# THE NATURE OF COLICIN K FROM PROTEUS MIRABILIS*

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Since the discovery of colicinogeny by Gratia in 1925, *Enterobacteriaceae* have been found to produce at least 17 different types of colicins (1, 2). Four of these have been isolated and characterized, colicins K, V, A, and SG 710 (3–7). In each instance the bacteriocin has proved to be a protein-lipocarbohydrate complex identical with the somatic O-antigen of the colicinogenic microorganism. The bactericidal activity of these substances is readily destroyed by proteolytic enzymes. In the case of colicin K it was shown that the activity was carried by the protein moiety of the complex (4). It has been suggested therefore that colicins are proteins or protein like substances which, in their native state, are conjugated with the O-antigens of the bacteriocin-producing bacilli (4, 8).

More recent studies of bacteriocins produced by microorganisms irradiated with ultraviolet light or grown in presence of mitomycin C have corroborated this hypothesis. Thus, after irradiation, *Escherichia coli* strain CA 42 secreted colicin F (now termed $E_2$-CA42) which proved to be a protein containing but a small quantity of a carbohydrate (9). After induction with mitomycin C, both the colicinogenic strains of *Proteus mirabilis* Col $K^+$ and of *E. coli* K 12 Col $K^+$ produced a bacteriocin, colicin K, which consisted only of protein (10, 11). Similarly, *E. coli* strains JC 411 Col $E_1^+$, W 3110 Col $E_2^+$ and W 3110 Col $E_3^+$ elaborated their bacteriocins as proteins of low molecular weight in presence of the antibiotic (12, 13).

The study to be reported here is concerned with the nature of the colicin K derived from *Proteus mirabilis* Col $K^+$. It will be shown that the bacteriocin elaborated by the colicinogenic *Proteus* in the presence of mitomycin C is a protein of low molecular weight having the same immunological and bactericidal specificities as colicin K derived from the *E. coli* K 235 bacillus. It differs, however, from the latter in that it is not conjugated with other antigens of the *Proteus* or K 235 bacilli. We shall see that the purified *Proteus* bacteriocin forms but a single boundary when tested by electrophoresis or by ultracentrifugation. Upon electrophoresis in polyacrylamide gel or electrofocusing it forms, how-

ever, several protein bands. It appears, therefore, that the colicin K produced by *Proteus mirabilis* exists in several forms which differ in electrical charge.

## Materials and Methods

*Microorganisms.*—The colicinogenic strain of *E. coli* K 235 L⁺O Col K⁺ which served as a source of the colicinogenic factor K and the *E. coli* B strain used as the indicator for colicin K were obtained from Dr. W. F. Goebel of The Rockefeller University. The strain of *E. coli* K 12 Hfr Me⁻ was given us by Dr. N. D. Zinder. *Proteus mirabilis* culture was provided by Dr. J. Marmur of the Albert Einstein College of Medicine, Bronx, N.Y. Mutants of these microorganisms resistant to colicin K, to streptomycin, or to sodium azide were isolated during the course of this study. The coliphages T1–T7 were originally obtained from Dr. Mark H. Adams, whereas the male specific phage f2 was given us by Dr. Zinder.

*Antisera and Bacteriocins.*—Antisera to the colicinogenic (Col K⁺) and noncolicinogenic (Col K⁻) strains of *Proteus mirabilis* were obtained by the intravenous injection of rabbits with graded doses of chloroform-killed bacteria containing $2 \times 10^8$–$10^{10}$ cells per dose. Injections were given two to three times weekly for a period of 2 months and the animals were then bled a week later. Antiserum to crude *Proteus mirabilis* colicin K was obtained from rabbits injected with graded doses of a bacteriocin solution containing 20–500 μg per dose Antisera to *E. coli* K 235 L⁺O Col K⁺ and L⁻O Col K⁻ and to the colicin K derived from mitomycin C induced *E. coli* K 235 L⁺T Col K⁺ were given us by Dr. Goebel, and was a sample of colicin K derived from the induced K 235 bacilli.

*Bacteriocin Assays and Immunological Tests.*—The assays of the bacteriocin were performed by the ring technique using *E. coli* B as an indicator (14). One unit of colicin K is defined as the minimum quantity of the bacteriocin contained in 1 ml of a solution, 0.02 ml of which, when deposited upon nutrient agar seeded with $5 \times 10^7$ *E. coli* B cells, would inhibit completely the growth of the indicator bacteria. The specific activity of a given preparation is defined as the number of units of bacteriocin per mg of material. Colicin neutralization and precipitin tests were carried out as described previously (6). The immunodiffusion tests were performed by the technique of Ouchterlony (15).

*Analytical Methods.*—Absorption spectra were measured in a Beckman recording spectrophotometer model DK 2. Free boundary electrophoresis was performed in a Tiselius apparatus using a 2 ml cell (16). Ultracentrifugal determinations were carried out in a Spinco analytical centrifuge, Model E. Gel electrophoresis was performed in a vertical cell (EC 470, E-C Apparatus Corp., Philadelphia, Pa.). Isoelectric fractionation was performed in an electrofocusing column of 440 ml capacity (LKB Producter, AB, Stockholm, Sweden) (17).

Nitrogen, carbon, and hydrogen were determined in a Perkin-Elmer Elemental Analyzer, Model 240 by the technique of Condon (18). Phosphorus was quantitated colorimetrically by the method of Allen (19). Amino acid analyses were carried out on hydrolysates of the colicin (6 N HCl, 20 hr, 110°C) in an automatic amino acid analyzer (Beckman, model 120C) using the procedure of Spackman, Stein, and Moore (20). For the determination of ethanolamine, the hydrolysates were chromatographed on a 50 cm column for the analysis of physiological fluids (20). Tryptophan was quantitated spectroscopically by the method of Goodwin and Morton (21). Lipids were determined gravimetrically by weighing the chloroform-soluble material liberated on hydrolysis of the preparation under investigation for 10 hr in 1 N HCl. Carbohydrates were quantitated by the anthrone reaction, using glucose as a standard (22).

## EXPERIMENTAL

*Isolation of a Colicinogenic Strain of Proteus mirabilis.*—One of the goals of this study was to ascertain the nature of the colicinogenic factor K, an extra-

chromosomal gene which controls the synthesis of colicin K. To facilitate its detection it was necessary to transfer this factor from its original carrier *E. coli* K 235 to a bacterium having deoxyribonucleic acid of a different buoyant density than that of the colon bacillus. For this purpose *Proteus mirabilis* was chosen. Attempts to conjugate the *Proteus* bacillus directly with *E. coli* K 235 were unsuccessful, for the frequency of transfer of the Col K factor was less than $10^{-6}$. A search was made for an intermediate host, a bacterium which would accept the Col K factor from the K 235 bacillus and which in turn would transfer it to *Proteus*. It was found that the factor could be transferred from the K 235 bacterium to the colicin-sensitive strain of *E. coli* K 12 in the following manner.

5 ml of an actively growing culture of a colicin-sensitive azide-resistant variant of *E. coli* K 12 Hfr ($2 \times 10^7$ B/ml) were mixed with an equal volume of *E. coli* K 235 L$^+$O Col K$^+$ ($10^6$ B/ml). The mixed culture was aerated for 16 hr at 37°C, and then diluted so as to contain $5 \times 10^6$ B/ml. 0.1 ml portions of the dilution were spread on nutrient agar plates containing 0.03 M sodium azide and the plates were incubated for 10 hr at 37°C. About 1000 microcolonies developed on each plate. To detect the colicinogenic colonies, the plates were overlayered with the indicator *E. coli* B and incubated for 6 hr. Only 1% were found to produce colicin. Of these 20 were isolated and purified by repeated single colony transfers. All were found to be sensitive to the T2, T3, T4, T6, and T7 viruses and resistant to T1 and T5, as was the original K12 strain. 80% of the isolates proved to be resistant, however, to the male specific phage f2, a fact which indicates that the Hfr factor of the K12 bacillus is partially excluded by the Col K factor.

