TRANSDUCTION IN

Staphylococcus aureus

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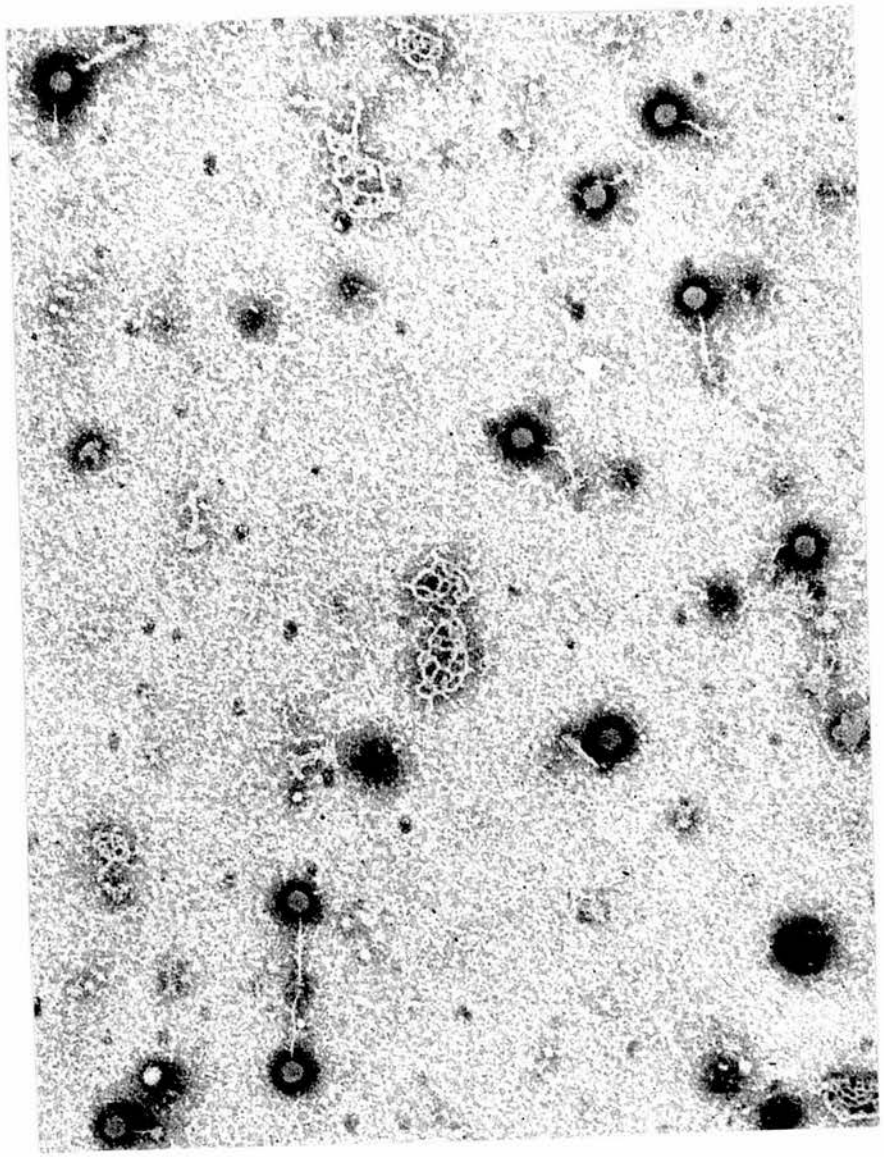

Transducing phage x 60,000

Electronmicrograph of phage AK72
stained with phosphotungstic acid.

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PREFACE

Transduction, in which phage acts as a vector in the transfer of genetic material from one bacterium to another, was reported first in *Salmonella*. Later the process was observed in *Escherichia coli* and *Shigella*. Only limited investigations had been made with *Staphylococcus aureus* when the present work began in 1959.

The first aim was to confirm that this type of genetic transfer did take place between strains of *Staph. aureus*. If positive results were obtained it was hoped to show that the process might play some part in the development of antibiotic-resistant strains of staphylococci *in vivo*.

The initial attempt to transduce streptomycin resistance by phage induced from artificially lysogenized streptomycin-resistant staphylococci was unrewarding; the results did not clearly indicate that transduction had taken place. As experience of staphylococcal cross infection and the prevalence of tetracycline-resistant staphylococci during hospital epidemics had aroused my interest, the possibility of transducing tetracycline resistance in staphylococci was chosen for detailed study.

In preference to artificially lysogenized strains, naturally lysogenic tetracycline-resistant staphylococci were used as the source of the phage. With two of these phages the transfer of tetracycline resistance to tetracycline-sensitive strains was achieved. At first it seemed as if lysogenic conversion of the recipient strain might be involved, but further work proved that transduction was taking place. At this stage the lytic spectra, serological group and morphological character of the two transducing phages were determined.

The results of the early experiments, in which a limited number of recipient strains were tested, suggested that a close similarity between the phage types of the donor and recipient strains is necessary for transduction to take place. To investigate this and to ascertain if the ability to be transduced is restricted to certain phage types, tetracycline-sensitive strains representative of the three main phage groups were collected and tested as potential recipient strains. When transduction of tetracycline resistance to the strains was attempted, the number of positive results was small. The strains had all been isolated from hospital

patients and a reasonable hypothesis was that they had remained tetracycline sensitive despite being in the hospital environment because of an inherent inability to be transduced. However, similar results were obtained when recipient strains isolated from nasal carriers unassociated with a hospital environment were used.

At this point, as developments in procedure had involved the incorporation of an increased amount of tetracycline in the selective medium, it was necessary to investigate the effect of varying the concentration of the antibiotic on the primary expression of the transduced cells. When the concentration of tetracycline was reduced, a high proportion of Group I and Group III staphylococcal strains were transduced whether they had been isolated from hospital patients or from nasal carriers. In contrast, no positive results were found when Group II strains were tested. As staphylococci of phage type 71 differ from other Group II strains in some respects, a small number of these organisms were tested as recipient strains. No positive results were obtained and it seems that type 71 strains resemble other Group II staphylococci in this respect at least.

If transduction plays any part in the emergence of epidemic strains, some of the characters commonly associated with such strains may be transducible. The characters associated with epidemicity include multiple antibiotic resistance, penicillinase production and resistance to mercuric chloride. The present work showed that tetracycline resistance is indeed transducible and attempts were then made to determine if transduction of penicillinase production and mercuric chloride resistance also took place. Penicillinase production was transduced by both of the phages used to transduce tetracycline resistance; co-transduction of mercuric chloride resistance was found with penicillinase production, but not with tetracycline resistance in the strains tested.

Evidence has recently been presented to show that penicillinase production and mercuric chloride resistance in staphylococci are determined by the presence of extrachromosomal elements. In the final part of this work, experiments are described in which the nature of the genetic determinants of the transduced characters is investigated. The results are in keeping with the conclusions of other workers that these determinants are situated on extrachromosomal elements.

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The results of some of the work included in this thesis have already been published.

The relevant reference is:

COLLINS, A.M., McDONALD, S. (1962) J. Path. Bact., 83, 399.

INTRODUCTION

The three mechanisms of genetic transfer in bacteria, conjugation, transformation and transduction, are all characterized by a one-way transfer of genetic material from donor to recipient cells and by the fact that usually only part of the genetic material of the donor cell is included in the zygote. Although the present study is primarily concerned with the phenomenon of transduction, it is necessary to outline the background knowledge of transformation and conjugation that contributed to the discovery of transduction. Before reviewing the work that has led to the elucidation of these mechanisms it is worthwhile to define the terms currently used to describe them.

Conjugation refers to the union of two sexually differentiated bacteria which is followed by the transfer of part, or occasionally the whole, of the genetic material from one cell to another. In transformation, the first process to be fully investigated, the agent of transfer is pure deoxyribonucleic acid (DNA) extracted from bacteria of one type and applied to organisms of another type differing from the first in one or more

characters. Transduction in the past has been defined as "the transmission of a (nuclear) genetic fragment from a donor cell (which in every case so far is destroyed in the process) to a recipient cell" (Lederberg 1955). This definition embraces both the processes that transfer small genetic fragments, these being transformation by free DNA and phage-mediated transduction. As the former already had a well established name the term transduction has become restricted by popular usage to mean the transmission of a fragment of bacterial DNA within a phage particle.

It is only recently that the different processes of genetic transfer have been investigated in detail but reports postulating the transfer of heritable characters are to be found in the early literature.

Kuhn and Ebeling (1916), whose paper is one of a group cited by Lederberg in 1948, described the phenomenon of para-agglutination in which Escherichia coli grown with organisms of the Shigella or Salmonella group acquired the ability to agglutinate when mixed with antisera prepared against the latter organisms. An account more suggestive of genetic transfer was given by Wollman and Wollman (1925) who found a rise in the agglutinating titre of

S. paratyphi B antiserum tested against Esch. coli after that organism had been grown with S. paratyphi B. Treatment of Esch. coli with cultures of S. typhi or filtrates of S. typhi or S. paratyphi B produced no rise in titre of the antiserum. In the same year Burnet (1925) claimed that the acquisition of 'paramelitensis' antigens by Brucella melitensis when grown in a culture filtrate of the former organism represents the action of a transforming virus, but later workers considered this to be an example of spontaneous change from the smooth to the rough phase in the same organism.

Reports that avirulent organisms become pathogenic when grown in sterile filtrates of virulent cultures are more difficult to assess. Legroux and Genevray (1933) reported that this took place between strains of Pseudomonas pyocyanea but they suggested that the change is an expression of latent pathogenicity in the avirulent strain and is not necessarily brought about by the acquisition of a filterable element.

Experiments with streptococci yielded results which can be taken to indicate genetic transfer. Cantacuzène and Bonciu (1926) found that streptococci that were not agglutinated by sera

from scarlet fever patients were rendered agglutinable by incubation for 24 hours with a sterile filtrate of pharyngeal exudate or urine from patients in the acute stage of scarlet fever. This property seemed to be a permanent character because it was retained by the recipient organisms after several subcultures. The authors noted that incubation of the exudate before filtration increased the degree of agglutinability acquired by the recipient streptococci but exposure to cold had the opposite effect. Somewhat similar work by Frobisher and Brown (1927) showed that the ability to form a toxin that could be neutralized by serum from scarlet fever patients, was gained by two strains of streptococci after incubation with toxigenic streptococci. Incubation with sterile filtrates of the toxigenic strains had the same effect, but with this method the recipient organisms did not become so strongly toxigenic.

Even when the work described in these early reports supports the claim that genetic transfer is taking place, the available evidence does not allow more than a guess to be made about the underlying mechanism responsible for the transfer. When the process requires direct contact between living donor and recipient cells as described by

Wollman and Wollman (1925) it may be thought that conjugation is implicated. In the experiments with streptococci the transfers take place when the recipient strains are exposed to sterile filtrates of material from infected patients, or to living cultures or sterile filtrates of donor strains. Therefore these changes may represent genetic transfer effected by a substance liberated during the growth of the donor strain or by phage. The latter is able to act by being a vector of bacterial genetic material or by attaching its own genetic material to the chromosome of the recipient cell.

Transformation

The discovery of transformation in 1928 by Griffith, during a study of virulence in pneumococci, proved to be a major advance in genetics because it showed clearly for the first time that hereditary characters can be transmitted from one bacterium to another and it led to the identification of the transforming material. Griffith found that suspensions of living, non-capsulated type II pneumococci or suspensions of heat-killed type I organisms are non-lethal when inoculated into separate groups of mice. However if comparable doses of these preparations were

inoculated together the mice died and blood culture yielded a pure growth of the capsulated type I organism. He suggested that the transforming agent might be the capsular polysaccharide which was clearly associated with the virulence of the organism. Although Griffith failed to transform pneumococci by in vitro methods this was done by Dawson and Sia in 1931. These workers did not agree with Griffith's suggestion that capsular polysaccharide was the transforming agent; they were unable to establish the identity of the agent (Sia and Dawson 1931). More than ten years later, Avery, MacLeod and McCarty (1944) finally identified the agent as DNA by chemical, enzymic and serological methods. Important evidence put forward was the failure of treatment with crystalline proteases or ribonuclease (RNase) to impair the agent's transforming ability. Confirmation of these findings was obtained later when treatment with minute amounts of purified deoxyribonuclease (DNase) resulted in an irreversible inactivation of the transforming substance (McCarty and Avery 1946).

In 1950 Alexander and Leidy showed that transformation takes place in species other than the Pneumococcus when they transformed non-typable

strains of Haemophilus influenzae to type b cells with type b DNA, or to type c cells with unpurified transforming substance from type c cells. Alexander and Redman extended this work in 1953 when they reported the transformation of type specificity in meningococci. Gillissen (1958) reported the development of penicillin resistance in penicillin-sensitive staphylococci after exposure to an extract of penicillin-resistant organisms. This transfer was inhibited by DNase and appears to be another example of transformation. Streptococci have also been used in studies of this nature and Perry and Slade (1962) described the transformation to streptomycin resistance in streptococci of different serological groups. In the Escherichia, Salmonella and Shigella groups, however, transformation has not been reproduced with sufficient consistency to establish that the phenomenon takes place in these organisms (Ravin 1961).

Although early workers on transformation studied characters dependent on the synthesis of polysaccharides the transforming process is not limited to that class of hereditary characters. More recently, characters such as antibiotic resistance and nutritional requirements have been widely exploited in quantitative studies as

methods are readily available with these systems to select the transformed bacteria from the untransformed recipient population. Such studies have proved useful in the demonstration of linkage between different genes on the bacterial chromosome. In transformation, linkage can only be demonstrated when two genes are transferred together on the same molecule of DNA. As the molecules of DNA transmitted in transformation are of relatively low molecular weight the demonstration of this linkage means that the genes must be situated very close to each other on the bacterial chromosome. Linkage is found in pneumococci between the determinants of streptomycin resistance and mannitol utilization. This was used by Hotchkiss and Marmur (1954) to demonstrate reciprocality and to confirm that in transformation genes in the exogenous DNA are exchanged with the allelic forms present in the recipient chromosome.

It is apparent that transformation can be used in the laboratory to perform genetic analysis of functions in many different bacterial species including some that are not amenable to conjugation or transduction. The process is also important as the only method available to correlate the effects of physical or chemical

alterations in the structure of DNA with its biological activity. In nature it is possible that transformation may provide a method of genetic transfer; Ottolenghi and Hotchkiss (1960) found that genetically active DNA is liberated spontaneously during exponential growth of pneumococci giving rise to transformation in untreated, mixed cultures.

Conjugation

This process, which differs from transformation in ways that relate it more closely to the sexuality found in higher organisms, was discovered by the carefully planned experiments of Lederberg and Tatum (1946 a, b). They recovered prototrophic recombinants from a mixture of different multiple auxotrophic derivatives of Esch. coli strain K-12 by plating the mixture on minimal agar on which neither of the parent strains could grow. The prototrophic colonies appeared at a frequency of about one per 10^6 to 10^7 parental bacteria plated. These colonies were present in such small numbers that a selective method was essential for their recognition. It was soon found that this form of genetic transfer requires cellular contact between intact bacteria of both parental strains;

treatment of cultures of one type with cell-free filtrates or extracts of the other fails to yield recombinants (Tatum and Lederberg 1947). It was also noted that treatment with DNase has no effect on the transfer. These findings made it clear that conjugation differs fundamentally from transformation.

In some of the early experiments reported the parent strains differed in resistance to phage T1 as well as in their auxotrophic requirements and this unselected marker segregated among the prototrophs as if it and the selected markers were arranged in a linear linkage group. As a wider range of unselected markers became available for study anomalies of segregation appeared. These anomalies were not in keeping with the idea held at that time that recombinants in bacteria arose by a process similar to meiosis in zygotes formed by the fusion of complete parental genomes. Analysis of the heterozygotes showed that they were incomplete and it was thought that the missing segment was eliminated after their formation, in other words by post-zygotic exclusion.

Evidence that parental cells do not play identical roles in zygote formation was provided by Hayes (1952a) who showed that the fertility of these crosses depends on the continued viability of only one of the two parents. It was concluded that these viable cells are essential to the fertility of the cross because they are exclusively recipients of genetic material contributed by the other parent. Hayes (1952b) found that ultraviolet light stimulates the fertility of donor but not recipient cells. This suggested that a virus or prophage might be the agent of transfer.

Work by Lederberg, Cavalli and Lederberg (1952) and Hayes (1953) revealed that the ability to act as a donor strain is dependent on the presence of a determinant F, the sex factor. If a cross is to be fertile one of the parental strains must be of the donor type. The sex factor F is transmitted with a very high frequency from donor (F+) cells to recipient (F-) cells in marked contrast to the low frequency of transfer of normal genes located on the bacterial chromosome. It was also shown that F can multiply autonomously in donor cells. While it can be lost spontaneously on rare occasions, it is never

spontaneously regained. Thus the loss of F is complete and is not the result of a mutation of a gene to a non-functional allele. As in the case of chromosomal transfer cell to cell contact between donor and recipient cells is required for the transfer of F and this indicates that its role is different from that of the phage vector in transduction.

The finding of an F- variant in a strain that had previously been F+ enabled Hayes (1953) and Cavalli, Lederberg and Lederberg (1953) to carry out reciprocal crosses. Only some of the donor markers were inherited by the recombinants which predominantly resembled the recipient strain; unselected markers that appeared in the recombinants were different in the two crosses. The interpretation of these findings aroused much controversy. Hayes (1953) suggested that donor bacteria transfer only part of their chromosome to the recipients so that incomplete zygotes are formed. This seemed a more likely explanation than that of post-zygotic exclusion and Hayes' hypothesis was confirmed later as a result of the work described below.

The isolation of a new type of donor cell in Esch. coli K-12 led to greater understanding of the

F factor. These new donor strains yield many more recombinants when mixed with F- cells than do the equivalent F+ strains and they are consequently termed high frequency (Hfr) donors. However, this high frequency of recombination is found only when a particular linked group of characters is selected. Wollman, Jacob and Hayes (1956) showed that cells of any one Hfr strain transfer their chromosomes in a specifically orientated way so that a particular extremity called the leading locus is always the first to penetrate the recipient cell and the genes enter the cell in the order of their arrangement on the chromosome. Random spontaneous breakage of the chromosome may interrupt the process so that the length of the chromosomal fragment transferred is variable and genes situated at a distance from the leading locus have a smaller probability of being transferred. It was also noted that, unlike F+ strains, Hfr strains do not convert recipient cells to the F+ state. They can, however, revert to that state themselves and therefore must retain F in some non-transmissable form. The Hfr character is transferred to a small proportion of the recipients: these being recombinants that are selected for donor markers

at the opposite end of the chromosome from the leading locus. This suggested that the Hfr determinant is linked to the terminal end of the transferred fragment of chromosome and that in Hfr strains F is attached to the chromosome. Hirota (1960) confirmed this when he showed that acridine orange treatment results in a permanent and rapid loss of F from F+ cells indicating that F is an extrachromosomal particle. In contrast Hfr cells are unaffected by the acridine orange treatment, a resistance accounted for by the association of F with the bacterial chromosome.

It would seem that the change from the Hfr state to the F+ state and vice versa corresponds to a change of F from a position of attachment to the chromosome to an autonomous cytoplasmic state. This allows F to be classified as an episome. Episomes were defined by Jacob, Shaeffer and Wollman (1960) as genetic elements which are not essential to the bacterial cell and may be present in two distinct, possibly mutually exclusive, states: the autonomous, independently replicating cytoplasmic state and the integrated or chromosomally attached state. The first member of this class to be recognised, temperate phage will be discussed later in this introduction.

When a variety of Hfr strains became available for investigation it was found that the cells of any one particular Hfr strain transferred a given set of markers at a high frequency and in a particular order. However, different strains transfer different groups of genes and in some cases the genes are transferred in the reverse direction. By comparing the genes transferred with a high frequency by different Hfr strains it is possible to determine the complete sequence of the known markers in Esch. coli K-12 and no interruption is found in this sequence. To account for these findings Jacob and Wollman (1958) suggested that the chromosome of the F+ donors is circular and the Hfr strains arise by opening up of the circle, at a point characteristic for each strain, to yield a linear transferable structure. The extremity of this structure to which F becomes attached is the tail and the other extremity becomes the leading locus.

In 1960 Adelberg and Burns discovered another type of donor strain whose properties are intermediate between those of Hfr and F+ strains; this new type is termed an intermediate donor F'. As a result of genetic exchange between the sex factor and the bacterial chromosome in this new

type of donor each carries a region of homology for the other. This exchange renders unstable the association between F and the chromosome so that F alternates rapidly between the bacterial cytoplasm and the chromosome. When F is integrated in the chromosome, intermediate donors act as Hfr strains. When F is in the cytoplasm and when the incorporated segment of bacterial chromosome carries known markers, these will be transferred to the recipient cells with high frequency under conditions that exclude chromosomal transfer. This phenomenon, which is called F-duction or sexduction, bears a close resemblance to restricted transduction by lambda phage although it differs from the latter in requiring cellular contact for the transfer and in transferring larger segments of chromosome than those carried by phage in restricted or generalized transduction.

All the work concerning conjugation that has been reviewed above was carried out with Esch. coli strain K-12 but conjugation studied in other systems is controlled by episomes similar to the F factor.

A small proportion of other Esch. coli strains give rise to recombinants and transmit

sex factors when crossed with strain K-12 recipients. These recipients behave quite differently from the F+ strains described above, indicating that the sex factors concerned are distinct from F. A larger proportion of Esch. coli strains are fertile when crossed with K-12 donors. Conjugation between Esch. coli K-12 donor strains and Shigella types has been reported by Luria and Burrous (1957) who found that the lower frequency of recombinants in this cross is accounted for by poor genetic homology between the Esch. coli and Shigella organisms and not by a failure of chromosome transfer. Esch. coli F+ cells will conjugate with Salmonella organisms and it is possible to isolate Hfr cells from irradiated Salmonella F+ strains. These have been used to transfer large segments of their chromosomes to Esch. coli recipients as well as to Salmonella recipients (Zinder 1960).

In 1963 Watanabe reviewed another system of conjugation comprising a wide range of gram-negative organisms in which multiple drug resistance is transferred by sex factors called resistance transfer factors (RTF). Resistance to streptomycin, chloramphenicol, tetracycline

and sulphonamide, is the commonest combination that is transferred. A variable number of determinants of drug resistance may be linked to an RTF and the whole element is termed an R factor. In individual bacteria the RTF can exist separately from the resistance markers of the R factor and this suggests an anology with the F and F' factors already discussed. Watanabe suggested that R factors may arise in the enteric bacteria by attachment of part of the bacterial chromosome to the RTF and he has classified the RTF as an episome. Datta (1965) was not convinced by the evidence for attachment of the RTF to the chromosome and she put forward an alternative hypothesis that R factors might arise by mutation in extrachromosomal elements that have no known biological activity.

The RTF system, in addition to being of considerable genetic interest, has important medical implications. There is epidemiological and experimental evidence that resistance transfer takes place readily in the intestines of man and animals (Anderson 1965).

