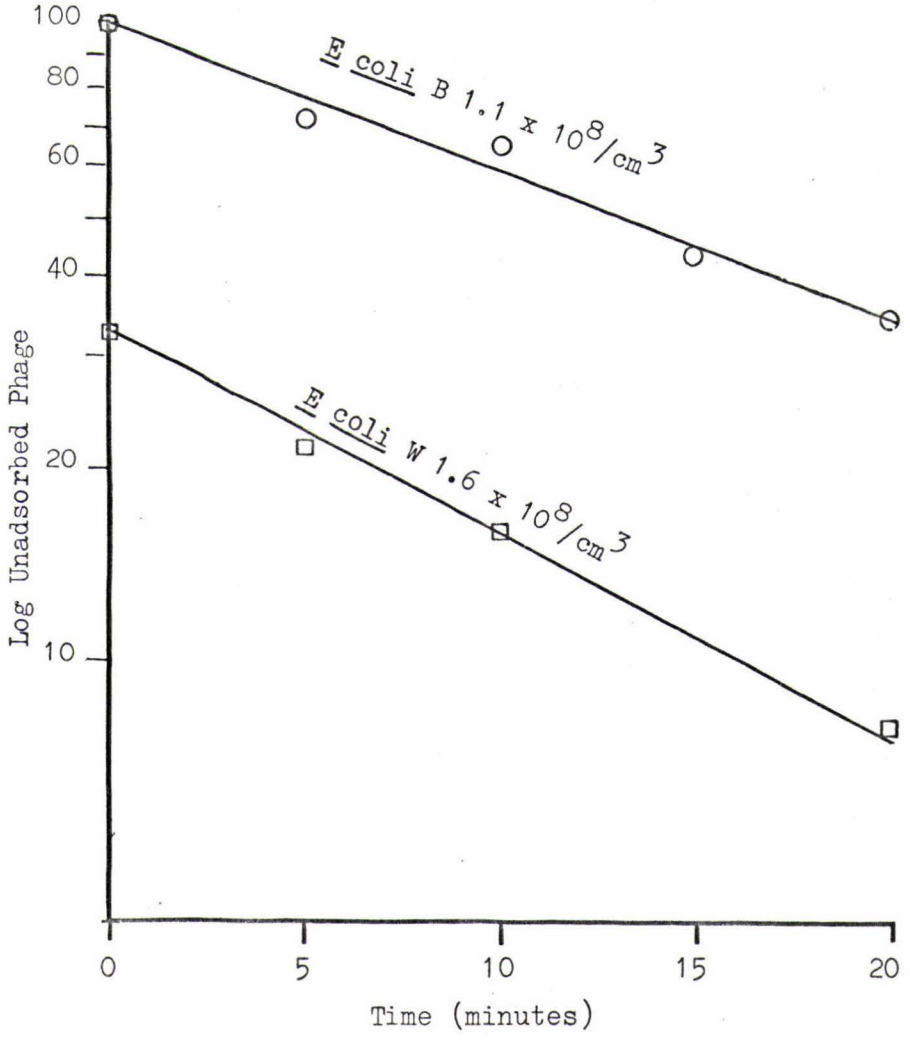


Figure 10. Adsorption Kinetics of Phage T5 to Escherichia coli strains B and W.

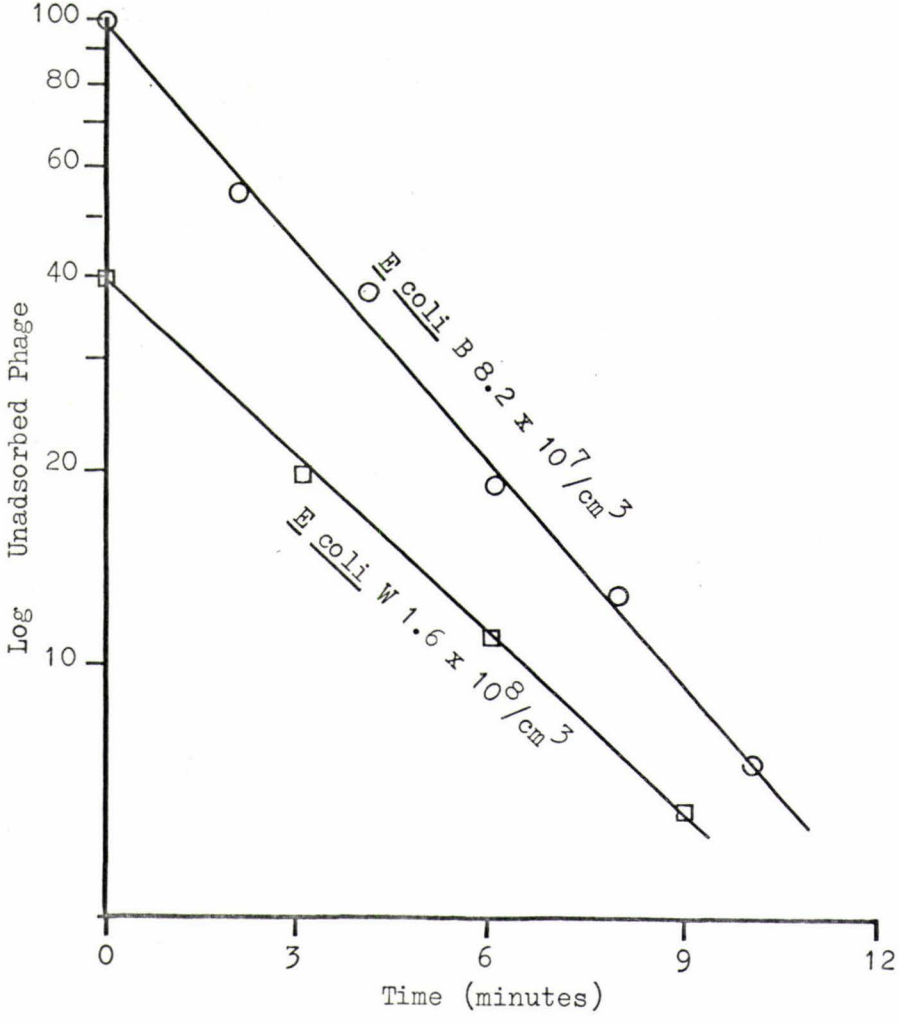


Figure 11. Adsorption Kinetics of Phage T6 to Escherichia coli strains B and W.

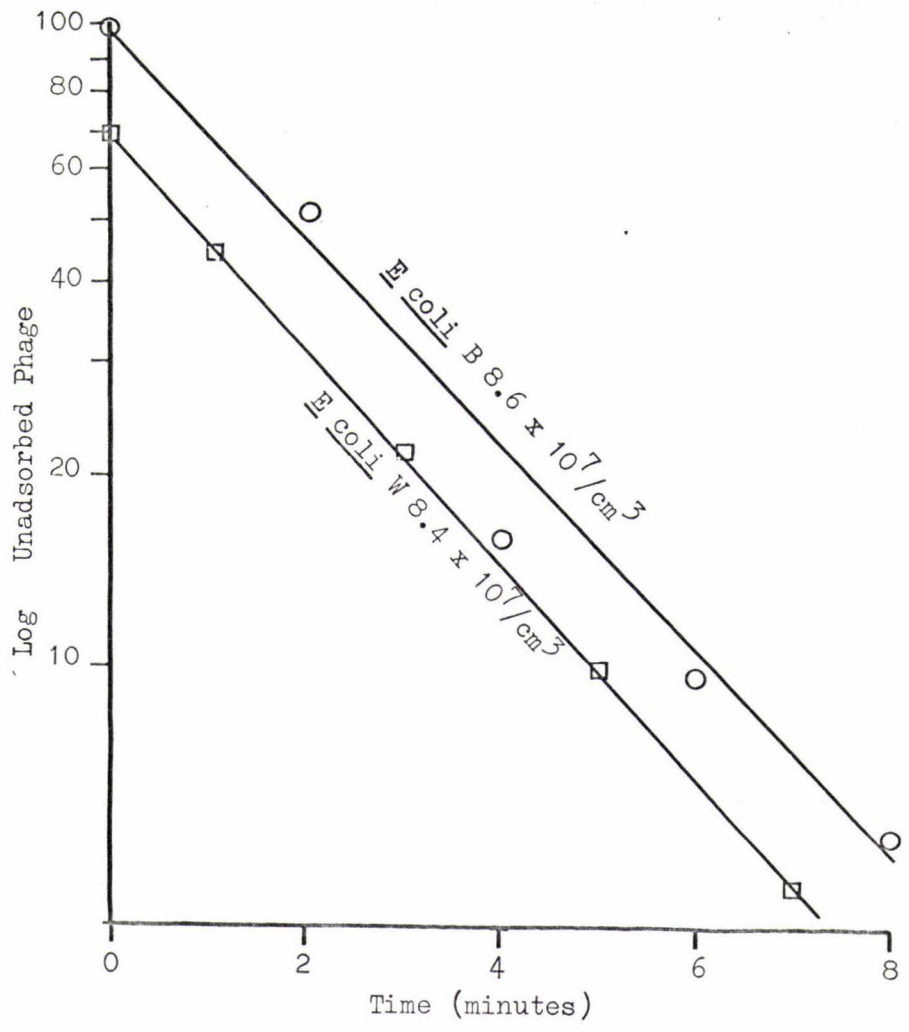


Figure 12. Adsorption Kinetics of Phage T7 to Escherichia coli strains B and W.

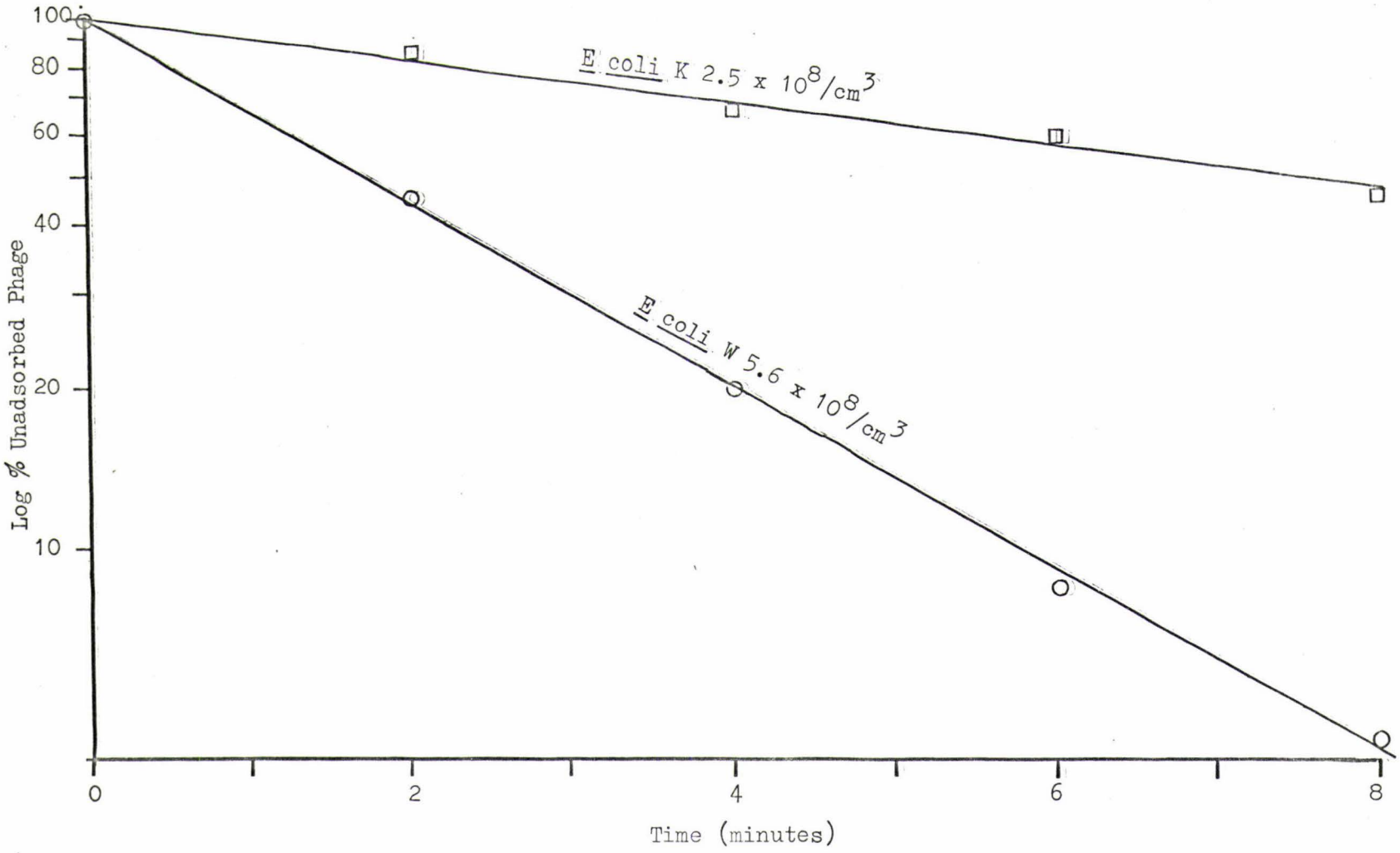


Figure 13. Adsorption Kinetics of Phage P1 to Escherichia coli Strains K and W.

II. ISOLATION OF PHAGE ABLE TO PROPAGATE ON ESCHERICHIA COLI STRAIN W

Most readily available laboratory phage strains with the exception of P2 (37), which is closely related to phage w, will not plate on *E coli* W. It was therefore decided to establish if phage able to do so could be isolated. Coliform isolates and sewage samples were obtained from various sources and screened for the presence of such phage.

