PHAGE AND BACTERIOCIN RECEPTOR ACTIVITY OF A *PROTEUS VULGARIS* STRAIN

by

Lenard Sidney Steinhardt

Submitted in partial fulfilment of the
requirements for the degree of
M.Sc.

in the Faculty of Science,
University of Pretoria,
Pretoria.

November, 1971

To my Parents

ACKNOWLEDGEMENTS

I wish to convey my gratitude to Professor J.N. Coetzee, Head of the Department of Microbiology, University of Pretoria and Director of the Medical Research Council Unit for Microbial Genetics for his sincere interest and inspiration; and for the provision of facilities for this study.

I am also indebted to Professor H.C. de Klerk for the many hours of assistance and guidance rendered by him throughout the duration of this thesis.

A word of thanks too, to Professor O.W. Prozesky for his encouragement and advice at all times.

Dr. C.R. Jansen of the Division of Life Sciences of the South African Atomic Energy Board is thanked for the use of laboratory facilities.

Mr. N. Hugo, also of the Division of Life Sciences, is thanked for invaluable assistance in respect of the electron microscopy studies.

The competent technical assistance of Miss H. Roos is gratefully acknowledged.

A special word of thanks to my wife for her understanding, assistance and unflagging encouragement.

CONTENTS

SUMMARY

Two hundred and sixty three Dienes incompatible strains of *Proteus vulgaris* were examined for the ability to produce bacteriocins active on *Proteus vulgaris* strain 69. This strain is the host organism in a transduction system utilizing a *P. vulgaris* generalized transducing phage, Ø107/69. Substances inhibitory for *P. vulgaris* strain 69 were obtained from twelve of the *P. vulgaris* strains upon induction by ultraviolet irradiation or Mitomycin C treatment. Electron microscopy of the active principle from the 12 inhibitor strains revealed the presence of phage-tail-like structures morphologically similar to the tail of phage 107/69.

Fifty mutants of strain 69 were selected for resistance towards each of the 12 bacteriocins obtained. All 50 x 12 bacteriocin-resistant mutants displayed simultaneous resistance towards the transducing phage 107/69. A number of mutants of strain 69 selected for resistance to phage 107/69 exhibited a similar cross-resistance towards all 12 bacteriocins.

The bacteriocins could be classified into 2 groups on the basis of activity patterns on many mutants of strain 69 and on numerous other strains of *P. vulgaris, P. mirabilis, P. morganii* and *Serratia marcescens.* Although all the bacteriocins were found to be morphologically similar, they were also classifiable serologically into the same 2 groups. A weak serological relationship was observed between the 2 groups of bacteriocins and phage 107/69.

The 12 bacteriocins are similar in another way. They all induce phage 107/69 development in *P. vulgaris* strain 107. This phenomenon which has been encountered with colicins and megacins was not further investigated.

It is concluded that the *P. vulgaris* phage-tail-like bacteriocins may represent the products of defective lysogeny, and that the members of the 2 groups and phage 107/69 adsorb to non-identical but closely linked receptor sites on the sensitive cell's surface.

SAMEVATTING

Twee honderd drie-en-sestig stamme van *Proteus vulgaris,* wat Dienes onverenigbaar is, is ondersoek vir die eienskap van bakteriosinogenie. As indikator is *Proteus vulgaris* stam 69, die gasheer vir die algemeen transduserende faag, Ø107/69, gebruik. Na behandeling met Mitomycin C of ultravioletbestraling produseer 12 stamme substanse wat stam 69 inhibeer. 'n Elektron mikroskopiese ondersoek van lisate van al 12 stamme het aan die lig gebring dat die aktiewe substans faagstertagtige strukture is. Hierdie strukture vertoon 'n morfologiese ooreenkoms met die stert van faag 107/69.

Bakteriosienbestande mutante van stam 69 is gebruik in kruis-bestandheidstoetse teen die 12 bakteriosiene. Vyftig mutante teen elke bakteriosien is bevind om gelyktydig bestand te wees teen faag 107/69. 'n Soortgelyke bestandheidspatroon is bespeur by mutante bestand teen faag 107/69.

Op grond van hul aktiwiteitspektra teen die mutante van stam 69 en verskeie ander stamme van *P. vulgaris, P. mirabilis, P. morganii* en *Serratia marcescens,* kon die 12 bakteriosiene in 2 groepe verdeel word. Die bakteriosiene van al 12 stamme vertoon morfologies identies, maar die gebruik van serologie ondersteun die klassifikasie van die bakteriosiene in 2 groepe. 'n Swak serologiese verwantskap is gevind tussen hierdie 2 groepe en faag 107/69.

Die feit dat al 12 bakteriosiene in staat is om faag 107/69 te induseer uit *P. vulgaris* stam 107, dui op 'n verdere onderlinge verwantskap. Hierdie eienskap, reeds waargeneem by sekere bakteriosiene van *Escherichia coli* en *Bacillus megaterium,* is nie verder ondersoek nie.

Dit word voorgestel dat die bakteriosiene van *P. vulgaris* moontlik produkte is van defektiewe lisogenie en dat die 2 groepe en faag 107/69 aan nie-identiese, maar nouverwante setels op die oppervlak van stam 69 adsorbeer.

CHAPTER I

INTRODUCTION

The term antibiotic is generally used to indicate an antibacterial substance derived from a living source. As early as 1889, Vuillemin introduced the term 'antibiosis' which means, literally, 'against life' (Barber & Garrod, 1963). It was ten years later, in 1899, that Emmerich & Löw described the antibacterial properties inherent in *Pseudomonas aeruginosa* (see Topley & Wilson, 1964). Among the antibacterial substances found, were those named pyocyanase (Emmerich & Löw, 1899), pyocyanin (Ehrismann, 1934) and α-hydroxy-phenazine (Schoental, 1941), all of which are active against a large variety of both Gram-positive and Gram-negative bacilli.

Thus it was, that although antagonism between different species and even members of the same species was known to be by no means a rare occurrence throughout nature, the study of bacteriocins as such, really dates back to 1925 when Gratia observed inhibition of *Escherichia coli* Ø by *E. coli* V (Gratia, 1925). One of the outstanding features of this case of antibiotic activity, in contrast to the earlier observation on *P. aeruginosa,* was its bactericidal specificity. The inhibitory substance, named colicin by Gratia and Fredericq in 1946, diffuses through agar and cellophane membranes, may be precipitated by acetone, is resistant to chloroform and is relatively thermostable, as well as being non-antigenic (Adams, 1959).

However, it is that specific property of bacteriocins which limits the range of their antibacterial spectra and which sets them in a class of their own with regard to the wider significance of the term 'antibiotic'. Jacob *et al.* (1953) defined bacteriocins as proteinatious substances, the biosynthesis of which is associated with a lethal consequence for the producing organism and non-occurrence of multiplication of the bactericide. The action of a bacteriocin is restricted to a limited number of related species, and some act only on certain strains of the same species, the action being determined by the presence of specific receptors. It should be stressed that it is this very narrow range of their antibacterial spectra which sharply delineates bacteriocins from the usual antibiotics (Ivanovics, 1962).

In recent years the induction of bacteriophage-like structures which exhibit the classical criteria afforded to bacteriocins but which do not multiply as bacteriophages,

have been isolated from many bacteria. Due to their conformance to the operational definition of bacteriocinogeny, the present tendency is to regard them as products of the bacteriocinogenic state, regardless of their unusual morphology with respect to the so-called 'classic' bacteriocins of relatively low molecular weight (Bradley, 1967). For the sake of brevity therefore, such particulate bactericidal objects would be referred to as high molecular weight bacteriocins.

According to Reeves (1965) and Bradley (1967) the generalized practice of naming the various bacteriocin families follows the example set by Gratia & Fredericq (1946) of basing the name of the bacteriocin concerned on the classification of its producing host bacterium. Due perhaps to the high degree of specificity of bacteriocins, the name is almost always based on the specific, rather than the generic name of the host organism. Thus, colicins are bacteriocins of *E. coli,* monocins of *Listeria monocytogenes,* and so on. A number of bacteriocins have been described from certain species which have not been named specifically according to the above-mentioned practice. An example of this are those obtained from the *Proteus* species (Cradock-Watson, 1965; Coetzee *et al.,* 1968; Taubeneck, 1963), which are merely referred to by the general term of bacteriocin.

Individual bacteriocins are usually referred to by the name of the producer strain, followed by the type designation. Thus, colicin K235–K is the colicin of type K, produced by *E. coli* K235 (Reeves, 1965).

The Family *Enterobacteriaceae* has for many years been recognised as a prolific source of bacteriocinogenic organisms. The genus *Proteus* was for a number of years conspicuous amongst the enteric bacteria with regards to its apparent lack of bacteriocinogeny. The discovery of bacteriocinogeny amongst strains of *Proteus hauseri* (Cradock-Watson, 1965) was followed by the demonstration of bacteriocinogenic organisms amongst strains of Providence and *Proteus morganii* by Coetzee in 1967.

This department has long been concerned with studies on the Proteus group of bacteria and their attendant bacteriophages. As a consequence of this interest, Coetzee, de Klerk, Coetzee & Smit (1968) investigated the incidence of bacteriocinogeny amongst many strains of locally isolated *P. vulgaris.* They observed that 57% of strains tested liberated high-molecular weight phage-tail-like bacteriocins. A striking observation which arose from this work was the remarkable morphological similarity of these structures to the tail of a *P. vulgaris* temperate transducing phage isolated from *P. vulgaris* strain 107, by Coetzee, de Klerk & Smit a year earlier. The host organism for this phage is

P. vulgaris strain 69. These two considerations, namely the possession of a *P. vulgaris* transduction system, and the morphological similarity between the *P. vulgaris* bacteriocins and this phage, provided the initial stimulus as motivation for this thesis. It was decided to undertake a search for *P. vulgaris* bacteriocins active on strain 69. By obtaining such bacteriocins it was hoped that mutants of strain 69 resistant to the bacteriocins could be isolated and utilized as donors in the transduction of the resistance determinant/s to bacteriocin-sensitive cells. Furthermore, an attempt was to be made to investigate qualitatively the possible relationship between phage 107/69 and the phage-tail-like structures. The morphological similarity between these particles and the tail of phage 107/69 has already been mentioned. The question which arose from this observation was whether there might not be other mutual characteristics inherent to these two entities, and it was on the basis of these considerations that this study evolved.

REFERENCES

ADAMS, M.H. (1959). *Bacteriophages.* Interscience Publishers Inc. New York.

BARBER, M. & GARROD, L.P. (1963). *Antibiotic and Chemotherapy.* E. & S. Livingstone Ltd., Edinburgh & London.

BRADLEY, D.E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriological Reviews 31,* 230.

COETZEE, J.N. (1967). Bacteriocinogeny in strains of Providence and *Proteus morganii. Nature, London* 213, 614.

COETZEE, H.L., DE KLERK, H.C., COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like particles associated with intra-species killing of *Proteus vulgaris. Journal of General Virology 2,* 29.

COETZEE, J.N., DE KLERK, H.C. & SMIT, J.A. (1967). A transducing bacteriophage for *Proteus vulgaris. Journal of general Virology 1,* 561.

CRADOCK-WATSON, J.E. (1965). The production of bacteriocines by *Proteus* species. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 385.

EHRISMANN, O. (1934). Pyocyanin und Bakerienantmung. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygeine: Erste Abteilung: Originale 116,* 209.

CHAPTER II

REVIEW OF LITERATURE

DISTRIBUTION OF THE BACTERIOCINS

Due to the early acceptance of the *Escherichia* species of coliform bacteria as convenient 'biological tools', it was from these organisms that the first bacteriocins were isolated (Reeves, 1965). It has subsequently been found that numerous genera of the Orders *Pseudomonadales* and *Eubacteriales* also contain strains which liberate similar substances besides the bacterial viruses (Bradley, 1967). No apparent correlation exists between the Gram-positive and Gram-negative bacteria with respect to bacteriocinogeny, both types being capable of displaying the property (Reeves, 1965).

The Family *Enterobacteriaceae* is by far the most comprehensive bacteriocinogenic taxonome. By 1963 every group of this family was shown to produce bacteriocins except the Proteus-Providentia group (Hamon & Perón, 1963). The discovery of bacteriocin production in strains of *Proteus hauseri* (Cradock-Watson, 1965) and in strains of Providence and *Proteus moganii* (Coetzee, 1967) completed this omission. Coetzee *et al.* (1968) demonstrated the production of high-molecular weight phage-tail-like bacteriocins from many species of *P. vulgaris,* which were associated with intra-species killing of this bacterium.

No bacteriocins have as yet been discovered from strains of *P. rettgeri* (Coetzee, 1967). A striking feature of bacteriocinogeny is its apparently high incidence amongst the genera studied in this connection (Bradley, 1967). The bacteriocins of the genus *Escherichia* were the first to be studied intensively. By 1948, Fredericq has grouped these "colicins" into 17 types, based on their spectrum of activity and the specificity of resistant mutants. Hamon (1964) later described 7 new types, designated E4, N, P, V2, V3, V4 and V5.

Fredericq's early studies also showed that within each species there is a tendency to produce only certain colicin types. Thus, *E. freundii* produces type A only, whilst Paracolobactrum produces only J or K; Shigella produces only the S types, and only colicin I, K and B are produced by the *Salmonella* species (Reeves, 1965).

Hamon & Perón (1963) have been responsible for the investigation and elucidation

of the properties of a large number of the 'families' of bacteriocins. They found that 30% of *Hafnia* spp. produce the alveicins which are active on many other strains of the same species. In addition, the caratovoracins and arizonacins produced by strains of Erwinia and *Paracolobactrum arizonae* respectively, were described in the same year. Two years previously, Hamon & Perón (1961) showed that 27% of *Enterobacter cloacae* strains studied produced the cloacins, active on certain *E. coli, Xanthomonas* and *Erwinia* spp. On the basis of activity spectra, six different cloacins have been identified. It was shown by Papavassiliou (1960) that certain colicin-resistant mutants of *E. coli* were also resistant to some cloacins, suggesting similarity of the receptor.

Hamon, Veron & Perón (1961) showed that 50% of *Pseudomonas fluorescens* produced fluocins. These bacteriocins had different activity spectra on the *P. fluorescens* studied, indicating a wide range of different types to be discovered. Only 5% were active on strains of *P. aeruginosa* and Erwinia strains tested (Hamon & Perón, 1962).

The pneumocins produced by 34% of *Klebsiella* spp. and aerocins produced by 75% of strains of *Aerobacter aerogenes* studied, were also described by Hamon & Perón in 1963. These two bacteriocins appear to be reciprocal with regard to their activity spectra, both being bactericidal for strains of the species producing the alternative type.

Jacob (1954) studied a pyocin produced by *Pseudomonas aeruginosa* and demonstrated several of its properties. Hamon (1956) and Hamon, Veron & Perón (1961) have subsequently shown that 94% of strains of *Pseudomonas aeruginosa* studied are bacteriocinogenic, active mainly on other *P. aeruginosa* strains. Some were active on strains of *P. fluorescens.* 17 types of pyocins have been described (Reeves, 1965). Hamon (1956) found that smooth strains of Salmonella, Shigella, and *E. coli* are generally resistant to pyocins, whereas rough strains are very sensitive.

The genus *Serratia* has proved to be highly bacteriocinogenic. Hamon & Perón (1961) found that 86% of strains produced bacteriocins which were named marcescins, due to the high incidence from *S. marcescens* in particular. Most of these were also bactericidal for *E. coli* B and *E. coli* K12. In a similar study, Mandel & Mohn (1962) showed 100% bacteriocinogeny amongst *Serratia* spp. studied. It is probable that there are two types of marcescin. One type appears to be relatively trypsin-sensitive and only capable of inhibiting *E. coli,* whilst the other is active only on *Serratia* spp. and is trypsin-resistant (Hamon & Perón, 1962).

Ben-Gurion & Hertman (1958) described a bacteriocin from *Pasteurella pestis,* with a 95% incidence of production amongst strains studied, all of which were inhibitory only for *P. pseudotuberculosis* and none for *P. pestis.* Brubaker & Surgalla (1961) found that 77 of 80 strains produced this antibiotic which has been named pesticin I. A second pesticin, named pesticin II, was also described by Brubaker & Surgalla (1961) which was active on 2 strains of *P. pestis,* both of which, interestingly, are pesticinogenic for pesticin II. The following year, Brubaker & Surgalla demonstrated that strains producing pesticin I produce an inhibitor to it, and fractionation to remove the inhibitor yields a 100- to 1000-fold increase in observed activity. The chemical nature of this inhibitor is not known (Brubaker & Surgalla, 1962).

Certain strains of *Alcaligenes faecalis* are able to produce bacteriocins(Maré & Coetzee, 1964). These bacteriocins are inhibitory for many members of the same species, as well as for a large number of Escherichia, Salmonella, Serratia, Staphylococcus and Proteus strains.

Farkas-Himsley & Seyfried (1963a) reported the production of a bacteriocin, which they named vibriocin, by strains of *Vibrio comma.* It was subsequently shown that vibriocin production was linked to Streptomycin sensitivity, whilst susceptibility to the bacteriocin was largely associated with Streptomycin resistant strains of *Vibrio comma* (Farkas-Himsley & Seyfried, 1963b).

Atkinson (1966) discovered a colicin-like antibiotic produced by Salmonella which was named salmonellin. In an investigation of 1825 strains of a wide variety of Salmonella serotypes and Kauffmann-White groups, between 5 and 10% were found to produce this antibiotic, The majority of these strains were sensitive to salmonellin, many of which were sensitive to at least one colicin. This combination was suggested as forming the basis for more exact strain identification of Salmonellas (Atkinson, 1970).

In an investigation of the incidence of lysogeny amongst 60 strains of Shigella, Fastier (1949) discovered a bacteriocin produced by a culture of *Shigella paradysenteriae* Type XI, which was found to have an antibiotic spectrum limited to certain members of the Paradysentery group.

Kingsbury (1966) described the production of an inducible meningocin by *Neisseria meningitidis.* These bacteriocins show a high degree of inhibitory specificity towards other strains of meningococcus. This property was used to type several serologically – identical meningococci into distinct bacteriocin groups (Kingsbury, 1966).

Mitchell, Newman & Eisenstark (1959) have demonstrated a substance produced by a strain of Brucella which behaves in a manner indicative of bacteriocinogeny. However, the 'bacteriocin' has the tendency to produce phage-like plaques on sensitive indicator strains of Brucella. Single plaque isolates do not give rise to additional phage and it is this absence of phage propagation, coupled with the absence of inhibition of the indicator at 100-fold dilution of the producer-lysate, which has prompted the tentative suggestion of bacteriocinogeny in this instance.

Amako, Tokiwa & Takeya (1970) demonstrated two types of bacteriocin released by the induction of *Shigella sonnei* strain 100052. The product of the lysis of this strain is unique in that only one host is known for this bacteriocin (Abbott & Shannon, 1958).

The Gram-positive bacteria have also contributed a number of types of bacteriocin.

The genus *Bacillus* has yielded the megacins and cerecins produced by strains of *Bacillus megaterium* and *B. cereus* respectively. Ivanovics & Nagy (1958) demonstrated a 48% incidence of bacteriocinogeny amongst 200 strains of *B. megaterium* examined for an antagonistic effect against members of the same species. Many were active only after induction by ultraviolet irradiation. As with pesticin II, megacinogenic strains exhibited no immunity to megacin. Ivanovics, Alföldi & Abraham (1955) showed sensitivity to these megacins amongst strains of some pigment-forming aerococci, in addition to certain *B. anthracis* and *B. subtilis* strains. A second megacin, type C, was described by Holland (1963), specific only for other strains of the same species.

McCloy (1951), investigating lysogeny amongst strains of *B. cereus,* described the first cerecin. In a more detailed study, Hamon & Perón (1963) found that they do not act on any known indicator strains for the bacteriocins of Gram-negative bacteria.

More than half of the strains of various *Streptococcus* species tested by Brock, Peacher & Pierson (1963) produced the bacteriocins known as enterococcins. They classified them into five types on the basis of their activity spectra, their sensitivity to heat, proteolytic enzymes and chloroform. Type I, produced by all strains of *S. zymogenes*, acts on all other strains of Enterococci and all other Gram-positive bacteria tested. It is probably identical with the hemolysin produced by all strains of this species (Reeves, 1965). Type 2, produced by some strains of *S. liquefaciens* acts on all strains of *S. faecium* and some *S. faecalis.* Types 3, 4 and 5 also showed some correlation on the basis of production and activity spectra to the classification of this group of strepto-

tericidal protein and not for the associated lipopolysaccharide moiety. (Nomura, 1967).

Ribi *et al.* (1964) reported that dissociation of phenol-extracted Gram-negative bacterial cell walls yielded a macro-molecular endotoxin complex consisting of protein, lipid and polysaccharide. The polysaccharide was associated with the O somatic antigen of the cell wall; the protein moiety apparently not contributing to the toxicity of the endotoxin complex. According to Westphal & Luderitz (1954), this toxicity resides in the lipid fraction and the polysaccharide and protein constituents act merely in an orientation and carrier capacity.

Kunugita & Matsuhashi (1970), using the techniques of Herschman & Helinski (1967) for the purification of colicins E2 and E3, found that purified colicin K consisted of a single protein free from polysaccharide, with a molecular weight of 70000. This is compatible with the K purification data of Dandeu & Barbu (1967) from *E. coli* K12 Co1 K^+. The colicin from this organism is a protein-free poly-saccharide which contains all the amino-acids but cysteine.

Jesaitis (1970) showed that the colicin K derived from *Proteus mirabilis* Co1 K^+ by means of Mitomycin C induction is a protein of low molecular weight, having the same immunological and bacterial specificities as colicin K derived from the *E. coli* K12 Co1 K^+ bacillus. The colicin obtained by Mitomycin C induction is a protein which is unconjugated with other antigens of the Proteus bacillus. It contains all the amino-acids save for cysteine (Jesaitis, 1970). This corroborates the findings of Tsao & Goebel (1969), who showed that the induced colicin K from *E. coli* K235 is not associated with the somatic antigen of the producing strain. In contrast, the colicins produced by non-inducing bacteria are protein-lipopolysaccharide complexes containing the somatic antigen of the colicinogenic micro-organisms (Goebel & Barry, 1958; Nüske, Hösel, Venner & Zinner, 1957; Barry, Everhart & Graham, 1963; Hinsdill & Goebel, 1966; Hutton & Goebel, 1962.) The processes which lead to the formation of the two types of bacteriocins are not fully understood (Jesaitis, 1970).

Colicin K357-V (Hutton & Goebel, 1961) and colicin SG-710 (Nüske *et al.*, 1957) have been shown to be protein-lipocarbohydrate complexes. Both these colicins are trypsin-sensitive, but it has not been shown whether their bactericidal activity resides in the protein fraction alone (Nomura, 1967). Further purification of colicin V (Hutton & Goebel, 1962) produced an electrophoretically and serologically homogenous substance which was considered to be analogous to the somatic antigen of the producer strain.

McGeachie & McCormick (1969), in a comparative study of primary extracts of colicins K and V, suggested that the antibiotic activity of colicin V is less closely bound to the endotoxin constituent of the producer strain than is the case with colicin K. The antibiotic activity of colicin V was found to be more soluble in alcohol, indicating a closer association of protein and lipid moieties for this colicin that is the case with colicin K.

Colicin A was shown by Barry, Everhart, Abbott & Graham (1965) to be a macromolecular substance constituted of carbohydrate, lipid and 67% protein, being free of nucleic acid. It is highly thermostable. Its bactericidal activity is destroyed by trypsin. Antiserum prepared against the micro-organism, neutralizes the biological activity of colicin A, which has been found to be highly antigenic.

Chemical analysis of colicin I (Keene, 1966) has shown that, like colicin A, it is a protein conjugated to a lipid-carbohydrate complex.

Senior (1968) showed that although B-type colicins from different colicinogenic strains have identical spectra of activity, they may be differentiated serologically. Their antigenic behaviour was shown to be indistinguishable from that attributed to the O-antigens of the strains that produced them (Senior & Emslie-Smith, 1969).

The purification of colicin CA42-E2 (Reeves, 1963) yielded a substance of relatively low molecular weight with a sedimentation constant of 3,6S. It consisted of 80% protein and 10% carbohydrate. No lipid fraction was detected. According to Reeves, (1965), this high protein content makes it unlikely to be the O antigen of *E. coli* strain CA42.

Herschman & Helinski (1967) purified another E2 type colicin, known as colicin P9—E2. This substance was shown to be a simple protein composed of all the amino-acids with a sedimentation constant of 4,0 and a molecular weight 60000.

Colicin CA38-E3 (Herschman & Helinski, 1967) has a similar composition and molecular weight as colicin P9—E2. Amino-acid and immunological analyses of colicins E2 and E3 suggested that there are regions of similar structure in the two proteins as well as regions unique to each (Herschman & Helinski, 1967).

Schwartz & Helinski (1968) investigated the nature of colicin El elaborated by *E. coli* strain JC411 and showed that it was also a basic protein of molecular weight 55000, being free of lipid or carbohydrate.

It is evident that there are two different kinds of low molecular weight colicins purified so far: one complexed with lipo-polysaccharide, and one a protein. The second group appears to be 10 to 100 times more active (Nomura, 1967).

An entirely different colicin-like substance called colicin 15 was discovered by Ryan, Fried & Mukai (1955). It was shown to have a morphology similar to that of many bacteriophages (Endo *et al.,* 1965; Menningmann, 1965; Sandoval, Reilly & Tandler, 1965). In addition to its high molecular weight, it has several features not found in other colicins. One of these is cellular lysis after induction with ultraviolet light or other inducing agents (Mukai, 1960; Mennigmann, 1965). This is in contrast to the absence of lysis in the induction of most other colicins (Kellenberger & Kellenberger, 1956; Ozeki, Stocker & de Margerie, 1959). It is considered that colicin 15 is a kind of defective phage resembling those discovered in *Bacillus subtilis* (Seaman *et al.,* 1964; Bradley, 1967; Ionesco, Ryter & Schaeffer, 1963; Stickler, Tucker & Kay, 1965).

An electron microscopic investigation of the colicin H activity of *E. coli* A10 also conceded particles similar to colicin 15 (Bradley & Dewar, 1966).

A study by Kellenberger & Kellenberger (1956) showed that colicin ML had two active components. One was very thermolabile, chloroform-resistant and sedimentable at 25,000 x g, suggesting phage components. The other was thermostable, chloroform-sensitive and not sedimentable, as are most colicins. Electron microscopy by means of the comparatively inefficient shadow-casting technique did not produce any definitive results concerning their detailed morphology. (Bradley, 1967).

Smit, de Klerk & Coetzee (1968) showed the *Proteus morganii* bacteriocin MR336 to be a thermolabile glycoprotein, consisting of 75% protein and 10% carbohydrate. This composition is similar to that found for colicin CA42-E2, also known as colicin F (Reeves, 1963). Bacteriocin 336 contains no sulfhydryl amino-acids, and no lipid moiety was detected. Extraction of the protein moiety with phenol destroyed all activity, as did treatment with proteolytic enzymes (Smit *et al.,* 1968).

A non-inducible bacteriocin from *Lactobacillus fermenti* was shown by de Klerk & Smit (1967) to be a thermostable, lipo-carbohydrate-protein complex, whose biological activity was dependant on its structural integrity. This bacteriocin consisted of 16 amino-acids, 4 sugars, hexosamine and phosphorus.

The bacteriocins produced by the induction of many strains of *P. vulgaris* were shown by Coetzee, de Klerk, Coetzee & Smit (1968) to be high molecular weight phage-tail-like particles which do not contain DNA. These bacteriocins appear similar to the structures discovered by Taubeneck (1963) from *P. mirabilis* strain 52, which are bactericidal for some *P. mirabilis* and *P. vulgaris* strains.

The bacteriocins of *Pseudomonas aeruginosa* appear varied in their physico-chemical nature (Nomura, 1967). Homma & Suzuki (1964; 1966) isolated a simple low molecular weight protein with pyocin activity from *P. aeruginosa* strain P I–III. The pyocin from *P. aeruginosa* strain R purified by Kageyama & Egami (1962) and later by Kageyama (1964) yielded a lipocarbohydrate-free protein, lacking nucleic acid. Electron microscopic examination (Ishii, Nishi & Egami, 1965) showed a phage-tail-like structure. As with colicin 15, synthesis of this bacteriocin is inducible and is accompanied by cellular lysis of the producer strain. (Ikeda, Kageyama & Egami, 1964). Bradley & Dewar (1966) studied the morphology of the pyocin from *P. aeruginosa* strain Götze and showed it to consist of a mixture of uncontracted and contracted phage-tail-like particles with the contracted form being more prolific. In contrast, studies of the original pyocin of Jacob (1954) from strain C10 indicated an excess of the uncontracted form. It has been suggested that the uncontracted form may contain some nucleic acid, probably DNA (Bradley, 1967). A bacteriocin liberated by *P. aeruginosa* C9, similar in nature, was described by Higerd, Baechler & Berk (1967).

An entirely different morphology has been shown for pyocin 28. This consists only of strands of polysheath-like material in the form of long flexible rods of variable length. They are thermolabile at 60°C and are usually hollow, but appear nevertheless, to constitute the active principle (Takeya *et al.*, 1967).

Hamon & Péron (1963) provided an indication of the nature of *Listeria monocytogenes* bacteriocins when they noted their thermolability and trypsin-resistance. These properties suggested their being phage components. In a later study, (Hamon & Péron, 1966), electron microscopy revealed many monocins to be phage-tail-like structures, most of which appeared contracted. They are the largest bacteriocins studied, being 3000Å long (Bradley, 1967).

A diffusable extracellular substance produced by a phage type 71 Staphylococcus was characterized by Dajani & Wannamaker (1969). It was shown to be a thermostable trypsin-sensitive protein. Production of the substance was inhibited by ultraviolet light or

Mitomycin C. This property and the extracellular location of the material are considered unusual for a typical bacteriocin (Dajani, Gray & Wannamaker, 1970).

Farkas-Himsley & Seyfried (1965). investigated the bacteriocin of *Vibrio comma*. This vibriocin was found to be of high molecular weight and was inactivated by many proteolytic enzymes in addition to trypsin. Chemical analysis of the substance indicated small amounts of nucleic acids, both DNA and RNA. The possibility of this bacteriocin being a defective phage was suggested (Farkas-Himsley & Seyfried, 1965).

Megacin 216 was shown by Ivanovics *et al.* (1959) to be antigenic and sensitive to proteolytic enzymes pepsin and chymotrypsin. Holland (1961) purified it as a simple protein of molecular weight 51000 which was resistant to pepsin, trypsin and chymotrypsin. Ozaki *et al.* (1966) have shown that megacin 216 has phospholipase A activity, and that both this activity and the megacin activity reside within the same substance. This indicates that megacin 216 is chemically distinct from other well-studied bacteriocins and may be a simple hydrolytic enzyme (Nomura, 1967). Nagy, Alföldi & Ivanovics (1959) have shown that all 17 type A megacins including megacin 216 are antigenically distinct, although all kill all strains of *B. megaterium*. It appears that these megacins, although they have the same activity spectrum, are chemically different (Reeves, 1965).

Brock & Davie (1963) have shown that the production or loss of ability to produce both bacteriocin activity and hemolytic activity from *Streptococcus zymogenes* are in parallel in many strains. Both activities were destroyed at the same rate by chloroform and heating to 45^{o}C and were antagonized by lecithin. The identity of this bacteriocin as a Group D Hemolysin was suggested (Brock & Davie, 1963).

The antibacterial substances classified as bacteriocins represent a heterogenous group of substances ranging from a simple protein through protein through protein-lipocarbohydrate complex structures. In each case the part responsible for killing activity seems to be the protein moiety (Nomura, 1967).

BACTERIOCINOGENIC FACTORS

The property of producing a bacteriocin is a hereditary characteristic of bacteriocinogenic organisms, governed by a genetic determinant, known as the Col factor (Fredericq, 1965). The Col factor was originally described by Jacob & Wollman (1958) as be-

ing of episomal nature. According to Nomura (1967), only two cases of a Col factor being integrated into the host chromosome have been reported.

It was first demonstrated by Fredericq (1953; 1954) that certain colicinogenic strains may transfer their colicinogenicity to non-colicinogenic strains by cell contact. The colicin produced by the recipient was found to be of the same type as that produced by the donor, and no linkage of chromosomal markers to the factor was observed (Fredericq, 1953; 1954). Nagel de Zwaig, Anton & Puig (1962) showed that a series of Hfr strains and an F^+ strain of *E. coli* K12 colicinogenic for colicins K94–V, K317–E2 and CA53–I, transfer the Col factor to F^- strains to an extent dependent only on the Col factor concerned and not on the 'origin' of the Hfr. This is in agreement with the results of Alföldi, Jacob, Wollmann and Mazé (1958) and of Clowes (1963) who showed that Col K30–E1 is transferred by different Hfr strains at a time independent of the origin of the Hfr donor. It has been suggested (Nagel de Zwaig *et al.*, 1962; Clowes, 1963; Nagel de Zwaig & Puig, 1964; Nomura, 1967) that in general Col factors are in extrachromosomal state and should be considered plasmids rather then episomes.

The first comparative study of different Col factors was undertaken by Ozeki, Stocker & Smith (1962). They investigated the transference of various Col factors from colicinogenic *Salmonella typhimurium* strain LT2 to non-colicinogenic LT2 bacteria. It was found (Ozeki *et al.*, 1962) that F^- –LT2 cells singly-colicinogenic for either Col K77–B or Col P9-Ib could conjugationally transfer these factors to non-colicinogenic recipients. The Col factors K30–E1, P9–E2 or K49–K could not be transmitted by singly-colicinogenic LT2 cells (Ozeki, Stocker & Smith, 1962). A LT2 cell containing either the Col P9–Ib or Col K77–B factors, in addition to either the E1, E2 or K, could transfer both factors efficiently (Ozeki *et al.*, 1962). Ozeki & Howarth (1961) demonstrated that the presence of the K30-E1 factor in *S.typhimurium* LT2 increase the promotor activity of the P9–Ib factor although alone it is devoid of promotor activity and is itself not transferred. It has been shown (Nagel de Zwaig & Anton, 1964; Meynell & Datta, 1966; MacFarren & Clowes, 1967) that K30–E1–containing cells are insensitive to male-specific phages, and that this factor does not exhibit mutual exclusion with F (Kahn & Helinski, 1964; MacFarren & Clowes, 1967). Clowes, Moody & Pritchard (1965) showed that Col K30–E1 can be eliminated by thymine deprivation but not by acridine orange. It was shown by Stocker, Smith & Ozeki (1963) that uninterrupted mating involving an F^-LT2 donor trebly colicinogenic for P9–Ib, K30–E1 and P9–E2, usually resulted in the conference of all three factors on the recipient. Interrupted mating indicated that transfer of these factors occurred independently of one

another at chronological random (Smith, Ozeki & Stocker, 1963). These results complement the findings of Ozeki & Howarth (1961) and of Clowes (1961), that the presence of the P9—Ib factor in *S. typhimurium* and *E. coli* K12 respectively, confers on these cells the ability to act as a donor of chromosomal markers. Both Clowes (1961) and Ozeki & Howarth (1961) observed that the donor ability of this Col factor was reminiscent of that type determined by the F factor of *E. coli,* in that large pieces of chromosome, which could include any gene, were transferred. Stocker *et al.* (1963) noticed that recipients of the Col I and B factors were only efficient donors of these plasmids for a few generations, the efficiency of plasmid donations declining within subsequent generations. It was suggested that the donor ability gradually becomes repressed by a mechanism analogous to the repression of phage λ in lysogenic cells (Stocker, Smith & Ozeki, 1963). Although the Col P9—Ib factor is infectious (Ozeki *et al.,* 1962) and cells harbouring it are rendered fertile (Ozeki & Howarth, 1961; Clowes, 1961), these cells are resistant to the male-specific phages. (Monk & Clowes, 1964; Meynell & Datta, 1966).