This review has dealt with the main advances in our knowledge concerning conjugation. The transfer of colicinogeny in the

Enterobacteriaceae (Frédéricq 1958) is mediated by genetic determinants called colicinogenic factors that share certain properties with sex factors and temperate phages but do not conform precisely to the classical definition of an episome. This system and the examples of intraspecific conjugation described in Pseudomonas pyocyanea (Holloway and Fargie 1960), Serratia marcescens (Belser and Bunting 1956) and Vibrio cholerae (Bhaskaran 1960) have many features in common with the processes described above. Studies of these systems have yielded no further information that is relevant to the understanding of transduction.

Temperate Phages and Phage Conversion

As temperate phages act as the vectors of genetic material in transduction, it is appropriate to consider their properties before discussing the phenomenon of transduction itself.

These phages may be present in their bacterial hosts in an autonomous or in an integrated state. In the autonomous or vegetative state the phage DNA determines its own replication at a faster rate than that of the bacterial genome and it controls the synthesis of the protein components of the

mature phage and the final production of infectious particles which are released when the host bacterium lyses. In the integrated or prophage state the phage DNA is closely associated with the bacterial chromosome and replicates in coordination with the host bacterium. This intimate relationship between phage and bacterium is called lysogeny and will be discussed more fully.

In the integrated state the phage does not express its viral potentialities and the synthesis of infectious particles does not take place. However, the lysogenic bacteria retain the information necessary for the production of mature phage particles and their progeny are capable of liberating infectious particles without additional infection. When a lysogenic culture is examined, free phage can always be found. For many years there was controversy regarding the manner of release of these phage particles. One theory proposed that the phage is secreted by the living and actively growing bacteria. The alternative theory held that each lysogenic bacterium inherits the potentiality to liberate phage, as in the vegetative phase, but has a very low probability of expressing this property. Lwoff and Gutmann (1950)

analysed the progeny from single cells of a lysogenic strain of Bacillus megaterium and established that free phage particles are liberated after lysis of a very few bacteria in the culture. They noted that the proportion of bacteria that liberate phage varies from one experiment to another and they suggested that certain external factors may induce phage production. In the same year Lwoff, Siminovitch, and Kjeldgaard found that ultraviolet light in doses too small to affect the growth of non-lysogenic bacteria produces almost complete lysis of a culture of lysogenic B. megaterium with liberation of at least 90 phage particles per bacterium. Later, other agents including X-rays, nitrogen mustards and hydrogen peroxide, were found to have a similar "inducing effect". Every bacterium of a lysogenic culture contains prophage and this suggests that the replication of prophage and its distribution to the daughter bacteria must be associated with the replication and distribution of the bacterial genome. Evidence for this was found by Wollman (1953) who carried out genetic crosses between strains of Esch. coli K-12 lysogenic and non-lysogenic for phage lambda. Lysogeny and non-lysogeny segregated among the

recombinants, and the prophage appeared to have a genetic locus close to that determining galactose fermentation. In 1954 Appleyard designed experiments with two different mutants of lambda phage to confirm that this prophage occupies a specific site on the bacterial chromosome.

Using the interrupted mating technique, Jacob and Wollman (1957) studied the location of 14 temperate phages in Esch. coli K-12. The prophages, seven of which are inducible, do not show any cross immunity; lysogenization with one strain does not confer immunity to infection by the others. Each of these phages was found to occupy a definite locus on the chromosome. The seven inducible prophages are grouped on a small segment of the chromosome near the Gal region and the seven non-inducible prophages are located on other regions. Thus these unrelated prophages occupy different specific sites on the bacterial chromosome. In Esch. coli K-12 one prophage (363) appears to lack a specific chromosomal location. This finding is of considerable interest as 363 can carry out generalized transduction and it may be that this multiple transducing property is related to an affinity for many sites on the bacterial chromosome.

The specificity of the connection between the prophage and the chromosome appears to be determined by a small region of the prophage genome but the precise structural nature of the connection is not yet defined. Two hypotheses have been put forward. In the attachment hypothesis part of the prophage is said to be in a persistent state of pairing with the homologous region of the chromosome. The most direct evidence in favour of this theory comes from the study of transductional heterogenotes in the phage lambda - Esch. coli system. The insertion hypothesis suggests that the prophage becomes integrated into the chromosome by a recombination event. Hayes (1964) considered that a clear decision between attachment or insertion of the prophage could not be made at that time although the available evidence seemed to favour attachment of the prophage.

One of the most interesting properties of the temperate phages is their capacity to modify various characters of the host bacterium. In some systems the only difference between the lysogenic and non-lysogenic bacteria is the heritable potentiality to liberate infectious phage particles and the possession of phage-specific

immunity. Such immunity allows lysogenic bacteria to survive infection by phage homologous to the carried prophage and appears to be responsible for the prevention of expression of phage viral functions whether such functions are controlled by the prophage genome or the superinfecting phage genome. It does not result from inability of the superinfecting phage to adsorb to the lysogenic cell or to inject its DNA; the immunity depends on the prevention of phage multiplication.

In other systems differences between lysogenic and non-lysogenic bacteria have been observed in properties that are unrelated to the lysogenic state. It seems that the DNA of a particular prophage can modify one or more host characters and this process has been termed lysogenic conversion or, more recently, phage conversion.

In 1951 Freeman found that if certain non-toxigenic strains of Corynebacterium diphtheriae are treated with phage from virulent toxigenic organisms of the same species a proportion of the survivors acquires the ability to synthesise toxin and simultaneously develops immunity to lytic infection by the phage. Groman (1955) put forward further evidence that lysogenization of a particular

strain of C. diphtheriae with a specific phage results in the acquisition of toxigenicity by that strain. He showed clearly that the process differs from transduction in two ways. Firstly, in the C. diphtheriae system there is a high ratio of conversion relative to phage concentration whereas in transduction this is low. Secondly, the converting ability of the phage is not dependent on propagation in a toxigenic host. This means that conversion depends on phage DNA and not on bacterial DNA carried by the phage particle. In these early experiments, there was correlation between lysogenization and toxin production and in consequence, this phenomenon became known as lysogenic conversion. Later workers (Uetake, Luria and Burrous 1958) working with a different system in the Salmonella group, and Barksdale (1959) using C. diphtheriae, found that virulent mutants of the converting phages can also induce changes in host functions very soon after infection in cells that are destined to lyse. This showed that lysogenization is not essential for conversion and Barksdale suggested the replacement of the name lysogenic conversion by the more general term phage conversion.

Examples of the alteration of the antigenic properties of the bacterial host by phage are to be found in the work on Salmonella cited above. Treatment of S. anatum with the temperate phage epsilon-15 resulted in a proportion of the cells becoming lysogenized and acquiring the ability to produce antigen 15 with a simultaneous loss of antigen 10 production. Lysogenization was not essential for this alteration in antigenic properties. The change also took place in cells treated with a virulent mutant of epsilon-15.

Phage conversion has also been demonstrated in Staphylococcus aureus. The phage-typing of Staph. aureus has been widely used to differentiate strains and thus to investigate outbreaks of staphylococcal infection. Any variation in the phage-typing pattern of individual strains of staphylococci is of obvious importance in the interpretation of phage-typing results. It was shown that changes in phage-typing patterns followed artificial lysogenization and as these changes comprised losses in phage sensitivity it seemed that they indicated acquisition of phage-specific immunity. It was not known if such changes took place in vivo. Later workers (Asheshov and Rippon 1959), investigating outbreaks

of infection caused by staphylococcus phage type 80/81, noted that strains with the phage-typing pattern 52/52A/80 appeared in some prolonged outbreaks. Using three temperate phages isolated from these latter strains they lysogenized type 80/81 strains and found a different typing pattern after lysogenization with each phage. In two cases losses of sensitivity to phage 81 were found, one of which could be accounted for by specific immunity as the lysogenizing phage was serologically related to phage 81. The gain in sensitivity to phages 52 and 52A is more difficult to explain. It is not due to a change in cell receptors as the 80/81 organisms adsorb both phages. Transduction is excluded as the change is independent of the type of strain used for propagation of the converting phage, and lysogenic conversion is not the explanation as the change remained after the loss of the converting phage. Prophage substitution was considered but could not be proved. These conversions are of considerable practical importance; if such a possibility is not taken into account when interpretating phage-typing results, close relationships between superficially different phage types may be overlooked.

More recently Winkler, de Waart and Grootsen (1965) described other changes in staphylococci following lysogenization. These workers have been able to convert staphylococci of phage groups I, II and III to staphylokinase production; an associated loss of β-toxin production was demonstrated. They also noted that certain 80/81 strains carry converting phages that are associated with staphylokinase production. The loss of these phages results in a loss of staphylokinase production but there is a simultaneous acquisition of β-toxin production and of sensitivity to Group III phages.

Transduction in the Enterobacteriaceae

The mechanism of transduction was first elucidated by detailed studies of the process in the enterobacteria. This work will therefore be discussed before the more recent papers describing the process in staphylococci are reviewed.

Two forms of transduction have been recognised. The first is termed generalized transduction and is typified by the S. typhimurium system described by Zinder and Lederberg in 1952. The second form, restricted transduction, is found in the phage lambda-Esch. coli system (Morse, Lederberg and Lederberg 1956).

In 1951 Lederberg, Lederberg, Zinder and Lively investigated the possibility that a genetic transfer similar to that found during conjugation between strains of Esch. coli K-12 might take place between auxotrophic mutants of S. typhimurium. They found that small numbers of prototrophic colonies can be recovered in this system. However, it was noted that prototrophs are recovered not only when cultures of the auxotrophs are mixed but also when a culture of one auxotroph is mixed with a cell-free extract of the other. This feature clearly differentiates the process from the conjugation system in Esch. coli K-12 in which, as has already been shown, contact between intact cells of both parental strains is essential for genetic transfer to take place. A preliminary test showed that the transfer process was not affected by DNase. The new process was called transduction and its mechanism was further studied by Zinder and Lederberg in 1952. They found that S. typhimurium, when treated with lysogenic phages or subinhibitory concentrations of antibiotics, lithium chloride or crystal violet, released a filterable agent (FA) which was capable of transferring heritable traits from one bacterial strain to another. The highest activity was detected in filtrates of cultures

treated with weakly lytic phages. As a rule a maximum of only one per million recipient cells acquired the new character. Any filtrate could transduce many different markers, including nutritional, fermentative, drug resistance and antigenic characters, from the donor cells but any particular recipient cell acquired only one new character. Thus, in these early experiments there is no evidence of the linkage that is a prominent feature of conjugation. Transduction was shown to be unaffected by the treatment of the filtrates with bacterial disinfectants such as chloroform, toluene and alcohol or with enzymes such as pancreatin, trypsin, RNase or DNase. The failure of DNase to inhibit the transfer process indicates the filterable agent is not free DNA and the process is different from transformation.

Even at this stage considerable evidence had accumulated to support the identification of FA as phage. Many of the transduced cells were found to become resistant carriers of the phage associated with the transducing lysate. This introduced the possibility of the process being an example of lysogenic conversion. Both FA and phage were shown to have a common filtration end point, to have a common specificity of adsorption on

Salmonella serotypes correlated with antigen 12 which is a phage receptor and to remain together during purification. It seemed, therefore, that in this system FA was closely associated with the particles of the temperate phage PTL-22 (now known as P-22). However lysogenic conversion was excluded as the underlying mechanism when the transduced characters were shown to depend on the genotype of the bacterium from which the lysate was prepared. It seemed more likely that the phage was acting as a passive carrier of the transducing material.

In 1953 Zinder compared the phenomena of transformation and transduction and investigated in more detail the role of phage in the latter process. He found that the ratio of FA to the plaque titre in different transducing lysates remained constant, thus indicating a correlation in the production of FA and phage and this ratio was maintained during purification. Attempts at differential inactivation were unsuccessful. Heat treatment produced the same reduction in plaque titre and transducing ability of the lysate. Antibacterial serum was without effect on either phage or transducing activity, but antiserum prepared by injection of purified

lysates into rabbits inactivated both the phage and the FA at the same rate. One agent that had a differential effect upon the two activities of the transducing lysates was ultraviolet irradiation. The phage was inactivated exponentially as the dose of ultraviolet light increased whereas the transducing activity increased at first and then subsided three to four times more slowly than the phage titre. It was suggested that the slow but definite inactivation of FA might indicate that a nucleic acid component was the active principle.

As was shown by Zinder and Lederberg (1953) both FA and phage P-22 are adsorbed by Salmonella organisms with the somatic antigen 12 and although many of these strains are not sensitive to the phage they are all transducible. On the other hand, strains that lack antigen 12 and cannot adsorb the phage are not transducible. In one strain of S. typhimurium both phage and FA reach saturation at the same level of 10 particles per bacterium, a factor which limits the maximum frequency of transduction. Other strains will adsorb many more phage particles and their maximum transduction frequencies can be correspondingly raised. The ability of a recipient organism to adsorb the phage from an

active lysate is therefore closely related to the frequency of its transduction. As some strains of Salmonella adsorb phage and are transduced but neither lyse nor become lysogenized it is clear that the transducing agent and phage can be separated at least within the bacterial cell. On the basis of the above evidence Zinder considered that the transducing agent and the phage are different biological entities which have a common adsorption mechanism. As already described, when a phage infects a bacterium its nucleic acid enters the host cell. Zinder suggested that transduction in Salmonella might represent the simultaneous injection of phage DNA with bacterial DNA acquired from the previous host.

Stocker, Zinder and Lederberg (1953) studied the transduction of flagellar characters in Salmonella strains by phage P22 and shed further light on the process. When non-motile recipients were treated with the appropriate transducing lysate they acquired motility and the cells swarmed across the plate. On many plates, in addition to the swarms, micro-colonies were found in large numbers at a distance from the site of inoculation. Subcultures from these colonies yielded non-motile forms yet their position on the

plate showed clearly that the cell from which each colony grew must have been motile. These so-called trails result from the failure of the transduced gene to replace its homologue in the recipient cell and therefore to replicate with the host chromosome. Thus, at each cell division there is segregation of the transduced gene to give a motile and non-motile daughter cell, the latter producing a micro-colony. This phenomenon is termed abortive transduction. It is of value in elucidating the tranduction process because its frequency indicates the transfer of genetic material whereas complete transduction corresponds to transfer with subsequent integration of the transduced material into the recipient chromosome. In such experiments the number of abortive transducants always exceeds that of complete transducants and this is good evidence that only a minority of transduced genes are ever integrated.

During these studies Stocker *et al* (1953) found many motile but non-lysogenic cells among the transducants. This confirmed that transduction can take place without lysogenization of the recipient cell and the observation provided a stimulus for further investigation of the nature of the transducing particles. In generalized

transduction the frequency of transfer of a donor marker is so low that an excess of non-transducing phages must be plated with the recipient bacteria. There is consequently a strong probability that a cell infected with a transducing phage will also be infected with a normal phage particle. If this probability is lessened by reducing the multiplicity of infection by phage P22 there is a resultant increase in the proportion of Salmonella transducants that are non-lysogenic. The phage P22-Salmonella system is not very suitable for this type of investigation because several generations may be required for the phage to establish lysogeny. Luria, Fraser, Adams and Burrous (1958) showed this when they isolated both phage sensitive and lysogenic cells from the same phage-infected parental cell. In the phage P1-Esch. coli system it is determined whether phage will lysogenize or not before the infected cell divides and pure lysogenic clones are formed. Phage P1 needs calcium for adsorption so that secondary infection on the transduction plate can be prevented. Using this system Lennox (1955) found that when transducants are selected on nutrient agar the majority are lysogenic but when selection is made on minimal agar the transducants

are non-lysogenic. These results show not only that transduction can take place without lysogenization but that transducing phages have defective genomes and are unable to establish lysogeny.

Phage P1 was also used by Lennox (1955) to transduce a variety of characters between strains of Esch. coli and Sh. dysenteriae. Thus genetic homology between these groups has been demonstrated by transduction as well as by conjugation studies. Lennox was able to show that joint transduction also takes place in this system. The characters that can be transduced together are those which behave as closely linked markers in conjugation experiments and the frequency of joint transduction decreases with increasing distance between the markers as measured by linkage in sexual crosses.

The transduction of lysogeny was demonstrated by Jacob (1955) in Esch. coli K-12 by means of phage 363, a phage that is closely related to P1. This confirmed the previous results of conjugation experiments which showed that lysogeny is controlled by a genetic determinant in the bacterium. Jacob's work also showed that a transducing phage can contain

the genetic material of one or more prophages in addition to homologous genetic material.

Thus in generalized transduction any bacterial gene can be transduced; these genes can be incorporated into the transducing phage during lytic infection as well as after induction of lysogenic cells; the transduced bacterial DNA usually, but not always, becomes integrated into the recipient chromosome; and transducants show no evidence of lysogenization.

Restricted transduction as described by Morse, Lederberg and Lederberg (1956) in the phage lambda-Esch. coli system differs in certain ways from generalized transduction and there is a close analogy between it and the phenomenon of sexduction described above. Lambda, a phage for which most strains of Esch. coli K-12 are lysogenic, transduces only certain genes, namely those responsible for galactose fermentation (Gal genes) Conjugation studies have shown the Gal genes to be very close to the specific site on the chromosome occupied by prophage lambda. The transducing ability of lambda was found to depend on its induction from lysogenic cells; lambda propagated on sensitive cells has no transducing activity. It seems, therefore, that the proximity of the

prophage site to the Gal loci determines the transducing ability of lambda. The majority of Gal+ transducants are unstable for the Gal character, thus implying the persistence of the recipient Gal- gene, with the result that the transducants are diploid for the Gal region (heterogenotes). This is in contrast to what normally happens in generalized transduction in which the transduced marker replaces the recipient allele. It was found that induction of these heterogenotes produces fewer phage particles than would be expected but almost all the particles have transducing activity. These results indicate that in the original lysogenic donor population rare phages arise that have incorporated the Gal+ region of the bacterial chromosome into their own genome and after transduction the phage and Gal+ gene remain associated in the heterogenote and are released together on induction giving high frequency transducing lysates. Later work showed that the integration of the Gal gene into the lambda genome results in a loss of phage function giving defective phage particles. Other inducible prophages which are situated close to the Gal region of Esch. coli are also able to transduce

this region and an inducible prophage closely linked to the tryptophan region has been reported to transduce it specifically. It would seem, therefore, that all inducible prophages that have a definite locus on the bacterial chromosome are potentially able to transduce neighbouring bacterial genes; that is, they can take part in restricted transduction.

These observations outline the essential difference between restricted and generalized transduction. In restricted transduction the transducible markers are situated close to the location of the prophage on the bacterial chromosome and become physically associated with a defective phage genome. This indicates a degree of homology between the phage and the bacterial host. In generalized transduction any bacterial marker may be picked up by the phage during lytic multiplication and may be transduced. Such a transduced marker is free of residual phage genome when it is released in the recipient cell. Thus there is no evidence of homology between the phage and the bacterial genome in this system.

Before transduction in staphylococci is reviewed a summary of the main features of the mechanisms of genetic transfer is given in Table 1.

TABLE 1

Mechanisms of genetic transfer

Process	Unit transferred	Agent of transfer	Cellular contact between donor and recipient bacterial cells required for transfer	Transfer inhibited by	
				DNase	Antiphage serum
Transformation	Small chromosomal fragments (free DNA)	None	No	Yes	No
Conjugation	Large chromosomal fragments Cytoplasmic elements	None	Yes	No	No
Transduction	Small chromosomal fragments Cytoplasmic elements	Phage	No	No	Yes
Phage Conversion	Phage chromosome	Phage	-*	No	Yes

* In phage conversion, transfer of genes from one bacterial cell to another does not take place. A phage chromosome enters the bacterial cell and changes the bacterial genotype.

Transduction in Staphylococcus aureus

Although the first report of transduction was published in 1952 by Zinder and Lederberg it was not until six years later that the phenomenon was described in Staph. aureus. In a communication given to the Society of American Bacteriologists in 1958 Ritz and Baldwin claimed to have transduced penicillinase production to a penicillin-sensitive strain of Staph. aureus by means of a typing phage (52) that had been propagated on a donor strain which produced penicillinase. The transducants were found to retain the biochemical properties and phage type of the recipient strain but became as resistant to penicillin as the donor strain. Ritz and Baldwin (1961) also reported similar findings when they propagated phage 80 on a penicillinase-producing strain of Staph. aureus (U-40) which was lysed by phages 80 and 81 and mutants of phages 42B, 47C, 44A and 52. Exposure of penicillin-sensitive staphylococci to filtrates of phage 80 propagated on this strain yielded a small number of penicillin-resistant transducants. These colonies were recognised on penicillin agar by their large size and the presence of a halo of small penicillin-sensitive colonies round each. It seemed, therefore, that inactivation of the

penicillin in the selective medium was taking place round each transduced colony. No confirmatory tests for penicillinase production were recorded in this paper. These workers drew attention to the importance of the amount of penicillin in the selective medium (0.12 units per ml). They found that if this is reduced undesirable background growth results; if it is even slightly increased there is a marked reduction in detectable transducants. Successful transductions were also reported using three of the mutant phages that lyse the donor strain but all attempts using phage 81 were unsuccessful. Nineteen penicillin-sensitive strains were tested as possible recipients in transduction experiments with phages 80 and 52 and four were found to acquire the penicillinase marker. These four strains were lysed by Group I typing phages whereas most of the strains that were not transduced were lysed by Group III phages. These observations indicate that a close relation between the donor and recipient strain may be necessary for transduction to take place. The authors suggested that the transduction of penicillinase production may well take place among mixed populations of staphylococci in nature and this may explain the emergence of penicillin-resistant strains.

Morse (1959) used a different system for the production of the transducing phage and he chose different antibiotic-resistance markers for transfer. He selected streptomycin or novobiocin-resistant mutants of the phage propagating strain 53 and he lysogenized them with phage 53. These artifically lysogenized donor strains were then induced by ultraviolet light and the resulting phage suspensions were used in attempts to transduce streptomycin or novobiocin resistance to the antibiotic-sensitive parent strain. Resistance to both of these antibiotics was transduced but it was found necessary to make allowance for four to six hours phenotypic delay in expression of the transduced genes that determined streptomycin resistance; in contrast 50 per cent of the genes for novobiocin resistance were shown to obtain phenotypic expression immediately. The frequency of transduction of these markers is slightly lower than that reported in the phage P22-Salmonella system as only one in 10^{7} -10^{8} recipient cells was found to be transduced. No linkage was found between the two resistance markers. Although the multiplicity of infection in these experiments was stated to be one phage or less per cell so that the transduced cells should have been infected with one phage only, many of these transducants were

found to be lysogenic. This is not in keeping with the observations on generalized transduction in the gram-negative bacilli.

