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[7] See (5.2.2) in ref. 3 above.

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*COLICINE V**

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Nearly a decade ago this laboratory began an investigation on the nature of colicines—antibacterial agents of remarkable potency which are elaborated by certain *Enterobacteriaceae* and which kill other microorganisms of the same species. These agents were first described by Gratia in 1925[1] who observed that cell-free filtrates of a virulent strain of colon bacillus with which he was working contained an agent, "principle V," which killed a sensitive host strain "coli ϕ." Gratia observed that his principle was thermostable and that it diffused through cellophane membranes. He pointed out that the agent bore a remarkable similarity to bacteriophage, yet differed in that it would not reduplicate upon serial passage.

A resurgence of interest in the colicines took place in the mid-forties, largely through the work of Frédéricq,[2] to whom most of our modern knowledge concerning the distribution, specificity, and tenuous relationship of the colicines to the bacteriophages can be attributed.[3] Extensive as our knowledge is in this regard, our understanding of their chemical nature has remained singularly enigmatic despite the efforts of a number of investigators.[4]

Several years ago we in this laboratory described the isolation of one of the colicines—colicine K.[5] This substance proved to be a lipocarbohydrate-protein complex, identical with the somatic O antigen of the microorganism from which it was derived. The material had exceedingly potent antibacterial properties. It was antigenic in rabbits and the antisera specifically precipitated the colicine and neutralized its antibacterial properties as well.[6] Although the colicines and bacteriophages show striking resemblances,[2] we were unable to demonstrate any serological relationship between our colicine K and the coli-dysentery phage T6, the virus to which this colicine is presumed to be related.

We have now isolated another and different colicine—colicine V. We chose to

study this substance not only because it was the first of the colicines to be described, the "principle V" of Gratia, but, if it is indeed readily dialyzable as he has said,[1] it should prove to be a far less complicated molecule than colicine K.

A colicine V producing bacillus, termed *E. coli* K357, was kindly sent us by Pierre Frédéricq of the University of Liège. Six variants were obtained from this microorganism which differed in colonial morphology. One of these, designated as *E. coli* K357 L-T, was selected for study because it liberated more colicine in the culture medium than did the others.

When this microorganism was grown at pH 7.0 in a medium containing only dialyzable constituents,[7] it elaborated some 400–800 units per ml of colicine V. After concentrating the cell-free medium *in vacuo* followed by dialysis, there was obtained by freeze drying the nondialyzable residue, a grayish-white, friable, amorphous powder. This substance, "crude colicine V," had striking antibacterial properties. A solution containing 1 to 2 μg per ml, when tested by a technique described in a former communication,[7] completely inhibited the growth of the test organism *E. coli* ϕ.

Purification of the colicine was effected by precipitating it at low ionic strength (0.02 *M* sodium acetate) with ethanol at 0°C. The substance which precipitated between 30 and 60 per cent ethanol concentration proved to be the colicine. The latter was further purified by passing a solution (1.6 gm in 100 ml of 0.05 *M* Tris buffer at pH 8.0) through a short column of DEAE cellulose (4 gm compressed in a column 1.9 × 10 cm). The last traces of pigment were thus removed without incurring an appreciable loss of the colicine.

Chemical Properties.—Purified colicine V is obtained as a white, water-soluble, amorphous substance. Its analysis is as follows: carbon, 49.95%; hydrogen, 7.75%; phosphorus, 2.02%; nitrogen, 3.80%; and acetyl, 5.90%. The material gives strong biuret and Folin tests indicating the presence of protein. On acid hydrolysis it yields 43 per cent of reducing sugars calculated as glucose and 11.3 per cent of a chloroform-soluble lipid. Colicine V is not precipitated by trichloroacetic or picric acids, nor does it precipitate with the salts of heavy metals, such as copper, uranium, lead, or silver. A solution of colicine V, when heated at 100°C with an equal volume of saturated picric acid yields a precipitate; presumably the complex is dissociated into its component parts and the protein component is precipitated as an insoluble picrate. Finally, it should be stated that we were unable to confirm Gratia's observation that colicine V is dialyzable. Our substance does not diffuse through cellophane membranes.

Toxic Properties of Colicine V.—Colicine V exhibits toxic properties both for bacteria and for mammals. A solution containing 1.5 μg of colicine per ml completely inhibits the growth of the test organism *E. coli* ϕ. *E. coli* B is also sensitive to colicine V, but it requires a concentration of 3 μg per ml to inhibit the growth of this bacillus. When colicine V is injected intraperitoneally into mice, it is found that the $L.D._{50}$ for the Collins-Nelson Rockefeller Institute strain of white mice is 0.90 mg. The intradermal injection of rabbits with 1 mg of colicine in saline occasionally results in death of the animal, but as a rule there occurs only a marked systemic reaction, accompanied by malaise and fever. There is a great deal of edema at the site of injection and usually within 24 hours a marked necrotic area appears.

Immunological Properties of Colicine V.—Colicine V is a potent antigen. The sera of rabbits which have received an intradermal injection followed by one or two courses of intravenous injections of minute quantities (200–500 μg), invariably show the presence of precipitating antibodies. The antisera also agglutinate at high dilutions (1:6400) the bacillus from which the colicine is obtained. These same sera neutralize the antibacterial properties of colicine V, so that the colicine is no longer capable of killing the sensitive organism *E. coli* ϕ. As a rule, 0.2 ml of a potent serum is sufficient to neutralize the antibacterial activity of 100 μg of colicine V.

A study of the immunological cross reactions of colicine V in colicine K antiserum and vice versa, has revealed that neither colicine is neutralized or precipitated by the heterologous serum. Furthermore, colicine V antisera do not agglutinate the colicine K producing bacillus *E. coli* K235, nor do colicine K antisera agglutinate the colicine V producing microorganism. The antigenic mosaics of the two bacilli appear to be quite unrelated.

Summary.—Colicine V had been isolated as an electrophoretically homogeneous substance. Chemical analyses indicate that it is a lipocarbohydrate-protein complex which is typical of many *Enterobacteriaceae*. Colicine V is toxic both for bacteria and for mammals. It is an excellent antigen and it elicits in rabbits antibodies which both precipitate and neutralize the colicine and which agglutinate the parent colicinogenic microorganism. Despite their close chemical similarity, colicine K and colicine V exhibit no cross serological relationships whatsoever.

This work is being continued in our laboratory. The isolation and characterization of still other colicines will be reported in subsequent communications.

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