Meynell & Lawn (1967) described a new type of pilus elaborated by Col Ib-containing cells which is morphologically distinct from the F-induced sex pilus. A direct correlation was observed between cells able to donate Col Ib and the ability to produce I-pili (Meynell & Lawn,1967). These I-pili were found to be unable to adsorb F-specific phages, but could adsorb an I-specific phage (Lawn, Meynell, Meynell & Datta, 1967). Clowes & Moody (1966) showed that chromosomal transfer mediated by the Col P9—Ib factor is not reduced in rec^- cells, in contrast to the findings for the F factor. They (Clowes & Moody, 1966) suggested that recombination between Col Ib and the chromosome is unnecessary for chromosomal transfer, in opposition to the recombinational requirement for F-mediated transfer. Both Col P9—Ib and F can co-exist stably in the same cell (Lawn *et al.,* 1967). According to Nomura (1967) the colicin I and B factors are distinct from the other Col factors in that, like the autonomous F factor, they promote the conjugation of cells through which genetic markers or inherently non-infectious colicin factors may be transmitted.

Only two instances of a Col factor integrating into the chromosome have been described (see Nomura, 1967). Fredericq, (1963; 1965) studied an *E. coli* K12 F^+ strain, doubly-colicinogenic for colicins K260—V and B. An Hfr derivative of this strain was found to have relinquished its V colicinogeny, but the Hfr and colicin B properties were transferred in crosses linked as the terminal markers (Fredericq, 1963; 1965).

Fredericq (1965) described a Col K260—B, V factor which carried the chromosomal genes cys B^+ and try^+, which were conjugationally co-transferable to a non-colicinogenic auxotrophic recipient.

The Col factor K94—V is also infectious and may be transferred efficiently in the absence of other 'helper' plasmids (Kahn & Helenski, 1964; Nagel de Zwaig & Anton, 1964; Nagel de Zwaig, 1966; MacFarren & Clowes, 1967). These workers have shown that, besides conferring fertility on F^- cells, the K94—V factor may act as a 'helper' in the transfer of non-infectious Col factors and displays mutual exclusion with the F factor and its derivatives, F'-lac and F'-gal. Cells possessing Col K94—V produce pili (Nagel de Zwaig, 1966; Caro & Schnös, 1966) which renders them sensitive to infection by male-specific phages (Nagel de Zwaig & Anton, 1964; Meynell & Datta, 1966; MacFarren & Clowes, 1967). Kahn & Helinski (1964) found that Col K94—V—containing cells may be cured of the factor by acridine orange, eliminating both colicin V production and accompanying fertility characteristics. Kahn & Helinski (1965) postulated a direct interaction of Col K94—V with the F integrating region of a Hfr strain. In crosses involving a V-colicinogenic Hfr strain with F^- bacteria, they (Kahn & Helinski, 1965) demonstrated that a large percentage of the Hfr V^+ donors transmitted the colicinogenic property closely linked to the origin of the Hfr. Consequently the occurrence of a genetic recombinational event between homologous regions on the integrated F factor and the fertility region of the Col 94—V factor was suggested (Kahn & Helinski, 1965). Nagel de Zwaig (1966) has proposed that the infective Col 94—V factor may have arisen from the genetic recombination of an F factor with a non-infectious Col factor.

Pasteurella pestis and *P. pseudotuberculosis* differ in that the former contains the fibrinolytic factor (F), the coagulase factor (C) and is non-motile (Brubaker, Surgalla & Beesley, 1965). These workers noted that the production of the F and C factors is correlated to the production of pesticin I in *P. pestis*. It was shown that the genetic determinants of the three activities are linked on a single extrachromosomal plasmid, and that a non-pesticinogenic strain of *P. pestis* resembles the wild-type *P. pseudotuberculosis*. It was suggested that loss of the plasmid function by *P. pestis* converts the organism to a *P. pseudotuberculosis*-like form and conversely, that donation of the plasmid to *P. pseudotuberculosis* converts it to a *P. pestis*-like form (Brubaker, Surgalla & Beesley, 1965).

Amati & Ozeki (1962) succeeded in transmitting the Col E1 and E2 factors from *S. typhimurium* LT2 to a strain of *Serratia marcescens*. Transfer of these two factors re-

quired the presence of a newly-introduced Col I factor in the LT2 donor cell. The presence of Col E1 in the donor was found to increase the transfer of factor E2 by 100-fold. The recipient Serratia cells carried the acquired factors stably, their presence not being eliminated by acridine orange (Amati & Ozeki, 1962). The transmittance of the bacteriocinogenic factor of *Enterobacter cloacae* DF 13 was studied by Tieze *et al.* (1969). This factor was transmitted by conjugation to a non-bacteriocinogenic *E. cloacae* strain and to *E. coli* K12 and Hfr cells. Transfer of chromosomal material was not observed. The bacteriocinogenic factor could not mediate its own transfer but required the presence of another transmissable plasmid (Tieze *et al.*, 1969). Coetzee (1964) demonstrated the transmittance of colicin factor D from *E. coli* strain CA 23 to five Providence strains and the transfer of an E1 Col factor from Paracolon strain CA 62 Col $E1^+$, I^+ to Providence NCTC 9295. The recipient strain 9295 cells displayed no colicin I production. Direct cell contact was found to be necessary for the transfer of the Col E1 and D factors (Coetzee, 1964).

There are several known interactions between Col factors and bacteriophages (Nomura, 1967). Watanabe & Okada (1964) found that the growth of phage W31 is restricted in cells harbouring Col K77–B or the F factor but not by Col K317–E2. Phage BF 23 on the other hand, is restricted by Col K317–E2 and by Col P9–Ib, but not by Col K77–B or the F factor (Strobel & Nomura, 1966). It was shown by Strobel & Nomura (1966) that phage BF 23 successfully injects its DNA which fails to replicate, but is not degraded by colicinogenic E2 or I cells. Successful transduction of colicinogeny in *E. coli* by phage P1 was demonstrated by Fredericq in 1958 and 1959 (see Fredericq, 1956b). Ozeki & Stocker (1958) reported the transduction of E-colicinogeny in *S. typhymurium* LT2 by phage PLT 22, and Vianu, (1969) described the transduction of bacteriocinogeny from Staphylococcus Strains 11 and 34 to two non-bacteriocinogenic Staphylococci.

Evidence of the deoxyribonucleic acid nature and size of Col factors was presented by Silver & Ozeki (1962) who observed a direct correlation between the transfer of colicinogeny and the transfer of radioactive DNA. By measuring the sensitivity of Col factors to P^{32} decay, Ozeki (1965) estimated the size of factors P9–Ib, K30–E1 and P9–E2 to be of the order of 1×10^5 phosphorus atoms per copy. De Witt & Helinski (1965) transferred the Col K30–E1 factor from *E. coli* to a non-colicinogenic *Proteus mirabilis*. By measuring the amount of DNA of buoyant density peculiar to *E. coli* obtained from the *P. mirabilis* recipient, they (de Witt & Helinski, 1965) calculated the molecular weight of the K30–E1 factor as 6×10^6 daltons per copy. The DNA of Col E1 isolated from

P. mirabilis has been shown to consist of three size classes of closed circular duplex molecules of molecular weights 4,2 8,5 and 12,7 X 10^6 daltons respectively (Roth & Helinski, 1967; Bazaral & Helinski, 1968a). According to Goebel & Helinski (1968) the higher circular forms in *P. mirabilis* resulted from a possible imbalance in the formation or concentration of Col E1 DNA duplicating enzymes rather than random recombination of plasmid monomers. Van Rensburg & Hugo (1969) studied the Col E1 isolated from a Providence strain which had obtained the Col factor from a Paracolon strain CA 62 (Coetzee, 1964). Three size classes of open and supercoiled DNA molecules were observed with molecular weights similar to those described in *P. mirabilis.* Bazaral & Helinski (1968b) isolated the Col E1, E2 and E3 factors from *E. coli* as single covalently intact supercoiled DNA molecules with molecular weights 4,2, 5,0 and 5,0 X 10^6 daltons respectively. The multiple forms characteristic of *P. mirabilis* Col E1 DNA were not observed. Clewell & Helinski (1969) showed that the supercoiled Col E1 factor from *E. coli* consists of a DNA-protein complex which may be induced to untwist and form an open circular double-stranded DNA 'relaxation complex'. Similar relaxation complexes were described for the Col E2 and E3 factors (Clewell & Helinski, 1970a) and for the Col P9—Ib factor (Clewell & Helinski, 1970b) with a molecular weight of 61,5 X 10^6 daltons (Bazaral & Helinski, 1968a). Inselburg & Fuke (1970) described replicating Col E1 DNA isolated from *E. coli* minicells (Inselburg, 1970) as circular molecules with two branched points suggestive of the 'rolling circle' model of DNA replication. Drygin, Bogdanova & Bogdanova (1971) demonstrated that *E. coli* Col E1 DNA exists as membrane-bound circular molecules. They (Drygin *et al.,* 1971) proposed that the general concept of the membrane attachment of the replication origin of bacterial and phage chromosomal DNA should be extended to exrachromosal plasmid DNA.

Colicinogenic cells are immune to the killing or biochemical action of homologous external colicins (Fredericq, 1957). Fredericq (1958) has shown that immune cells retain receptors for the colicins they produce and that immunity is distinct from resistance to adsorption of the colicin. Similar results for the adsorption of colicin E2 to E2-colicinogenic cells were obtained by Nomura (1963), who suggested that immunity must be due to some process after the adsorption step. Nomura (1963) demonstrated that immunity to colicin E2 is not due to an alteration in the properties of host DNA, the biochemical target of E2. Both Fredericq (1957) and Nomura & Maeda (1965) observed that colicinogenic cells are not immune to high concentrations of homologous bacteriocin. Utilizing colicins Ia and Ib, Levisohn, Konisky & Nomura (1967) established that immunity breakdown by high concentrations of bacteriocins exhibited similar biochemical

effects on the homologous immune cells as the effects found in sensitive cells exposed to low multiplicities of bacteriocin.

Nomura & Maeda (1965) proposed that immunity is due to an alteration in the mechanism responsible for the initiation and/or the transmission of the specific stimulus which effects the target in sensitive cells. The synthesis of a specific immunity substance which interferes with the proposed transmission system has been suggested (Nomura, 1963). Two instances of cells being sensitive to the bacteriocins they produce have been reported. Brubaker & Surgalla (1961) found that certain strains of *P. pestis* which are sensitive to pesticin II are also pesticinogenic for pesticin II, but not for pesticin I. Ryan, Fried & Mukai (1955) reported the isolation of a colicin from ultraviolet-irradiated *E. coli* strain 15h- cells. The only strain found to be sensitive to this colicin is the same h- strain that produces it (Ryan *et al.*, 1955).

PRODUCTION OF BACTERIOCINS

According to Jacob *et al.* (1953) one of the criteria of colicins is their lethal biosynthesis — the production of colicin involving the death of the bacteriocinogenic organism.

The observation of Jacob, Siminovitch & Wollman (1952) that colicin ML—E1 elaborated by *E. coli* strain ML could be induced by ultraviolet light, prompted Fredericq (1954) and Hamon & Lewe (1955) to apply this method of induction for the production of other colicins in different strains of *E. coli*. Jacob *et al.* (1952) emphasized the analogy between colicinogeny and lysogeny as a result of the lysis of the induced *E. coli* ML producers. It was later shown by Kellenberger & Kellenberger (1956) that *E. coli* strain ML is also lysogenic and that the lysis occurs due to the lysogeny rather than the colicinogeny. Fredericq (1955) showed that ultraviolet irradiation can enhance colicin production without lysis.

In a study of the kinetics of colicin production, Ozeki, Stocker & de Margerie (1959) investigated a *S. typhimurium* strain made colicinogenic for colicin P9—E2, and observed the release of colicin from single bacteria as minute areas of inhibition, termed 'lacunae' in a lawn of sensitive organisms. Isolation of single colicinogenic cells by micro-manupulation showed that cells which produce colicin do not multiply and are killed, but without lysis. Amati (1964) found that ultraviolet irradiation increases the number of lacunae in addition to the total amount of colicin produced by a colicinogenic

culture. According to Nomura (1967): "Colicin production is thus a lethal biosynthesis, and the function of the structural gene for colicin is repressed in the majority of colicinogenic cells under ordinary conditions".

Ultraviolet irradiation is not unique as an inducing agent of bacteriocin production (Reeves, 1965). Mitomycin C was shown by Iijima (1962) to induce the production of colicin in *E. coli* K30 and in colicinogenic strains of *E. coli* K12. Kohiyama & Nomura (1965) described the induction of colicin E2 in a DNA temperature-sensitive mutant of *E. coli* K12 by elevated temperatures. These workers suggested that the heat induction of colicin E2 in hosts with temperature-sensitive DNA synthesis results in an abnormal state in the regulatory system of bacterial DNA synthesis which, in turn, interferes with the regulated replication of the Col factor. The thermal induction of colicin Ia from a strain of *Shigella sonnei* has been described by Gromkova (1971). Upon transfer of the Col factor to non-colicinogenic cells, a similar effect was observed in those cells acquiring colicinogeny. Gromkova (1971) has proposed that the colicin induction by elevated temperature is due to a thermosensitive colicin repressor. Luzzati & Chevallier (1964) were able to induce colicin production by the addition of thymine to starved thymineless mutants of colicinogenic *E. coli.* Pritchard & Lark (1964) showed that the addition of thymine to starved thymineless mutants results in an abnormal state of the bacterial DNA upon the resumption of DNA synthesis. Kohiyama & Nomura (1965) suggested this abnormality as being responsible for colicin induction by thymine.

Iijima (1962) found that the addition of chloramphenicol after Mitomycin C induction of colicinogenic *E. coli* K12 cells resulted in a concommitant loss of detectable bacteriocin production. On the basis of this it was suggested that colicin production is a *de novo* synthesis (Iijima, 1962). Ben-Gurion (1965) showed that the addition of chloramphenicol for a short period after irradiation of bacteriocinogenic cells increased the production of colicin after resumption of protein synthesis. Similar results were obtained for non-irradiated cells by treatment with chloramphenicol and puromycin (Ben-Gurion, 1970). The addition of fluorouacil and thymidine (Ben-Gurion, 1965) to irradiated colicinogenic cells resulted in the prevention of colicin production, suggesting the necessity of *de novo* RNA synthesis for this function. Studying a strain of *Proteus mirabilis* colicinogenic for K30—E1, de Witt & Helinski (1965) demonstrated a 30— to 100-fold increase in the amount of 'satellite' DNA corresponding to the Col K30-E1 factor upon induction of colicin E1 production by Mitomycin C. They (de Witt

& Helinski, 1965) supported the proposal by Amati (1964) that the increased production of colicin E1 is at least partly due to the derepression and subsequent increase in copies of the genetic determinants of colicin E1.

Hamon & Peron (1962), studying colicinogenic derivatives of *E. coli* K12 either lysogenic or non-lysogenic for phage λ, showed that the release of bacteriocin is continuous after induction in non-lysogenic cells. In lysogenic cells, the presence of phage λ prevented release of colicin before lysis, resulting in a sudden release of colicin at the moment of lysis (Hamon & Peron, 1962). *Pseudomonas aeruginosa* strain C10 is non-lysogenic but pyocin C10 accumulates intracellularly upon induction, being released by cellular lysis one hour after induction (Hamon, 1956). Similar results were shown for pyocin R by Ikeda, Kageyama & Egami (1964). No DNA synthesis was detected, although pyocin R could only be obtained after induction of *P. aeruginosa* strain R. *Bacillus megaterium* strain 216 does not normally produce megacin (Ivanovics, 1962) but produces large amounts of the bacteriocin upon induction by ultraviolet light. No megacin is detectable intracelluarly until one hour after induction, being suddenly released on cell lysis, although this cell appears to be non-lysogenic (Ivanovics & Alföldi, 1957). Ivanovics & Nagy (1958) found that other megacinogenic strains undergo lysis spontaneously, releasing megacin without induction. Pesticin I production was shown by Hertman & Ben-Gurion (1958) to be dependent on induction although the pesticin is released without lysis of the producing cells.

Besides being susceptible to induction in certain cases, the production of bacteriocins is also very dependant on growth conditions (Reeves, 1965). Hertman & Ben-Gurion (1958), working on pesticin I production, and Goebel *et al.* (1956) and Matsushita *et al.* (1960) studying colicin K235-K production, have shown that the culture medium and other growth conditions of the bacteriocinogenic culture influences the amount of bacteriocin elaborated by the cells. Many bacteriocinogenic strains which produce zones of inhibition on agar overlayed with sensitive cells, do not produce the bacteriocin in broth (Reeves, 1965). Lachowicz (1965) demonstrated that staphylococcin production could only be observed on solid medium, with no detectable titer being obtained in broth. Krcmery, Hurwitz & Fredericq (1970) found that the introduction of resistance (R) factors into colicinogenic *E. coli* abolishes the colicin production of certain Col $^{+}$ strains, possibly due to elimination of the colicin determinant by the R determinant. Recombination-deficient mutants of E1 or E2-colicinogenic *E. coli* are unable to produce these colicins, although Col V^{+} cells are not prevented from producing colicin V (Helinski & Herschman, 1967). MacPhee (1970) was

able to isolate recombination-deficient mutants of *S. typhimurium* from nitroso-guanidine-treated cultures of colicinogenic cells by detecting the failure of cells to produce colicin E1.

BACTERIOCIN-RECEPTORS OF BACTERIAL CELLS

The first suggestion pertaining to the existence of specific receptors for the adsorption of bacteriocins was made by Fredericq in 1946 in an attempt to classify the colicins. The adsorption of colicins to sensitive cells was shown by the disappearance of colicin activity from solution after the mixing of the colicin with bacterial cells (Jacob, Siminovitch & Wollman, 1952; Hamon & Peron, 1960; Mayr-Harting, 1964). Bordet & Beumer (1948) observed that cell-wall extracts of colicin-sensitive bacteria inhibit bactericins *in vitro.* Utilizing radioactive colicins Nomura & Maeda (1965) and Maeda & Nomura (1966) demonstrated that the bacteriocin adsorbs onto the surface of sensitive cells, remaining at this site and initiating its bactericidal activity from this point. This concept was endorsed by Konisky & Nomura (1967) who showed that mixing ribosomes *in vitro* with colicin CA38—E3 does not cause the ribosome inactivation. The specificity of receptors for each type of colicin is indicated by the attainment of non-adsorption of particular colicins per mutation of the sensitive host (Luria, 1964). Trypsin rescue of treated cells confers renewed susceptibility to adsorption of the particular bacteriocin concerned on the sensitive cell (Nomura & Nakamura, 1962). By means of trypsin-rescue experiments, Reynolds & Reeves (1969) demonstrated that functional adsorption occurs in two stages in that after initial adsorption to the cell surface, a secondary adsorbance effect is necessary for the initiation of metabolic arrest of the sensitive cell.

The nature of the cell surface structures which specifically adsorb or inactivate the biological agent *in vitro* is poorly understood (Weltzien & Jesaitis, 1971).

Working with stable L-forms of *E. coli* and *P. mirabilis,* Smarda & Taubeneck (1968) demonstrated that the cytoplasmic membrane contains effective bacteriocin receptors. The adsorbing capacity of the cytoplasmic membrane of *E. coli* spheroplasts for colicins E2, E3 and K was shown to be of the same magnitude as the adsorbance by intact cells (Nomura & Maeda, 1965). The observation that spheroplasts effectively adsorbed bacteriocin but were insensitive to its lethal effect, led Nomura & Maeda (1965) to propose that the primary functional receptors are analogous to the lipopoly-saccharide surface receptors for bacteriophage. This is in contradiction to the subsequent results of Smarda & Taubeneck (1968) that L-forms are equally susceptible to the killing action of colicins as are their parent bacteria. Smarda & Taubeneck (1968) proposed that the lethal adsorption of colicins is to receptors in the cyto-

plasmic membrane, and that the cell wall cannot be regarded as a compulsory initial step leading to the killing of the cell. Guterman & Luria (1969) found that colicin B is inactivated by lipopolysaccharides of *E. coli* strains which are sensitive to the bacteriocin. Chang & Hager (1970) however, found that it binds the colicin. On the basis of the findings of Goebel & Barry (1958) that purified colicin K consists of a LPS-protein complex with the colicin activity residing in the protein moiety, they (Chang & Hager, 1970) concluded that LPS has a natural affinity to bind non-specifically with protein. It was suggested that the specific functional binding of colicin to LPS *in vivo* requires a specific covalently linked chemical structure found only in native LPS (Chang & Hager, 1970). Weltzien & Jesaitis (1971) reported that the cell walls of colicin K-sensitive cells of *E. coli* strains B and Cullen are potent inhibitors of this bacteriocin, whilst the walls of resistant mutants are not. Upon separation of spheroplasts of these bacteria into cytoplasmic and outer membranes, the receptor activity was found only in the latter fraction, suggesting that the initial colicin receptor is a constituent of the bacterial cell wall. Beppu & Arima (1970), studying DNA-membrane complexes isolated from protoplasts of sensitive *E. coli,* observed the dissociation of all membrane-bound DNA, RNA and protein from the membrane complex on the addition of colicin E2. These workers suggested that the cytoplasmic membrane of the sensitive cell has a functional importance in the transmission of colicin action from the primary cell surface receptor sites to a specific and lethal intracellular target. According to de Graaf & Stouthamer (1970) both the cell wall and the cytoplasmic membrane operate co-operatively in effecting functional adsorption and subsequent expression of the lethal action of the bacteriocin. It has been possible to distinguish two kinds of bacteriocin-resistant mutants: one which has lost bacteriocin receptors, and another which retains them but is still resistant to colicin action (Nomura, 1967). Both Clowes (1965) and Nomura (1964) made this distinction on isolating mutants of the second type which have been designated as being 'tolerant' of bacteriocin (Nagel de Zwaig & Luria, 1967; Nomura & Witten, 1967). Several groups of workers have initiated studies on tolerant mutations utilizing the E group and K colicins. A number of different groups of tolerant mutants showing different tolerance patterns have been found in *E. coli,* many of them mapping close to the galactose operon (Nagel de Zwaig & Luria, 1967; Nomura & Witten, 1967; Hill & Holland, 1967). Clowes (1965) described a *tol* mutant resistant to E1 only, which mapped near the histidine locus. Hill & Holland (1967) suggested that the successful fixation of bacteriocins involves a dual role for the cell surface 'receptor'; the first for the binding of the protein and the second for the correct orientation of the bound molecule relative to the cytoplasmic membrane.

Nagel de Zwaig & Luria (1967) have interpreted tolerance mutations as affecting some components of the cytoplasmic membrane which mediates between the adsorbed bacteriocin

molecules and the target sites of their biochemical effects in the bacterial cell.

Recent evidence for the involvement of an altered cytoplasmic membrane in tolerant mutants was provided by Bhattacharyya, Wendt, Whitney & Silver (1970). These workers have shown that membrane vesicles prepared from tolerant mutants do not release accumulated radioactive proline whilst those from both sensitive and receptor-negative cells do, indicating an altered cytoplasmic membrane in tolerant cells. Burman & Nordström (1971) described a new type of tolerance mutation in which the defect resides in the cell wall composition although the bacteriocin is still able to effect adsorption. It was proposed (Burman & Nordström, 1971) that disturbances to the steric conformation of cell wall promotors may lead to increased trapping or repulsion of various molecules such as colicins, which reduces the probability of initiating the lethal interaction consequential to normal adsorption.

Certain colicins and bacteriophages appear to adsorb onto similar receptors, and there are a few instances of cross-resistance between colicins and bacteriophages, suggesting a possible common receptor (Reeves, 1965). A certain measure of cross-resistance between colicin K and phage T6, and between colicin M and phage T1 and T5 has been noted (Fredericq & Gratia, 1950; Fredericq, 1951). Similarly, cases of cross-resistance between colicins C, I, V and B have been observed (Reeves, 1965; Nomura, 1967; Bradley, 1967). Both Reeves (1965) and Smarda & Schuhmann (1967) have emphasized that the above-mentioned correlation has in no instance been absolute; certain colicin-resistant mutants retaining the ability to adsorb phage and vice versa. Taubeneck (1963) has demonstrated that stable L-forms of *Proteus mirabilis* which are devoid of their cell walls, have lost their phage receptors and are absolutely resistant to the action of phages. Smarda & Schuhmann (1967) have shown that stable L-forms of *E. coli* B. cells which are normally sensitive to both colicin K and phage T6, are likewise unable to adsorb phage whilst retaining full sensitivity to colicin K. They (Smarda & Schuhmann, 1967) concluded that phage T6 requires adsorption to the cell wall for the expression of its biological potential, whilst colicin K does not. Weltzien & Jesaitis (1971) undertook a comparative study of the cell walls and cytoplasmic membranes of *E. coli* cells doubly-sensitive for phage T6 and colicin K and of their resistant mutants. These workers observed that phage-resistant mutants were also resistant to colicin K and suggested that the genes coding for colicin K and T6 receptors are closely linked. Cell wall fractions of sensitive cells inhibited both the colicin and phage T6, whilst cell walls of resistant mutants inhibited neither. Cytoplasmic membrane isolates only inhibited colicin K to a minor degree and did not inhibit phage T6, suggesting that the primary receptors for the colicin and phage form

part of the cell wall. Weltzien & Jesaitis (1971) have shown that although the two receptors appear biologically linked, they differ in their sensitivity to enzymes and chemical reagents and hence must be of different chemical nature. On the basis of these observations it was suggested that the specific chemical groupings of the cell wall which react with the bacteriocin are distinct from those which combine with the virus, but that these chemical configurations might be linked as integrals of the same receptor macromolecule (Weltzien & Jesaitis, 1971).

MODE OF ACTION OF BACTERIOCINS

Bacteriophages are thought to kill sensitive cells after first adsorbing onto a specific receptor (Nomura, 1967). Fredericq (1948) introduced the word 'receptor' on the basis of his results in connection with a classification of the colicins. The specificity of adsorption was demonstrated (Hamon & Peron, 1960) by non-adsorption to resistant mutants. Actual adsorption to the surface of sensitive cells, but not to resistant mutants has been demonstrated using purified radioactive colicins (Maeda & Nomura, 1966). The number of receptor sites on a sensitive cell has been measured by several workers. Mayr-Harting (1964) and Mayr-Harting & Shimeld (1965), using colicin P9—E2 or colicin CA42—E2 at saturating levels, found that one sensitive cell adsorbed 11 killing units of colicin. Maeda & Nomura (1966) measured the adsorption of 20 to 30 killing units of colicin E2 on sensitive cells. Reeves (1965) found that 30 to 90 killing units are adsorbed to sensitive bacteria. A killing unit is defined as the amount of colicin necessary to kill a single sensitive cell, as measured by the number of colony-forming survivors(Nomura, 1967). Maeda & Nomura (1966) estimated that one killing unit corresponds to 100 colicin molecules and therefore that the actual number of receptors is two to three thousand. According to Reeves (1965) one killing unit corresponds to one colicin molecule, indicating that the number of receptors is 30 to 90. The reason for the discrepancy is not yet clear, but it is certain that there are many receptors on a cell (Nomura, 1967). Data produced by Nomura (1964) and Nomura & Maeda (1965) indicates that most, if not all, of these receptors are potentially capable of responding to adsorbed colicins.

The kinetics of killing by bacteriocins was first studied by Jacob, Siminovitch & Wollman (1952). The initial rate of killing of a given concentration of bacteria by colicin ML—E1 was shown to be proportional to the concentration of colicin, as was the final number of bacteria killed. Similar results were obtained for pyocin C10 by Jacob (1954) who suggested that the killing action of most bacteriocins is a single-hit process. This conclusion was confirmed by the results of Reeves (1965) and Nomura (1963). According to Nomura (1963) bacteriocins behave like particles, their adsorption to sensitive cells follows Poisson's distribution and their killing titer can be assayed in terms of 'killing particles' or 'killing units'.

Luria (1964), Nomura & Nakamura (1962) and Nomura (1964) proposed that a cell attacked by a single colicin has a definite probability of being killed. They suggested that the effect of a single colicin molecule could be transmitted through the cytoplasmic membrane from a local site of action. Nomura & Maeda (1965) proposed a model whereby the adsorption of a single colicin molecule causes an irreversible change at the receptor site which is transmitted to the biochemical target by some specific transmission mechanism. Evidence has been presented (Nomura, 1963; 1964; Maeda & Nomura, 1966) that bacteriocins remain at the bacterial surface and act from there. Changeux & Thiery (1967) and Changeux *et al.* (1967) suggested a mechanism for the spreading of the local action of a single colicin molecule. Membranes are constituted by the association of repeating globular lipoprotein units (Green & Perdue, 1966). According to Changeux & Thiery (1967) conformational changes may be propagated throughout the entire co-operative lattice structure of the cytoplasmic membrane as a result of the allosteric interdependence of individual membrane submits on one another.

Different colicins exert different biochemical effects on sensitive cells (Nomura, 1963). Nomura & Nakamura (1962) and Nomura (1963) studied colicin K235—K. They found that it inhibits DNA, RNA and protein synthesis, but not respiration of sensitive cells. This is in agreement with the pioneer studies of Jacob *et al.* (1952) on colicin ML—E1. Colicin K does not cause leakage of P^{32}-containing substances from P^{32}-labelled cells or of β-galactosidase from fully induced cells (Nomura, 1963). The active uptake of radio-active potassium by sensitive cells is inhibited (Nomura & Maeda, 1965; Wendt, 1970). Colicin K was also shown (Nomura & Maeda, 1965) to induce the leakage of radioactive potassium from sensitive cells. Luria (1964) discovered that colicin K and colicin E1 block the function of certain permeases. This effect was shown to be specific and no substantial impairment of the permeability function of the membrane was observed (Fields, Ruby & Luria, 1966). Colicins K and E1 were shown to interfere with the energy supply of the sensitive cell, whether by inhibiting the formation or enhancing the breakdown of ATP. (Luria, 1964; Nomura & Maeda, 1965; Maeda & Nomura, 1966; Levinthal & Levinthal in Luria, 1964). Cells treated with high multiplicities of K235—K exhibited some physical degradation of ribosomes under certain conditions (Konisky & Nomura, 1967). They suggested this as a secondary effect of this colicin on sensitive cells. It has been proposed (Nomura & Maeda, 1965; Reeves, 1965; Nomura, 1967; Konisky & Nomura, 1967; Wendt, 1970) that the inhibition of oxidative phosphorylation is the main biochemical effect of colicin K. This condition leads to the inhibition of macromolecular synthesis as well as the inhibition of the active transport through the cell surface of certain substances (Nomura, 1967). Nomura & Nakamura (1962) demon-

strated that the inhibition of macromolecular synthesis by colicin K can be reversed by treatment of the colicin-cell complex with trypsin. Using resting cells in the absence of a carbon source, they were able to show that 'colicin-killed' cells could be completely rescued by trypsin in the presence of CN^-, 2,4-dinitrophenol, chloramphenicol or azauracil. The rescued cells showed no metabolic impairment (Nomura & Nakamura, 1962). Endo *et al.* 1969) showed that colicin K235-K does not cause DNA degradation or induce the development of phage λ in lysogenic *E. coli.* The colicin inhibits the reproduction of virulent phage T4 when added to cells soon after infection by T4 (Nomura & Nakamura, 1962).

Colicin P9—E2 has been shown to specifically affect DNA synthesis and induce DNA degradation, leading to subsequent inhibition of synthesis of other macromolecules (Nomura, 1963; Nomura & Maeda, 1965). Nomura (1964) was unable to demonstrate any deoxyribonuclease activity from purified P9—E2 preparations. Infection of P9—E2—inhibited cells by phage T4 causes a resumption of macromolecular synthesis. Phage T4 or T5, infecting cells 25 minutes after colicin P9—E2 adsorption, could still initiate phage DNA synthesis (Nomura, 1963). These findings indicate (Nomura, 1967) that all of the synthesizing machinery of the cell, including the energy-supplying system, remains intact. Endo *et al.* (1963) found that colicin P9—E2 induced the production of phage λ from lysogenic E. coli. The efficiency of induction was high and the burst size nearly normal. It was suggested (Nomura, 1967) that the effect of colicin P9-E2 is specific to bacterial DNA inhibition and that the synthetic machinery necessary for λ production remains intact. Persiel (1965) showed that a high multiplicity of P9—E2 may inhibit respiration of sensitive cells as a secondary effect. Slow degradation of ribosomal DNA as a secondary effect of colicin E2 treatment was suggested by Nose, Mizuno & Ozeki (1966).

Colicin CA42—E2 was studied by Reeves (1963). It stops DNA synthesis within 2 minutes RNA within 5 minutes and protein synthesis within 10 minutes (Reeves, 1963). Reynolds & Reeves (1963) found that degradation of bacterial DNA could be detected within 5 minutes of colicin CA42—E2 adsorption. The lethal effects of the colicin are reversible by trypsin up to 15 minutes after its addition to cells (Reynolds & Reeves, 1963). Sensitive cells pretreated with 2,4-dinitrophenol before the addition of colicin CA42—E2, can be rescued indefinitely (Reeves, 1965). Nomura (1963) reported similar results for colicin P9—E2 and suggested that dinitrophenol protected sensitive bacteria by the inhibition of DNA degradation. Colicin CA42—E2, like P9—E2, induces the development of vegetative phage λ from lysogenic *E. coli* and displays little effect on the propagation of phage T4 by sensitive cells (Reeves, 1965). Obinata & Mizuno (1970) investigated the possibility that deoxyribonucleases were

activated or that their subcellular location changed in colicin E2–induced cells. They found no concomitant synthesis of specific deoxyribonuclease to be detectable. Endonucleolytic breakage of both DNA strands was indicated in a sensitive strain lacking endonuclease I. It was suggested by Obinata & Mizuno (1970) that colicin E2 induces the activation of a specific endonuclease which causes initial breakage of DNA strands, followed by degradation by other nucleases which have no specific function until broken DNA strands are formed. Colicin E2 has no effect on the oxidative phosphorylation system or on the active transport of potassium (Reeves, 1965; Nomura, 1967).