One of these strains, *E. coli* K12 Col K$^+$f2$^R$ Az$^R$, was used as a donor of the colicinogenic factor K to the streptomycin resistant variant of *Proteus mirabilis*. A nutrient broth culture containing $5 \times 10^8$ cells per ml of each donor and recipient strains was aerated for 2 hr at 37°C and portions containing $10^5$ cells were spread on nutrient agar plates containing 200 $\mu$g of streptomycin per ml. Upon incubating the plates for 10 hr, about 25,000 microcolonies grew out on each. The plates were replicated, then sterilized with chloroform vapor, and overlayered with soft agar seeded with the colicin sensitive strain of *E. coli* B Str$^R$. After incubation, 17 zones of inhibition were found on 8 of the plates. Thus the cells containing the Col K factor occurred in the Proteus progeny with a frequency of $8 \times 10^{-5}$. The location of the inhibition plaques was marked on the replicate plates, colonies were then picked from these areas and suspended in broth. Samples of each suspension containing about 200 cells were spread on streptomycin-containing plates and 8 colicinogenic colonies were isolated by the replica technique. The isolates were then purified by streaking on agar plates and single colonies were transferred to slants.

One of the isolates was selected for further study. This strain proved to be resistant to the T phages which lyse *E. coli* K 12 bacillus. The heat killed cells of this bacterial strain were agglutinated by an antiserum to the noncolicinogenic *Proteus mirabilis* at the same titer as were heated homologous bacteria. The colonies of this strain formed an inhibition zone of 10–14 mm in diameter when overlayered with *E. coli* B; the inhibition zone was absent or very narrow when the colonies were overlayered with colicin-resistant strain of the colon bacillus, or with agar containing the colicin-sensitive indicator bacteria and

antiserum to colicin K derived from *E. coli* K 235. Thus, the strain in question proved to be *Proteus mirabilis* which elaborated a bacteriocin having the same specificity as the colicin K produced by the K 235 bacillus.

The colonies of the colicinogenic *Proteus* were found to contain between 5 and 20% of noncolicinogenic cells. The latter could not be completely eliminated by repeated transfer of the colicinogenic colonies. Apparently the colicinogenic factor is not transmitted to all progeny when the bacillus divides. Since the proportion of noncolicinogenic cells increased steadily on subculturing, the colicinogenic variant must occasionally be reisolated in order to maintain the strain bacteriocinogenic.
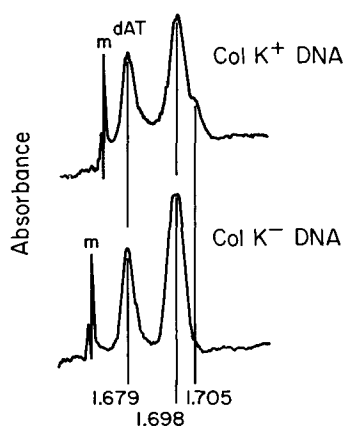


Fig. 1. Microdensitometer tracings of banding patterns of DNA derived from *Proteus mirabilis* Col K$^+$ and Col K$^-$ bacilli in CsCl gradient. Col K$^+$ DNA, DNA from *P. mirabilis* Col K$^+$; Col K$^-$ DNA, DNA from *P. mirabilis* Col K$^-$; dAT, reference band containing copolymer of deoxyadenylic and deoxythymidylic acids; m, meniscus.

*Properties of the Deoxyribonucleic Acid of Proteus mirabilis.*—To ascertain whether the colicinogenic *Proteus* contains genetic elements of the colon bacillus, the deoxyribonucleic acids of both the colicinogenic and noncolicinogenic variants of the *Proteus* were isolated and analyzed by density gradient centrifugation in CsCl solution. Since mitomycin C increases the quantity of the colicinogenic factor in the *Proteus* bacilli, induced cultures were used for the isolation of DNA (23).

2 liter cultures of the colicinogenic and noncolicinogenic variants of the *Proteus* bacillus were grown in casamino acid–glucose medium (4) at pH 7.0 to a concentration of $2 \times 10^9$ cells per ml. An aqueous solution of mitomycin was then added (1 mg/liter) and the aeration was continued for an additional 2 hr. The cells were collected and washed with 0.15 M NaCl containing 0.1 M ethylenediaminetetraacetic acid (EDTA) at pH 8.0. The deoxyribonucleic acid was isolated from the cells according to the procedure of Marmur (24). The yield varied between 3 and 5 mg.

For ultracentrifugal analyses stock solutions of the *Proteus* nucleic acids were diluted with 0.15 M NaCl–0.015 M Na citrate buffer at pH 7.0 to contain 4 μg DNA/ml. The dilution was adjusted to a specific density of 1.718 by adding 1.324 g of CsCl/ml. To this was added 1 μg of copolymer of deoxyadenylic and thymidylic acids as a marker. The mixture was spun in a Spinco analytical ultracentrifuge at 44,000 rpm for 24 hr at 20°C. Photographic recordings of the ultracentrifugal patterns were made and scanned in a Joyce, Loebl microdensitometer. The tracings are shown in Fig. 1.[1]

It may be seen that the DNA of the noncolicinogenic *Proteus* formed but a single symmetrical band having a buoyant density of 1.698. The DNA of the colicinogenic strain, on the other hand, separated partially into two bands. The major component had the density of the *Proteus* DNA, but the minor compo-
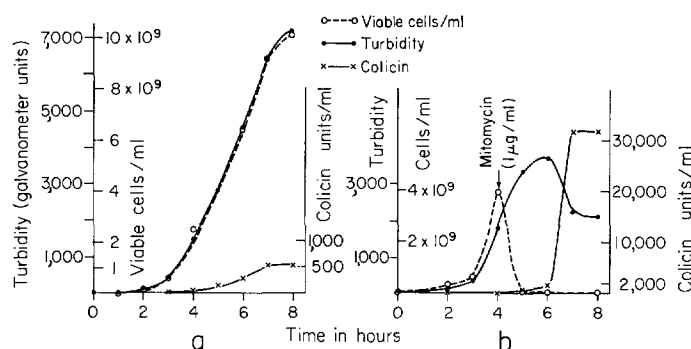


Fig. 2. Effect of mitomycin C on the production of colicin K in *Proteus mirabilis* Col K⁺ cultures. (*a*) Production of colicin in absence of mitomycin. (*b*) Production of bacteriocin in cultures induced with 1 μg/ml of mitomycin. In each instance the colicin content was determined in the cell-free supernatant of the culture.

nent had a slightly higher density of 1.705. Since the buoyant density of the deoxyribonucleic acid of the colon bacilli is higher than that of *Proteus*, we assume that the satelite DNA present in *P. mirabilis* Col K⁺ is nucleic acid derived from *E. coli* K 235 containing the colicinogenic factor K.

*Isolation of Crude Proteus Colicin K.*—When the colicinogenic *Proteus mirabilis* is grown in casamino acids–glucose medium at pH 7.0, it secretes from 500 to 1000 units of the bacteriocin per ml during its late phase of logarithmic growth (Fig. 2 *a*). When the culture is induced with mitomycin C, however, the yield of the bacteriocin increases to 5,000–40,000 units/ml in the cell-free medium (Fig. 2 b). The bacteria were grown and the crude bacteriocin was obtained in the following manner.

Two 2 liter portions of casamino acid–glucose medium (4) were seeded with 10 ml of a glycerinated colicinogenic *Proteus* culture containing 5 × 10¹⁰ cells. The cultures were aerated

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at a rate of 4 liters/min at 37°C and the pH was maintained at 7.0 by adding 1 M Na$_2$CO$_3$ with automatic titrators (Radiometer Co., Copenhagen, Denmark). When the concentration of cells reached 4 $\times$ 10$^9$ bacteria/ml, 2 mg of mitomycin C in aqueous solution was added to each. Tributyl phosphate was used to control foaming. Aeration was continued for 4 hr and the bacterial growth then terminated by adding 20 ml of chloroform. The cultures were stored overnight at 4°C and then spun to remove the bacteria. The cell-free supernatant was adjusted with HCl to pH 4.3 and the precipitate was collected by centrifugation. The sediment was suspended in water, adjusted to pH 8.6, and spun to remove insoluble material. The supernatant was now fractionated by adding ammonium sulfate to 33, 66, and 100% saturation. After each addition, the precipitate was collected, redissolved at pH 8.6, dialyzed, and lyophilized. Fraction II, which precipitated between 33 and 66% saturation of ammonium sulfate, contained the major portion of the bacteriocin.