Pattee and Baldwin (1960, 1961, 1962) confirmed the transduction of novobiocin resistance and penicillinase production in Staph. aureus. They also transduced resistance to chlortetracycline and to certain macrolide antibiotics. As before phage 80 propagated on the appropriate donor strain was used in the majority of the experiments though phages 29, 52A, 79 and 53 were also shown to have transducing ability. Phage 81 failed to transduce any of these markers; a result in keeping with the findings of Ritz and Baldwin (1961) when penicillinase production was the only marker tested. The recipient strains were found to be lysed by Group I phages or phage 81 and, therefore, in many cases were sensitive to the transducing phage. However some positive results were obtained in systems where the recipient strain was not lysed by the transducing phage. These results showed that the ability of a recipient strain to be transduced does not depend on its sensitivity to the transducing phage. In systems in which the recipient strain was sensitive to the transducing phage the authors found that transducants were not

recovered unless superinfection was prevented. This was done by preparing the selective medium with brain heart infusion broth. In contrast to the high proportion of lysogenized transducants that Morse reported, Pattee and Baldwin found that the transducing phages they used did not lysogenize the recipient strains. They did not detect linkage between the markers for novobiocin resistance, chlortetracycline resistance or penicillinase production. In transduction of resistance to the macrolide antibiotics two related markers could be distinguished; one controls an inducible enzyme system which is specifically induced by erythromycin and determines resistance to erythromycin, oleandomycin, spiramycin and carbomycin. The other marker determines resistance to the same group of antibiotics but shows no evidence of being dependent on an inducible mechanism. The frequency of transduction of resistance to the macrolide antibiotics to certain recipient strains was very much lower than the frequency of transduction of the other markers tested. The authors put forward the theory that the chromosomal region where these markers are sited may not be genetically compatible in all strains of

staphylococci thus accounting for the variation in their results.

In 1961 Edgar and Stocker grew phage 53 and other serologically related phages on their respective propagating strains which were not nutritionally exacting. With these phage preparations they were able to transduce the non-exacting character to all threonine-requiring strains that they tested and to some strains that required tryptophan, tyrosine, alanine, lysine or hypoxanthine. Linkage was shown between loci for successive steps in biosynthetic pathways. They did not observe any minute colonies which would have indicated that abortive transduction had taken place.

Using the same method as Morse for the preparation of transducing phage, Korman and Berman (1962) confirmed the transduction of streptomycin resistance and the need to allow for phenotypic expression of that marker. They succeeded in transducing the markers for lactose, mannitol, or maltose fermentation. They also claimed that coagulase negative mutants of Staph. aureus are amenable to transduction of the coagulase positive character but difficulties were encountered in selecting the coagulase-positive transducants.

Among their streptomycin-resistant transducants a rare class of heterogeneous clones was found. It was suggested that the genetic fragment bearing the streptomycin resistance marker is not regularly integrated in the recipient cell. The stable resistant segregants derived from these clones are those in which integration of the locus for streptomycin has taken place.

The transducing ability of the lysates was increased by ultraviolet irradiation, a finding similar to that of Zinder (1953) who studied the transducing lysates of phage P22. This enhancement was found to be most marked for lysates derived from the heterogeneous clones. It would seem that irradiation increases the likelihood of integration of the transduced material.

In later papers Korman (1962, 1963) described the transduction of a pleiotropic carbohydrate locus and a locus governing coagulase production, reaction to mannitol and galactose assimilation. She suggested that the most likely explanation for such pleiotropy is an alteration in the structure or organisation of the cell wall.

These papers all describe features of transduction in Staph. aureus which indicate that the system resembles generalized transduction rather

than the type of restricted transduction reported in Esch. coli K-12. Such features include the transduction of a wide variety of markers and the production of transducing lysates by lytic growth of the phages on the donor strains as well as by induction of lysogenic donor strains.

The nature of staphylococcal phages that had been shown to have transducing ability was investigated by Dowell and Rosenblum (1962a). They drew attention to the fact that the typing phages used by previous workers in successful transduction experiments all belong to serological group B. An exception appeared to be the mutants of phages 42B and 47C; these phages belong to serological group A, but Ritz and Baldwin (1961) claimed that they possessed transducing ability. Dowell and Rosenblum (loc. cit.) examined the propagating strain of these phages (PS42B/47C) and found it carried a phage which was neutralized by B and F antisera; this phage had transducing ability. They suggested that the phage may well have been the mutant described by Ritz and Baldwin. Because of the strong reaction of this phage with F antiserum Dowell and Rosenblum tested two typing phages belonging to group F, (42D and 77) for transducing ability. Positive results were

obtained only with phage 42D, a serological variant of a phage originally belonging to group B. In addition four typing phages, 54, 81, 3A and 3C belonging to group A and five typing phages, 44, 31, 53, 42C and 80 belonging to group B were all tested for ability to transduce novobiocin resistance. Only phages 53 and 80 gave positive results. The other group B phages which gave negative results were found to be atypical in their calcium requirements and were able to lyse cells on brain heart infusion agar. Ritz and Baldwin emphasised the need to inhibit lysis of recipient cells sensitive to the transducing phage and it may be of significance that lysis was not inhibited with these atypical group B phages. Dowell and Rosenblum (1962a) then induced novobiocin-resistant mutants of 24 staphylococcal strains representing all the phage-typing groups and they screened the lysates for ability to transduce novobiocin resistance to 85 novobiocin-sensitive staphylococci. Two of these lysates were found to have transducing ability and this was markedly reduced by treatment with group B antiserum. All these results indicate that transducing ability is associated with staphylococcal phages belonging to serological group B or the closely related group F.

In their second paper Dowell and Rosenblum (1962b) investigated the ability of transducing phage particles to lysogenize the recipient cells. They found that if superinfection is prevented lysogenization of the transducants does not take place. This suggests that, as in the systems examined in the gram-negative bacilli, transducing phages are defective. It seems probable that the large number of lysogenized transducants obtained by Morse (1959) can be accounted for by a failure to prevent superinfection.

The transduction of other antibiotic resistance markers was reported by several groups of workers who also investigated the incidence of strains that can act as recipients. Kuwahara and his colleagues (1963) used phages 80 or 81 propagated on staphylococci of phage type 80/81 or 81 that were resistant to tetracycline and erythromycin. Both these phages were found to transduce tetracycline and erythromycin resistance. The tetracycline marker was transduced to 50-70 per cent of the 165 recipient strains tested. Positive results were given by strains representative of all phage groups although the majority of strains which gave negative results were non-typable or belonged to Group II. The erythromycin marker was transduced to only

10-17 per cent of the strains and no correlation was found between ability to act as recipient for this marker and phage type. There were two unusual features about these results. Firstly other workers have been unsuccessful when attempting transduction with phage 81. The authors record that the phage 81 used in their experiments was obtained from a Japanese source. It is possible that this phage differs from the phage 81 used by the American workers and it would be interesting to know its serological group. The other unexpected finding is the ability of Group II strains to act as recipients. The transduction frequencies are not given for any individual Group II strains but in some experiments the authors accept the finding of a single colony on a selective plate as proof that transduction took place.

Collins and Roy (1963) transduced chloramphenicol resistance with phages induced from two lysogenic, antibiotic-resistant strains of staphylococci. The phages differed from each other in that one was induced from a Group III strain and it transduced recipient strains of that phage group but failed to transduce strains of Groups I and II. The other phage was induced from a donor of type 80/81/82 and was most active in transducing other

Group I strains. The results again indicate that a close relation is required between donor and recipient strains for transduction to take place although the recipient strain does not have to be sensitive to the transducing phage. These authors did not find any linkage between the markers for chloramphenicol and tetracycline resistance. Experiments were also carried out to demonstrate the transduction of novobiocin resistance but the results were difficult to interpret because of the natural emergence of novobiocin-resistant mutants. The work of Morse (1959) was confirmed but in four other phage-staphylococcus systems transduction of this marker could not be demonstrated satisfactorily.

In 1963 Novick used transduction to analyse mutations affecting penicillinase production in Staph. aureus. He found that reversion to penicillinase production does not take place with penicillinase-negative mutants isolated from a strain of Staph. aureus inducible for penicillinase or with naturally occurring penicillinase-negative strains. He used transduction experiments to show that crosses between these penicillinase-negative mutants do not yield penicillinase-positive

recombinants; neither do transductional crosses between these mutants and strains with point mutations in the penicillinase region yield penicillinase-positive recombinants. Unlike the transducing ability of phage lysates for other markers in staphylococci which is stimulated by ultraviolet irradiation, the penicillinase-transducing activity is reduced exponentially by irradiation. These findings and the high frequency of spontaneous loss of the ability to produce penicillinase (Barber 1949) have given rise to the theory that the determinants of penicillinase production in Staph. aureus are sited on an extrachromosomal fragment. Against this theory is Novick's own observation that treatment with acridine orange does not increase the rate of loss of the determinant controlling penicillinase production. Acridine orange treatment has been shown to eliminate extrachromosomal factors from gram-negative bacilli.

Mitsuhashi, Morimura, Kono and Oshima (1963) found that the resistance to erythromycin and the other macrolide antibiotics which they were able to transduce with phage 80 or 81, can be eliminated by acriflavine and they suggested that it is controlled by a cytoplasmic element. The penicillin resistance of their strains was unaffected

by this treatment. However, in 1964 Harmon and Baldwin reported the elimination of the marker for penicillinase production from certain strains of Staph. aureus but admitted that the frequency of elimination is low compared to that reported by Hirota (1960) for elimination of the F factor. They obtained the highest rates of elimination when the pH of the medium was adjusted to 7.6 and when acridine orange was used in preference to proflavine. Their findings were supported by Hashimoto, Kono and Mitsuhashi (1964) who claimed that acriflavine treatment eliminated the marker for penicillinase from 17 out of 18 strains tested but again the frequency of elimination, 0.1-3.5 per cent, was low. Some colonies were noted that had lost resistance to the macrolide group as well as loss of the ability to produce penicillinase. Further studies by these Japanese workers (Mitsuhashi, Hashimoto, Kono and Morimura 1965) confirmed that penicillinase production and resistance to the macrolide antibiotics are jointly eliminated from seme strains of staphylococci by treatment with acriflavine. Using phage lysates prepared by ultraviolet irradiation of antibiotic resistant lysogenic donor strains they found that joint transduction of these markers also takes place. Mitsuhashi, Oshima, Kawaharada and Hashimoto (1965)

transduced tetracycline resistance with phage lysates obtained by ultraviolet irradiation of 15 resistant donor strains. Apart from three exceptions which were non-typable, these donor strains all belonged to phage group I. The strains that were transduced included several Group II strains but the frequency of transduction to these Group II recipient strains appeared to be very low and the transducants lost their sensitivity to Group II phages and became non-typable. There were minor changes in the phage types of other transducants comprising a loss of one or two reactions and it was suggested that these changes may be caused by lysogenization of the recipient.

A character associated with the antibiotic-resistant staphylococci so often responsible for epidemics of infection is resistance to mercuric chloride (Moore 1960), and this marker was shown to be most closely correlated with high penicillinase production (Richmond, Parker, Jevons and John 1964). Joint transduction of penicillinase production and mercuric chloride resistance was demonstrated by Richmond and John (1964); in every mercury resistant strain of Staph. aureus that they examined, the loss of ability to produce penicillinase was accompanied by the loss

of mercury resistance. These results all indicate that the penicillinase marker and the mercury resistance marker are closely linked.

Novick and Richmond (1965) have investigated the genetic elements controlling the production of penicillinase in Staph. aureus. They have confirmed that all of the genetic information required for penicillinase synthesis is carried on an extrachromosomal element. As we have no evidence at present that there is integration of this element with the staphylococcal chromosome, it is best referred to as a plasmid (Lederberg 1952). A number of related but genetically distinct plasmids have been described by Novick and Richmond. All carry the penicillinase locus but differ in the other markers present which may control the degree of extracellularity of the enzyme, resistance to mercuric chloride, or, rarely, resistance to the macrolide group of antibiotics. The ability of any two plasmids to co-exist in one bacterial cell was investigated by transduction experiments. Each of the plasmids in staphylococci of phage groups I and III examined in this way can be placed in one of two groups. A plasmid is incompatible with all members of its own group but is compatible with

members of the other group. Novick and Richmond examined three plasmids in detail and found that two belonged to compatibility group I and one to compatibility group II. Several other naturally occurring plasmids were examined by Richmond (1965a) and all were assigned to compatibility group I. Richmond (1965b) found that the penicillinases of different staphylococci can be differentiated into three types (A, B or C) on the basis of serological and enzymic criteria. Penicillinase type B is synthesized only by phage group II staphylococci and plasmids carrying this marker may form a third compatability group. Attempts to transduce penicillinase genes from phage group II strains to phage group I or III strains have been unsuccessful. Therefore it has not been possible to determine the compatibility of plasmids from phage group II staphylococci with plasmids known to be members of compatibility groups I or II.

It is clear that transduction is now being widely used to investigate the genetics of Staph. aureus, particularly in relation to studies of the cytoplasmic elements, the analysis of chromosomal regions (Kloos and Pattee 1965) and the elucidation of control mechanisms of such enzymes as penicillinase (Richmond 1965c). However there

is also considerable interest in the part that transduction and lysogenization may play in the spread of staphylococci with multiple antibiotic resistance that have a limited range of phage-typing patterns.

MATERIALS AND METHODS

Organisms

For the early part of this work, tetracycline-sensitive and tetracycline-resistant strains of *Staphylococcus aureus* were chosen from a stock collection in the Bacteriology Department, Hospital for Sick Children, Toronto. These included the propagating strains of the International Typing Series and other strains isolated from diagnostic specimens. Later, when a greater variety of organisms was required, strains representing the main phage-typing groups were selected from clinical sources in Scotland. Another collection of strains which were not associated with the hospital environment was made by culturing nasal swabs from science and pre-clinical medical students in Edinburgh. A small number of phage type 71 strains were kindly supplied by Dr. M.P. Jevons of the Cross-infection Reference Laboratory, Colindale, London.

Culture media

In early experiments trypticase soy agar (Baltimore Biological Laboratories) was used for poured plates; brain heart infusion broth (Baltimore Biological Laboratories) was routinely used as the fluid medium and the 0.7 per cent agar medium was nutrient agar (Difco) dissolved in brain heart infusion

broth. In later work tryptone soya agar (Oxoid) or brain heart infusion agar (brain heart infusion broth, Difco, solidified with 1.2 per cent Davis agar) was used for poured plates and Oxoid no.2 nutrient broth or brain heart infusion broth (Difco) was the fluid medium.

Antibiotic solutions

The antibiotics were dissolved in distilled water to give stock solutions of the following concentrations; streptomycin, tetracycline and chloramphenicol 2 x 10^3 μg per ml and penicillin 10^3 μg per ml. The solutions were stored frozen in 5 ml volumes.

Antibiotic sensitivity tests

Standard drops of 18-hour broth cultures were spot-inoculated with a fine needle on to freshly prepared agar plates containing doubling dilutions of the antibiotic. The plates were examined for the presence or absence of confluent growth in the drop area after incubation at 37°C for 24 hr.

Mercuric chloride resistance tests

The method described by Moore (1960) was used but each test strain was spotted on to a series of peptone agar plates containing different concentrations of mercuric chloride. The concentrations were 1 in 27,500, 1 in 38,020 and

1 in 61,350. A mercury-sensitive and a mercury-resistant strain were inoculated on each plate and the readings were taken from the plate on which these control strains gave the correct result. The controls were necessary as minor variation between batches of medium influenced the results.

Tests of natural lysogenicity

Supernatant fluids of centrifuged broth cultures of tetracycline-resistant staphylococci were inoculated as drops on to plates that had been flooded with inocula of tetracycline-sensitive strains. The plates were incubated overnight at 30°C and they were then examined for plaque formation.

Artificial lysogenization

A typing phage active on a streptomycin-resistant staphylococcus was dropped on to a plate flooded with this strain and, after incubation, phage-resistant colonies were picked from the area of lysis. Lysogenic colonies were selected by tests of their ability to lyse the homologous phage-propagating staphylococcus.

Induction

The naturally lysogenic and artificially lysogenized donor cultures were induced by ultra-

violet irradiation. Cultures were grown in broth at 37°C for 18 hr and were mechanically shaken during the last 3 hr to reduce clumping of the cells. After centrifugation and resuspension in an equal volume of physiological saline, 8-ml amounts were pipetted into petri dishes. The open plates were gently agitated for periods of 20-40 sec at a distance of 56 cm from an ultraviolet lamp (a 30 watt General Electric germicidal lamp emitting rays of wavelength 254 mμ). The suspensions were centrifuged, resuspended in broth and incubated on a shaker at 37°C for 4 hr. They were then refrigerated overnight, and they were finally filtered through sintered glass filters with a maximum pore size of 1.4 μ. Before use, the sterility of the filtrates was proved by culture.

Propagation of phages

Phages obtained by the induction of lysogenic strains were later propagated on tetracycline-resistant strains. One ml of a 3-hour broth culture of the propagating strain and 1 ml of a suspension of the phage to be propagated, titre 10^5 particles per ml were inoculated into 100 ml of nutrient broth to which 1 ml of $CaCl_2$ had been added. This mixture was incubated at 37°C overnight, and it was then filtered through a

sintered glass filter. The sterility of these filtrates was proved by culture.

Phage titrations

These were performed by the Gratia agar layer method (Gratia 1936). The phage-sensitive staphylococcus and a measured amount of the phage dilution were mixed with 2 ml of warm 0.7 per cent agar at 46°C. The mixture was poured over the surface of an agar plate and allowed to solidify to form a thin layer. The bacteria grew in this layer and the phage plaques appeared as clear areas in the opaque layer of bacterial growth. Plaque counts were made after overnight incubation at 30°C. Only filtrates with a titre of more than 10^7 plaque forming units per ml (p.f.u. per ml) were used for transduction, although difficulty was sometimes experienced in attaining this titre.

Phage-typing

Phage-typing of the donor and recipient strains was carried out as described by Williams and Rippon (1952). The typing phages used in Canada were supplied by Dr.E.T.Bynoe, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa. The phages used in Scotland were supplied to the department in Edinburgh by the Public Health Reference Laboratory, Colindale, London.

Transduction

The recipient staphylococci were chosen from strains that were sensitive to the appropriate antibiotic and, in the early experiments, sensitive to the phage induced from the lysogenic strain. Standard cultures of the recipient strains were prepared by adding 2.5 ml of an 18-hour broth culture to 50 ml broth and shaking at 37°C for 3 hr. These cultures contained 10^9 viable bacteria per ml. One ml of a standard culture of the recipient strain was mixed with 0.5 ml of phage filtrate to give a mixture containing slightly fewer phage particles than bacteria. The mixture was made up to 5 ml with broth. A control mixture of an equal number of staphylococci in broth without phage was also set up. In several experiments, an additional control mixture was prepared in which deoxyribonuclease (20 μg per ml), obtained from the Nutritional Biochemicals Corporation, was added to the staphylococcus-phage mixture. The mixtures were incubated at 37°C for 30 min to allow adsorption of phage.

The test. Aliquots (0.5 ml) of these mixtures were mixed with 2 ml of 0.7 per cent agar at 46°C and layered over agar plates. When firm, a second 2 ml layer of 0.7 per cent agar containing the

appropriate antibiotic was poured over the plate. In the preliminary attempts to transduce streptomycin resistance a concentration of 100 μg per ml of streptomycin was used in the second layer. This concentration did not inhibit all the untransduced cells of the recipient strain. In the other experiments the concentration of antibiotic in the second layer, tetracycline 50 μg per ml or chloramphenicol 100 μg per ml, was such as would allow the donor strains to grow but these concentrations inhibited the growth of the untransduced cells of the recipient strains. When it became apparent that no allowance need be made for phenotypic delay in the expression of tetracycline resistance this antibiotic was incorporated into the base layer of agar. At first the same concentration of tetracycline was used as in the overlay i.e. 50 μg per ml but this was later reduced to 25 or 12.5 μg per ml; all of these concentrations prevented growth of the untransduced recipient cells. In each early experiment nine control plates and nine transduction plates were used, but the number of plates was reduced in later experiments to one control plage and four transduction plates. All plates were incubated at 37°C for 48 hr. The colonies on the transduction plates were counted and in certain

experiments 100 colonies were subcultured to antibiotic-free plates. Later, transfers of these colonies were made to broth for testing of antibiotic sensitivity and lysogenicity.

Transduction of penicillinase production

A different method was used to demonstrate the transduction of penicillinase production. A 0.5 ml volume of a standard culture of the recipient strain was mixed with 1 ml of the phage filtrate. The mixture was made up to 2.5 ml with nutrient broth. A control mixture of an equal number of staphylococci in broth without phage was set up. These mixtures were incubated on a shaker at 37°C for 1 hr to allow adsorption of the phage to take place. Then the mixtures were centrifuged and the deposit in each case was resuspended in 1 ml of brain heart infusion broth. Aliquots (0.2 ml) of these suspensions were spread over the surface of brain heart infusion agar plates containing 0.06 μg or 0.12 μg of penicillin per ml. The plates were incubated at 37°C for 48 hr. The colonies on the transduction plates were counted and a number from each experiment were subcultured to penicillin-free plates. Later transfers of these colonies were made to broth for testing of antibiotic sensitivity and resistance to mercuric chloride.

Preparation of phage antiserum

Two phages, which had transduced tetracycline resistance, were propagated on tetracycline-sensitive staphylococci. Each phage was propagated three times, the inoculum for the second and third propagation being a suitably diluted sample from the previous propagation. The phage suspensions were then titrated and filtered through sintered glass filters. The filtrates were shown to have no residual transducing ability although each had a titre of more than 10^8 p.f.u. per ml.

Each filtrate was then used to immunize two rabbits; the dose was 1 ml injected intravenously and repeated three weeks later. The rabbits were bled one month after the second injection.

Effect of phage antiserum on plaque-forming titres of phages

Dilutions of each phage antiserum were incubated with a suspension of each transducing phage for 4 hr at 37°C. The plaque-forming titres of the phage suspensions treated in this way were compared with the titres of control phage suspensions which had been incubated alone for 4 hr at 37°C. The effect of each phage antiserum on the plaque-forming titres of typing phages 6, 52A and 77 were tested in the same way. These typing phages were selected as

representatives of the serological groups A, B and F.

Effect of phage antiserum on the transduction of tetracycline resistance

The effect of phage antiserum and normal rabbit serum on the transduction process was tested by incubating 1 ml of the filtrate of the transducing phage for 1 hr at 37°C with the phage antiserum or with normal rabbit serum. The transduction experiments were then carried out in the usual way.

The lytic spectra of the transducing phages

The lytic spectrum of each transducing phage was determined as described by Blair and Williams (1961). This method is used to check the lytic spectra of the typing phages. The lytic activity of each typing phage on its propagating staphylococcus is compared with its activity on a series of other indicator strains; in this way each typing phage is characterized by a particular pattern of reactions. Neither of the transducing phages used in the present work had a recognised propagating strain of staphylococcus. Therefore the indicator strain on which each phage had shown its highest plaque-forming titre was regarded as the propagating strain for that phage. The lytic activity of each transducing phage on its

"propagating strain" was then compared with its lytic activity on the series of other indicator strains.