Source	N° isolates	Species	Number of strains producing: Lytic zones on the initial screening (Plate 5)	Particles able to propagate on W	Particles producing reduced areas when plated on W
Palmerston North hospital - human urine and faeces	12	*E coli*	5	0	0
Massey University Veterinary clinical pathology lab.	31	*E coli*	20	4	*4
	7	*S. typhimurium*	5	0	0
	10	*S. pullorum*	0	0	0
Palmerston North sewage treatment plant	N° Samples		N° of samples producing the above phenomena		
	3		-	1	0

Table 9. Screening of *E coli* isolate and sewage samples for the presence of phage able to propagate on strain W (p.41).

* These reduced areas may be produced by phage similar to V7, V10 and V11.

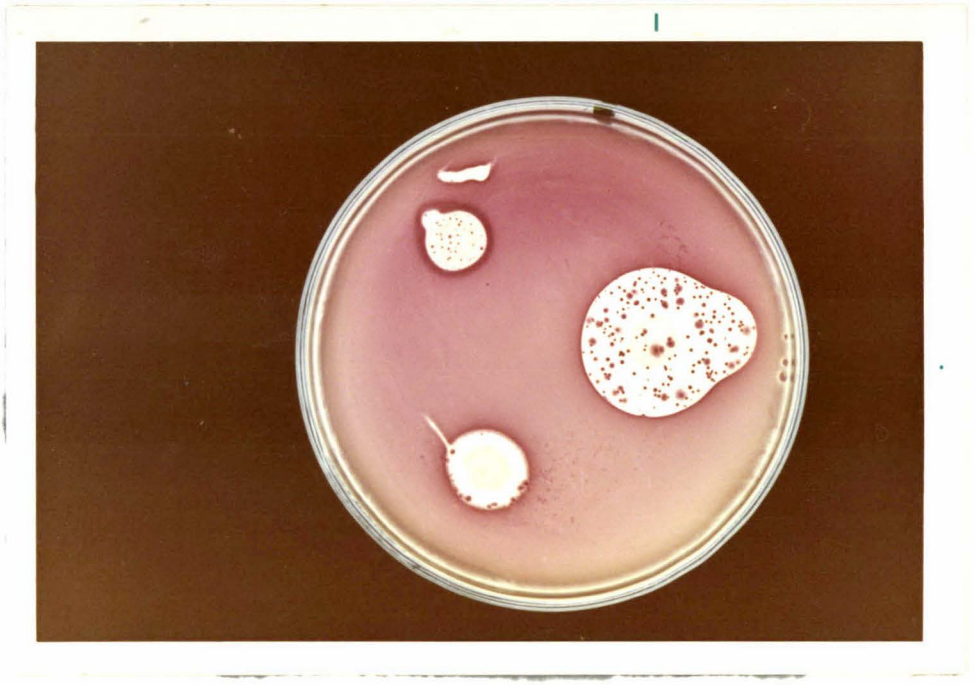


Plate 5. The screening method used to detect phage and colicins active against E coli W.

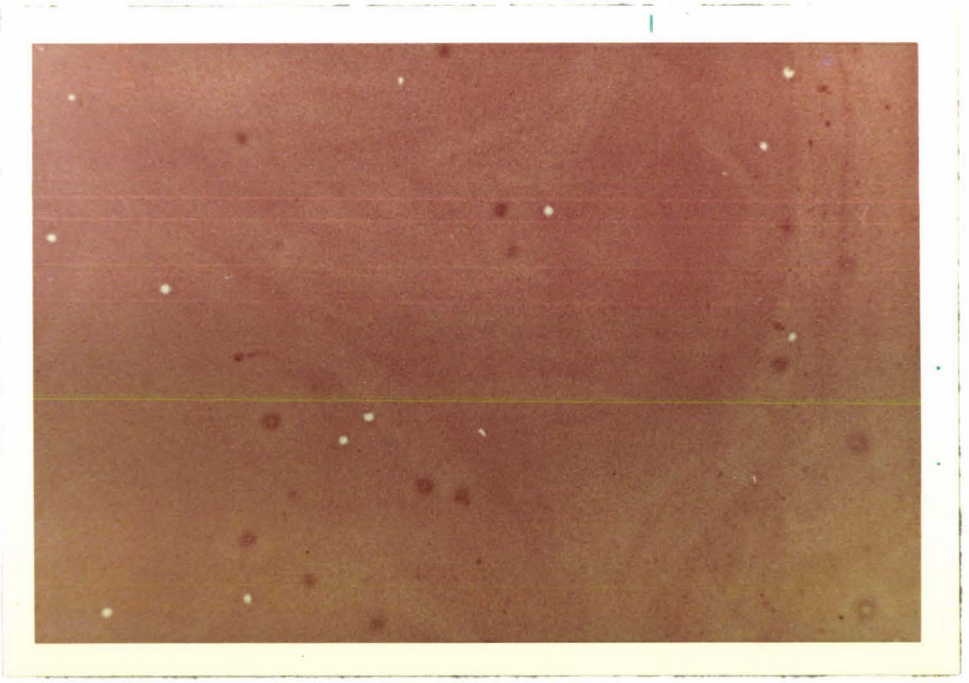


Plate 6. Plaques formed by phage V11 on E coli W (x3)

The supernatants from broth cultures of three strains of E coli obtained from the Massey University Veterinary Clinical Pathology Laboratory were plated on strain W. Each supernatant contained 10^4 - 10^5 plaque forming units per cm^3. Lysates were prepared from single plaque isolates and the phages designated V7, V10, and V11. These phages could be propagated on strain W by the methods used to propagate lytic phage and titers in excess of 10^{10} particles per cm^3 were obtained. Each lysate gave rise to three different plaque types in approximately equal proportions (Plate 6):

1) Plaques with sharply defined edges 0.3mm in diameter and lacking 'halos';

2) Plaques with clear centres surrounded by a ring of intense tetrazolium reduction or 'halo' 0.6mm in diameter;

3) Areas of intense tetrazolium reduction with no cleared centre, about 0.3mm in diameter.

When any one of these three plaque types was stabbed with a sterile needle and propagated in strain W, all three types were again produced by the progeny in approximately equal proportions.

The efficiencies of plating of V7, V10, and V11 were determined on strains B, C and K. It was less than 10^{-9} on strain B and less than 10^{-7} on strains C and K; measurements of plating efficiencies of V-phages on C and K below 10^{-7} were prevented by the presence of phage w in the lysates. Phage w was shown not to adsorb to strain B and hence a lower frequency could be determined in this case. It appears from electron micrographs (Plates 11,12) that the failure of the V-phages to plate on strains B, C and K is due to their failure to adsorb to these strains.

The three phages were observed under the electron microscope, (Plates 7-10) and all three were seen to be similar in size and morphology to phages T5 and λ. No base plates or tail fibers were seen and the sheaths were never seen to contract, even on infection of strain W.

Table 10. Dimensions of the V-phages.

	head(nm)	tail(nm)
V7	85 x 85	170 x 10
V10	85 x 70	170 x 10
V11	80 x 65	165 x 10
λ*	65 x 65	170 x 10

*from Bertani (9)

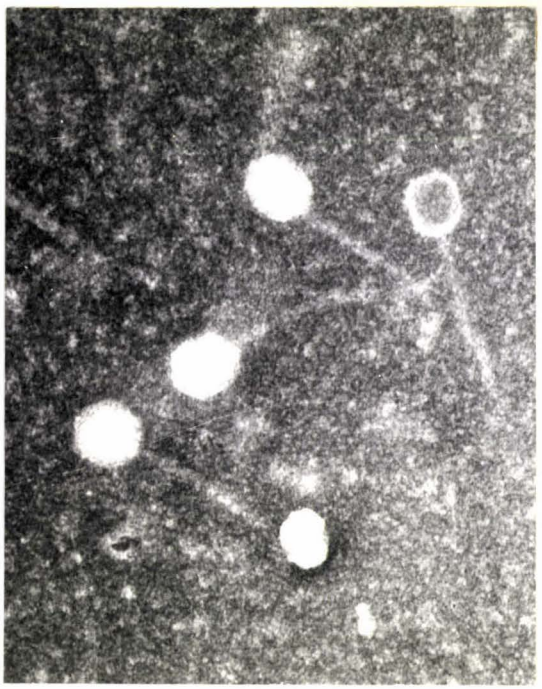


Plate 7. Phage V7 (x116,000)

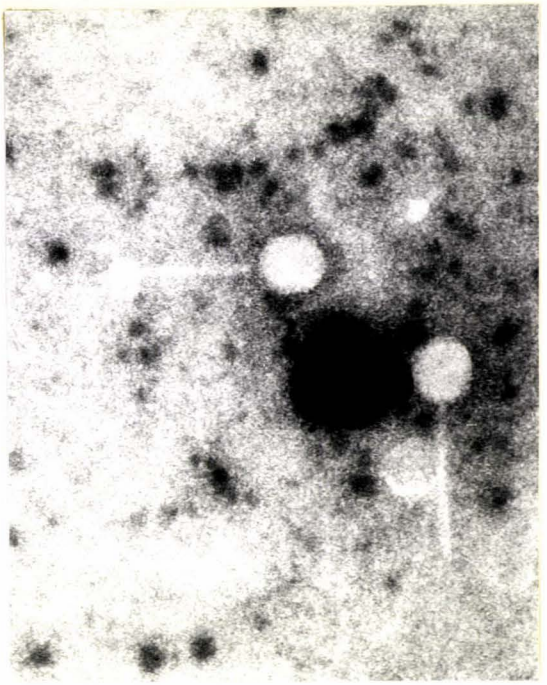


Plate 8. Phage V10 (x116,000)

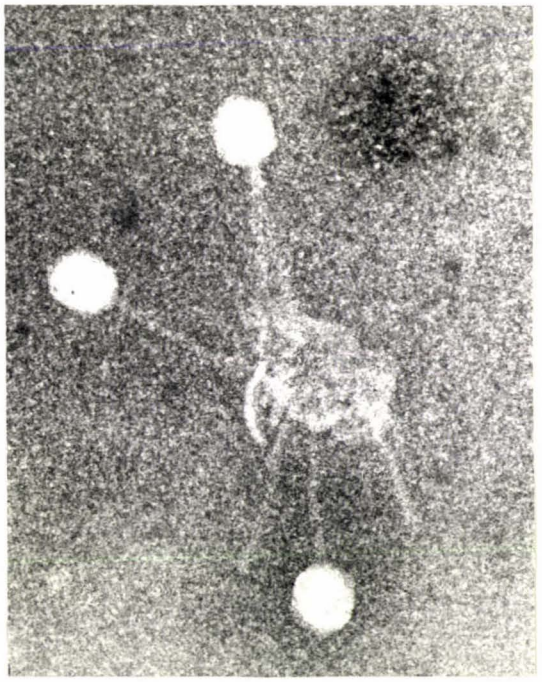


Plate 9. Phage V11 attached to bacterial debris (x116,000)

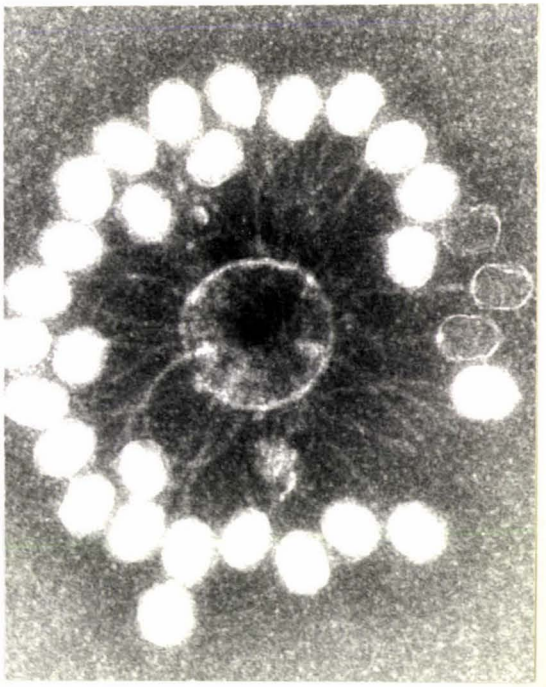


Plate 10. Phage V11 attached to cell debris (x116,000)

Plate 11. Phage V11 adsorbed to *E coli* W; approximate phage input ratio = 50 : 1 (x85,000)

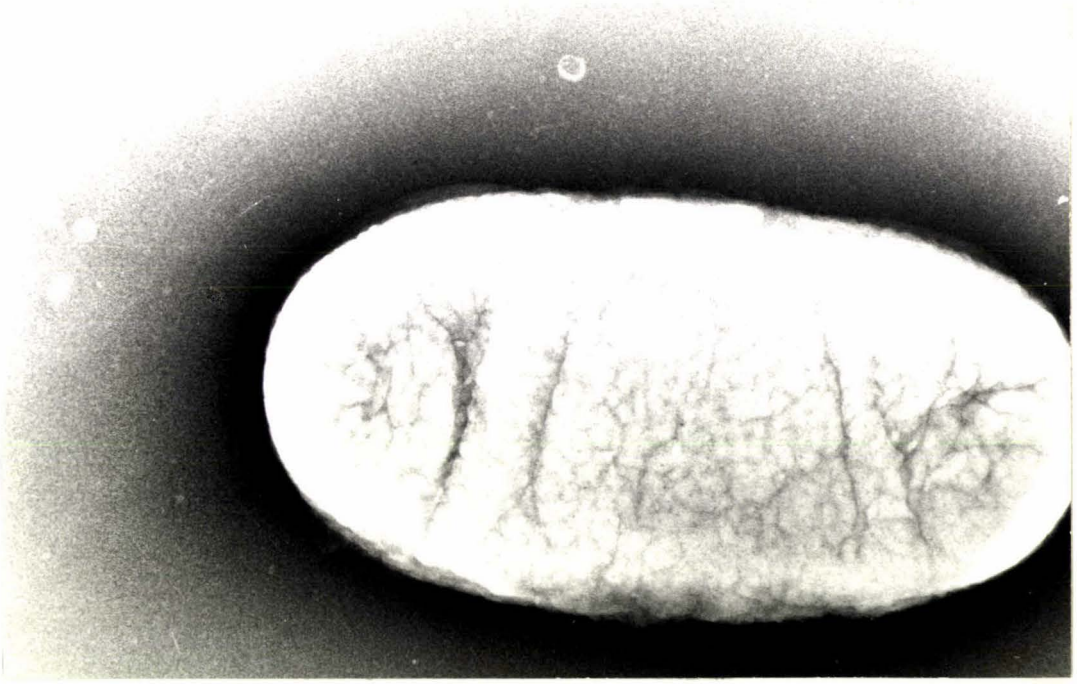


Plate 12. Phage V11 and *E coli* K; approximate phage input ratio = 50 : 1 (x48,000)

III. PLATING EFFICIENCIES OF PHAGE w ON E COLI STRAINS C AND K

Supernatants from overnight cultures of E coli W were shown to contain approximately 10^5-10^6 plaque forming units per cm^3 able to plate on both strains C and K. The number detected in a particular supernatant varied slightly depending on whether the supernatant was plated on strain C or K; the ratio of plaque forming units on K to plaque forming units on C varied from 1.0 to 10^{-1}. The plaque types on both strains C and K were typical of temperate phages, often having a growth of lysogenised cells in the centre (Plate 16). Virulent mutants appeared in the population at a frequency of about 10^{-5}.