The yield of the crude colicin varied between 50 and 200 mg and its specific activity ranged between 25,000 and 400,000 units per mg.[2]
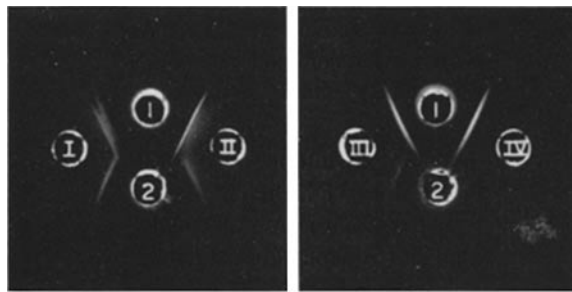


FIG. 3. Gel diffusion reactions of *Proteus mirabilis* colicin K at various stages of purifica tion. Wells I–IV contain solutions of colicin preparations at concentrations of 1 mg/ml. I crude colicin; II, colicin purified on Sephadex G-200; III, colicin purified on DEAE-Sephadex; IV, colicin purified on CM-Sephadex. Wells 1 and 2 contain antisera to *P. mirabilis* Col K$^+$ and Col K$^-$ bacilli, respectively.

*Chromatographic Purification of Proteus Colicin K.*—Crude *Proteus* colicin K is heavily contaminated with other constituents of the bacillus. The impure substance produces several precipitin bands when tested by immunodiffusion with antisera to the colicinogenic and noncolicinogenic variants of the *Proteus* bacillus (Fig. 3, well I). To remove the contaminating antigens, the crude colicin was purified chromatographically on G-200, diethylaminoethyl (DEAE)- and carboxymethyl (CM)-Sephadex columns as shown in Fig. 4. In each instance, the effluents having a high bactericidal activity were combined, as indicated in each diagram by a bar, and precipitated with ammonium sulfate at 70% saturation. The precipitates were redissolved at pH 8.6, dialyzed, and lyophilized.

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[2] An additional amount of crude bacteriocin can be obtained by extracting the bacterial pellet with 1 M NaCl containing 0.01 M potassium phosphate buffer at pH 7.5 (13). This material can be purified by precipitation with ammonium sulfate as described above. Usually, about 100 mg of crude bacteriocin is obtained from the cells harvested from 4 liters of an induced *Proteus* culture.
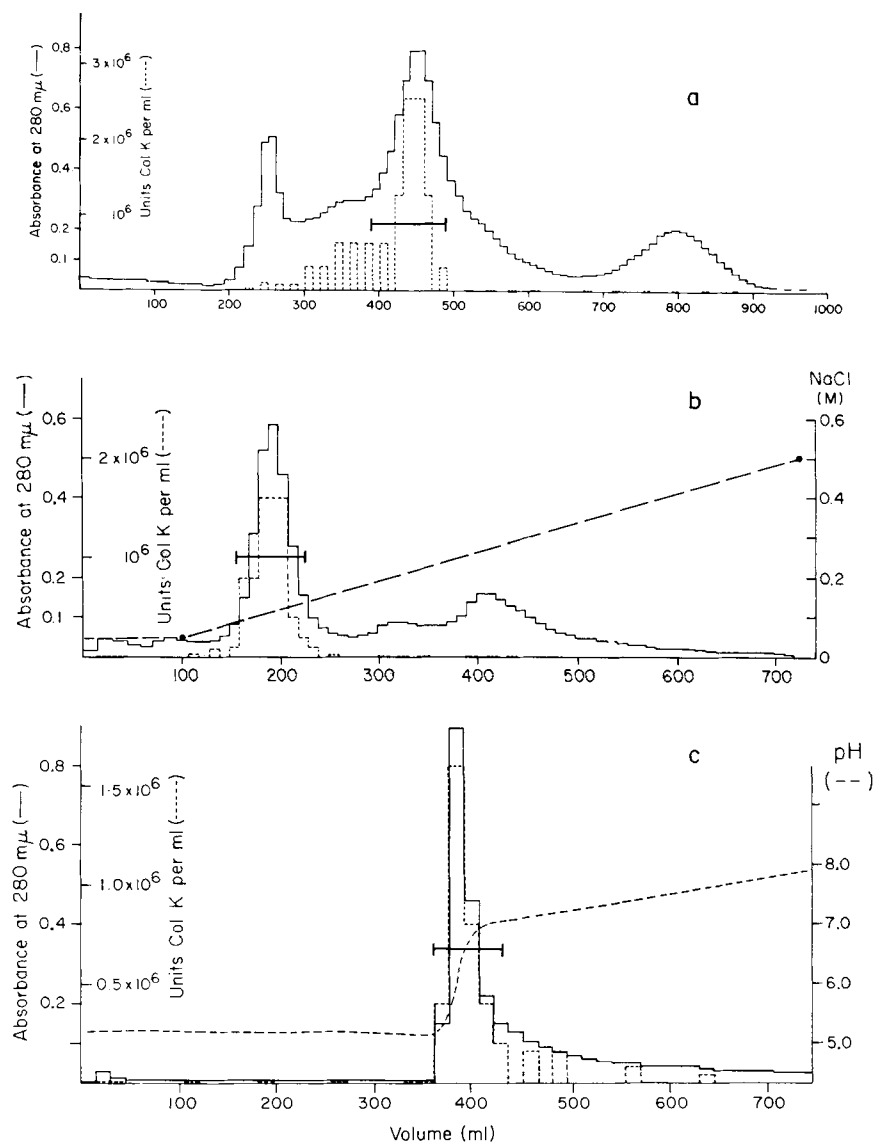
FIG. 4. Chromatographic purification of Colicin K from *P. mirabilis.* (*a*) Elution profile of crude colicin from Sephadex G-200 column. Bed dimensions: 4.5 × 50 cm. Eluant: 0.025 M Tris buffer, pH 7.5, containing 0.3 M NaCl. Sample: 250 mg of crude colicin K in 11 ml buffer. Yield: 72.7 mg. Specific activity: 400,000 units/mg. (*b*) Elution profile of Sephadex G-200 purified colicin from DEAE-Sephadex A-50 column. Bed dimensions: 2 × 12 cm. Eluant: gradient from 0.05 M NaCl–0.05 M Tris buffer, pH 7.5, towards 0.5 M NaCl–0.05 M Tris Buffer, pH 7.5. Sample: 70.0 mg of Sephadex G-200 purified colicin in 10 ml of the first buffer. Yield: 31.0 mg. Specific activity: 800,000 units/mg. (*c*) Elution profile of DEAE-Sephadex purified colicin K from CM-Sephadex C25 column. Bed dimensions: 2 × 12 cm. Eluant: gradient formed by mixing 300 ml 0.05 M NaH$_2$PO$_4$ and two 300 ml portions 0.05 M Na$_2$HPO$_4$ in three chamber gradient mixer. Sample: 83.2 mg DEAE-purified colicin in 10 ml of 0.01 M NaCl at pH 4.0. Yield: 28.6 mg CM-Sephadex-purified colicin. Specific activity: 800,000 units/mg.

The yields and the specific activities of the substances so obtained are given in Fig. 4. The immunodiffusion patterns of these materials are presented in Fig. 3. As may be seen in the photograph, the colicin obtained upon filtration through Sephadex G-200 still contained several antigens (Fig. 3, well II). The major part of the contaminating antigens could be removed by fractionation on DEAE-Sephadex. The colicin eluted from this column formed a heavy precipitin line with an antiserum to the colicinogenic *Proteus* and a weak line with an antiserum to the noncolicinogenic variant of this bacillus (Fig. 3, well III). Apparently the former was produced by the colicin, whereas the latter was formed by an antigen common to both variants of the *Proteus* bacillus. The latter antigen could be separated by fractionation on the cation exchanger, CM-Sephadex. In this instance, the contaminating *Proteus* antigen was not absorbed, whereas the colicin was eluted from the column when the pH of the effluent changed from 5.1 to 7.0. As shown in the Fig. 3, well IV, the colicin purified in this manner now formed but a single precipitin line when tested against an antiserum to the colicinogenic *Proteus* bacillus and failed to react with an antiserum to the noncolicinogenic variant.