Preparation of transducing phages for electronmicroscopy

The phage filtrate was spun for 10 min at 10,000 rev/min (12,000G) on a Spinco preparative ultracentrifuge with a swing-out head. The supernatant fluid was then spun for 2 hr at 20,000 rev/min (50,000G). After this centrifugation, the supernatant fluid was discarded and the deposit was resuspended in distilled water and spun again for 2 hr at 20,000 rev/min. After the final centrifugation the deposit was mixed with a few drops of 2 per cent phosphotungstic acid at pH 7.0 and examined on an A.E.I. (Em 6) electron microscope using a carbon collodion membrane.

A second filtrate was treated in the same way but 0.1 M ammonium acetate was used for resuspending the deposit instead of distilled water.

Testing of transduced organisms

Resistance to antibiotics and mercuric chloride were measured as described above.

Lysogenicity was demonstrated as follows: the ability of the transduced strain to produce lysis

of the parent recipient strain was shown by spotting the supernatant fluid of an 18-hour broth culture on to a plate flooded with the parent strain, and the resistance of the transduced strain to lysis by the transducing phage was determined by spotting a drop of this phage on to a plate flooded with cells from the transduced colony.

Phage-typing of colonies from early transduction experiments was carried out as described above.

Penicillinase production was demonstrated by a modification of the screening method described by Foley and Perret (1962). Each transduced colony to be tested was subcultured on to a tryptone soya agar plate and incubated for 18 hr at 37°C. Single colonies were emulsified in 0.2 ml of a freshly prepared solution containing 10,000 units of benzyl penicillin per ml in 0.05 M phosphate buffer at pH 6.5 to give a faintly opalescent suspension. These suspensions were left for 1 hr at room temperature. One drop (about 0.02 ml) of a freshly prepared mixture composed of equal parts of 1 per cent starch solution and 0.04 M iodine was mixed with the contents of each tube and the time of disappearance of the blue colour was noted. Control cultures of a penicillinase-producing staphylococcus and a penicillin-sensitive

strain were included with each batch of tests. Positive results were recorded if the indicator solution was decolourized within 45 min provided the negative control test remained coloured.

Acridine orange treatment of transduced organisms

Acridine orange (George T. Gurr Ltd.) was dissolved in water to give a stock solution of 500 μg per ml. This was stored at room temperature in the dark and replaced by a fresh solution after 1 week. For use, the stock solution was diluted with nutrient broth pH 7.6 to give a final concentration of 10 μg per ml. A 5 ml volume of the diluted acridine orange solution was inoculated with 0.1 ml of a diluted 18-hour broth culture of the test strain; the culture was diluted 1 in 2 with sterile nutrient broth at pH 7.6. A control culture in 5 ml of nutrient broth at pH 7.6 without acridine orange was set up in the same way. These cultures were incubated at 37°C for 18 hr. Then each culture was diluted 1 in 10^6 in nutrient broth and 0.2 ml portions were spread on tryptone soya agar plates. The plates were incubated at 37°C for 18 hr and a representative number of the colonies were subcultured. Each colony was inoculated on to a

plate of tryptone soya agar containing 100 μg per ml of penicillin or 50 μg per ml of tetracycline and then on to a plate of antibiotic-free medium to confirm that failure to grow on the first plate was caused by the antibiotic.

Ultraviolet irradiation of transducing phage AK72

The filtrate was centrifuged as described for the EM studies for 1½ hr at 39,000 rev/min (125,000G). The supernatant fluid was discarded and the deposit was resuspended in physiological saline. The saline suspension was irradiated for periods of 30-180 sec in a petri dish as described previously in the subsection on induction.

Samples were withdrawn during irradiation and the plaque-forming titre and transducing titre of the phage was estimated.

EXPERIMENTAL OBSERVATIONS

A preliminary attempt to demonstrate transduction in staphylococci

Transduction of streptomycin resistance to Staphylococcus aureus was attempted with a system similar to that described by Morse (1959). A streptomycin-resistant mutant of the propagating strain 53 was lysogenized with its homologous phage, induced by ultraviolet irradiation and filtered. When this phage filtrate was used to transduce streptomycin resistance to the parent strain, PS53, a large number of streptomycin-resistant mutants appeared spontaneously on the control plates. It was impossible to determine if the organisms that became resistant to streptomycin after exposure to the phage had been transduced or if they were spontaneous streptomycin-resistant mutants.

While useful experience had been gained during this preliminary work it had not been possible to reproduce the work of Morse and the model was considered unsuitable for further study. It was decided to prepare the transducing phage for the next experiments by the induction of naturally lysogenic staphylococci and tetracycline resistance was selected as the character for possible transduction.

Induction of phage from naturally lysogenic staphylococci

Forty tetracycline-resistant staphylococci isolated from clinical sources were examined for lysogenicity. The indicator staphylococci for these experiments were chosen from the propagating strains of the International Typing Series and from tetracycline-sensitive strains isolated from clinical sources. Twenty-one of the 40 tetracycline-resistant staphylococci carried phages which lysed one or more of the indicator strains. Induction of 12 of these lysogenic cultures was carried out by ultraviolet irradiation. Further study of two of the lysates was undertaken by a colleague and the results of these investigations are not included in the present work. The other ten lysates were filtered and their plaque-forming titres were estimated. Several titrations, using different tetracycline-sensitive indicator strains, were carried out for each filtrate. In some cases the titre varied according to the indicator strain that was used; the highest titre obtained for each filtrate and the relevant indicator strains are recorded in Table 2. Five of the phage filtrates had titres of less than 10^7 p.f.u. per ml and these filtrates were discarded. The others were retained for use in transduction experiments.

TABLE 2

Plaque-forming titres of filtrates of lysogenic staphylococci after induction by ultraviolet irradiation

Lysogenic strain	Indicator strain	Titre p.f.u. per ml*
AS36	AS11	$< 10^7$
AK72	74426	16×10^8
12386	PS6	3×10^7
14214	PS7	$< 10^7$
14161	13346	$< 10^7$
16487	12737	$< 10^7$
15844	13346	6×10^8
12888	360	2×10^9
14438	PS54	7×10^9
15494	PS82	$< 10^7$

*plaque-forming units per ml.

Transduction of tetracycline resistance

Transduction of tetracycline resistance was attempted with each of the five phage filtrates that had a titre of more than 10^7 p.f.u. per ml; these had been induced from donor strains that were resistant to more than 50 μg of tetracycline per ml.

The sterility of the phage filtrates was proved by culture before they were used in transduction experiments. The recipient strains were staphylococci that had been previously used as indicators of lysogenicity and they were known to be sensitive to less than 6 μg of tetracycline per ml.

In experiments with four of the phage filtrates the transduction mixtures consisted of 0.5 ml of the filtrate, 1 ml of a 3-hour culture of the recipient strain and 3.5 ml of broth. The titre of one phage, that induced from donor strain 12386, was only 3×10^7 p.f.u. per ml. Therefore the transduction mixture for this system was made up with 4 ml of the filtrate and 1 ml of a 3-hour culture of the recipient strain. Thus all of the transduction mixtures contained over 10^8 phage particles. In each experiment, transduction was demonstrated by observing the control plates containing only the recipient staphylococcus in comparison with the plates containing the staphylococcus-phage mixture. After incubation at 37°C no colonies of staphylococci were seen on the control plates where the tetracycline agar had been layered over the tetracycline-sensitive recipient strain alone. In contrast, many colonies grew through the antibiotic layer on the transduction

plates which contained the staphylococcus-phage mixture (Plate 1). Early experiments included plates that were set up to allow for phenotypic delay in expression of the transduced character. The tetracycline agar layer was added to these plates after they had been incubated at 37°C for 4 hr. There was no difference between the results obtained with plates on which the tetracycline agar had been layered immediately and the results from plates which had been incubated for 4 hr before the addition of the tetracycline agar. This indicated that there was no delay in the phenotypic expression of tetracycline resistance and in later experiments the tetracycline agar was added immediately to all plates.

Two of the phages were each shown to transduce tetracycline resistance to two different recipient strains. Phage AK72 transduced the marker to strain 74426 and to strain 12861. Phage 15844 transduced the marker to strain 13346 and to strain PS47. The transduction of PS47 by phage 15844 yielded fewer transducants than were recovered from the other systems. When the amount of tetracycline in the overlay was reduced from 50 μg per ml to 25 μg per ml the number of transducants

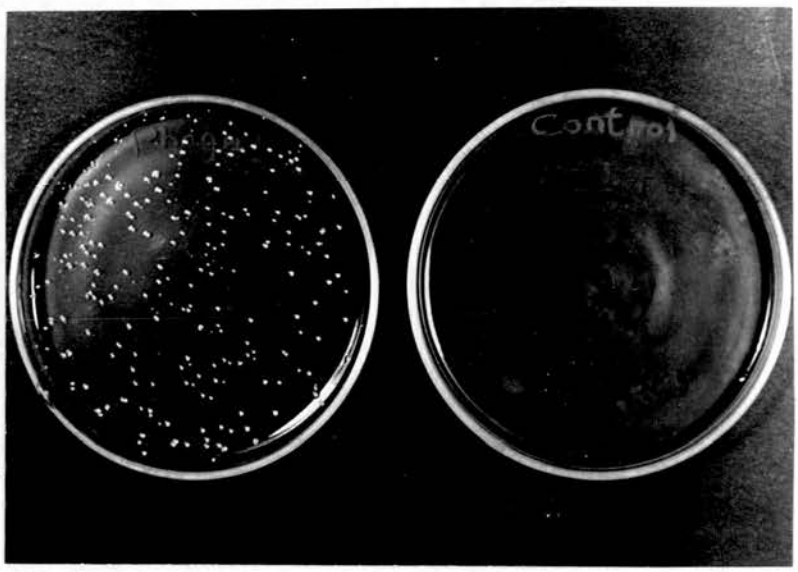


Plate 1. Transduction of tetracycline resistance

Left: a transduction plate seeded with the staphylococcus-phage mixture. Many colonies have grown through the tetracycline agar.

Right: a control plate seeded with the recipient staphylococcus alone. Growth has been completely inhibited by the tetracycline agar.

increased although the recipient strain on the control plates was still completely inhibited. The other phage filtrates did not show any transducing ability with the recipient strains tested (Table 3).

TABLE 3

Transduction of tetracycline resistance by phages induced from lysogenic donor strains of staphylococci

Donor strain	Recipient strain	Transduction
AK72	74426	+
	12861	+
12386	PS6	-
	360	-
15844	13346	+
	PS47	+
12888	360	-
	12737	-
14438	PS7	-
	PS54	-

These transductions were all repeated several times with similar results. After transduction the recipient strains were found to be resistant to more than 50 μg of tetracycline per ml. Before transduction they had been sensitive to less than

6 μg per ml therefore their resistance had increased at least eight-fold as a result of transduction. The donor and recipient strains were phage-typed and, as can be seen from Table 4, there was some similarity between the phage types of the donor and recipient strains in each transduction system. These results also show that transduction was not restricted to one particular phage type or phage group of staphylococcus. Phage AK72 was induced from a donor strain of phage type 82 and transduced strain 74426, phage type 80/81+, and strain 12861, phage type 80. Thus in this system all the strains belong to Group I. Phage 15844 was induced from a donor strain of phage type 53/77+ and transduced strain 13346, phage type 79/53/77+, and strain PS47, phage type 47/75+. In this system all the strains have typing patterns belonging to Group III predominantly.

TABLE 4

Tetracycline sensitivities and phage types of donor and recipient strains in transduction experiments

Donor strain			Recipient strain			
No	Phage type	Inhibitory concentration of tetracycline (μg per ml)	No	Phage type	Inhibitory concentration of tetracycline (μg per ml)	
					before transduction	after transduction
AK72	82	>50	74426	80/81+	<6	>50
			12861	80	<6	>50
15844	53/77+	>50	13346	79/53/77+	<6	>50
			PS47	47/75+	<6	>50

The frequencies with which phage AK72 and phage 15844 transduced tetracycline resistance to each recipient strain are shown in Table 5. In each system the colonies on the transduction plates were counted and, from this figure the number of transducants present in the total 5 ml of the transduction mixture was calculated. As the number of phage particles was known and as these were mixed in each transduction mixture with a constant number of recipient staphylococci, the number of transducants yielded by 10^8 phage particles could be estimated for each recipient strain. The frequency of transduction for phage AK72 was similar for the two recipient strains tested, namely 289-426 transducants per 10^8 phage particles for strain 74426 and 279-478 transducants per 10^8 phage particles for strain 12861. However, the transducing activity of phage 15844 appeared to be less than that of phage AK72. With recipient strain 13346 the number of transducants recovered was 213-233 per 10^8 phage particles. With strain PS47 the number recovered was 410-821 per 10^8 phage particles, but in this system the tetracycline in the agar overlay had been reduced from 50 μg per ml to 25 μg per ml. These observations suggest that different phages vary in the frequency with which they can transduce

tetracycline resistance. It also appears that the frequency of expression of transduction is influenced by the amount of tetracycline in the selective medium.

TABLE 5

Frequency of transduction of tetracycline resistance by phage AK72 and phage 15844

Transducing phage	Recipient strain of staphylococcus	Transduction frequency*
AK72	74426	289 - 426
AK72	12861	279 - 478
15844	13346	213 - 233
15844	PS47	410 - 821+

*number of transducants per 10^8 phage particles

+transducants recovered from plates with 25 μg of tetracycline per ml in agar overlay.

The effect of various factors on the transduction of tetracycline resistance

The following investigations were carried out to confirm that the characteristics of the transfer

that was taking place in the experiments described above were consistent with those described for transduction.

Effect of heat

When the filtrates of phages AK72 and 15844 were heated at 60°C for 30 minutes, their plaque-forming titres fell from more than 10^8 p.f.u. per ml to 10^2 p.f.u. per ml. Attempts to transduce tetracycline resistance to the appropriate recipient strain with these heated phage filtrates were completely unsuccessful. Thus, the agent concerning was thermolabile at 60°C and its activity was related to the concentration of active phage.

Effect of deoxyribonuclease

DNase was added to the transduction mixtures to give a concentration of 20 μg per ml. The number of transducants recovered from these experiments was the same as the number recovered when DNase was not added to the transduction mixture. This indicates that free DNA was not the agent involved and that the mechanism was not one of transformation.

Propagation of phage AK72 and phage 15844 on tetracycline-resistant staphylococci

Staphylococci that were isolated from clinical sources and were resistant to more than 50 g of tetracycline per ml were tested for sensitivity to phages AK72 and 15844. Strain TR4, a staphylococcus of phage type 80/81, was chosen as the propagating strain for phage AK72. Strain TR8, a staphylococcus of phage type 6/7/47/53/75/77+ was chosen as the propagating strain for phage 15844. Propagation on these strains yielded phage filtrates with plaque-forming titres similar to the titres of the filtrates obtained by induction of the lysogenic donor strains. Both the propagated phages transduced tetracycline resistance to the appropriate recipient strain. This finding shows that the transducing ability of these phages does not depend on their location on the chromosome of the lysogenic donor strains; the phages are able to pick up the marker for tetracycline resistance during lytic multiplication. This may take place by propagation on a phage-sensitive strain or by induction of a lysogenic donor strain and is characteristic of generalized transduction.

Propagation of phage AK72 and phage 15844 on tetracycline-sensitive staphylococci

A tetracycline-sensitive strain 1/23, a staphylococcus of phage type 52/52A/80/81, was chosen as the propagating strain for phage AK72. Phage 15844 was propagated on the recipient strain 13346. Both these strains were sensitive to less than 6 μg of tetracycline per ml. Three serial propagations were carried out for each phage, and filtrates of both phages were obtained with plaque-forming titres of more than 10^8 p.f.u. per ml. When these filtrates were used in transduction experiments with recipient strains 74426 or 13346 no transducants were obtained. Thus the transducing ability of the phages is shown to depend on the presence of the marker for tetracycline resistance in the donor bacterium. The process described here is clearly not phage conversion; in phage conversion the phage genome alone is able to change the character of the host bacterium.

Effect of phage antiserum

The phage filtrates that were prepared by propagation on tetracycline-sensitive staphylococci and were shown to be without transducing ability were used to immunize rabbits

(see Methods). One ml of each phage antiserum was incubated for 1 hour at 37°C with 1 ml of the homologous transducing phage suspension. Then the transduction mixtures were made up as before with 1 ml of the antiserum-phage mixture replacing 0.5 ml of the phage filtrate included in previous experiments. No transducants were recovered from experiments in which the transducing phage had been treated with phage antiserum. If the transducing phages were treated with normal rabbit serum instead of phage antiserum, transducants were recovered in the usual numbers. The inhibitory effect of phage antiserum on the transfer of tetracycline resistance indicates that the marker is being carried by the phage particle. It might be argued that an unknown factor that is released with the phage from the donor bacterium at the time of lysis is responsible for the transfer of tetracycline resistance. This factor could have given rise to antibodies when the phage filtrate was used to immunize the rabbits. Thus the inhibitory effect of the antiserum could be produced not by its action on the phage but by its action on this factor. If such a theory were correct any phage filtrate shown to have no transducing effect would lack the factor. In the present experiment, therefore, a phage filtrate

without transducing ability was deliberately chosen as the antigen so that the above possibility was excluded.

These results are summarized in Table 6 and they confirm that the transfer of tetracycline resistance described in the present work is brought about by transduction.

TABLE 6

The effect of various treatments on the ability of phage AK72 and phage 15844 to transduce tetracycline resistance

Treatment of transducing phage	Transduction of tetracycline resistance	
	by phage AK72 to recipient strain 74426	by phage 15844 to recipient strain 13346
Exposure to 60°C for 30 minutes	-	-
Incubation with DNase	+	+
Propagation on tetracycline-resistant staphylococci	+	+
Propagation on tetracycline-sensitive staphylococci	-	-
Incubation with normal rabbit serum	+	+
Incubation with phage antiserum	-	-

Changes in the recipient organisms after transduction

One hundred transducants from two representative transductions of each recipient strain were subcultured for further investigation. Their resistance to tetracycline was tested and all were found to be resistant to more than 50 μg per ml. This included the PS47 transducants that had been recovered from plates where the agar overlay contained only 25 μg of tetracycline per ml. The increase in resistance of the transducants appeared to be a stable character since it was maintained through at least five subcultures in tetracycline-free medium.

Table 7 shows the results of testing the transducants for resistance to tetracycline, for ability to lyse their parent strain, and for resistance to lysis by the transducing phage. With recipient strains 74426, 12861 and 13346 more than half of the transducants were lysogenic, being both lytic for their parent strain and immune to lysis by the transducing phage. Most of the remainder of the transduced colonies from these systems were non-lysogenic, being non-lytic for the parent strain and sensitive to the transducing phage. A small number of 12861 transducants did not fall into either of these categories. These

colonies were either lytic for the parent strain and phage-sensitive, or non-lytic for the parent strain and phage-immune. The pattern of results with recipient strain PS47 was very different, the majority of these transducants lysed the parent strain and remained sensitive to the transducing phage. In all systems there was a variation in results when the transduction was repeated. It would seem from the above findings that the acquisition of tetracycline resistance by the recipient strains does not depend upon the establishment of lysogeny by the transducing phage.

TABLE 7

Characters of recipient strains of staphylococci after transduction of tetracycline resistance

Transducing phage	Recipient strain of staphylococcus	Percentage of transducants that were				
		resistant to 50 μg of tetracycline per ml.	lytic for parent strain and phage-immune+	lytic for parent strain but phage-sensitive	not lytic for parent strain but phage-immune	not lytic for parent strain and phage-sensitive*
AK72	74426	100	100	0	0	0
AK72	74426	100	78	0	0	22
AK72	12861	100	82	4	13	1
AK72	12861	100	50	6	12	32
15844	13346	100	44	0	0	56
15844	13346	100	72	0	3	25
15844	PS47	100	0	100	0	0
15844	PS47	100	13	71	5	11

\+ i.e. typically lysogenic

* i.e. typically non-lysogenic

Changes in the phage-typing patterns of the recipient strains after transduction

The phage type of a recipient strain might be expected to change as a result of transduction. To investigate this possibility phage-typing of the transduced colonies shown in Table 7 was carried out. The 178 lysogenic transducants of the recipient staphylococcal strain 74426 were found to be non-typable or to be lysed by phage 82 only. As the phage type of strain 74426 was 81/82+ these changes in typing pattern represented a loss in sensitivity to typing phage 81. In contrast, the 22 non-lysogenic transducants did not show this loss and were either phage type 80/81/82 or 81/82. The 116 lysogenic transducants of recipient strain 13346 were found to be lysed by Group III phages only. As the phage type of strain 13346 was 79/53/77+ this change represented a loss in sensitivity to the Group I typing phage 79. The 81 non-lysogenized transducants retained their sensitivity to both Group I and Group III phages. With recipient strain 12861, phage type 80, all the transducants were non-typable. This loss of sensitivity to typing phage 80 took place whether the transducants had been lysogenized or not. The transducants of PS47 gave such a variety of reactions with Group III phages that no general

conclusions could be drawn from the results in that system.

Thus the phage-typing patterns of recipient strains 74426, 13346 and 12861 were found to have altered after transduction. In the first two systems the changes in phage type were not found in all the transducants examined. When a change in phage type did take place in recipient strain 74426 or 13346 it was clearly associated with the lysogenization of the transducant by the transducing phage. The results found in the other system were quite different; every transducant of 12861 tested had become non-typable but the change was not always associated with lysogenization of the transducant by the transducing phage.

Transduction of resistance to penicillin

If the transfer process described here is an example of generalized transduction the transducing phages should be able to transfer a variety of markers. Since donor strain AK72 was resistant to penicillin as well as to tetracycline it was used in an attempt to transduce penicillin resistance to strain 2371. This recipient strain, a staphylococcus of phage type 29 at 1000 RTD, was sensitive to 0.06 μg of penicillin per ml. The

method that was selected to demonstrate the transduction of penicillin resistance differed in some aspects from that used to demonstrate the transduction of tetracycline resistance. The proportion of phage particles to recipient cells in the transduction mixture was increased and a longer period of incubation at 37°C was allowed for the adsorption of phage to take place. In preliminary experiments before these changes were made transduction was not observed. It was found that the transduced colonies in this system were more easily recognised if the transduction mixture was spread directly on to the surface of the selective medium. In previous transduction experiments the mixture had been poured on to the surface of the selective medium in an agar overlay.

After incubation at 37°C for 48 hr the control plates that contained 0.06 μg of penicillin per ml and had been inoculated with the penicillin-sensitive recipient strain alone, yielded a growth of small colonies; on the control plates containing 0.12 μg of penicillin per ml there was no growth. On the transduction plates containing 0.06 μg of penicillin per ml the transduced colonies could be distinguished from the background growth by their larger size and a surrounding halo of small penicillin-sensitive

colonies (Plate 2). On the transduction plates containing 0.12 μg of penicillin per ml there was no background growth and there were fewer penicillin-sensitive satellites round each transduced colony (Plate 3).

