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The Bacteriocins

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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 et al. (73). Bacteriocins produced by various species of bacteria, in contrast to all other antibiotics, act only on strains of the same or closely related species; also, they are protein in nature.

The study of bacteriocins really dates back to 1925 when Gratia observed inhibition of *Escherichia coli* ϕ by *E. coli* V (39). Although inhibition of one bacterial strain by another had been observed many times, it was the thorough way in which this observation was followed up by Gratia and then Fredericq which led to our current knowledge of the subject. The development of the study of colicins has been reviewed several times by Fredericq (17, 27, 30), and bacteriocins in general were reviewed recently by Ivánovics (64) and Fredericq (31). Readers are referred to these reviews for references to the detailed literature, and to Hayes' recent book (55) for details and discussion of points pertaining to bacterial and bacteriophage genetics. Fredericq (27, 31) also reported the methods used to demonstrate and study bacteriocins. The present review leans heavily on these excellent prior reviews, and will attempt only to discuss some of the more recent findings and to examine critically some of the generalizations made about bacteriocins.

SPECIFIC ADSORPTION AND CLASSIFICATION OF COLICINS

Colicins were the first bacteriocins to be studied in detail. The classification of colicins put forward by Fredericq has been used as a model in classifying other bacteriocins, and has contributed significantly to our understanding of bacteriocins. The classification of colicins is treated in all Fredericq's reviews; that of 1948 (17) presents the most detail, although that of 1963 obviously presents a later view. Fredericq 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 (14). He was able, by the use of these mutants, to group the colicins into 17 types named colicins A, B, C, D, E, F, G, H, I, J, K, V, S_1, S_2, S_3, S_4, and S_5, each characterized by the observation that, in general, a mutant resistant to one was resistant to all of that group, but not necessarily to colicins of other types (17). Other colicins not fitting into this scheme have since been discovered (21, 88), and Hamon and Péron refer to 23 types (50).

Colicins are thought to kill after first adsorbing onto a specific receptor, with mutation to colicin resistance involving loss of the receptor. When Fredericq introduced the word "receptor," he was careful to add that he was not prejudging whether this was a fixation of colicin comparable to that of antibody on an antigen or some other specific mechanism of action (17). However, time has confirmed the literal interpretation, and colicins are now thought to be adsorbed onto specific receptors on the sensitive cell surface; the direct evidence for this is discussed later. Thus, the classification of colicins by Fredericq attempts to group them into types according to the receptor they adsorb onto.

The detailed classification by Fredericq (17) was complicated by the frequent occurrence of strains producing more than one colicin and of mutants resistant to more than one colicin type. These difficulties were largely overcome by the judicious use of particular indicator strains and other special techniques. However, certain problems of colicin classification remain. Thus, the six colicin types E, F, J, S_2, S_3, and S_5 have been amalgamated in a new type E (18). These alternative meanings of the category "type E" can cause confusion. The term $\overline{E}$ is used in this review where the wider interpretation was clearly intended. It has been suggested that all colicins of type $\overline{E}$ adsorb onto the same specific receptor, although it is difficult to reconcile this hypothesis with the resistant mutants which retain sensitivity to some colicins of type $\overline{E}$ but not others. The difficulty can be appreciated if one examines Table 9 of Fredericq's review (17), which summarizes the cross-resistance of 250 mutants.

The difficulty of classifying the colicins of type $\overline{E}$ has been specifically mentioned, because in the original publications the data are compiled to enable one to see the distinction between colicins E, F, J, S_2, S_3, and S_5. Unfortunately, the full details, which run into thousands of interactions, have never been published, and the analysis of the data does not indicate the frequency of other examples of colicins of the same type not demonstrating full cross-resistance. It is, in any case, necessary to remember that, in view of the difficulties of classifying certain colicins and the necessity to hypothesize the frequent occurrence of mutants which simultaneously lose receptors to two or more colicin types, even the best established colicin type may include colicins adsorbing onto two different receptors which are almost invariably lost simultaneously.

Colicins and bacteriophages both appear to adsorb onto similar receptors, and there are a few instances of cross-resistance between colicins and bacteriophages, suggesting a common receptor. One example of cross-resistance is that between colicin $\overline{E}$ and bacteriophages of group II, in particular phage BF23 (15, 22, 33). Forty-one mutants of 7 strains selected for resistance to one of six bacteriophages of group II were all resistant to the colicin of type E tested, but not to all the other colicins now included in type $\overline{E}$. In the reverse case of 175 mutants selected as resistant to 1 of the 42 colicinogenic strains used (of types E, F, J, S_2, S_3, or S_5), only 149 were resistant to bacteriophage II (15). Thus, although there is a very strong correlation between resistance to colicins of type $\overline{E}$ and bacteriophages of group II, this correlation is not absolute.

In the case of cross-resistance between colicins of type K and bacteriophages of group III, also described by Fredericq (16), there is once again a strong but not absolute correlation; all 16 phage-resistant mutants resist colicin K, and 39 of 53 colicin-resistant mutants also resist bacteriophages of group III. In both examples, the majority, but not all, of the mutants not showing cross-resistance, were derived from *Shigella sonnei* E.90.

Fredericq and Gratia (34), searching for further cross-reactions, found that bacteriophage T6 was a member of their group III, and that mutants of either *S. sonnei* E.90 or *E. coli* B (selected for resistance to several colicin K-producing strains, bacteriophage T6, or other phages of group III) all resist both T6 and colicin K. This absolute correlation between T6 and colicin K found by Fredericq and Gratia (34), is difficult to reconcile with the data given earlier by Fredericq (16), although direct comparison is difficult as precise details of the colicinogenic strains and phage are not given in either case.

Other cases of cross-resistance between colicins C and M and bacteriophages T1, T5, and T7 (19, 20, 21), and between colicins I, V, and B and bacteriophages T1 and T5 (35), again do not display absolute cross-resistance.

Thus, there are exceptions to both the examples of cross-resistance on which the classification of colicins is based, and to the examples of cross-resistance between bacteriophages and colicins. These exceptions cannot be explained on the hypothesis that the same receptor is involved and that this is lost in resistant mutants. Unfortunately, there appear to have been no studies of these particular mutants to determine whether their resistance is, in fact, due to lack of a receptor. If this point were established, it would be difficult to retain the hypothesis that two colicins, or a colicin and a bacteriophage, adsorbed onto the same receptor, in the face of a single exceptional bacterial mutant capable of adsorbing only one of them.

It is likely that at least some of the receptors involve specific carbohydrate patterns, and mutants selected for the loss of one receptor might well lose others as a result of a single change in their carbohydrate metabolism. Indeed, one of the cases of cross-resistance between bacteriophages has already been shown to have such a cause (93), and similar mechanisms probably underlie the cross-resistances observed with colicins. The difficulty of distinguishing the colicins, now all classified as colicin $\overline{E}$, may perhaps be accounted for by a greater frequency of mutants involving the loss of several receptors than of mutants involving only one.

When a particular colicin is being studied or referred to, the producing strain should be specified, because it is only in this way that a colicin can be adequately defined. Perhaps the best way would be to give each colicin a name and to follow this by the type, if this is known. In this review, the more general terms such as colicin E will only be used as generalizations. Although it would be impossible to overrate the value of Fredericq's classification in facilitating communication on the subject of colicins, it should not be overlooked that, despite its value, there are exceptions to the implied generalizations. It is not, in fact, possible to be certain that all colicins in one group adsorb onto the one receptor, and it is known that they may differ in other respects such as their activity spectra (22).

NOMENCLATURE

The literature on colicins and C-factors is now reaching sufficient proportions for nomenclature to be of great importance. In this review, individual colicins are referred to, where possible, by the name of the producer strain followed by the type designation. Thus, colicin K235-K is colicin of type K produced by *E. coli* K235 or by any other strain which originally derived its colicinogeny from *E. coli* K235. Colicinogenic factors (C-factors) have the same symbol but are enclosed in parentheses, and the strain designation indicates the strain from which this C-factor was first derived. Thus, *E. coli* K-12 (K235-K) refers to a strain of *E. coli* K-12 made colicinogenic for colicin K235-K by a C-factor originally derived from *E. coli* K235. This nomenclature, although cumbersome, does not make the probably invalid assumptions that colicin K or its

C-factor are the same regardless of their origin. The same principles of nomenclature are applied to the other bacteriocins.

VARIOUS FAMILIES OF BACTERIOCINS

Colicins

The distribution of colicinogeny was fully reviewed by Fredericq (17, 31). Producing strains have been found in *E. coli* (20 to 25% of strains), *E. freundii*, *Paracolobactrum*, *Shigella*, and less frequently in *Salmonella*, *Aerobacter*, and possibly *Serratia* (see section on Marcescins). As discussed above, colicins have been divided into 17 types based on their spectrum of activity and the specificity of resistant mutants.

Fredericq also systematically studied other properties of the 17 types of colicin and found correlations between the classification based on specificity of resistant mutants and such properties as sensitivity to heat and proteolytic enzymes and the dialyzability of colicins. Readers are referred to Fredericq's reviews (27, 31) for more details.