It was possible that the colicin purified as CM-Sephadex might still be contaminated with serologically inert material.

A solution of 20 mg of the bacteriocin in 1.2 ml of 0.3 M NaCl-0.025 M Tris (hydroxymethyl)-aminomethane buffer at pH 7.5 was therefore filtered through a Sephadex G-200 column. As shown in Fig. 5 a, the effluents were combined to form four fractions. Fractions I–III were dialyzed and lyophylized. The recovered material weighed 3.6, 11.6, and 1.1 mg and had a specific activity of 80,000, 1,300,000, and 40,000 units/mg, respectively. Fraction IV contained dialyzable material which was not bactericidal.

As may be seen in Fig. 6 a, fractions I and II formed but a single precipitin line with the antiserum to the colicinogenic *Proteus*. The line formed by fraction I was much weaker than that of fraction II, a fact which indicates that fraction I contained only traces of colicin. Fraction III, however, reacted weakly with the antisera to both the colicinogenic and noncolicinogenic bacteria. Apparently this material contained small amounts of bacteriocin and an antigen common to both variants of the *Proteus* bacillus. Thus, the filtration of the CM-Sephadex-purified colicin through a Sephadex G-200 column results in the removal of additional impurities which were found in fractions I, III, and IV. Fraction II, on the other hand, contained chromatographically purified colicin K having a high specific activity.

Finally, to ascertain whether the bacteriocin so obtained is homogeneous, another sample of CM-Sephadex-purified colicin (27.0 mg) was filtered through Sephadex G-200 as described above. To avoid artifacts which might arise on lyophylization, the effluents forming fraction II were concentrated to a volume of 1.1 ml by filtration through a collodion bag (No. 100, Schleicher & Schuell, Inc., Keene, N. H.). The concentrate was then filtered through a Sephadex G-200 column (1.0 × 90 cm), the effluents were assayed and combined to form three fractions, as indicated in Fig. 5 b. After dialysis and lyophilization, 12.2 mg of the bacteriocin

was recovered in fraction II. The other two fractions (I and III) contained but a trace of material and each was therefore dissolved in 1.0 ml of 0.01 M sodium bicarbonate. The solutions contained 0.6 and 0.4 mg of protein, as determined by the Folin test. The bactericidal activity of fraction II proved to be 1,600,000 units/mg, whereas that of fractions I and III was but 160,000 and 80,000 units per mg of protein, respectively. Upon immunodiffusion, each of the fractions formed identical single precipitin lines with an antiserum to the colicino-
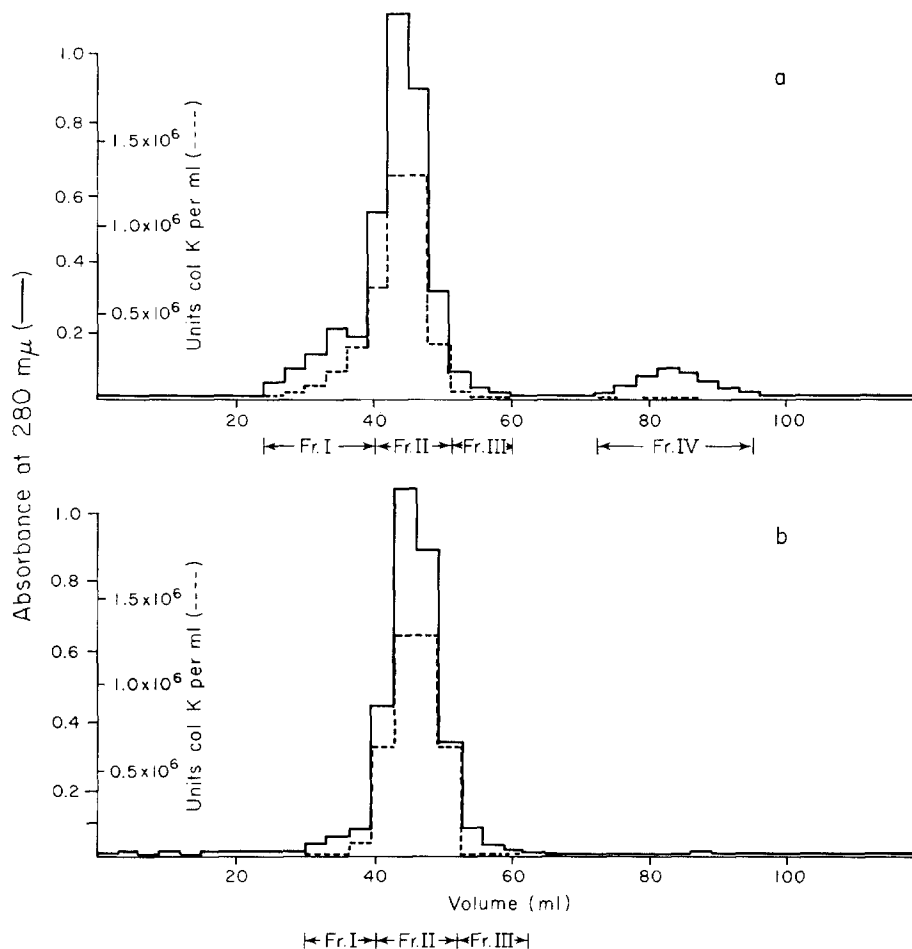


FIG. 5. Gel filtration of *P. mirabilis* colicin K. (*a*) Elution profile of CM–Sephadex-purified colicin from Sephadex G-200 column. Bed dimensions: 1.0 × 90 cm. Eluant: 0.025 M Tris buffer, pH 7.5, containing 0.3 M NaCl. Sample: 20.0 mg of CM–Sephadex-purified colicin in 1.2 ml of the buffer. Yield: 11.6 mg of chromatographically purified colicin K (fraction [Fr.] II). (*b*) Refractionation of chromatographically purified colicin K (Fr. II) on Sephadex G-200. Conditions same as in Fig. 5 a. Sample: 1.1 ml of fraction II concentrate. Yield: 12.2 mg of colicin K.

genic strain of the *Proteus* bacillus, but failed to react with the antiserum to the noncolicino-genic variant. (Fig. 6 b).

Thus it is evident that the *Proteus* colicin K purified by chromatography on G-200, DEAE-, and CM-Sephadex and refractionated on Sephadex G-200 contains but a single antigen. The bacteriocin exhibits considerable uniformity in regard to its molecular size, for it emerges from the column as a narrow symmetrical band. The bactericidal activity of various preparations ranged between 400,000 and 1,600,000 units per mg. The yield of purified colicin was low and varied between 2 and 4 % of the crude colicin used in the chromatographic procedure.
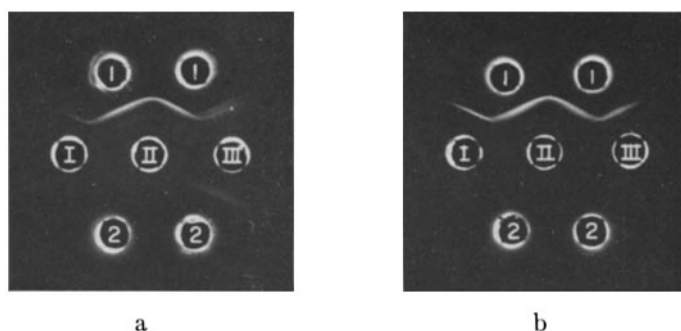


a                                                b

FIG. 6. Gel diffusion reactions of various fractions of *P. mirabilis* colicin K. (*a*) Immuno-diffusion patterns of fractions I–III obtained upon fractionation of CM-Sephadex purified colicin on Sephadex G-200. Wells I–III contain solutions of fractions I–III (1 mg/ml), respectively. Well 1, antiserum to *P. mirabilis* Col K$^+$. Well 2, antiserum to *P. mirabilis* Col K$^-$. (*b*) Immunodiffusion patterns of fractions I–III obtained upon refractionation of chromatographically purified colicin on Sephadex G-200. Wells I–III contain solutions of fractions I–III (0.6, 1.0, and 0.4 mg/ml), respectively. Well 1, antiserum to *P. mirabilis* Col K$^+$. Well 2, antiserum to *P. mirabilis* Col K$^-$.