Frequency of transduction

The frequency of transduction of penicillin resistance to strain 2371 by phage AK72 was calculated. Twenty-nine transducants per 10^8 phage particles were recovered. This is a much lower frequency of transduction than the frequencies observed with the systems in which the marker for tetracycline resistance was transduced by phage AK72. The difference is greater than the variation in frequency noted when these experiments were repeated with the same systems. Thus not only does the frequency of transduction vary between different phages but it also varies when different markers are transduced.

The character of the transduced organisms

Fifty colonies were transferred to broth and tested for resistance to penicillin, mercuric chloride and tetracycline and for the ability to produce penicillinase. The organisms from 49 of these 50 colonies were found to have become

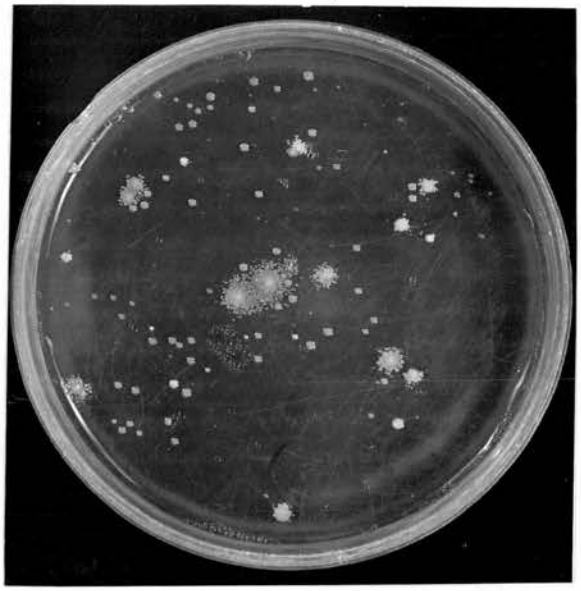


Plate 2. Transduction of penicillin resistance

A transduction plate, containing 0.06 μg of penicillin per ml of agar, on which the transduced colonies can be distinguished from the background growth by their larger size and haloes of penicillin-sensitive colonies.

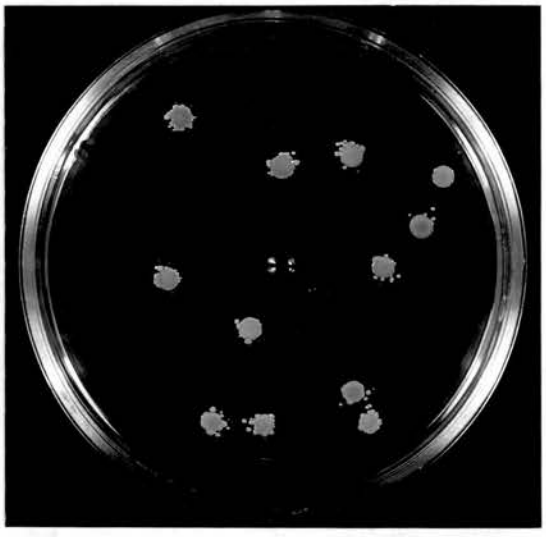


Plate 3. Transduction of penicillin resistance

A transduction plate, containing 0.12 μg of penicillin per ml. of agar, on which the transduced colonies are easily recognised as there is no background growth.

resistant to more than 100 μg of penicillin per ml, to have become resistant to mercuric chloride and to have gained the ability to produce penicillinase. None of the 50 colonies had become resistant to tetracycline. The results show that the transduction of penicillin resistance is dependent on the acquisition of the ability to produce penicillinase. Co-transduction of the markers for penicillinase production and resistance to mercuric chloride was shown to take place but the markers for penicillinase production and tetracycline resistance were not co-transduced.

Transduction of resistance to chloramphenicol

The transduction of chloramphenicol resistance was also investigated to confirm the finding that in staphylococci a variety of markers can be transduced. As the two transducing phages used in the previous experiments were induced from chloramphenicol-sensitive donor strains they could not be used to transduce chloramphenicol resistance. Two chloramphenicol-resistant strains of staphylococci, CB50 and S29, were examined for lysogenicity using ten chloramphenicol-sensitive strains as indicators of lysogenicity. Strain CB50, phage type 6/7/47/53/54, was found to carry a phage that lysed indicator strains 13346 and

PS47. A culture of the lysogenic strain CB50 was induced by ultraviolet irradiation and the phage filtrate had a titre of 1×10^7 p.f.u. per ml when titrated on strain 13346. Transduction experiments with strain 13346 or PS47 as recipients were carried out as described for the transduction of tetracycline resistance. The transduction mixtures were made up with 4 ml of the phage filtrate and 1 ml of a 3-hour culture of the recipient strain. Thus each transduction mixture contained 4×10^7 phage particles. The antibiotic overlay contained 100 μg of chloramphenicol per ml. This completely inhibited growth on the control plates on which the chloramphenicol-sensitive recipient strain had been plated alone. On the transduction plates on which the staphylococcus-phage mixture had been plated, numerous colonies grew through the chloramphenicol overlay. The frequency of transduction in this system was found to be much higher than the frequencies observed in the other systems. With recipient strain 13346 the number of transducants recovered was 3,305 per 10^8 phage particles, and with strain PS47 the number recovered was 2,302 per 10^8 phage particles. The results show that it is possible to transduce several different antibiotic-resistance markers in staphylococci. They confirm that the frequency of transduction

varies when different phages and different markers are used.

On subculture, the transduced colonies were resistant to 50 μg of chloramphenicol per ml. This represented an eightfold increase in resistance to chloramphenicol and the increase was maintained after five subcultures in chloramphenicol-free medium. Co-transduction of the markers for chloramphenicol resistance and tetracycline resistance was not found.

The character of the transducing phages

Since phage AK72 and Phage 15844 had been isolated from naturally lysogenic strains of staphylococci nothing was known about their characters; these had to be investigated before the phages could be compared with the transducing phages used by other workers.

Lytic spectra

The lytic spectrum of each transducing phage was determined. Dilutions of the phage were spotted on to a series of plates flood-seeded with the appropriate propagating strain and 18 other test strains. The ability of the phage to lyse each test strain was compared with its ability to lyse the propagating strain. A grade 5 reaction

was recorded when a strong reaction (++) was given by the same dilution of phage acting on the test strain as on the propagating strain. A grade 4 reaction was recorded when it required a tenfold or hundredfold increase in phage concentration to produce a strong reaction with the test strain in comparison with the concentration that was effective against the propagating strain. A grade 3 reaction required a thousandfold or ten-thousandfold increase in phage concentration to produce a strong reaction with the test strain in comparison with the concentration that was effective against the propagating strain. Table 8 shows that phage AK72 gave grade 5 reactions with test strains 52, 80, 81 and 82 and grade 3 reactions with test strains 47 and 2009. This pattern of reactions resembles the lytic spectrum of typing phage 80. Table 9 shows that phage 15844 gave grade 5 reactions with test strains 47, 53, 54 and 77 and a grade 3 reaction with test strain 75. These results resemble the lytic spectrum of typing phage 53 or of typing phage 77.

Serological group

As already described phage antisera were prepared against phages AK72 and 15844. The effect of these antisera on the plaque-forming

TABLE 8

The lytic spectrum of phage AK72

Test strain of staphylococcus	Suspension of phage AK72 diluted 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
74426(propagating strain)	++*	++	++	++	++	+
29	-	-	-	-	-	-
52	++	++	++	++	++	+
52A/79	-	-	-	-	-	-
80	++	++	++	++	++	+
82	++	++	++	++	++	+
2009	++	++	+	±	-	-
3A	-	-	-	-	-	-
3B	-	-	-	-	-	-
71	-	-	-	-	-	-
8719	-	-	-	-	-	-
42C	-	-	-	-	-	-
42E	-	-	-	-	-	-
47	++	++	+	±	-	-
53	-	-	-	-	-	-
54	-	-	-	-	-	-
75	-	-	-	-	-	-
77	-	-	-	-	-	-
81	++	++	++	++	++	+

*++ represents confluent lysis, semi-confluent lysis or more than 50 plaques

\+ represents 20-50 plaques

± represents less than 20 plaques

TABLE 9

The lytic spectrum of phage 15844

Test strain of staphylococcus	Suspension of phage 15844 diluted 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
13346 (propagating strain)	++*	++	++	++	++	++
29	-	-	-	-	-	-
52	-	-	-	-	-	-
52A/79	-	-	-	-	-	-
80	-	-	-	-	-	-
82	-	-	-	-	-	-
2009	-	-	-	-	-	-
3A	-	-	-	-	-	-
3B	-	-	-	-	-	-
71	-	-	-	-	-	-
8719	-	-	-	-	-	-
42C	-	-	-	-	-	-
42E	-	-	-	-	-	-
47	++	++	++	++	++	++
53	++	++	++	++	++	++
54	++	++	++	++	++	++
75	++	++	++	+	±	-
77	++	++	++	++	++	++
81	-	-	-	-	-	-

*++ represents confluent lysis, semi-confluent lysis or more than 50 plaques.

\+ represents 20-50 plaques

± represents less than 20 plaques

titres of the transducing phages was investigated. Equal volumes of the phage and tenfold dilutions of the antiserum under test were incubated for 4 hr at 37°C. A sample of the phage suspension alone was incubated for the same time. After incubation, titration of the plaque-forming ability of the phage that had been treated with the antiserum was carried out and this was compared with the titre of the untreated phage control. The results are shown in Table 10. It is clear that the antisera produce the same reduction in the plaque-forming titres of the heterologous phage and the homologous phage. This suggests that these phages belong to the same serological group. The antisera were tested against typing phages 6, 52A and 77 representative of serological groups A, B and F respectively. The ability of the antisera to reduce the plaque-forming titres of suspensions of these phages is recorded in Table 11. These results show that both antisera are active in reducing the plaque-forming titre of the group B phage only and this provides good evidence that the phages that were used to prepare these antisera belong to serological group B.

TABLE 10

The effect of antisera prepared against phage AK72 or phage 15844 on the plaque-forming titres of suspensions of these phages

Procedure		Phage AK72 p.f.u.per ml	Phage 15844 p.f.u.per ml.
Untreated		3×10^2	10^3
Treated with antiserum AK72			
Diluted	10^{-6}	2×10^2	10^3
	10^{-5}	2×10^2	10^3
	10^{-4}	10^2	10^3
	10^{-3}	10	10^2
	10^{-2}	0	0
	10^{-1}	0	0
Treated with antiserum 15844			
Diluted	10^{-6}	2×10^2	10^3
	10^{-5}	2×10^2	8×10^2
	10^{-4}	2×10^2	10^2
	10^{-3}	10	3
	10^{-2}	0	0
	10^{-1}	0	0

TABLE 11

The effect of antisera prepared against phage AK72 or phage 15844 on the plaque-forming titres of suspensions of typing phages 6, 52A and 77

Procedure	Phage 6 p.f.u.per ml	Phage 52A p.f.u.per ml	Phage 77 p.f.u.per ml
Untreated	10^3	10^3	10^3
Treated with antiserum AK72			
Diluted 10^{-4}	10^3	3×10^2	10^3
10^{-3}	10^3	10^2	10^3
10^{-2}	10^3	0	10^3
10^{-1}	10^3	0	10^3
Treated with antiserum 15844			
Diluted 10^{-4}	10^3	10^2	10^3
10^{-3}	10^3	5	10^3
10^{-2}	10^3	0	10^3
10^{-1}	10^3	0	10^3

RESULT	UNCHANGED	NEUTRALIZED	UNCHANGED

Morphology

The morphology of the two transducing phages was found to be similar when they were examined on the electron microscope after staining with phosphotungstic acid. With this negative staining method, phage particles appear as electron-transparent objects set against an electron-dense background of phosphotungstic acid. If the phage head has lost its DNA this is replaced by the stain which diffuses through the head membrane. Thus the contrast between an empty phage head and the background is much less than that provided by a head filled with DNA.

Plate 4 is an electronmicrograph of phage AK72 which was stained after having been washed in distilled water. In this preparation the phage is empty but there is some angularity in its outline. The cross-striations in the phage tail are clearly seen and there is a tail knob present. Plate 5 is an electronmicrograph of phage 15844 which was stained after having been washed in 0.1 M ammonium acetate. In this preparation the phage head has retained its content of DNA but structural detail is ill-defined. The cross-striations of the phage tail are not so clearly seen and there is no tail kbon present. The phage heads in both preparations

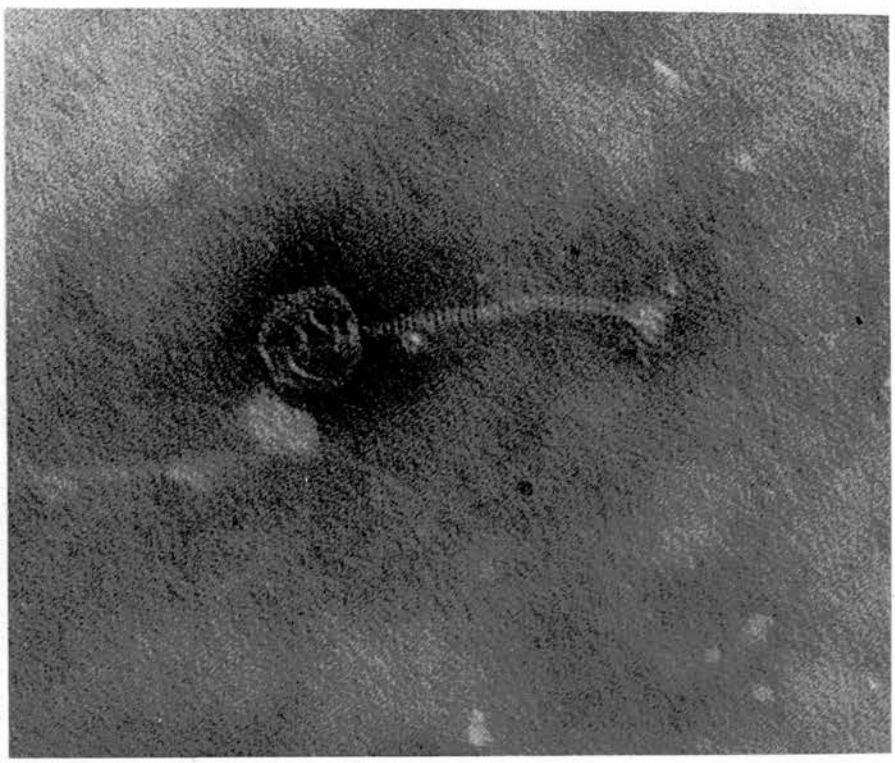


Plate 4 Transducing phage AK72 x 250,000
Electronmicrograph of phage AK72. The phage head is empty. Cross-striations are seen in the tail and a tail knob is present. (The preparation was initially suspended in distilled water and stained with phosphotungstic acid.)

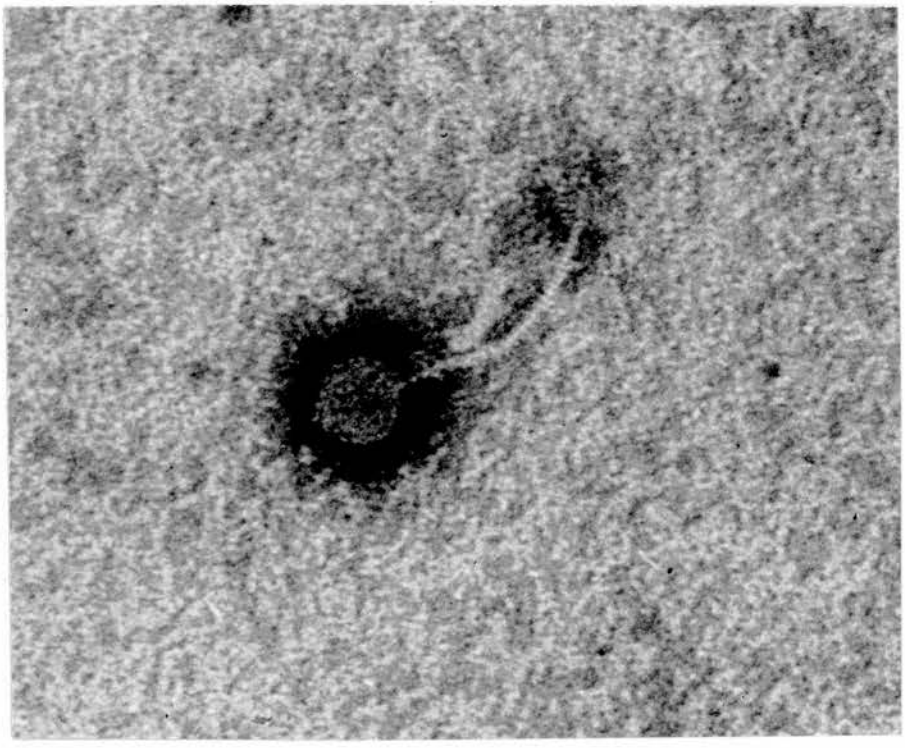


Plate 5. Transducing phage 15844 x 250,000
Electronmicrograph of phage 15844. The phage head has retained its content of DNA. No tail knob is seen.
(The preparation was initially suspended in 0.1 M ammonium acetate and stained with phosphotungstic acid.)

measure 52 mμ in diameter and the tails measure 152 mμ in length.

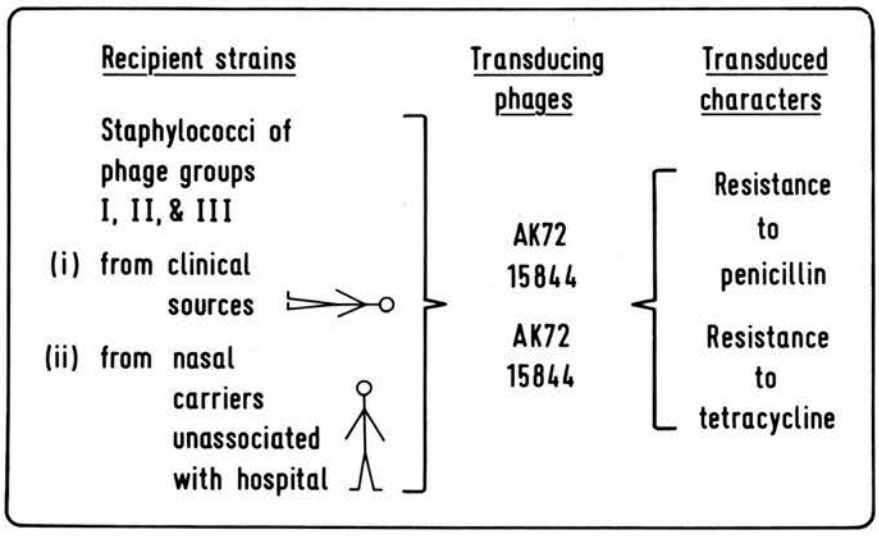
Bradley (1963) studied the structure of several staphylococcal phages representing the serological groups A, B, F and L. He found that phages belonging to group F had heads measuring 55 mμ in diameter and tails measuring 220-240 mμ in length. The only group L phage examined had a head of 60 mμ in diameter and a tail of 170 mμ in length. The group B phages had heads of 50-60 mμ in diameter and tails of 150 mμ in length. The group A phages differed markedly from the other phages examined; their heads were elongated and with some minor variations they measured 96 x 55 mμ. All the tails were long (300 mμ). As the transducing phages used in the present work had heads measuring 52 mμ in diameter and tails measuring 152 mμ in length their morphological appearance indicated that they belonged to serological group B. This finding is in keeping with the results of the neutralization experiments with the phage antiserum.

Transduction of antibiotic resistance by phage AK72 and phage 15844 to staphylococci of different phage groups isolated from different sources.

The results of earlier experiments, in which tetracycline resistance and penicillin resistance had been transduced to a small number of recipient strains, indicated that a similarity between the phage types of the donor and recipient strains was necessary for transduction to take place. To confirm this finding, antibiotic sensitive staphylococci representing the three main phage groups were collected from clinical and non-clinical sources. An outline of the following experiments is given in Figure 1. Transduction of each recipient strain was attempted with phage AK72, induced from a Group I donor strain, and with phage 15844, induced from a Group III donor strain, as follows:

Transduction of tetracycline resistance to Group I staphylococci isolated from clinical sources

The transduction of tetracycline resistance was carried out essentially as in previous experiments but with one alteration in procedure. Since it had been demonstrated that phenotypic delay was unnecessary for the expression of the transduced character in this system, the tetracycline was incorporated into the base layer of agar instead of



Outline of transduction experiments with staphylococci derived from clinical sources and from nasal carriers unassociated with a hospital environment.

FIGURE 1.

being poured on to the plate as overlay. It became evident that the expression of the transduced colonies was influenced by the amount of tetracycline present. If the concentration of tetracycline in the base layer was 50 μg per ml, few recipient strains appeared to be transduced. With concentrations of 25 μg or 12.5 μg per ml the number of positive transductions was increased. With a concentration of 6.25 μg per ml the non-transduced recipient cells were not completely inhibited. In this series of experiments, selective media containing 50 μg, 25 μg and 12.5 μg of tetracycline per ml were used to test for transduction of each recipient strain. Before transduction the recipient strains were all sensitive to less than 6 μg of tetracycline per ml. They were phage-typed, their sensitivity to both transducing phages was determined, and they were tested for resistance to mercuric chloride. The characters of each recipient strain and the results of the transduction experiments are recorded in Table 12.

These results show that phage AK72 was able to transduce tetracycline resistance to 22 out of 31 Group I staphylococci tested. The ability to be transduced by phage AK72 does not appear to be associated with any particular phage type within

Group I or with the ability of phage AK72 to lyse the recipient strain. In contrast, the amount of tetracycline in the selective medium had a marked influence on the number of positive results obtained. When a concentration of 50 μg per ml was used in the selective medium, the transduction of only eight recipient strains was recognised. It is of interest to note that five of these eight strains were resistant to mercuric chloride, and that the five strains were the only mercury-resistant strains in the Group I series. When the results of the transduction experiments with phage 15844 are considered the picture is very different. Only ten recipient strains were transduced by this phage and the transduced character was expressed on the selective medium containing the lowest level of tetracycline. Nine of these were sensitive to typing phage 29 and it seems that this phage type was associated with the ability to be transduced by phage 15844. No correlation was found between the resistance of the recipient strains to mercury and their ability to be transduced by phage 15844.