Lysates were prepared from single plaques of phage from the W supernatant on strain C and plated on C and K. The efficiency of plating of these lysates on K was 10^{-5} as compared to 1.0 on C. Lysates were then prepared from single plaques of phage w.C on strain K and replated on K and on C. A plating efficiency of between 1.0 to 10^{-1} was obtained on C compared to 1.0 on K. Further lysates were prepared from single plaques of phage w.CK on C and the procedure repeated for several more alternating cycles on C and K, (Figure 14, Table 11). This procedure was duplicated with plaques produced by phage from the W supernatant which plated on K and a totally different pattern of behavior was observed; the phage grew best in the host in which it was last propagated.

The above procedure was carried out with seventeen different plaques of the phage from the W supernatant plated on strain C and with twelve different plaques from the W supernatant plated on strain K. In all cases, the observed behavior was consistant with that depicted in Figure 14.

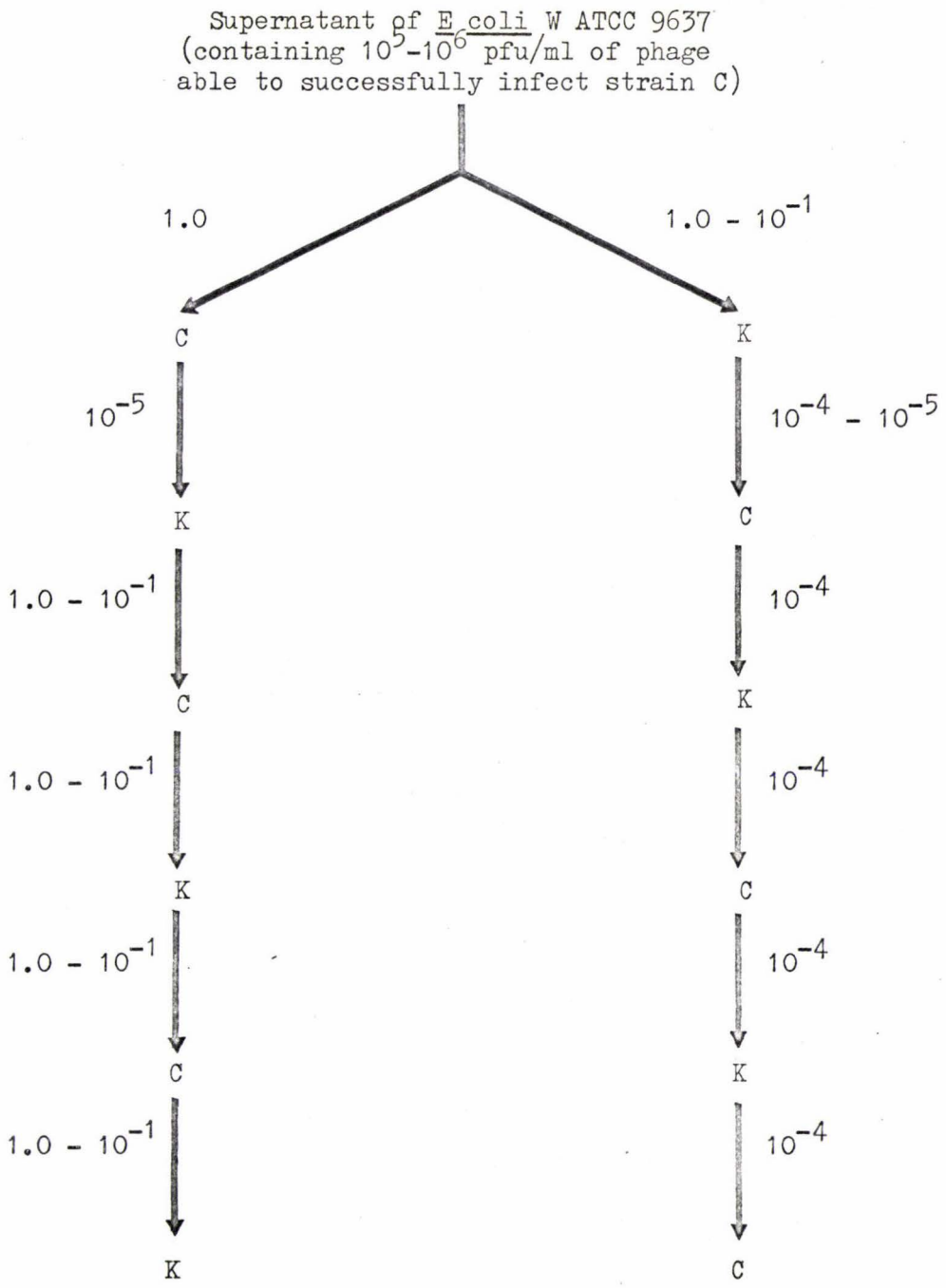


Figure 14. Plating efficiencies of phage from the supernatant of E coli W on strains K and C

Table 11. The relative efficiencies of plating of phage from an E coli, W supernatant on E coli strains C & K.

Phage origin*	on C	on K
E coli W supernatant	1.0	$1.0\text{-}10^{-1}$
w.K	$10^{-4}\text{-}10^{-5}$	1.0
w.KC	1.0	10^{-4}
w.KCK	10^{-4}	1.0
w.KCKC	1.0	10^{-4}
w.KCKCK	10^{-4}	1.0
w.C	1.0	10^{-5}
w.CK	$1.0\text{-}10^{-1}$	1.0
w.CKC	1.0	$1.0\text{-}10^{-1}$
w.CKCK	$1.0\text{-}10^{-1}$	1.0
w.CKCKC	1.0	$1.0\text{-}10^{-1}$

* The letters following w denote the history of the phage population and should not be confused with those denoting host specificity; w.K represents the phage progeny from a single plaque on E coli strain K propagated on K; w.KC represents the progeny from a single plaque when phage w.K was plated on C etc.

It was suggested by Glover (37,38) that phage w.CK is a host range mutant wk able to plate on both K and C. This hypothesis is supported by an experiment in which the adsorption kinetics of phage w.C to strains C and K were determined (Figure 15). The phage adsorbed to C but no adsorption to K could be demonstrated. The derivative of the phage (w.CK) however, adsorbed to both strains C and K. This is to be contrasted with the adsorption kinetics of phages w.K and w.KC (Figures 16,17, Table 12). Phage w.K could not be demonstrated to

adsorb to C, nor could phage w.KC be demonstrated to adsorb to K. Phage w.CK thus acted like a typical host range mutant able to adsorb to both strains C and K, but phage w.KC did not, as on gaining the ability to adsorb to K it lost the ability to adsorb to C.

Table 12. Adsorption coefficients of w phage to E coli strains C and K.

Phage	adsorption coefficient (K_{ADS}) on C	on K
w.C	1.0×10^{-9}	-*
w.K	-*	1.1×10^{-9}
w.KC	1.1×10^{-9}	-*

The simplest hypothesis to explain the observed behaviour is that of two different phages in the W supernatant; one able to plate on strain C and the other on strain K. From the relative plating efficiencies it seems unlikely that two phages are present and that both can plate on C and K, or that one can plate on C and K but the other only on K, or again that one phage can plate on C and K, but the other only on C. In addition, all seventeen phage obtained from plaques of the W supernatant plated on strain C exhibited one identical pattern of behavior and all twelve phage obtained from plaques of the W supernatant on strain K exhibited another, alternative pattern of behaviour(Figure 14).

In the light of this conclusion various properties of phages w.KC and w.CKC were compared to determine their degree of similarity.