Colicin CA38–E3 inhibits protein synthesis but not DNA or RNA synthesis (Nomura & Nakamura, 1962). Neither respiration nor energy-supplying reactions were found to be inhibited by colicin CA38–E3 under conditions in which the primary effect of the colicin was pronounced (Nomura & Maeda, 1965). This colicin is unable to act as an inducer of phage λ from lysogenic *E. coli* (Endo *et al.*, 1963). Konisky & Nomura (1967) showed that the ribosomes from E3-inhibited cells are physically intact, but are inactive when the activity is assayed by means of a poly U-directed polyphenylalanine incorporation system. The induced alteration of the ribosome was shown by Hosokowa *et al.* (1966) and Staehelin & Meselson (1966) to reside in the 23S 'core' of the 30s ribosomal subunit. The subunit was shown to retain the ability to bind messenger RNA, but does not show messenger RNA-directed transfer RNA binding. The 50S ribosomal subunit remains active (Staehelin & Meselson, 1966). Senior, Kwasniak & Holland (1970) reported that the polyribosomes of E3–treated cells were rendered unstable and dissociated at low magnesium ion concentration *in vitro*. They suggested that the reduced protein synthesizing ability of E3-treated ribosomes, reported by Konisky & Nomura (1967), is due to a modification in 70S ribosomal structure. Bowman *et al.* (1971) isolated the proteins and RNA from 30S ribosomes of bacteria exposed to colicin E3. They showed that the defect in the 30S subunit resides in the 16S ribosomal RNA and not in the complement of ribosomal proteins. Several oligonucleotides were found to be cleaved from the 16S ribosomal RNA, including that which contains the 3' terminal base (Bowman *et al.*, 1971). Senior & Holland (1971) reported similar results and showed that colicin E3-treated 16S ribosomal RNA sedimented at 15S and that this RNA lacks the 3' terminal sequence of intact 16S RNA. It was suggested that a primary effect of colicin E3 is the activation of a highly specific ribonuclease that degrades 16S ribosomal RNA *in situ* (Senior & Holland, 1971).

It has been shown (Maeda & Nomura, 1966) that colicins P9–E2 and CA38–E3 share a common receptor, even though their apparent modes of action are different. According to

Nomura (1967) the specificity of biochemical action therefore cannot be accounted for by the specificity of adsorption.

The mode of action of colicin A was studied by Nagel de Zwaig (1969). It was shown to block the function of certain permease activities. The uptake of radioactive isoleucine was found to be blocked and accumulated isoleucine was released. Similar effects were observed on the accumulation of thio-methyl–β D–galactoside (Nagel de Zwaig, 1969). As with colicins E1 or K (Fields & Luria, 1969) these activities of colicin A are exerted in the presence of chloramphenicol. Colicin A was shown to arrest the motility of actively motile *E. coli* K12 cells (Nagel de Zwaig, 1969). Colicin A does not induce vegetative replication of phage λ in a lysogenic-sensitive strain (Nomura & Maeda, 1965). Nagel de Zwaig (1969) has suggested that colicin A, like colicins E1 and K (Fields & Luria, 1969; Nomura & Maeda, 1965) affects all macromolecular synthesis and the ATP-dependant active transport systems through an impairment of the production, utilization or availability of ATP.

Persiël (1965) showed that colicin S3 reduces the oxygen uptake of actively multiplying indicator-bacteria. Colicin S action on the glucose oxidation of resting bacteria was indicated. Non-adapted stationary-phase cells, treated with this colicin, were found to be less capable of oxidizing lactose. (Persiel, 1965).

The killing of cells with pyocin R is a single hit process (Kageyama *et al.*, 1964). Kaziro & Tanaka (1965) found that a single killing unit corresponds to only a few phage-tail-like particles (Kageyama & Egami, 1962; Kageyama, 1964). Addition of pyocin R to sensitive cells resulted in leakage of ultraviolet-absorbing material through the cell wall (Kageyama *et al.*, 1964). The significance of the leakage is not clear (Nomura, 1967) since it was noted that a lytic enzyme is synthesized together with the pyocin after induction of *P. aeruginosa* strain R cells (Kageyama *et al.*, 1964). Kaziro & Tanaka (1965) showed that pyocin inhibits DNA, RNA and protein synthesis in a manner similar to colicin K235–K. It was observed by Kaziro, Tanaka & Shimazono (1964) that ribosomes isolated from pyocin R-treated cells are inactive *in vitro*. Kaziro & Tanaka (1965) demonstrated that these ribosomes are physically degraded. Pyocin C10, studied by Jacob (1954), causes the rate of respiration to decrease, leading to non-multiplication and subsequent death. The pyocin was shown to destroy the plaque-forming ability of sensitive cells infected with virulent phage T2 (Jacob, 1954). Ohnishi, Takade & Takeya (1971) studied the morphological changes in *P. aeruginosa* treated with pyocin 28. This pyocin was shown to induce dispersion of the nuclear material throughout the cytoplasm within 15 minutes of adsorption. They suggested

that the bacteriocin induces the release of membrane-bound DNA, resulting in nuclear disorganisation and non-viability.

Ivanovics & Alföldi in 1955, observed that the addition of megacin A to sensitive cells led to a drop in their respiration. Studies by Ivanovics *et al.* (1959) and Holland (1962) have proved that this megacin causes a disruption of the permeability barrier of cells and the conversion of protoplasts to ghosts. Ozaki, Higahsi, Saito & Amano (1966) showed that megacin A has phospho-lipase A activity. Adsorption of megacin A to sensitive cells has been shown to be very weak, if occurring at all (Ivanovics *et al.*, 1959; Holland, 1962). It was suggested by Ozaki *et al.* (1966) that this megacin may be a simple hydrolytic enzyme. Megacin C, studied by Holland (1963; 1965) does not cause cellular leakage. It affects DNA synthesis, leading to DNA degradation and induces the development of phage λ in lysogenic-sensitive cells. Ribonucleic acid and protein synthesis ceases, but there is no breakdown of RNA (Holland, 1965).

Jayawardene & Farkas-Himsley (1970) studied the mode of action of a vibriocin purified (Jayawardene & Farkas-Himsley, 1969) from *Vibrio comma*. This bacteriocin inhibited DNA synthesis, while RNA and protein synthesis continued at a reduced rate. The vibriocin *per se* displayed no detectable deoxyribonuclease activity, but bacterial DNA degradation was demonstrable 10 minutes after addition to the cells. Radioactive potassium-efflux studies indicated a damaged bacterial membrane. Tryptic digestion of the bacteriocin reversed the lethal action. Chloramphenicol was shown to protect sensitive cells from the lethal action. Impairment of membrane function occurred in the presence of chloramphenicol. Jayawardene & Farkas-Himsley showed, in 1969, that sensitive cells could be protected by inhibitors of oxidative phosphorylation and by incubation at low temperature after adsorption of bacteriocin. It was suggested (Jayawardene & Farkas-Himsley, 1970) that the first step of vibriocin activity is damage to the bacterial membrane, followed by the induction of a previously repressed deoxyribonuclease which degrades the host DNA.

Elgat & Ben-Gurion (1969) studied a bacteriocin from *Pasteurella pestis*. Deoxyribonucleic acid synthesis was arrested and ribonucleic acid was degraded. Little effect was observed on protein synthesis. The pesticin was shown to induce phage P1 from lysogenic *E. coli* strain Ø. Trypsin was found to reverse pesticin action on sensitive *E. coli* Ø. The bacteriocin was shown to be active in the presence of dinitrophenol, and cells which were unable to grow due to nicotinamide starvation, were sensitive to the pesticin (Elgat & Ben-Gurion, 1969). Brubaker & Surgalla (1961) and Elgat & Ben-Gurion (1969) demonstrated that the presence of

calcium ions is necessary for the action of pesticin on *P. pseudotuberculosis*. Brubaker & Surgalla (1962) suggested that calcium ions affect the action of an inhibitor of pesticin produced by certain strains. Guterman & Luria (1969) described mutants of *E. coli* strains, B, B4 and K–12 which excreted a substance that afforded protection to cells sensitive to colicins A, B, Ia, Ib, E1, E2, E3 and K. The protecting material was found to be filterable, non-dializable, heatstable and insensitive to trypsin and ribonuclease (Guterman & Luria, 1969). They suggested that the substance is lipopolysaccharide which competes with cells for colicin adsorption. Elgat & Ben-Gurion (1969) demonstrated that sensitive *P. pestic* cells do not produce the inhibitor and that calcium ions are not necessary for pesticin action. It was suggested by Elgat & Ben-Gurion (1969) that pesticin activity is independant of the energy metabolism of sensitive cells and that the presence of calcium ions facilitates the formation of more functional receptors.

Studies by de Graaf, Speckman & Stouthamer (1969) showed that a bacteriocin produced by *Enterobacter cloacae* strain DF 13 inhibited protein synthesis of sensitive *Klebsiella edwardsii* bacteria. No effect on RNA and DNA synthesis, nor on respiration and fermentation was found. The degree of sensitivity to the bacteriocin depended on the medium in which the cells were grown. De Graaf *et al.* (1969) have suggested that this diversity in sensibility may be due to differing numbers of ribosomes in cells grown from different media, as shown by Maaløe & Kjeldgaard (1966). The bacteriocin stimulated RNA synthesis in a leucine-deficient mutant after leucine deprivation (de Graaf *et al.*, 1969). This effect stimulates the action of several antibiotics which also inhibit protein synthesis, such as chloramphenicol (Fraenkel & Niedhardt, 1961), and streptomycin (Brock, 1964). Growth of bacteriocin cultures could be restored by trypsin treatment (de Graaf *et al.*, 1969).

De Klerk (personal communication, 1971) has found that the bacteriocin from *Proteus morganii* strain 336 causes inhibition of protein synthesis and of RNA and DNA synthesis in sensitive *P. morganii* strain MR 47 cells. Degradation of RNA was also observed.

CLASSIFICATION OF BACTERIOCINS

One of the major criteria for the definition of a bacteriocin as recommended by Jacob *et al.* (1953) is the specific fixation of bacteriocins by sensitive bacteria (Ivanovics, 1962). It is this property which was utilized by Fredericq (1948) in the first attempt at some classification of the colicins (Reeves, 1965).

Fredericq (1948) found that whenever sensitive cells are treated with colicin, resistant

mutants grow up, but these remain sensitive to the colicins of most, but not all other strains (Fredericq, 1946). By the judicious use of such mutants, he was able to group the colicins into 17 types, named colicins A, B, C, D, E, F, G. H, I, J, K, V, S1, S2, S3, S4, and S5; each characterized by the observation that, in general, a mutantresistant to one, was resistant to all of that group but not necessarily to colicins of other types (Reeves, 1965). Unfortunately, this system was complicated by the frequent occurrence of strains producing more than one colicin and of mutants resistant to more than one colicin (Fredericq, 1950). By the use of particular indicator strains and other special techniques, the six colicin types E, F, J, S2 and S5 have been amalgamated in a new type E; all of them appearing to adsorb to the same specific receptor (Fredericq, 1956). Colicins of Group E can, however, be further separated into subtypes E1, E2 and E3, according to the specificity of the immunity conferred by transfer of colicinogenic factors E to indicator strains having the corresponding receptor (Fredericq, 1965). Similarly, colicins Ia and Ib apparently share a common receptor and belong to Group I, but can be distinguished from each other by their immunity specificity (Stocker, 1965; in Nomura, 1967).

Fredericq (1965) has pointed out that the present procedure for the classification of bacteriocins according to the specificity of their receptors is by no means sufficiently efficient to be absolute. This is partly due to the fact that many bacteriocinogenic strains produce more than one type of colicin, and many indicator mutants appear to be more or less unstable. In addition some mutants may not be strictly specific of only one colicin, and quantitative differences may arise due to differences in the degree of resistance of the mutants and in the amount of colicins produced by different colicinogenic strains (Fredericq, 1965).

A problematic situation concerning the taxonomy of the bacteriocins has arisen in the years subsequent to Fredericq's original considerations regarding the classification of bacteriocins (Bradley, 1967). The original definition of a bacteriocin (Jacob *et al.*, 1953) omits any reference as to the origin or morphological characteristics which must be adhered to by a bactericidal particle in order to qualify for the definition (Bradley, 1967). The discovery of the high-molecular weight phage-tail-like particles such as the pyocins (Jacob, 1954), monocins (Sword &Pickett, 1961), and those from Proteus (Taubeneck, 1963; Coetzee *et al.*, 1967) for example, has raised some fundamental taxonomic questions. These particles are protein in nature, do not multiply within a sensitive indicator, are generally restricted in their action to members of the same or closely-related species and adsorb to specific receptors. They thus fulfil the operational definition of a bacteriocin, despite their rather obvious morphological similarity to bacteriophages (Bradley & Dewar, 1966; Bradley, 1967; Ivanovics, 1962; Hamon & Peron, 1966).

Nomura (1967) has proposed that an antibacterial substance, regardless of its morphology, may be called a bacteriocin if it can be shown to require a specific receptor for its action. According to Bradley (1967) one possible taxonomic criterion is the natural division of bacteriocins into two basic types which could conveniently be designated as low and high molecular weight forms. Some of their respective properties, which admittedly have been studied in only a few cases, are as follows:—

> The low molecular weight bacteriocins are non-sedimentable, colicin CA42—F, for example, having an S value of only 3,6 (Reeves, 1965); they are generally trypsin-sensitive, are thermostable and cannot be resolved in the electron microscope. The high molecular weight bacteriocins are sedimentable at 50,000 X g., colicin 15, for example has a molecular weight exceeding 200,000 (Sandoval, Reilly & Tandler, 1965). They are trypsin-resistant, relatively thermolabile and are visible in the electron microscope as phage-like objects or components.
>
> A common feature of the two types is that they are essentially protein in nature (Reeves, 1965).

The aforementioned taxonomic problem has further been aggravated by the observation in recent years of numbers of inducible objects from many bacteria, a number of which exhibit no biological activity, but which possess certain properties indicative of a possible bacteriophagic origin (Bradley, 1967).

Seaman *et al.* (1964) described what they called a 'protophage' obtained as a result of the induction of a strain of *Bacillus subtilis*. This complete phage-like particle was able to kill a sensitive cell without multiplying within it. They considered it as being a defective temperate bacteriophage rather than a bacteriocin, due to the presence of DNA in the head. Ionesco, Ryter & Schaeffer (1964), who described a similar 'killer particle' from *B. subtilis*, shared this view. Bradley (1965) and Stickler, Tucker & Kay (1965) who studied similar particles from *B. subtilis* and *B. licheniformis* tend on the other hand towards their classification as bacteriocins, their contention being that even certain pyocins, such as pyocin C10, contain a small amount of DNA.

If the definition of a bacteriocin, according to Bradley (1967), is accepted as 'bactericidal particles which are unable to multiply in a sensitive indicator', then the Bacillus particles must be included.

Lewin (1963) described a form of RNA-containing particle which is in the form of headless contracted phage tails. They are known as Rhapidosomes, and are produced by the autolysis of the gliding organism *Saprospira grandis.* They do not appear to have any biological activity. Correll & Lewin (1964) demonstrated that these rhapidosomes may frequently be encountered in the form of polysheaths as well.

Pate, Johnson & Ordal (1967) have reported rhapidosomes from *Chondrococcus columnaris,* and Reichenbach (1965) and Gräf (1965) showed similar particles occurring in the myxobacterium *Archangium violaceum* and in cells of *Sporocytophaga myxococcoides* respectively. Pate *et al.* (1967) postulated the origin of *C. columnaris* rhapidosomes as being the breakdown products of the compound membranes of the mesosomes.

The rhapidosomes resemble the so-called microtubules found in *Proteus mirabilis* by von Iterson, Hoeniger & van Zanten (1967), which are also polysheath-like in appearance and are believed to be of bacteriophage origin (Bradley, 1967).

Mennigmann (1965) proposed a scheme whereby antibacterial agents may be differentiated into bacteriocins, as distinct from bacteriophages and defective bacteriophages on the basis of a number of criteria:—

1. Production of the antibacterial agent inducible by ultraviolet irradiation, Mitomycin C or thymine deprivation.

2. Lysis of the bacterial culture on induction.

3. No loss of antibacterial activity after storage at $5^{o}C$ for more than one year.

4. No loss of antibacterial activity on exposure to 8,7 kiloergs UV/mm^2.

5. Loss of antibacterial activity of heating at $70^{0}C$ for 30 minutes.

6. Loss of antibacterial activity on treating with trypsin.

7. No antibacterial activity left in supernatent after high-speed centrifugation.

8. No loss of anti-bacterial activity by dialysis.

9. No transmissibility of the antibacterial activity.

10. Antibacterial activity very limited with regards to the number of strains which can be inhibited.

All the above criteria merely indicate the presence of an antibacterial agent. Numbers 6 and 9 favour the agent as being a bacteriocin, whilst 2 and 7 are indicative of a bacteriophage or defective phage. No distinction can be made as to the nature of the agent on the basis of the criteria nos. 1, 3, 4, 5, 8, and 10 (Mennigmann, 1965).

In summary, our present knowledge of many of the products liberated by certain organisms is insufficient to justify any absolute taxonomic criteria regarding the bacteriocins. For the time being, it is thought best to place the two groups of high and low molecular weight bactericidal particles, including the 'defective phages' or 'killers' under the one heading of bacteriocins (Bradley, 1967).

REFERENCES

ABBOTT, J.D. & SHANNON, R. (1958). A method for typing *Shigella sonnei,* using colicine production as a marker. *Journal of Clinical Pathology 11,* 71.

ALFÖLDI, L., JACOB, F., WOLLMAN, E.L. & MAZÉ, R. (1958). Sur le déterminisme génétique de la colicinogénie. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences. 246,* 3531.

AMAKO, K., TOKIWA, H. & TAKEYA, K. (1970). A bacteriocin induced from a strain of *Shigella sonnei. Japanese Journal of Microbiology 14,* 505.

AMANO, T., GOEBEL, W.F. & MILLER-SMIDTH, E. (1958). Colicin K. III. The immunological properties of a substance having colicin K activity. *Journal of Experimental Medicine 108,* 731.

AMATI, P. (1964). Vegetative multiplication of colicinogenic factors after induction in *Escherichia coli. Journal of Molecular Biology 8,* 239.

AMATI, P. & OZEKI, H. (1962). Transfer of colicinogenic factors to *Serratia marcescens. Abstract: VIIIth International Congress of Microbiology A. 51,* 26.

ANACKER, R.L. & ORDAL, E.J. (1959). Studies on the Myxobacterium *Chondrococcus columaris. Journal of Bacteriology 78,* 33.

ATKINSON, N. (1966). Salmonella antibiotics. I. Salmonellin, a new colicin-like antibiotic. *Australian Journal of Experimental Biology and Medical Science 44,* 559.

ATKINSON, N. (1970). Colicin-like antibiotics and bacteriophages of Salmonellas. *Australian Journal of Experimental Biology and Medical Science 48,* 199.

BARRY, G.T. EVERHART, D.L., ABBOTT, V. & GRAHAM, M.G. (1965). Preparation, Properties and Relationship of Substances Possessing Colicine A Activity Obtained from *Enterobacteriaceae. Zentralblatt fur Bakteriologie, Parasitenkunde, Infekionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 248.

BARRY, G.T., EVERHART, D.L. & GRAHAM, M. (1963). Colicin A. *Nature, London 198,* 211.

BAZARAL, M. & HELINSKI, D.R. (1968a). Characterization of multiple circular DNA forms of colicinogenic factor E1 from *Proteus mirabilis. Biochemistry 7,* 3513.

BAZARAL, M. & HELINSKI, D.R. (1968b). Circular DNA forms of colicinogenic factors E1, E2 and E3 from *Escherichia coli. Journal of Molecular Biology 36,* 185.

BEERENS, H. & BARON, G. (1965). Mise en évidence de Bactériocines élaboreés par les bactéries anaérolies a gram negatif appartenant au genre Eggerthella. *Annales de l'Institut Pasteur, Paris 106,* 225.

BEERENS, H., CASTEL, M.M., & FIEVEZ, L. (1962). Classification des *Bacteroidaceae. VIIIth International Congress for Microbiology. Abstract A1.14,* 120.

BEN-GURION, R. (1965). Induction of Colicines. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene: Erste Abteilung: Originale 196,* 183.

BEN-GURION, R. (1970). Chloramphenicol and Puromycin as inducers of colicinogenic bacteria. *Biochemical and Biophysical Research Communications, 40,* 1281.

BEN-GURION, R. & HERTMAN, I. (1958). Bacteriocin-like material produced by *Pasteurella pestis. Journal of General Microbiology, 19,* 289.

BEPPA, T. & ARIMA, K. (1970). Dissociating activity of purified colicin E2 on the isolated membrane complex of *Escherichia coli. Biochimica et Biophysica Acta 219,* 512.

BHATTACHARYYA, P., WENDT, L., WHITNEY, E. & SILVERS, S. (1970). Colicin-tolerant mutants of *Escherichia coli:* Resistance of membranes to colicin E1. *Science 168,* 998.

BORDET, P. & BEUMER, J. (1948) Inhibition de l'action d'antibiotiques par des extraits des bactéries sensibles. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales. 142,* 259.

BOWMAN, C.M., DAHLBERG, J.E., IKEMURA, T., KONISKY, J. & NOMURA, M. 1971). Specific Inactivation of 16S Ribosomal RNA Induced by Colicin E3 *In Vivo. Proceedings of the National Academy of Sciences of the United States of America 68,* 964.

BRADLEY, D.E. (1965). Techniques for mounting, dispersing and disintegrating specimens. In *Techniques for Electron Microscopy.* 2nd Edition. Kay, D.H. (Ed.) Blacwell Scientific Publications, Oxford.

BRADLEY, D.E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriological Reviews 31,* 230.

BRADLEY, D.E. & DEWAR, C.A. (1966). The structure of phage-like objects associated with non-induced bacteriocinogenic bacteria. *Journal of General Microbiology 45,* 399.

BROCK, T.D. (1964). Action of Streptomycin and related antibiotics. *Federation of American Societies for Experimental Biology. 23,* 965.

BROCK, T.D. & DAVIE, J.M. (1963). Probable Identity of a Group D Hemolysin with a Bacteriocine. *Journal of Bacteriology 86,* 708.

BROCK, T.D., PEACHER, B. & PIERSON, D. (1963). Survey of the bacteriocines of Enterococci. *Journal of Bacteriology 86,* 702.

BRUBAKER, R.R. & SURGALLA, M.J. (1961). Pesticins. I. Pesticin-bacterium interrelationships and environmental factors influencing activity. *Journal of Bacteriology 82,* 940.

BRUBAKER, R.R. & SURGALLA, M.J. (1962). Pesticins. II. Production of pesticins I and II. *Journal of Bacteriology 84,* 539.

BRUBAKER, R.R., SURGALLA, M.J. & BEESLEY, E.D. (1965). Pesticinogeny and bacterial virulence. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 302.

BURMAN, L.G. & NORDSTRÖM, K. (1971). Colicin Tolerance induced by Ampicillin or Mutation to Ampicillin resistance in a strain of *Escherichia coli* K12. Journal of Bacteriology 106, 1.

CARO, L.G. & SCHNÖS, M. (1966). The attachment of male-specific bacteriophage fl to sensitive strains of *Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America 56,* 126.

CHANG, Y. & HAGER, L.P. (1970). Inhibition of colicin E2 by bacterial lipopolysaccharide. *Journal of Bacteriology 104,* 1106.

CHANGEUX, J.P. & THIÉRY, J. (1967). On the mode of action of colicins: A model of regulation at the membrane level. *Journal of Theoretical Biology 17,* 315.

CHANGEUX, J.P., THIÉRY, J., TUNG, Y. & KITTEL, C. (1967). On the co-operativity of biological membranes. *Proceedings of the National Academy of Sciences of the United States of America 57,* 335.

CLARK-WALKER, G.D. (1969). Association of microcyst formation in *Spirillum itersonii* with the spontaneous induction of a defective bacteriophage. *Journal of Bacteriology 97,* 885.

CLEWELL, D.B. & HELINSKI, D.R. (1969). Supercoiled circular DNA-protein complex in *Escherichia coli:* Purification and induced conversion to an open circular DNA form. *Proceedings of the National Academy of Sciences of the United States of America 62,* 1159.

CLEWELL, D.B. & HELINSKI, D.R. (1970a). Evidence for the existence of the colicinogenic factors E2 and E3 as supercoiled circular DNA-protein relaxation complexes. *Biochemical and Biophysical Research Communications 40,* 608.

CLEWELL, D.B. & HELINSKI, D.R. (1970b). Existence of the Colicinogenic factor — sex factor Col Ib-P9 as a supercoiled circular DNA-protein relaxation complex. *Biochemical and Biophysical Research Communications 41,* 150.

CLOWES, R.C. (1961). Colicine factors in bacteria. *Escherichia coli* K12. *Nature, London 190,* 988.

CLOWES, R.C. (1963). Colicin factors and episomes. *Genetical Research, Cambridge 4,* 163.

CLOWES, R.C. (1965). Transmission and elimination of colicin factors and some aspects of immunity to colicin E1 in *E. coli. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 152.

CLOWES , R.C. & MOODY, E.E.M. (1966). Chromosomal Transfer from "Recombinational-Deficient" Strains of *Escherichia coli* K12. *Genetics 53,* 717.

CLOWES, R.C., MOODY, E.E.M. & PRITCHARD, R.H. (1965). The elimination of extrachromosomal elements in thymineless strains of *Escherichia coli* K12. *Genetical Research, Cambridge 6,* 147.

COETZEE, J.N. (1964). Transmission of colicinogeny to Providence strains. *Nature London 203,* 897.

COETZEE, J.N. (1967). Bacteriocinogeny in strains of Providence and *Proteus morganii. Nature, London 213,* 614.

COETZEE, H.L., DE KLERK, H.C., COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like Particles associated with Intra-species killing of *Proteus morganii*. *Journal of General Virology 2,* 29.

COETZEE, J.N., DE KLERK, H.C. & SMIT, J.A. (1967). A transducing bacteriophage for *Proteus vulgaris*. *Journal of General Virology 1,* 561.

CORRELL, D.L. & LEWIN, R.A. (1964). Rod-shaped ribonucleoprotein particles from Saprospira. *Canadian Journal of Microbiology 10,* 63.

CRADOCK-WATSON, J.E. (1965). The production of bacteriocines by Proteus species. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale, 196,* 385.

DAJANI, A.S., GRAY, E.D. & WANNAMKER, L.W. (1970). Bactericidal substance from *Staphylococcus aureus*. Biological properties. *Journal of Experimental Medicine 131,* 1004.

DAJANI, A.S. & WANNAMAKER, L.W. (1969). Demonstration of a bactericidal substance against β-hemolytic streptococci in supernatent fluids of Staphylococcal cultures. *Journal of Bacteriology 97,* 985.

DANDEU, P. & BARBU, E. (1967). Purification de la colicine K. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences 26,* 774.

DE GRAAF, F.K., SPECKMAN, E.A.S. & STOUTHAMER, A.H. (1969). Mode of action of a bacteriocin produced by *Enterobacter cloacae* DF 13. *Antonie van Leeuwenhoek: Journal of Microbiology and Serology 35,* 287.

DE GRAAF, F.K. & STOUTHAMER, A.H. (1970). Isolation and properties of bacteriocin-tolerant mutants of *Klebsiella edwardsii var. edwardsii*. *Antonie van Leeuwenhoek: Journal of Microbiology and Serology 36,* 217.

DE KLERK, H.C. & COETZEE, J.N. (1961). Antibiosis among Lactobacilli. *Nature, London 192,* 340.

DE KLERK, H.C. & SMIT, J.A. (1967). Properties of a *Lactobacillus fermenti* bacteriocin. *Journal of General Virology 48,* 309.

DE WITT, W. & HELINSKI, D.R. (1965). Characterization of colicinogenic factor E1 from a non-induced and a Mitomycin C-induced Proteus strain. *Journal of Molecular Biology, 13,* 692.

DRYGIN, Y.F., BOGDANOVA, S.L. & BOGDANOV, A.A. (1971). Membrane association of circular forms of Col E1 DNA: Electron microscopic examination. *Febs Letters 12,* 201.

ELGAT, M. & BEN-GURION, R. (1969). Mode of action of pesticin. *Journal of Bacteriology 98,* 359.

ENDO, H., AYABE, K., AMAKO, K. & TAKEYA, K. (1965). Inducible phage of *Escherichia coli 15. Virology 25,* 469.

ENDO, H., KAMIYA, T. & ISHIZAWA, M. (1963). λ Phage induction by colicine E2. *Biochemical and Biophysical Research Communications 11,* 477.

FARKAS-HIMSLEY, H. & SEYFRIED, P.L. (1963a). Lethal biosynthesis of a bacteriocin, vibriocin, by *V. comma.* I. Conditions affecting its production and detention. *Canadian Journal of Microbiology 9,* 329.

FARKAS-HIMSLEY, H. & SEYFRIED, P.L. (1963b). Lethal biosynthesis of a bacteriocin, by *V. comma.* II. Vibriocin production and sensitivity in relation to redox potentials and Streptomycin resistance. *Canadian Journal of Microbiology, 9,* 339.

FARKAS-HIMSLEY, H. & SEYFRIED, P.L. (1965). Vibriocin and nucleic acids. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale, 196,* 298.

FASTIER, L.B. (1949). An antibiotic substance produced by a member of the *Shigella* group. *Journal of Immunology 62,* 399.

FIELDS, K.L. & LURIA, S.E. (1969). Affects of colicins E1 and K on cellular metabolism. *Journal of Bacteriology, 97,* 64.

FIELDS, K.L., RUBY, R.H. & LURIA, S.E. (1966). Separation of transport and accumulation of β-galactosides by colicins. *Federation Proceedings; Federation of American Societies for Experimental Biology 25,* 591.

FISK, R.T. (1942). Studies on staphylococci. I. Occurrence of bacteriophage carriers among strains of *Staphylococcus aureus. Journal of Infectious Diseases 71,* 153.

FRAENKEL, D.G. & NIEDHARDT, F.C. (1961). Use of chloramphenicol to study control of RNA synthesis in bacteria. *Biochemica et Biophysica Acta 53,* 96.

FREDERICQ, P. (1946). Sur la pluralité des rècepteurs d'antibiose de *E. coli. Comptes Rendus de Seances de la Societe de Biologie et de ses Filiales 140.* 1189.

FREDERICQ, P. (1948). Actions antibiotiques réciproques chez les *Enterobacteriaceae. Revue Belge de Pathologie et de Medicine Experimentale (Suppl. 4) 19,* 1.

FREDERICQ, P. (1950). Analogies entre colicines E et K et bacteriophages II et III. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 144,* 437.

FREDERICQ, P. (1951). Récherches sur l'origine des mutants de *E. coli* V produisant la colicine M. *Comptes Rendus de Seances de la Societe de Biologie et de ses Filiales 145,* 930.

FREDERICQ, P. (1952). Action bactericide de la colicine K. *Comptes Rendus de Seances de la Societe de Biologie et de ses Filiales 146,* 1295.

FREDERICQ, P. (1953). Colicines et bactériophages. *Annales de l'Institut Pasteur, Paris, 84,* 294.

FREDERICQ, P. (1954). Induction de la production de colicine par irradiation ultraviolette de souches colicinogenes *d'Escherichia coli. Comptes Rendus de Seances de la Societe de Biologie et de ses Filiales 148,* 1276.

FREDERICQ, P. (1955). Induction de la production de colicine et de bactériophages par irradiation ultraviolette de souches colicinogenes et lysogènes *d'Escherichia coli. Comptes Rendus de Seances de la Societe de Biologie et de ses Filiales 149,* 2028.

FREDERICQ, P. (1956). Resistance et immunité aux colicines. *Comptes Rendus de Seances de la Societe de Biologie et de ses Filiales 150,* 1514.

FREDERICQ, P. (1957). Colicins. *Annual Review of Microbiology 11,* 7.

FREDERICQ, P. (1958). Colicins and colicinogenic factors. *Symposia of the Society for Experimental Biology 12,* 104.

FREDERICQ, P. (1963). On the nature of colicinogenic factors: A review. *Journal of Theoretical Biology 4,* 159.

FREDERICQ, P. (1965a). A note on the classification of colicines. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 141.

FREDERICQ, P. (1965b). Genetics of colicinogenic factors. *Zentralblatt fur Bakteriolofie, Parasitenkunde, Infektionskrankheiten und Hygiene 196,* 142.

FREDERICQ, P. & GRATIA, J.P. (1950). Rapports entre colicines et bactériophages du Groupe T.1—T.7. *Antonie van Leeuwenhoek; Journal of Microbiology and Serology 16,* 119.

GOEBEL, W.F. & BARRY, G.T. (1958). Colicin KII. The preparation and properties of a substance having colicin K activity. *Journal of Experimental Medicine 107,* 185.

GOEBEL, W.F., BARRY, G.T. & SHEDLOVSKY, M. (1956). Colicine K.I. The production of colicine K in media maintained at constant pH. *Journal of Experimental Medicine 103,* 577.

GOEBEL, W.F. & HELINSKI, D.R. (1968). Generation of higher multiple circular DNA forms in bacteria. *Proceedings of the National Academy of Sciences of the United States of America 61,* 1406.

GRÄF, W. (1956). Bewegungsorganellen bei myxobakterien. *Archiv fur Hygiene und Bakteriologie 149,* 518.

GREEN, D.E. & PERDUE, J.F. (1966). Membranes as expressions of repeating units. *Proceedings of the National Academy of Sciences of the United States of America 55,* 1295.

GROMKOVA, R.H. (1971). Induction of colicin Ia at high temperature. *Journal of Bacteriology 106,* 720.

GUTERMAN, S.K. & LURIA, S.E. (1969). *Escherichia coli:* strains that excrete an inhibitor of colicin B. *Science 164,* 1414.

HAMON, Y. (1956). Contribution á l'étude des pyocines. *Annales de l'Institut Pasteur, Paris, 92,* 489.

HAMON, Y. (1964). Les bacteriocines. *Annales de l'Institut Pasteur, Paris 107,* 18.

HAMON, Y. & LEWE, Z. (1955). Etude de l'induction par l'iiradiation ultraviolette de quelques cultures d'*E. coli* K12 péralablement rendues colicinogènes par transduction. *Annales de l'Institut Pasteur 89,* 336.

HAMON, Y. & PÉRON, Y. (1960). Etude du mode de fixation des colicines et des pyocines dur les bacteries sensibles. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 251,* 1840.

HAMON, Y. & PÉRON, Y. (1961). Les proprietes antagonistes reciproque parmi les Erwinia. Discussion de la position taxonomique de ce genre. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 253,* 914.

HAMON, Y. & PÉRON, Y. (1962). Sur la cinétique de la libération de leur antibiotique pour les divers types de microbes bacteriocinogènes. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 255,* 2210.

HAMON, Y. & PÉRON, Y. (1963). Individualisation de quelques nouvelles familles d'enterobacteriocines. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 257,* 309.

HAMON, Y. & PÉRON, Y. (1966). Sur la nature des bactériocines produites par *Listeria monocytogenes. Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences 263,* 198.

HAMON, Y., VERON, M. & PÉRON, Y. (1961). Contribution à l'etude des propriétés lysogènes et bacteriocinogènes dans le genre *Pseudomonas. Annales de l'Institut Pasteur, Paris 101,* 738.

HELINSKI, D.R. & HERSCHMAN, H.R. (1967). Effect of Rec mutations on the activity of colcinogenic factors. *Journal of Bacteriology 94,* 700.

HERSCHMAN, H.R. & HELINSKI, D.R. (1967). Purification and characterization of colicin E2 and colicin E3. *Journal of Biological Chemistry 242,* 5360.

HERTMAN, I. & BEN-GURION, R. (1958). A study of pesticin biosynthesis. *Journal of General Microbiology 21,* 135.

HIGERD, T.B., BAECHLER, C.A. & BERK, R.S. (1967). *In vitro* and *in vivo* characterization of pyocin. *Journal of Bacteriology 93,* 1976.

HILL, C. & HOLLAND, I.B. (1967). Genetic basis of colicin E susceptibility in *Escherichia coli. Journal of Bacteriology 94,* 677.