Fredericq's early studies, summarized in 1948 (17), also showed that within each species group there is a tendency to produce only certain colicin types. Thus, *E. freundii* produced type A only; *E. coli* produced only types B, C, D, E, F, G, H, I, or V; *Paracolobactrum* produced J or K; *Shigella* produced S_1, S_2, S_3, S_4, and S_5; and *Salmonella* produced I, K, or B (31, 42, 97). Even within these species, there was sometimes a correlation between biochemical or other properties and the type of colicin produced (17), although in most cases the correlation was not very high. However, an exception to this was discovered by Hamon and Nicolle (42), who found that in *S. typhi* both of two strains of lysotype 36, and 50 of 51 strains of lysotype 40, produced colicin B. This is the only known occurrence of colicin B in *Salmonella*, and is unusual in exhibiting such a high correlation with other genetic properties.

The patterns of susceptibility also differ (17, 31). Thus, *Escherichia* and *Shigella* were more often sensitive to one or more colicins than were the other genera, and all 17 types of colicins acted on at least one strain of *Escherichia* and *Shigella* whereas only a limited number acted on the others. Five types (B, C, D, H, and V) were found to act on *Salmonella*, four (A, B, C, and D) on *Aerobacter*, and only colicin type H acted on *Proteus*. In subsequent studies, sensitive strains were found among *Paracolobactrum arizonae* (type E) (50), *Erwinia* (types E, I, S_4, and S_5) (45), and *Pasteurella* (type G) (94). The colicins produced by *Shigella* and *Escherichia* showed a distinctly higher probability of killing strains of the same genus (17). Thus, even within these closely related genera, where bacteriocins are all called colicins, we can see the tendency to act on closely related strains.

Alveicins

This family of bacteriocins, like most of those other than colicins, was discovered recently by Hamon and Péron (50). Their approach was to take a series of strains of one genus or group and test each for activity against some or all of the others. Ultraviolet irradiation was also used to induce bacteriocin production and increased the number of strains scored as bacteriocinogenic. One or a few strains which are sensitive to most of the bacteriocins of the group were then used as indicator strains to test for sensitivity to bacteriocins produced by other groups. The bacteriocins with a wide spectrum of activity against other strains of the group were, in general, those tested against the indicator strains of other groups.

Hamon and Péron (50) showed that 30 of 98 (or 30%) strains of *Hafnia* spp. produced bacteriocins active on *Hafnia*. Only two *Hafnia* strains were used as indicators and, hence, it is not possible to say whether alveicins can be subdivided into types in the way that colicins have been. Alveicins acted on *E. coli* 36 and some acted on *Shigella paradysenteriae* Y6R, *E. coli* K-12, or *E. coli* B in addition. Some acted only on K-12 or Y6R. It was suggested that the majority of alveicins had an affinity with colicin E.

Caratovoricins

Hamon and Péron (45) found that seven of nine strains of six *Erwinia* species produced bacteriocins when tested against the same nine strains and indicator strains of other genera. Two of the caratovoricins were active against the same strains and may be identical; the others had different activity spectra. Of the six caratovoricins, three did not kill any of the nine *Erwinia* strains and were detected only by their activity on other genera. *E. coli* K-12S was sensitive to two, *E. coli* B to one, and *S. paradysenteriae* Y6R to none of the six caratovoricins. These three strains are in general sensitive to all colicins. Other strains sensitive to some caratovoricins were strains of *Pseudomonas fluorescens*, two of *Serratia* sp., and one of *Xanthomonas*. The pyocin indicator, *P. pyocyanea* P.10S, was not sensitive.

Arizonacins

Hamon and Péron (50) found that 33 of 220 (15%) strains of *Paracolobactrum arizonae*

produced bacteriocins when tested against 25 *P. arizonae* strains. A few acted only on one *P. arizonae* strain, although the majority acted on several, in addition to one, two, or three of the four colicin indicator strains K-12S, B, 36, and Y6R. Among the *P. arizonae* strains found to be sensitive to arizonacins, some were sensitive to colicin E, but to no other colicins.

Cloacins

Hamon and Péron (51) found that 8 of 29 (27%) strains of *Enterobacter cloacae* produced bacteriocins. The effect of ultraviolet induction was to change rather than to extend the activity spectrum, suggesting that different bacteriocins are produced. Taking these into account, Hamon and Péron identified at least six different cloacins. Some of these act on either *Escherichia coli* B or K-12 to high titer and show some activity against strains of *Xanthomonas* and *Erwinia*. No activity was detected against *Pseudomonas pyocyanea* or *Serratia*. Papavassiliou (87) showed that certain colicin-resistant mutants of *E. coli* were also resistant to some cloacins, suggesting similarity of the receptor.

Marcescins

The study of the bacteriocins of *Serratia* spp. has revealed some points of particular interest. Hamon and Péron (44) found that 73 of 85 (mostly *S. marcescens*) or 86% of strains produced bacteriocins and thought the percentage might be higher if only freshly isolated strains were studied. Each preparation had, in general, its own activity spectrum, suggesting the existence of many different types of marcescins. The majority of these preparations were also bactericidal for *E. coli* B and K-12S. Twelve of these preparations, when treated with trypsin, lost their activity for *E. coli* but retained it for *Serratia*. Two other preparations retained activity against *E. coli* after trypsin treatment. Thus, most strains produce a trypsin-sensitive bacteriocin active on *E. coli* only and a trypsin-resistant bacteriocin active on *Serratia* only. Typical colicins were inactive on *Serratia*, and K-12S mutants resistant to the *Serratia* bacteriocins retained their sensitivity to the various type colicins (specific colicins not detailed). One marcescin was active on *Erwinia* strains (46).

In a similar study, Mandel and Mohn (78) found all strains to be bacteriocinogenic, and all inhibited *E. coli* B and K-12 in addition to a range of *Serratia* strains specific for each bacteriocinogenic strain studied. They found the bacteriocins to be trypsin-sensitive and considered them to be subsets of colicin K, because resistant mutants of *E. coli* also resisted colicin K and vice versa.

In view of the high proportion of bacteriocinogenic cultures in each study, it is probable that both groups were studying similar substances; the cause of the minor discrepancies is not obvious, but it appears that *Serratia* strains can produce two types of bacteriocins, one very similar to known colicins and the other best considered as a separate group, the marcescins.

Pneumocins

Hamon and Péron (50) found that 38 of 112 (34%) *Klebsiella* species (96 *K. pneumoniae*) were bacteriocinogenic. Their activity was generally limited to the other strains of *Klebsiella*, usually to a very small number of the 62 strains tested for sensitivity. The great variety of activity spectra suggests the existence of many types of pneumocins. They did not act on any of the colicin indicator strains but acted occasionally on the aerocin indicator strains of *Aerobacter aerogenes*.

Aerocins

Of 28 strains of *A. aerogenes*, 21 (75%) were bacteriocinogenic (50). Aerocins often acted on many of the 28 *A. aerogenes* strains, and the activity spectra indicated several different types of aerocins. They did not act on any of the colicin indicator strains, but they frequently acted on strains of both *Enterobacter cloacae* and *Klebsiella*.

Pyocins

Jacob (71) studied a bacteriocin produced by *Pseudomonas pyocyanea* and demonstrated several of its properties. Hamon (40) and Hamon, Veron, and Péron (53) showed that a large proportion (18 of 19) of *P. pyocyanea* strains produced bacteriocins active mainly on other *P. pyocyanea* strains, but some were also active on strains of *P. fluorescens*. Only two had the same activity spectrum on a series of *P. pyocyanea* strains, indicating a wide range of types. The 17 pyocins studied inhibited from 1 to 7 (average, 4) of the 15 *P. pyocyanea* strains tested, but only 8 inhibited either 1 or 2 of 18 *P. fluorescens* strains studied (53). Smooth strains of *Salmonella*, *Shigella*, and *E. coli* are generally resistant to pyocins, whereas rough strains are very sensitive (40).

Fluocins

Hamon, Veron, and Péron (53) found that 12 of 28 strains of *P. fluorescens* (19 strains) and other *Pseudomonas* spp. (9 strains) produced

bacteriocins. All 12 had different activity spectra on the *P. fluorescens* studied, indicating a wide range of different types to be discovered. Only one was bactericidal for any of the 19 strains of *P. pyocyanea* tested, and this one was bactericidal only for strain P.10S. One fluocin was active on strains of *Erwinia* (46).

Pesticins

Ben-Gurion and Hertman (3) found that of 23 strains of *Pasteurella pestis* examined, 22 produced an antibiotic active on a strain of *P. pseudotuberculosis*. Brubaker and Surgalla (5) found 77 of 80 strains to produce this antibiotic, which has been named pesticin I. A mutant selected for resistance to the pesticin of one strain was resistant to the pesticins of the other 21 strains tested (3), suggesting that one substance was involved, and that this was produced by most strains of *P. pestis*. Burrows and Bacon (7) showed further that sensitivity was confined to and characteristic of all strains of the serological group A of *P. pseudotuberculosis*. Brubaker and Surgalla (5) extended the range by showing that some strains of *E. coli* are sensitive to pesticin I. Of nine pesticin-resistant mutants of *E. coli* ϕ, four also lost their sensitivity to colicins B, D, I, and S_1; the other five retained their sensitivity to the 16 colicins tested. However, the *P. pseudotuberculosis* strains sensitive to pesticin I were resistant to the 16 colicins. This finding suggests that pesticin may be adsorbed onto receptors similar to those involved in colicin action, although it was not rigorously established that the actions against *E. coli* and *P. pseudotuberculosis* were due to the same agent. Strains producing pesticin I also produce an inhibitor to it (5, 6), and fractionation to remove the inhibitor can give a 100- to 1,000-fold increase in observed activity. The chemical nature of the inhibitor is not known.