*Biological Properties of Proteus Colicin K.*—Colicins differ in their bactericidal and serological specificities. To ascertain whether the colicin produced by *P. mirabilis* is identical with that derived from mitomycin induced *E. coli* K 235 bacilli, the activity spectra of the two were compared using a variety of test microorganisms. The results are shown in Table I. Here it is seen that *Proteus* colicin and the colicin of *E. coli* K 235 inhibit the growth of the same bacterial strains. Moreover, the *Proteus* colicin fails to inhibit the growth of those microorganisms which are resistant to the *Coli* bacteriocin. Although the two bacteriocins have identical antibacterial specificities, they differ in potency; the *Proteus* colicin inhibits the growth of *E. coli* B at concentration of $0.62 \times 10^{-6}$ mg/ml, wheras the K 235 bacteriocin exhibits the same effect but at a concentration 50 times higher.

The serological properties of the colicins derived from the *Proteus* and K 235 bacilli were compared by neutralization and by precipitin tests (6.) The neutralization tests, shown in Table II, reveal that both colicins failed to inhibit the growth of the indicator bacteria on those plates which contained an anti-

TABLE I

*Activity Spectra of Colicins Derived from P. mirabilis and E. coli K 235*

| Strain tested | Pm colicin K ($10^{-6}$ mg/ ml) | | | K 235 colicin K ($10^{-6}$ mg/ml) | | |
|---|---|---|---|---|---|---|
| | 2.5 | 0.62 | 0.16 | 125 | 31 | 8 |
| *E. coli* B | 4 | 4 | 2 | 4 | 4 | 2 |
| *E. coli* B/2 | 4 | 4 | 3 | 4 | 4 | 3 |
| *E. coli* B/4 | 4 | 4 | 3 | 4 | 4 | 3 |
| *E. coli* B/6 | 1 | 0 | 0 | 2 | 1 | 0 |
| *E. coli* C-1 | 4 | 3 | 1 | 4 | 3 | 2 |
| *E. coli* Cullen | 4 | 3 | 2 | 4 | 3 | 2 |
| *E. coli* K12 | 4 | 3 | 1 | 4 | 3 | 2 |
| *Shigella sonnei* E90 | 2 | 1 | 0 | 3 | 2 | 1 |
| *P. mirabilis* | 0 | 0 | 0 | 0 | 0 | 0 |

4, complete inhibition of bacterial growth; 2, partial inhibition; 0, no inhibition; Pm, *Proteus mirabilis*; K 235, *E. coli* K 235.

TABLE II

*Neutralization of P. mirabilis and E. coli K 235 Colicins by Various Antisera*

| Colicin tested | Antisera of rabbits immunized with: | Units of colicin/ml | | | | |
|---|---|---|---|---|---|---|
| | | 64 | 16 | 4 | 1 | 0.5 |
| Pm colicin K | Pm colicin K | 0 | 0 | 0 | 0 | 0 |
| | *P. mirabilis* Col K$^+$ | 0 | 0 | 0 | 0 | 0 |
| | *E. coli* K 235 Col K$^+$ | 0 | 0 | 0 | 0 | 0 |
| | *P. mirabilis* Col K$^-$ | 4 | 4 | 4 | 4 | 3 |
| | *E. coli* K 235 Col K$^-$ | 4 | 4 | 4 | 4 | 3 |
| K 235 colicin K | Pm colicin K | 0 | 0 | 0 | 0 | 0 |
| | *P. mirabilis* Col K$^+$ | 0 | 0 | 0 | 0 | 0 |
| | *E. coli* K 235 Col K$^+$ | 0 | 0 | 0 | 0 | 0 |
| | *P. mirabilis* Col K$^-$ | 4 | 4 | 4 | 4 | 3 |
| | *E. coli* K 235 Col K$^-$ | 4 | 4 | 4 | 4 | 3 |

4, complete inhibition of bacterial growth; 2, partial inhibition; 0, no inhibition; Pm, *P. mirabilis*; K 235, *E. coli* K 235.

serum to the *Proteus* colicin or antisera to the colicinogenic strains of the *Proteus* and K 235 bacteria. Both bacteriocins were neutralized alike by the antibodies present in the homologous and heterologous sera, a fact which indicates that the two colicins are serologically indistinguishable. As one might

expect, the antisera to the noncolicinogenic variants of these bacilli had no effect upon the activity of the two bacteriocins.

The results of the precipitin reactions of the two colicins in these same antisera are summarized in Table III. It may be seen in the table that *Proteus* colicin was precipitated by its homologous antiserum and by the antisera to the two colicinogenic strains as well. It did not react with the antisera to the noncolicinogenic variants of the two microorganisms. This fact indicates that *Proteus* colicin is precipitable by colicin-specific antibodies and is not associated with any antigens of the noncolicinogenic variants of the *Proteus* and colon bacilli. Similarly, the K 235 colicin is precipitated by antisera to the *Proteus* colicin and to the colicinogenic variants of the two bacilli in question. How-

TABLE III

*Precipitin Reaction of P. mirabilis and E. coli K 235 Colicins with Various Antisera*

| Colicin tested | Antisera of rabbits immunized with: | Final dilution of antigen | | | | |
|---|---|---|---|---|---|---|
| | | $2 \times 10^3$ | $1 \times 10^4$ | $5 \times 10^4$ | $2.5 \times 10^5$ | $1.2 \times 10^6$ |
| Pm colicin K | Pm colicin K | 2 | 3 | 4 | 2 | $\frac{1}{2}$ |
| | *P. mirabilis* Col K$^+$ | 3 | 4 | 3 | 2 | $\frac{1}{2}$ |
| | *E. coli* K 235 Col K$^+$ | 1 | 3 | 4 | 2 | $\frac{1}{2}$ |
| | *P. mirabilis* Col K$^-$ | 0 | 0 | 0 | 0 | 0 |
| | *E. coli* K 235 Col K$^-$ | 0 | 0 | 0 | 0 | 0 |
| K 235 colicin K | Pm colicin K | 2 | 3 | 2 | 1 | 0 |
| | *P. mirabilis* Col K$^+$ | 3 | 3 | 2 | 1 | 0 |
| | *E. coli* K 235 Col K$^+$ | 3 | 4 | 3 | 1 | $\frac{1}{2}$ |
| | *P. mirabilis* Col K$^-$ | 2 | 1 | $\frac{1}{2}$ | 0 | 0 |
| | *E. coli* K 235 Col K$^-$ | 3 | 4 | 2 | $\frac{1}{2}$ | 0 |

4, heavy precipitation, clear supernatant; 2, partial precipitation; 0, no precipitation; Pm, *P. mirabilis*; K 235, *E. coli* K 235.

ever, this substance reacts also with antisera to the noncolicinogenic strains of these bacteria, a fact which indicates that the K 235 colicin contains, in addition, antigens of the noncolicinogenic K 235 bacillus and that certain of these are serologically related to those present in the *Proteus* bacillus.

The results of the immunodiffusion tests confirmed these observations, as one may see in Fig. 7 a. The *Proteus* colicin forms but a single precipitin line with the antisera to the *Proteus* and K 235 colicins and with the antisera to the colicinogenic variants of the two bacilli. Since these lines merge, the colicin antibodies of the four sera must have the same specificity. The fact that this colicin fails to react with antisera to the noncolicinogenic variants of the two microorganisms indicates that *Proteus* colicin is unconjugated with other antigenic constituents of the noncolicinogenic variants of these two bacteria. Moreover, it may be seen in Fig. 7 b that the bacteriocins of the *Proteus* and

K 235 bacilli form single and identical precipitin lines with an antiserum to the *Proteus* colicin and that the lines merge. Similarly, the *Proteus* bacteriocin forms a single line with an antiserum to the K 235 colicin, which fuses with an identical line given by the K 235 colicin. The latter, however, forms two additional lines with this antiserum. Apparently, this material contains antigenic components of *E. coli* K 235 which are not present in the *Proteus* bacteriocin.