HINSDILL, R.D. & GOEBEL, W.F. (1966). Colicine K. VII. The transfer of Type K colicinogeny to *Shigella sonnei. Journal of Experimental Medicine 123,* 881.

HOLLAND, I.B. (1961). The purification and properties of megacin, a bacteriocin from *Bacillus megaterium. Biochemical Journal 78,* 641.

HOLLAND, I.B. (1962). Further observations on the properties of megacin, a bacteriocin formed by *Bacillus megaterium. Journal of General Microbiology 29,* 603.

HOLLAND, I.B. (1963). Effect of a bacteriocin preparation (megacin C) on DNA synthesis in *Bacillus megaterium. Biochemical and Biophysical Research Communications 13,* 246.

HOLLAND, I.B. (1965). A bacteriocin specifically affecting DNA synthesis in *Bacillus megaterium. Journal of Molecular Biology 12,* 429.

HOMMA, J.Y. & SUZUKI, N. (1964). 'Cell wall protein A' of *Pseudomonas aeruginosa* and its relationship to 'original endotoxin protein'. *Journal of Bacteriology 87,* 630.

HOMMA, J.Y. & SUZUKI, N. (1966). The protein moiety of the endotoxin of *Pseudimonas aeruginosa. Annals of the New York Academy of Sciences 133,* 508.

HOSOKOWA, K., FUJIMURA, K. & NOMURA, M. (1966). Reconstitution of functionally active ribosomes from inactive subparticles and proteins. *Proceedings of the National Academy of Sciences of the United States of America. 55,* 198.

HUTTON, J.J. & GOEBEL, W.F. (1961). Colicin V. *Proceedings of the National Academy of Sciences of the United States of America 47,* 1498.

HUTTON, J'J. & GOEBEL, W.F. (1962). The isolation of colicine V and a study of its immunological properties. *Journal of General Physiology 45,* 125.

IIJIMA, T. (1962). Studies on the colicinogenic factor in *Escherichia coli* K12 induction of colicine production by Mitomycin C. *Biken Journal: Journal of the Research Institute for Microbial Diseases 5,* 1.

IKEDA, K., KAGEYAMA, M. & EGAMI, F. (1964). Studies of a pyocin. II. Mode of production of the pyocin. *Journal of Biochemistry 55,* 54.

INSELBURG, J. (1970). Segregation into and replication of plasmid deoxyribonucleic acid in chromosomeless segregants of *Escherichia coli. Journal of Bacteriology 102,* 642.

INSELBURG, J. & FUKE, M. (1970). Replicating DNA: Structure of colicin factor E1. *Science 169,* 590.

IONESCO, H., RYTER, A. & SCHAEFFER, P. (1964). Sur un bactèriophage hèbergé par la souche marbug de *Bacillus subtilis. Annales de l'Institut Pasteur, Paris 107,* 764.

ISHII, S., NISHI, Y. & EGAMI, F. (1965). The fine structure of a pyocin. *Journal of Molecular Biology 13,* 428.

IVANOVICS, G. (1962). Bacteriocins and bacteriocin-like substances. *Bacteriological Reviews 26,* 108.

IVANOVICS, G. & ALFÖLDI, L. (1955). Observation on lysogenesis in *Bacillus megaterium* and on megacin, the antibacterial principle of this *Bacillus* species. *Acta Microbiologica Academiae Scientiarum Hungaricae 2,* 275.

IVANOVICS, G. & ALFÖLDI, L. (1957). Bacteriocinogenesis in *Bacillus megaterium. Journal of General Microbiology 16,* 522.

IVANOVICS, G., ALFÖLDI, L. & ABRAHAM, E. (1955). Das antibacterielle spektrum des megacins. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung; Originale 163,* 274.

IVANOVICS, G., ALFÖLDI, L. & NAGY, E. (1959). Mode of action of megacin. *Journal of General Microbiology 21,* 51.

IVANOVICS, G. & NAGY, E. (1958). Hereditary aberrancy in growth of some *Bacillus megaterium* strains. *Journal of General Microbiology 19,* 407.

JACOB, F. (1954). Biosynthése induit et mode d'action d'une pyocine antibiotique de *Pseudomonas pyocyanea. Annales de l'Institut Pasteur, Paris 86,* 149.

JACOB, F., LWOFF, A., SIMINOVITCH, A. & WOLMANN, E. (1953). Définition de quelques termes relatifs à la lysogènie. *Annales de l'Institut Pasteur, Paris 84,* 222.

JACOB, F., SIMINOVITCH, L. & WOLLMANN, E. (1952). Sur la biosynthése d'une colicine et sur son mode d'action. *Annales de l'Institut Pasteur, Paris 83,* 295.

JACOB, F. & WOLLMANN, E. (1958). Les épisomes, éléments génétiques ajoutés. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences 247,* 154.

JAYAWARDENE, A. & FARKAS-HIMSLEY, H. (1969). Vibriocin: a bacteriocin from *Vibrio comma.* I. Production, purification, morphology and immunological studies. *Microbios 1B,* 87.

JAYAWARDENE, A. & FARKAS-HIMSLEY, H. (1970). Mode of action of vibriocin. *Journal of Bacteriology 102,* 382.

JESAITIS, M.A. (1970). The nature of colicin K from *Proteus mirabilis. Journal of Experimental Medicine. 131,* 1016.

KAGEYAMA, M. (1964). Studies of a pyocin. *Journal of Biochemistry 55,* 49.

KAGEYAMA, M. & EGAMI, F. (1962). On the purification and some properties of a pyocin, a bacteriocin produced by *Pseudomonas aeruginosa. Life Sciences; Part II: Biochemistry, General and Molecular Biology No. 9,* 471.

KAGEYAMA, M., IKEDA, K. & EGAMI, F. (1964). Studies of a pyocin. III. Biological properties of the pyocin. *Journal of Biochemistry 55,* 59.

KAHN, P. & HELINSKI, D.R. (1964). Relationship between colicinogenic factors E1 and V and F factor in *Escherichia coli. Journal of Bacteriology 88,* 1573.

KAHN, P.L. & HELINKSI, D.R. (1965). Interaction between colicinogenic factor V and the intergrated F factor in an Hfr strain of *Escherichia coli. Journal of Bacteriology* 90, 1276.

KAZIRO, Y. & TANAKA, M. (1965). Studies on the mode of action of pyocin. I. Inhibition of macromolecular synthesis in sensitive cells. *Journal of Biochemistry 57,* 689.

KAZIRO, Y., TANAKA, M. & SHIMAZONO, N. (1964). Mode of action of pyocin. Inactivation of ribosomes in supporting poly U directed incorporation of phenylalanine. *Biochemical and Biophysical Research Communications 17,* 624.

KEENE, J.H. (1966). Preparation and chemical properties of colicine V. *Canadian Journal of Microbiology 12,* 425.

KELLENBERGER, G. & KELLENBERGER, E. (1956). Etude de souches coliogènes au microscope électronique. *Schweizerische Zeitschrift fur Allgemeine Pathologie und Bakteriologie 19,* 582.

KINGSBURY, D.T. (1966). Bacteriocin production by strains of *Neisseria meningitidis. Journal of Bacteriology 91,* 1696.

KOHIYAMA, M. & NOMURA, M. (1965). DNA synthesis and induction of colicine E2 as studied with a temperature-sensitive mutant of colicinogenic *E. coli* strain. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 211.

KONISKY, J. & NOMURA, M. (1967). Interaction of colicins with bacterial cells. II. Specific alteration of *Escherichia coli.* ribosomes induced by colicin E3 *in vivo. Journal of Molecular Biology 26,* 181.

KRcMÉRY, V., HURWITZ, C. & FREDERICQ, P. (1970). Loss of colicinogeny in *Escherichia coli* strains infected by certain resistance factors. *Journal of Bacteriology 102,* 521.

KUNUGITA, K. & MATSUHASHI, M. (1970). Purification and properties of colicin K. *Journal of Bacteriology 104,* 1017.

LACHOWICZ, T. (1965). Investigations on staphylococcins. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 340.

LAWN, A.M., MEYNELL, G.G., MEYNELL, E. & DATTA, N. (1967). Sex pili and the classification of sex factors in the *Enterobacteriaceae. Nature, London 216,* 343.

LEVISOHN, R., KONISKY, J. & NOMURA, M. (1971). Interaction of colicins with bacterial cells. IV. Immunity breakdown studied with colicins Ia and Ib. *Journal of Bacteriology 96,* 811.

LEWIN, R.A. (1963). Rod-shaped particles in Saprospira. *Nature, London, 198,* 103.

LURIA, S.E. (1964). On the mechanisms of action of colicins. *Annales de l'Institut Pasteur Paris 107,* 67.

LUZZATI, D. & CHEVALLIER, M.R. (1964). Induction, par carence en thymine, de la production de colicine par des bactéries colicinogènes thymine-exigeantes. *Annales de l'Institut Pasteur Paris* 107, 152.

MAALØE, O. & KJELDGAARD, N.C. (1966). Steady states of growth. *Control of Macromolecular Synthesis* p.70–96. W.A. Benjamin Inc., New York & Amsterdam.

MACFARREN, A.C. & CLOWES, R.C. (1967). A comparative study of two F-like colicin factors, Col V2 and Col V3, in *Escherichia coli* K–12. *Journal of Bacteriology 94,* 365.

MAC PHEE, D.G. (1970). Recombination-deficient mutants of colicinogenic *Salmonella typhimurium* detected by their failure to produce colicin. *Journal of Bacteriology 104,* 345.

MAEDA, A. & NOMURA, M. (1966). Interaction of colicines with bacterial cells. I. Studies with radioactive colicines. *Journal of Bacteriology 91,* 685.

MANDEL, M. & MOHN, F. (1962). Colicins in *Serratia marcescens.* Microbial *Genetics Bulletin 18,* 15.

MARE, I.J. & COETZEE, J.N. (1964). Antibiotics of *Alcaligenes faecalis. Nature, London 203,* 430.

MATSUHASHI, H., FOX, M.S. & GOEBEL, W.F. (1960). Colicine K. IV. The effect of metabolites upon colicine synthesis. *Journal of Experimental Medicine 112,* 1055.

MAYR-HARTING, A. (1964). The adsorption of colicine. *Journal of Pathological Bacteriology 87,* 255.

MAYR-HARTING, A. & SHIMELD, C. (1965). Some observations on colicine receptors. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 263.

MC CLOY, E.W. (1951). Studies on a lysogenic Bacillus strain. I.A bacteriophage specific for *Bacillus anthracis*. *Journal of Hygiene 49,* 114.

MC GEACHE, J. & MC CORMICK, W. (1969). A comparison of colicines K and V extracted from solid medium. *Antonie van Leeuwenhoek: Journal of Microbiology and Serology 35,* 97.

MEITERT, E. (1969). Bacteriocins in *Corynebacterium diphtheriae.* II. A study of the bacteriocins produced by *C. diphtheriae, C. ulceranus, C. hoffmani, C.xerose* and by atypical Corynebacteria. *Archives Roumaines de Pathologie Experimentale et de Microbiologie 28,* 1086.

MENNIGMANN, H.D. (1965). On the nature of the inducible antibacterial agent of *E. coli* 15. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 207.

MEYNELL, E. & DATTA, N. (1966). The nature and incidence of conjugation factors in *Escherichia coli. Genetical Research, Cambridge 7,* 141.

MEYNELL, G.G. & LAWN, A.M. (1967). Sex pili and common pili in the conjugational transfer of colicin factor Ib by *Salmonella typhimurium. Genetical Research Cambridge 9,* 359.

MITCHELL, R., NEWMAN, F. & EISENSTARK, A. (1959). Lytic agent for Brucella: phage or bacteriocin? *Bacteriological Proceedings 600,* 46.

MONK, M. & CLOWES, R.C. (1964). Transfer of the colicin I factor in *Escherichia coli* K12 and its interaction with the F fertility factor. *Journal of General Microbiology 36,* 365.

MORA, E.C. & EISENSTARK, A. (1958). Production of bacteriocin-like substances by strains of Mycobacterium. *Bacteriological Proceedings 58th General Meeting,* 81.

MUKAI, F.H. (1960). Interrelationship between colicin sensitivity and phage resistance. *Journal of General Microbiology 23,* 539.

NAGEL DE ZWAIG, R. (1966). Association between colicinogenic and fertility factors. *Genetics 54,* 381.

NAGEL DE ZWAIG, R. (1969). Mode of action of colicin A. *Journal of Bacteriology 99,* 913.

NAGEL DE ZWAIG, R. & ANTON, D.N. (1964). Interactions between colicinogenic and fertility factors. *Biochemical and Biophysical Research Communications 17,* 358.

NAGEL DE ZWAIG, R., ANTON, D.N. & PUIG, J. (1962). The genetic control of colicinogenic factors E2, I and V. *Journal of General Microbiology 29,* 473.

NAGEL DE ZWAIG, R. & PUIG, J. (1964). The genetic behaviour of colicinogenic factor E1. *Journal of General Microbiology 36,* 311.

NAGEL DE ZWAIG, R. & LURIA, S.E. (1967). Genetics and physiology of colicin-tolerant mutants of *Escherichia coli. Journal of Bacteriology 94,* 1112.

NAGY, E., ALFÖLDI, L. & IVANOVICS, G. (1959). Megacins. *Acta Microbiologica Academiae Scientiarum Hungaricae 6,* 327.

NOMURA, M. (1963). Mode of action of colicins. *Cold Spring Harbour Symposia on Quantitative Biology 28,* 315.

NOMURA, M. (1964). Mechanism of action of colicins. *Proceedings of the National Academy of Sciences of the United States of America 52,* 1514.

NOMURA, M. (1967). Colicins and related bacteriocins. *Annual Review of Microbiology 21,* 257.

NOMURA, M. & MAEDA, A. (1965). Mechanism of action of colicins. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 216.

NOMURA, M. & NAKAMURA, M. (1962). Reversibility of inhibition of nucleic acids and protein synthesis by colicin K. *Biochemical and Biocphysical Research Communications 7,* 306.

NOMURA, M. & WITTEN, C. (1967). Interaction of colicins with bacterial cells. III. Colicin-tolerant mutations in *Escherichia coli. Journal of Bacteriology 94,* 1093.

NOSE, K., MIZUNO, D. & OZEKI, H. (1966). Degradation of Ribosomal RNA from *Escherichia coli* induced by colicine E2. *Biochimica et Biophysica 119,* 636.

NÜSKE, R., HÖSEL, G., VENNER, H. & ZINNER, H. (1957). Uber ein Colicin aus *Escherichia coli* SG 710. *Biochemische Zeitschrift 329,* 346.

OBINATA, M. MIZUNO, D. (1970). Change in deoxyribonuclease activities in colicin E2-induced cells. *Biochemical and Biophysical Research Communications 199,* 330.

OHNISHI, Y., TAKADE, A. & TAKEYA, K. (1971). Morphological changes in *Pseudomonas aeruginosa* treated with rod-shaped pyocin 28. *Japanese Journal of Microbiology 15,* 201.

OZAKI, M., HIGASHI, Y., SAITO, H.O., AN, T. & AMANO, T. (1966). Identity of megacin A with phospholipase A. *Biken Journal: Journal of the Research Institute for Microbial Diseases 9,* 201.

OZEKI, H. (1965). The behaviour of colicinogenic factors in *Salmonella typhimurium. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene: Erste Abteilung: Originale 196,* 160.

OZEKI, H. & HOWARTH, S. (1961). Colicine factors as fertility factors in bacteria. *Salmonella typhimurium* strain LT 2. *Nature, London 190,* 986.

OZEKI, H. & STOCKER, B.A.D. (1958). Phage-mediated transduction of colicinogeny in *Salmonella typhimurium. Heredity 12,* 525.

OZEKI, H., STOCKER, B.A.D. & DE MARGERIE, H. (1959). Production of colicine by single bacteria. *Nature, London 184,* 337.

OZEKI, H., STOCKER, B.A.D. & SMITH, S.M. (1962). Transmission of colicinogeny between strains of *Salmonella typhimurium* grown together. *Journal of General Microbiology 28,* 671.

PAPAVASSILIOU, J. (1960). Colicinogènie et sensibilité aux colicines de *Escherichiceae* d'origines humaine et animale. *Archives de l'Institut, Pasteur, Tunis 37,* 103.

PATE, J.L., JOHNSON, J.L. & ORDAL, E.L. (1967). The fine structure of *Chondrococcus columnaris.* II. Structure and formation of Rhapidosomes. *Journal of Cell Biology 35,* 15.

PERSIEL, I. (1965). Contribution to the mode of action of colicine S3. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 275.

PRITCHARD, R.H. & LARK, K.G. (1964). Induction of replicaition by thymine starvation at the chromosome origin in *Escherichia coli. Journal of Molecular Biology 9,* 288.

REEVES, P.R. (1963). Preparation of a substance having colicin F activity from *Escherichia coli* CA 42. *Australian Journal of Experimental Biology 41,* 163.

REEVES, P.R. (1965). The bacteriocins. *Bacteriological Review 29,* 24.

REICHENBACH, H. (1965). Rhapidosomen bei myxobakterien. *Archiv fur Mikrobiologie 50,* 246.

REYNOLDS, B.L. & REEVES, P.R. (1963). Some observations on the mode of action of colicin F. *Biochemical and Biophysical Research Communications 11,* 140.

REYNOLDS, B.L. & REEVES, P.R. (1969). Kinetics and adsorption of colicin CA 42—E2 and reversal of its bactericidal activity. *Journal of Bacteriology 100,* 301.

RIBI, E., ANACKER, R.L., FUKUSHI, K., HASKINS, W.T., LANDY, M. & MILNER, K.C. (1964). *Relationship of chemical composition to biological activity in bacterial endotoxins.* Eds. Landy, M. & Braun, W. Rutgers Univ. Press, New Brunswick, N.J.

ROTH, T.F. & HELINSKI, D.R. (1967). Evidence for circular DNA forms of a bacterial plasmid. *Proceedings of the National Academy of Sciences of the United States of America 58,* 650.

RYAN, F.J., FRIED, P. & MUKAI, F.H. (1955). A colicin produced by cells that are sensitive to it. *Biochimica et Biophysica Acta 18,* 131.

SANDOVAL, H.K., REILLY, H.C. & TANDLER, B. (1965). Colicin 15: Possibly a defective phage. *Nature, London 205,* 522.

SCHWARTZ, S.A. & HELINSKI,D. (1968). Purification and characterization of colicin E1. *Bacteriological Proceedings 68,* 153.

SEAMAN, E., TARMY, E. & MARMUR, J. (1964). Inducible phages of *Bacillus subtilis. Biochemistry 3,* 607.

SENIOR, B.W. & EMSLIE-SMITH, A. (1969). Serological studies on Group—B colicines and organisms producing them. *Journal of Medical Microbiology 2,* 507.

SENIOR, B.W. & HOLLAND, I.B. (1971). Affect of colicin E3 upon the 30S ribosomal subunit of *Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America 68,* 959.

SENIOR, B.W. KWASNIAK, J. & HOLLAND, I.B. (1970). Colicin E3-directed changes in ribosome function and polyribosome metabolism in *Escherichia coli* K12. *Journal of Molecular Biology 53,* 205.

SILVER, S. & OZEKI, H. (1962). Transfer of deoxyribonucleic acid accompanying the transmission of colicinogenic properties by cell mating. *Nature, London 195,* 838.

SMARDA, J. & SCHUHMANN, E. (1967). Do certain colicins and bacteriophages share common receptors? *Nature, London. 213,* 614.

SMARDA, J. & TAUBENECK, U. (1968). Situation of colicin receptors in surface layers of bacterial cells. *Journal of General Microbiology 52,* 161.

SMITH, S.M., OZEKI, H. & STOCKER, B.A.D. (1963). Transfer of Col E1 and Col E2 during high frequency transmission of Col I in *Salmonella typhimurium. Journal of General Microbiology 33,* 231.

SMIT, J.A., DE KLERK, H.C. & COETZEE, J.N. (1968). Properties of a *Proteus morganii* bacteriocin. *Journal of General Microbiology 54,* 67.

STAEHELIN, T. & MESELSOHN, M. (1966). *In vitro* recovery of ribosomes and of synthetic activity from synthetically inactive ribosomal subunits. *Journal of Molecular Biology 15,* 245.

STICKLER, D.J., TUCKER, R.G. & KAY, D. (1965). Bacteriophage-like particles released from *Bacillus subtilis* after induction with hydrogen peroxide. *Virology 26,* 142.

STOCKER, B.A.D., SMITH, S.M. & OZEKI, H. (1963). High ihfectivity of *Salmonella typhimurium* newly infected by the Col I factor. *Journal of General Microbiology 30,* 201.

STROBEL, M. & NOMURA, M. (1966). Restriction of the growth of bacteriophage BF 23 by a colicine I (Col I—P9) factor. *Virology 28,* 763.

SWORD, C.P. & PICKETT, M.J. (1961). The investigation and characterization of bacteriophage from *Listeria monocytogenes. Journal of General Microbiology 25,* 241.

TAKEYA, K., MINAMISHIMA, Y., AMAKO, K. & OHNISHI, Y. (1967). A small rod-shaped pyocin. *Virology 31,* 166.

TAUBENECK, U. (1963). Uber die Produktion biologisch aktiver Phagen-Schänze durch einen defect lysogene *Proteus mirabilis*-stamm. *Zeitschrift fur Naturforschung; ten B. 18b,* 989.

TCHAN, Y.T. & GIUNTINI, J., (1950). Action antagoniste chez les Cytophagaceae. *Annales de l'Institut Pasteur Paris 78,* 451.

TIEZE, G.A., STOUTHAMER, A.H., JANSZ, H.S., ZANDBERG, J. & VAN BRUGGEN, E.F.J. (1969). A bacteriocinogenic factor of *Enterobacter cloacae. Molecular and General Genetics 106,* 48.

TSAO, S.S. & GOEBEL, W.F. (1969). Colicin K. VIII. The immunological properties of Mitomycin-induced colicin K. *Journal of Experimental Medicine 130,* 1313.

TUBYLEWICZ, H. (1966). Experimental studies on bacteriocinogeneity in *Clostridium perfringens* type A. I. Isolation of bacteriocines and their antibacterial spectrum. *Bulletin de'Academie Polonaise des Sciences Biologiques 14,* 31.

VAN ITERSON, W., HOENIGER, J.F.M. & VAN ZANTEN, E.N. (1967). A 'microtubule' in a bacterium. *Journal of Cell Biology 32,* 1.

VAN RENSBURG, A.J. & HUGO, N. (1969). Characterization of DNA of colicinogenic factor E1 in a Providence strain. *Journal of General Microbiology 58,* 421.

VIANU, I. (1969). Preliminary investigations concerning the transfer of bacteriocin formation in Staphylococci. *Archives Roumaines de Pathologie Experimentale et de Microbiologie 28,* 1097.

WATANABE, T. & OKADA, M. (1964). New type of sex factor-specific bacteriophage of *Escherichia coli. Journal of Bacteriology 87,* 727.

WELTZIEN, H.U. & JESAITIS, M.A. (1971). The nature of the colicin K receptor of *Escherichia coli Cullen. Journal of Experimental Medicine 133,* 534.

WENDT, L. (1970). Mechanism of colicin action: early events. *Journal of Bacteriology 104,* 1236.

WESTPHAL, O. & LUDERITZ, O. (1954). Chemische Erforschung von Lipopoly-sacchariden Gramnegativer Bakterien. *Angewandte Chemie 66,* 407.

CHAPTER III

DETECTION OF BACTERIOCINS ACTIVE ON *PROTEUS VULGARIS* STRAIN 69

SUMMARY

Two hundred and sixty three strains of *Proteus vulgaris* were investigated in an attempt to isolate bacteriocins active on *P. vulgaris* strain 69. Twenty strains (7,6%) yielded inhibitory agents with a non-transmissible killing effect on strain 69. Of these strains, only twelve (4,5%) produced inhibitory titres in fluid medium. Electron microscopy of the inhibitory preparations revealed the active principle as phage-tail-like structures typical of certain high-molecular weight bacteriocins. Broth cultures of bacteriocinogenic strains are inducible by ultraviolet light and Mitomycin C. Induced cultures yielded bacteriocin titres ranging from 1::128 to 1:512. The activity is sedimentable by high-speed centrifugation and may be precipitated by ammonium sulphate.

INTRODUCTION

The bacteriocins appear to be a natural class of antibiotics, distinguished from all others by sufficient properties to merit the distinctive name given to them by Jacob, Lwoff, Siminovitch & Wollman in 1953.

Although the first detailed study of these bactericidal substances (Gratia, 1925) was as early as three decades prior to the definition of Jacob *et al.* (1953), it was only in 1963 that the first indication of the occurrence of bacteriocinogeny in the genus *Proteus* was observed. Taubeneck (1963) noted the liberation by *Proteus mirabilis* strain 52 of phage-tail-like structures which were contractile and killed some *P.mirabilis* and *P. vulgaris* strains. In 1965 Cradock-Watson described the occurrence of bacteriocinogenic strains amongst Proteus organisms by demonstrating the production of bacteriocins by 61% of strains of *P. mirabilis* and 10% of *P. vulgaris* tested. Further studies by Coetzee (1967) on Providence and *P. morganii* and by Coetzee, de Klerk, Coetzee & Smit (1968) on *P. vulgaris* confirmed the frequency of bacteriocin production among these Enterobacteria. The phage-like structures from *P. vulgaris* described by Coetzee *et al.* (1968) are reminiscent of similar objects liberated by other bacterial types, which

comply with the operational definition of bacteriocinogeny. Colicin 15 produced by *Escherichia coli* WT15 was shown to consist of small-headed phage-like particles (Endo, Ayabe, Amako & Takeya, 1965; Sandoval, Reilly & Tandler, 1965). Pyocin R elaborated by *Pseudomonas aeruginosa* strain R was seen (Ishii, Nishi & Egami, 1965) to resemble headless contractile tails of bacteriophages. The colicin H activity of *E. coli* A10 was found to reside in phage-like objects similar to those described for colicin 15 (Bradley & Dewar, 1966). Numerous other pyocinogenic strains of *P. aeruginosa* in addition to a monocin from *Listeria monocytogenes* were shown to resemble contractile phage-like tails (Bradley & Dewar, 1966; Bradley, 1967; Takeya, Minamishima, Amako & Ohnishi, 1967).

Although some bacteriocinogenic organisms release bacteriocin spontaneously, the function of the structural gene governing bacteriocin production is repressed in the majority of bacteriocinogenic cells under ordinary conditions (Nomura, 1967). The discovery by Jacob, Siminovitch & Wollman (1952) that ultraviolet light acts as an inducer of colicin ML—E1 from colicinogenic cells, prompted the adoption of this method of induction to the applied to the study of other bacteriocinogenic organisms. Subsequently, other methods of inducing bacteriocins have been reported (Reeves, 1965). Thus, Mitomycin C was found to be an efficient inducing agent of numerous bacteriocins from strains of *E. coli* (Iijima, 1962). Induction by elevated temperatures on temperature-sensitive bacteriocinogenic cells (Kohiyama & Nomura, 1965; Gromkova, 1971) and by the addition of thymine to thymineless mutants (Luzzati & Chevallier, 1964) has been demonstrated. Both ultraviolet light irradiation and Mitomycin C were employed as inducing agents in this study.

Taubeneck (1963) considered the phage-tail-like bacteriocins liberated by *P. mirabilis* strain 52 to be the products of defective lysogeny. The phage-tail-like structure from *P. vulgaris* strains described by Coetzee *et al.* (1968) were occasionally associated with complete phage-like particles. The possibility of these particles representing defective phages was considered. A similar contention was propounded by Bradley & Dewar in 1966 for certain other bacteriocins. The *P. vulgaris* tail-like structures (Coetzee *et al.*, 1968) also closely resembled the sheathed contractile tail section of a temperate generalized transducing phage (Ø107/69) of *P. vulgaris* described by Coetzee, de Klerk & Smit (1967). Coetzee *et al.* (1968) suggested that a state of defective lysogeny may possibly have arisen by selection for the genes favourable for this condition by transduction (Coetzee, Smit & Prozesky, 1966; Coetzee *et al.*, 1967) or conversion Coetzee

(1961) in previously lysogenic Proteus cells.

As a result of the discovery of the *P. vulgaris* transducing phage 107/69 (Coetzee, 1967) and its striking morphological resemblance to the 'vulgaricins' (Coetzee *et al.*, 1968), the following investigations were initiated:

An attempt was made to demonstrate the transduction of the genetic determinant governing resistance to the phage-tail-like bacteriocins utilizing the Ø107/69–*P. vulgaris* strain 69 transduction system. In addition the feasibility of the possible evolutionary relationship between the above-mentioned phage and phage-tail-like bacteriocins was considered, with due regard to their respective characteristics concerning activity spectra, morphology and serology.

With this end in mind, a search for typical phage-tail-like vulgaricins active on *P. vulgaris* strain 69 was undertaken.

MATERIALS AND METHODS

Bacterial cultures. Two hundred and sixty four strains of *Proteus vulgaris* were used in this investigation. These strains were isolated locally over a period of 4 years in this laboratory. The inducer strain employed in the detection of bacteriocins was a mutant of the parent strain *P. vulgaris* 69 (Coetzee *et al.*, 1967), selected for resistance towards a virulent phage (named Ø69*vir*) occasionally liberated spontaneously by strain 69. This mutant strain 69 remains sensitive to infection by the temperate transducing phage 107/69 (Coetzee *et al.*, 1967). Strains were maintained on nutrient agar slopes at 4°. Cultures were incubated at 37°C.

Media.
Nutrient broth
Nutrient agar
S S agar
MacConkey agar
Phage agar
Phage agar top-layer

Reagents. Mitomycin C

Details are given in the appendix.

Detection of bacteriocin production.

A. Spontaneous production.

A modification of the method of Abbott & Shannon (1958) was used. Overnight cultures of the strains to be tested for bacteriocin production were diluted 1:5 in fresh broth and incubated for 90 min. Each culture was then inoculated in a broad streak across an S S or MacConkey-agar plate. The plates were incubated for 7,0 hrs. to give a confluent streak of growth. The organisms were then killed by inverting the plate over a dish containing a few ml. of chloroform for 15 min. The killed organisms were scraped off towards the edge of the plate with a clean glass slide and removed together with a small portion of the agar. An overnight culture of *P. vulgaris* strain 69 to be tested for sensitivity towards any bacteriocin liberated was then inoculated across the plate at right angles to the position formerly occupied by the primary inoculum. The plates were incubated for 16 hr. Production of inhibitory agents active on strain 69 was noted by observing an area of growth inhibition of this indicator at the region of intersection of the primary and secondary inocula.

B. Ultraviolet light irradiation.

Method 1.

The above-mentioned method of detection of inhibitor production was repeated as in the foregoing experiment except that the primary inoculum was incubated for 4 hr. after which the plates were subjected to varying time-exposures of ultraviolet light irradiation. The plates were then incubated overnight in the dark to minimise the effects of photoreactivation (Kelner, 1949; Newcombe, 1955) and thereafter treated in a manner similar to that described above (Plate 1).

Method 2.

Strains producing inhibitory agents detected as per Method 1 were incubated overnight in broth. These stationary-phase cultures were diluted 10-fold in fresh warm broth and incubated for 90 min. Ten ml. aliquots were then centrifuged at 6037 g for 10 min. The supernatent fluid was discarded and the bacterial pellet resuspended in 5 ml. of physiological saline. These samples were then pipetted into sterile Petri dishes and irradiated for varying degrees of time, ranging from 0 min. to 7 min. with continuous agitation. The irradiated samples were poured into flasks containing 100 ml. of fresh broth and incubated overnight in the dark. After the incubation period the cultures

were centrifuged at 6037 g for 10 min. and the supernatent assayed for inhibitory activity on strain 69.

The source of ultraviolet radiation for both methods 1 and 2 was a 30—W Hanovia sterilamp delivering 80% of its output at a wavelength of 2537 Å. Cultures were irradiated from a distance of 25 cm. from the source of energy (23 ergs/mm.^{2}/sec.).

C. Mitomycin C.

A modification of the method of Seaman, Tarmy & Marmur (1964) was used. Overnight broth cultures of strains to be tested for bacteriocinogeny were diluted 1:10 and incubated for 30 min. Mitomycin C was added to give a final concentration of 2 µg./ml., and the cultures incubated for a further 30 min. The drug was removed by centrifuging the cells and resuspending them in an equal volume of fresh warm broth. The resultant culture was incubated for a further 3 hrs. to allow for lysis to occur. The culture was then centrifuged and the supernatent fluid assayed for activity. All supernatents obtained by Methods B and C were stored over chloroform at 4°.

Assay of bacteriocin activity. Inhibitor activity in fluid medium was assayed by a spotting technique (Coetzee, 1967).

Method 1.

Serial dilutions of the inhibitory agent to be assayed were made in nutrient broth. A 1:10 dilution of an overnight culture of strain 69 was made in a sterile Petri dish. Filter paper discs which had been sterilized in chloroform were immersed in the indicator solution and applied to either S S or MacConkey-agar plates for 5 min. After removal of the discs the plates were allowed to dry for 10 min. Single drops of the serial dilutions were spotted on the plates and incubated for 6 hrs. The highest inhibitory dilution expressed the titre.

Method 2.

Indicator plates were prepared by a modification (Coetzee, 1958) of the double-agar layer method of Herschey, Kalmanson & Bronfenbrenner (1943). A 1:10 dilution of overnight indicator organism was incorporated into 2,5 ml. of melted (45°) phage agar top-layer to give a final vol. of 3 ml. This was poured over a phage agar plate and left for 30 min. to set. Inhibitory activity was assayed on these plates by the serial dilution spotting technique as described above in Method 1. (Plate II, fig. 1).

Transmissibility. Serial transmission of the bactericidal activity was tested by cutting out a small piece of the area of inhibition from an O dilution spot with a sterile wire loop. This was then gently resuspended in a small amount of broth to which a few drops of chloroform was added. The suspension was centrifuged at 6037 g for 10 min. The excess chloroform was bubbled off from the resultant supernatent and dilutions of this were spotted on a lawn of the indicator strain 69 organism. All of the *P. vulgaris* substances active on strain 69 were treated in the same way as described, except that the supernatents obtained were also spotted on lawns of the remaining 262 *P. vulgaris* strains utilized in this study.

Purification. Purification of the inhibitory agents was achieved by several cycles of differential centrifugation. Crude fluid preparations of these substances were centrifuged at 6037 g for 30 min. to sediment bacterial debris. The supernatent was then subjected to centrifugation at 54333 g for 120 min. The supernatent was discarded and the pellet resuspended in 0,1 N-ammonium acetate (pH 7,2). This procedure of alternate high and low-gravitational force centrifugation was repeated for 6 cycles before the bacteriocin activity was finally assayed.

Concentration. Inhibitory agents were concentrated by salting out-precipitation with ammonium sulphate (Coetzee *et al.*, 1968). The fluid inhibitor-containing medium was centrifuged at 12350 g for 30 min. to remove excess bacterial debris. The supernatnent was placed into a sterile flask around which crushed ice was packed. Finely powdered ammonium sulphate was slowly added to the suspension up to 40% (w/v). The mixture was continually agitated on an electromagnetic stirrer. The solution was left to stand at 4^{o} overnight. The precipitated material was then collected by centrifugation at 6037 g for 15 min. The resultant pellet was resuspended in 0,1 N-ammonium acetate (pH 7,2) and assayed for activity.