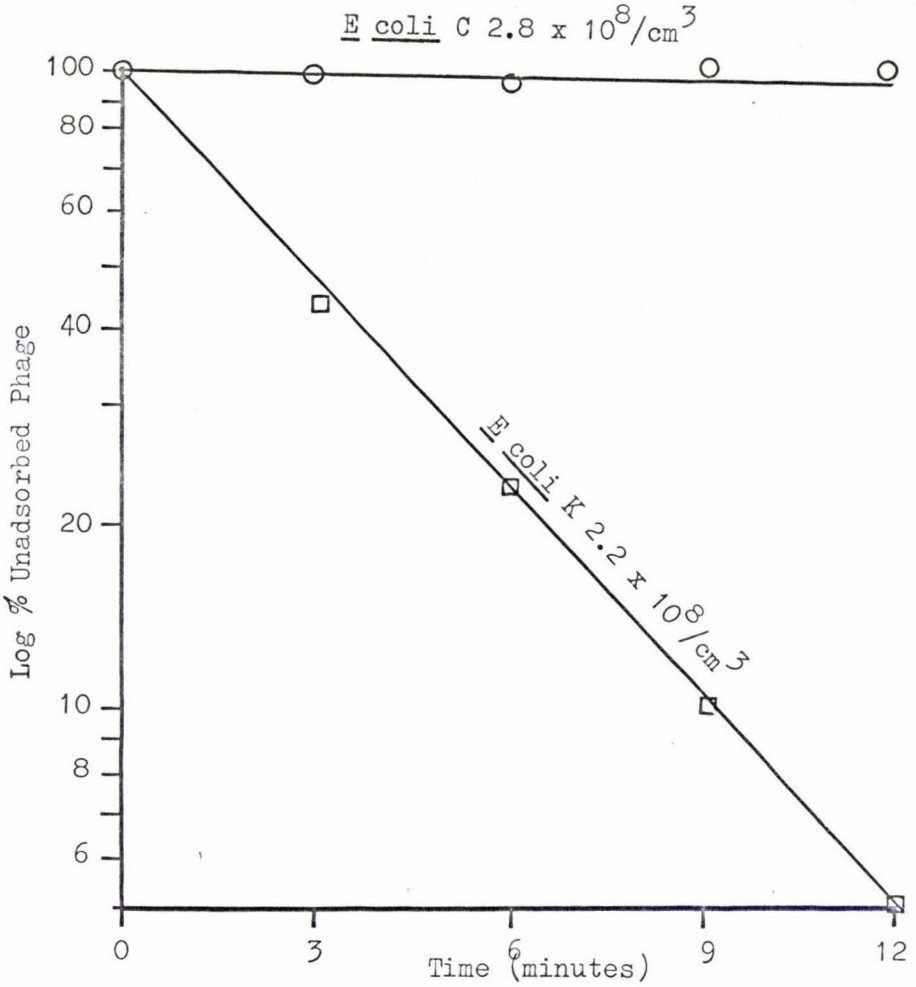


Figure 15. Adsorption Kinetics of Phage w.K to E coli Strains C and K

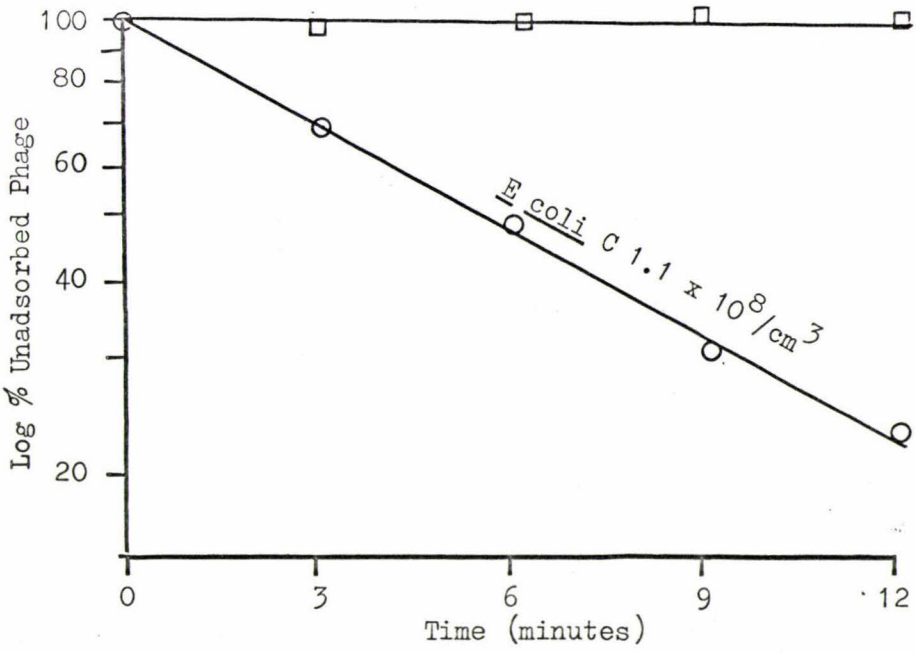


Figure 16. Adsorption Kinetics of Phage w.KC to E coli Strains C and K

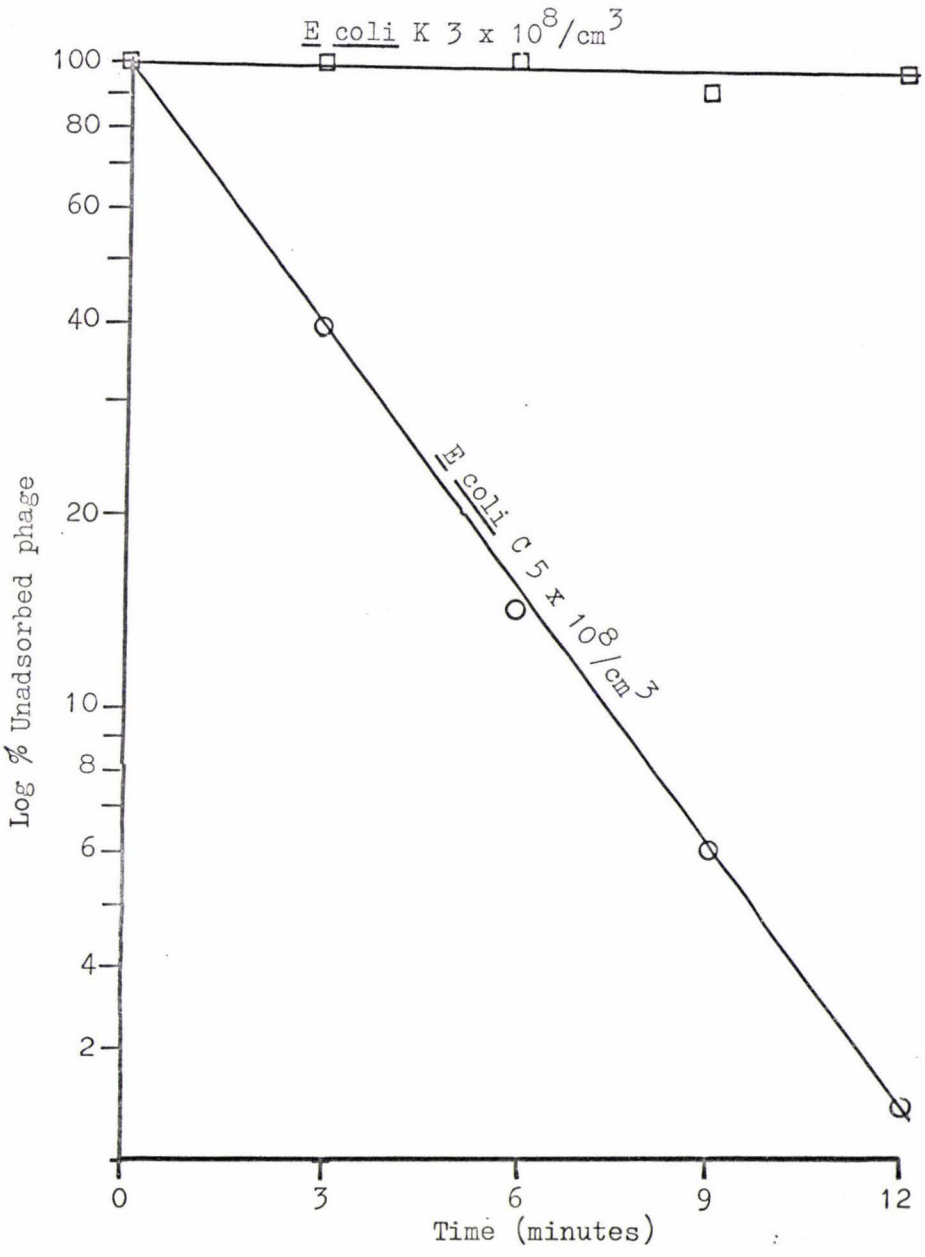


Figure 17. Adsorption Kinetics of Phage w.C to E coli W Strains C and K

IV. COMPARISON OF THE PROPERTIES OF PHAGES w.KC AND w.CKC

(i) Heat sensitivity.

Within experimental error, both phages had identical heat sensitivities at a wide range of temperatures and calcium concentrations (Tables 13,14, Figures 18,19). Lack of calcium ions resulted in a marked increase in heat sensitivity of both phages, suggesting calcium ions are involved in stabilizing the w phage protein coat.

Table 13. Heat sensitivities of phages w.CKC and w.KC in 0.1M NaCl + 0.5M $CaCl_2$.

Temperature (°C)	pfu/ml on C	
	w.CKC	w.KC
20	1.4×10^7	7.6×10^6
60	1.4×10^7	7.1×10^6
65	8.0×10^6	5.4×10^6
70	1.4×10^6	1.3×10^6
75	$<10^5$	$<10^5$

Table 14. Heat sensitivities of phages w.CKC and w.KC in 0.1M NaCl.

Temperature (°C)	pfu/ml on C	
	w.CKC	w.KC
20	1.8×10^7	1.4×10^7
45	8.3×10^6	8.9×10^6
50	1.0×10^5	$<10^5$
55	$<10^5$	$<10^5$

The data in Tables 13 and 14 were obtained by subjecting phage at approximately 10^7 pfu/cm^3 to the indicated temperatures for

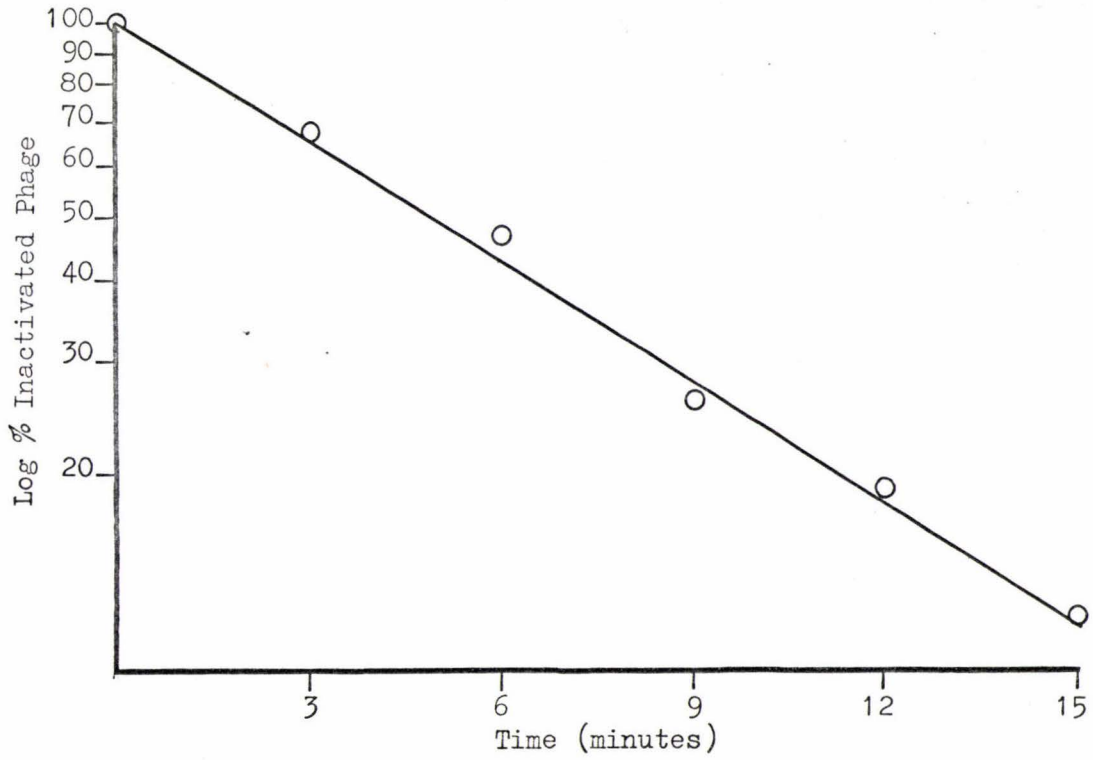


Figure 18. Kinetics of Heat Inactivation of Phage w.CKC at 70°C in 0.1M NaCl + 0.5M $CaCl_2$

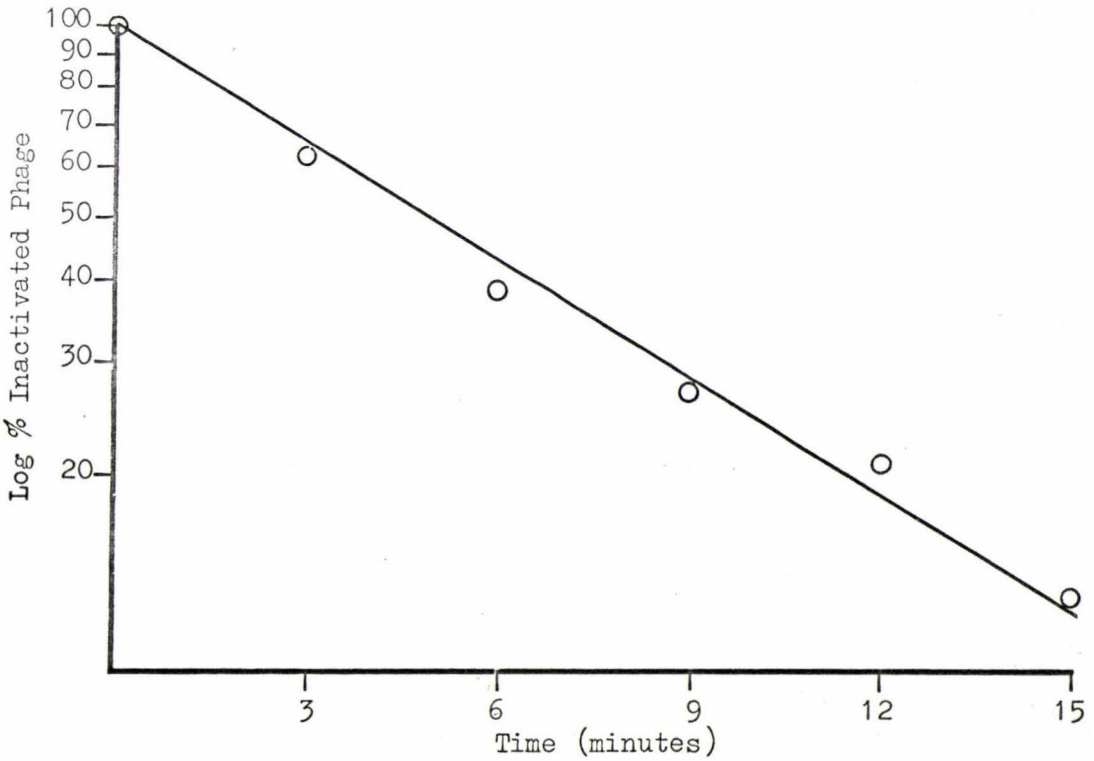


Figure 19. Kinetics of Heat Inactivation of Phage w.KC at 70°C in 0.1M NaCl + 0.5M $CaCl_2$

10 minutes then immediately diluting 0.1 cm^3 samples into 9.9 cm^3 of BHI broth and tilrating the resultant suspensions.

(ii) Serological properties.

Antisera were prepared against phages w.CKC and w.KC. The two phages were shown to be serologically identical (Figures 20,21).

(iii) Morphology.

Both phages are identical in size and morphology and are very similar in these respects to phage P2. The phage heads are spherical, about 65 nm across, the tails 180 nm long, and16 nm in diameter. On contraction, the sheaths are 26 nm in diameter and the exposed tail cores 8 nm in diameter.

At high concentrations these phage tend to clump together to form rosettes (Plate 13). The phage in this preparation have intact heads and uncontracted tail sheaths. Previously published electron micrographs have depicted the phage with contracted tail sheaths and empty heads. The preparation used to prepare this plate contained 10 percent BHI broth. This led to a 'dirty' preparation; it was again centrifuged and resuspended in distilled water to give a final broth concentration of about one percent. This gave clearer pictures but broke up the rosettes and disrupted the phage to give many particles with empty heads and contracted sheaths. Plate 14 depicts two w.KC phage particles, both with empty heads, and one with a contracted tail sheath. The base plate can be seen on one particle but can not be seen attached to the particle with the contracted sheath. Plate 15 shows two w.CKC phage particles and two other objects which may each consist of two contracted tail sheaths joined end to end. A dividing line between the two units can be seen. These particles

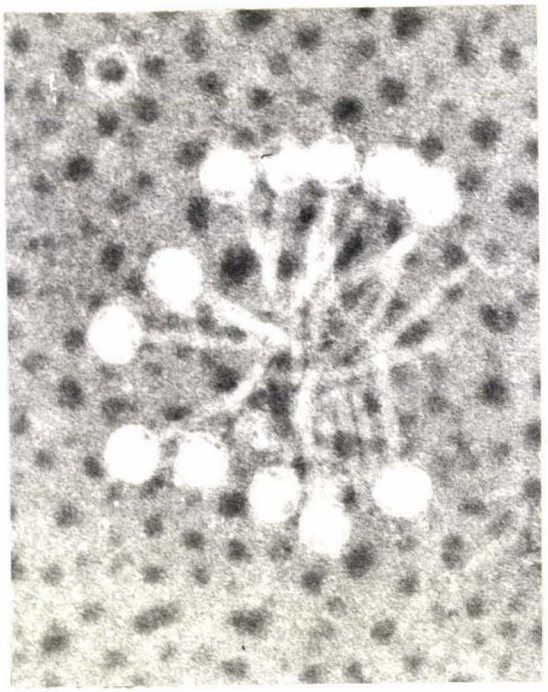


Plate 13. A rosette of phage w. (x116,000)