*Physical and Chemical Properties of Proteus Colicin K.*—Purified *Proteus* colicin K is a water soluble substance which has a solubility minimum lying between pH 5.0 and 5.6. Its ultraviolet absorption spectrum is typical of proteins, for its exhibits a minimum at 250 m$\mu$ and a maximum at 278 m$\mu$ when meas-


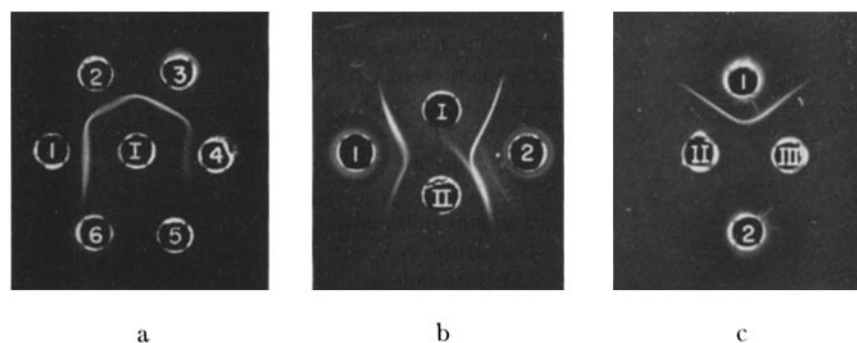
a             b             c

FIG. 7. (a) Gel diffusion reactions of *P. mirabilis* colicin K in various antisera. Center well, I, colicin K (1 mg/ml). Wells 1–6 contain antisera to (1) *E. coli* K 235 colicin K, (2) *P. mirabilis* colicin K, (3) *P. mirabilis* Col K$^+$ bacilli, (4) *E. coli* K 235 Col K$^+$ bacilli, (5) *P. mirabilis* Col K$^-$ bacilli, (6) *E. coli* K 235 Col K$^-$ bacilli. (b) Gel diffusion reactions of colicins K from *P. mirabilis* and *E. coli* K 235. Well I, *P. mirabilis* colicin K (1 mg/ml); well II, *E. coli* K 235 colicin K (4 mg/ml). Wells 1 and 2, antisera to *Proteus* and K 235 colicins, respectively. (c) Gel diffusion reactions of isoelectrically purified *P. mirabilis* colicin K fractions. Well II, colicin K, fraction II (1 mg/ml). Well III, colicin K, fraction III (1 mg/ml). Wells 1 and 2 contain antisera to *P. mirabilis* Col K$^+$ and Col K$^-$ bacilli, respectively.

ured in 0.05 M sodium phosphate buffer at pH 8.0. The extinction coefficient of a 0.1% solution at 280 m$\mu$ is 0.77–0.79. The colicin has all the characteristics of a typical protein. It contains approximately 15.8% nitrogen, 48.4% carbon, and 6.8% hydrogen (Table IV). The content of phosphorus, lipids, and carbohydrates is less than 0.3%. Color reactions for pentoses, deoxypentoses, hexoses, methylpentoses, hexosamines (25–28), as well as those for uronic, sialic, and ketodeoxyoctonic acids (29–31) are essentially negative. The content of volatile acids, calculated as acetyl, is less than 0.1% (32). On hydrolysis of the bacteriocin with 6 N hydrochloric acid at 110° C, amino acids and ammonia are liberated. Amino acid residues account for 89% of the total material, whereas ammonia accounts for 2.0%. The bacteriocin produces color with protein reagents. Using bovine serum albumin as a standard, the protein content is 87–

TABLE IV

*Chemical Properties of Colicin K Derived from P. mirabilis*

|  |  | % |
| --- | --- | --- |
| Nitrogen |  | 15.8 ± 0.2* |
| Carbon |  | 48.4 ± 0.5 |
| Hydrogen |  | 6.8 ± 0.2 |
| Phosphorus |  | 0–0.1 |
| Lipids |  | 0–0.3 |
| Saccharides‡ |  | 0–0.3 |
| Amino acids§ |  | 89.2 ± 1.4 |
| Ammonia |  | 2.0 ± 0.3 |

* The data presented are averages, or range, of the analyses of at least three colicin K preparations; they are corrected for the ash content.
‡ Determined by anthrone reaction.
§ Sum of amino acid residues.

TABLE V

*Amino Acid Composition of P. mirabilis Colicin K*

| Amino acid | Colicin K |
| --- | --- |
| Lysine | 10.6 ± 0.2* |
| Histidine | 1.2 ± 0.05 |
| Arginine | 2.1 ± 0.1 |
| Aspartic acid | 13.2 ± 0.1 |
| Threonine | 4.3 ± 0.4 |
| Serine | 9.3 ± 0.4 |
| Glutamic acid | 14.5 ± 0.4 |
| Proline | 1.7 ± 0.1 |
| Glycine | 7.7 ± 0.3 |
| Alanine | 8.7 ± 0.1 |
| Cysteine | 0.2 ± 0.2 |
| Valine | 7.4 ± 0.5 |
| Methionine | 1.6 ± 0.04 |
| Isoleucine | 4.0 ± 0.2 |
| Leucine | 6.8 ± 0.1 |
| Tyrosine | 2.5 ± 0.1 |
| Phenylalanine | 2.7 ± 0.1 |
| Tryptophan | 1.2 ± 0.05 |

*Moles per 100 moles of amino acids. The data presented are averages of the amino acid analyses of three colicin K preparations. The values for threonine, serine, and tyrosine are corrected for loss on hydrolysis (48). Tryptophan was determined spectrophotometrically (21).

90% as determined by the biuret reaction (33), and 107–110% when measured by the Folin procedure (34). As shown in Table V, lysine, aspartic, and glutamic acids are the major constituents of the bacteriocin, whereas histidine, proline, methionine, and tryptophan occur only in small amounts. Traces of cysteine

were found in one of the colicin preparations, but not in others, and, hence, this amino acid does not appear to be an essential constituent. Basic and acidic amino acids account for 14 and 28 moles/100 moles of all amino acids, respectively. Acid hydrolysates of the bacteriocin were tested for ethanolamine (20), aliphatic diamines (35), choline, and diethylaminoethanol (36), but the tests were negative. Thus, the known components of the colicin account for only some 90–93% of its weight. The substance apparently contains about 10%
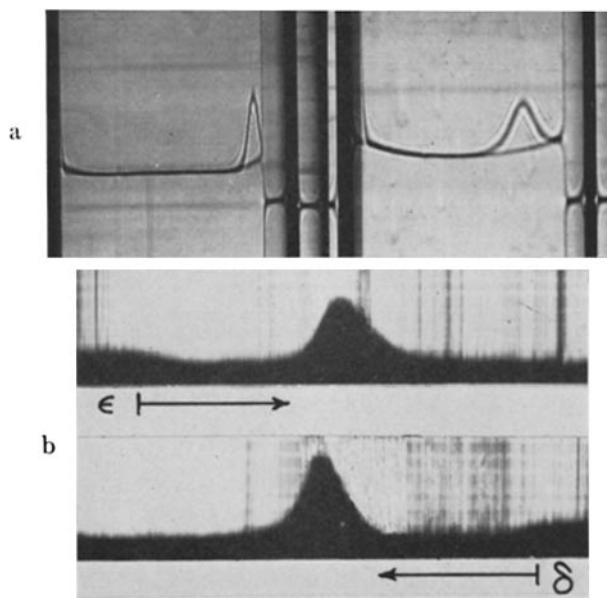


Fig. 8. Sedimentation and electrophoresis patterns of *P. mirabilis* colicin K. (*a*) Sedimentation patterns of colicin K (6.6 mg/ml) in 0.1 M sodium Veronal buffer at pH 8.58. Photographs were taken 14 and 78 min after rotor reached maximum speed of 52,000 rpm. (*b*) Electrophoretic pattern of colicin K (6.6 mg/ml) in 0.1 M sodium Veronal buffer at pH. 8.58 after electrophoresis for 228 min at a potential gradient 8.3 volt/cm.

unidentified constituents; whether these are acid-resistant peptides, or other chemical compounds, is not yet known.