Dienes phenomenon. All the inhibitor-producing strains as well as strain 69 were tested by means of the Dienes phenomenon to determine the differences between the strains (Dienes, 1947). Three of the cultures were stabbed onto a nutrient agar plate at 120^{o} intervals and incubated at 25^{o} to allow for swarming.

Electron microscopy. Samples of the inhibitory substances were purified for electron microscopic examination by differential centrifugation as described above. 0,01 ml. of the sample was mixed with an equal volume of a 2% potassium phosphotungstate acid solution (pH 7,4) (Brenner & Horne, 1959) on a glass slide. A freshly-prepared carbon

support grid (Veco 400 mesh/in.) was then touched onto the surface of the mixture. Excess liquid was removed by filter paper, leaving a thin film of the sample spread over the grid (Bradley, 1962). After drying, the specimen was examined in a Philips EM 200 electron microscope.

Carbon support grids were prepared according to the following method:

(a) A standard microscope slide is cleaned by immersion in chloroform and polished with a soft cloth.

(b) The slide is dipped into a 0,3% (w/v) Formvar solution. The excess fluid is drained off and the slide dried in an ethylene dichloride evaporation chamber.

(c) The Formvar film is floated off the slide onto the surface of distilled water by gradual immersion of the slide, and film picked up onto the surface of the grid by surface tension.

(d) After drying each coated grid on filter paper it is placed in a carbon vapourizing unit. A 20–100 Å thick carbon layer is evaporated onto the grid.

(e) The Formvar film substrate is then dissolved away by immersion of the grid in chloroform, leaving the single layer of carbon intact.

RESULTS

Incidence of inhibitory agents active on strain 69. Twenty (7,6%) of the 263 strains tested yielded inhibitory agents active on strain 69. Only twelve (4,5%) of these strains were capable of producing inhibitors of detectable titre in fluid medium (Plate III). The remaining eight strains with inhibitory activity on agar medium only, were disregarded for the purposes of this study (Table 1). Two of the twelve producer strains yielded inhibitory titres without induction. Ten strains required induction by either Mitomycin C treatment or ultraviolet irradiation. The dose of irradiation necessary to induce release of bactericide was specific for each strain (Table 2). Inhibitory titres obtained by both Mitomycin C and ultraviolet irradiation were found to be identical, although lysis of the drug-induced cells was more pronounced. All the cultures were subsequently induced by ultraviolet light for the production. of inhibitors in fluid medium.

Table 1. Bacteriocinogenic strains which only inhibited strain 69 on agar.

P. vulgaris strain	Inhibition on agar	Titre in broth
2	+	0
3	+	0
13	+	0
88	+	0
101	+	0
123	+	0
131	+	0
133	+	0

Table 2. Ultraviolet irradiation exposures required for induction of bacteriocin production

	Bacteriocinogenic Strain											
Exposure time	35	36	41	46	49	52	75	115	121	127	223	226
0 sec.											+	+
3 sec.											+	+
8 sec.										+	+	+
16 sec.										+	+	+
32 sec.							+			+	+	+
60 sec.							+			+	+	+
90 sec.							+			+	+	+
2 min.					+	+	+	+		+	+	+
3 min.				+	+	+	+	+				
4 min.	+	+	+	+	+	+	+	+	+	+	+	+

+ denotes bacteriocin production in fluid medium.

Assay of activity. Inhibitory titres were found to be variable, depending on the type of agar medium employed in the test. The highest titres were observed by means of the spotting technique utilizing the agar-layer method. Titres on both S S plates and MacConkey medium were 8 x lower (Table 4). Five of the strains (Nos. 41, 49, 52, 75, 127) yielded titres of 1:512, whilst strains 223, 226, 115, 121 and 35 inhibited strain 69 up to a dilution of 1:256. The remaining two strains 36 and 46 were inhibitory only up to a dilution of 1:128 (Table 3.).

Transmissibility. None of the twelve inhibitor preparations were able to transmit their biological activity from excised areas of inhibition on agar to fresh indicator cultures of strain 69. Fluid suspensions of inhibitors were completely neutralized by the addition of sensitive cells. Supernatents of the resuspended areas of inhibition by all 12 substances were unable to form plaques on any of the remaining 262 *P. vulgaris* strains tested.

Purification. It was found that the inhibitory material could be sedimented out of suspension in fluid medium by high speed centrifugation. No activity was detectable in the supernatents after centrifugation at 54000 g for 2 hr.

Concentration. The active principle from all 12 producer-strains was precipitable by salting-out with 40% (w/v) ammonium sulphate. By means of precipitation the bactericidal activity of crude inhibitor preparations could be concentrated to yield titres of 1:2048.

Dienes test. The twelve producer-strains differed from one another as well as from the indicator strain 69 in that they exhibited the Dienes demarcation line when matched against one another on agar plates (Plate II, fig. 2) in all combinations.

Electron microscopy. Visual examination of the active principle from all twelve producer-strains revealed masses of structures resembling sheathed phage tails. Occasional complete phage-like particles were also seen. Most of the tail-like objects were completely sheathed while in a few the sheaths were contracted revealing a hollow core. The particles are reminiscent of the phage-tail-like bacteriocins from *P. vulgaris* described by Coetzee *et al.* (1968), and are similar to the tail of a *P. vulgaris* transducing phage (Coetzee, de Klerk & Smit, 1967).

Table 3. Ultraviolet irradiation as a prerequisite for bacteriocin production.

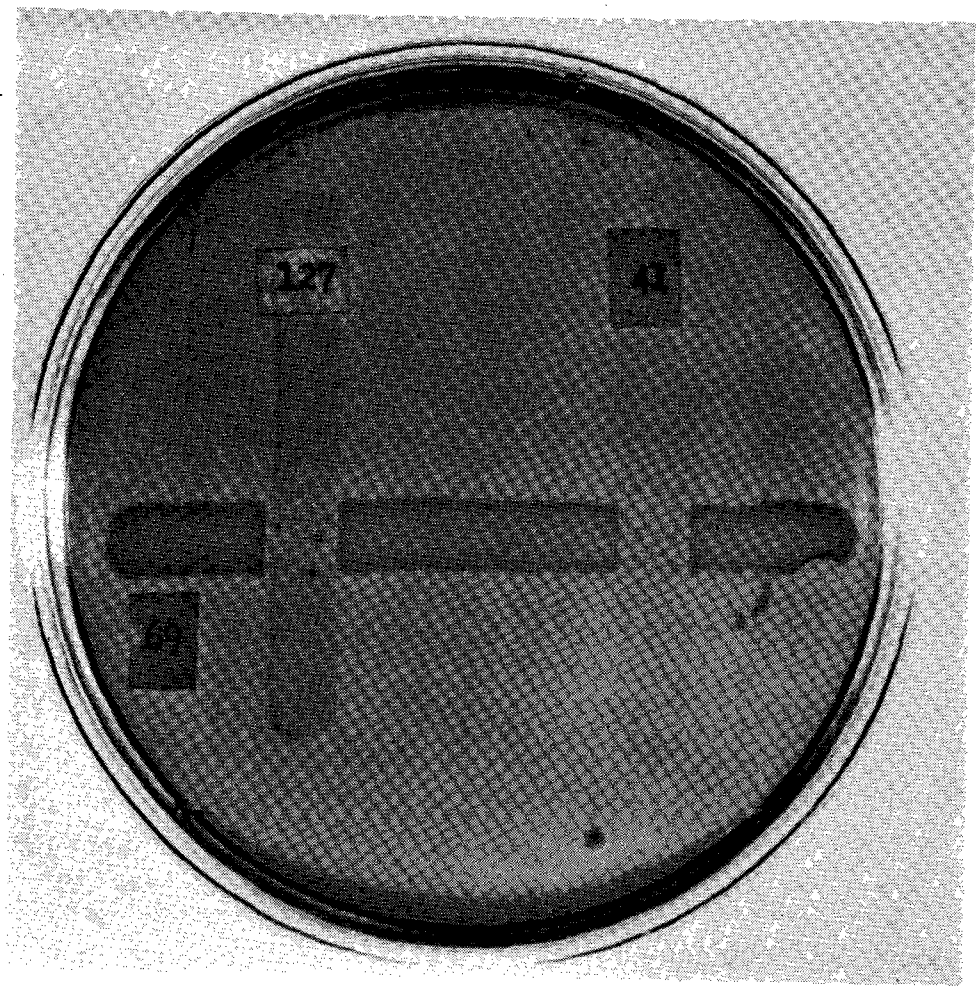
P. vulgaris strain	Bacteriocin titre*	
	Without induction	U.v.-induced
36	0	1:128
46	0	1:128
35	0	1:256
115	0	1:256
121	0	1:256
223	1:256	1:256
226	1:256	1:256
41	0	1:512
49	0	1:512
52	0	1:512
75	0	1:512
127	0	1:512

*Titres are expressed as the highest dilution of bacteriocin inhibiting the indicator organism on agar.

Table 4. Bacteriocin activity assayed on different agar media.

P. vulgaris Bacteriocin	Bacteriocin titre	
	Assay on phage-agar double layer	Assay on SS & MacConkey agar
36	1:128	1:16
46	1:128	1:16
35	1:256	1:32
115	1:256	1:32
121	1:256	1:32
223	1:256	1:32
226	1:256	1:32
41	1:512	1:64
49	1:512	1:64
52	1:512	1:64
75	1:512	1:64
127	1:512	1:64

PLATE I



Bacteriocinogenic *Proteus vulgaris* strains 41 and 127 cross-streaked on MacConkey agar plates according to the method of Abbott & Shannon (1958) with *P. vulgaris* strain 69 as indicator. Inhibition of growth of strain 69 at the intersection with the primary inocula indicates its sensitivity to the bacteriocins.

PLATE II

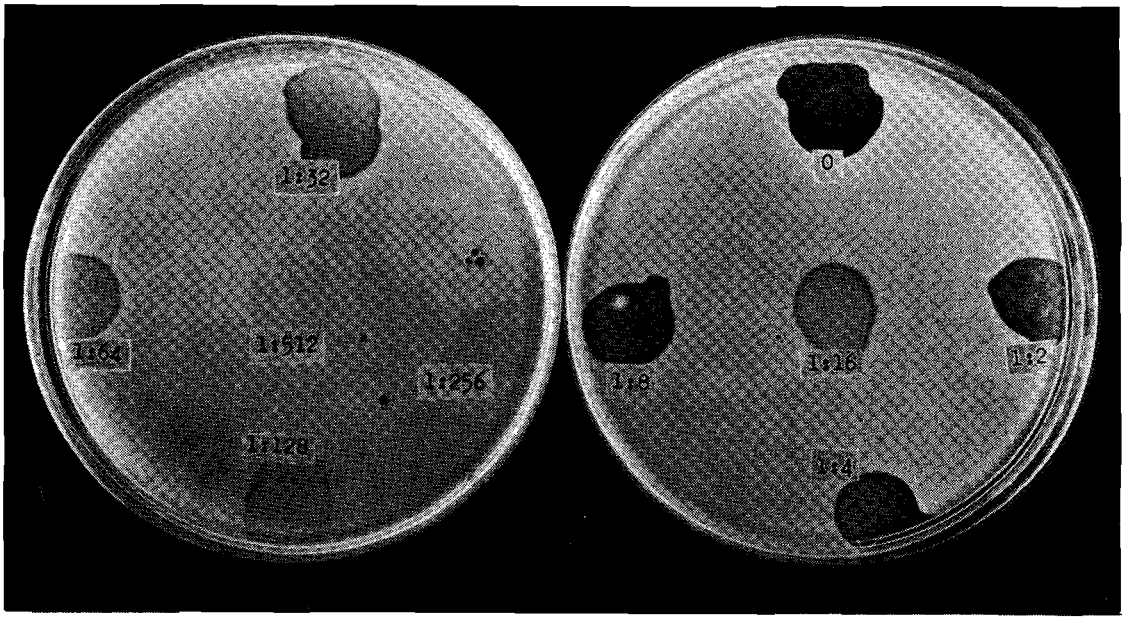


Fig. 1. Assay of bacteriocin 41 on strain 69. The highest dilution which gives a zone of inhibition in the confluent indicator lawn expresses the titre (1:512).

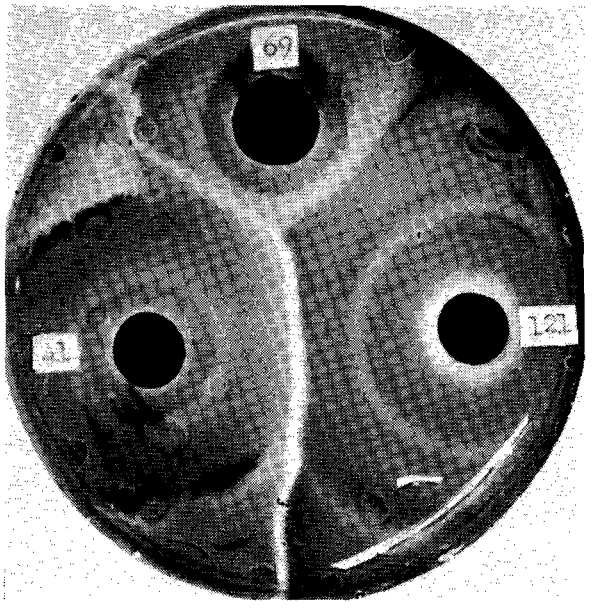
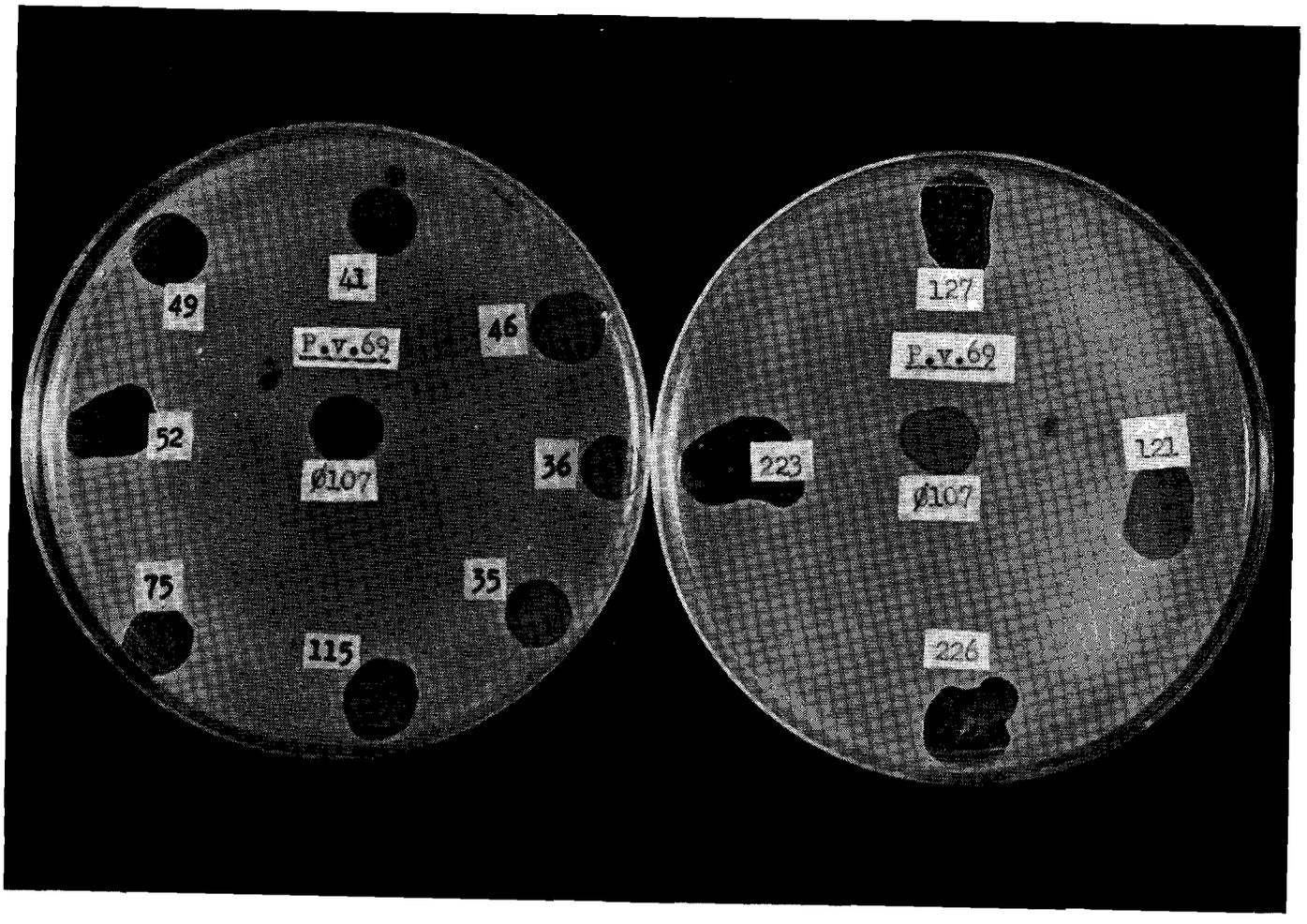


Fig. 2. The bacteriocinogenic strains 41 and 121 and strain 69 inoculated on nutrient agar. The three demarcation lines between the swarms denotes a positive Dieries phenomenon.

PLATE III



Agar-layer plates seeded with indicator strain 69 bacteria. Single drops of the 12 bacteriocins spotted on the lawn. Zones of inhibition are indicative of sensitivity towards the bacteriocins.

DISCUSSION

In the course of this investigation, 20 strains (7,6%) were found to produce inhibitory agents active on strain 69. 8 of these strains were unable to yield inhibitory substances of detectable titre in fluid medium. Electron microscopy of the inhibitory agents produced in fluid medium revealed headless phage-tail-like structures similar to the *Proteus vulgaris* bacteriocins described by Coetzee *et al.* (1968). No inhibitory activity was detectable in the supernatents of inhibitor preparations subjected to high-speed centrifugation. These results suggest that the inhibitory agents are representative of the class of phage-tail-like bacteriocins as previously described (Coetzee *et al.,* 1968) in strains of *P. vulgaris.*

It is known that the production of certain bacteriocins is highly dependant on growth conditions (Reeves, 1965) and culture medium. (Hertman & Ben-Gurion, 1958; Goebel *et al.,* 1956; Matsushita *et al.,* 1960). Lachowicz (1965) demonstrated that staphylococcin production could only be observed on solid medium, with no detectable titre being obtained in broth. No attempt was therefore made on further characterization of those strains inhibitive on agar alone.

Two of the remaining twelve strains produced bacteriocin spontaneously, the other ten requiring induction prior to the liberation of bactericide. Many bacteriocinogenic strains have been shown to require some means of induction as a prerequisite for bacteriocin production (Mukai, 1960; Kageyama & Egami, 1962; Kageyama, 1964; Reeves, 1965). It is possible that the function of the structural gene responsible for bacteriocin production is in a constitutive state in the former two *P. vulgaris* strains. Both ultraviolet light irradiation and Mitomycin C were found to be efficient inducers of bacteriocin production. Titres obtained as a result of either method of induction were identical for all twelve bacteriocinogenic strains. The precise mechanism whereby ultraviolet light and Mitomycin C induces bacteriocin production is not clear. Mitomycin C selectively inhibits bacterial DNA synthesis (Shiba, Terawaki, Taguchi, & Kawamata, 1959) in addition to inducing the development of prophages in lysogenic bacteria (Otsuji, Sekiguchi, Iijima & Takagi, 1959) and increasing the chromosomal recombination process in mated recipient cells (Yuki, 1962). These characteristic effects are similar to those of ultraviolet light (Iijima, 1962). It would appear that both inducing agents may possibly initiate a common reaction resulting in unregulated bacteriocin production in treated cells. This explanation would explain the elaboration of similar titres of bacteriocin through either induction agent as observed in this study.

It is interesting to note the different results obtained concerning the assay of the inhibitory agents on the three agar media employed. No explanation can be given for the decreased titres observed on S S or MacConkey medium. The possibility that these media may contain substances partially detrimental to the biological activity of certain bacteriocins was not excluded.

It would appear as if the bacteriocins studied here are primarily proteinatious as they are precipitatable with ammonium sulphate. This result is similar to the findings for colicins A (Barry, Everhart & Graham, 1963), E2 (Reeves, 1963), I (Keene, 1966), V (Hutton & Goebel, 1962) and K (Goebel & Barry, 1958). A number of pyocins studied by Jacob (1954) and Kageyama & Egami (1962) were also found to be precipitable with ammonium sulphate.

The observation that the inhibitory agents are sedimentable by centrifugation at 54000 g, designates them to the class of high-molecular weight bacteriocins. The *P. vulgaris* phage-tail-like bacteriocins described by Coetzee *et al.* (1968) were similarly classified.

Although no DNA estimates were carried out on the phage-tail-like structures involved in the study, Coetzee *et al.* (1968) have shown that the phage-tail-like bacteriocins from other strains of *P. vulgaris* do not contain any deoxyribonucleic acid.

Although antigenically different strains of *P. vulgaris* do not necessarily produce a Dienes demarcation line when matched together on agar, a demarcation line indicates some or other difference between the strains involved (Story, 1954; Skirrow, 1969). The observation that the twelve bacteriocin-producing strains and strain 69 display the Dienes phenomenon was thus accepted as evidence of their inherent difference.

REFERENCES

ABBOTT, J.D. & SHANNON, R. (1958). A method for typing *Shigella sonnei,* using colicine production as a marker. *Journal of Clinical Pathology 11,* 71.

BARRY, G.T., EVERHART, D.L. & GRAHAM, M. (1963). Colicin A. *Nature, London 198,* 211.

BRADLEY, D.E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriological Reviews 31,* 230.

BRADLEY, D.E. & DEWAR, C.A. (1966). The structure of phage-like objects associated with non-induced bacteriocinogenic bacteria. *Journal of General Microbiology 45,* 399.

BRENNER, S. & HORNE, R.W. (1959). A negative staining method for high resolution electron microscopy of viruses. *Biochimica et Biophysica Acta 34,* 103.

COETZEE, J.N. (1958). The characterization of a series of Proteus bacteriophages. *S.A. Journal of Laboratory and Clinical Medicine 4,* 147.

COETZEE, J.N. (1961). Lysogenic conversion in the genus *Proteus. Nature, London 189,* 946.

COETZEE, J.N. (1967). Bacteriocinogeny in strains of Providence and *Proteus morganii. Nature, London 213,* 614.

COETZEE, H.L., DE KLERK, H.C., COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like particles associated with intra-species killing of *Proteus vulgaris. Journal of General Virology 2,* 29.

COETZEE, J.N., DE KLERK, H.C. & SMIT, J.A. (1967). A transducing bacteriophage for *Proteus vulgaris. Journal of General Virology,* 561.

COETZEE, J.N., SMIT, J.A. & PROZESKY, O.W. (1966). Properties of Providence and *Proteus morganii* transducing phages. *Journal of General Microbiology 44,* 167.

CRADOCK-WATSON, J.E. (1965). The production of bacteriocins by Proteus species. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteiling: Originale 196,* 385.

DIENES, L. (1947). Further observations on the reproduction of bacilli from large bodies in Proteus cultures. *Proceedings of the Society for Experimental Biology and Medicine, New York 66,* 97.

ENDO, H., AYABE, K., AMAKO, K. & TAKEYA, K. (1965). Inducible phage of *Escherichia coli* 15. *Virology 25,* 469.

GOEBEL, W.F., & BARRY, G.T. (1958). Colicin K. II. The preparation and properties of a substance having colicin K activity. *Journal of Experimental Medicine 107,* 185.

GRATIA, A. (1925). Sur un remarquable exemple d'antagonisme entre deux souches de colibacille. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 93,* 1040.

GROMKOVA, R.H. (1971). Induction of colicin Ia at high temperature. *Journal of Bacteriology 106,* 720.

HERSHEY, A.D., KALMANSON, G. & BRONFENBRENNER, J. (1943). Quantitative methods in the study of phage-antiphage reaction. *Journal of Immunology 46,* 267.

HUTTON, J.J. & GOEBEL, W.F. (1962). The isolation of colicine V and a study of its immunological properties. *Journal of General Physiology 45,* 125.

IIJIMA, T. (1962). Studies on the colicinogenic factor in *Escherichia coli* K12 induction of colicin production by Mitomycin. C. *Biken Journal; Journal of the Research Institute for Microbial Disease 5,* 1.

ISHII, S., NISHI, Y. & EGAMI, F. (1965). The fine structure of a pyocin. *Journal of Molecular Biology 13,* 428.

JACOB, F. (1954). Biosynthése induit et mode d'action d'une pyocine antibiotique de *Pseudomonas pyocyanea. Annales de l'Institut Pasteur, Paris 86,* 149.

JACOB, F., LWOFF, A., SIMINOVITCH, A. & WOLLMAN, E. (1953). Definition de quelques termes relatifs à la lysogénie. *Annales de l'Institut Pasteur, Paris, 84,* 222.

JACOB, F., SIMINOVITCH, L. & WOLLMAN, E. (1952). Sur la biosynthese d'une colicine et sur son mode d'action. *Annales de l'Institut Pasteur, Paris 83,* 295.

KAGEYAMA, M. (1964). Studies of a pyocin. *Journal of Biochemistry 55,* 49.

KAGEYAMA, M. & EGAMI, F. (1962). On the purification and some properties of a pyocin, a bacteriocin produced by *Pseudomonas aeruginosa. Life Sciences; Part II: Biochemistry, General and Molecular Biology no. 9,* 471.

KEENE, J.H. (1966). Preparation and chemical properties of colicine V. *Canadian Journal of Microbiology 12,* 425.

KELNER, A. (1949). Photoreactivation of ultraviolet-irradiated *Escherichia coli* with special reference to the dose-reduction principle and to ultraviolet induced mutations. *Journal of Bacteriology 58,* 511.

KOHIYAMA, M. & MOMURA, M. (1965). DNA synthesis and induction of colicine E2 as studied with a temperature-sensitive mutant of colicinogenic *E. coli* strain. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196, 211.*

LUZZATI, D. & CHEVALLIER, M.R. (1964). Induction par carence en thymine, de la production de colicine par de bactéries colicinogènes thymine-exigeantes. *Annales de l'Institut Pasteur, Paris 107,* 152.

MUKAI, F.H. (1960). Interrelationship between colicin sensitivity and phage resistance. *Journal of General Microbiology 23,* 539.

NEWCOMBE, H.B. (1955). The timing of induced mutations in Streptomyces. *Brookhaven Symposia in Biology 8,* 88.

OTSUJI, N., SEKIGUCHI, M., IIJIMA, T. & TAKAGI, Y. (1959). Induction of phage formation in the lysogenic *Escherichia coli* K12 by Mitomycin C. *Nature, London 184,* 1079.

REEVES, P.R. (1963). Preparation of a substance having colicin F activity from *Escherichia coli* CA 42. *Australian Journal of Experimental Biology 41,* 163.

REEVES, P.R. (1965). The bacteriocins. *Bacteriological Reviews 29,* 24.

SANDOVAL, H.K., REILLY, H.C. & TANDLER, B. (1965). Colicin 15: Possibly a defective bacteriophage. *Nature, London 205,* 522.

SEAMAN, E., TARMY, E. & MARMUR, J. (1964). Inducible phages of *Bacillus subtilis. Biochemistry 3,* 607.

SHIBA, S., TERAWAKI, A., TAGUCHI, T. & KAWAMATA, J.(1959). Selective inhibition of deoxyribonuclei acid in *Esherichia coli* by Mitomycin C. *Nature, London 183,* 1056.

SKIRROW, M.B. (1969) The Dienes (mutual inhibition) test in the investigation of Proteus infections. *Journal of Medical Microbiology 2,* 471.

STORY, P. (1954). Proteus infections in hospital. *Journal of Pathology 68,* 55.

TAKEYA, K., MINAMISHIMA, Y., AMAKO, K. & OHNISHI, Y. (1967). A small rod-shaped pyocin. *Virology 31,* 166.

TAUBENECK, U. (1963). Uber die Produktion biologisch aktiver Pahgen-Schänze durch einen defekt lysogenen *Proteus mirabilis*-stamm. *Zeitschrift fur Naturforschung; Ten B 18b,* 989.

YUKI, S. (1962). The effect of Mitomycin C on the recombination in *Escherichia coli* K12. *Biken Journal; Journal of the Research Institute for Microbial Diseases 5,* 47.

CHAPTER IV

CLASSIFICATION OF BACTERIOCINS

SUMMARY

Six hundred mutants of *P. vulgaris* strain 69, comprising 50 mutants resistant to each of 12 *P. vulgaris* bacteriocins were isolated. The 12 bacteriocins could be classified into 2 groups comprising 8 and 4 respectively on the basis of their receptor specificity to many of the mutants obtained. This group classification scheme was substantiated by the activity of the members of the respective groups of bacteriocins on many other strains of *P. vulgaris, P. mirabilis, P. morganii* and *S. marcescens.* Cross-resistance between the transducing phage 107/69 and the bacteriocins was observed on all the bacteriocin-resistant mutants of *P. vulgaris.* A number of mutants of strain 69 resistant to Ø107/69 were isolated. These mutants also exhibited cross-resistance to the 12 bacteriocins. The probability of the existence of different but closely-linked receptor sites for the two groups of bacteriocins and the bacteriophage is discussed

INTRODUCTION

As has already been stipulated in the introduction to this thesis, the underlying motivation for undertaking this study was of a dual nature. Firstly, an attempt was to be made to transduce the genetic determinant governing resistance to bacteriocin from bacteriocin-resistant cells of *Proteus vulgaris* to sensitive organisms of the same species. Due to the fact that *P. vulgaris* strain 69 is the host organism in the transduction system utilizing a *Proteus vulgaris* generalized transducing phage, Ø107/69 (Coetzee, de Klerk & Smit, 1967), it was therefore this bacterium against which bacteriocins were sought from amongst other strains of *P. vulgaris* which would be active on this particular strain. Consequently, as has been described, 12 bacteriocins of the high molecular weight type were subsequently isolated from amongst 263 strains of *P. vulgaris* which were inhibitory for strain 69.

Having obtained bacteriocins active on the phage-host organism, the logical consequence was deemed as the necessity to isolate mutants of strain 69 resistant to these bacteriocins. It was reasoned that by obtaining mutants of strain 69 resistant to bacteriocin, these mutants could be utilized as genetic donors in the possible transduction of the property of bacteriocin-resistance to bacteriocin-sensitive cells of the parent organism.

On the basis of this consideration a search was undertaken for mutants of strain 69 resistant to each of the 12 bacteriocins isolated.

Secondly, an attempt was to be made to initiate some system of classification of the bacteriocins obtained from *P. vulgaris,* and to investigate the relationship, if any, between these phage-tail-like structures and phage 107/69, both of which are active on *P. vulgaris* strain 69. This latter consideration was inspired by the observation of Coetzee *et al.* (1968) that other phage-tail-like structures isolated from strains of *P. vulgaris* bore a remarkable morphological resemblance to the tail of this *P. vulgaris* transducing bacteriophage. The classification of most bacteriocins follows the scheme originally devised by Fredericq (1965) which is based on the specificity of adsorption of bacteriocins to sensitive cells. By means of the judicious use of bacterial mutants isolated as being resistant to certain bacteriocins, most bacteriocins have thereby lent themselves to a system of classification based on adsorption patterns (Reeves, 1965). It was reasoned that by obtaining a large number of mutants of the indicator strain *P. vulgaris* 69, some similar system of classification might be applied to those bacteriocins concerned with this study. It was also considered necessary to test these bacteriocins for activity or lack of activity on bacteria other than strain 69 in order to investigate further the host range of these bacteriocins which were selected due to their activity on one strain of *P. vulgaris* only, namely strain 69. It was hoped that any initial classification of the bacteriocins as based on their adsorption patterns in respect of mutants of strain 69, might be confirmed by their respective activities on strains other than this particular organism. To this end, 100 strains of each of the species of the Proteus group were tested for sensitivity to each of the bacteriocins. In addition, a number of strains of Providence, Serratia and Salmonella were tested.

Bacteriocinogenic cells are immune to the killing action of homologous bacteriocin (Fredericq, 1958). Immunity is not due to a lack of adsorption, as immune bacteriocinogenic cells have been shown by the use of radioactive colicins to retain receptors for the adsorption of the bacteriocin to which they are immune (Maeda & Nomura, 1966). By employing the phenomenon of immunity, Fredericq (1965) was able to subdivide the colicins of a particular group from his original classification scheme based on adsorption, into further subgroups based on their sensitivity to a specific immunity substance (Nomura, 1963) produced by colicinogenic cells.

The bacteriocins involved with this study were thus also tested against their respective producer strains in addition to the remaining bacteriocinogenic organisms, in order to

investigate any possible relationship existing between them, and to test the validity of any classification scheme based on adsorption specificity which may have arisen during the course of this study.

MATERIALS AND METHODS

Bacterial strains. The twelve bacteriocinogenic strains as described in Chapter III (page 62) and the indicator strain 69 were used. For host range studies, the following number of strains isolated locally were employed:—

P. vulgaris 100;
P. mirabilis 100;
P. morganii 100;
P. rettgeri 100
Providence 34

Eleven strains of *Serratia marcescens* and two strains of *Salmonella typhimurium* were also tested for sensitivity to bacteriocins and phage.

All strains were stored on nutrient agar slopes at 4°. Incubation temperatures was 37°.

Media. Nutrient broth
MacConkey agar
Phage agar
Phage agar top-layer

Details are given in the Appendix. General phage techniques are those of Adams (1959).

Preparation of Ø107/69 lysates. Fresh lysates of the *P. vulgaris* transducing phage 107/69 (Coetzee, de Klerk & Smit, 1967) were made by a modification (Adams, 1959) of the agar layer method of Hershey, Kalmanson & Bronfenbrenner (1943). Phage agar plates were overlaid with molten phage agar top-layer containing sufficient indicator organism strain 69 and phage 107/69 to give confluent lysis. After overnight incubation at room temperature, 2 ml. broth was added to each plate and the top layer scraped off with a sterile glass rod. Agar and bacterial debris were removed by centrifugation at 6000 g for 20 min. and the lysate sterilized with 0,1 vol. chloroform. In this manner, phage lysates containing 1×10^7 p.f.u./ml. were obtained.

Isolation of a clear plaque mutant of Ø107/69. Phage 107/69 is a temperate phage yielding small turbid plaques on *P. vulgaris* strain 69. In order to isolate phage-resistant

mutants of strain 69, it was considered advisable to isolate a virulent or clear plaque mutant of this phage in order to facilitate easier isolation of phage-resistant mutants of the indicator organism. To this end the following method was employed:—
One hundred phage agar plates were overlaid with phage agar top-layer containing indicator organism strain 69 and sufficient phage 107/69 to yield approximately 1000 plaques on each plate. After overnight incubation at room temperature, each plate was examined for the presence of one or more clear plaque mutants amongst the turbid plaques of the wild-type phage. In this manner a clear plaque mutant of phage 107/69 was obtained. This phage proved virulent for the indicator *P. vulgaris* strain 69, but is unable to plate on strain 69 made lysogenic for Ø107/69. This indicates the mutated phage as being of the type incapable of producing its own repressor, and is subsequently classed as a "clear" plaque mutant as opposed to a virulent mutant where the lesion is presumed to be in the operator gene involved. Lysates of this phage with titres of *ca* 1 x 10^9 p.f.u./ml. were prepared on phage agar top-layer plates as described above for preparation of phage 107/69.