Brubaker and Surgalla (5) discovered a second pesticin (II) produced by all tested strains of *P. pseudotuberculosis* and *P. pestis*, which was active on the avirulent *P. pestis* strains A 12 and Java, both nonpesticinogenic for pestin I. Interestingly, both these strains are pesticinogenic for pesticin II as well as being sensitive to it.

Megacins

Iványovics and Nagy (69) examined 200 strains of *Bacillus megaterium* for an antagonistic effect against 1 strain of the same species. Of the 92 strains that produced an effect, some were active only after induction by ultraviolet irradiation. All those tested were active on all of hundreds of *B. megaterium* strains tested for sensitivity, including the megacinogenic strains (64). However, the antibacterial titers for six megacins against 11 sensitive strains showed that some did not have high titers against any, whereas others, such as 216, were highly active against several strains (80). The relative sensitivities suggest that in addition to any variation in the amount of megacin produced by each strain, there are qualitative differences in the megacins themselves.

Of the many strains of other species tested, both gram-negative and gram-positive, only some pigment-forming aerococci were sensitive, in addition to 8 of 43 *B. anthracis* strains and 2 of 13 *B. subtilis* strains (68). Recently, Holland (59) described some of the properties of a new bacteriocin, megacin C, which is produced by several strains of *B. megaterium*, and is specific for other strains of the species.

Monocins

Bacteriocins active against several cultures of the same species were produced by 25 of 51 strains of *Listeria monocytogenes* (48). Of the 51 strains, 9 were sensitive to some or all of the 25 monocins. It has been possible to classify the monocins into two types, A and B, on the basis of cross-resistance. All but one of the monocins were of type A, and the resistant mutants obtained to five of these were resistant to all the others of the type. The one monocin of type B was distinguished because it acted on one of the series of mutants resistant to all type A monocins (49).

The majority of type A monocins also have the same activity spectrum, acting on 9 of the 51 strains tested. However, two of the nine sensitive strains (700X and 10574) produce monocins, and these same strains are also resistant to the monocin of 10905 (subtyp A_1). Since strains 700X and 10574 are resistant each to its own monocin, as well as to that of 10905, monocins 700X and 10574 constitute subtypes A_2 and A_3. The remaining 21 monocins are subdivided on the basis of physical properties into subtypes A_4 to A_6 (49). The monocins have no effect on the gram-negative bacteria, nor on any of 18 *Streptococcus* cultures; but the culture supernatant did inhibit 34 of 88 strains of *Staphylococcus* and 6 of 20 strains of *Bacillus* (48); because these activities had thermolability similar to that of the effect against *Listeria*, it seems that both effects are due to the same agent (49).

Cerecins

The first cerecin was discovered in a study of *Bacillus* phages by McCloy (77). Hamon and

Péron (52) studied eight cerecins produced by *B. cereus* as part of a more general study. The only detail of the activity spectra given was that they do not act on any known indicator strains for the bacteriocins of gram-negative bacteria.

Enterococcins

Brock, Peacher, and Pierson (4) found that more than half of 100 strains of various species of *Streptococcus* of the enterococcus group produce a bacteriocin. They were able to classify these bacteriocins into five types on the basis of their sensitivity or resistance to proteolytic enzymes, heat, and chloroform, and their activity spectra. Type 1 was produced by all strains of *S. zymogenes*, and is probably identical with the hemolysin produced by definition by all strains of this species. It acts on all other strains of enterococci and all other gram-positive bacteria tested. Type 2 is produced by some strains of *S. liquefaciens* and acts on all strains of *S. faecium* and some strains of *S. faecalis*. Type 3 is produced by some strains of *S. faecalis* and *S. faecium* and acts on all strains of *S. zymogenes* and *S. liquefaciens* and some strains of *S. faecalis* and *S. faecium*. Type 4 is produced by some strains of *S. faecium* and acts on some strains of *S. faecium* and *S. faecalis* and all strains of *S. zymogenes* and *S. liquefaciens*. Type 5 is produced by only one strain of *S. zymogenes*, which also produces type 1 and is active against other *S. zymogenes*. Thus, the production and sensitivity to enterococcins is correlated, but not absolutely, with the classification of this group of streptococci. Type 1 acts against all of a wide range of gram-positive bacteria, whereas the other types act only against closely related species, in addition to those listed above from the producing species group. In no instance does an enterococcin act on any strain producing the same type of enterococcin.

Staphylococcins

Fredericq (13) first studied the bacteriocins of *Staphylococcus*; he observed five staphylococcins, each with its own activity spectrum against other *Staphylococcus* strains. They were also active against *Bacillus* strains. Hamon and Péron (52), studying 20 staphylococcins, found that *Listeria*, *Bacillus*, and *Corynebacterium* were often sensitive to staphylococcins.

Different Families of Bacteriocins

In the preceding description, some of the various families of bacteriocins have been discussed separately. Hamon and Péron (49) list in addition some which are not so well studied. The discovery of the colicins, alveicins, caratovoricins, arizonacins, cloacins, marcescins, pneumocins, and aerocins has shown that every group of the Enterobacteriaceae except *Proteus-Providencia* can produce bacteriocins (50), and bacteriocins are being discovered in other bacterial families.

The production of bacteriocins thus appears to be a widespread phenomenon. In most cases, each family of bacteriocins consists of many different types, as indicated by the variation in the detailed activity spectra. The main activity of each family is within the group of species producing it, and the spectrum of activity of bacteriocins has been used by Hamon and Péron (46) as an aid in bacterial classification. They argue that because bacteriocins usually act only on strains closely related to the producing strain, the production of bacteriocins by one species active on another indicates close relationship. They use this principle in a discussion of the taxonomy of *Serratia*, *Erwinia*, *Aeromonas*, and *Xanthomonas uredovorus*.

However, some activity outside the group of producing species is not uncommon. Thus, some alveicins, caratovoricins, cloacins, and arizonacins act on one or more of the four colicin indicator strains *E. coli* K-12S, B, and 36, and *S. paradysenteriae* Y6R. However, no individual bacteriocin of these families acts on all four indicators, differentiating them from most colicins. The pneumocins and aerocins are perhaps closely related, as both act to some extent on the other's indicator strains, but not on the colicin indicator strains. Of the bacteriocins of the Enterobacteriaceae, only the caratovoricins have been shown to have any activity on the Pseudomonadaceae.

The pyocins and fluocins seem in general to be very similar to the bacteriocins of the Enterobacteriaceae although, of course, active on a different range of bacteria.

The pesticins differ from the other bacteriocins discussed so far in two important respects. First, the production of a particular pesticin is, in general, a characteristic property of all strains of a species, and sensitivity to pesticin I is, likewise, characteristic of a particular serological group. There are, however, some exceptions to this generalization and, in addition to those previously mentioned in the section on pecticins, there is the example of a few strains of *P. pestis* sensitive to pesticin I (5), and the observation that nonpesticinogenic mutants of *P. pestis* are sensitive to pesticin I (56). Second, there seem to be only a very limited variety of pesticins, only two having been discovered during the examination of many strains. Brubaker and Surgalla (6) found it impossible to state, on the basis of available data, whether or not pesticins should be

considered bacteriocins. Only further work on all bacteriocins can answer this.

Of the five families of bacteriocins produced by gram-positive bacteria, the megacins and monocins differ from the majority of the bacteriocins in that most members of each family have the same activity spectrum, and except for serological and similar differences each family would be almost monotypic. This situation obviously resembles that found for pesticins, of which only two types exist. Hamon and Péron (52) consider the bacteriocins of the gram-positive bacteria to be distinct from those of the gram-negative bacteria on several counts, most of which will be discussed more fully later. Of importance here is the suggestion that their activity spectra are not limited, like those of the gram-negative bacteria, to closely related species. Thus, the monocins act on a large proportion of *Bacillus* and *Staphylococcus* strains; the staphylococcins also act on strains of several gram-positive genera, and enterococcin 1 acts on all gram-positive bacteria. However, megacins, although active against some *B. anthracis* and *Micrococcus* strains, were ineffective against many other species (68), and the other four enterococcin types are very restricted in activity. The studies so far undertaken on bacteriocins of gram-positive bacteria certainly suggest that they can have a wider activity spectrum than those of the gram-negative bacteria, although it must be remembered that some of the latter can act on unrelated species, as in the case of a pesticin or pyocins acting on *E. coli*, and of some caratovoricins acting on *Pseudomonas*.

PRODUCTION OF BACTERIOCINS

Bacteriocinogenic strains, although they possess the stable genetic ability to produce a bacteriocin, do not do so all the time or under all conditions. The factors controlling the synthesis are only incompletely understood. Jacob et al. (74) discovered that colicin ML-E could be induced by ultraviolet light, and this observation has been extended to various chemical agents, such as peroxides, known previously either as inducing agents for lysogenic bacteria or as carcinogenic or mutagenic agents (31).