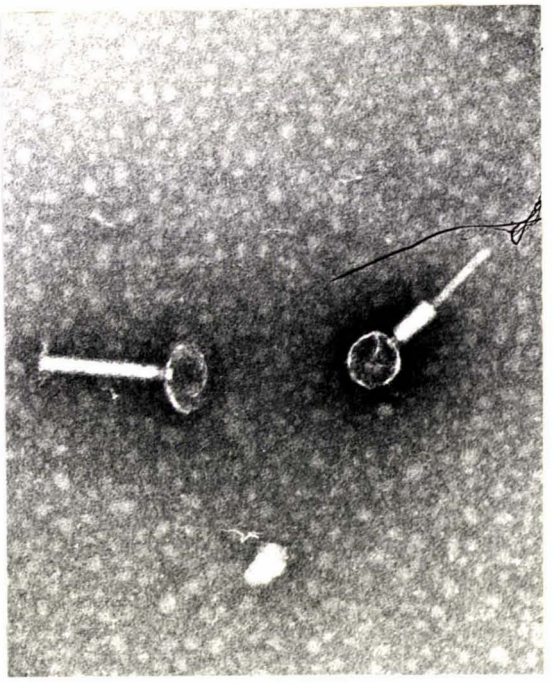


Plate 14. Phage w.KC (x116,000)

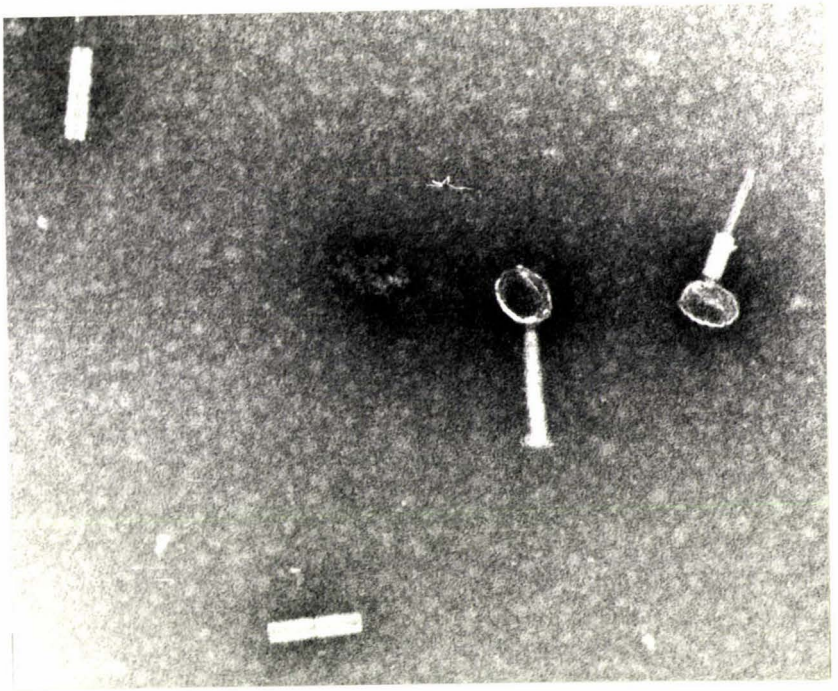


Plate 15. Phage w.CKC (x116,000)

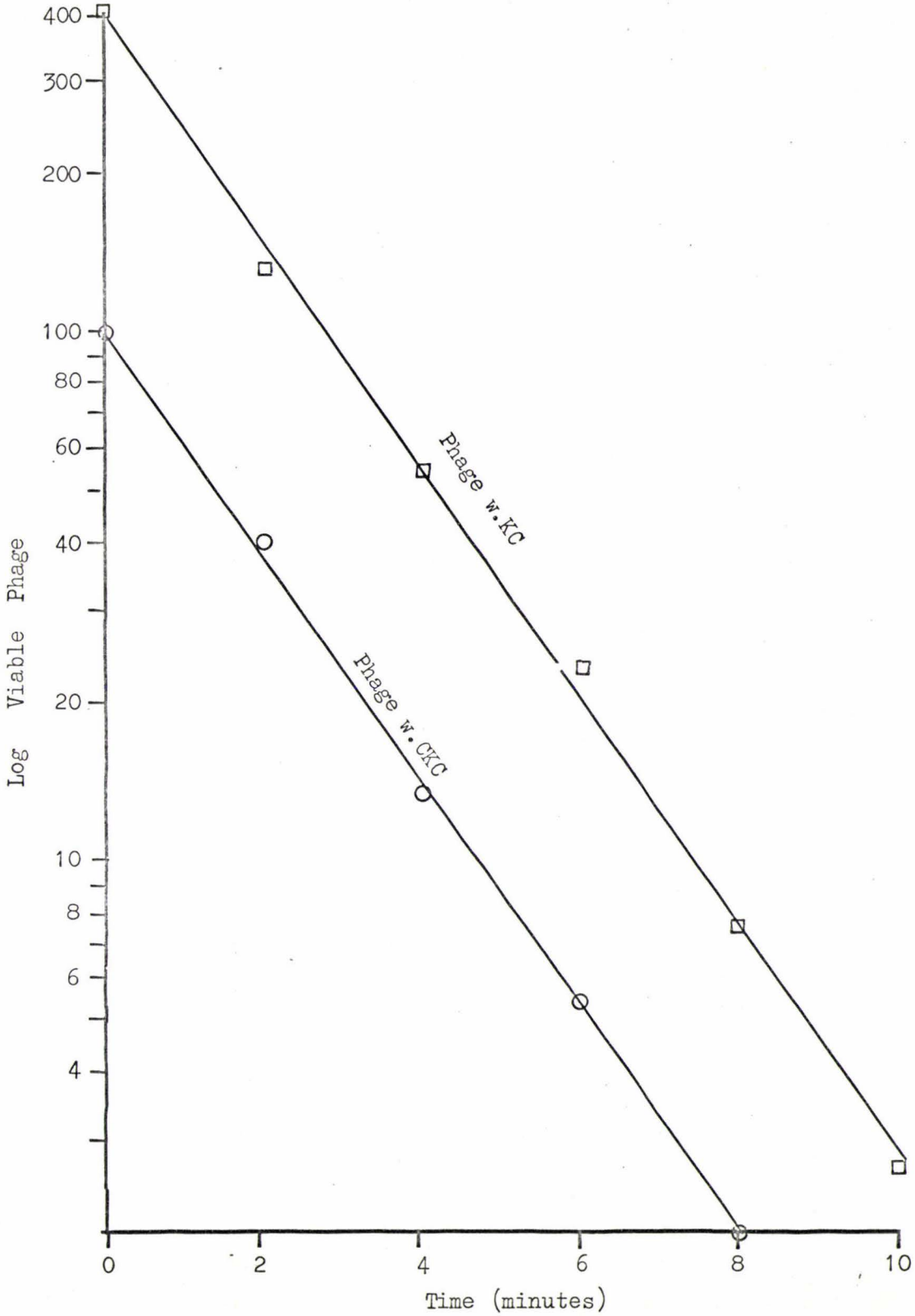


Figure 20. Inactivation of Phage w.CKC and Phage w.KC by w.CKC Antiserum.

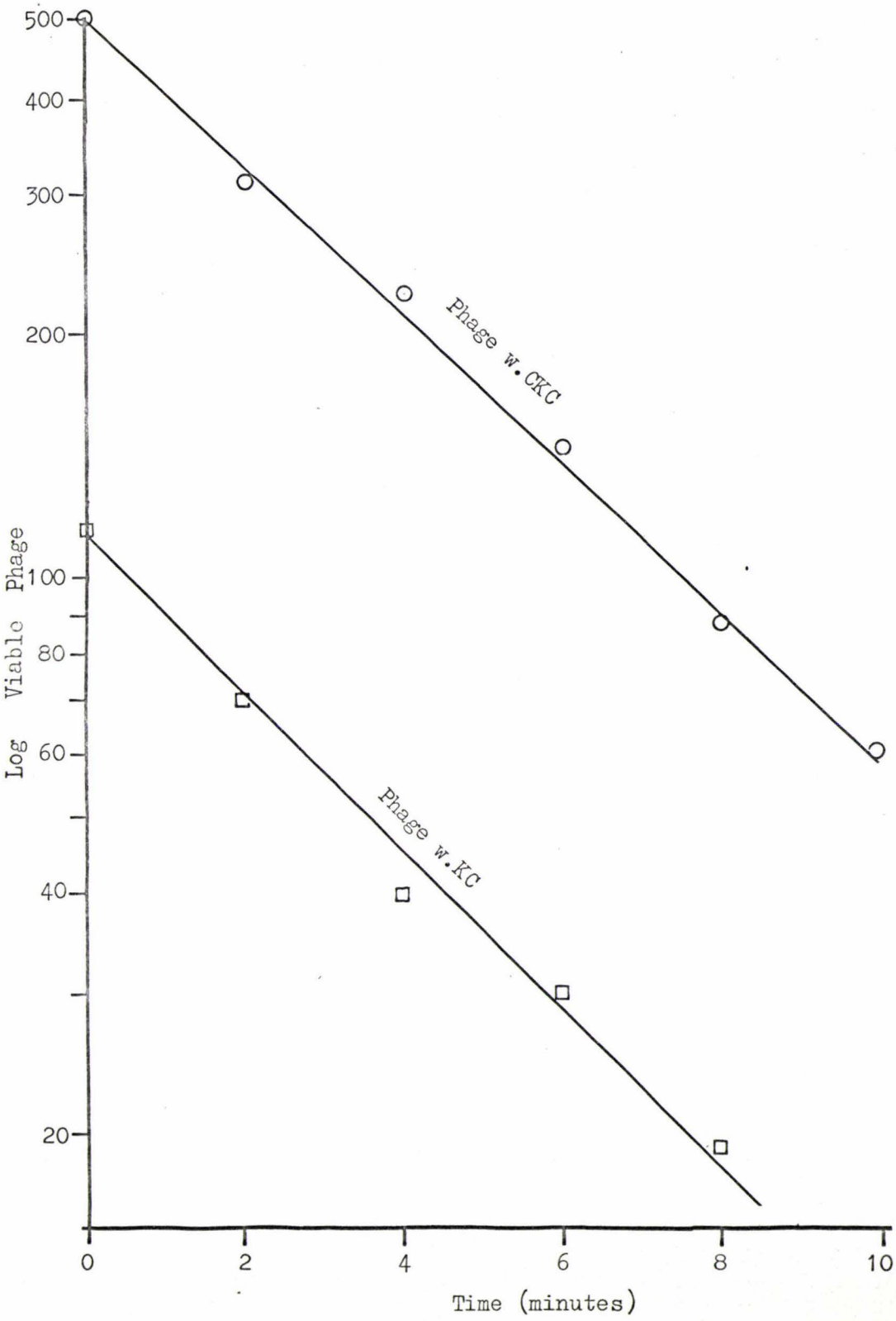


Figure 21. Inactivation of Phage w.CKC and Phage w.KC by w.KC Antiserum.

occur in both phage w.CKC and phage w.KC lysates. They also occur singly and never more than two are joined together.

(iv) Co-immunity.

E coli C lysogenic for phage w.KC will not plate phage w.CKC or phage w.KC; nor will strain C lysogenic for phage w.CKC plate phage w.KC or phage w.CKC; the efficiency of plating in each case was less than 10^{-8}. Adsorption of the phages to the lysogenised cells occurs in both instances (Table 15) and hence it seems likely that the two phages are co-immune.

Table 15. Adsorption of phages w and P1 to lysogens of E coli C.

Phage	Lysogen	pfu/cm^3 at:		
		0 min	10 min	20 min
P1	C(w.KC)	3.3×10^7	5.8×10^6	2.5×10^6
w.CKC	C(w.KC)	4.3×10^7	4.0×10^6	2.6×10^6
w.KC	C(w.KCK)	2.8×10^7	3.2×10^6	9.1×10^5

Phage at approximately 6×10^7 pfu/cm^3 were mixed with an equal volume of bacterial cells at approximately 5×10^8/cm^3. 0.1 cm^3 samples were withdrawn from the mixture at 10 and 20 minute intervals, placed in 9.9 cm^3 diluent containing chloroform to kill infected cells and titrated at suitable dilutions.

(iv) Properties of E coli strains C and K lysogenic for phage w.

Substrains of E coli C and K were lysogenised with phage w (method p.44) and their ability to support propagation of various phages investigated (Table 16).

Table 16. Ability of E coli K and C lysogenised with phage w to support the propagation of various phages.

Lysogen	Number screened	Plating efficiency of phage P1 on the lysogens	Plating efficiency of phage λ on the lysogens	Plating efficiency of phages T1-T7 on the lysogens
E coli C				
C(w.C)	1	$<10^{-7}$	$<10^{-7}$	1.0
	7	$<10^{-7}$		
C(w.CKC)	4	1.0	1.0	1.0
C(w.KC)	15	$<10^{-7}$		1.0
C(w.KCKC)	8	1.0		
E coli K				
AB 266 (w.KCK)	1	1.0	1.0	1.0
	6	1.0		
AB 266 (w.CK)	1	1.0	1.0	1.0
T186 (w.K)	2	1.0		

Each lysogen was isolated from bacteria growing in a single plaque of the w phage; no two lysogens were isolated from the same plaque.

Table 16 indicates that the exclusion of phage P1 exactly parallels that of phage λ. Phage λ has been reported by Glover et al (38) to have adsorbed normally to phage w lysogens of strain C and where exclusion occured, breakdown of DNA was observed. Phage P1 was shown to adsorb to the lysogens (Table 15) but no experiment was undertaken to demonstrate the breakdown of P1 DNA.

The pattern of abortive infection of the lysogens by P1 is summarised in Table 17. The pattern suggests that

(i) lysogens of K do not exclude P1 and

(ii) once having gained the ability to plate on strain K, a phage on lysogenisation of strain C loses its ability to restrict P1, except in the case of the lysogen C(w.KC).