Phage from sewage. Fresh sewage was centrifuged at 6000 g for 20 min. and the supernatent sterilized with 0,1 vol. chloroform. Ten millilitres of this supernatent was added to 100 ml. of fresh broth enriched with 10 ml. of an overnight culture of *P. vulgaris* strain 69. After overnight incubation the suspension was centrifuged and the supernatent titrated for infectious centres on lawns of strain 69. By this method a phage yielding large clear plaques on strain 69 was isolated. This phage was named ØD. It does not plate on any of the 12 bacteriocinogenic organisms and was thus utilized as a control in experiments designed to obtain mutants of strain 69 resistant to bacteriocin as described hereunder. Lysates of phage D with titres of 2 x 10^9 p.f.u./ml. were prepared as described above for preparation of Ø107/69.

Isolation of bacteriocin-resistant mutants. Spontaneous mutants of strain 69 resistant to each of the bacteriocins were isolated by the following method: Overnight cultures of each of the bacteriocinogenic strains were diluted 1:5 in fresh warm broth and incubated for 90 min. Each culture was then inoculated in a broad streak across a MacConkey agar plate and the plates incubated at 37^o for 4,0 hr. after which the plates were subjected to 4,0 min. of ultraviolet irradiation. After overnight incubation in the dark to prevent photoreactivation (Kelner, 1949; Newcombe, 1955) the organisms were killed by inverting the plates over chloroform for 30 min. After removing the killed bacteriocinogenic organisms by means of a clean glass slide, as described in Chapter III

(page 57), overnight cultures of strain 69 were streaked across the plates at right angles to the primary inoculum and incubated overnight. After overnight incubation each plate was examined for the presence of single colonies within the area of inhibition of strain 69. If present, such colonies were picked off and tested for resistance to bacteriocin by the spotting technique as described in Chapter III (page 58). All 12 bacteriocins in addition to phages 107/69 and D were spotted on each mutant thus obtained.

Properties of bacteriocin-resistant mutants. In order to test whether mutants which were insensitive to bacteriocin were able to adsorb bacteriocin i.e. tolerant mutants, or whether they were of the receptor minus type, the following experiment was performed: Five ml. of an overnight culture of the mutant to be tested was centrifuged at 6000 g for 10 min. The supernatent was discarded and the cells resuspended in 0,5 ml. fresh broth. 0,5 ml. of undiluted bacteriocin was added and the sample incubated for 1 hr. After incubation the cells were sedimented by centrifugation and the supernatent fluid sterilized with chloroform and assayed for bacteriocin activity on lawns of strain 69. Control experiments were run with the bacteriocin-adsorbing parent strain.

Bacteriocin-resistant mutants were tested for their ability to adsorb phage 107/69 by the method of Adams (1959). Phage adsorption was measured by the titration of unadsorbed phage in mixtures of phage and organism at time zero and at 5 min. intervals. The assay of free phage in adsorption mixtures was done by treating the latter with chloroform.

Isolation of phage 107/69-resistant mutants. In order to isolate mutants of strain 69 resistant to phage 107/69, a clear plaque mutant of this phage obtained by the method already described, was employed. An overnight culture of strain 69 was diluted 1:3 in fresh broth. To 1,0 ml. of this sample, 2,0 ml. of the phage at a titre 1 x 10^9 p.f.u./ml. was added and the mixture incubated for 2 hr. The cells were then centrifuged and resuspended in 1,0 ml. of fresh broth to which a further 2,0 ml. sample of the phage was added and reincubated for another 2 hr. After incubation, the mixture was centrifuged and the resultant pellet obtained suspended in 0,5 ml. physiological saline. 0,1 ml. aliquots of this suspension were streaked out on MacConkey agar plates for single colonies. After overnight incubation, single colonies were picked out and tested for resistance to the temperate transducing phage 107/69, by performing phage adsorption experiments on the colonies obtained by the method described above for bacteriocin-resistant mutants. Phage-resistant mutants were tested for their sensitivity towards all

twelve bacteriocins and for their ability to adsorb the latter.

Host range experiments. All strains to be tested were inoculated into nutrient broth and diluted 1:5 after overnight incubation. 0,5 ml. aliquots of each strain were incorporated into 2,5 ml. molten phage agar top-layer and evenly spread on phage agar plates which were then left for 45 min. to gel. All bacteriocins and both phage 107/69 and phage D were spotted on these plates and left overnight at room temperature.

Immunity test. All the bacteriocinogenic strains as well as *P. vulgaris* strain 107, the strain lysogenic for phage 107/69, were tested by preparing lawns in phage agar top-layer and spotting the 12 bacteriocins on them. In addition both phages 107/69 and D were tested for activity on these strains by the spotting technique.

RESULTS

Bacteriocin-resistant mutants. Six hundred bacteriocin-resistant mutants of strain 69, comprising 50 mutants selected for resistance to each of the 12 bacteriocins were obtained. All 600 of these mutants were found to have acquired insensitivity to the transducing phage 107/69 (Table 1). Each of these mutants was tested for sensitivity or resistance towards all of the remaining bacteriocins besides that one from which it was selected as being resistant to. 500 of these mutants were found to be resistant to all 12 bacteriocins. Of the remaining 100 mutants, 91 were resistant to 8 of the bacteriocins i.e. bacteriocins 41, 49, 52, 75, 115, 35, 36 and 46, whilst retaining sensitivity to the remaining 4 bacteriocins 127, 223, 226 and 121. Conversely, the last 9 mutants displayed a similar but reciprocal group-resistance pattern, being resistant to the 4 bacteriocins 127, 223, 226 and 121, whilst remaining sensitive to the aforementioned 8 bacteriocins. From these results it is evident that 3 types of bacteriocin resistant mutant were obtained. The majority (500) are resistant to all the bacteriocins, but the remaining mutants are divisable into 2 types; one displaying resistance towards 8 of the bacteriocins whilst retaining sensitivity to the remaining 4, and a second type displaying resistance to these latter 4 bacteriocins whilst retaining sensitivity to the other 8. On the basis of these group-resistance patterns exhibited by many of the mutants, the bacteriocins themselves are divisable into 2 groups comprising 8 and 4 respectively. Bacteriocins 41, 49, 52, 75, 115, 35, 36 and 46 were subsequently allocated as representatives of one group which was designated as the Group I bacteriocins, and bacteriocins 127, 223, 226 and 121 grouped together as the Group II bacteriocins (Plate IV, Figs 1 and 2). Table 2 shows the group-resistance patterns of the mutants of strain 69 towards the 12 bacteriocins.

Table 1. Bacteriocin-resistant mutants of *P. vulgaris* strain 69

Bacteriocins	No. of resistant mutants		Sensitivity to phage 107/69
41	50		Negative
49	50		"
52	50		"
75	50		"
115	50		"
35	50		"
36	50	600	"
46	50		"
127	50		"
223	50		"
226	50		"
121	50		"

Bacteriocin adsorption experiments were performed on 20 of the mutants resistant to all 12 bacteriocins, in addition to 10 mutants resistant to the 8 Group I bacteriocins and on the 9 Group II-resistant mutants. All mutants tested did not adsorb the bacteriocins to which they were resistant while sensitive strain 69 cells adsorbed all bacteriocin from suspensions of cells and bacteriocin. No mutants of the tolerant class were encountered. These same bacteriocin-resistant mutants tested for non-adsorption of bacteriocin were similarly unable to adsorb phage 107/69 in phage adsorption experiments, whilst strain 69 adsorbed *ca* 90% of free phage in 20 min.

Phage 107/69-resistant mutants. Seven mutants of strain 69 resistant to phage 107/69 were obtained. These mutants were unable to adsorb the phage in phage adsorption experiments. The mutants were insensitive to all 12 bacteriocins and were unable to adsorb the bacteriocins in bacteriocin adsorption experiments.

Host range. The bacteriocins exhibited a broad spectrum of activity against many strains of the Proteus group. None of the 100 strains of *P. rettgeri* tested were sensitive to any of the bacteriocins or either phage. The 34 Providence strains tested were likewise insensitive to any of these agents as were the 2 Salmonella strains tested. Of the 11 *Serratia marcescens* strains tested, 8 were sensitive to all of those bacteriocins which constitute the Group I bacteriocins and insensitive to the 4 Group II bacteriocins. All 11 strains were insensitive to either phage 107/69 or phage D (Table 3). Of the 100 *P. vulgaris* strains tested, 52%

PLATE IV

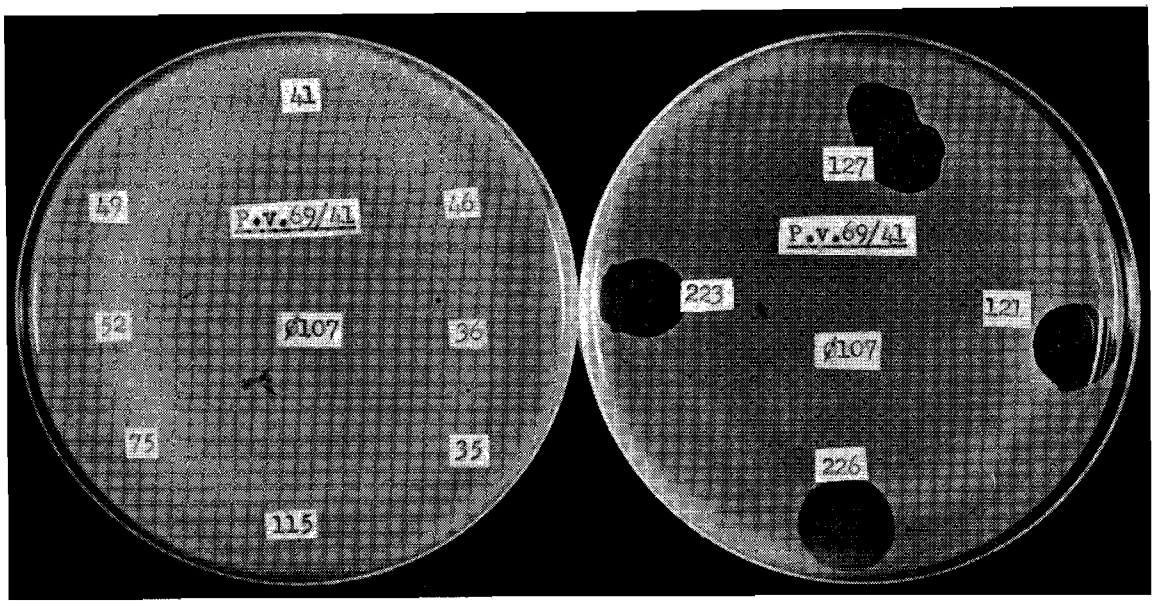


Fig. 1. Assay of bacteriocins and Ø107/69 on a mutant of strain 69 resistant to bacteriocin 41. The mutant is also insensitive to the remaining 7 Group I bacteriocins but still sensitive to the 4 Group II bacteriocins. The mutant is insensitive to Ø107/69.

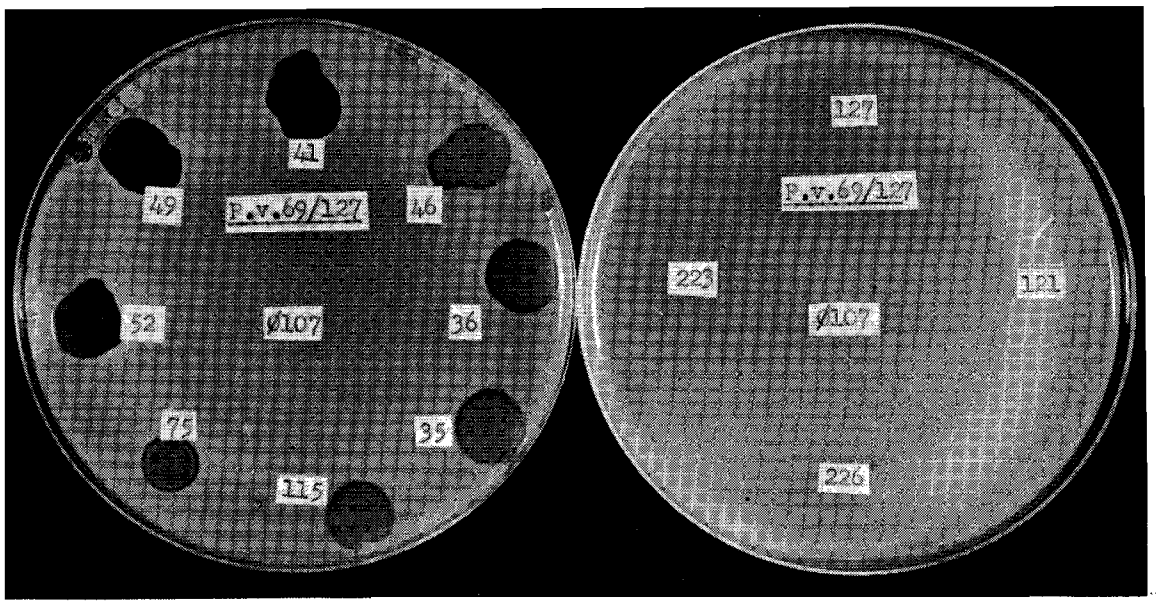
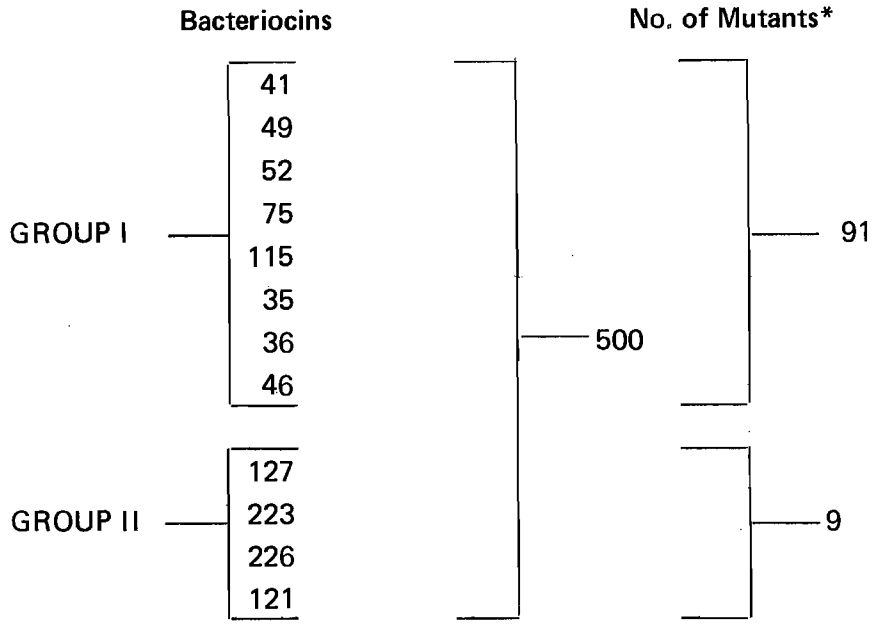


Fig. 2. Assay of bacteriocins and Ø107/69 on a mutant of strain 69 resistant to bacteriocin 127. The mutant is also insensitive to the remaining 3 Group II bacteriocins but still sensitive to the 8 Group I bacteriocins. The mutant is insensitive to Ø107/69.

Table 2. Bacteriocin-resistant mutants of *P. vulgaris* strain 69



* 500 of the mutants are resistant to all 12 bactericins. 91 mutants are resistant to the 8 Group I bacteriocins whilst retaining sensitivity to the 4 Group II bacteriocins. 9 mutants are resistant to the 4 Group II bacteriocins whilst remaining sensitive to the 8 Group I bacteriocins.

Table 3. Activity spectrum of bacteriocins and bacteriophages active on 11 *Serratia marcesens* strains

S. marcesens strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
1	+	+	+	+					+	+	+	+		
2	+	+	+	+					+	+	+	+		
3	+	+	+	+					+	+	+	+		
4														
5	+	+	+	+					+	+	+	+		
6	+	+	+	+					+	+	+	+		
7	+	+	+	+					+	+	+	+		
8														
9														
10	+	+	+	+					+	+	+	+		
11	+	+	+	+					+	+	+	+		

\+ indicates activity

were sensitive to bacteriocin. Although most of these strains (31) were sensitive to all the bacteriocins, 40% displayed the same bacteriocin group-sensitivity pattern as was displayed by many mutants of strain 69 and the Serratia strains as mentioned. For example, 19 of the sensitive strains exhibited group sensitivity towards the 8 Group I bacteriocins alone and were insensitive to the 4 Group II bacteriocins. Conversely, strains 40 and 90 were sensitive to the 4 Group II bacteriocins and insensitive to the 8 Group I agents. Eleven of the bacteriocin-sensitive strains besides strain 69, were also sensitive to phage 107/69. Interestingly, all these strains were of the type which were sensitive to all 12 bacteriocins, as is strain 69. Strain 107, which is lysogenic for Ø107/69 (Coetzee, de Klerk & Smit, 1967) was one of the strains which displayed areas of inhibition on lawns of this strain when spotted with all bacteriocins. It was uncertain whether this organism is in fact sensitive to the bactericidal activity of the bacteriocins, or whether the areas of inhibition were due to the induction of phage 107/69 from these cells by the bacteriocins. Certain bacteriocins such as colicin E2 (Endo, Kamiya & Ishizawa, 1963) and megacin C (Holland 1963; 1965) for example, are known to induce phage λ from lysogenic *E. coli*. Besides strain 69, strain 107 was also the only *P. vulgaris* strain inhibited by phage D. Here again it was uncertain whether strain 107 is productively infected by the sewage phage or whether Ø107/69 was being induced. Table 4 shows the sensitivity of the 100 *P. vulgaris* strains tested, to both bacteriocins and phage. In order to ascertain whether strain 107 was being killed by the bacteriocins or whether the transducing phage was being induced from these cells, the areas of inhibition from lawns of strain 107 spotted with the bacteriocins were cut out with a sterile wire loop and resuspended in 2,0 ml. of broth to which a few drops of chloroform was added. After centrifugation to remove excess bacterial debris and agar, the supernatents were titrated on strain 69. After overnight incubation the plates were examined for the presence of plaques of phage 107/69.

In order to test whether phage D productively infects strain 107, dilutions of the phage were made and titrated on strain 107 cells by the agar layer method. After overnight incubation, the plates were examined for the presence of infectious centres of ØD. This phage was found to productively infect strain 107, yielding large plaques on lawns of this strain which are typical of the plaque morphology of phage D as produced on strain 69.

The bacteriocins on the other hand, appear to be responsible for the induction of phage 107/69 from cells of strain 107. Areas of inhibition from lawns of strain 107

Table 4. Activity spectrum of bacteriocins and bacteriophages active on 100 *P. vulgaris* strains

P. vulgaris strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
1														
2														
3														
4	+	+	+	+					+	+	+	+		
6	+	+	+	+					+	+	+	+		
7	+	+	+	+					+	+	+	+		
10														
11														
12	+	+	+	+					+	+	+	+		
13														
14	+	+	+	+					+	+	+	+		
16	+	+	+	+	+	+	+	+	+	+	+	+		
18														
19	+	+	+	+					+	+	+	+		
20														
22	+	+	+	+					+	+	+	+		
23														
24	+	+	+	+	+	+	+	+	+	+	+	+		
26														
27	+	+	+	+					+	+	+	+		
28														
29	+	+	+	+					+	+	+	+		
30														
31														
33														
34	+	+	+	+					+	+	+	+		
35														
36														
37														
38	+	+	+	+	+	+	+	+	+	+	+	+		
39					+	+	+	+						
40														
41														
42	+	+	+	+					+	+	+	+		
43														
44														
45														
46														
47														
48														
49														
50														
52														
53	+	+	+	+	+	+	+	+	+	+	+	+	+	
54	+	+	+	+	+	+	+	+	+	+	+	+	+	
55	+	+	+	+	+	+	+	+	+	+	+	+		
58														

\+ indicates activity

Table 4 (continued)

P. vulgaris strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
59	+	+	+	+	+	+	+	+	+	+	+	+	+	
60	+	+	+	+	+	+	+	+	+	+	+	+		
61														
62	+	+	+	+	+	+	+	+	+	+	+	+	+	
63	+	+	+	+	+	+	+	+	+	+	+	+	+	
64														
65	+	+	+	+	+	+	+	+	+	+	+	+	+	
66	+	+	+	+	+	+	+	+	+	+	+	+	+	
67	+	+	+	+	+	+	+	+	+	+	+	+		
68														
69	+	+	+	+	+	+	+	+	+	+	+	+	+	+
70														
71	+	+	+	+					+	+	+	+		
72	+	+	+	+	+	+	+	+	+	+	+	+		
74														
75														
76	+	+	+	+	+	+	+	+	+	+	+	+		
77	+	+	+	+	+	+	+	+	+	+	+	+	+	
78	+	+	+	+	+	+	+	+	+	+	+	+		
79	+	+	+	+	+	+	+	+	+	+	+	+		
80														
82	+	+	+	+	+	+	+	+	+	+	+	+		
83	+	+	+	+	+	+	+	+	+	+	+	+		
84														
85														
86														
87	+	+	+	+					+	+	+	+		
88	+	+	+	+	+	+	+	+	+	+	+	+		
89														
90					+	+	+	+						
91	+	+	+	+	+	+	+	+	+	+	+	+		
92	+	+	+	+					+	+	+	+		
93	+	+	+	+					+	+	+	+		
94														
95														
96														
97														
98	+	+	+	+	+	+	+	+	+	+	+	+	+	
99	+	+	+	+					+	+	+	+		
100														
101	+	+	+	+	+	+	+	+	+	+	+	+	+	
102	+	+	+	+	+	+	+	+	+	+	+	+	+	
103	+	+	+	+					+	+	+	+		
104	+	+	+	+					+	+	+	+		
105	+	+	+	+	+	+	+	+	+	+	+	+		
106														
107	+	+	+	+	+	+	+	+	+	+	+	+		+
108	+	+	+	+	+	+	+	+	+	+	+	+		
109	+	+	+	+	+	+	+	+	+	+	+	+		
110	+	+	+	+					+	+	+	+		
114	+	+	+	+	+	+	+	+	+	+	+	+		
115														
116														

+ indicates activity

which had been spotted with bacteriocin and resuspended in broth which had then been titrated on strain 69, yielded plaques typical of the transducing phage 107/69. This is indicative of the actual induction of Ø107/69 from strain 107 (which is lysogenic for the phage) by the bacteriocins.

The percentage of *Proteus mirabilis* strains sensitive to the bacteriocins was approximately of the same magnitude as that of the *P. vulgaris* strains. Of 100 strains tested, 48% were found to be sensitive to bacteriocin. Once again, the phenomenon of group-activity of the bacteriocins was noticed as observed on both *P. vulgaris* and *S. marcescens* strains. In contrast to the relatively high percentage within *P. vulgaris* strains tested, only 7 of the 48 *P. mirabilis* bacteriocin-sensitive strains were sensitive to all 12 bacteriocins. Of the remaining 41 sensitive strains, 35 displayed sensitivity to the 8 Group I bacteriocins, whilst only 6 were sensitive to the 4 Group II bacteriocins. An unexpected but striking observation was that made with regard to the response of the *P. mirabilis* strains to the 2 bacteriophages. Thirty eight per cent of these strains exhibited sensitivity to phage D. Eleven of these strains were not sensitive to any of the bacteriocins or phage 107/69. Seven of the 100 *P. mirabilis* strains were sensitive to phage 107/69. In contrast to the results obtained with the *P. vulgaris* cells, these 7 phage 107/69-sensitive strains were not sensitive to all bacteriocins. It has been noted that any strain of *P. vulgaris,* including the 600 bacteriocin-resistant mutants of strain 69, which was resistant to any of the bacteriocins, was always simultaneously resistant to phage 107/69. Two of the 7 *P. mirabilis* phage 107/69-sensitive strains, i.e. strains 18 and 98, were in fact insensitive to any of the bacteriocins (Table 5).

Of the 100 *P. morganii* strains tested, 22 were sensitive to bacteriocin, all of which, bar one strain, i.e. strain 57, were sensitive to the Group I bacteriocins. Strain 57 was the only strain sensitive to the Group II agents and resistant to the rest. None of the 100 strains tested were sensitive to either of the bacteriophages (Table 6).

Immunity test. None of the 12 bacteriocinogenic strains displayed sensitivity to any of the bacteriocins or either of the bacteriophages. *P. vulgaris* strain 107, lysogenic for the transducing phage 107/69, exhibited areas of inhibition on plates when lawns of this organism were spotted with all the bacteriocins and phage D (Table 7). As mentioned before, it would appear that these inhibited growth areas represent the induction of phage 107/69 from strain 107 by the bacteriocins. The area of inhibition caused by a spot of phage D represents the productive infection of strain 107 by this phage.

Table 5. Activity spectrum of bacteriocins and bacteriophages active on 100 *P. mirabilis* strains

P. mirabilis strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
1	+	+	+	+					+	+	+	+	+	+
2	+	+	+	+					+	+	+	+		+
3														
4														
5														+
6	+	+	+	+					+	+	+	+		+
7	+	+	+	+					+	+	+	+		
8														
9	+	+	+	+					+	+	+	+		+
10														
11	+	+	+	+					+	+	+	+		+
12														
13														
14	+	+	+	+					+	+	+	+		+
15	+	+	+	+					+	+	+	+	+	+
16														
17					+	+	+	+						
18													+	
19	+	+	+	+					+	+	+	+		
20														
21	+	+	+	+					+	+	+	+		+
22														+
23														
24	+	+	+	+					+	+	+	+		
25														
26					+	+	+	+					+	
27														+
28														
29					+	+	+	+						+
30														
31	+	+	+	+					+	+	+	+	+	+
32														
33														
34														
35														+
36														+
37	+	+	+	+					+	+	+	+		
38														
39														+
40	+	+	+	+					+	+	+	+	+	+
41														+
42														
43														+
44	+	+	+	+					+	+	+	+		+
45	+	+	+	+					+	+	+	+		
46														
47														
48	+	+	+	+					+	+	+	+		

+ indicates activity

Table 5 (continued)

P. mirabilis strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
49	+	+	+	+					+	+	+	+		
50														
51	+	+	+	+					+	+	+	+		
52														
53														
54														
55	+	+	+	+					+	+	+	+		+
56														
57	+	+	+	+					+	+	+	+		+
58	+	+	+	+					+	+	+	+		
59														
60														
61														
62														
63	+	+	+	+					+	+	+	+		+
64														
65	+	+	+	+					+	+	+	+		+
66														
67					+	+	+	+						
68	+	+	+	+					+	+	+	+		+
69					+	+	+	+						
70	+	+	+	+					+	+	+	+		
71	+	+	+	+	+	+	+	+	+	+	+	+		+
72	+	+	+	+					+	+	+	+		+
73	+	+	+	+					+	+	+	+		+
74	+	+	+	+					+	+	+	+		+
75					+	+	+	+						
76														
77														+
78	+	+	+	+					+	+	+	+		+
79														
80														
81														
82	+	+	+	+					+	+	+	+		
83														+
84	+	+	+	+	+	+	+	+	+	+	+	+		+
85														
86														+
87	+	+	+	+	+	+	+	+	+	+	+	+		+
88	+	+	+	+	+	+	+	+	+	+	+	+		+
89	+	+	+	+	+	+	+	+	+	+	+	+		
90	+	+	+	+	+	+	+	+	+	+	+	+		
91	+	+	+	+	+	+	+	+	+	+	+	+		+
92														
93	+	+	+	+					+	+	+	+		
94	+	+	+	+					+	+	+	+		
95														
96	+	+	+	+					+	+	+	+		
97														
98													+	+
99	+	+	+	+					+	+	+	+		
100														

+ indicates activity

Table 6. Activity spectrum of bacteriocins and bacteriophages active on 100 *P. morganii* strains

P. morganii strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
1														
2														
3														
4														
5														
6														
7														
8														
9														
10	+	+	+	+					+	+	+	+		
11	+	+	+	+					+	+	+	+		
12														
13	+	+	+	+					+	+	+	+		
14														
15														
16														
17														
18														
19														
20														
21														
22														
23														
24														
25														
26														
27														
28	+	+	+	+					+	+	+	+		
29														
30	+	+	+	+					+	+	+	+		
31														
32														
33														
34	+	+	+	+					+	+	+	+		
35														
36														
37														
38														
39														
40														
41	+	+	+	+					+	+	+	+		
42														
43														
44														
45	+	+	+	+					+	+	+	+		
46														
47														
48														
49	+	+	+	+					+	+	+	+		
50														

\+ indicates activity

Table 6 (continued)

P. morganii strain no.	Bacteriocins												Phages	
	41	49	52	75	127	223	226	121	115	35	36	46	Ø107	ØD
51														
52														
53														
54														
55														
56														
57					+	+	+	+						
58														
59														
60														
61														
62														
63	+	+	+	+					+	+	+	+		
64														
65	+	+	+	+					+	+	+	+		
66														
67														
68	+	+	+	+					+	+	+	+		
69														
70														
71														
72	+	+	+	+					+	+	+	+		
73														
74														
75														
76	+	+	+	+					+	+	+	+		
77														
78														
79														
80														
81														
82														
83														
84														
85														
86	+	+	+	+					+	+	+	+		
87	+	+	+	+					+	+	+	+		
88	+	+	+	+					+	+	+	+		
89														
90	+	+	+	+					+	+	+	+		
91														
92														
93														
94														
95	+	+	+	+					+	+	+	+		
96														
97	+	+	+	+					+	+	+	+		
98	+	+	+	+					+	+	+	+		
99														
100														

+ indicates activity

Table 7. Activity of bacteriocins and bacteriophages on bacteriocinogenic strains and on *P. vulgaris* strain 107

		Bacteriocinogenic strains												*P. vulgaris* lysogenic for Ø107/69
		41	49	52	75	127	223	226	115	121	35	36	46	107
Bacteriocins	41													+
	49													+
	52													+
	75													+
	127													+
	223													+
	226													+
	115													+
	121													+
	35													+
	36													+
	46													+
Bacterio-phages	Ø107 / 69													
	ØD													+

+ indicates activity.

Blank space indicates no activity.

DISCUSSION

Fifty bacteriocin-resistant mutants of *P. vulgaris* strain 69 were selected for resistance to each of the 12 bacteriocins involved in this study. All of the 600 mutants obtained were found to be insensitive to the *P. vulgaris* transducing phage 107/69. One of the primary considerations which prompted this study was to be an attempt at the transduction of the genetic determinant responsible for the property of resistance to bacteriocin from bacteriocin-resistant mutants of strain 69 to the bacteriocin-sensitive cells of the parent organism. Due to the fact that mutants of strain 69 selected for resistance to any one of the 12 bacteriocins always simultaneously displayed insensitivity to the transducing phage, it was subsequently not possible to effect the transduction of bacteriocin resistance to sensitive cells of the parent organism by utilizing this phage.

It was postulated by Fredericq as early as 1946 that bacteria which are sensitive to a certain bacteriocin contain a specific receptor substance on their surface which serves as the site of attachment for the bacteriocin. The presence of specific receptor macromolecules possessed by sensitive bacteria for the adsorption of both bacteriocins and bacteriophages is by now an established fact although the precise chemical nature and molecular configuration of many of these receptors is still largely unknown. This holds particularly with regard to the receptors of bacteriocins (Weltzien & Jesaitis, 1971). A number of cases have been reported in which microorganisms which were sensitive to certain bacteriocins were always attacked by particular bacteriophages. Thus colicins M, K or E were always attacked by bacteriophages T1, T6 and BF23 respectively, and that mutants of sensitive bacteria which had lost their sensitivity to one of the colicins were always simultaneously resistant to the corresponding phage. It was suggested therefore that in each of these instances the receptor for the bacteriocins and virus was identical (Fredericq, 1953; 1956). On the basis of these considerations it was considered possible that the phenomenon of cross-resistance observed between the *P. vulgaris* phage tail-like bacteriocins and phage 107/69, as exhibited by both phage and bacteriocin-resistant mutants of strain 69, reflects a similar situation whereby these two types of particle utilize the same receptor site on the sensitive cell's surface.

A complication which frustrates this relatively straight-forward hypothesis however, is the observation that the 12 bacteriocins involved in this study apparently do not constitute a homologous entity with regard to their adsorption to a single common receptor site. Of the 600 bacteriocin-resistant mutants of strain 69 isolated, the majority, namely 500, are resistant to all 12 of the bacteriocins, irrespective of which bacteriocin

the mutants were obtained as being specifically resistant to. However, the remaining 100 mutants displayed a distinct pattern of resistance towards only certain of the bacteriocins, retaining sensitivity to the rest. Indeed, it was on the basis of this property of being resistant to certain of the bacteriocins whilst retaining sensitivity to the rest that the bacteriocins could be classified into 2 groups. For example, 91 of the bacteriocin-resistant mutants of strain 69 displayed resistance to those 8 bacteriocins designated as the Group I bacteriocins, whilst remaining sensitive to the 4 agents designated as the Group II bacteriocins. Conversely, 9 of the mutants exhibited the reciprocal group-resistance pattern, being resistant to the 4 Group II bacteriocins and sensitive to the 8 Group I structures. All these mutants were however resistant to phage 107/69, irrespective of which group-resistance pattern they exhibited.

From these results it would appear that 2 receptor sites apparently exist on the strain 69 cell surface with regard to the adsorption of the members of each of the 2 groups of bacteriocin, that is, one receptor site responsible for the adsorption of each group.

On the basis of the observation that any mutant which is resistant to the Group I bacteriocins whilst still being sensitive to the 4 Group II bacteriocins, was always also resistant to phage 107/69, implies that this phage utilizes certain adsorption factors in common with the Group I bacteriocins. On the other hand, the existence of mutants which displayed simultaneous resistance to the 4 Group II bacteriocins and the phage whilst retaining sensitivity to the 8 Group I bacteriocins, offers in turn the contradictory implication that the phage and the Group II structures in fact share common adsorption factors. A possible explanation to account for these apparently contradictory results is the hypothesis that the 2 receptor sites concerned with the successful fixation of the 2 groups of bacteriocin both contain certain functional chemical groupings essential for the successful fixation of phage 107/69 to the surface of the sensitive cell. This proposal implies that the 2 bacteriocin receptor sites may possibly be closely linked or overlapping, and that any conformational change on the surface of the cell acquired and expressed as the result of the attainment of resistance on the part of strain 69, is sufficient to prevent the functional fixation of the phage, even though this particular change need not necessarily confer resistance to both groups of bacteriocin. If this be the case, as it would appear to be from the results obtained, then certain receptor molecules on the strain 69 cell surface responsible for the adsorption of phage 107/69, possibly consist of constituents or chemical groupings which are also shared to some extent by both bacteriocin receptor sites. A conformational change to the macromolecular configuration of the cell surface receptor responsible for the

normal adsorption of the Group I bacteriocins, may not be stringent enough to prevent the activity of the Group II bacteriocins but is sufficient to prevent the successful fixation of phage 107/69. Similarly, a change on the cell surface sufficient to cause resistance to the Group II bacteriocins but not the Group I bacteriocins may nevertheless be sufficient to confer resistance on the bacterium to the phage.

It was interesting to note that the adsorption patterns of the 2 respective groups of bacteriocin was maintained on all the other bacteriocin-sensitive strains of *P. vulgaris* tested, as well as on the many sensitive *P. mirabilis* strains, and on those strains of *P. morganii* and *S. marcescens* which proved to be bacteriocin-sensitive. No strain of *P. vulgaris* which was resistant to either group of bacteriocins was ever sensitive to phage 107/69, suggesting the possibility of a similar linked receptor phenomenon for other strains of *Proteus vulgaris* as was found for strain 69.