Colicin E_2 production is a lethal biosynthesis and under normal cultural conditions only a few cells produce colicin; the effect of ultraviolet light is to induce a large proportion of cells to produce colicin. The cells which actually produce colicin die in the process. These results of Ozeki, Stocker, and Margerie are discussed in recent reviews (31, 64).

The analogy between colicin and temperate phage production is, thus, very close. As with lysogenic bacteria, only some bacteriocins are inducible. These include examples of several colicin types (31) and at least some marcescins (44), caratovoricins (45), cloacins (51), megacins (66, 69), monocins (48), pyocins (40, 53, 71), and fluocins (53), and both pesticins (3, 5). In some cases, there is no detectable bacteriocin before induction, and in others the level is greatly increased.

The kinetics of synthesis and release after ultraviolet induction of several colicins, one pyocin, one megacin, and one pesticin have been studied. It appears that synthesis of colicin (47, 74) and pyocin C.10 (71) begins a few minutes after irradiation and continues for 1 hr or more. In nonlysogenic *E. coli* strains, release of colicin is continuous. In the case of a bacteriocinogenic strain which is also lysogenic, the release, instead of being continuous, is abrupt at the time of lysis. Hamon and Péron (47), studying various colicinogenic derivatives of *E. coli* K-12, either lysogenic or nonlysogenic for phage λ, showed that the presence of λ prevented release of colicin before lysis and resulted in a sudden release of colicin at lysis, as opposed to a steady release beginning soon after induction of nonlysogenic strains. Pyocin C.10 accumulated intracellularly and was released by cell lysis, although strain C.10 is apparently nonlysogenic. However, R-mutants of C.10 produce pyocin on induction but do not lyse (40), although the kinetics of pyocin release in this instance are not given.

Megacin 216 (64, 70) is not detectable intracellularly until 45 to 60 min after induction, and is released suddenly on cell lysis after about 90 min. Strain 216 appears to be nonlysogenic (66). Other megacinogenic strains undergo mass lysis of the culture spontaneously and release megacin (69).

Pesticin I was detected 60 min after induction by ultraviolet light (56), but unfortunately no distinction is made between extra- and intracellular pesticin. The production of bacteriocins, in addition to being susceptible to induction by certain agents in some cases, is also very dependent on growth conditions, as exemplified by detailed studies by Hertman and Ben-Gurion (56) on pesticin I synthesis, and by Goebel et al. (38) and Matsushita et al. (79) on synthesis of colicin K235-K. In each case, the medium and other growth conditions had to be carefully controlled for optimal titers; relatively minor modifications sometimes depressed the titer 100-fold. Many of the colicinogenic strains which produce good zones of inhibition if grown on agar and overlayered with a sensitive strain, do not produce any colicin in broth, again indicating the significance of cultural conditions. The role of

the factors influencing bacteriocin production in these cases is not at all understood.

COLICINOGENIC PARTICLE

Transduction of Colicinogeny

Colicinogeny is generally a stable character, although it is sometimes lost (31), and under certain conditions a large proportion of some strains can lose their colicinogeny (54).

Some colicinogenic strains, when grown together with other strains, can transfer to them the ability to produce the colicin (24, 25, 31). This colicin is always of the same type as that of the donor, the transfer requires cell contact, and the character is stable. The new colicinogenic strain normally resembles the noncolicinogenic parent in all other properties. This indicated that colicin production is determined by a plasmid (a genetic determinant not associated with the chromosome) which was being transferred on cell contact. This was confirmed in crosses between *E. coli* K-12 strains, of which the donor had been made colicinogenic, when transfer of colicinogeny is unlinked to any other segregating locus. Plasmids determining colicinogeny are termed C-factors. C-factors can also be transferred by transduction by phage PLT22. These phenomena were discussed by Fredericq (31).

Further confirmation of the plasmid nature of C-factors comes from the recent demonstration (98) that a series of Hfr strains and an F^+ strain of *E. coli* K-12 made $(K94\text{-}V)^+$, $(K317\text{-}E2)^+$, or $(CA53\text{-}I)^+$ all transfer the C-factor to F^- strains to an extent dependent only on the C-factor concerned and not on the origin of the Hfr, whereas, were a C-factor to attach to a specific chromosomal locus, it would be transferred at a much higher frequency from the Hfr strains in which this locus was transferred early.

This shows that the C-factor is not associated with the chromosome. In the case of (K94-V), the time of transfer was also shown to be independent of the origin of the Hfr. Clowes (10) showed that ($K30\text{-}E_1$) is also transferred by different Hfr strains at a time independent of the origin of the Hfr and that some earlier experiments (1), thought at the time to show that the ($K30\text{-}E_1$) factor attached to the chromosome in K-12 strains, have an explanation unconnected with the C-factor.

Thus, in the four cases studied, a C-factor transferred to a K-12 strain does not regularly become attached to a specific site on the chromosome, and again the only satisfactory explanation is that in a large percentage, if not all, of the organisms of the culture the C-factors are being transferred independently of the chromosome.

Ozeki and Howarth (85) showed that the (P9-I) C-factor could confer on *S. typhimurium* LT2 the ability to act as a genetic donor; Clowes (9) repeated this with the same C-factor and *E. coli* K-12. In both cases, donor ability was of the type determined by the F-factor of *E. coli*, in that large pieces of chromosome, which could include any gene, were transferred. This fertility system, like that determined by the F-factor of *E. coli* K-12, also confers the ability to transfer other C-factors. In LT2, it enabled the transfer of the ($P9\text{-}E_2$) and (K49-K) C-factors and increased the transfer of ($K30\text{-}E_1$) from 0.1 to 5% and of (K77-B) from 20 to 60% (86). Smith et al. (95) showed that, in normal crosses with a donor LT2 strain trebly colicinogenic, $(P9\text{-}I)^+$, $(P9\text{-}E_2)^+$, and $(K30\text{-}E_1)^+$, recipients receiving one C-factor usually receive the other two, but that if mating is interrupted early they commonly receive only one, but any one, of the three C-factors. This suggests that, although transferred independently, without interruption all three are eventually transferred, giving an appearance of linkage. The presence of the ($K30\text{-}E_1$) C-factor greatly increases the promoter activity of the (P9-I) C-factor in LT2 (85) by an unknown mechanism, although alone it is devoid of promoter activity and is not itself transferred (86). The (P9-I) C-factor can itself exist in two different states in LT2, a recently transferred factor being much more efficient at promoting fertility.

The C-factors, in addition to determining the ability to produce colicin, also confer immunity to that colicin and usually to other colicins of the same type, although the immunity may not be complete (24, 26). This immunity does not involve loss of the receptor for the colicin, and thus bears a strong resemblance to the immunity conferred by a prophage against superinfection by the same or a closely related phage. In the case of C-factors of type $\bar{E}$, the immunity conferred is only against certain of the colicins of that type; this enabled Fredericq (26) to subdivide the $\bar{E}$ colicins into two types, E_1 and E_2; C-factors of each type only confer immunity against colicins of the same type. The term, type E_3, has also been used (81), presumably for those colicins of type $\bar{E}$ which Fredericq describes as active against both $(Col\ E_2)^+$ and $(Col\ E_1)^+$ strains.

Fredericq (29, 30, 31) showed that the three strains (K94, K260, and K30) able to transfer their colicinogeny V to *E. coli* $K\text{-}12F^-$ transferred plasmids which also conferred fertility on the K-12 strains. *E. coli* K260 also produces

colicin B, and both colicinogenic properties and fertility are linked in transfer. An Hfr derivative of a K-12 (K260-V,B,F+) strain was found to have lost its colicinogeny V, but the colicinogeny B and Hfr properties were transferred in crosses linked as the terminal markers (29, 30). Thus, the two colicinogenic properties and fertility are probably determined by the one plasmid, although the three properties are occasionally separated in crosses (29, 31).

Plasmid or Episomal Nature of Colicinogeny

C-factors were one of the three types of episomal elements originally described by Jacob and Wollman (75), episomes being genetic factors which can exist either independently of the chromosome or attached to it. In the latter case, they replicate strictly in phase with the chromosome. However, the evidence that C-factors are episomes has since been reinterpreted, and the subsequent work described above has revealed only one instance of a C-factor attaching to the chromosome, and in this instance the factor has fertility promoter activity as well (29, 30).

The two well-established types of episomes, temperate bacteriophages and fertility promoters or F-factors, differ in certain respects. Temperate bacteriophages can exist in stable equilibrium with the cell only when attached to the chromosome; when in the autonomous state, they always replicate faster than the bacteria and cause cell lysis and death. When the phage genome becomes integrated with the bacterial chromosome, it usually does so at a specific site. On infection with a temperate phage, a large proportion of bacteria may become lysogenic and, since the others die, all the survivers have the prophage attached at the specific site. There are, however, some temperate phages which can attach at any of a large number of sites, if not anywhere, on the chromosome (96).

On the other hand, the F-factor can replicate in phase with the bacterial genome in both the autonomous and attached states. Integration is rare and, because it confers no marked natural selective advantages, special selective techniques are required to isolate the clones with an integrated F-factor. The F-factor can attach at any of a large number of sites, if not anywhere, on the chromosome. However, there are exceptional F-factors which, having incorporated a piece of bacterial chromosome, attach specifically to the homologous region of the host chromosome (55).