TABLE 12

Transduction of tetracycline resistance by phage AK72

and phage 15844 to Group I staphylococci isolated from clinical sources

Recipient strain of staphylococcus	Phage type**	Lysed by transducing phage		Resistant to $HgCl_2$	Tetracycline resistance transduced by phage					
					AK72			15844		
					with transducants selected by tetracycline at					
		AK72	15844		12.5	25	50µg/ml	12.5	25	50µg/ml*
1/1	29	-	-	-	+	+	+	+	-	-
1/2	29/52/81	+	+	-	+	+	-	+	-	-
1/3	29/80	-	-	-	+	+	-	+	-	-
1/4	29	-	-	-	+	+	+	+	+	-
1/5	52A/79+	-	-	-	-	-	-	-	-	-
1/6	29/52	+	-	-	+	+	-	+	-	-
1/7	29	-	-	-	+	+	-	+	-	-
1/9	52A/79+	-	-	-	-	-	-	-	-	-
1/10	52A/79+	-	-	-	-	-	-	-	-	-
1/11	81+	-	-	-	+	+	-	-	-	-
1/12	80/81	-	-	-	+	+	-	-	-	-
1/13	81	-	-	-	-	-	-	-	-	-
1/14	52	-	-	-	+	+	-	-	-	-
1/15	80	-	-	-	+	+	-	-	-	-
1/16	29	-	-	-	+	+	-	+	-	-
1/17	52A/79	-	-	-	-	-	-	-	-	-
1/18	29w	+	-	-	-	-	-	-	-	-
1/19	29	+	-	-	+	+	-	+	-	-
1/20	52/80	+	+	-	+	+	+	-	-	-
1/21	52A	-	-	-	+	+	-	-	-	-
1/23	52/52A/80/81	+	-	+	+	+	+	-	-	-
1/24	52/52A/80/81	+	-	+	+	+	+	-	-	-
1/25	52/52A/80/81	+	-	+	+	+	+	-	-	-
1/26	52A	-	-	-	-	-	-	-	-	-
1/27	81+	+	-	-	+	+	-	+	-	-
1/28	29/52/80/81+	+	-	-	+	+	-	+	-	-
1/29	52/52A/80/81	+	-	-	+	+	-	-	-	-
1/30	81	+	-	+	+	+	+	-	-	-
1/31	52/52A/80	-	-	-	-	-	-	-	-	-
1/32	80/81	+	-	+	+	+	+	-	-	-
1/33	52/79	-	-	-	-	-	-	-	-	-

** Reactions at RTD or 1000 RTD

* Concentration of tetracycline in selective medium

Failure of transduction of tetracycline resistance to Group II staphylococci isolated from clinical sources.

A similar series of transduction experiments was carried out with recipient strains from Group II. The characters of these strains and the results of the transduction experiments are recorded in Table 13.

The results are completely different from those obtained with the Group I recipient strains. No Group II recipient strains were transduced by phage AK72 or by phage 15844 and the same negative results were obtained with the three different levels of tetracycline in the selective medium.

TABLE 13

Failure of transduction of tetracycline resistance by phage AK72 and phage 15844 to Group II staphylococci isolated from clinical sources

Recipient strain of staphylococcus	Phage type**	lysed by transducing phage		Resistant to $HgCl_2$	Tetracycline resistance transduced by phage	
					AK72	15844
					with transducants selected by tetracycline at	
		AK72	15844		12.5 25 50 µg/ml	12.5 25 50 µg/ml*
2/1	3C/71+	-	-	-	- - -	- - -
2/2	3C+	-	-	-	- - -	- - -
2/3	3C/55/71	-	-	-	- - -	- - -
2/5	3B/3C/55/71	-	-	-	- - -	- - -
2/6	55	-	-	-	- - -	- - -
2/7	55w	-	-	-	- - -	- - -
2/8	55	-	-	-	- - -	- - -
2/9	55	-	-	-	- - -	- - -
2/10	55w	-	-	-	- - -	- - -
2/11	55	-	-	-	- - -	- - -
2/12	3A	-	-	-	- - -	- - -
2/13	55/71w	-	-	-	- - -	- - -
2/14	3C/55	-	-	-	- - -	- - -
2/15	55/71	-	-	-	- - -	- - -
2/16	3B/3C/55/71	-	-	-	- - -	- - -
2/17	3C/55	-	-	-	- - -	- - -
2/18	3C/55w	-	-	-	- - -	- - -
2/19	55+	-	-	-	- - -	- - -
2/20	3B/55	-	-	-	- - -	- - -
2/21	3A	-	-	-	- - -	- - -
2/22	3Bw/55w	-	-	-	- - -	- - -
2/23	3A/3B/3C/55/71	-	-	-	- - -	- - -
2/24	3A	-	-	-	- - -	- - -
2/25	3C	-	-	-	- - -	- - -
2/26	3Aw	-	-	-	- - -	- - -
2/27	3A	-	-	-	- - -	- - -
2/28	3A	-	-	-	- - -	- - -
2/29	71	-	-	-	- - -	- - -
2/30	55	-	-	-	- - -	- - -
2/31	3A	-	-	-	- - -	- - -
2/32	3C	-	-	-	- - -	- - -

** Reactions at RTD or 1000 RTD

* Concentration of tetracycline in selective medium

Transduction of tetracycline resistance to Group III staphylococci isolated from clinical sources.

A further series of transduction experiments was carried out with recipient staphylococci of phage group III. Details of the recipient strains and the results of the transduction experiments are presented in Table 14.

TABLE 14

Transduction of tetracycline resistance by phage AK72 and phage 15844 to Group III staphylococci isolated from clinical sources

Recipient strain of staphylococcus	Phage type**	lysed by transducing phage		Resistant to $HgCl_2$	Tetracycline resistance transduced by phage		
					AK72		15844
					with transducants selected by tetracycline at		
		AK72	15844		12.5 25 50 μg/ml		12.5 25 50 μg/ml*
3/1	42E	-	+	-	- - -		- - -
3/2	42E	-	+	-	- - -		- - -
3/7	6/7/42E/54	-	+	-	- - -		- - -
3/8	7/47/53w/54/75+	-	-	+	- - -		- - -
3/9	54+	+	+	-	+ + -		+ - -
3/10	6/7/53	+	+	-	+ + -		+ - -
3/11	53	-	+	-	+ + -		+ - -
3/12	7/53/54/75/77	-	-	+	- - -		+ - -
3/13	7/47/53/54/75/77	-	+	+	- - -		- - -
3/14	53/75w	-	-	-	- - -		- - -
3/15	47w/53w	-	+	-	- - -		- - -
3/16	6/7/47/53/54/75	-	+	-	- - -		- - -
3/17	47/53w/75/77	-	+	+	- - -		- - -
3/18	6/47/53/75/77	-	+	-	+ + -		+ - -
3/19	47/75	-	+	-	- - -		- - -
3/20	7/47/54/75	-	+	-	+ + -		+ - -
3/21	6/7/47/53/54/75	-	+	-	- - -		+ - -
3/22	53	-	+	-	- - -		+ - -
3/23	75/77	-	-	-	- - -		+ - -
3/24	6/47/53	-	+	-	- - -		- - -
3/25	6/47/53w/75/77	-	+	-	+ + -		+ - -
3/26	6/47w/53/54/75+	-	+	-	- - -		- -

**Reaction at RTD or 1000 RTD

*Amount of tetracycline in selective medium

The results show that both phage AK72 and phage 15844 are capable of transducing tetracycline resistance to Group III staphylococci. As might be expected, a greater number of these strains were transduced by phage 15844 which had been induced from a Group III donor strain, than by phage AK72 which had been induced from a Group I donor strain. Phage 15844 transduced tetracycline resistance to ten out of 22 recipient strains, and phage AK72 transduced the marker to six of these recipient strains. It seems that, although similarity between the phage type of the donor and recipient strain is not essential for transduction to take place, such a similarity increases the likelihood of transduction taking place. From the results it appears that the tetracycline resistance marker transduced by phage AK72 is capable of primary expression on a selective medium containing 25 μg of tetracycline per ml whereas the marker transduced by phage 15844 is only expressed when the amount of tetracycline is reduced to 12.5 μg per ml. When the transducants were subcultured and tested for resistance to tetracycline all were found to be resistant to more than 50 μg per ml; no difference was found between the transducants

isolated from the selective medium containing the smaller amount of tetracycline and those transducants recovered from the medium containing the larger amount of tetracycline (q.v.). No correlation was found between the phage type of the recipient strains, their sensitivity to the transducing phages, and their ability to be transduced by either phage. The four mercury-resistant strains in this series did not show any special pattern of behaviour.

Transduction of tetracycline resistance to Group I staphylococci isolated from nasal carriers unassociated with a hospital environment.

It was suggested that the results of transduction experiments might depend on the source of the staphylococci used as recipient strains; antibiotic-sensitive staphylococci isolated from the hospital environment might have retained their sensitivity because of an inherent inability to be transduced. To investigate this possibility it was decided to carry out a series of transduction experiments with recipient strains selected from tetracycline-sensitive staphylococci that had been isolated outside the hospital environment. Nasal swabs were taken from 294 science and pre-clinical medical students. Staph. aureus was isolated from

114 of these swabs and these strains were all found to be sensitive to less than 6 μg of tetracycline per ml. Phage-typing showed that 60 strains belonged to Group I, 15 to Group II and 14 to Group III. The remaining 25 were non-typable. The characters of 31 of the Group I strains and the results of transduction experiments carried out with these recipient strains are shown in Table 15.

TABLE 15

Transduction of tetracycline resistance by phage AK72 and phage 15844 to Group I staphylococci isolated from nasal carriers unassociated with a hospital environment

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Tetracycline resistance transduced by phage					
					AK72			15844		
					with transducants selected by tetracycline at					
		AK72	15844		12.5 μg/ml	25 μg/ml	50 μg/ml	12.5 μg/ml*	25 μg/ml*	50 μg/ml*
S1/1	29/52/80/81	-	-	-	+	+	-	+	-	-
S1/2	52/80/81/7	-	-	-	+	+	-	+	-	-
S1/3	52/80/81	-	-	-	+	+	-	-	-	-
S1/4	52/80/81	-	-	-	+	+	-	-	-	-
S1/5	29/52/6/54/75	-	-	-	+	+	-	+	+	-
S1/6	29/52/52Aw/80	+	-	-	+	+	-	+	+	-
S1/7	52/52A/80	+	-	-	+	+	-	-	-	-
S1/8	29	-	-	-	+	+	-	+	+	-
S1/9	29/52/80/81	-	-	-	+	+	-	+	+	-
S1/10	29	-	-	-	+	+	-	+	+	-
S1/11	81	-	-	-	+	+	-	-	-	-
S1/12	81	+	+	-	+	+	-	+	-	-
S1/13	29/52/80/81/77	-	-	-	+	+	-	+	-	-
S1/14	29/52/80/81	-	-	-	+	+	-	+	-	-
S1/15	29/52	+	-	-	+	+	-	+	+	-
S1/16	29/52/80/81	+	-	-	+	+	-	-	-	-
S1/17	52/81	-	-	-	+	+	-	+	+	-
S1/18	52/80	+	-	-	+	+	-	+	-	-
S1/19	29/52/80	-	-	-	+	+	-	+	-	-
S1/20	52/52Aw/80	+	-	-	+	+	-	-	-	-
S1/21	29/52/80	-	-	-	+	+	-	-	-	-
S1/22	81	+	-	-	+	+	-	-	-	-
S1/23	81	-	-	-	+	+	-	+	-	-
S1/24	29/79/42E/53	+	-	-	-	-	-	-	-	-
S1/25	29/52/80	-	-	-	+	+	-	+	-	-
S1/26	29/52/80	+	-	-	+	+	-	+	-	-
S1/27	52	+	+	-	+	+	-	-	-	-
S1/28	52/80/81	+	-	-	+	+	-	+	-	-
S1/29	52	+	-	-	+	+	-	-	-	-
S1/30	52/80/81	-	-	-	+	+	+	+	+	-
S1/31	29/52/52A/80	-	-	-	-	-	-	-	-	-

+ Reaction at RTD or 1000 RTD

* Amount of tetracycline in selective medium

Tetracycline resistance was transduced to 29 out of the 31 recipient strains by phage AK72. The marker was transduced to 19 of the 31 strains by phage 15844. In the experiments with hospital staphylococci phage AK72 transduced tetracycline resistance to 22 of 31 recipient strains and phage 15844 transduced the marker to ten of the 31 strains. Thus the number of positive transductions obtained with recipient strains isolated from outside the hospital environment is slightly higher than that obtained with recipient strains isolated from clinical sources. The ability of recipient strains to be transduced was not correlated with their sensitivity to the transducing phages. The association between sensitivity to the typing phage 29 and the ability to be transduced by phage 15844 was not so marked as in the series of Group I hospital staphylococci.

Failure of transduction of tetracycline resistance to Group II staphylococci isolated from nasal carriers unassociated with a hospital environment

A similar series of transduction experiments was carried out with 15 Group II recipient strains. The characters of these strains and the results of the transduction experiments are recorded in Table 16.

TABLE 16

Failure of transduction of tetracycline resistance by phage AK72 and phage 15844 to Group II staphylococci isolated from nasal carriers unassociated with a hospital environment

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Tetracycline resistance transduced by phage	
					AK72	15844
					with transducants selected by tetracycline at	
		AK72	15844		12.5 25 50 µg/ml	12.5 25 50 µg/ml*
S2/1	55/71	-	-	-	- - -	- - -
S2/2	3Bw/55/71	-	-	-	- - -	- - -
S2/3	3Bw/55/71	-	-	-	- - -	- - -
S2/4	71	-	-	-	- - -	- - -
S2/5	55	-	-	-	- - -	- - -
S2/6	55	-	-	-	- - -	- - -
S2/7	3C	-	-	-	- - -	- - -
S2/8	55/71	-	-	-	- - -	- - -
S2/9	3A/3B/3C/55/71	-	-	-	- - -	- - -
S2/10	55/71	-	-	-	- - -	- - -
S2/11	3B/3C/55	-	-	-	- - -	- - -
S2/12	3B/3C/71	-	-	-	- - -	- - -
S2/13	55	-	-	-	- - -	- - -
S2/14	3A	-	-	-	- - -	- - -
S2/15	3B/3C/71	-	-	-	- - -	- - -

+Reaction at RTD or 1000 RTD

*Amount of tetracycline in the selective medium.

The attempts to transduce tetracycline resistance to the 15 Group II staphylococci were completely unsuccessful. Negative results were obtained with both the transducing phages. These results are consistent with the negative findings obtained when attempts were made to transduce Group II hospital strains of staphylococci.

Transduction of tetracycline resistance to Group III staphylococci isolated from nasal carriers unassociated with a hospital environment.

Only a small number of Group III staphylococci were isolated from nasal carriers. The characters of these recipient strains and the results of the transduction experiments are shown in Table 17.

Phage 15844 transduced tetracycline resistance to seven of the 14 recipient strains and phage AK72 transduced the marker to four of the strains. These findings are very similar to the results obtained when the Group III hospital strains were tested.

The results are summarized in Tables 18 and 19.

TABLE 17

Transduction of tetracycline resistance by phage AK72 and phage 15844 to Group III staphylococci isolated from nasal carriers unassociated with a hospital environment

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Tetracycline resistance transduced by phage					
					AK72			15844		
					with transducants selected by tetracycline at					
		AK72	15844		12.5	25	50 µg/ml	12.5	25	50 µg/ml*
S3/1	6/7/47/53/54/75	-	-	-	-	-	-	+	+	-
S3/2	6/47/53/83A	-	+	-	-	-	-	+	-	-
S3/3	6/7/42E/47/53/54/83A	-	+	-	-	-	-	-	-	-
S3/4	42E	-	-	-	-	-	-	-	-	-
S3/5	42E	+	-	-	-	-	-	-	-	-
S3/6	42E	+	+	-	-	-	-	-	-	-
S3/7	54/75	-	-	-	+	+	-	-	-	-
S3/8	42E/47/53/77/81	-	-	-	+	+	-	+	-	-
S3/9	7/42E/47/53/54/75/83A	-	+	-	-	-	-	+	-	-
S3/10	187	-	-	-	-	-	-	-	-	-
S3/11	53/77	-	+	-	-	-	-	+	-	-
S3/12	53	-	-	-	+	+	-	+	-	-
S3/13	7/42E/81	-	-	-	+	+	-	+	+	-
S3/14	42E/47/81	-	-	-	-	-	-	-	-	-

+Reaction at RTD or 1000 RTD

*Amount of tetracycline in the selective medium

TABLE 18

Transduction of tetracycline resistance by phage AK72 to staphylococci of the three main phage groups isolated from clinical sources or from nasal carriers unassociated with a hospital environment

	Staphylococci of phage groups					
	I		II		III	
	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains
Number of strains tested	31	31	31	15	22	14
Number of strains transduced	22	29	0	0	6	4
Percentage of tested strains transduced	71%	94%	-	-	27%	28%

TABLE 19

Transduction of tetracycline resistance by phage 15844 to staphylococci of the three main phage groups isolated from clinical sources or from nasal carriers unassociated with a hospital environment

	Staphylococci of phage groups					
	I		II		III	
	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains
Number of strains tested	31	31	31	15	22	14
Number of strains transduced	10	19	0	0	10	7
Percentage of tested strains transduced	32%	61%	-	-	45%	50%

A striking feature of these results is the failure to transduce tetracycline resistance to any of the 46 staphylococci tested from Group II. These strains had been isolated from clinical sources and from healthy carriers therefore they included a variety of Group II strains. However, there was a subgroup of Group II staphylococci that was not well represented in the collection. Staphylococci of phage type 71 differ in some respects from other members of Group II; they have been associated with outbreaks of impetigo and are frequently resistant to penicillin. It was decided to investigate the possibility that these strains differ from other Group II staphylococci in their ability to be transduced. A collection of 14 tetracycline-sensitive type 71 staphylococci were sent to the author by Dr M.P. Jevons. These strains were resistant to penicillin but, in general, the level of resistance was lower than that observed in the Group I and Group III staphylococci tested. When attempts were made to transduce tetracycline resistance to these 14 Group II strains no positive results were obtained. Thus phage type 71 strains resemble other Group II staphylococci in their inability to be transduced

by phages induced from Group I or Group III donor strains.

Changes in the recipient strains after transduction of tetracycline resistance by phage AK72 or phage 15844

In the early transduction experiments with recipient strains 74426, 12861, 13346 and PS47 the transducants were found to be resistant to more than 50 μg of tetracycline per ml. As these recipient strains were all resistant to penicillin before transduction it had been impossible to test for co-transduction of the marker for tetracycline resistance and the marker for penicillin resistance. Now that a larger series of recipient strains had been transduced it was possible to confirm the level of tetracycline resistance transduced and to investigate the occurrence of co-transduction. Transducants were subcultured from each transduction in which the recipient strain before transduction had been sensitive to penicillin as well as to tetracycline. The results of testing the resistance of these transducants to tetracycline, penicillin and mercuric chloride are recorded in Table 20.

TABLE 20

Changes in recipient strains after transduction of tetracycline resistance by phage AK72 or phage 15844

	Number tested	Number resistant to		
		Tetracycline	Penicillin	Mercuric chloride
Recipient strains before transduction	60	0	0	0
Transducants	640	640*	0	3

* Minimum inhibitory concentration = 50 μg tetracycline/ml.

The results show that all of the 640 transducants tested were resistant to more than 50 μg of tetracycline per ml; no difference in resistance was found between the transducants recovered from the selective medium containing 25 μg per ml and those transducants recovered from the selective medium containing only 12.5 μg of tetracycline per ml. There was no evidence of co-transduction of the marker for tetracycline resistance and the markers for resistance to penicillin or mercuric chloride.

Transduction of penicillin resistance by phage AK72 and phage 15844 to staphylococci of different phage groups isolated from different sources

A proportion of the tetracycline-sensitive recipient strains, that had been collected for the previous experiments were sensitive to penicillin. Among the tetracycline-resistant staphylococci collected for induction and propagation experiments were a few penicillin-sensitive strains. These various staphylococci were all sensitive to 0.06 μg of penicillin per ml and it was decided to use them as recipient strains for the transduction of penicillin resistance. When the recipient strain was sensitive to both antibiotics the ability of each

phage to transduce penicillin resistance could be compared with its ability to transduce tetracycline resistance to the same recipient strain.

Transduction of penicillin resistance to Group I staphylococci isolated from clinical sources.

The transduction of penicillin resistance was carried out as described in the experiment with recipient strain 2371 and the results are recorded in Table 21.

The results show that the majority of penicillin-sensitive recipient strains that gave positive results when phage AK72 was used to transduce tetracycline resistance gave positive results when penicillin resistance was the marker; phage AK72 transduced penicillin resistance to 15 out of the 22 strains tested. With phage 15844 the results were very different; although this phage had transduced tetracycline resistance to eight of the penicillin-sensitive recipient strains no positive results were obtained when penicillin resistance was used as the marker in the experiments.

TABLE 21

Transduction of penicillin resistance by phage AK72 and phage 15844 to Group I staphylococci isolated from clinical sources

Recipient strain of staphylococcus	Phage type[+]	Lysed by transducing phage		Resistant to $HgCl_2$	Penicillin resistance transduced by phage	
		AK72	15844		AK72	15844
1/1	29	-	-	-	+	-
1/2	29/52/81	+	+	-	+	-
1/3	29/80	-	-	-	+	-
1/4	29	-	-	-	+	-
1/5	52A/79+	-	-	-	-	-
1/6	29/52	+	-	-	+	-
1/7	29	-	-	-	+	-
1/8	29+	-	-	-	+	-
1/9	52A/79+	-	-	-	-	-
1/10	52A/79+	-	-	-	-	-
1/11	81+	-	-	-	+	-
1/12	80/81	-	-	-	+	-
1/13	81	-	-	-	-	-
1/14	52	-	-	-	+	-
1/15	80	-	-	-	+	-
1/16	29	-	-	-	+	-
1/17	52A/79	-	-	-	-	-
1/18	29w	+	-	-	-	-
1/19	29	+	-	-	+	-
1/20	52/80	+	+	-	+	-
1/21	52A	-	-	-	-	-
1/22	8/w	-	-	-	+	-

[+]Reactions at RTD or 1000 RTD

Failure of transduction of penicillin resistance to Group II staphylococci isolated from clinical sources

A similar series of transduction experiments was carried out with recipient strains from Group II. The results are shown in Table 22.

No positive results were obtained in the transduction experiments when 18 Group II strains were tested as recipients of the marker for penicillin resistance. This is in keeping with negative findings when the transduction tetracycline resistance was attempted. It would appear that the inability of Group II staphylococci to be transduced by phage AK72 or phage 15844 is not restricted to one marker.

TABLE 22

Failure of transduction of penicillin resistance by phage AK72 and phage 15844 to Group II staphylococci isolated from clinical sources

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Penicillin resistance transduced by phage	
		AK72	15844		AK72	15844
2/1	3C/71+	-	-	-	-	-
2/2	3C+	-	-	-	-	-
2/3	3C/55/71	-	-	-	-	-
2/5	3B/3C/55/71	-	-	-	-	-
2/6	55	-	-	-	-	-
2/7	55w	-	-	-	-	-
2/8	55	-	-	-	-	-
2/9	55	-	-	-	-	-
2/10	55w	-	-	-	-	-
2/11	55	-	-	-	-	-
2/12	3A	-	-	-	-	-
2/13	55/7w	-	-	-	-	-
2/14	3C/55	-	-	-	-	-
2/15	55/71	-	-	-	-	-
2/16	3B/3C/55/71	-	-	-	-	-
2/17	3C/55	-	-	-	-	-
2/18	3C/55w	-	-	-	-	-
2/19	55+	-	-	-	-	-

+ Reaction at RTD or 1000 RTD

Transduction of penicillin resistance to Group III staphylococci isolated from clinical sources.