The high incidence of bacteriocin-sensitive strains amongst the *P. vulgaris* (52 %) and *P. mirabilis* (48%) strains tested, is considered significant and indicative of the close relationship existing between these 2 separate species (Kauffmann, 1966) of the Proteus group "hauseri" (Ewing, 1958).

A point of interest is the response of the bacteriocinogenic organisms and the lysogenic strain 107 to the bacteriocins and both phages 107/69 and D. All the bacteriocinogenic strains were immune to all 12 bacteriocins and both bacteriophages. *Proteus vulgaris* strain 107 was however productively infected by the sewage phage D. It was also observed that the bacteriocins, when spotted on lawns of strain 107, appear to induce the development of the prophage 107/69 from these lysogenic cells. This activity is reminiscent of the inducing ability of certain other bacteriocins for phage λ from lysogenic *E. coli* (Endo *et al.*, 1963; Holland, 1963; 1965). This observation may provide a clue to the mode of action of the *P. vulgaris* phage-tail-like structures. The modes of action of both colicin E2 and megacin C for example, both of which induce phage λ from *E. coli,* is to induce the specific degradation of the host DNA and to bring about the cessation of RNA and protein synthesis. Jayawardene & Farkas-Himsley (1970), working with the high-molecular weight phage-tail-like structures from *Vibrio comma,* demonstrated a similar mode of action for the vibriocins.

In conclusion it would appear that the bacteriocins involved in this study may be divisable into 2 groups on the basis of their respective activity spectra, and that the receptor sites on the strain 69 cell surface involved in the adsorption of both groups of bacteriocin and the transducing phage 107/69, are closely linked or overlapping.

REFERENCES

ADAMS, M.H. (1959). *Bacteriophages.* Interscience Publishers Inc. New York.

COETZEE, H.L., DE KLERK, H.C., COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like particles associated with intra-species killing of *Proteus vulgaris. Journal of General Virology 2,* 29.

COETZEE, J.N., DE KLERK, H.C. & SMIT, J.A. (1967). A transducing phage for *Proteus vulgaris. Journal of General Virology 1,* 561.

ENDO, H., KAMIYA, T. & ISHIZAWA, M. (1963). λ Phage induction by colicine E2. *Biochemical and Biophysical Research Communications 11,* 477.

EWING, W.H. (1958). The nomenclature and taxonomy of the Proteus and Providence groups. *International Bulletin of Bacteriological Nomenclature and Taxonomy 8,* 17.

FREDERICQ, P. (1946). Sur la pluralité des récepteurs d'antibiose de *E. coli. Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 140,* 1189.

FREDERICQ, P. (1953). Colicines et bactériophages. *Annales d l'Institut Pasteur, Paris, 84,* 294.

FREDERICQ, P. (1956). Resistance et immunité aux colicines. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales 150, 1514.*

FREDERICQ, P. (1958). Colicins and colicinogenic factors. *Symposia of the Society for Experimental Biology 12,* 104.

FREDERICQ, P. (1965). A note on the classification of colicines. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 141.

HERSCHEY, A.D., KALMANSON, G. & BRONFENBRENNER, J. (1943). Quantitative methods in the study of phage-antiphage reaction. *Journal of Immunology 46,* 267.

HOLLAND, I.B. (1963). Effect of a bacteriocin preparation (megacin C) on DNA synthesis in *Bacillus megaterium. Biochemical and Biophysical Research Communications 13,* 246.

HOLLAND, I.B. (1965). A bacteriocin specifically affecting DNA synthesis in *Bacillus megaterium. Journal of Molecular Biology 12,* 429.

JAYAWARDENE, A. & FARKAS-HIMSLEY, H. (1970). Mode of action of vibriocin. *Journal of Bacteriology 102,* 382.

KAUFFMANN, F. (1966). *The Bacteriology of Enterobacteriaceae.* Copenhagen: Munksgaard.

KELNER, A. (1949). Photoreactivation of ultraviolet-irradiated *Escherichia coli* with special reference to the dose-reduction principle and to ultraviolet induced mutations. *Journal of Bacteriology 58,* 511.

MAEDA, A. & NOMURA, M. (1966). Interaction of colicines with bacterial cells. I. Studies with radioactive colicines. *Journal of Bacteriology 91,* 685.

NEWCOMBE, H.B. (1955). The timing of induced mutations in *Streptomyces. Brookhaven Symposia on Biology 8,* 88.

NOMURA, M. (1963). Mode of action of colicins. *Cold Spring Harbour Symposium on Quantitative Biology 28,* 315.

REEVES, P. (1965). The bacteriocins. *Bacteriological Reviews 29,* 24.

WELTZIEN, H.U. & JESAITIS, M.A. (1971). The nature of the colicin K receptor of *Escherichia coli Cullen. Journal of Experimental Medicine 133,* 534.

CHAPTER V

COMPARATIVE MORPHOLOGY OF PHAGE 107/69 AND *P. VULGARIS* BACTERIOCINS

SUMMARY

An investigation of the morphologies of the 2 groups of bacteriocins comprising 4 and 8 respectively, and the tail component of temperate transducing phage 107/69 was made. The morphologies of the 12 bacteriocins with respect to length and breadth dimensions were shown to be identical. The length of the tail of phage 107/69 was found to be dissimilar to that of the bacteriocins. The width dimentions of these two entities are identical.

INTRODUCTION

The electron microscope was employed in conjunction with photographic techniques as early as 1934 by Marton in the successful electron micrography of bacteria (Marton, 1941). It required a further five years before Kaushe, Pfankuch & Ruska (Williams & Wyckoff, 1945) were able to demonstrate and photographically record particles of tobacco mosaic virus. The specimen support material employed by the early electron microscopists was a colloidal suspension of nitrocellulose which tended to obscure the fine structure of viral-sized particles. Schaeffer & Harker (1945) introduced the use of another substance known as polyvinyl formal (Formvar) which was stronger and more temperature stable. The relatively inefficient 'shadow-casting' technique of Williams & Wyckoff (1945) was used for many years until Brenner & Horne (1959) described the negative staining technique for high resolution electron microscopy of viruses. The method consists of 'embedding' the specimen particles in an electron dense material such as potassium phosphotungstate. This technique as modified by Bradley & Kay (1960) and Bradley (1962) provides the necessary contrast between specimen and substrate required for effective photography and is the technique employed in the observation of bacteriocins and phage for this study.

The bacteriocins studied here appear to be classifiable into two distinct groups on the basis of their positive or negative response with regard to adsorption to resistant mutants of the indicator strain. It would appear, as pointed out in the preceeding chapter, that eight

of the bacteriocins under investigation form a collective entity by virtue of their adsorption or lack of adsorption to certain of the indicator mutants described. Conversely, the remaining four bacteriocins are apparently representative of a second group, differentiated from the former by their common ability or inability to effect adsorption to those cells which are indicative of the collectivity assigned to the first group of particles. Furthermore, as has been pointed out, there appears to be some degree of similarity concerning the adsorption site between phage 107/69 and all twelve bacteriocins. The evidence is suggestive of the possibility that certain chemical groups concerned with efficient adsorption of phage 107/69 may also be common to both groups of bacteriocin. The striking morphological resemblance between the phage-tail-like bacteriocins and the tail section of phage 107/69 has already drawn comment. Indeed a similar observation has previously been made concerning this phage and other bacteriocins liberated by bacteriocinogenic strains of *P. vulgaris*(Coetzee *et al.*, 1968). On the basis of these observations and bearing in mind the proposal of Coetzee *et al.* (1968) that the *P. vulgaris* phage-tail-like structures may possibly constitute the products of defective lysogeny, it was considered justifiable in undertaking the following:—

(a) A comparison of the morphology of the twelve bacteriocins with due consideration to their group-behavioural pattern regarding adsorption to mutants of strain 69. It was hoped that the bacteriocins might fall into 2 groups on the basis of common morphology in a manner complementary to the above-mentioned adsorption patterns.

(b) A morphological comparison of the tail of phage 107/69 and the tail-like bacteriocins concerned with this study. The motivation for this was the possibility that the similar adsorption response of the phage to that of the bacteriocins might be reflected in a morphological conformation common to the phage-tail and the bacteriocins. In addition, the contention that the phage-tail-like particles may be representative of defective phages was considered on the basis of possible morphological similarities between these structures and this temperate transducing phage. It was felt that a morphological similarity between these two entities, coupled with their similar adsorption response to strain 69, might lend substance to the hypothesis that the *P. vulgaris* bacteriocins are defective phages, possibly related in origin to this phage.

The above-mentioned considerations were thus instrumental in determining an investigation of the morphological structure of the respective particles.

METHODS

Electron Microscopy.

Samples of the twelve bacteriocins and phage 107/69 were prepared for electron microscopy by several cycles of differential centrifugation as described in Chapter III (page 59). Final pellets of the active material were suspended in 0,1 N-ammonium acetate (pH 7,2) and negatively stained with an equal volume of a 2% potassium phosphotungstate acid solution. These suspensions were then mounted on carbon support grids by the spreading technique (Bradley, 1962) as described in Chapter III (page 60). Preparations were mounted for electron microscopy on the same day as that on which electron micrographs were to be taken.

Dimensions of Particles.

All samples were examined in a Philips EM 200 electron microscope. Electron micrographs of each preparation were taken at the same standard magnification of 200,00 x. Photographic prints of each micrograph were then enlarged to yield a final magnification of 3,039 x 10^5 x for each particle observed. Approximately 3 to 4 micrographs of each preparation were taken, each from a different region of the sample grid. The dimensions of both the width and length of individual phage-tail-like particles were measured by means of mathematical dividers. The measurements of ten particles from each preparation was finally calculated as a mean value of the dimensions representative of the bacteriocin type. The unit length of each phage-tail-like structure was expressed as the greatest distance between the origin of the base-plate-like region and the opposite extremity. The width of each particle was measured across the base-plate-like region i.e. the widest point. Dimensions were only taken for fully sheathed structures, although partly contracted sheaths were occasionally observed. The length of the tail of phage 107/69 was measured from the base-plate region to the origin of the neck-piece, i.e. the sheath-length. The width of the tail was measured across its widest point at the distal extremity in addition to various points along the sheath-length.

RESULTS

Dimensions of bacteriocins.

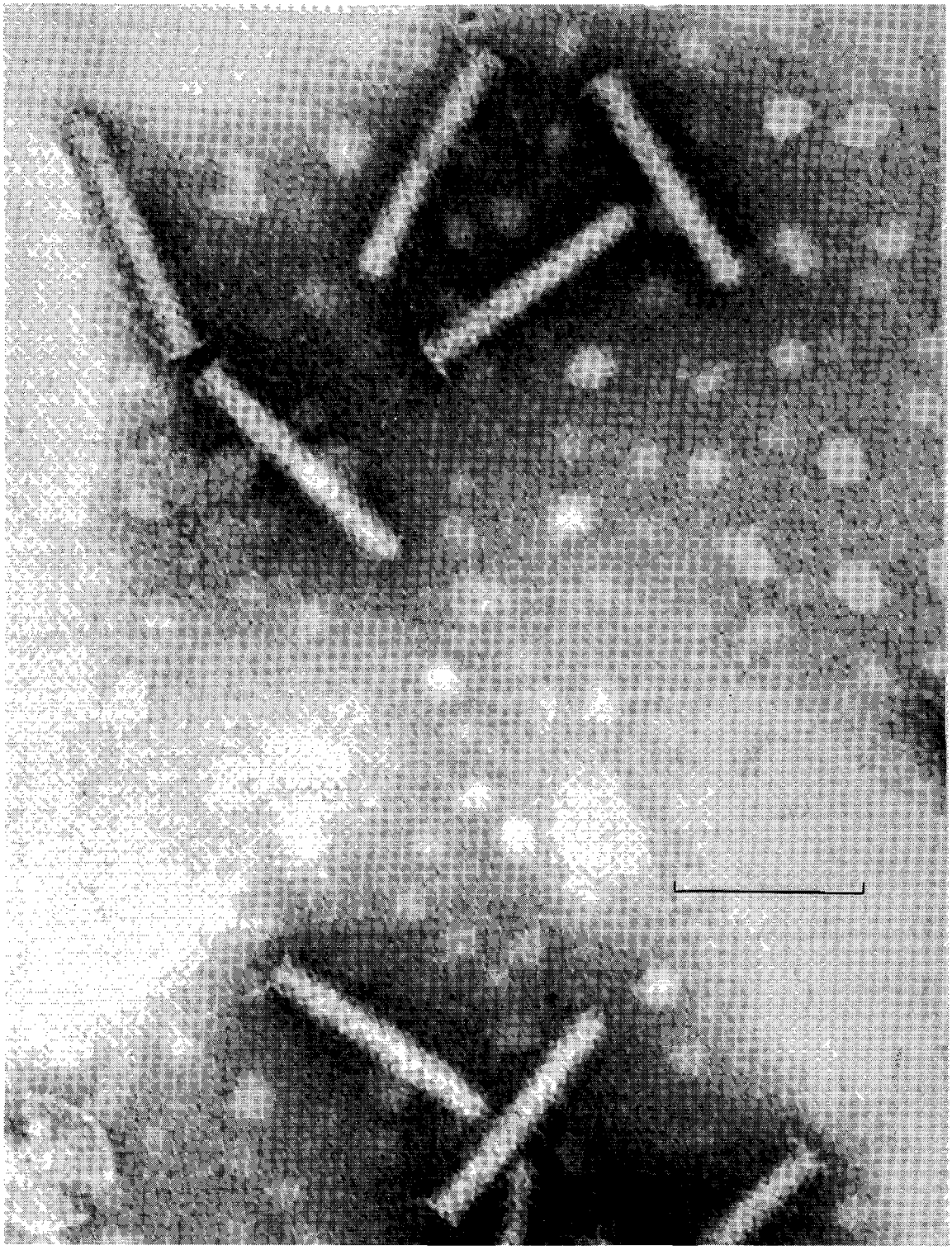
The majority of phage-tail-like structures in any one of the preparations studied were of a uniform size. Closer examination of micrographs representative of all twelve

bacteriocins yielded an occasional structure in all the preparations which appeared to deviate in length from that of the greater mass of particles. These occasional fully-sheathed structures were always shorter than the average particle. Very few contracted sheaths were seen, and no distinctive tail fibres, tail pins or base plates were observed. There appeared to be a thickening of the tail-like sheathed rod at the extremity opposite to that at which the tail core was always visible as a small hollow projection. This thickened end was 40 Å wider than the overall width of the paricle. Diagonal cross-striations suggestive of helical symmetry could be seen on particles from all twelve bacteriocin preparations. The lengths of fully sheathed particles from all twelve bacteriocin samples were found to be identical. The average length of each particle measured from all 12 bacteriocins was 1380 Å. The average width of the particles was also identical for all the bacteriocins, being approximately 220 Å at the widest point (Plate V). Three of the preparations, viz. Nos. 223, 226 and 36 contained occasional complete phage-like particles closely resembling phage 107/69. (Coetzee, 1967). The length and width dimensions of the tails of these phage-like objects were almost identical to those of the bacteriocin particles, being 1340 Å wide. The head appears to be octahedral with an inter-apex distance of approximately 660 Å (Plate VI). In addition, preparations of bacteriocin 127 (Plate VII) contained an occasional phage-like object reminiscent of the *Proteus rettgeri* phage 7476/332 (Prozesky, de Klerk & Coetzee, 1965). Bacteriocin 127 also contained the only particles with contracted sheaths. These contracted sheath particles revealed the core of the tail-like structures as a hollow tube 60 Å wide. It would appear from measurements of the contracted entities that the cavity containing the core is 90 Å wide, indicating a slight gap between the core and the inner wall of the sheath. A similar observation was made by Coetzee *et al.* (1968) for other *P. vulgaris* bacteriocins.

Dimensions of phage 107/69.

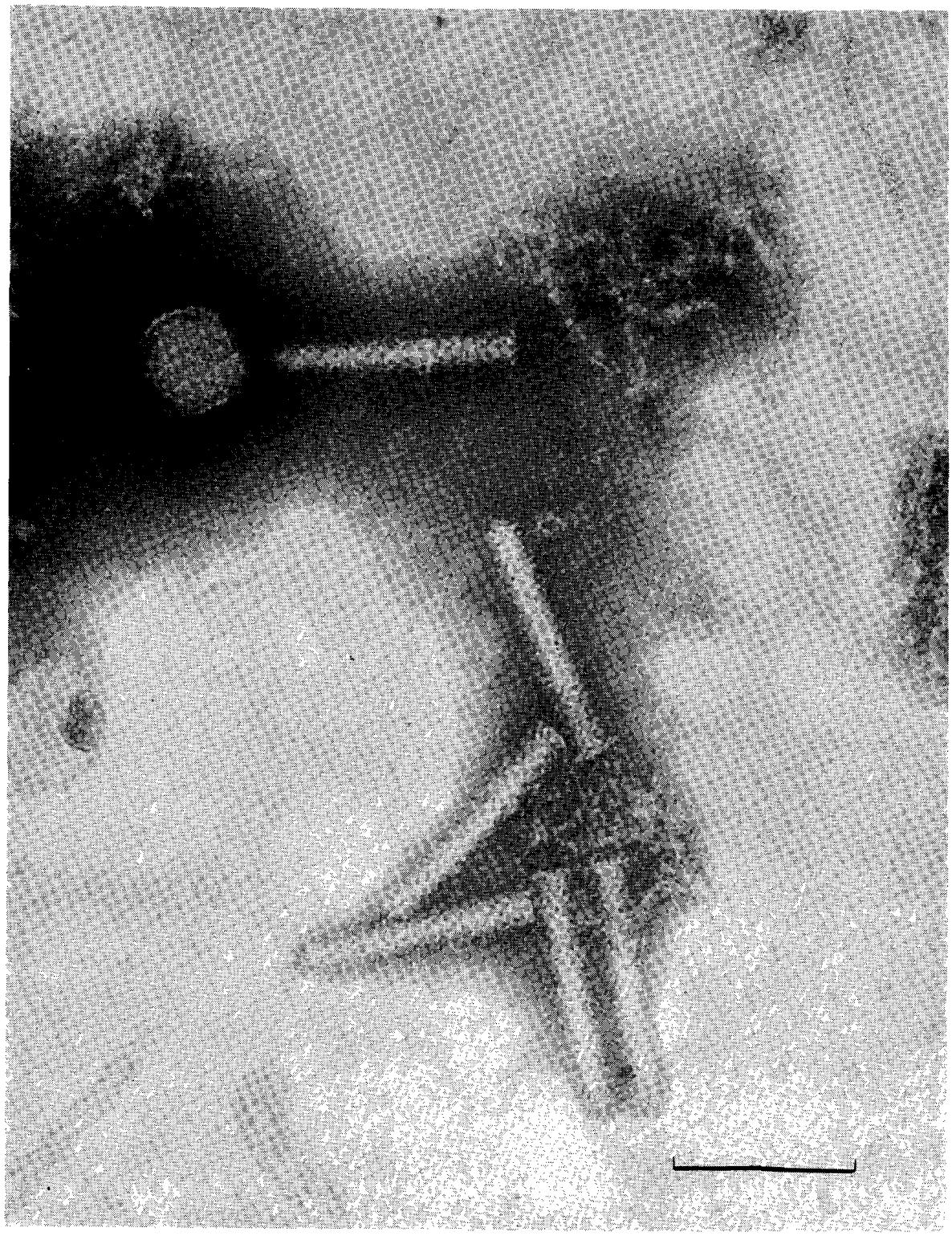
The length of the tail of phage 107/69 was found to be approximately 60 Å shorter than the length dimensions applicable to the twelve bacteriocins. The sheath-length of the tail of this phage, measured from the distal extremity to the origin of the neck section was found to be 1320 Å. (Plate VIII). The same value in respect of the length of this phage tail was reported by Coetzee in 1967. The overall width of the tail expressed as the average of three values taken at points independant of the distal extremity is 180 Å. This result is similar to that obtained for the bacteriocins. In addition, the width of the

PLATE V



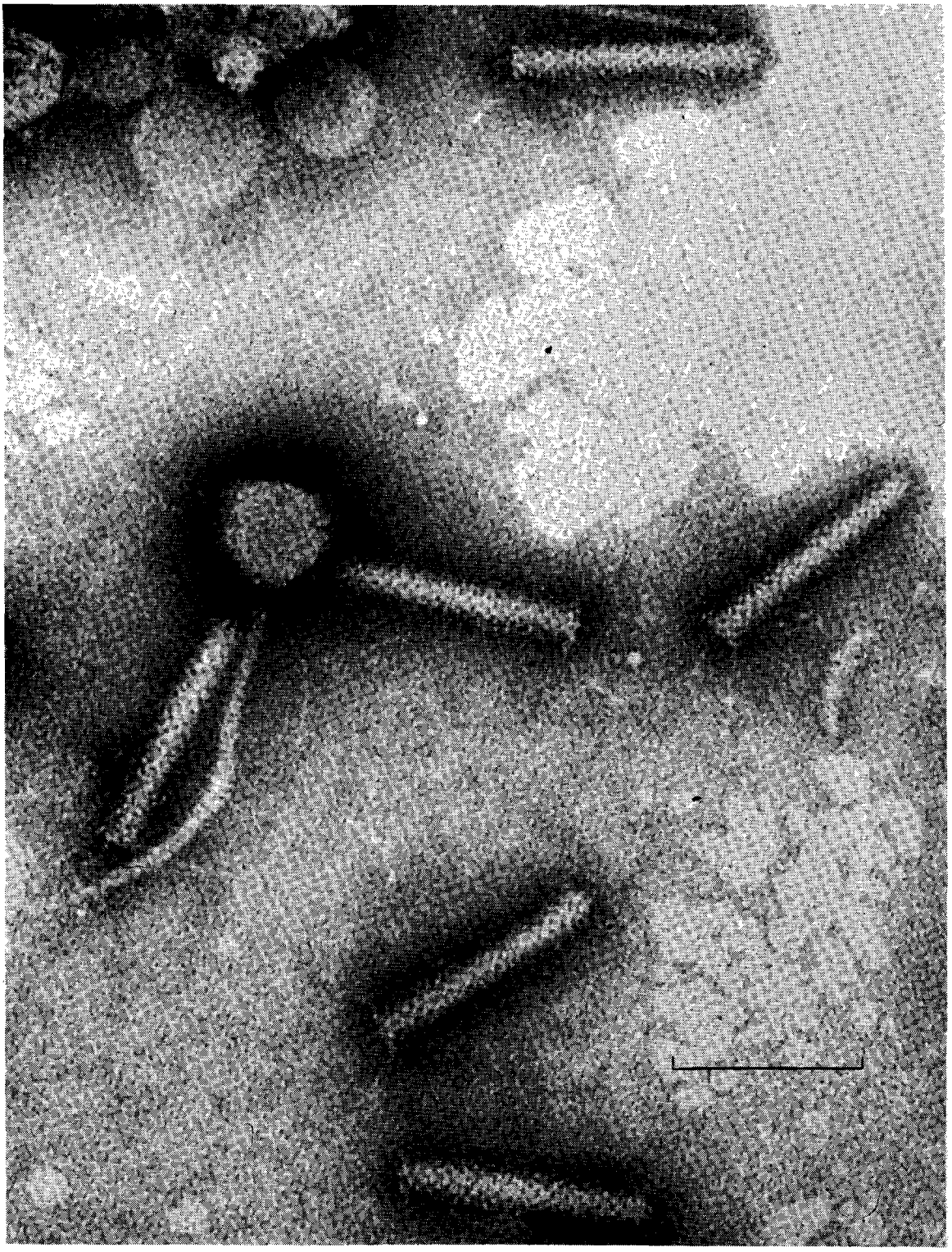
Phage tail-like structures of bacteriocin 35. The magnification is x300,000. The bar represents 1,000 Å.

PLATE VI



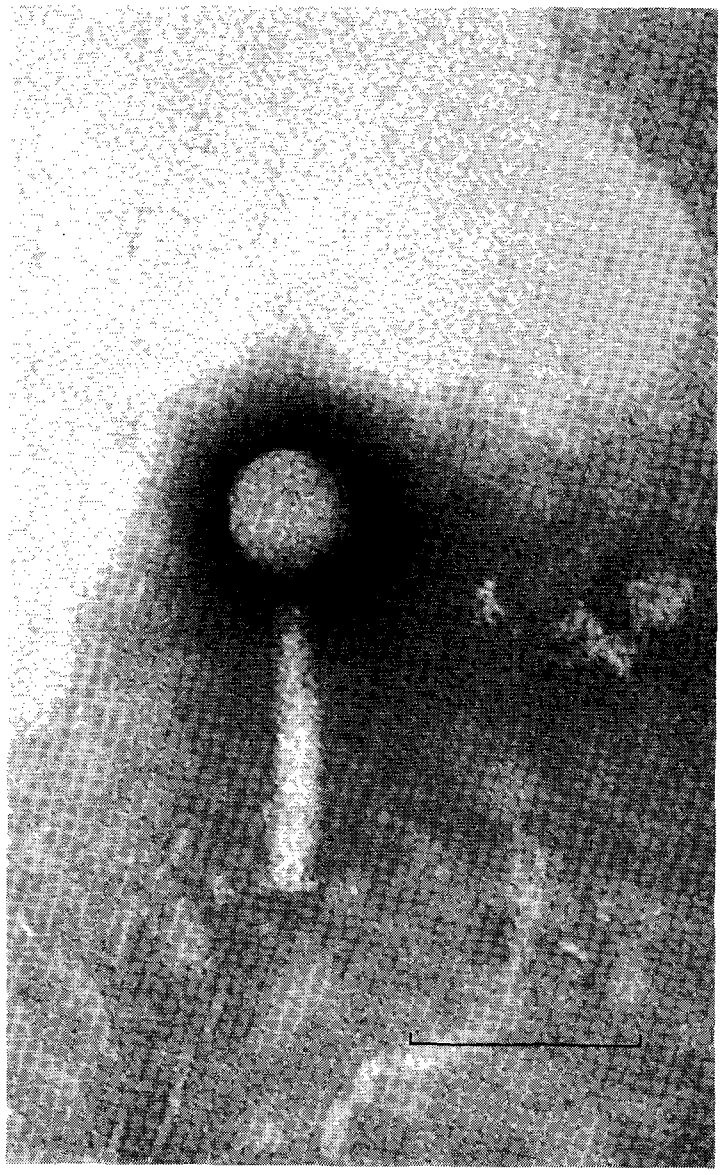
Phage tail-like particles of bacteriocin 223 and a phage 107/69-like structure. Magnification is x 300,000. The bar represents 1,000 Å.

PLATE VII



Bacteriocin 127 and a complete phage-like object. Magnification is x 300,000. The bar represents 1,000 Å.

PLATE VIII



Phage 107/69. Magnification is x 300,000. The bar represents 1,000 Å.

phage tail at the basal region i.e. the widest point, was found to be 220 Å. This value is likewise in agreement with that found for all 12 bacteriocins.

DISCUSSION

It was both interesting and disappointing to observe that the dimensions of the phage-tail-like structures, with respect to both length and width, were the same for all twelve preparations. This observation however, was not entirely unexpected, as Coetzee *et al.* (1968) were similarly unable to demonstrate any individuality of dimension amongst 30 *P. vulgaris* bacteriocins studied. On the basis of this result it would thus appear that the group specificity with regards to the adsorption of these phage-tail-like structures to the strain 69 cell surface wall is not expressed in any readily visible morphological difference between the two groups of bacteriocin. The conclusion can therefore be drawn that the essential functional difference between the two groups of bacteriocin is not reflected in any morphological difference but would appear to be infinitely more subtle in nature.

The dimensions of the *P. vulgaris* bacteriocins are very similar to those obtained for the pyocin phage-tail-like structures liberated by *Pseudomonas aeruginosa* strain TTC. These particles were found to be 1400 Å long and 180 Å wide (Bradley & Dewar, 1966). They are however, considerably larger than either the pyocin 28 (Takeya, Minamashima, Amako & Ohnishi, 1967) or colicin 15 (Mennigmann, 1965) structures.

The complete phage-like objects seen in preparations of bacteriocins 223, 226 and 36 were similar in appearance to phage 107/69 although the tail was slightly longer. No explanation can be given for the presence of these occasional particles. Although the possibility has not been excluded that they may be viable temperate bacteriophages harboured within the above-mentioned strains, no sensitive host has yet been found for them. The possibility was considered that these structures might represent the vestiges of the hypothetical defective phage from which the tail-like structures within these particular strains originated, but the difference of 40 Å in length makes this doubtful.

A comparison of the length dimensions of the phage-tail-like structures with that of phage 107/69 indicates that these bacteriocins are likewise morphologically distinct from the tail of this particular bacteriophage. However, it is interesting to note that the width

of the respective tails measured across the 'basal' region is identical. It is possible that this specific similarity may well reflect the observation that certain molecules involved in the adsorption of phage 107/69 and both groups of phage-tail-like structure appear to be common to both entities.

Although the bacteriocins and the tail of phage 107/69 are not identical, it should be emphasized that instances of morphological variance of bacteriophage have been reported. Walker & Anderson (1970) described morphological variants of phage P1 in which 3 different head sizes were observed for this phage. These variants were shown to be a function of the P1 genome. It is therefore conceivable that defective phage-tail-like bacteriocins, such as those studied here, could have been coded for by genes contained within the genome of a phage 107/69-like organism.

In conclusion, it would appear that although the *P. vulgaris* phage-tail-like bacteriocins are structurally distinct from the tail of phage 107/69, particularly with regard to their respective lengths, the morphological evidence nevertheless supports the proposal that these bacteriocins may well have originated from an unknown defective bacteriophage closely related phylogenetically to phage 107/69.

REFERENCES

BRADLEY, D.E. (1962). A study of the negative staining process. *Journal of General Microbiology 29,* 503.

BRADLEY, D.E. & DEWAR, C.A. (1966). The structure of phage-like objects associated with non-induced bacteriocinogenic bacteria. *Journal of General Microbiology 45,* 399.

BRADLEY, D.E. & KAY, D. (1960). The fine structure of bacteriophages. *Journal of General Microbiology 23,* 553.

BRENNER, S. & HORNE, R.W. (1959). A negative staining method for high resolution electron microscopy of viruses. *Biochimica et biophysica acta 34,* 103.

COETZEE, H.L., DE KLERK, H.C., COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like particles associated with intra-species killing of *Proteus vulgaris*. *Journal of General Virology 2,* 29.

COETZEE, J.N., DE KLERK, H.C. & SMIT, J.A. (1967). A transducing bacteriophage for *Proteus vulgaris*. *Journal of General Virology 1,* 561.

MARTON, L. (1941). The electron microscope. A new tool for bacteriological research. *Journal of Bacteriology 41,* 397.

MENNIGMANN, H.D. (1965). Electron microscopy of the antibacterial agent produced by *Escherichia coli* 15. *Journal of General Microbiology 41,* 151.

PROZESKY, O.W., DE KLERK, H.C. & COETZEE, J.N. (1965). The morphology of Proteus bacteriophages. *Journal of General Microbiology 41,* 29.

SCHAEFFER, V.J. & HARKER, D. (1942). Surface replicas for use in the electron microscope. *Journal of Applied Physiology 13,* 427.

WALKER, D.H. & ANDERSON, T.F. (1970). Morphological variants of coliphage P1. *Journal of Virology 5,* 765.

WILLIAMS, R.C. & WYCKOFF, R.W.G. (1945). Electron shadow-micrography of virus particles. *Proceedings of the Society for Experimental Biology and Medicine 58,* 265.

CHAPTER VI

SEROLOGY OF *P. VULGARIS* BACTERIOCINS AND PHAGE 107/69

SUMMARY

A study of the serological properties of 12 *P. vulgaris* phage-tail-like bacteriocins and the *P. vulgaris* transducing phage, 107/69, was undertaken. Antiserum was prepared against 2 of the bacteriocins, one of each group, and against the phage. Some measure of cross-neutralization of phage and bacteriocin could be demonstrated with the respective sera obtained. The 12 bacteriocins could be classified into 2 groups on the basis of their response to the 2 bacteriocin antisera. The grouping of the 12 bacteriocins on the basis of antigenic similarity is corroborative of a classification system of these entities based on adsorption patterns.

INTRODUCTION

A natural consequence of the relatively early discovery of the now 'classical' low-molecular weight colicins in contrast to the later interest afforded to the phage-tail-like bacteriocins, is the unfortunate imbalance existing at present with regard to the knowledge obtained regarding the serological properties of these two types of bacteriocin. In this particular respect, once again, it is the colicins about which most is known. This is in spite of the now widely held contention (Bradley, 1967; Taubeneck, 1963; Coetzee *et al.*, 1968) that a close relationship would appear to possibly exist between the phage-tail-like structures and as yet unidentified bacteriophages.

Colicins are antigenic in accordance with their protein character and when injected into rabbits, they give rise to antibodies that both neutralize the bactericidal effect and give a specific precipitate with colicin (Ivanovics, 1962). This antigenic property possessed of most colicins studied, has been utilized by numerous workers in attempts at both classification and group identity studies (Hamon & Peron, 1963; Senior & Emslie-Smith, 1969) and in investigations pertaining to the chemical nature of colicins and their origin (Goebel, Barry & Amano, 1957; Hutton & Goebel, 1962; Barry, Everhart & Graham, 1963; Barry, Everhart, Abbott & Graham, 1965; Hinsdill & Goebel, 1966).

A number of cases are known in which colicins and bacteriophages appear to utilize the same or closely linked receptor molecules on the sensitive bacterium's cell

wall (Reeves, 1965; Nomura, 1967; Weltzien & Jesaitis, 1971). The most intensively studied of these examples is that of colicin K and phage T6. Colicin K and phage T6 exhibit absolute cross-reaction with regard to their adsorption response to sensitive and resistant mutants of *E. coli* cells (Weltzien & Jesaitis, 1971). The killing abilities of these two agents are in addition destroyed at similar rates by X-rays (Latarjet & Fredericq, 1955), a result which would suggest that the colicin and the protein of the tip of the tail of the phage might be similar in size and chemical nature (Latarjet & Fredericq, 1955). However, Goebel, Barry, Jesaitis & Miller (1955) subsequently demonstrated that these respective proteins are antigenically unrelated, just as phage T6 is serologically distinct from other phages with the same specificity of action (Fredericq, 1957).

Although the low-molecular weight bacteriocins have commanded the greatest amount of interest, in particular with regards to seriological studies, a few instances of serological investigations carried out on the high-molecular weight particles have been reported. According to Hamon, Maresz & Perón (1968; see Maresz, Hamon & Peron, 1969), certain lethal so-called 'defective phages' produced by some bacteria are good antigens and that specific immune sera containd high titres of antibodies. Several pyocins and monocins have been shown to be antigenic and in general the antisera were specific (see Reeves, 1965). Hamon & Perón (1963) tested 3 different monocins, all of which were found to be antigenic. The antiserum of these 3 substances neutralized each of the 14 monocins tested, suggesting that all monocins are serologically identical, despite the fact that they can be divided into several types on the basis of other criteria (Reeves, 1965).