The data available on the C-factors which have been studied show that they can replicate in phase with the bacterial chromosome while in the autonomous state without killing the cell and that, if they can become integrated, they do so only rarely. Thus, the C-factors appear to be more similar to the F-factors than to temperate phage. The induction of colicin production by ultraviolet light and certain chemicals has reasonably been likened to the induction of prophage, and may likewise be effected by interference with host deoxyribonucleic acid (DNA) synthesis. However, it is not possible to use this similarity as evidence for the episomal nature of C-factors, because, if C-factors are episomal, they resemble the F-factors rather than the bacteriophage; therefore, induction of colicin production cannot be simply the "release" of the C-factor from the integrated state, because C-factors can exist in the autonomous state stably and without colicin production.

It has been suggested that the reduction in promoter activity by a (P9-I) C-factor after it has been in the LT2 cell for a few generations is due to its having attached to the chromosome, but there is no direct evidence for this. The nature of the C-factor has also been discussed recently by Fredericq (30). In the absence of convincing evidence that a particle is episomal, it is best called a plasmid; thus, it would seem that at present the majority of C-factors are best referred to as plasmids.

Chemical Nature of the C-Factors

C-factors, being plasmids, are likely to be composed of nucleic acid. Silver and Ozeki (92) measured the transfer of radioactive DNA accompanying the transfer of C-factors between two bacterial strains. They showed that *S. typhimurium* LT2 (P9-I), known to transfer the (P9-I) C-factor to *E. coli* K-12, also transferred some of its DNA at the same time. The use of noncolicinogenic LT2 established that the transfer was due to the C-factor. The addition of the (K30-E_1) or (P9-E_2) C-factor to the LT2 donor strain (both of which can be transferred to *E. coli* K-12 if the donor is also (P9-I)+, increased the amount of DNA transferrable to *E. coli* K-12. Making the reasonable assumption that the DNA transferred was, in fact, the various C-factors, they were able to estimate the molecular size of the DNA associated with each of the particles as (P9-I) = 6×10^4, (K30-E_1) = 7×10^4, and (P9-E_2) = 3×10^4 nucleotide pairs. They pointed out that, as they were working near the limit of detectability, these are only order of magnitude estimates. The genetic aspects of colicinogeny were discussed by Hayes (55).

Genetic Determination of Other Bacteriocins

The genetic basis of the production of bacteriocins has only been established in the case of

the colicins determined by C-factors. In the case of the colicinogenic properties not transferrable on cell contact, no evidence has been adduced to suggest that they are determined by chromosomal loci, although the discovery by Fredericq (29, 30) of a C-factor stably attached to the chromosome suggests one possibility. It is possible that many of them are determined by plasmids, but they are not observed to be transferrable to other strains, because of the absence of any recombination mechanism in the strains. However, as Jacob et al. (72) pointed out, because there is almost no tranfser of cytoplasm during conjugation, the transferred nonchromosomal factors must transfer by some selective mechansim. Thus, even in those cases of multiply colicinogenic strains where not all are transferrable (25) the nontransferrable ones may be determined by plasmids which do not have the specific requirements for transfer. There is, in fact, no evidence as yet to state whether all colicinogenic properties are determined by C-factors or whether some are determined by chromosomal genes.

In the case of the other bacteriocins, the genetic determination has hardly been examined, although genetic recombination mechanisms exist in *Pseudomonas* (61), and it should be possible to determine whether or not pyocinogeny has a chromosomal location. Hamon (40) attempted to demonstrate pyocinogenic factors, but was unable to do so because of the existence of genetic recombination of other characters in his experiments. Ben-Gurion and Hertman (4) failed to transfer the pesticinogeny I property. Holland and Roberts (60) looked for evidence of transmission of a megacinogenic factor during mixed growth with a nonmegacinogenic strain of *B. megaterium*. They examined a total of 600 colonies involving 7 different megacinogenic strains with no success. In no other case does there appear to have been any analysis of the genetic basis of bacteriocin formation, and our knowledge is still confined to colicins.

CHEMICAL NATURE OF BACTERIOCINS

All the bacteriocins which have been studied in sufficient detail have been found to be macromolecular and to include, if not to consist of, polypeptide or protein. It is this, in addition to activity against closely related species, which sets the bacteriocins apart from other antibiotics, which are all of relatively low molecular weight. However, many groups of bacteriocins have not been studied in sufficient detail even to establish whether they are macromolecular, and they are included in the list of bacteriocins only on the basis of their type of activity spectrum.

Many of the early papers on the chemical structure of colicins were discussed by Cocito et al. (11) and in recent reviews (31, 64); only a few will be discussed here.

Colicins K235-K, K357-V, SG710, and CA31-A

Colicin K235-K has been shown by Goebel's group (2, 37) to be the "O" somatic antigen of the producer strain. Their preparations are electrophoretically homogeneous when tested by free electrophoresis or on a polyvinyl block. By diethylaminoethyl (DEAE)-cellulose chromatography, Goebel et al. were able to demonstrate heterogeneity in the O somatic antigen of *E. coli* K235, but even under these conditions they were unable to purify the colicin, because each fraction had the same specific activity (36). Thus, the colicin is either an integral part of, or firmly attached to, the O somatic antigen. The O somatic antigen is a lipocarbohydrate protein complex and, on dissociation, the colicin activity is associated with the protein moiety (37).

Comparative studies (2, 91) with the colicin and products obtained from a noncolicinogenic mutant of *E. coli* K235 showed that the protein moiety of the O antigen, which is derived from the colicinogenic strain and has all the colicin activity, has an antigenic site not possessed by the corresponding protein of the noncolicinogenic mutant. Antibody directed against this antigenic site is adsorbed out by the colicin, neutralizes its activity, and precipitates the free protein, although not the complete O antigen. The antibody has no affinity for the O somatic antigen of the noncolicinogenic mutant and is not adsorbed out by it. Chemical studies show that the two O somatic antigens are indistinguishable except for this antigenic difference in the protein moiety and the presence of slightly more protein in the antigen derived from the colicinogenic strain. The extension of the detailed chemical analysis of these two antigens to their protein moieties should be expected to reveal the difference between them (91). However, it must not be overlooked that the "noncolicinogenic" mutant may, in fact, produce an altered, nonactive "colicin," analogous to the inactive enzymes produced by some bacterial and other mutants. If this were the case, the differences between the two proteins would not reflect the full extent of the colicin component; determination of this may have to await the study of strains rendered colicinogenic by transfer of a C-factor.

Serological studies on the colicin produced by *E. coli* strain K-12 (K235-K), a mutant of *E. coli* K-12 which has acquired the ability to produce colicin K235-K by growth with *E. coli* K235 and

which was referred to by Amano et al. (2), as K8C+ suggest that this colicin also resides in the O antigen, in this instance, of course, having the antigenic specificity of strain K-12 (2). However, although *E. coli* K235 antiserum will not precipitate this O somatic antigen, it will completely neutralize its colicin activity, suggesting that the protein component, which has the colicin activity, also has the antigenic site found in the colicin produced by *E. coli* K235. Colicin K235-K, then, is a protein normally associated with, if not a property of, the protein moiety of this antigen. Further study of this most interesting system may tell us whether the colicin molecule as determined by the (K235-K) plasmid constitutes the whole of the protein moiety of the antigen or only a part.

Colicins K357-V (62, 63) and SG-710 (83) have been shown to be lipocarbohydrate protein complexes, as was colicin K. However, in these two instances it has not been shown whether the activity resides in only one fraction, such as the protein, although colicin SG710 and K357-V are trypsin-sensitive. Nüske et al. (83) purified the colicin of *E. coli* SG710, unfortunately not classified in Fredericq's system, and the material obtained, having a molecular weight of 10^6 to 10^7 and a composition of 45 to 55% protein, 20 to 25% carbohydrate, 15 to 20% lipid, and 5 to 10% inorganic material, could well be the O somatic antigen.

Hutton and Goebel (62, 63) purified colicin K357-V as an electrophoretically and serologically homogeneous substance. The purified colicin is a lipocarbohydrate-protein complex, containing about 10% protein, 20 to 40% carbohydrate, and 11% lipid; it is toxic for mice and is the O somatic antigen of the producing strain.

Colicin CA31-A has been purified by Barry et al. (2*a*) and appears to be the "O" somatic antigen of the strain. It contains 67% protein, 7.6% reducing sugar, 5.6% hexosamine, and 1.1% phosphorus.

Colicin CA42-E₂

Colicin CA42-E₂, also known as CA42-F, was purified by Reeves (89) to give a substance fairly homogeneous on starch-gel electrophoresis (89) and immunoelectrophoresis (*unpublished data*), which contained 75% protein with approximately 10% carbohydrate. This high protein content makes it unlikely to be the O somatic antigen, and its relatively low molecular weight, evidenced by its ability to move on starch gel (89) and a sedimentation coefficient of 3.6*S* (*unpublished data*), suggest that not all colicins will fit into the pattern established by colicin K.