The number of penicillin-sensitive staphylococci isolated from clinical sources was so small that it is difficult to generalize from the results recorded in Table 23.

Two out of the four recipient strains available for testing were transduced by phage AK72. The one positive result obtained with phage 15844 indicates that this phage is capable of transducing penicillin resistance but the results from this small series and from the Group I series suggest that phage 15844 is less active in the transduction of penicillin resistance than in the transduction of tetracycline resistance.

TABLE 23

Transduction of penicillin resistance by phage AK72 and phage 15844 to Group III staphylococci isolated from clinical sources

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Penicillin resistance transduced by phage	
		AK72	15844		AK72	15844
3/1	42E	-	+	-	-	-
3/3	7/47	-	+	-	+	-
3/5	6/47/53/54	-	+	-	-	+
3/6	7/47	-	-	-	+	-

+ Reaction at RTD or 1000 RTD

Transduction of penicillin resistance to Group I staphylococci isolated from nasal carriers unassociated with a hospital environment

Twenty-four out of the 31 strains that were used as recipients for the transduction of tetracycline resistance were sensitive to penicillin and could be used as recipient strains for the transduction of penicillin resistance. The results are recorded in Table 24.

TABLE 24

Transduction of penicillin resistance by phage AK72 and phage 15844 to Group I staphylococci isolated from nasal carriers unassociated with a hospital environment

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Penicillin resistance transduced by phage	
		AK72	15844		AK72	15844
S1/1	29/52/80/81	-	-	-	+	-
S1/3	52/80/81	-	-	-	+	-
S1/4	52/80/81	-	-	-	+	-
S1/5	29/52/6/54/75	-	-	-	+	+
S1/7	52/52A/80	+	-	-	+	-
S1/8	29	-	-	-	+	-
S1/9	29/52/80/81	-	-	-	+	-
S1/10	29	-	-	-	+	-
S1/11	81	-	-	-	+	-
S1/12	81	+	+	-	+	-
S1/14	29/52/80/81	-	-	-	+	-
S1/16	29/52/80/81	+	-	-	+	-
S1/17	52/81	-	-	-	+	-
S1/18	52/80	+	-	-	+	-
S1/20	52/52Aw/80	+	-	-	+	-
S1/21	29/52/80	-	-	-	+	-
S1/22	81	+	-	-	+	-
S1/24	29/79/42E/53	+	-	-	-	-
S1/26	29/52/80	+	-	-	-	-
S1/27	52	+	+	-	-	-
S1/28	52/80/81	+	-	-	+	-
S1/29	52	+	-	-	+	-
S1/30	52/80/81	-	-	-	+	-
S1/31	29/52/52A/80	-	-	-	-	-

+Reaction at RTD or 1000 RTD

The results show that phage AK72 transduced penicillin resistance to 20 out of the 24 recipient strains tested. All except two of the strains to which tetracycline resistance had been transduced by phage AK72 gave positive results when penicillin resistance was the marker. Phage 15844 transduced penicillin resistance to only one of the 24 strains. These findings are very similar to the results obtained with the Group I strains isolated from clinical sources and they confirm the marked difference in the ability of the two phages to transduce penicillin resistance to Group I staphylococci.

Failure of transduction of penicillin resistance to Group II staphylococci isolated from nasal carriers unassociated with a hospital environment

Thirteen of the 15 recipient strains that were used for the transduction of tetracycline resistance were sensitive to penicillin. The results of the attempts to transduce penicillin resistance to these recipient strains are shown in Table 25.

The results confirm the failure of phage AK72 or phage 15844 to transduce penicillin resistance to Group II staphylococci.

TABLE 25

Failure of transduction of penicillin resistance by phage AK72 and phage 15844 to Group II staphylococci isolated from nasal carriers unassociated with a hospital environment

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Penicillin resistance transduced by phage	
		AK72	15844		AK72	15844
S2/1	55/71	-	-	-	-	-
S2/4	71	-	-	-	-	-
S2/5	55	-	-	-	-	-
S2/6	55	-	-	-	-	-
S2/7	3C	-	-	-	-	-
S2/8	55/71	-	-	-	-	-
S2/9	3A/3B/3C/55/71	-	-	-	-	-
S2/10	55/71	-	-	-	-	-
S2/11	3B/3C/55	-	-	-	-	-
S2/12	3B/3C/71	-	-	-	-	-
S2/13	55	-	-	-	-	-
S2/14	3A	-	-	-	-	-
S2/15	3B/3C/71	-	-	-	-	-

+ Reaction at RTD or 1000 RTD

Transduction of penicillin resistance to Group III staphylococci isolated from nasal carriers unassociated with a hospital environment.

As with the Group III hospital strains, only a few out of this series of staphylococci were sensitive to penicillin. The results of the transduction experiments with the five penicillin-sensitive recipient strains are shown in Table 26.

In this small series only one positive result was obtained with phage AK72. No positive results were given by phage 15844.

These results are summarized in Tables 27 and 28.

TABLE 26

Transduction of penicillin resistance by phage AK72 and phage 15844 to Group III staphylococci isolated from nasal carriers unassociated with a hospital environment

Recipient strain of staphylococcus	Phage type+	Lysed by transducing phage		Resistant to $HgCl_2$	Penicillin resistance transduced by phage	
		AK72	15844		AK72	15844
S3/4	42E	-	-	-	+	-
S3/8	42E/47/53/54/81	-	-	-	-	-
S3/10	187	-	-	-	-	-
S3/13	7/42E/81	-	-	-	-	-
S3/14	42E/47/81	-	-	-	-	-

+ Reaction at RTD or 1000 RTD

TABLE 27

Transduction of penicillin resistance by phage AK72 to staphylococci of the three main phage groups isolated from clinical sources or from nasal carriers unassociated with a hospital environment

	Staphylococci of phage groups					
	I		II		III	
	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains
Number of strains tested	22	24	18	13	4	5
Number of strains transduced	15	20	0	0	2	1
Percentage of tested strains transduced	68%	83%	-	-	-*	-*

* Inadequate numbers to allow percentage estimations

TABLE 28

Transduction of penicillin resistance by phage 15844 to staphylococci of the three main phage groups isolated from clinical sources or from nasal carriers unassociated with a hospital environment

	Staphylococci of phage groups					
	I		II		III	
	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains	Hospital strains	Non-hospital strains
Number of strains tested	22	24	18	13	4	5
Number of strains transduced	0	1	0	0	1	0
Percentage of tested strains transduced	-	4%	-	-	-*	-*

* Inadequate numbers to allow percentage estimations

The outstanding feature of these results is the failure of phage 15844 to transduce penicillin resistance to more than two out of the 86 recipient strains. This might be explained by the fact that only a few penicillin-sensitive Group III staphylococci were available for testing, but phage 15844 was shown to transduce tetracycline resistance frequently to both Group I and Group III staphylococci. It may be that a closer relation between donor and recipient strain is required for the transduction of penicillin resistance than for the transduction of tetracycline resistance. The donor strains of staphylococci, from which both the transducing phages were induced, and the strains on which these phages were propagated were investigated by Dr. M.H. Richmond. He found that the type of penicillinase produced by each of these strains was penicillinase type A. Therefore the difference in the ability of phages AK72 and 15844 to transduce penicillin resistance could not be explained by a difference in the enzyme produced by the staphylococci from which they had been derived.

The inability of Group II staphylococci to be transduced, already noted when tetracycline resistance was used as the marker, was also found

when attempts were made to transduce penicillin resistance with phages induced from Group I or Group III donor strains. It has been noted that the penicillinase type B, produced by Group II staphylococci differs from the penicillinases produced by Group I and Group III strains. This could account for the failure to transduce penicillin resistance to Group II strains by phages derived from staphylococci that produce type A penicillinase.

Changes in the recipient strains after transduction of penicillin resistance by phage AK72 or phage 15844

In the preliminary transduction experiment with recipient strain 2371, 49 out of 50 transducants were found to have become resistant to more than 100 μg of penicillin per ml, to have become resistant to mercuric chloride and to have acquired the ability to produce penicillinase None of the transduced strains had become resistant to tetracycline. To confirm these findings transducants from a variety of recipient strains were investigated. Transducants were subcultured from each transduction in which the recipient strain before transduction had been sensitive to tetracycline as well as to penicillin. The

results of testing 459 colonies recovered from the transduction plates in experiments with 35 recipient strains are recorded in Table 29.

TABLE 29

Changes in the recipient strains after transduction of penicillin resistance by phage AK72 or phage 15844

	Number tested	Number resistant to		
		Tetracycline	Penicillin	Mercuric chloride
Recipient strains before transduction	35	0	0	0
Transducants	459	0	355*	356

* Minimum inhibitory concentration = 100 μg penicillin/ml. 344 colonies were shown to be penicillinase producers.

The results show that only 355 out of the colonies tested were resistant to more than 100 μg of penicillin per ml. It seems likely that the penicillin-sensitive colonies were picked from the transduction plates containing 0.06 μg of penicillin per ml. On these transduction plates there was always a background growth of penicillin-sensitive colonies that could have contaminated subcultures made from these plates. Of the 355 penicillin-resistant transducants tested for penicillinase production 344 gave unequivocally positive results. Thus the acquisition of penicillin resistance generally depended on the acquisition of the ability to produce penicillinase. It was confirmed that co-transduction of the markers for penicillinase production and resistance to mercuric chloride took place. Transduction of the marker for tetracycline resistance did not accompany transduction of the markers for mercuric chloride resistance or penicillinase production.

Treatment of transduced colonies with acridine orange.

It was decided to investigate the nature of the genetic determinants of the characters transduced by phage AK72. Recently it has been suggested that penicillinase production in staphylococci is determined by the presence of extrachromosomal elements. Several groups of workers have reported the elimination of this marker by treatment with the acridine dyes but it appears that strains of penicillin-resistant staphylococci vary in their response to this treatment. In experiments with enterobacteria, the elimination of genetic determinants by the acridine dyes is taken as evidence for the extrachromosomal position of such determinants. The elimination of the marker for penicillinase production from staphylococci in this manner may similarly indicate that the production of the enzyme is governed by cytoplasmic elements.

An attempt was made to use acridine orange treatment to eliminate the factors conferring resistance to penicillin and tetracycline from transducants obtained with transducing phage AK72. Penicillin-resistant or tetracycline-resistant transducants from five recipient strains were

grown in nutrient broth containing 10 μg of acridine orange per ml at pH 7.6. After overnight incubation subcultures were made and between 800 - 1000 colonies from each recipient strain were tested for resistance to penicillin or tetracycline. Colonies from control cultures in nutrient broth were also tested. Table 30 compares the number of penicillin-sensitive colonies recovered after growth of each transduced strain in the presence of acridine orange with the number of penicillin-sensitive colonies recovered after growth in nutrient broth alone. In Table 31 a similar comparison is made between the number of tetracycline-sensitive colonies recovered after treatment of the five transduced strains with acridine orange and the number recovered from the untreated strains. It is clear that neither penicillin resistance or tetracycline resistance was eliminated from the five transduced strains by acridine orange treatment. Two of the transduced strains 1/4 and S1/3 show a spontaneous loss of the marker for penicillin resistance at a rate of 0.8 per cent and 0.9 per cent. Thus in five transduced strains the response to acridine orange treatment is not in keeping with the theory that the

markers for penicillin resistance or tetracycline resistance occupy an extrachromosomal position.

TABLE 30

The failure of acridine orange treatment to eliminate penicillin resistance from staphylococcal transducants

Recipient strain transduced by phage AK72	Colonies derived from control cultures in nutrient broth		Colonies derived from broth cultures containing acridine orange 10 μg/ml.	
	Total number tested	Number penicillin sensitive	Total number tested	Number penicillin sensitive
1/1	974	0	995	2
1/4	863	7	838	8
1/11	999	2	953	2
1/12	939	1	999	1
S1/3	999	9	1000	7
S1/10	1000	0	1000	0

TABLE 31

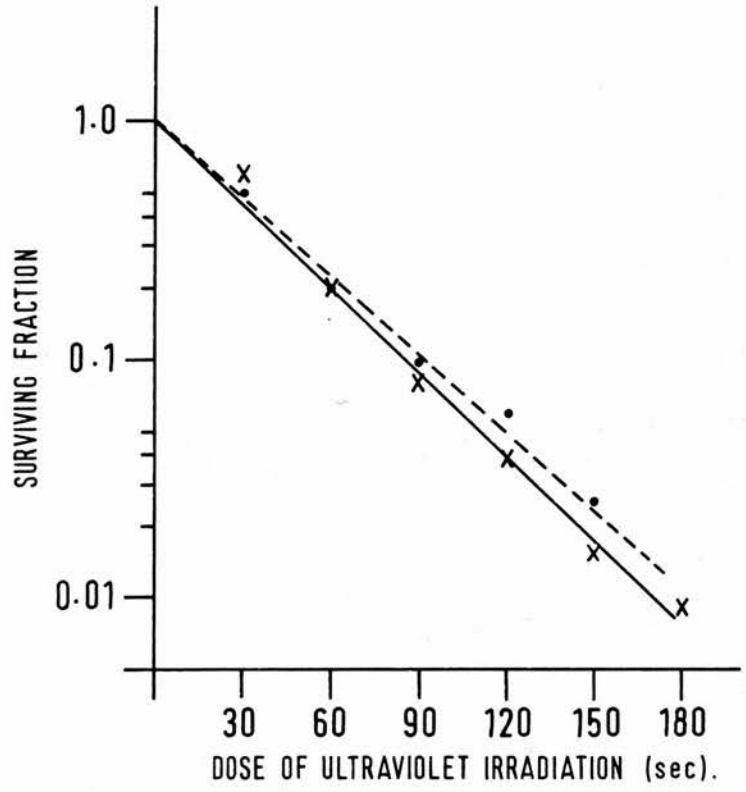
The failure of acridine orange treatment to eliminate tetracycline resistance from staphylococcal transducants

Recipient strain transduced by phage AK72	Colonies derived from control cultures in nutrient broth		Colonies derived from broth cultures containing acridine orange 10 μg/ml	
	Total number tested	Number tetracycline sensitive	Total number tested	Number tetracycline sensitive
1/1	930	0	930	0
1/4	811	3	849	1
1/11	972	0	905	1
1/12	1000	0	1000	0
S1/3	1000	1	1000	2
S1/10	1000	0	1000	0

Effect of ultraviolet irradiation on the plaque-forming titre and transducing ability of phage AK72

In the enterobacteria it has been found that ultraviolet irradiation of suspensions of transducing phages increases the frequency of production of stable transducants for chromosomal genes but does not increase the observed rate of transduction of cytoplasmic factors. Ultraviolet irradiation has been shown to increase the transducing ability of staphylococcal phage lysates for some markers but not for the marker for penicillin resistance. To confirm this finding the effect of ultraviolet irradiation on the transduction of penicillin resistance by phage AK72 was investigated.

After centrifugation, the phage AK72 was resuspended in physiological saline to give a suspension containing 10^9 p.f.u. per ml. This suspension was irradiated for three minutes and samples were withdrawn at intervals for the estimation of their plaque-forming titres and their transducing ability for the marker for penicillinase production. The results are shown in Figure 2. This graph indicates that both the plaque-forming ability and the transducing ability of phage AK72



The effect of ultraviolet irradiation on transducing phage AK 72 suspended in saline to give a titre of 10^9 p.f.u. per. ml. Plaque-forming titre (X) and transducing titre for penicillinase (•) were determined for each dose of ultraviolet irradiation (semilogarithmic plot)

FIGURE 2.

are reduced exponentially with increasing exposure to ultraviolet irradiation. There is no increase in the transducing ability of the phage. This indicates that the transduced marker is not integrated into the bacterial chromosome. The result of this experiment appears to be at variance with the result of the previous experiment. (see Discussion).

DISCUSSION

This work was undertaken to investigate three aspects of transduction in Staphylococcus aureus: (i) the character of the process, so that it could be compared with transduction in the enterobacteria; (ii) the extent of its occurrence between different strains of Staph. aureus; and (iii) the nature of some of the transduced fragments.

The transduction process.

Although transduction had been extensively studied in the enterobacteria only limited investigations had been made to determine if it could take place in Staph. aureus when the present work began in 1959. The first description of the process in staphylococci was given to the Society of American Bacteriologists by Ritz and Baldwin in 1958. They claimed to have transduced penicillinase production to a penicillin-sensitive strain of Staph. aureus by means of typing phage 52 but few details are given in the abstract of the communication. A second report followed in 1959 when Morse transduced resistance to streptomycin or novobiocin with typing phage 53. This transducing phage was induced from artificially lysogenized streptomycin-resistant or novobiocin-resistant mutants of the phage-propagating staphylococcus.

As a preliminary to the present work it was decided to attempt the transduction of streptomycin resistance as described by Morse. The results of these experiments were extremely difficult to interpret because large numbers of streptomycin-resistant mutants grew on the control plates on which the streptomycin-sensitive recipient strain had been plated alone so that it was impossible to determine whether the colonies on the transduction plates were transducants or spontaneous mutants. Morse does not record any difficulties of this kind in regard to the transduction of streptomycin resistance. When he used streptomycin-sensitive cultures as the source of the phage no streptomycin-resistant colonies were found on the plates. He does mention that a small number of novobiocin-resistant colonies were found when novobiocin-sensitive cultures were used as the source of the phage but very many more novobiocin-resistant colonies were obtained when the phage was induced from novobiocin-resistant staphylococci. It is difficult to explain the discrepancy between the results reported by Morse and those recorded in the present work. The conditions appear to be comparable as the time allowed for phenotypic expression and the amount of streptomycin in the agar overlay were the same in both sets of experiments.

This preliminary work gave valuable experience but the model was not considered suitable for further study. As it was hoped to show that transduction might play a part in the emergence of antibiotic-resistant staphylococciin nature it was decided to use naturally lysogenic staphylococci as the source of the transducing phage. Staphylococci resistant to tetracycline had been implicated in many outbreaks of staphylococcal infection in hospitals (Williams 1959). This resistance marker was therefore chosen for possible transduction as it seemed to be an important character from the clinical point of view.

The phage filtrates used in the transduction experiments each had a plaque-forming titre of more than 10^7 p.f.u. per ml. As Morse reported that only one phage particle in 10^7 to 10^8 carried the genes for resistance to streptomycin or novobiocin it did not seem to be reasonable to attempt transduction experiments with filtrates that had lower titres. Each phage was tested for the ability to transduce tetracycline resistance to two different strains of tetracycline-sensitive staphylococci. These staphylococci were sensitive to the transducing phage as it was thought that such a relationship might be necessary for transduction to take place. Two phages, induced

from donor strains AK72 and 15844, were found to transduce tetracycline resistance to both the recipient strains tested. With these systems there was no difficulty in interpreting the results of the transduction experiments. When the recipient staphylococci were seeded alone on the control plates their growth was completely inhibited by the tetracycline agar overlay; no spontaneous tetracycline-resistant mutants were found. When the recipient staphylococci were mixed with the appropriate transducing phage and seeded on the transduction plates many tetracycline-resistant colonies grew through the tetracycline agar overlay. All the transducants tested for tetracycline resistance showed an eight-fold increase in resistance to the antibiotic which was maintained after at least five subcultures on tetracycline-free medium and therefore this acquisition of tetracycline resistance appeared to be a permanent change.

The other three phages gave negative results with each of the two recipient strains tested. The negative result obtained with the phage induced from donor strain 12386 might be explained on the grounds that insufficient phage particles were present in the transduction mixture; the titre of the phage filtrate was 3×10^7 p.f.u. per ml.

and it was possible to include only 1 x 10^8 phage particles in the mixture. This was not the explanation for the failure of transduction with the other phage filtrates; both had titres of over 10^9 p.f.u. per ml. Positive results might have been obtained if a series of recipient strains had been tested but this is unlikely as three of the recipient strains tested, 360, PS7 and PS54, were transducing by other phages (Collins and McDonald 1962). A more probable explanation is that the two phages induced from strains 12888 and 14438 lacked transducing ability.

The systems in which positive results had been obtained were more closely examined. Phage AK72 which had been induced from a donor staphylococcus belonging to phage group I transduced tetracycline resistance to recipient strains 74426 and 12861 both of which also belonged to phage group I and all were sensitive to one or other of the closely related phages 80, 81 and 82. In the other system the donor strain 15844 and the recipient strains 13346 and PS47 had typing patterns belonging to Group III predominantly. Thus it appeared that a close relationship, as shown by similarity in phage type, was required between donor and recipient staphylococci before transduction could take place.

Before making a definite conclusion about the relationship between donor and recipient strains required for transduction it was necessary to test a series of recipient staphylococci representing the different phage groups and including strains both sensitive and immune to lysis by the transducing phage.

When the frequency of transduction of the tetracycline marker was calculated there was a certain amount of variation in the same system if the experiments were repeated. The frequency of transduction for phage AK72 was similar for the two recipient strains tested (289-426 transducants per 10^8 phage particles for strain 74426 and 279-478 transducants for strain 12861). These figures resemble the frequencies reported by Pattee and Baldwin (1961). They transduced chlortetracycline resistance by phage 80 to 14 recipient staphylococci that were sensitive to lysis by Group I phages. From 13 of the 14 strains 1000-9000 transducants per 10^9 phage particles were recovered. The other recipient strain, phage type 79/55, yielded only 34 transducants per 10^9 phage particles.

The transducing activity of phage 15844 appeared to be less than that of phage AK72.

With recipient strain 13346 the number of transducants recovered was 213-233 per 10^8 phage particles. With strain PS47 the number recovered was 410-821 per 10^8 phage particles but these transducants were obtained from plates with a reduced amount of tetracycline in the agar overlay. Pattee and Baldwin used phage 53, propagated on a donor strain sensitive to both Group I and Group III phages, to transduce chlortetracycline resistance to 13 recipient strains sensitive to Group I phages. Five of the 13 strains gave negative results and the number of transducants recovered from the other nine strains ranged from 200-600 per 10^9 phage particles. The 13 recipient strains were all transduced by phage 52A giving 1000-6000 transducants per 10^9 phage particles. Therefore these recipient strains were capable of being transduced at higher frequencies when the appropriate phage was used. The negative results and low frequencies could be attributed to low transducing activity of the phage or lack of a close relationship between the donor and recipient strain. In the present work, phage 15844 was tested with recipient strains belonging to Group III and of similar phage types to the donor strain; therefore a close relationship

was present. This suggests that phages may vary in their transducing activity even when they are related to the recipient staphylococcus.

The increase in the number of PS47 transducants recovered when the concentration of tetracycline was reduced in the agar overlay shows clearly that the observed frequency of transduction can be markedly influenced by this factor. The higher concentration of tetracycline inhibits the primary expression of certain transducants. It is not a question of transduction of different levels of tetracycline resistance because, on subculture all the transducants tested were resistant to over 50 μg of tetracycline per ml even if they had been isolated from the selective medium containing the lower concentration of tetracycline.