On ultracentrifugation the colicin forms a single and relatively symmetric peak (Fig. 8 a). The sedimentation rate of the bacteriocin ($s_{20,w}$) is 2.81S when determined in 0.1 M sodium Veronal buffer at pH 8.58 and at a concentration of 6.6 mg/ml. A value of 2.86S was obtained when the rate was measured in 0.05 M sodium phosphate buffer at pH 8.0 and at a concentration of 2.4 mg/ml. The partial specific volume of the bacteriocin in this buffer is 0.71 ml/g. The solution of the material is more viscous, however, than are those of globular

proteins. The intrinsic viscosity of the colicin in the phosphate buffer is 9.8 ml/g, a fact which indicates that the colicin molecule may be asymmetric.

The molecular weight of the bacteriocin was estimated from its Stokes radius (the radius of the equivalent sphere) as described by Siegel and Monty (37). To determine the radius, a solution of 6.4 mg of colicin K in 0.5 ml of 0.025 M Tris
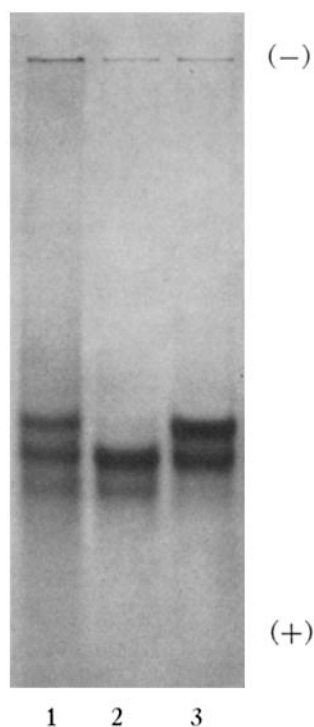


Fig. 9. Electrophoresis of *P. mirabilis* colicin K in polyacrylamide gel. 1, colicin K purified on CM-Sephadex. 2, colicin K purified by electrofocusing (fraction II). 3, colicin K purified by electrofocusing (fraction III). 20 μl samples of solutions containing 200 μg of colicin were deposited into slots of vertical electrophoresis cell, containing 5% polyacrylamide gel in 0.077 M glycine–0.01 M Tris buffer at pH 7.9. After electrophoresis for 4 hr at 400 volts, the gel was stained with 0.25% amido black.

buffer at pH 7.5, containing 0.3 M NaCl, was filtered through a Sephadex G-200 column (1 × 91 cm) which had been equilibrated with the same buffer. The exclusion volume of the column, $V_o$, was 24.9 ml and its total volume, $V_t$, was 80.5 ml. On filtration, the colicin emerged from the column at maximum concentration when the elution volume, $V_e$, was 45.0 ml. From these data, $K_{av}$, the fraction of the gel volume available for diffusion of the material, was calculated to be 0.36. The column was calibrated with bovine serum albumin (5.0

mg/0.5 ml). The monomer form of this protein was eluted from the column when the $V_e$ was 50.0 ml; the $K_{av}$ for this material was found to be 0.45. Since ($-\log K_{av})^{1/2}$ of proteins is proportional to their Stokes radii, and since the radius of bovine serum albumin is known to be $3.5 \times 10^{-7}$ cm (37), the Stokes radius of colicin K molecule was calculated to be $3.96 \times 10^{-7}$ cm. Using this value, as well as the sedimentation rate and the partial specific volume of colicin, the molecular weight of the bacteriocin was found to be 44,900. Its frictional coefficient, $f/f_0$, was computed to be 1.70. Thus, *Proteus* colicin K consists of highly asymmetric molecules having a molecular weight of approximately 45,000.
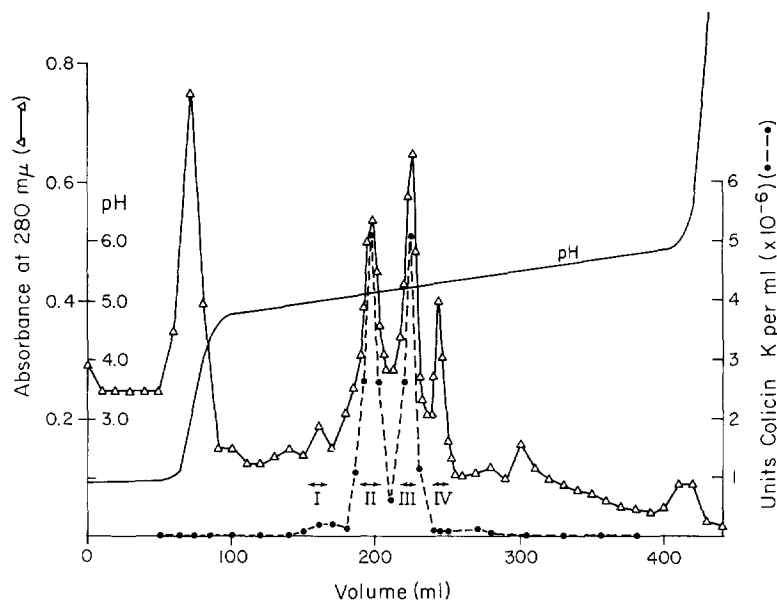


Fig. 10. Isoelectric fractionation of *P. mirabilis* colicin K in ampholine-sucrose gradient. This figure shows the absorbance, the pH, and the bactericidal activity of the effluents from the gradient column. A 38.7 mg sample of colicin was electrofocused in a 440 ml column containing 0.8% carrier ampholytes (pH 4.8-5.9) for 63 hr at 800 volts and 4°C.

*Electrophoresis of Proteus colicin K.*—

To learn more of the nature of the *Proteus* colicin, the substance was analyzed by several electrophoretic methods. The patterns obtained on free boundary electrophoresis of a solution of colicin K (0.66%) in 0.1 M sodium Veronal buffer at pH 8.58 are shown in Fig. 8 b. It may be seen that the bacteriocin forms but a single boundary which migrates towards the anode ($u_D = -2.3 \times 10^{-5}$ cm$^2$/volt sec). The boundary is asymmetrical, however, a fact which indicates slight heterogeneity. Because of this, the bacteriocin was analyzed by electrophoresis in a polyacrylamide gel at pH 7.9. As may be seen in Fig. 9, the substance formed three bands which migrated anodically at relative rates of 1.0, 1.09, and 1.17.

In order to isolate the substances forming the bands, a sample of partially purified colicin

was fractionated by electrofocusing in an ampholine-sucrose gradient column (17, 38). The ampholine used for this purpose had a pH range 4.8–5.9. The results of the fractionation of 38.7 mg of colicin, purified on DEAE-Sephadex, are presented graphically in Fig. 10. As indicated in the diagram, four absorption peaks were associated with bactericidal activity. The effluents underlying each peak were combined as shown by arrows I–IV to form four fractions. The solutions were concentrated by ultrafiltration through a collodion bag (No. 100, Schleicher and Schuell), dialyzed and lyophilized. The substances in fractions I–IV weighed 0.9, 2.7, 3.3, and 0.7 mg, respectively. The bactericidal activity of fractions I and IV was 80,000 and 160,000 units/mg, respectively, whereas that of fractions II and III was in each instance 800,000 units/mg.

It is apparent that the *Proteus* colicin is separated by electrofocusing into two minor and two major constituents. The latter (fractions II and III) had a high bactericidal activity and formed identical single precipitin lines with an antiserum to the colicinogenic *Proteus* bacillus. (Fig. 7 c). They differed, however, in their isoelectric points (pI) when determined in ampholine-sucrose solution. The pI of fraction II was 5.14, whereas that of fraction III was 5.21. On subsequent electrophoresis in polyacrylamide gel, each substance produced two bands (Fig. 9). Fraction II formed bands migrating at relative rates of 1.09 and 1.17; fraction III formed bands which migrated at rates of 1.00 and 1.09. *Proteus* colicin consists therefore of at least two major components, each having identical immunological properties and the same bactericidal potency but differing in net electrical charge. Since fraction II and fraction III form double bands migrating at different rates upon gel electrophoresis, even after lyophilization and resolution, it is, therefore, unlikely that they are aggregates or interconvertible conformational forms of the bacteriocin. They may rather be regarded as stable modifications of the colicin molecule. Just why these substances form double bands on gel electrophoresis is not yet known. Their study is being continued.