Megacin 216

Megacin 216 was studied by Iványovics et al. (70) and found to be antigenic. Holland (57) showed it to be a protein of a molecular weight of approximately 51,000 (based on sedimentation coefficient and assumed partial specific volume). The purified megacin could not be shown to be inhomogeneous by examination in an analytical ultracentrifuge, a Kern microelectrophoresis apparatus, or a Perkin-Elmer electrophoresis apparatus.

The fact that all attempts to purify the product failed to give an increase in specific activity strongly suggests that the product is indeed chemically pure. Megacin 216 was found by some investigators (70) to be sensitive to proteolytic enzymes (pepsin and chymotrypsin), and by others (57) to be resistant (pepsin, trypsin, and chymotrypsin).

Chemical Nature of Other Bacteriocins

In addition to previously mentioned studies, there are many which have used impure fractions to show that bacteriocin activity has some, at least, of the properties of proteins. Thus, many colicins, some pyocins, one fluocin, some enterococcins, and both pesticins are destroyed by some proteolytic enzymes. Data on the antigenicity, dialyzability, thermostability, and precipitability by salts and organic solvents has been published for several colicins and other bacteriocins [see reviews by Fredericq (27, 31) and Cocito et al. (11)].

Several colicins and pyocins have been shown to be antigenic, and in general the antisera were specific (2*a*, 41, 63). Three monocins were tested; all were found to be antigenic, and the antiserum of all three 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 other grounds (49), as discussed in the section on monocins.

It is possible to recognize different antigenic specificities among megacins of type A, all of which kill all strains of *B. megaterium*. Antiserum to megacin 216 had no neutralizing activity against 16 other megacin preparations (80). Thus, it appears that these megacins, although they have the same activity spectrum, are in fact chemically different.

Conclusions on Chemical Nature of Bacteriocins

Although only a few bacteriocins have been chemically characterized, the data are probably sufficient to justify the generalization that bacteriocins are protein in nature. Emphasis has at

times been placed on the fact that three colicins are lipocarbohydrate-protein complexes, but the detailed analysis of K235-K shows that in this instance the activity resides entirely in the protein component. Some colicins have been shown to be dialyzable and, hence, cannot be associated with the O somatic antigen; in the case of colicin CA42-E_2, there are other indications of its lower molecular weight and nonassociation with the O somatic antigen.

The fact that one and perhaps many colicins are associated with the protein of the O somatic antigen of the cell wall raises the interesting question of whether this protein (a characteristic component of the O somatic antigen of all gram-negative bacteria) can be determined entirely by a plasmid. It would seem more probable that the colicin is an extra component which becomes finally associated with this O antigen protein.

MODE OF ACTION

Adsorption and Kinetics of Killing

Some of the evidence that bacteriocins adsorb onto specific receptors on the cell surface has already been discussed. However, very few studies have been undertaken to determine whether bacteriocins are actually removed from solution by sensitive bacteria.

Fredericq (23) showed that colicin K is adsorbed from solution by sensitive bacteria, but it remained for Hamon and Péron (43) to show that this adsorption was specific. They showed that six colicins and five pyocins could be adsorbed by sensitive strains but not by resistant mutants. To demonstrate the adsorption, it was necessary to use a low concentration of the bacteriocin, and suggestions by other authors that bacteriocins are not adsorbed may be due to the use of excess bacteriocin.

Holland (58) produced data which clearly showed that megacin 216 is adsorbed to the sensitive strain *B. megaterium* 207M. However, there was a lesser amount of adsorption to an *E. coli* strain and the megacin producer strain (also sensitive). In the absence of mutants resistant to megacin for comparative studies, it is difficult to eliminate the possibility that the adsorption of 4.4×10^{-8} g/ml of protein are nonspecific, although by analogy with colicins and pyocins one would expect specific adsorption.

If one accepts that, as a prerequisite for killing, bacteriocin must be firmly bound to the bacterial surface, then it is reasonable to ask how many molecules of bacteriocin are required to kill. This question has been most commonly approached by studying the kinetics of killing by bacteriocins. Bacteria and bacteriocin at various concentrations are allowed to interact, at intervals a sample is diluted sufficiently to dilute out the unadsorbed bacteriocin, and a viable count is done. In this way, one determines the proportion of bacteria which have absorbed sufficient bacteriocin to kill them. This type of experiment, of course, only determines the kinetics of the initial bacterium-bacteriocin interaction and not of any subsequent aspect of killing. Jacob, Simonovitch, and Wollman (74), using colicin ML-E, showed that the initial rate of killing of a given concentration of bacteria was proportional to the concentration of colicin, as was the final number of bacteria killed. Both these results suggest that one colicin particle kills one bacterium. Similar data were given by Jacob (71) for pyocin C.10.

Using colicin CA38-E, Fredericq and Delcour (32) were also able to show that the initial rate of killing was proportional to the amount of colicin present. When they plotted the logarithm of the proportion of survivors at a given time against the amount of colicin added, they obtained a final logarithmic fall, but they claimed that there was an initial shoulder, indicating a "multi-hit" curve. It is difficult to evaluate this claim, as no details are given of the method of determining the viable count, and the statistical error involved here could be critical. In fact, if the points for the zero concentration of colicin are given precedence after those for the lowest concentrations of colicin, there is a reasonable fit to a "one-hit" curve. Fredericq (23), in a comparison of the kinetics of killing of bacteriophage T6 and colicin K, obtained data for colicin K which have not as yet been adequately explained, but which are completely incompatible with a one-hit curve. He found that, when a given number of bacteria were treated for 10 min with various dilutions of colicin K, the proportion of bacteria surviving was inversely proportional to the amount of colicin used.

Claims have been made that other bacteriocins show a one-hit killing curve or that kinetic data show that the bacteriocin has a particulate nature. Unfortunately, in most of these cases the data are not presented (44, 45, 48, 51, 81).

Two factors should be considered in interpreting kinetic data; first, one must take into account the statistical difficulty of distinguishing between the one-hit and other low-order reactions when only the surviving bacteria (and not the killed) can be scored directly. Jacob et al. (74) measured the number of survivors after 1 min with varying levels of colicin and for low levels extrapolated back from the amount of killing after longer times. In this way, they obtained an excellent fit to the hypothesis of one-hit killing over an 80-fold range of colicin concentrations. The

observation that colicin E_2 at low multiplicities induces λ phage (12) provides a method of directly assaying the colicin-killed bacteria. Nomura (80) considers that colicin E_2 does indeed induce λ phage with first-order kinetics. The kinetic data of Holland (57, 58) indicate exponential killing, again suggesting a one-hit or at least low-order reaction for killing by megacin 216 (although this was not suggested by the author). The megacin example brings us to the second consideration in interpreting these data. The most that kinetic data alone can show is that death results from the interaction of a certain number of colicin molecules and one bacterium. Should the data suggest that one molecule kills, this does not imply that the adsorption of a colicin molecule has a probability of one of leading to cell death, but only that the probability is unaffected by the adsorption of more colicin molecules. Holland (57) has shown that more than 100 molecules of megacin are required to kill a cell; the discrepancy between this observation and the apparent first-order kinetics could, in fact, be explained if each molecule on adsorption had only 1 in 100 probability of leading to cell death, owing to a constant probability of 1 in 100 for each interaction, to only 1 in 100 receptors being sensitive, or to only 1 in 100 molecules of his purified material being active. These alternate interpretations apply equally strongly in the other cases, but we do not have the purified material on which to make any quantitative estimates.

Effect of Bacteriocins on the Sensitive Cells

In this area, once again, it is the colicins about which most is known. Although apparently only four or five have been studied, some variation in their mode of action is already apparent and it is difficult to generalize. Although not the first to be studied, colicin K235-K, which was investigated by Nomura and Nakamura (82) and Nomura (81), has perhaps given the least complicated results.

Colicin K235-K. Colicin K235-K at high multiplicities stops DNA, ribonucleic acid (RNA), and protein synthesis in a sensitive strain, and at low multiplicities it inhibits these activities in proportion to the number of cells killed; thus, presumably it inhibits them completely in all killed cells. Unfortunately, no data are given except for DNA synthesis (81, 82), which stops in less than 5 min. This colicin inactivates the plaque-forming ability of T4-infected cells with the same kinetics as for killing sensitive cells, even when the T4 infection has progressed to the stage where there are 30 to 50 vegetative phage chromosomes. Thus, there is no direct inhibitory effect of a single colicin molecule on a chromosome. The effect of K235-K then might be a complete metabolic shutdown and, unfortunately, as in all other such studies, there is very little information except on macromolecular syntheses. However, no leakage was observed of either P^{32} from cells grown in $P^{32}O_4$ or β-galactosidase from fully induced cells treated with excess colicin. Whatever the nature of the inhibition, it is reversible by trypsin (82), because the addition of trypsin 30 min after the addition of colicin leads to a resumption of both DNA and β-galactosidase production. The data do not indicate what proportion of the bacteria are rescued, but it must be considerable to account for the observed recovery of the syntheses. Using resting cells, i.e., cells in the absence of a carbon source, Nomura and Nakamura 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, and that these cells showed no metabolic impairment. It was claimed, on the basis of these data, that, since trypsin rescue requires little or no metabolic activity, the "killing" in the first place involved little direct cellular damage. However, this applies only to the action of colicin on resting cells. In fact, the data on growing cells show that either trypsin does not rescue all cells or that recovery takes time. In summary, colicin K235-K stops all macromolecular synthesis, but the effects are reversed if the colicin is removed by trypsin.