Table 17. Abortive infection of phage w lysogens of C by phage P1.

Lysogen	Ability to exclude P1
C(w.C)	+
K(w.CK)	-
C(w.CKC)	-
K(w.K)	-
C(w.KC)	+
K(w.KCK)	-
C(w.KCKC)	-

If two phages are present the W supernatant, one able to plate on C, the other on K, then no mutational event would be necessary for this latter phage to plate directly on K and hence its ability to exclude phage P1 on lysogenisation of strain C would not be lost. This may explain the ability of lysogen C(w.KC) to exclude P1.

The above evidence seems to support the presence of two very closely related phages in the W supernatant. A mutation of either enabling it to adsorb to strain K seems to be associated with a loss in ability of the phage, on lysogenisation of strain C, to exclude P1 and λ.

It was found, however, on isolation of six lysogens from a single plaque of phage w.CKC on strain C, that one half of these lysogens were able to exclude phage P1. This brings to mind the observation by Glover (37,38) who reported that different strains of C lysogenised by phage w restricted λ to varying degrees. He suggested that it was possible that phage w, like P2, may integrate at different sites on the chromosome of strain C and that restriction of λ depended upon the location of the integration site. This hypothesis could be extended in the following manner:

(1) Two prophages are integrated into the E coli W genome at different sites, and on spontaneous induction, one is able to plate on strain K, the other on strain C.

(2) More than one site of integration may exist on the E coli C chromosome; the phages may mediate exclusion of P1 only when integrated at certain of the sites and these sites may be the preferred sites of integration.

(3) Phage w - mediated exclusion of P1 and λ may not occur on integration of the w phage into any site on the chromosome of E coli K.

(4) Phage from the W supernatant able to plate on C probably have to mutate before being able to plate on strain K and this mutation may result in a reduced probability of the phage occupying a 'restrictive' site on the E coli C chromosome.

This hypothesis was tested in the following experiments which involve the employment of conjugal crosses.

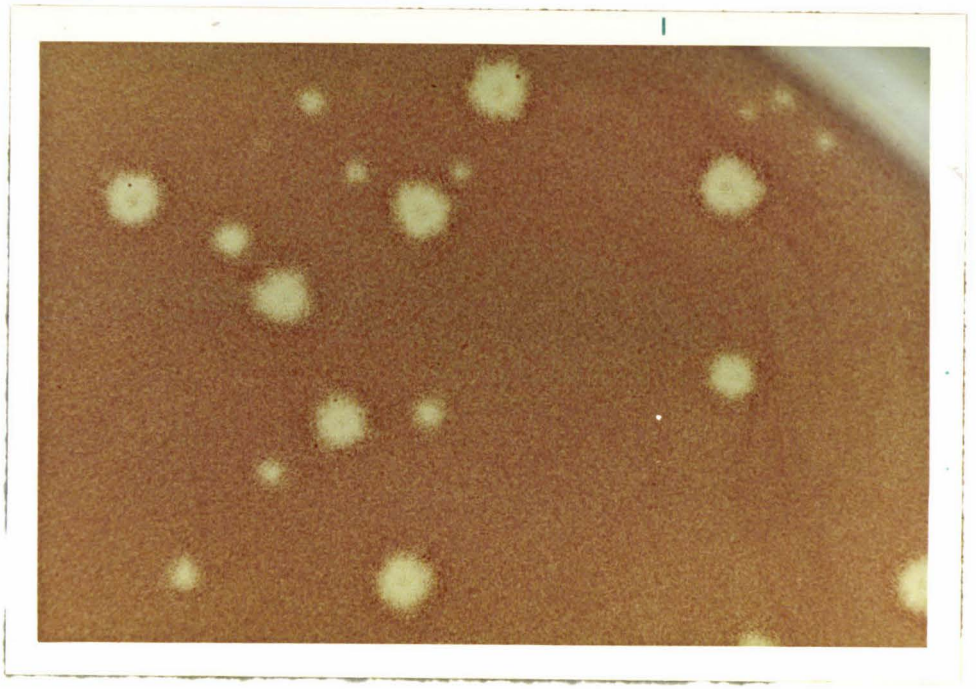


Plate 16. Plaques of phage w.CKC on *E coli* C (x3)

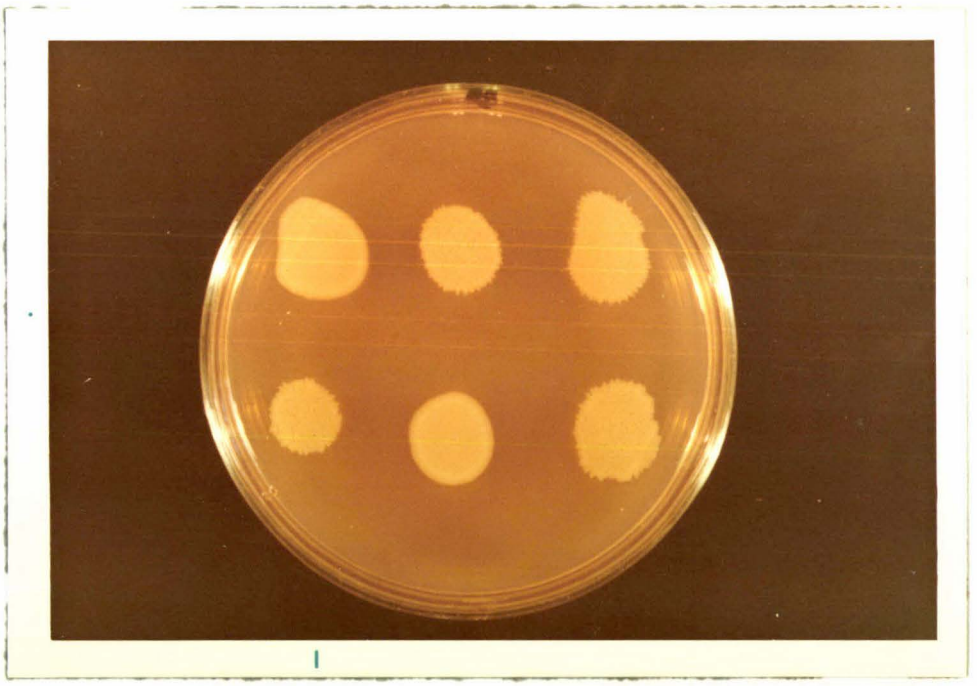


Plate 17. Screening of lysogens for ability to exclude phage P1. Lysogens are spotted onto a plate spread with 10^4–10^5 particles of phage P1. From left to right:
top - C(w.C), K(w.K), C(w.CKC); bottom - K(w.KCK), C(w.KC), C(w.KCKC)

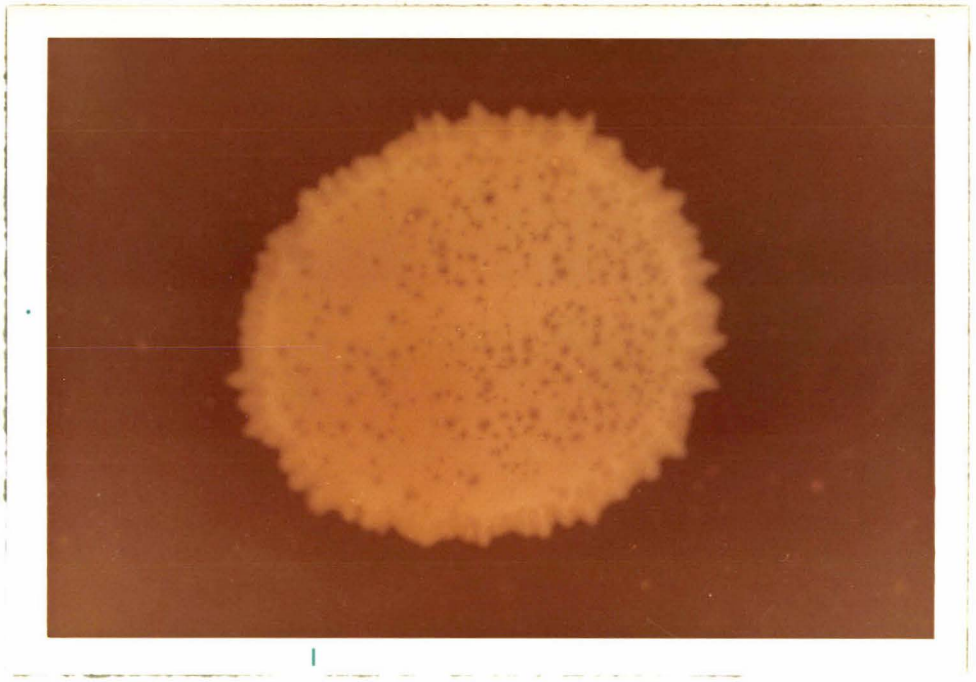


Plate 18. A lysogen of E coli K,K(w.CK) spotted on a plate previously spread with phage P1 particles; strains of K lysogenic for phage w have never been shown to exclude P1

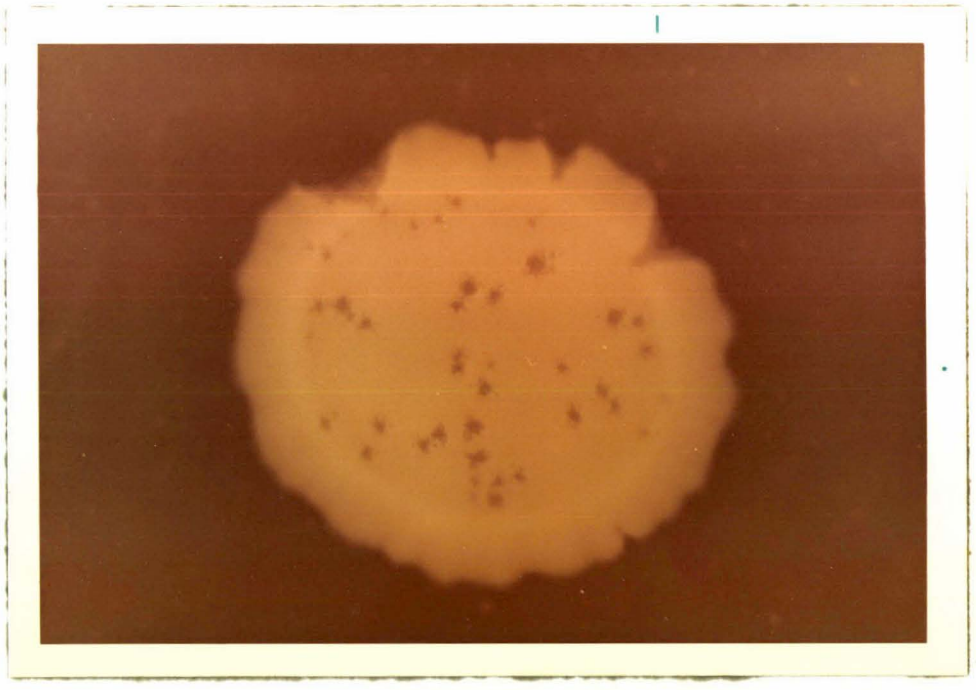


Plate 19. The method employed to screen for phage w lysogens of E coli strains K and C; the strain depicted is not lysogenic

V. MAPPING OF THE SITES OF INTEGRATION OF THE w PROPHAGES ON THE CHROMOSOMES OF E COLI STRAINS C AND W

In order to map the integration sites of the w prophages on the chromosome of E coli W it was decided to cross the E coli W Hfr F2-3-27 with an F^- strain of C. Before the cross, the supernatant from a broth culture of F2-3-27 was checked for the presence of phage which could plate directly on both strains K and C. It was found that the supernatant contained phage able to plate on C but no phage able to plate on K. Hence a number of substrains of W were screened, including several Hfrs with origins different from that of F2-3-27, (Table 18, Figures 3 and 4).

Of those substrains screened, only supernatants of broth cultures of Hfrs which have an origin at 5 minutes and transfer markers in a clockwise direction did not contain phage able to plate on K. These Hfrs were obtained by Bacon (51) who crossed Hfr K12 808 with an F^- substrain of W which was pro^-. Proline is transferred as a late marker by Hfr 808 and hence in order to obtain Hfr zygotes of W, pro^+ recombinants were selected. It appears that on selection for pro^+, part of the W genome which mediates plating of the induced w prophage on strain K was lost. Presumably this section of the W chromosome carries the site of integration for the phage which plates directly on strain K. It is not known whether the pro A or pro B marker is involved as there is no simple biochemical test to distinguish the two, both having a block prior to L-glutamate semialdehyde (36).

Table 18. Presence of phage able to plate on strains K and C in the supernatants of broth cultures of substrains of E coli W.

Substrain	Mating type	Origin(mins) and direction of marker transfer	Presence of phage in the supernatant able to plate on C	Presence of phage in the supernatant able to plate on K
160-37-D2	F^-	-	+	+
F2	F^-	-	+	+
F2-3	F^-	-	+	+
D2-18-1-O^+	F^-	-	+	+
D2-8	F^-	-	+	+
F2-3-27	Hfr	5,cw*	+	-
F2-3-19	Hfr	5,cw	+	-
D2-175/4	Hfr	5,cw	+	-
G1-53	Hfr	59,aw*	+	+
G1-53-O^+	Hfr	59,aw	+	+
G1-1	Hfr	59,aw	+	+
G12-O^+-9	Hfr	74,cw	+	+

* aw = anticlockwise cw = clockwise

The conjugal cross Hfr H x D2-18-1-O^+ was employed to determine if the linkage of this integration site to proline was a real phenomenon. The strain K Hfr H has its origin located at about 87 minutes and transfers its genome in the opposite direction to Hfr 808 (Figure 4) with proline as an early marker. The E coli W F^- strain D2-18-1-O^+ is pro^-, leu^-, Sm^r and carries both phage w.C and phage w.K.