Notwithstanding the antigenicity discovered for both types of bacteriocin, the point of prime importance to be considered concerning studies on phages and bacteriocins on the comparative level, is the observation as expressed by Jacob & Wollmann (see Adams, 1959; 2nd printing, 1966), as late as 1966, that: "In particular no case has been as yet found of antigenic similarities between bacteriocins and bacteriophages". Unfortunately, it would appear that no subsequent contradictory result has to date been reported.

The interest in serology with regard to this study stems from the observation that certain of the *Proteus vulgaris* phage-tail-like bacteriocins and *P. vulgaris* phage 107/69, appear to adsorb to similar receptors on the same host organism. Although the bacteriocins

and the tail of this phage are not morphologically identical, they nevertheless exhibit a remarkable degree of similarity in this respect. These two observations, particularly when contemplated in conjunction with one another, were considered as supplying sufficient justification for a serological investigation of these two possibly related entities to be undertaken. In addition a serological comparison of bacteriocins of each of the 2 groups mentioned was considered necessary in order to investigate further the validity of this grouping scheme bestowed upon them.

MATERIALS AND METHODS

Bacterial strains. *Proteus vulgaris* strain 69 was used as the indicator of both bacteriocin and phage activity.

Bacteriocins. Fresh suspensions of the 12 bacteriocins were prepared in nutrient broth medium by ultraviolet light induction as described in Chapter III (Page 57). Two of the bacteriocins i.e. bacteriocins 41 and 127 were chosen at random as being representative of their respective groups. It was against these two bacteriocins that anti-bacteriocin serum was prepared.

Bacterial cultures were stored on nutrient agar slopes at 4^{o}. Bacteriocins were stored over 0,1 volume chloroform in sterile flasks at 4^{o}. Incubation temperature was 37^{o}.

Media.
- Nutrient broth
- Phage agar
- Phage agar top-layer
- Minimal medium (supplemented with 40 µg./ml. uracil, and 2% (w/v) casamino acids)

Reagents.
- Saline-Citrate buffer
- Ammonium sulphate
- Physiological saline

Details are given in the Appendix.

General phage techniques are those of Adams (1959).

Phage neutralization experiments were according to the method in Adams (1959).

Preparation of phage antigen. Lysates of phage 107/69 with titres of 1 x 10^7 p.f.u./ml. were made by a modification (Adams, 1959) of the agar layer method of Herschey, Kalmanson & Bronfenbrenner (1943). Phage agar plates were overlaid with 2,5 ml. phage agar top-layer seeded with 0,5 ml. of a 1:10 dilution of an overnight culture of *P. vulgaris* strain 69. 0,1 ml. of a sufficient dilution of phage 107/69 to give confluent lysis was incorporated into the agar top-layer. After overnight incubation at 37°, approximately 2,0 ml. of broth was added to each plate and the top-layer was scraped off with a sterile glass rod. Agar and bacterial debris was removed by centrifugation at 6000 g for 20 min. and the ensuing lysate sterilized with 0,1 vol. chloroform. Approximately 1000 ml. of this lysate was subjected to several cycles of differential ultracentrifugation. The final phage pellet was washed a number of times in Saline-Citrate buffer (pH 7,2) before being finally resuspended in 25,0 ml. of the same solution. The resultant buffered suspension contained phage with a titre of 2 x 10^8 p.f.u./ml. This suspension was bottled and stored at 4° before injection into rabbits.

Preparation of bacteriocin antigen. Antiserum was to be prepared against two of the twelve bacteriocins. Bacteriocin 41 was chosen randomly as a representative of the Group I bacteriocins. Bacteriocin 127 was chosen to represent the Group II bacteriocins Strains 41 and 127 were grown overnight in nutrient broth at 37°. Each overnight culture was diluted 1:5 into fresh warm broth and incubated for 1 hr. One hundred millilitres of each culture was divided into 10 samples and the cells centrifuged at 6000 g for 10 min. The resultant bacterial pellets were resuspended in 5 ml. physiological saline and pipetted into sterile Petri dishes. Each aliquot was then subjected to ultraviolet light irradiation for 4 min. with continuous agitation. Each sample of irradiated cells was transferred into 100 ml. quantities of minimal medium which had been supplemented with 40 µg./ml. of uracil (Coetzee, pers. comm.) and incubated in the dark to prevent photoreactivation (Kelner, 1949; Newcombe, 1955) for 16 hr. Minimal medium was used as a growth substrate to obviate unwanted broth proteins. The cultures were then centrifuged at 6000g for 20 min. to remove excess bacterial debris. By the above method 1,0 litre quantities of bacteriocins 41 and 127 in fluid suspension at titres of 1:512 were obtained.

The bacteriocins were further concentrated by a modification (Goebel, Barry, Jesaitis & Miller, 1955) of the ammonium sulphate precipitation method of Green & Hughes (1962) as described in Chapter III (Page 59). The precipitated material was centrifuged and washed a number of times in Saline-Citrate buffer (pH 7,2). The final pellets, resuspend-

ed in 25,0 ml. buffered solution, yielded inhibitory titres of 1:2048 for both bacteriocins. These suspensions were used as antigens for obtaining anti-bacteriocin serum.

Preparation of phage antiserum. One ml. of concentrated phage 107/69 at a titre of 2×10^8 p.f.u./ml. and an equal volume of Complete Freund's adjuvant were well mixed and injected subcutaneously into rabbits. Ten injections were administered over a period of 4 weeks and the rabbits bled by cardiac puncture after a further 10 days. The serum was decomplemented at 56^o for 30 min. and absorbed twice with viable strain 69 cells at 37^o for 2 hrs. The K-value of the serum was determined according to Adams (1959). Serum was diluted 1:10, 1:100 and 1:1000 in nutrient broth. To 0,9 ml. diluted serum 0,1 ml. of phage 107/69 at a titre of 1×10^7 p.f.u./ml. was added and the mixture incubated. At times zero and at 5 min. intervals, 0,1 ml. of the phage-serum mixture was removed and diluted into 9,9 ml. nutrient broth to minimise phage-antibody collisions. Incubation of phage-serum mixtures was continued for 30 min. Samples were then titrated on strain 69 for infectious centres. The K-value was determined according to the formula of Burnet, Keogh & Lush (1937; see Adams, 1959) where $K = 2,3 \times D/t \times \log P^o/P^t$. In this D = dilution of serum; t = time; P^o = number of infectious centres at zero time and P^t = number of infectious centres at time t.

Preparation of bacteriocin antiserum. One ml. samples of concentrated bacteriocins 41 and 127 at inhibition titres of 1:2048 were mixed with equal volumes of Complete Freund's adjuvant and injected subcutaneously into rabbits. Ten injections of each bacteriocin were administered over a period of 4 weeks. Serum was collected ten days after the last injection as described above for phage antiserum preparation. The respective sera were absorbed with strain 41 and 127 cells and with strain 69 organisms. Due to the inability of bacteriocin particles to form infectious centres, the bacteriocin-neutralizing potency of the two anti-bacteriocin sera was determined as the highest dilution of serum able to reduce the titre of homologous bacteriocin to zero. 0,5 ml. of undiluted serum, as well as equal volumes of serum dilutions of 1:10, 1:100, 1:1000, and 1:10000 were used. To these samples 0,5 ml. of homologous bacteriocin was added and the mixture incubated for 15 min. After incubation, two-fold dilutions of each sample was made and the dilutions assayed for bacteriocin activity by the spotting technique described in Chapter III (Page 58).

In addition to the homologous bacteriocin, all the bacteriocins of Group I and Group II were tested against the two anti-bacteriocin sera.

Neutralization of bacteriocin by phage antiserum. The following experiment was designed to determine the sensitivity of the *P. vulgaris* phage-tail-like structures to neutralization by phage 107/69 antiserum: Undiluted serum and serum dilutions in broth of 1:10, 1:100 and 1:1000 were made. To 0,5 ml. serum samples, 0,5 ml. of fluid suspensions of the bacteriocins to be tested was added. The mixture was incubated for 2 hr. after which two-fold dilutions were made and the dilutions then assayed for bacteriocin activity by the spotting technique previously described.

Neutralization of phage by bacteriocin antiserum. A comparison of the dilution, D, of phage antiserum and the two anti-bacteriocin sera required to inactivate 99% of the phage in 15 min. was to be made. Phage neutralization experiments using both anti-bacteriocin sera were carried out as described above for phage neutralization by homologous antiserum. K-values of the respective bacteriocin antisera were calculated according to Adams (1959) and the values substituted in the equation to calculate the dilutions of the two sera required to inactivate two logs of phage 107/69.

RESULTS

Phage 107/69 antiserum. A K-value of 200 min.$^{-1}$ was obtained for anti-107/69 serum. The dilution, D, required to inactivate 99% of phage 107/69 in 15 min. was calculated as 1:650.

Bacteriocin antiserum. The highest dilution of bacteriocin antiserum able to reduce the titre of homologous bacteriocin to zero was 10^{-2} for both bacteriocin 41 and bacteriocin 127 antisera. The highest dilution of bacteriocin 41 antiserum able to reduce to zero the titre of the remaining Group I bacteriocins was of the same magnitude as for neutralization of the homologous bacteriocin 41 (Group I). The highest dilution of the same antiserum able to reduce to zero the titre of the 4 Group II bacteriocins was 1 log lower i.e. 10^{-1}. A similar result was obtained for the Group II antiserum, anti-127, for inactivation of the remaining bacteriocins of the same Group, and for the 8 Group I bacteriocins (Table 1).

Neutralization of phage by bacteriocin antiserum. The velocity constant, K, of phage 107/69 inactivation by bacteriocin 41 antiserum was determined as 2 min.$^{-1}$. By

Table 1. Neutralization of bacteriocins by bacteriocins 41 and 127 antisera

Serum	Serum dilution	Titre of bacteriocin											
		Group I								Group II			
		* 41	49	52	75	115	35	36	46	127	223	226	121
–	–	1:256	1:256	1:256	1:256	1:128	1:128	1:64	1:64	1:256	1:128	1:128	1:128
Anti–41 (Group I)	0	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-1}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-2}	0	0	0	0	0	0	0	0	1:32	1:16	1:16	1:8
	10^{-3}	1:8	1:16	1:16	1:8	1:16	1:32	1:16	1:16	1:128	1:64	1:64	1:32
	10^{-4}	1:256	1:256	1:256	1:256	1:128	1:128	1:64	1:64	1:256	1:128	1:128	1:128
Anti-127 (Group II)	0	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-1}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-2}	1:32	1:32	1:32	1:64	1:32	1:16	1:16	1:16	0	0	0	0
	10^{-3}	1:128	1:128	1:128	1:256	1:128	1:64	1:64	1:64	1:32	1:32	1:32	1:16
	10^{-4}	1:256	1:256	1:256	1:256	1:128	1:128	1:64	1:64	1:256	1:128	1:128	1:128

* Numbers in this row designate bacteriocins.

Table 2. Neutralization of bacteriocins by phage 107/69 antiserum

Bacteriocin	Titre	Dilution (log_{10}) phage 107/69 antiserum				
		–	0	−1	−2	−3
41	1:256	1:256	0	1:64	1:256	1:256
49	1:256	1:256	0	1:64	1:256	1:256
52	1:256	1:256	0	1:64	1:256	1:256
75	1:256	1:256	0	1:64	1:256	1:256
115	1:128	1:128	0	1:128	1:128	1:128
35	1:128	1:128	0	1:32	1:128	1:128
36	1:64	1:64	0	1:32	1:64	1:64
46	1:64	1:64	0	1:32	1:64	1:64
127	1:256	1:256	0	1:128	1:256	1:256
223	1:128	1:128	0	1:32	1:128	1:128
226	1:128	1:128	0	1:32	1:128	1:128
121	1:128	1:128	0	1:32	1:128	1:128

substituting the K-value in the equation of Burnet, Keog & Lush (1937) the dilution of anti-41 serum giving 99% of phage 107/69 neutralization in 15 min. was calculated as *ca* 1:6,2. A similar K-value of 2,5 $min.^{-1}$ was found for phage 107/69 inactivation by bacteriocin 127 antiserum. From this a dilution of *ca* 1:8 was calculated for 99% neutralization of this phage in the same time.

Neutralization of bacteriocins by phage 107/69 antiserum. The only concentration of phage antiserum capable of reducing to zero the titres of the 12 bacteriocins was that of undiluted serum. A 1:10 dilution of phage antiserum reduced the titres of each bacteriocin by only 2 two-fold dilutions (Table 2).

DISCUSSION

It has already been shown that the twelve bacteriocins concerned with this study, although morphologically indistinguishable, appear to be heterologous with respect to their adsorption response to the indicator organism strain 69. Mutants of strain 69 were obtained which were sensitive to certain of the bacteriocins whilst being simultaneously

resistant to the remaining bacteriocins. It was on the basis of this phenomenon that the 12 bacteriocins under investigation were divided into 2 groups of 8 and 4 respectively. Furthermore, any mutant resistant to bacteriocin was also found to be resistant to the transducing phage 107/69, indicating some common adsorption factors for the 2 types of particle.

It was gratifying to find that, as with the other phage-tail-like bacteriocins studied, such as the monocins (Hamon & Perón, 1963) and pyocins (Kageyama, Ikeda & Egami, 1964), the bacteriocins studied here appear to be good antigens, a log 2 dilution of antiserum neutralizing to zero the titre of homologous bacteriocin. In addition, it was interesting to observe that those bacteriocins which constitute a group as described above, appear to be classifiable into the same group on the basis of their response to antiserum prepared against a member of that group. Thus it was that antiserum prepared against bacteriocin 41 (a Group I bacteriocin) was able to neutralize the 7 remaining bacteriocins of that group to an extent comparable to that of its inactivation of the homologous bacteriocin. This same antiserum was however approximately 10 x less efficient in neutralizing the 4 Group II bacteriocins. A similar result was obtained when antiserum was prepared against bacteriocin 127, a Group II bacteriocin (see Table 1). From these results it would therefore appear as if the bacteriocins of Group I are serologically similar to one another and related to, but not identical to, the 4 Group II bacteriocins which likewise form a serological entity of their own. This serological grouping of the 12 bacteriocins into 2 groups of 8 and 4 respectively, thus corroborates the classification based formerly only on activity or lack of activity towards mutants of the indicator strain.

An additional point of great interest is the observation that a measure of serological relatedness appears to exist between phage 107/69 and the phage-tail-like bacteriocins. Bacteriocin 41-antiserum was found to have a K-value of 2 min.$^{-1}$ for neutralization of phage 107/69, whilst that of bacteriocin 127-antiserum was slightly higher at 2,5 min. $^{-1}$. Conversely, undiluted phage antiserum was able to neutralize to zero the titres of all 12 bacteriocins. These results indicate the existence of some weak antigenic relatedness between the bacteriocins and the phage and might explain the observation that resistance of strain 69 to bacteriocin is reflected by simultaneous resistance to phage 107/69 and vice versa. It is possible that the attainment of the property of resistance on the part of the indicator organism is an expression

of a change in nature or conformation of certain specific receptor molecules on the strain 69 cell surface, and that one or more of these particular molecular groupings are normally involved in the fixation of both phage 107/69 and the phage-tail-like structures. This implies that at least one or more molecules complementary to those on the cell surface would be common to both the tail of phage 107/69 and the bacteriocins. The observation that phage antiserum is able to inactivate these bacteriocins and that the reciprocal experiment of bacteriocin antiserum neutralizing phage also holds true, thus appears to bear out the validity of the assumption that these two types of particle possess certain antigens in common.

In conclusion, it is apparent from the results of this serological study that the 12 bacteriocins concerned with this study constitute 2 serologically separable units, and that this grouping is corroborative of their group-behavioural pattern with regard to activity on the indicator strain. In addition, the observation that some measure of serological analogy exists between the phage-tail-like structures and phage 107/69 apparently lends substance to the hypothesis that these high molecular weight bacteriocins could conceivably represent the products of defective lysogeny.

REFERENCES

ADAMS, M.H. (1959). *Bacteriophages.* New York: Interscience Publishers Inc.

ADAMS, M.H. (1966). *Bacteriophages.* New York: Interscience Publishers Inc. (2nd. Printing).

BARRY, G.T., EVERHART, D.L., ABBOTT, V. & GRAHAM, M.G. (1965). Preparations, properties and relationship of substances possessing colicine A activity obtained from *Enterobacteriaceae. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene: Erste Abteilung: Originale 196,* 248.

BARRY, G.T., EVERHART, D.L. & GRAHAM, M. (1963). Colicin A. *Nature London 198,* 211.

BRADLEY, D.E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriological Reviews 31,* 230.

COETZEE, H.L., DE KLERK, H.C. COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like particles associated with intra-species killing of *Proteus vulgaris. Journal of General Virology 2,* 29.

FREDERICQ, P. (1957). Colicins. *Annual Review of Microbiology 11,* 7.

GOEBEL, W.F., BARRY, G.T. & AMANO, T. (1957). Colicine K. *Science 126,* 1231.

GOEBEL, W.F., BARRY, G.T., JESAITIS, M.A. & MILLER, E.M. (1955). Colicin K. *Nature, London 176,* 700.

GREEN, A.A. & HUGHES, W.L. (1962). Protein fractionation on the basis of solubility in aqueous solutions of salts and organic solvents. *Methods in Enzymology 3,* 67.

HAMON, Y. & PÉRON, Y. (1963). Etude du pouvoir bactériocinogènie dans le genre *Listeria.* II. Individualité et classification des bactériocines en cause. *Annales de l'Institut Pasteur, Paris 104,* 55.

HERSCHEY, A.D., KALMANSON, G. & BRONFENBRENNER, J. (1943). Quantitative methods in the study of phage-antiphage reaction. *Journal of Immunology 46,* 267.

HINSDILL, R.D. & GOEBEL, W.F. (1966). Colicine K. VII. The transfer of Type K colicinogeny to *Shigella sonnei. Journal of Experimental Medicine 123,* 881.

HUTTON, J.J. & GOEBEL, W.F. (1962). The isolation of colicine V and a study of its immunological properties. *Journal of General Physiology 45,* 125.

IVANOVICS, G. (1962). Bacteriocins and bacteriocin-like substances. *Bacteriological Reviews 26,* 108.

KAGEYAMA, M., IKEDA, K. & EGAMI, F. (1964). Studies of pyocin. III. Biological properties of the pyocin. *Journal of Biochemistry 55,* 59.

KELNER, A. (1949). Photoreactivation of ultraviolet-irradiated *Escherichia coli* with special reference to the dose-reduction principle and to ultraviolet-induced mutations. *Journal of Bacteriology 58,* 511.

LATARJET, R. & FREDERICQ, P. (1955). An X-ray study of a colicine and of its relationship to bacteriophage T6. *Virology 1,* 100.

MARESZ, J., HAMON, Y. & PERÒN, Y. (1969). Biological properties of lethal factors produced by *Enterobacter aerogenes. Archivum Immunologiae et Therapiae Experimentalis 17,* 718.

NEWCOMBE, H.B. (1955). The timing of induced mutations in *Streptomyces. Brookhaven Symposia in Biology 8,* 88.

NOMURA, M. (1967). Colicins and related bacteriocins. *Annual Review of Microbiology 21,* 257.

REEVES, P. (1965). The bacteriocins. *Bacteriological Reviews 29,* 24.

SENIOR, B.W. & EMSLIE-SMITH, A. (2969). Serological studies on Group—B colicines and organisms producing them. *Journal of Medical Microbiology 2,* 507.

TAUBENECK, U. (1963). Uber die Produktion biologisch aktiver Phagen-Schänze durch einen defekt lysogenen *Proteus mirabilis*-stamm. *Zeitschrift fur Naturforschung: Ten B 18b,* 989.

WELTZIEN, H.U. & JESAITIS, M.A. (1971). The nature of the colicin K receptor of *Escherichia coli Cullen. Journal of Experimental Medicine 133,* 534.

CHAPTER VII

GENERAL DISCUSSION

This department has for many years devoted particular interest and attention to the study of the Proteus group of bacteria and their attendant bacteriophages and plasmids.

By 1963, the Proteus-Providence group of enteric bacteria had become conspicuous in that they were the only organisms of this taxonomic entity from which bacteriocins had not been reported. Two years later, Cradock-Watson (1965) described the production of bactericidal substances which fulful the operational definition applicable to bacteriocins, by 139 strains of *Proteus mirabilis* and 1 strain of *P. vulgaris* which were inhibitory for 18 strains of *P. mirabilis.* He was unable to demonstrate the production of bacteriocins by any of the *P. rettgeri, P. morganii* or Providence strains tested.

Coetzee in 1967 was successful in demonstrating that strains of both Providence and *Proteus morganii* do in fact possess the inherent potential for the production of bacteriocins. To date no bacteriocins have been described from strains of *P. rettgeri.*

The demonstration by Cradock-Watson (1965) of only a single bacteriocinogenic strain from *P. vulgaris,* prompted workers in this laboratory to undertake a more comprehensive investigation into the incidence of bacteriocinogeny amongst strains of this particular species. Thus it was that Coetzee, de Klerk, Coetzee & Smit (1968) reported the production of high-molecular weight phage-tail-like structures by 57% of *P. vulgaris* strains tested, which were found to be bactericidal for many members of the same species. An interesting observation which arose from this study was the striking morphological resemblance of these bacteriocins to the tail of a *P. vulgaris* generalized transducing phage, Ø107/69, isolated by Coetzee, de Klerk & Smit a year earlier. As has previously been contended, Coetzee *et al.* (1968) ventured the opinion that the high-molecular phage-tail-like bacteriocins may possibly represent the products of defective lysogeny. The proposal has been offered that this phenomenon of defective lysogeny (Campbell, 1961; Neubauer, 1967) could possibly arise through the selection only of genes which are beneficial to the host bacterium. These genes may have been acquired by transduction (Coetzee, Smit & Prozesky, 1966) or by conversion (Coetzee, 1961); both systems conceivably capable of conferring a selective advantage on the host organism. Genes thus acquired could lead to the gradual accumulation of defective prophages into

originally lysogenic Proteus.

The discovery and possession of a *P. vulgaris* transduction system (Coetzee *et al.*, 1967) as mentioned, and the high incidence of bacteriocinogeny observed amongst strains of this species, coupled with the morphological similarity displayed between these "vulgaricins" and the tail of the transducing phage, where collectively instrumental in providing a motivation for undertaking the present study. An attempt was to be made at the transduction of the genetic determinant governing the property of bacteriocin-resistance from bacteriocin-resistant cells of the phage's host (*P. vulgaris* strain 69) to sensitive cells of the parent organism. To this end bacteriocins were sought from amongst many strains of *P. vulgaris,* which would be active on strain 69 and against which bacteriocin-resistant mutants (of strain 69) could be isolated and utilized as genetic donors of the resistance markers to the bacteriocin-sensitive cells of the parent. Consequently, 12 *P. vulgaris* phage-tail-like bacteriocins active on strain 69 were isolated, and 50 mutants of this strain were selected for resistance towards each of the bacteriocins obtained. Unfortunately, all 600 of these mutants of strain 69 were subsequently found to have acquired simultaneous resistance to the transducing phage 107/69, thereby ruling out the possibility of transduction by means of this particular bacteriophage. The reciprocal experiment in which mutants of strain 69 were specifically selected for their resistance to the phage also displayed a similar cross-resistance phenomenon towards all the bacteriocins. These results implied that the phage and the bacteriocins possibly utilize the same bacterial cell wall receptor site. However, a contra-indication to this relatively straight-forward hypothesis was the observation that the bacteriocins themselves do not appear to constitute a homogenous entity with regard to their common adsorption to the same receptor site. Although most of the bacteriocin-resistant mutants displayed resistance to all 12 bacteriocins and the phage, many of these mutants exhibited a distinct pattern of resistance towards only certain of the bacteriocins whilst retaining sensitivity to the remainder. By means of these group-resistance patterns it was possible to classify the 12 bacteriocins into 2 groups of 8 and 4, designated as the Group I and Group II bacteriocins respectively. Certain of the mutants displayed resistance to the Group I bacteriocins and the phage whilst remaining sensitive to the Group II structures. Conversely, others exhibited resistance towards the Group II bacteriocins and the phage whilst retaining sensitivity to the Group I bacteriocins. From this it became apparent that the bacteriocins themselves do not utilize a single common receptor which they share with the phage, but that at least 2 bacteriocin receptors probably exist; one for each bacteriocin group.

A number of cases have been reported in which certain bacteriocins and bacteriophages would appear to adsorb onto the same or similar bacterial cell wall receptor sites. Examples of these are the cases of cross-resistance exhibited towards colicins M, K and E and phages T1, T6, and BF23 respectively (see Reeves, 1965). Unfortunately, the precise chemical nature and molecular configuration of many of these receptor sites is largely unknown, particularly with regard to those concerned with the adsorption of bacteriocins (Weltzien & Jesaitis, 1971). The importance of the structural configuration of chemical groupings in the functional binding of biological macromolecules such as bacteriocins and phages to bacterial cell walls has often been emphasized. Hill & Holland (1967) have suggested that the successful fixation of bacteriocins involves a dual role for the cells surface receptor; the first for the successful binding of the protein and the second for the correct orientation of the bound molecule relative to the cytoplasmic membrane. Burman & Nordström (1971) proposed that the successful reception or repulsion of various molecules by the cell wall is dependant on the actual steric conformation of specific cell wall protomers. In the course of an investigation into the marked cross-resistance displayed by cells of *E. coli* towards colicin K and phage T6, Weltzien & Jesaitis (1971) proposed that the genes coding for colicin K and T6 receptors are closely linked. These workers undertook a chemical and enzymatic analysis of the cell wall receptors from *E. coli* for colicin K and phage T6, and concluded that these receptors are not in fact identical, but that the specific chemical groupings which react with the bacteriocin and the phage are closely linked and might form part of the same basic receptor macromolecule (Weltzien & Jesaitis, 1971).

From the results obtained in this study it seems possible that a similar situation is operative concerning the 2 groups of bacteriocin and phage 107/69. Due to the fact that mutants of *P. vulgaris* strain 69 were obtained which always displayed resistance to the phage regardless of to which group of bacteriocins they were simultaneously resistant, it would appear that the specific receptor sites involved in the adsorption of either group of bacteriocins are closely linked or possibly overlapping, and that those molecules necessary for the adsorption of the phage are integrals of both bacteriocin receptors.

The classification scheme bestowed upon the bacteriocins on the basis of their group-activity patterns on mutants of strain 69, was substantiated by the observation of the same patterns of activity on numerous other strains of *P. vulgaris, P. mirabilis* and *P. morganii* as well as on some *Serratia marcescens* strains. This classification scheme was also found to be reflected in the response of the bacteriocins to antiserum prepared

against members of either group. The two groups of 8 and 4 bacteriocins respectively were shown to constitute 2 serologically separable entities, even though the 12 bacteriocins were shown to be morphologically indistinguishable from one another. In addition there would appear to be some measure of serological relatedness between the phage-tail-like structures and phage 107/69. When considered in conjunction with their similar adsorption responses, it is felt that this finding enhances the feasibility of the idea of a close evolutionary relationship possibly existing between these 2 entities, and supports the proposal that phage-tail-like bacteriocins may well represent the products of defective lysogeny.

An additional point of interest was the observation that all 12 *P. vulgaris* bacteriocins appear to be capable of inducing the development of the prophage 107/69 from cells of strain 107 lysogenic for this phage. This observation may provide a clue to the mode of action of these bacteriocins. Endo, Kamiya & Ishizawa (1963) demonstrated the induction of phage λ from lysogenic *E. coli* by colicin E2, and a similar observation was made by Holland (1963; 1965) using megacin C. These bacteriocins specifically induce the degradation of the host DNA with concomitant cessation of both RNA and protein synthesis. A similar mode of action was shown for the high molecular weight phage-tail-like vibriocins of *Vibrio comma* by Jayawardene & Farkas-Himsley (1970). If the phage-induction ability of the *P. vulgaris* bacteriocins is a reflection of the sequence of events as initiated by other phage-inducing bacteriocins, then it is conceivable that the "vulgaricins" possess a similar mode of action to that of the aforementioned bacteriocins. This consideration merits further investigation.

From the results obtained in this study, a number of other considerations have arisen which may justify further investigation. It is considered worthwhile to undertake a study into the probable biochemical differences existing between the various types of bacteriocin-resistant mutants obtained. An investigation into the qualitative and/or quantitative differences apparently existing between the cell walls of the various types of mutant and the parent organism, may contribute further to our knowledge on the role of the membrane in biological systems.

By means of the acquisition of another *P. vulgaris* transducing phage which may utilize a different receptor to that of Ø107/69 and the bacteriocins, it may still be possible to transduce the property of bacteriocin-resistance to bacteriocin-sensitive cells and to determine whether the transductants simultaneously acquire resistance to phage 107/69.

Cradock-Watson (1965) was able to group strains of *P. mirabilis* into 3 types on the basis of production of bacteriocin and susceptibility of organisms to bacteriocin. A similar typing scheme for *P. vulgaris* utilizing the bacteriocins and Ø107/69 is envisaged.

REFERENCES

BURMAN, L.G. & NORDSTRÖM, K. (1971). Colicin tolerance induced by Ampicillin or mutation to Ampicillin resistance in a strain of *Escherichia coli* K12. *Journal of Bacteriology 106,* 1.

CAMPBELL, A. (1961). Conditions for the existence of bacteriophage. *Evolution 15,* 153.

COETZEE, J.N. (1961). Lysogenic conversion in the genus *Proteus. Nature, London, 189,* 946.

COETZEE, J.N. (1967). Bacteriocinogeny in strains of Providence and *Proteus morganii. Nature, London 213,* 614.

COETZEE, H.L., DE KLERK, H.C., COETZEE, J.N. & SMIT, J.A. (1968). Bacteriophage-tail-like particles assoicated with intra-species killing of *Proteus vulgaris. Journal of General Virology 2,* 29.

COETZEE, J.N., DE KLERK, H.C. & SMIT, J.A. (1967). A transducing bacteriophage for *Proteus vulgaris. Journal of General Virology 1,* 561.

COETZEE, J.N., SMIT, J.A. & PROZESKY, O.W. (1966). Properties of Providence and *Proteus morganii* transducing phages. *Journal of General Microbiology 44,* 167.

CRADOCK-WATSON, J.E. (1965). The production of bacteriocines by *Proteus* species. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene; Erste Abteilung: Originale 196,* 385.

ENDO, H., KAMIYA, T. & ISHIZAWA, M. (1963). λ Phage induction by colicine E2. *Biochemical & Biophysical Research Communications 11,* 477.

HILL, C. & HOLLAND, I.B. (1967). Genetic basis of colicin E susceptibility in *Escherichia coli. Journal of Bacteriology 94,* 677.

HOLLAND, I.B. (1963). Effect of a bacteriocin preparation (megacin C) on DNA synthesis in *Bacillus megaterium. Biochemical & Biophysical Research Communications 13,* 246.

HOLLAND, I.B. (1965). A bacteriocin specifically affecting DNA synthesis in *Bacillus megaterium. Journal of Molecular Biology 12,* 429.

JAYAWARDENE, A. & FARKAS-HIMSLEY, H. (1970). Mode of action of vibriocin. *Journal of Bacteriology 102,* 382.

NEUBAUER, Z. (1967). Brief consideration of the meaning of lysogenic conversion in *Salmonella anatum* phage system. *Nature, London 213,* 1263.

REEVES, P. (1965). The bacteriocins. *Bacteriological Reviews 29,* 24.

WELTZIEN, H.U. & JESAITIS, M.A. (1971). The nature of the colicin K receptor of *Escherichia coli Cullen. Journal of Experimental Medicine 133,* 534.

CHAPTER VIII

APPENDIX

MEDIA AND CHEMICALS

Media:

1. **Nutrient Broth:** This is a modification of the broth of Coetzee & Sacks (1960). Difco nutrient broth, 16 g.; NaCl, 10 g.; Oxoid Lab-Lemco broth, 16 g.; Difco tryptose broth, 52 g. Dissolve in 2 litres distilled water; steam for 45 min.; add 2 ml. N $CaCl_2$ solution; adjust to pH 7,4 with 4% NaOH; bottle and autoclave.

2. **Nutrient agar:** 2 lb of minced lean meat is soaked overnight in 2 litres tapwater at 4^{o}; filter through cheesecloth; steam for 1 hr.; filter through filter paper; steam for 1 hr.; leave overnight. To 2000 ml. of this meat extract add Ocean Gold agar, 30 g.; steam until dissolved; add Difco peptone, 20 g. and NaCl, 10 g. Adjust to pH 7,4 with 4% NaOH; add 14 ml. 3,5% Na_2CO_3 solution; steam for 30 min.; filter through cheesecloth and autoclave. Nutrient agar slants are prepared in 20 ml. screw-top bottles.

3. **Phage agar:** Dissolve the following in 2 litres distilled water:— Bacto agar, 22 g.; Bacto tryptose broth 26 g.; NaCl, 16 g.; glucose, 3 g. Autoclave.

4. **Phage agar top-layer:** Dissolve in 2 litres distilled water: Bacto agar, 12 g.; Bacto tryptose broth, 20 g.; NaCl, 16 g.; glucose, 6 g. Steam for 1 hr. to dissolve; bottle in 100 ml. quantities and autoclave. Steam for 90 min. before use.

5. **Difco MacConkey agar.**

6. **Difco S.S. agar.**

7. **Minimal medium:** This is a modification (Grabow & Smit, 1967) of the medium of Davis & Mingioli (1950).

(a) Salt solution: In 1000 ml. distilled water dissolve K_2HPO_4, 105 g.; KH_2PO_4, 45 g.; Na-Citrate. $2H_2O$, 4,7 g. and $(NH_4)_2SO_4$, 10 g.; add $MgSO_4.7H_2O$, 1,02 g.; autoclave and bottle in 80 ml. quantities.

(b) Carbohydrate: Glucose or Lactose (20% w/v) is bottled in 20 ml. quantities and sterilised in boiling water for 30 min.

(c) Supplements:

(i) nicotinic acid: Dissolve 0,4 g. in 200 ml. distilled water. Bottle in 2,5 ml. quantities. Sterilise by placing in boiling water for $1^1/2$ hr. One 2,5 ml. quantity is added to the minimal medium to give a final concentration of 0,625% (w/v).

(ii) casamino acids: 16 g. of Difco vitamin-free casamino acids is added to the minimal medium to give a final concentration of 2% (w/v).

(iii) uracil: Dissolve 3,2 g. uracil in 100 ml. sterile distilled water. Add 1,0 ml. of this solution to the minimal medium to give a final concentration of 40 µg./ml.

The minimal medium is made up by adding to 720 ml. sterile distilled water, 80 ml. of salt solution, 20 ml. carbohydrate solution and the appropriate supplements.

8. **Physiological saline:** NaC1 (0,85% w/v) in distilled water is bottled in convenient quantities and autoclaved.

9. **Saline Citrate Buffer:** Na Citrate 0,015 M
Na C1 0,015 M

Adjust to pH 7,2 with NaOH and sterilise by Seitz filtration.

10. **Inducing agents:** Mitomycin C was obtained from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. The drug is always freshly prepared and used at a concentration of 2 µg./ml.

REFERENCES

COETZEE, J.N. & SACKS, T.G. (1960). Lysogeny in the genus *Proteus*. *Nature, London 184,* 1340.

DAVIS, B.D. & MINGIOLI, E.S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B12. *Journal of Bacteriology 60,* 17.

GRABOW, W.O.K. & SMIT, J.A. (1967). Methionine synthesis in *Proteus mirabilis*. *Journal of General Microbiology 46,* 47.