Colicin ML-E_1. The first colicin to be studied in any detail was ML-E_1 by Jacob et al. (74) in 1952. They showed that excess colicin inhibited DNA and RNA synthesis and cell growth immediately. Respiration ceased to rise and remained constant for 20 min before falling. The reproduction of a virulent phage was also inhibited if colicin was added soon after infection. Thus, this colicin may act similarly to colicin K235-K.

Colicin E_2. Colicin CA42-E_2 was studied by Reynolds and Reeves (90), and an unspecified colicin E_2 was studied by Endo et al. (12) and Nomura (81). Colicin CA42-E_2 (previously classified as type F) stops DNA synthesis within 2 min, RNA within 5 to 6 min, and protein after 10 to 15 min. Thus, in this instance the metabolic shut-down is not immediate. Following the work of Nomura and Nakamura (82), the degradation of DNA in the same system was studied, and was first detected at 5 min and continued steadily for 50 min when 50% of the DNA was degraded. The remaining 50% remained precipitable by

the Schmidt-Thannhauser technique for up to 150 min (*unpublished data*). The lethal effects of this colicin are reversible by trypsin up to 10 to 15 min after the addition of colicin, the viable count returning to the original within experimental error. If the cells are pretreated with 2,4-dinitrophenol before addition of colicin, they can be rescued indefinitely, and Nomura (81) suggested that this may be owing to the inhibition of DNA degradation by this agent by analogy with his observations on colicin E_2.

Nomura (81) showed that at a high multiplicity E_2 inhibits DNA and RNA syntheses within 5 min and protein within 10 to 15 min, and, in contrast to colicin K235-K and E_3, extensive DNA degradation is observed, only 10% of the DNA remaining precipitable after 80 min. At low multiplicities, these syntheses are not detectably inhibited, although unfortunately detailed data are not given. Colicin E_2 has little effect on the propagation of T4 by sensitive cells, and T4 or T5 infecting cells 25 min after colicin adsorption leads to a marked DNA synthesis. The use of labeled phage showed that there was very little degradation of T4 DNA after infection of colicin-killed cells. In addition, colicin E_2 and colicin CA42-E_2 actually induce the development of λ in lysogenic strains; at low multiplicities, this accounts for all the cells killed. At higher multiplicities, however, the plaque-forming ability was also destroyed. In the case of E_2 and CA42-E_2, the primary effect appears to be on bacterial DNA; both its synthesis and its transcription to RNA are inhibited, leading to its degradation. The ability of T4 to propagate in such cells indicates that there is no further derangement, the cessation of bacterial protein synthesis probably being a secondary effect.

Once again our knowledge is restricted to macromolecular synthesis and break-down, and the nature of the colicin action on DNA is unknown, although the rapidity of the cessation of RNA synthesis makes it unlikely that this is due directly to the break-down of the DNA.

The fact that sensitive cells become immune when infected with other E_2 C-factors must also be taken into account. Nomura (81) presented evidence that this immunity is due to a cytoplasmic factor, because its development in newly colicinogenic cells takes about 15 min and requires metabolism but not DNA synthesis. Since there are several chromosomes per cell and almost certainly not as many C-factors are transferred, the immunity cannot be due to direct association of a C-factor and the chromosome.

Colicin E_3. Colicin E_3 was studied by Nomura (81) and compared with E_2 and K. Very little experimental detail is available, but it is stated that at a high multiplicity E_3 inhibits protein synthesis but not DNA and RNA synthesis, both of which continue for at least 30 min. As with E_2, this inhibition did not occur at low multiplicities, and the reproduction of T4 was relatively resistant to colicin. Like colicin K235-K, colicin E_3 did not lead to DNA degradation and did not induce phage λ, and its lethal effects were reversible by trypsin over long periods of time. In this instance, the fact that colicin specifically stops bacterial protein synthesis but not that associated with T4 infection is of particular interest, as the two metabolisms are not known to differ at the protein synthesis level.

Pyocin C.10. Jacob's data (71) for the only pyocin which has been studied fit into the same general pattern, the normal rise in optical density stopping immediately after pyocin C.10 was added to a log-phase culture of a sensitive strain of *P. pyocyanea*. The respiration rate actually fell slowly from the moment pyocin was added. Pyocin was also able to completely destroy the plaque-forming ability of the sensitive strain previously infected with the virulent phage P_2. In all aspects where the two sets of data are comparable, the action of pyocin C.10 and colicin K are the same.

Bacteriocins of gram-positive bacteria. It has been suggested, partly on the basis of their mode of action, that bacteriocins of gram-positive bacteria differ from those of the gram-negative bacteria (52). It is thought that megacin acts on the cell membrane of sensitive cells (58, 67). Within 10 to 15 min after addition of megacin 216 to sensitive cells, there is a marked drop in respiration (65) and leakage of cell contents (67), leading eventually to conversion to ghosts. Both whole cells and protoplasts are affected, but there is no effect on isolated cell membranes or cell walls (58, 67). There have been no studies on other aspects of metabolism.

In the case of megacin C, studied by Holland (59), there is a break-down of the sensitive cells' DNA and induction of lysogenic bacteriophage. RNA and protein synthesis stop, but there is no break-down. Thus, megacin C resembles colicin E_2 in its mode of action, and suggests that at least some of the bacteriocins of the gram-positive bacteria may resemble those of the gram-negative bacteria.

Conclusions on Mode of Action of Bacteriocins

It has been necessary to summarize the data on the effects of each bacteriocin studied because of the difficulty of drawing any final clear-cut conclusion.

The studies on the kinetics of killing by colicins, one pyocin, and one megacin, show that killing

begins as soon as the bacteriocin is added to a culture, and suggest that all bacteriocins are bactericidal, as opposed to bacteriostatic. These and other studies suggest that the first stage in killing is specific irreversible adsorption of the bacteriocin, and that, in some instances at least, only one molecule may be required to kill a sensitive cell.

The studies on the effect of several colicins, pyocin C.10, and megacin C, all show a very rapid effect on some or all macromolecular syntheses. However, there appear to have been no studies on the effect of bacteriocins on intermediary metabolism, which may, in part at least, account for the belief that they act primarily on macromolecular syntheses. The different colicins, although they all apparently act on macromolecular syntheses, display considerable variation in detail, suggesting different primary effects. The fact that, in the three cases studied, sensitive cells can be rescued by removal of colicin from the cell surface by trypsin suggests that colicin exerts its action while attached to the outside of the cell. In two cases, K235-K and E_3, the trypsin rescue was still possible long after colicin was added.

In the case of studies on megacin 216, the long lag before megacin causes lysis and the absence of any observed effect on isolated protoplast membranes suggest that the effect on the membrane may be indirect. Studies on metabolism of megacin-treated cells may reveal the primary effect.

It is impossible at this stage to generalize very much on this most important aspect of bacteriocins, although their mode of action will form a basis for some of the speculations below.

GENERAL CONCLUSIONS

Concept of Bacteriocins

The colicins were early recognized as being quite different from other antibiotics, and as more bacteriocins have been discovered the concept has developed of bacteriocins as a distinct class of biological compounds. This review, like all previous ones, has relied on the few well-studied examples and, on the basis of these, the concept arises of bacteriocins as a class of specific proteins, associated with lipopolysaccharide in the case of some gram-negative bacteria, and active generally on certain other strains of the same and closely related species. Some bacteriocins are known to be determined by cytoplasmic factors, the presence of which not only determines the ability to synthesize the bacteriocin, but also confers on the cell resistance to the action of the particular bacteriocin. The synthesis of many bacteriocins is inducible by ultraviolet irradiation and certain chemicals known to be mutagenic or carcinogenic, or to induce lysogenic bacteriophage in other circumstances.

Where studied, synthesis of a bacteriocin seems to be lethal for the producing cell. Bacteriocins kill by adsorption onto the cell surface and disruption of the cells' macromolecular syntheses (megacins, staphylococcins, and cerecins may be an exception here). The disruption is perhaps maintained only as long as the bacteriocin remains adsorbed to the surface. Kinetic studies show that only very few molecules are required to kill.

Most of the bacteriocins have been classified as such only on the basis of the type of activity spectrum, and only time will tell whether all the above properties will apply to all of them. Suffice it to say that so far no real exceptions have been found.

The bacteriocins, then, differ markedly from the "classical" antibiotics, most of which are of relatively low molecular weight and are probably analogues of some metabolite, acting by competitively occupying the active sites of enzymes, and sometimes acting on distantly related species by interference with some specialized part of their metabolism.

Bacteriocins probably do not act in this way, and their biological significance is discussed in the next section.

General Similarities among Bacteriocins, Their Determinant Factors, and Other Entities

Many authors have pointed to the similarities among bacteriocins, their bacteriocinogenic factors, bacteriophages, and fertility factors (17, 30, 31). These similarities fall into three general types. All bacteriocins, bacteriophages, and fertility factors probably function through the use of specific "receptors" on the bacterial cell surface. These receptors are of many specificities and in some instances the same receptor may serve for both a bacteriophage and a colicin's adsorption or for a fertility system and a bacteriophage (76). It is probable that more such cross-reactions will be discovered. The second similarity lies in the fact that in some cases, at least of bacteriocins, as in the case of fertility factors and bacteriophages, the genetic determinant is a small piece of DNA capable of replicating independently of the bacterial chromosome. In some cases, these genetic units can be identified as episomes. Finally, there is the similarity in the induction behavior of the inducible bacteriophages and bacteriocins.