Recombinants were selected which were either pro^+ or leu^+ on minimal agar plates supplemented with 50 ug/cm^3 of leucine or proline respectively. The Hfr parent was eliminated by adding 100 ug

of streptomycin per cm^3 to the plates.

The recombinants were grown overnight in 2.5 cm^3 BHI broth cultures, centrifuged, and the supernatants titrated for the presence of phage able to plate on strain C or K. The cells were washed twice in saline and spotted onto minimal agar plates to score for unselected nutritional markers.

The elimination of phage w.C from the E coli V chromosome was not observed but the results obtained (Table 19) indicated that prophage w.K could be eliminated utilising Hfr H and that this prophage is integrated at a site very closely linked to the proline locus. With the distal, proline locus as the selected marker, the w.K^- character was coinherited at a frequency of 77 percent. On selection of the proximal leucine marker the gradient of transmission of proline and the w.K^- character again indicated that the two are very closely linked; the coinheritance of each of these distal unselected markers with leucine is very similar. The data were not comprehensive enough to determine on which side of the proline locus the phage integration site is situated; a three factor cross could not be employed as inheritance of the w.K^- character in a recombinant could not be used as a selective marker.

From the results of the previous cross it appeared possible that the integration site of the w phage able to plate on K (phage w.K) might also be closely linked to the arg F or citrilline locus if the site was somewhere between leucine and proline. Hence the cross Hfr H x D2-8 was carried out. D2-8 is pro^- cit^- and again carries both phage types. Recombinants were selected on minimal agar supplemented with proline or citrilline at 50 ug/cm^3. The

Table 19. Frequency of selected and unselected markers in the cross Hfr H x D2-18-1-O$^+$

Selected marker	Recovery of selected markers as a % of Hfr input	Number of recombinants screened	Unselected markers scored	Recovery of unselected markers	
				number	frequency(%)
pro$^+$	0.16%	46			
			leu$^+$	17	37
			w.C$^-$	0	0
			w.K$^-$	35	77
			leu$^+$ w.K$^+$	2	4
			leu$^+$ w.K$^-$	15	33
			leu$^-$ w.K$^+$	9	20
			leu$^-$ w.K$^-$	20	43
leu$^+$	0.14%	48			
			pro$^+$	16	33
			w.C$^-$	0	0
			w.K$^-$	14	29
			pro$^+$ w.K$^+$	4	8
			pro$^+$ w.K$^-$	12	25
			pro$^-$ w.K$^+$	30	63
			pro$^-$ w.K$^-$	2	4

Hfr parent was again eliminated by using streptomycin at a concentration of 100 ug/cm^3 in the plate agar. Recombinants were cultured overnight in BHI broth, centrifuged, and the supernatant titrated for phage plating on strain K or C; the cells were washed twice in saline and spotted onto minimal agar plates to score for unselected nutritional markers.

Table 20. Frequency of selected and unselected markers in the cross Hfr H x D2-8

Selected marker	Recovery of selected markers as a % of Hfr input	Number of re-combinants screened	Unselected markers scored	Recovery of unselected markers	
				number	frequency (%)
pro^+	1.7%	40			
			cit^+	40	100
			$w.C^-$	0	0
			$w.K^-$	32	80
			cit^+ $w.K^+$	8	20
			cit^+ $w.K^-$	32	80
			$cit^-w.K^+$	0	0
			cit^- $w.K^+$	0	0
cit^+	3.1%	40			
			pro^+	18	45
			$w.C^-$	0	0
			$w.K^-$	17	42
			pro^+ $w.K^+$	1	3
			$pro^+w.K^-$	17	42
			pro^- $w.K^+$	22	55
			pro^- $w.K^-$	0	0

The results depicted in Table 20 demonstrate the very close linkage of the phage w.K integration site to the proline locus. As expected the citrilline locus, in comparison to that of leucine was more closely linked to the phage integration site, but this linkage was still not as high as that of proline to the integration site. On selection of the proximal citrilline marker the gradient of transmission of proline and the $w.K^-$ character again demonstrated the close linkage of the two loci.

The gradient of transmission of proline, citrilline and the w.K$^-$ character in both the above crosses on selection of leucine, the proximal marker, is consistant with a situation in which the phage is integrated at a site distal to proline; in both cases the w.K$^-$ character was recovered at a lower frequency than either the proline or citrilline markers.

The cross Hfr F2-3-27 x C1-a/50 was employed to attempt to map the site of integration of phage w.C on the E coli W chromosome (57). The origin and direction of transfer of markers of F2-3-27 are the same as those for Hfr 808 (Figure 4). F2-3-27 is methionine$^-$, histidine$^-$ and was eliminated on the plates by the omission of histidine. Methionine was added at 50 ug/cm^3. As a broth culture of F2-3-27 contains about 10^6 pfu per cm^3 of phage w.C the possibility existed that infection of zygotes by free phage might have occured. Antiserum against the w phage was added and the level of phage in the cross supernatant after one hour was 10^5 pfu per cm^3, titrated on strain C. Hence infection by free phage might be expected to occur with a frequency of less than one per 10^4 recombinants. In addition, none of the sixteen F$^-$ cells isolated after one hour on minimal agar supplemented with threonine and proline carried phage w.C as evidenced by their ability to propagate phage w.C (Plate 19).

Strain C1-a/50 is theonine$^-$ proline$^-$; thr$^+$ recombinants were selected on minimal agar plates supplemented with proline at 50 ug/cm^3. Recombinants were grown overnight in BHI broth, centrifuged and the supernatants titrated for the presence of phage able to plate on strain C. The pelleted cells were resuspended, spotted onto a 'lawn' of about 5 x 10^4 particles of phage w.C which were spread on a plate of ML agar, and incubated overnight. Each

spot covered about 10^3 pfu of phage w.C. When the recombinant was non-lysogenic, the growth which developed in these spots had nibbled edges and an irregular surface with overlapping lysogenic plaques. Where the recombinant was lysogenic the growth developed fully, with a smooth surface and regular edges. In some cases where the growth developed fully, ten or twenty single distinct plaques appeared. This was attributed to the introduction by recombination of the E coli W genome hs system into the recipient (E coli C) thus restricting the unmodified phage, w.C; approximately one in 10^2 phage could escape this W genome specified restriction, become modified and initiate a plaque.

Table 21. Frequency of selected and unselected markers in the cross Hfr F2-3-27 x C1-a/50.

Selected marker	Recovery of selected markers as a % of Hfr input	Number of re-combinants screened	Unselected markers scored	Recovery of unselected markers number	Recovery of unselected markers frequency (%)
thr^+	0.05%	38			
			$w.C^+$	25	66
			$w.C^-$	13	34
			$w.C^-$ *hs$^+$	8	21
			$w.C^-$ *hs$^+$	5	13

* hs : the E coli W genome host specificity locus

The prophage w.C was transmitted at a high frequency by Hfr F2-3-27; 66 percent of thr^+ recombinants carried phage able to plate on strain C. (Table 21) As the w.C phage integration site appeared not to be eliminated in either cross Hfr H x D2-18-1-O^+ or Hfr H x D2-8, it appeared likely that this second site lay to the left of the Hfr H origin (Figure 22) and as the site was also closely linked to leucine, it could not lie very far to the left of the Hfr H origin.

As previously mentioned, it appears that the E coli W genome hs locus is also transfered by the E coli W Hfr F2-3-27, possibly at a high frequency, but this possibility could not be readily investigated as the phenotype w.C^+ hs^+ could not be identified; the immunity endowed upon the cell by the w phage masked the expression of the E coli W genome hsr restriction activity. A mutant of phage P1 able to propagate on lysogens such as C(w.C) would facilitate this investigation; such a P1.C mutant would propagate normally on w.C^+ hs^- recombinants but would be restricted in those of phenotype w.C^+ hs^+.

It seems likely however, that the W genome hs site may be allelic with the hs sites of E coli K, B, 15 and A which all map one minute to the left of the theonine locus on the linkage map of E coli K (14).

To determine if the site of integration of the w phage in strains C and K determined the ability of the lysogen to mediate exclusion of phages P1 and λ an attempt was made to eliminate the integrated prophage by recombination in conjugal crosses of Hfr H with the lysogens C(w.CKC) and K(w.CKC) which do not exclude P1 or λ and with C(w.KC) which does.

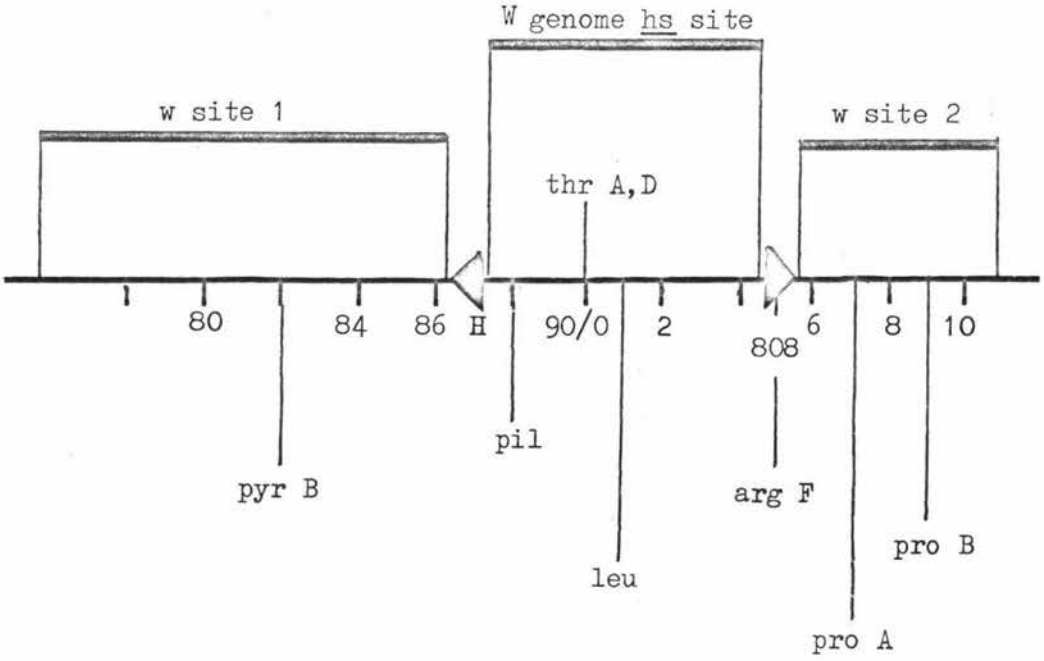


Figure 22. The tentative locations on the chromosome of E coli W of: (i) the W genome hs site; (ii) two possible integration sites of phage w.

Table 22. Frequency of selected and unselected markers in crosses of Hfr H with lysogens of *E coli* strains C & K

Cross	Selected marker	Recovery of selected markers as a % of Hfr input	Number or recombinants screened	Unselected markers scored	Recovery of unselected markers	
					number	frequency(%)
Hfr H x C(w.CKC)	pro^+	30%	25	$w.K^-$	0	0
				$w.C^-$	0	0
				thr^+	7	28
	thr^+	11%	25	$w.K^-$	0	0
				$w.C^-$	0	0
				pro^+	9	36
Hfr H x C(w.KC)	pro^+	15%	40	$w.K^-$	*-	*-
				w.C	0	0
				thr^+	8	20
	thr^+	11.5%	25	$w.K^-$	*-	*-
				$w.C^-$	0	0
				pro^+	7	28
Hfr H x AB 266 (w.CKC)	pro^+	20%	24	$w.K^-$	0	0
				$w.C^-$	0	0
				leu^+	10	42
	leu^+	33%	23	$w.K^-$	0	0
				$w.C^-$	0	0
				pro^+	13	57

* This could not be measured as the eop on K of phage arising from spontaneous induction of the lysogen C(w.KC) is 10^{-4}

The substrains lysogenised were C1-a/50 ($thr^-pro^-sm^r$) and AB 266 (thr^- leu^- pro^- $B1^-$ Sm^r). The level of phage able to plate on strains K and C in the cross supernatants after one hour was less than 10^5 pfu/cm^3 in all three cases and hence the frequency of reinfection of zygotes would be expected to be less than 10^{-4}.

Thr^+, leu^+ and pro^+ recombinants were selected on minimal agar supplemented with the appropriate amino acids and 100 ug of streptomycin per cm^3 to eliminate the Hfr parent. Recombinants were grown overnight in BHI broth, centrifuged and the supernatants titrated for phage able to plate on strains C or K. The cells were washed twice in saline and spotted onto minimal agar plates containing the appropriate amino acids at concentrations of 50 ug/cm^3 to score for unselected nutritional markers.

It was anticipated that those lysogens excluding phage P1 might have the w prophage integrated at an alternate site to that which is occupied by the prophage in lysogens failing to exclude P1 and that these sites might coincide with those tentatively mapped on the chromosome of E coli W (Figure 22). The results of the cross however, were inconclusive as it appears that the w phages integrated into the chromosome of E coli C are not eliminated at a high frequency in conjugation with Hfr H.

An attempt was made to obtain a 'cured' strain of E coli W lacking both phage w.C and phage w.K which might then fail to exclude phages λ and P1 and thus permit mapping of the W chromosome by transduction. The strain K Hfr 808 was crossed with a pro^+ sm^r $w.K^-$ derivative of the W strain D2-18-1-O^+, from which the w.K phage had been eliminated by conjugation with Hfr H. The level of w phage able

to plate on strain C in the cross supernatant after one hour was less than 10^5 pfu/cm^3 and hence the frequency of reinfection of zygotes would again be expected to occur at a frequency of less than 10^{-4}.

Leu^+ recombinants were selected on minimal agar plates supplemented with 100 ug of streptomycin per cm^3 to eliminate the Hfr parent. Recombinants were grown overnight in BHI broth, centrifuged and the supernatants titrated for the presence of phage able to plate on strain C.