Since these experiments have shown that it is possible to transfer tetracycline resistance from one strain of staphylococcus to another, the evidence for considering this transfer to be an example of transduction will be discussed. The mechanisms of genetic transfer are (i) conjugation, (ii) transformation, (iii) phage conversion, and (iv) generalized and restricted transduction, Conjugation requires cellular contact between donor and recipient cells before transfer of genetic

material can take place. In the process described in the present study, a cell-free filtrate from the donor staphylococcus was able to transfer the marker for tetracycline resistance to the recipient strain. Clearly this mechanism of transfer is not conjugation. In transformation the unit transferred is free deoxyribonucleic acid which is inactivated by the enzyme deoxyribonuclease. When deoxyribonuclease was added to the mixture of the recipient staphylococcus and the filtrate from the donor strain the transfer of tetracycline resistance was not inhibited. This proves that free deoxyribonucleic acid was not the unit being transferred and that the process is not transformation.

Phage plays a part in the other three mechanisms of genetic transfer, phage conversion, generalized and restricted transduction. The effect of heat on the activity of the filtrates from the donor staphylococci was in keeping with the theory that phage was implicated in this transfer of tetracycline resistance. Heating the filtrates for 30 min at $60^{o}C$ removed their ability to transfer the resistance marker and reduced their plaque-forming titres. This showed that the agent concerned with the transfer

was thermolabile at 60°C and that its activity was related to the concentration of active phage present in the filtrate. If phage conversion was involved in the transfer the phage genome alone would be able to change the character of the recipient bacterium. It was shown that neither phage AK72 nor phage 15844 was able to transfer tetracycline resistance after propagation on tetracycline-sensitive staphylococci. Thus the ability of these phages to transfer tetracycline resistance depended on the presence of that resistance marker in the donor staphylococcus. This finding is evidence against the process being that of phage conversion. Moreover, the relatively low frequency of transfer of the resistance marker is not in keeping with the usual findings in phage conversion.

If the transfer of tetracycline resistance is an example of transduction, the process should be inhibited by phage antiserum. The phage filtrates obtained by propagation of each phage on tetracycline-sensitive staphylococci were used to immunize rabbits. Antisera prepared in this way completely inhibited the transfer of tetracycline resistance. This inhibitory effect of phage antiserum seems to prove that the marker for

tetracycline resistance was being carried by the phage particle. However, it could be argued that an unknown factor is released with the phage at the time of lysis of the donor bacterium and that this factor is responsible for the transfer of tetracycline resistance. If such a factor were present it could give rise to antibodies when the phage filtrate was used to immunize the rabbits. The inhibitory effect of the antiserum would then be produced not by its action on the phage but by its action on the factor. This possibility is excluded by using as the antigen a phage filtrate that is devoid of transducing ability. Such a filtrate could not contain the resistance factor and it would stimulate the production of antibodies to the phage only.

These results of my investigations prove that the transfer of tetracycline resistance is an example of transduction. The transducing phages for the experiments were induced from lysogenic donor staphylococci by ultraviolet irradiation. This method of production of the transducing phage might be taken as evidence that the process is one of restricted transduction. The marker for tetracycline resistance could have become incorporated into the transducing phage while the phage was attached to the bacterial chromosome

as a prophage or the marker could have been picked up during lytic multiplication after induction. However, the phages were shown to have transducing ability after propagation on suitable tetracycline-resistant staphylococci. This proved that the marker was picked up during lytic multiplication, therefore the transducing ability of these phages did not depend on their attachment to the bacterial chromosome. Thus this type of transduction resembles the generalized transduction mechanisms manifested in the phage P22-Salmonella and phage P1-Esch. coli systems rather than the restricted transduction that is found in the phage lambda-Esch. coli K-12 system.

Certain changes were observed in the recipient strains after transduction. Although all the transducants had become resistant to more than 50 μg of tetracycline per ml varying results were obtained when they were tested to determine if lysogenization with the transducing phage had taken place. Phage AK72 lysogenized a large percentage of the recipients but even in this system lysogenization was not completely correlated with the transfer of tetracycline resistance. Phage 15844 lysogenized a much smaller percentage of the transducants. In some transducants the two

characters that were used to define lysogenicity were not found together. This made it difficult to classify these transducants as lysogenic or non-lysogenic. When the transducants were found to be immune to the transducing phage but non-lytic for the parent staphylococcus, the explanation may be either that the methods were inadequate to demonstrate the bacterium's ability to release lytic phage or that the prophage was present in a defective state. An explanation is also required for the finding that a large number of PS47 transducants were lytic for the parent strain but still sensitive to the transducing phage. It may be that many of the transduced colonies were composed either of a mixture of lysogenic and non-lysogenic cells or of cells in an unstable state. This is not unlikely as many workers, including Luria and his colleagues (1958), have reported that during lysogenization and transduction there is a transitional period before integration of the phage genome or the transduced fragment of bacterial genome takes place.

From these results it is reasonable to conclude that the transfer of tetracycline resistance did not depend on the lysogenization of the recipient cells by the transducing phage. This is in keeping

with the results of Pattee and Baldwin (1961) who did not observe lysogenization of the recipient strains during the transduction of novobiocin or chlortetracycline resistance: Mitsuhashi and his colleagues (1965) on the other hand record that a proportion of transducants were immune to the transducing phage after the transduction of tetracycline resistance. These results probably reflect a difference in the multiplicity of infection or a difference in the selective medium; both of these factors influence the likelihood of superinfection taking place. None of the results show clearly that staphylococcal phage particles with transducing ability are defective. Dowell and Rosenblum (1962b) found that if novobiocin-resistant transducants were isolated under conditions that allowed superinfection almost all the clones were lysogenic. If superinfection was prevented the resultant transducants were non-lysogenic. Their work indicates that the transducing phage particle is defective as has been shown for the transducing phages in the systems investigated in the enterobacteria.

The changes in the phage types of the transducants in two systems in the present study were correlated with lysogenization of these

organisms by the transducing phage and comprised a loss of sensitivity to one or two of the typing phages. Similar findings are recorded by Mitsuhashi and his colleagues (1965).

A property of phages capable of generalized transduction is their ability to transduce different markers. As the donor strains from which phage AK72 and phage 15844 had been derived were resistant to penicillin as well as to tetracycline, attempts were made to transduce resistance to penicillin. Positive results were not obtained until the number of phage particles in the transduction mixture was increased and a longer incubation time was allowed for adsorption of the phage to the recipient cells. Ritz and Baldwin (1961) drew attention to the critical level of penicillin in the selective medium. In the present work plates containing 0.06 μg and 0.12 μg of penicillin per ml were used. On the plates containing the lower concentration of penicillin there was an undesirable background growth. This probably consisted of the 1st step mutants described by Ritz and Baldwin. These mutants do not produce penicillinase.

Even with the adjustments in procedure described above, the frequency of transduction, 29

transducants per 10^8 phage particles, was markedly lower than any of the frequencies obtained for the transduction of tetracycline resistance. It may be that cells were lost during centrifugation. Certainly Ritz and Baldwin, using phage 80 or phage 52 to transduce resistance to penicillin, record a frequency of between 1-2 transducants per 10^7 phage particles. Pattee and Baldwin (1961) found a much lower frequency of transduction of penicillin resistance compared with that for tetracycline resistance in comparable systems. The frequencies for the transduction of penicillin resistance reported by Mitsuhashi and his colleagues (1965) are even lower. The extremely low figures may be explained by the high concentration of penicillin (3.1 units per ml.) used in the selective medium or by a difference in the penicillin resistance marker being transduced. In these experiments the marker for penicillin resistance was linked to the marker that determined resistance to the macrolide group of antibiotics.

Ritz and Baldwin (1961) do not record any confirmatory test for penicillinase production in their paper. Mitsuhashi et al (1965) state that all transducants resistant to penicillin were shown

to produce penicillinase. In the present work penicillinase production was demonstrated by the screening method of Foley and Perret (1962) and in the first experiment 49 out of 50 transducants were found to be resistant to penicillin and to produce penicillinase. Ayliffe and Barber (1963) described a quantitative modification of the screening method. This allowed grading of staphylococci according to the rate of the reaction which indicated the amount of penicillinase produced by each strain. They found that a high proportion of staphylococci that were resistant to several antibiotics were very active penicillinase producers. This association between high penicillinase production and multiple antibiotic resistance was confirmed by Richmond and his colleagues in 1964. Correlation was also found between high penicillinase production and resistance to mercuric chloride. As the donor strains of staphylococci used in the present study were active penicillinase producers, resistant to tetracycline and resistant to mercury salts the possibility was investigated that the markers for these characters might be linked and therefore transduced together. When the 49 transducants that had been shown to produce penicillinase were tested for

resistance to mercuric chloride, all gave positive results but none was found to be resistant to tetracycline. These findings were confirmed in later experiments when transducants from a variety of recipient strains were available for testing. Thus the markers for penicillinase production and mercuric chloride are co-transduced and it is very likely that they are closely linked. Richmond and John (1964) reported similar findings.

The transduction of resistance to chloramphenicol confirmed that a variety of antibiotic resistance markers can be transduced in staphylococci. However, no co-transduction was demonstrated between tetracycline resistance and chloramphenicol resistance. The failure to demonstrate co-transduction between these markers is in keeping with the findings of Collins and Roy (1963).

The results of my experiments show that the systems of genetic transfer in staphylococci that have been studied here have the characters of generalized transduction. It has been clearly demonstrated that phage is acting as a carrier of genetic material from one strain of staphylococcus to another, that different markers may be transduced and that phage particles pick up the bacterial

genetic material during lytic multiplication. The frequencies of transduction are variable, and marked differences have been demonstrated when different markers are transduced.

The transducing phages

The possibility that all staphylococcal phages might not possess transducing ability made it of interest to investigate the characters of the transducing phages used in the present study and to compare them with the transducing phages described by other workers. Many of the workers investigating transduction in staphylococci have used typing phages of the International Typing Series. Ritz and Baldwin (1961) used phage 80 to transduce resistance to penicillin. They also reported positive transductions with mutants of phages 52, 42B and 47C. Pattee and Baldwin (1961) used phages 29, 52A, 79, 80 and 53 for the transduction of resistance to penicillin, chlortetracycline and novobiocin. Phage 53 and other phages serologically related to it were used by Edgar and Stocker (1961) to transduce non-exacting nutritional characters. The determination of the lytic spectra of the transducing phages used in the present work showed that phage AK72 resembles phage 80 in this respect and phage 15844

resembles phage 53. Dowell and Rosenblum (1962a) suggested that there might be a correlation between the serological group of a phage and its transducing ability. The majority of the transducing phages that have been described belonged to serological group B or F. It appeared at first that the mutants of phages 42B and 47C to which Ritz and Baldwin has ascribed transducing ability might be exceptions to this theory. These typing phages belong to serological group A. However Dowell and Rosenblum found that the propagating staphylococcus for phages 42B and 47C carries a phage that was neutralized by B and F antiserum and this phage was shown to have transducing ability. They suggested that the "mutant" phages isolated from phages 42B and 47C by Ritz and Baldwin might in reality be the phage carried by the propagating strain.

The antisera prepared against the transducing phages used in the present work neutralized phage 52A, a phage belonging to serological group B, and had no action against phages belonging to group A or F. This indicated that phage AK72 and phage 15844 belong to serological group B.

Staphylococcal phages do not form a homogeneous group and the most significant division has

probably been made on the basis of serology. This is correlated with differences in morphology. Bradley (1963) divided staphylococcal phages into two main groups on the basis of their structure as seen on the electron microscope. Phages belonging to the same serological group were found to belong to the same morphological group. The structure of phage AK72 and phage 15844 places them in the same morphological subgroup as other group B phages. Thus it seems that the transducing phages described in the literature and in the present work are either typing phages of Group I or Group III or have lytic spectra resembling these phages and belong to serological groups B or F. One exception appears to be phage 81 which Kuwahara and his colleagues (1963) used to transduce tetracycline and erythromycin resistance. Ritz and Baldwin (1961) state that they were unable to demonstrate transduction with phage 81. It may be that the phage 81 used by the Japanese workers which came from a local laboratory differs from the typing phage 81 used in other areas. It is of interest to note that there are no reports in the literature of Group II typing phages having transducing ability and this is in keeping with the behaviour of Group II staphylococci in transduction experiments.

The occurrence of transduction among staphylococci of different phage types.

One of the aims of the present work has been to investigate the occurrence of transduction among staphylococci of different phage types that had been isolated from different sources. The results obtained after testing representative collections of staphylococci show that phage AK72, which resembles phage 80, is able to transduce tetracycline and penicillin resistance to the majority of Group I staphylococci isolated from clinical or non-clinical sources. Differences in the phage types of these recipients and in their immunity to lysis by the transducing phage ware not related to their ability to be transduced. A higher proportion of the strains from the non-clinical sources gave positive results but the number of strains in each group was small and the difference was found to be barely significant. Fewer of the Group III strains were transduced by phage AK72 and there was no difference in results between the strains isolated from clinical and non-clinical sources in this group. From these results it would appear that a phage is most likely to transduce recipient strains that are sensitive to lysis by closely related phages. However phage 15844, although resembling a Group III typing

phage, also transduced more Group I staphylococci than Group III staphylococci. The ability of phage 15844 to transduce penicillinase production was very limited. This finding is similar to that obtained by Ritz and Baldwin (1961) with phage 53. The most outstanding result with both phages was the failure to transduce any of the Group II recipients tested. The failure to transduce antibiotic resistance to Group II staphylococci has been noted by other workers. A few positive results with such strains have been recorded by Mitsuhashi and his colleagues (1965) but the numbers of transducants recovered were extremely small. It appears that Group II staphylococci differ very markedly from other staphylococci in their failure to be transduced by phages derived from Group I or Group III organisms. Few Group II staphylococci are found to be resistant to antibiotics or to be associated with epidemics of hospital infection. It may be that these characters are related to the organisms' inability to be transduced and this point is discussed in more detail below.

Staphylococci and the Enterobacteriaceae are groups of organisms in which the emergence of antibiotic-resistant strains has become a serious clinical problem. Recently the investigation of

resistance transfer factors in the Enterobacteriaceae has indicated a possible mechanism for the development of antibiotic-resistant strains in that group (Anderson 1965). It may well be that transduction plays a similar role in the development of antibiotic-resistant strains of staphylococci. Direct proof of this hypothesis will be difficult to obtain but the results of the present work support such a theory. The antibiotic-resistant strains associated with epidemics of staphylococcal infection have phage-typing patterns belonging to the 52/52A/80/81 complex or to Group III. These are the strains that have been shown to be readily transducible. The very small number of antibiotic-resistant staphylococci found in Group II could be a result of their inability to be transduced. The Group II staphylococci that are found to be resistant to penicillin usually belong to phage type 71. Type 71 staphylococci have been associated with outbreaks of impetigo and have certain properties that differentiate them from other Group II strains (Parker 1958). However these type 71 strains do not show multiple antibiotic resistance and are not associated with hospital infections. When tested as recipient strains by the writer in transduction experiments they gave the same negative results as other Group II strains.

Thus staphylococci of the phage types associated with outbreaks of staphylococcal infection in hospitals are able to acquire the ability to produce penicillinase and to acquire resistance to other antibiotics by transduction. As Hayes (1964) wrote "it is surely one of the more bizarre manifestations of evolutionary adaptation, that a potentially lethal virus should acquire the redeeming function of a gamete, rescuing some of its victims' genes for posterity!"

The nature of the transduced markers

Anderson (1965) considered that the ease with which the determinants of antibiotic resistance in the enterobacteria are mobilized by transfer factors indicates that these determinants are cytoplasmic at the time of their mobilization. In staphylococci, the high frequency of spontaneous loss of the ability to produce penicillinase reported by Barber (1949) and the analysis of mutations affecting penicillinase production carried out by Novick in 1963 gave rise to the theory that the determinants of penicillinase production are sited on extrachromosomal elements.

In the present work transducants from five recipient strains were grown in the presence of acridine orange to determine if this treatment increased the rate of loss of the penicillinase

marker. Two of these strains were shown to have a high rate of spontaneous loss of the determinant but acridine orange treatment did not increase the rate of loss in any of the five transduced strains. Hirota (1960) found that the sex factor F was eliminated by acridine orange but this elimination has not been found for all extrachromosomal elements. Other workers have experienced variable results when the acridine dyes were used to eliminate the penicillinase marker from staphylococci. Novick (1963) did not find any increase in the frequency of loss of the marker from four strains of staphylococci after they were grown in the presence of acridine orange. Harmon and Baldwin (1964) claimed that exposure to acridine or proflavine increased the rate of loss of penicillin resistance in two strains but these workers admitted that the frequencies were low. Hashimoto and his colleagues (1964) reported the loss of the penicillinase marker from 17 out of 18 strains of staphylococci grown in the presence of acriflavine but again the frequencies of loss were low (0.1 - 3.5 per cent.). It would appear that the response to treatment with the acridine dyes by staphylococci is variable and cannot be taken as evidence for or against the extrachromosomal position of the

marker for penicillinase production. May, Houghton and Perret (1964) found that growth of a tetracycline-resistant penicillinase-producing staphylococcus at 43-44°C resulted in the emergence of progressively increasing proportions of tetracycline-sensitive penicillinase-negative staphylococci. The losses of the two determinants appeared to be independent; this finding is in keeping with my failure to demonstrate linkage between the two markers in transduction experiments. The authors suggest that not only is the marker for penicillinase production sited on an extrachromosomal element but that the marker for tetracycline resistance is sited on a different cytoplasmic element. However, in the present work acridine orange treatment was shown to have no effect on the rate of loss of the marker for tetracycline resistance.

Ultraviolet irradiation of phage AK72 produced an exponential reduction in transducing ability for the penicillinase marker. This result is in keeping with Novick's finding for the penicillinase marker but it is not in agreement with the results reported by Korman and Berman (1962) who found that the transduction of

other staphylococcal markers is increased by ultraviolet irradiation. Zinder (1953) and Arber (1960) studied the effects of ultraviolet irradiation of phages in transduction experiments in the enterobacteria. These experiments showed that such irradiation increases the frequency of transduction of markers that are integrated into the recipient chromosome. In contrast ultraviolet irradiation produced an exponential reduction in the frequency of transduction of markers that are not integrated into the chromosome of the recipient cell. If this response is the same for transduction in staphylococci as in the enterobacteria these results indicate that the marker for penicillinase production is sited on an extrachromosomal element and that the markers studied by Korman and Berman are sited on the bacterial chromosome.

Richmond (1965a) has shown that there are several different extrachromosomal elements or plasmids present in staphylococci. These plasmids carry other genetic markers as well as the penicillinase marker and he has divided the penicillinase plasmids in staphylococci of phage groups I and III into two groups depending on the compatibility of the plasmids with each other. The penicillinase marker that has been studied

in the present work is linked to the marker for mercury resistance but not to the marker for resistance to erythromycin and type A penicillinase is produced. If this penicillinase marker is associated with a plasmid then these characters indicate that the plasmid would correspond to the ∠ plasmid described by Richmond.

It would be of value to determine if any incompatibility exists between the penicillinase plasmids and extrachromosomal elements carrying other antibiotic resistance markers. It may be that the presence of a particular penicillinase plasmid in a staphylococcus prevents that organism from acquiring other antibiotic resistance markers. It would be of great academic and clinical interest if this mechanism were found to operate because it would explain why some strains of staphylococci remain sensitive to most antibiotics while other strains are well known to develop multiple antibiotic resistance.

SUMMARY

1. The known mechanisms of microbial transfer of genetic information, transformation, conjugation, phage conversion and transduction have been outlined and the literature concerning the process of transduction in Staphylococcus aureus has been considered in detail.

2. Preliminary studies on the transduction of streptomycin resistance to streptomycin-sensitive staphylococci revealed technical difficulties of interpretation which were not previously described. The possibility of transducing tetracycline resistance in staphylococci was chosen for further study.

3. Ten naturally lysogenic, tetracycline-resistant staphylococci were induced by ultraviolet irradiation. Five of these lysates contained more than 10^7 phage particles per ml after filtration, and two of the five filtrates were found to transfer tetracycline resistance to tetracycline-sensitive strains of staphylococci.

4. The transfer of tetracycline resistance was shown to be mediated by phage and the mechanism had the characters of transduction.

5. In each of these transduction systems there was a similarity between the phage type of the staphylococcus from which the phage had been induced and the phages types of the recipient strains.

6. A variable number of the transducants were found to be lysogenized by the transducing phages. Lysogenization of two recipient strains was accompanied by changes in the phage type of the transducants.

7. The donor staphylococci from which these two transducing phages had been derived were resistant to penicillin as well as to tetracycline. Both phages were shown to transduce penicillin resistance. The acquisition of penicillin resistance by the receipient strains was correlated with the acquisition of the ability to produce penicillinase.

8. Co-transduction of the marker for penicillinase production and the marker for resistance to mercuric chloride took place. Transduction of tetracycline resistance did not accompany the transduction of penicillinase production.

9. The transduction of chloramphenicol resistance was demonstrated by means of a phage induced from a naturally lysogenic, chloramphenicol-resistant staphylococcus.

10. Transduction of chloramphenicol resistance was not accompanied by the transduction of tetracycline resistance.

11. The number of transducants recovered in relation to a given number of phage particles depended on the transducing phage used and on the marker being transduced. The highest frequency was obtained for the transduction of chloramphenicol resistance and the lowest for the transduction of penicillinase production.

12. The characters of the two phages that were capable of transducing tetracycline resistance and the ability to produce penicillinase were investigated. The phage that had been induced from a donor staphylococcus belonging to phage group I had a lytic spectrum resembling the

spectrum of typing phage 80. The other phage which had been induced from a staphylococcus of phage group III had a lytic spectrum resembling that of typing phage 53.

13. Both of the transducing phages had the serological and the morphological characters of group B phages.

14. Collections of staphylococci representative of the three main phage groups were drawn from clinical and non-clinical sources.

15. Both of the phages transduced tetracycline resistance to the majority of 62 strains of staphylococci belonging to Group I and to a smaller percentage of the 36 Group III strains that were tested.

16. The phage derived from the Group I donor staphylococcus also transduced penicillinase production to the majority of Group I staphylococci but the phage induced from the Group III staphylococcus had a very limited ability to transduce penicillinase production.

17. Both of the phages failed to transduce any of the 60 strains of staphylococci belonging to Group II.

18. The nature of the penicillinase marker was studied. Treatment with acridine orange failed to eliminate the marker from five transduced strains. This finding is not in keeping with the theory that the marker for penicillinase production is sited on an extrachromosomal element.

19. Ultraviolet irradiation produced an exponential reduction in the transducing ability for the marker for penicillinase production. This finding indicates that the transduced marker is not integrated into the chromosome of the recipient bacterium.

20. The significance of these results is discussed in relation to the hypothesis that transduction may play a part in the emergence of antibiotic-resistant strains of staphylococci in nature.

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