These similarities suggest some relationship between the three entities, but the details are

difficult to elucidate. The remainder of this review is speculation on this point, much of it, of course, profiting from the speculations of previous authors.

Biological Significance of Bacteriophages, Bacteriocinogenic Factors, and Fertility Factors

Before considering their interrelationship, we should perhaps briefly note the biological nature of each of these factors, all three of which, when determined by transmissible genetic factors, can have an independent existence and respond to natural selection.

From an ecological viewpoint, virulent bacteriophages are clearly parasites, their only effect on a bacterial cell being to kill it, with death usually followed by self-replication within its cytoplasm. Their existence must lead to continual selection among bacteria for the loss of any receptor involved.

Bacteriocinogeny is probably of considerable selective advantage to bacteria, although suitable ecological studies have yet to be done. The few in vivo studies which have been carried out with colicins were discussed by Fredericq (31). The fact that the actual synthesis of bacteriocin may be lethal would not detract much from its selective advantage, because, if only a very small percentage produce bacteriocin and die, a very small advantage to the other members of the clone will offset this and give the bacteriocinogenic property a selective advantage. Because in most environments dispersal of a clone will show a strong tendency to be localized, the production of colicin by a few will, by killing nearby competing bacteria, improve the prospects of the clone and give the bacteriocinogenic property a selective advantage. C-factors also confer immunity to the same bacteriocin produced by other clones. Thus, bacteriocinogenic factors will survive because of the selective advantages they confer on their hosts. Any chromosomal genes determining colicinogeny would, of course, be selected in the same way. The existence of bacteriocins must also lead to selection for the loss of the receptors on the sensitive cells.

Temperate phages are also parasites and kill a cell when they replicate in the vegetative phase. In contrast to virulent phages, they also confer some advantages on their hosts, because when they become prophage, their presence confers on the lysogenic strain the same advantages discussed above for bacteriocinogeny. Since the bacteriophage adsorbs by the same receptor, regardless of whether it enters the vegetative or prophage phase, there may be selection exerted on the bacteria both for and against the presence of the receptor. The ability of some temperate phages to transduce host genes may be of some, perhaps paramount, importance here.

The F-factor of *E. coli* K-12, the only one of which we have much detailed knowledge, confers on cells the ability to conjugate and act as a genetic donor to compatible cells. This involves complementary recognition sites on the two cells, enabling mate recognition and facilitating appropriate responses to ensure chromosome transfer and integration. The advantages of genetic recombination to a species have long been appreciated by students of plants and animals, and presumably apply equally to bacteria. These advantages are basically the ability to rearrange the genetic material of the species' gene pool, which gives the species flexibility in response to natural selection, enabling the rapid formation and selection of desirable character combinations. The effect of natural selection at the level of the F-factor itself is not clear, but its ability to confer fertility on cells has presumably led to its maintenance by natural selection. The topic of episomes and evolution was discussed recently by Hayes (55).

Specific Receptor

The primary function of the receptors on the cell surface is not known. They may be the inevitable surface structure of the cell wall, their use as receptors being superimposed on this. In this case, their variety may be a polymorphism maintained by the interactions of the various bacteriophages and the host strains carrying the various specific receptors. However, they do, as receptors, perform some beneficial functions, and may be primarily specific recognition sites. If this is so, then it must be their role as recognition sites in conjugation and perhaps for temperate phage which maintains them under natural selection, their use by virulent phage and bacteriocins being purely deleterious to the bacterium.

Interrelation of the Various Episomes and Plasmids

Jacob's (72) clarification of the genetic units of bacteria brought about in part by the coining of the word "replicon," has considerably simplified this section. He suggested that there is a locus called a replicator controlling the replication of each genetic unit of bacteria, be it a chromosome, plasmid, or episome, such as a phage genome, F-factor, or bacteriocinogenic factor. It is this locus, together with any regulator locus affecting it, which determines when the replicon actually replicates. It is their different regulatory

mechanism at this level which distinguishes the chromosome and the various episomes or plasmids, determining, for instance, that an F-factor or C-factor can replicate in phase with the host when unattached to the chromosome, whereas a bacteriophage cannot. Since translocation (or recombination) within the cell can lead to genes being transferred from one replicon to another, any replicon may carry genes which originated on a different type of replicon. Thus, genes determining bacterial enzymes have been observed to become part of an F-factor or a bacteriophage genome, although they were originally on the chromosome (55).

Some of these translocations are observed to occur relatively readily, but there may be no limit to those which can occur. For this reason, the fact that some episomes can determine both fertility and colicinogeny may be fortuitous, due to two independent factors being combined by past recombination. Likewise, the fact that all F-factors and bacteriocinogenic factors which have been studied are determined either by plasmids or episomes could also be due to translocation of some of the properties from one type of replicon to another, and does not necessarily indicate any homology between the two types of factor. One should also bear in mind that the methods used to determine the genetic basis of bacteriocinogeny are more likely to succeed with those that are episomal or plasmid in nature; any determined by chromosomal loci would probably remain unstudied.

The fact that genes can be transferred from one replicon to another means that the presence of a gene on an episome or chromosome is probably the result of present natural selection for the most favored arrangement and does not indicate the situation in which the gene originated. In considering the origin of bacteriocins, then, we are on better ground if we confine ourselves to their nature and activities rather than their genetic determination.

Possible Relationships of Bacteriocins to Other Entities

A striking fact about bacteriocins, which needs explanation, is that, with the exception of some produced by gram-positive bacteria, they can only act against closely related species. Since one would imagine that bactericidal properties against other unrelated species would also be advantageous to the producing cell, it seems that this limitation must be inherent and that most bacteria are incapable of making bacteriocins except those active on their relatives. This implies that the specific interaction between bacteriocin and sensitive cell is a result of evolution within the species, perfected as a result of natural selection for some mutual advantage accruing to both cells with the complementary specificity. Because it is inconceivable that this could apply to the observed effects of the interaction between bacteriocin and sensitive cell, this specificity must have arisen for some other recognition system, probably a fertility system. On this hypothesis, bacteriocins are fertility recognition sites, which would have to have been protein in nature, modified by subsequent natural selection to perform a purely bactericidal function.

This hypothesis would account for the remarkable sensitivity of bacteria to bacteriocins. A single protein molecule which remains attached to the outside of the cell can interfere so grossly with the cell's metabolism as to kill the cell. It seems incredible that bacteria would be sensitive to such an adsorbed molecule unless their death were due to a specific active response which is in some way miscarried and leads to death. If this hypothesis is correct, then the same may apply, in part at least, to the initial disruption of cell functions by the T-even phages, as this can apparently be produced merely by an adsorbed phage ghost without any DNA content (8), and is thus similar to the effect of bacteriocins.

Colicin, if derived from a fertility recognition factor, must have been modified to elicit a response from the sensitive cell, which, instead of leading to satisfactory recombination, results in cell death. It is, of course, the advantage of retaining the ability to mate and undergo genuine recombination which maintains the specific receptors and responses of the sensitive cell, despite their concurrent disadvantages.

The observation (90*a*) that conjugating bacteria produce less β-galactosidase than nonconjugating and the recent observation of Clowes (10) that some Hfr strains can kill F^- strains during conjugation lends some support to this hypothesis.

If colicins have indeed evolved from fertility recognition sites, then the occurrence of colicin activity associated with cell-wall components is readily understood, this being the natural site of any cell recognition system. The association with cell-wall material may itself explain the fact that colicin synthesis is often a lethal synthesis occurring naturally in only a few bacteria of a clone, as perhaps only dead and dying cells liberate cell-wall components.

If this hypothesis can withstand experimental study, it would suggest that the existence of many different receptors on the cell surface indicates a complex compatibility system for conjugation. This would also be indicated by the fact that the known colicins act equally on F^+

and F⁻ strains, and, in fact, it has not even been possible to speculate whether colicins simulate donor or recipient receptors.

Fredericq (30), in addition to discussing the similarities between C-factors and F-factors, considered their relation to bacteriophage. Here one is in the dilemma that, although the C-factor produces a lethal protein as does a virulent phage, it can replicate in phase with the chromosome like a temperate phage, although apparently remaining separate from it. These two properties never coexist in one phage. Although the C-factor appears to be closest to the F-factor, there may well have been considerable genetic exchange between the various genetic elements, and Fredericq (30) suggests that virulent phage may have arisen by addition of a C-factor to the genome of a temperate phage.

To summarize, then, it is hypothesized that bacteriocins are derived from fertility recognition sites, are protein in nature, and are modified so as to elicit a lethal response from cells with the complementary site, and that the synthesis of these bacteriocins is now controlled so as to occur to excess, but in only some cells, leading to cell death and release of the bacteriocin free in the medium by this minority of cells. It is suggested that this accounts for the restriction of their activity to related species, their occurrence associated with cell-wall components, and their observed mode of action.

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