Table 23. Frequency of recovery of selected and unselected markers in the cross Hfr 808 x D2-18-1-0$^+$(pro^+ w.K^-)

Selected marker.	Recovery of selected markers as a % of Hfr input	Number of re-combinants screened	Unselected markers scored	Recovery of unselected markers	
				number	frequency(%)
leu^+	0.8%	25			
			w.C^-	0	0
			w.C^+	25	100

The failure to obtain a 'cured' derivative of E coli W suggests strongly that in addition to the two sites of integration of the w phage already tentatively mapped, (Figure 22), at least one other site may occur into which w.C may integrate and that the phage integrated at this site does not appear to be eliminated at a high frequency by either Hfr 808 or Hfr H.

As a result of the postulated presence of this third site, the position of the second postulated site (site 2, Figure 22) on the chromosome of E coli W must be modified. Site 2 may now occur in the

region between the origin of Hfr H and the origin of Hfr 808, as well as in the region indicated in the figure, as possible elimination of the phage at this site in the crosses Hfr H x D2-18-1-O^+ and Hfr H x D2-8 may have been masked by the presence of phage from the postulated third integration site.

CONCLUSIONS

None of the phages of the T series with the exception of T1 could be shown to form plaques on E coli W although all, with the exception of phage T4, readily adsorbed to this strain. Hence the abortive infection of E coli W by the T phages may not be due to the presence or absence of glucosylated DNA (which occurs in the T-even phages) or the ability of the so-called intemperate phages (T5 and the T-evens) to degrade the DNA of the infected host.

Phage T7, like T2, was shown to produce some phage progeny on infection of strain W but these few particles were unmodified and were thus unable to plate on strain W. Killing of the host cell by phages T2, T3, T5, T6 and T7 appeared to occur and the results obtained indicated the possibility that one phage particle adsorbed to an E coli W cell may be sufficient to cause cell death, and the mechanism of abortive infection may be similar for each of these five phages.

Phage T1.B gives rise to atypical plaques with an efficiency of 10^{-4} when plated on E coli W. On propagation of phage from these plaques on strain C, their ability to plate on W is not lost or reduced, suggesting the mechanism allowing propagation of T1.B on strain W is not host controlled modification but a mutation which involves either the nucleotide sequence recognised by the w prophage restriction system or that recognised by the W genome restriction system. It is unlikely that both sequence types mutate considering the high proportion of mutants in the T1.B population. This suggests either the W genome restriction system or the w prophage restriction

system is not active against the DNA of phage T1.B.

In contrast to the results of Smith and Pizer (42) no exclusion of T2 or any other T phage (except T4) was observed on infection of phage w lysogens of E coli C. However, a reduced plating efficiency of 0.5 by the phages T2 and T6 was observed on both strain C and on C(w) lysogens compared to a relative efficiency of plating of 1.0 on strain B, and the plaques produced on C and the C(w) lysogens were much smaller than those produced on B (Plates 3,4).

The ability of the T phage series other than T4 to plate on the E coli C lysogens C(w.C) and C(w.KC) which prevent propagation of phages P1 and λ raises two possibilities with respect to E coli W;

i) that in E coli W the w phage exclusion system is active against phages P1, λ and the T series but that w phage lysogens of E coli C, although able to exclude P1 and λ, are in some way unable to exclude the T series, or

ii) that an additional exclusion system exists in E coli W in addition to the W genome and w phage mediated exclusion systems discussed in this report.

Phage P1 readily adsorbed to but did not kill E coli W; no plaques were produced on plating 6 x 10^8 P1 particles with 10^8 E coli W cells. To this extent P1 resembles phage λ, but to attribute this similarity to conventional restriction would require confirmation of the penetration and subsequent degradation of P1 DNA.

If conventional restriction does occur, a mutant of phage P1 similar to that of the λ mutant (λw) may possibly be isolated. This isolation may be facilitated by the use of the lysogen C(w.C) which lacks the strain W genome hsr activity. Such a mutant would be

extremely useful in the mapping of the E coli chromosome by transduction as no transducing phages have yet been isolated able to successfully propagate on strain W.

Phages able to propagate on strain W were readily isolated as temperate phage from other coliforms and from sewage samples. These phages must be able to bypass any mechansim causing abortive infection in strain W and it appears likely that these phages, unlike λ, lack the specific nucleotide sequence recognised by the w prophage - mediated exclusion system and that the mechanism causing abortive infection by some of the T phages in strain W does not operate; the infection of strain W by these isolated phages is similar in these respects to that of infection of strain W by the mutant λw.

Unfortunately, host range mutants of V7, V10 or V11 could not be isolated which could plate on strains B, C or K as no more than 10^7 particles of these phages could be plated on strains K or C before plaques of phage w appeared. This hindrance may possibly be removed by the use of w antisera, but no plaques appeared when 10^9 particles of any of these phages were plated with 5×10^8 E coli B cells (which do not allow adsorption of phage w). If a host range mutant of V7, V10 or V11 could be obtained which was able to plate on strain C, it could then be determined to what extent these phages are restricted by the E coli W genome hsr activity.

The reason for the production of three plaque types by a single lysate of either V7, V10 or V11 is not known, but it is thought that this effect may reflect the different times at which cells

in the E coli W lawn were infected. Further investigation possibly involving the infection of cells in a broth culture and the plating of these infected centres on a lawn of E coli W may help clarify the situation.

It appears that two very closely related phages exist in the supernatants of broth cultures of E coli W, one phage able to plate on strain K, the other on strain C and that these phage have a characteristic pattern of plating efficiencies on C and K when propagated alternatively in these hosts. The phage w.K which was initially isolated from a plaque of phage from the W supernatant on strain K has the unusual property of growing best in the host in which it was last propagated. It seems that a mutation enabling this phage to plate on K prevents it from plating on C and vice versa. This behaviour is not typical of host range mutants; after having mutated to adsorb to an alternative host, a typical host range mutant, as phage w.CK seems to be, typically retains its ability to plate on its original host. The behaviour exhibited by phages w.K and its derivatives however, seems to be similar to that of phage able to produce host range mutants in that phage w.K will not adsorb to strain C and phage w.KC will not adsorb to strain K (Figures 15,16).

It is interesting to note that the exclusion of phage P1 in phage w lysogens of E coli C is paralleled by a similar exclusion of λ, supporting the earlier postulate that the exclusion of P1 in strain W may well be due to conventional restriction. A mutation enabling either phage w.C or phage w.KC to plate on strain K often seems to be associated with a loss of the ability to exclude P1 on lysogenisation of strain C. It was suggested that this mutation may decrease the probability of a phage integrating into a 'restrictive'

site on the E coli C chromosome but no concrete evidence has yet been obtained in support of the presence of restrictive and non-restrictive sites on the E coli C chromosome. The observation that three of six lysogens isolated from a single plaque of phage w.CKC could still exclude phage P1 supports the view that the phage integration sites in strain C may be involved in determining the ability of the lysogen to exclude the phage.

It has been established that phage w.K integrates at a site adjacent to one of the proline loci on the E coli W chromosome and that phage w.C may integrate at a site close of the pil locus (88 minutes) although this w.C integration site may not be the only site of integration of this phage.

It might be argued that the site of integration of a w phage into the E coli W chromosome may determine the ability of the spontaneously induced prophage to plate on either strain C or K and may also determine the subsequent behaviour of the phage when alternatively propagated on strains C and K, but no evidence has been obtained either for or against this hypothesis which tends to depart from the simpler and more conventional interpretation of events; that of the presence of two closely related phages.

It is interesting to note that the E coli hs gene locus probably maps in the same region as the hs locus of E coli strains K, B, 15 and A. More accurate mapping of this site in strain W would be facilitated by the use of a transducing phage able to propagate on W; the possibility exists that a mutant of P1 able to do so might now be isolated.

Certain anomalies appeared in the results of some crosses. For example, in the cross Hfr H x D2-8, on selection of pro^+, the unselected cit^+ marker was coinherited at a frequency of 100 percent. In addition, the coinheritance of the unselected marker thr^+ in the cross Hfr H x C(w.CKC) (thr^- pro^-) and again of that same marker in the cross Hfr H x C (w.KC) (thr^- pro^-) was significantly lower than the figure of 50 percent expected. These anomalies might tentatively be explained by the presence of mispairing in the leucine-proline region between the DNA of the heterologous strains employed in these crosses.

BIBLIOGRAPHY

1. BERTANI, G. (1958) Adv. Virus. Res. 5, 151
2. JACOB, F., MONOD, J. (1961) J.Mol.Biol. 3, 318
3. JACOB, F., MONOD, J. (1961) Cold Spr. Harb. Symp. Quant. Biol. 26, 193
4. THOMAS, R. (1968) Symp. Soc. Gen. Microbiol. 18, 315
5. Echoles, H. (1971) Ann. Rev. Biochem. 40, 827
6. WALSH, J., MEYNELL, G.G. (1967) J. Gen. Virol. 1, 581
7. RAO, R.N. (1968) J.Mol.Biol. 35, 607
8. SCHELL, J., GLOVER, S., STACEY, K., BROADA, P., SYMONDS, N. (1963) Genet. Res. Camb. 4, 483
9. MAKELA, O., SOIKKELI, S. (1964) Ann. Med. Exp. Fenn. 42, 188
10. DETTORI, R., MACCACARO, G., PICCININ, G. (1961) Giron. Microbiol 9, 141
11. CUZIN, F. (1965) C.R. Acad. Sci. 260, 6482
12. WATANABE, R., OKADA, M. (1964) J.Bact. 87, 727
13. MORRISON, T., MALAMY, M. (1971) Nature (New Biol.) 231, 37
14. ARBER, W., LINN, S. (1969) Ann. Rev. Biochem. 38, 467
15. ARBER, W. (1965) Ann. Rev. Microbiol. 19, 365
16. ARBER, W. (1968) Symp. Soc. Gem. Microbiol. 18, 295
17. REVEL, H., LURIA, S. (1970) Ann. Rev. Genetics 4, 177
18. STACEY, K. (1965) Brit. Med. Bull. 21, 211
19. HATTMAN, S., REVEL, H., LURIA, S. (1966) Virology 30, 427
20. DUSSOIX, D., ARBER, W. (1962) J.Mol.Biol. 5, 37
21. DUSSOIX, D., ARBER, W., HATTMAN, S. (1963) Virology 21, 30
22. TERZI, M. (1968) J. Mol. Biol. 34, 165
23. ARBER, W., MORSE, M. (1964) Genetics 51, 137
24. WOOD, W. (1966) J. Mol. Biol. 16, 118
25. GLOVER, S., COLSON, C. (1964) Genet. Res. Camb. 6, 153
26. COPELAND, J., BRYSON, V. (1966) Genetics 54, 441

27. SMITH, H.O., WILCOX, K.W. (1970) J. Mol. Biol. 51, 379

27a. SMITH, H.O., KELLY, T.J. (1970) J. Mol. Biol. 51, 393

28. MESELSON, M., YUAN, R. (1968) Nature 217, 1110

29. ROULLAND-DUSSOIX, D., BOYER, H.W. (1969) Biochem. Biophys. Acta. 195, 219

30. SCHELL, J., GLOVER, S.W. (1966) Genet.Res.Camb. 7, 277

31. MOHOLT, B., FRAZER, D. (1965) Biochem. Biophys. Res. Commun. 19, 571

32. SCHELL, J., GLOVER, S.W. (1965) J. Gen. Micro. 45, 61

33. SCHELL, J., GLOVER, S.W. (1966) Genet. Res. 7, 273

34. HIRSCH-KAUFFMANN, M., SAUERBIER, W. (1968) Mol. Gen. Genetics 102, 89

35. MAMELAK, L., BOYER, H.W. (1970) J. Bact. 104, 57

35a. BOYER, H.W., FORD, E. (1970) J. Bact. 104, 594

36. TAYLOR, A.L., TROTTER, C.D. (1967) Bact. Rev. 31, 332

37. GLOVER, S.W., KERSZMAN, G. (1967) Gen. Res. Camb. 9, 135

38. KERSZMAN, G., GLOVER, S.W., ARONOVITCH, J. (1968) J. Gen. Virol. 1, 333

39. GLOVER, S.W., ARONOVITCH, J. (1967) Gen. Res. Camb. 9, 129

40. BERTANI, G. (1958) Virology 6, 357

41. BERTANI, G. (1963) Virology 19, 32

42. PIZER, L.I., SMITH, H.S., MIOVIC, M., PYLKAS, L. (1968) J. Virol. 2, 1339

42a. SMITH, H.S., MIOVIC, M., PIZER, L.I. (1969) J. Virol.4, 195

43. BENZER, S. (1955) Proc. Nat. Acad. Sci. USA. 41, 344

44. LEDERBERG, S. (1957) Virology 3, 496

45. FIELDS, K.L. (1968) J. Bact. 97, 78

46. SMITH, H.W., PIZER, L.I. (1968) J.Mol.Biol. 37, 131

47. SMITH, H.S., PIZER, L.I., PYLKAS, L., LEDERBERG, S. (1969) J. Virology 4, 162

48. KRYLOV, V.N. (1970) Virology 41, 711

49. PUCK, T.T., LEE, H.H. (1954) J. Exp. Med. 99, 481

50. DUCKWORTH, D.H. (1970) Virology 40, 673

51. BACON, D.F. (1962) Genetics 47, 939

52. HAYES, W. (1968) The Genetics of Bacteria and their Viruses p.666

53. ADAMS, M.H. (1959) Bacteriophages

54. GORINI, L., KAUFMAN (1960) Science 131, 604

55. PUCK, T.T. J. Exp. Med. (1951) 93, 65

56. STENT, G.S. (1963) Molecular Biology of Bacterial Viruses p.91

57. JACOB, F., WOLLMAN, E.L. (1961) Sexuality and the Genetics of Bacteria p.86