## DISCUSSION

The hereditary information of an organism is encoded not only in its chromosomal genes but in genes located in the cytoplasm of the cell, as well. In higher organisms, cytoplasmic genes determine the properties of the cell organelles, such as mitochondria, chloroplasts, or centrioles (39). In bacteria, they are responsible for sex characteristics, drug resistance, and for the ability of the microorganism to produce bacteriocins (40). The cytoplasmic genes controlling the synthesis of colicins, the colicinogenic factors, have been recently isolated and characterized. DeWitt and Helinski have found that the DNA of *Proteus mirabilis* which had acquired the colicinogenic factor $E_1$, contained a small amount of satellite DNA having a buoyant density of the nucleic acid of the colon bacillus which had served as a donor (23). They suggested that the satellite DNA contains the colicinogenic factor. Roth and Helinski separated this DNA and showed that it was a circular double-stranded molecule having a

particle weight of 4.5 million daltons (41). The colicinogenic factors $E_2$ and $E_3$ were isolated from *E. coli* W 3110 by dye-buoyant centrifugation and were found to have a particle weight of 5 million daltons (42).

The results of the study reported in this communication are in agreement with these findings. We have shown that the DNA of *Proteus mirabilis*, carrying the colicinogenic factor K, contains a small amount of satellite nucleic acid having a buoyant density of 1.705. This DNA is absent in the noncolicinogenic variant of the *Proteus* bacillus and hence we assume that the satellite DNA must contain the colicinogenic factor. We are aware, however, that this assumption still requires direct proof. We hope that substantiating evidence can be obtained by determining whether the satellite DNA of the *Proteus* bacillus can serve as a template for the formation of colicin K in the DNA-dependent protein synthesis system.

The nature of the bacteriocin specified by the colicinogenic factor K was first investigated by Goebel and his coworkers in 1955. They showed that the colicin K elaborated by *E. coli* K 235 is a protein-lipopolysaccharide complex identical with the somatic antigen of the bacillus (3, 4). Upon dissociating the complex into its protein and lipopolysaccharide components, the bactericidal activity was found to accompany the protein moiety (4). The separation of the bactericidal protein from other protein constituents of the somatic antigen has as yet not been achieved and its nature remains to be ascertained.

Our work is primarily concerned with the isolation and characterization of the bacteriocin produced by the colicinogenic strain of *Proteus mirabilis*. We have seen that this bacillus in the presence of mitomycin C, secretes considerable amounts of colicin K into the culture medium. The bacteriocin can be separated from other bacterial constituents by chromatographic methods and is obtained as an immunologically homogeneous substance unconjugated with other antigens of the *Proteus* bacillus. The colicin is a protein containing all the usual amino acids save for cysteine, and is free of lipid and carbohydrate. Since amino acid residues account for but 90% of the dry weight of the material, it is still possible that the bacteriocin might contain some 10% of acid-resistant peptides or other unidentified constituents. Colicin K is a relatively homogeneous substance having a molecular weight of approximately 45,000. However, when the material is subjected to electrofocusing, it can be separated into two major components, each having the same bactericidal activity and immunological specificity. Thus, the colicinogenic factor K produces at least two variants of colicin K which differ in electrical charge but which have the same biological properties. Whether these differences can be attributed to a slight variation in amino acid composition or to an unidentified substituent is not yet known.

Colicin K has also been isolated from the colicinogenic strain of *E. coli* K 12 by Dandeu and Barbu (11). As one would expect, the properties of the colicin produced by this microorganism in the presence of mitomycin C resemble closely

those of the bacteriocin derived from the *Proteus* bacillus. Colicin K derived from the K12 bacillus is also a protein which contains all usual amino acids but cysteine and is free of polysaccharide. Its molecular weight, estimated by gel filtration, was found to be 75,000. Since the molecular weight of the *Proteus* colicin was calculated from its Stokes radius, sedimentation rate, and partial specific volume, it is quite possible that the discrepancy between the two figures can be attributed to difference in the methods employed. The immunological properties of partially purified colicin K derived from mitomycin-induced *E. coli* K 235 have been recently described by Tsao and Goebel (43). They found in this instance that the colicin is not associated with the somatic antigen of the K 235 bacillus. Other colicins derived from mitomycin-induced cultures have also proved to be proteins of low molecular weight. Thus, colicin $E_1$ elaborated by *E. coli* JC 411 is a basic protein having an isoelectric point at pH 10.5–11.0 and a molecular weight of 55,000 (12). The well-characterized colicins $E_2$ and $E_3$ derived from *E. coli* W 3310, have molecular weights of 60,000 and isoelectric points close to neutrality (pI 7.5 and pI 6.6, respectively) (13). Their amino acid compositions differ considerably from that of colicin K for they contain less lysine and glutamic acid and more arginine and proline. The two colicins contain also one cysteine residue per molecule.

From the work which has been presented here, and from that of others as well, it is evident that the colicins formed by bacteria induced with mitomycin C are proteins of a low molecular weight. In contrast, the bacteriocins produced by noninduced bacteria are protein-lipopolysaccharide complexes containing the somatic antigen of the colicinogenic microorganisms (4–8). The processes which lead to the formation of the two types of bacteriocins are not yet fully understood. It is known, however, that bacteria grown in the absence of mitomycin C contain but one, or at best a few molecules of the colicinogenic factor per cell (23), and that, under optimal conditions, they produce only small quantities of colicin (20–2000 units per ml) (14, 44). Furthermore, it has been shown that colicin is formed in the bacterial cytoplasm as a heat labile protein and it has been suggested that the latter might subsequently be linked to the somatic antigen in the cytoplasmic membrane (45). The nature of the bond between the bacteriocin and antigen has not yet been ascertained. It is possible, however, that the two substances are bound by their hydrophobic groupings, for they can be separated only after treatment with reagents containing organic solvents, such as phenol or cyclohexylamine and propanol (4). On the other hand, when bacteria are exposed to low concentrations of mitomycin C (0.1–1.0 $\mu$g/ml), the division of bacterial cells is inhibited; the cells form filaments and the number of the viable cells in the culture declines (46). Since the filaments are more fragile than normal cells, it has been suggested that mitomycin interferes with the formation of the bacterial cell wall. (47). The synthesis of other bacterial constituents, such as DNA, RNA, and proteins, is however inhibited

only slightly (46). The antibiotic apparently has but little effect upon the replication of the colicinogenic factor, for the amount of the latter increases up to 10% of the total bacterial DNA, and as a result, induced cells might contain as many as 30–100 molecules of the factor. (23). Since induced cells are still capable of synthesizing both RNA and proteins, and since they now contain multiple copies of the colicinogenic factor, they elaborate large amounts of bacteriocin (15,000–200,000 units/ml) (44). Some of the latter apparently combines with the cell wall constituents, whereas the excess bacteriocin accumulates in the cell or escapes into the medium as an unconjugated protein. Thus, colicins derived from mitomycin-induced bacteria might be considered to be products of protein synthesis directed by the colicinogenic factors. The bacteriocins obtained from noninduced microorganisms, on the other hand, might be regarded as substances which are formed when the protein-like colicin combines in vivo with the constituents of the bacterial cell wall.

## SUMMARY

The colicinogenic factor K has been transferred from *E. coli* K 235 to *Proteus mirabilis*. The DNA of the colicinogenic *Proteus* has been shown to contain a small amount of a satellite DNA which presumably harbors the Col K factor.

In the presence of mitomycin C the colicinogenic *Proteus* secretes colicin K into the growth medium. The bacteriocin has been purified by chromatography and obtained as an immunologically homogeneous substance unconjugated with other antigens of the *Proteus* bacillus.

*Proteus* colicin K is a protein of relatively low molecular weight. It contains all of the usual amino acids except cysteine and is free of lipids and polysaccharides. The bacteriocin can be separated by electrofocusing into two major components. The latter have the same biological properties but differ in their specific electrical charges.

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