ON THE ENHANCEMENT OF BACTERIAL VIRULENCE BY GASTRIC MUCIN

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Introduction

Bacterial pathogens of human origin which are either highly virulent or highly toxic for laboratory animals present a ready opportunity for immunological investigation. On the other hand, bacteria of human origin which are neither highly virulent nor highly toxic for laboratory animals offer immediate obstacles to the study of active and passive immunity. Among organisms of this latter group may be mentioned certain of the respiratory bacteria such as meningococci, and certain of the enteric bacteria, such as *B. typhosus*. Except for a low degree of virulence and the ability to produce so-called endotoxin, these organisms are usually entirely without spectacular effect when inoculated into experimental animals. However, their importance as human pathogens demands increased attention to the devising of special methods whereby either high virulence or high toxicity may be exhibited, at least temporarily, in experimental animals.

Nungester, Wolf, and Jourdonais (1) and Miller (2) have devised procedures for augmenting the low-degree mouse virulence which many bacteria naturally exhibit. By coating organisms with a certain form of gastric mucin, it was found possible to bring about perhaps a million fold increase in virulence. This increase in virulence appeared so great and of such promise that experiments were carried out in the Lilly Laboratories, in the winter of 1933-34, to attempt to repeat these original works. These experiments as conducted by Miss Lucille Wade, and as yet unpublished, appeared to verify the original results. However, due to interruption, this study was not continued at that time.

Subsequently Miller (3, 4, 5) and Rake (6, 7) have reported the results of additional experiments on enhancing the virulence of meningococci by use of gastric mucin and attempting to protect animals with antiserum against bacteria of such enhanced virulence. Similar methods and experiments have been applied to *B. typhosus* later by Rake (8).

It is obvious that the mucin coating of human pathogens provides knowledge of certain potential capacities of bacteria otherwise unobtainable and also offers a means for testing of antiserum or actively immune conditions in immunized subjects. For these reasons it appeared of interest to investigate this subject further in this laboratory, dealing with both the meningococcus and the typhoid bacillus.

Experimental

Our experiments on mucin-coating have dealt with the mucin-virulence of meningococci and serum protection tests in mice and mucin-virulence of *B. typhosus* and immunity tests of mice receiving typhoid vaccines.

Mucin Virulence of the Meningococcus. Two groups of cultures available for study were subjected to mucin coating according to Miller (3), using a special lot of mucin from The Wilson Laboratories.

We obtained the first group of cultures from Mr. F. A. Miller of the Lilly Laboratories, Greenfield, Indiana. Cultures "123", "55", "57", and "60" of this group came from the Rockefeller Series and have been cultivated for many years. The other cultures of this group, "331", "173", "146", "302", and "158", have been cultivated from two to four years. Cultures "57" and "302" showed spectacular virulence as noted in Table I. The others were either irregular or of low virulence.

TABLE I

Mucin-virulence of older cultures of meningococci for mice

C.C. of Culture	Culture								
	I 123	I 331	II 55	II 173	III 57	III 146	III 302	IV 158	IV 60
	S	S	S	S	D	S	D	D	D
10^{-2}	D	S	D	S	D	S	D	D	S
	S	S	S	S	D	S	D	D	S
10^{-3}	S	S	S	S	D	S	D	S	S
	S	S	S	S	D	S	D	S	D
10^{-4}	D	S	D	S	D	S	D	S	S
	S	S	S	S	D	S	S	S	S
10^{-5}	D	S	S	S	D	D	D	S	S
	S	S	S	S	D	S	D	D	S
10^{-6}	S	D	S	S	D	S	D	S	S

Legend: D = Died; S = Survived for 2 days.

The meningococcus strains in the second group were recently isolated. Cultures known as "Rockwell I" and "Rockwell 5" of this group were obtained from Dr. G. E. Rockwell, University of Cincinnati. Cultures known as "M. Smith", "E. Roller", and "L. Thoine", also of this group, were obtained from Dr. G. F. Kempf, Indianapolis City Hospital. The results in Table II show that all cultures of this latter group except "Rockwell 5" exhibit high virulence. It is to be recalled, of course, that the cultures in this latter group had been under cultivation less than a year.

Of a total of fourteen meningococcus cultures tested for mouse virulence, when coated with mucin, six exhibited a high degree of virulence. The recently isolated cultures were the more likely to be virulent; however, one fully virulent culture i. e. "57 III," was found in the oldest group.

TABLE II

Mucin-virulence of newer cultures of meningococci for mice

C.C. of Culture	Culture				
	I Rockwell 1	I Rockwell 5	I M. Smith	I E. Roller	III L. Thoine
10^{-2}	D D	D D	D D	D D	D D
10^{-3}	D D	D D	D D	D D	D D
10^{-4}	D D	S S	D D	D D	D D
10^{-5}	D D	S S	D D	D D	D D
10^{-6}	D D	D S	D D	D D	D D

Legend: D = Died; S = Survived for 2 days.

Serum Protection of Mice Against Mucin-Virulent Meningococci. Serum was obtained from twelve horses being immunized with meningococci. These twelve sera were administered in constant doses of 0.1 c.c. intraperitoneally to twelve series of mice, and one-half hour later each series of mice received graded doses of living mucin-coated meningococci intraperitoneally. The results of these mouse protection tests are shown in Table III. All of the sera showed protection against large multiples of a fatal dose, and about half the sera protected against the largest test dose of culture used, i. e., 0.1 cubic centimeter.

Mucin-Virulence of B. typhosus. Utilizing the same mucin-coating technique as for the meningococcus, we tested the following strains of *B. typhosus:* "220" and "222", separately cultivated, Rawlings strains, used for vaccine preparation; and "Sumori", a freshly isolated culture obtained from Dr. G. F. Kempf. Cultures "Sumori" and "222" exhibited a high degree of virulence while culture "220" was of low virulence for mice as shown in Table IV. The culture "Sumori" appeared to be the best of the three.

Since it appeared rather easy to impart high mouse virulence by mucin-coating certain typhoid bacilli, attempts were made to immunize groups of mice with various vaccines, then after an interval, to subject them to tests with graded doses of living mucin-coated culture. The vaccines used comprised two lots made from culture "220", one lot from

TABLE III

Meningococcus serum mouse protection tests

C.C. of Culture 57 III	Horse Sera												
	732 153	825 156	1198 44	1200 10	1358 7	1375 34	1514 18	1519 17	1671 TB17	1679 10	1750 14	1800 8	Control
10^{-1}	S	S	S	S	D	D	D	D	D	S	S	S	D
10^{-2}	S	D*	S	S	S	S	S	S	S	S	S	S	D
10^{-3}	S	S	S	S	S	S	S	S	S	S	S	S	D
10^{-4}	S	S	S	D	S	S	S	S	S	S	S	S	D
10^{-5}	S	S	S	S	S	S	S	S	S	S	S	S	D
10^{-6}	S	S	S	S	S	S	S	S	D	S	S	S	D

Legend: D = Died; S = Survived for 2 days. *Accident.

TABLE IV

Mucin-virulence of B. Typhosus for mice

C.C. of Culture	Culture		
	Sumori	222	220
10^{-1}	D	D	D
10^{-2}	D	D	D
10^{-3}	D	D	S
10^{-4}	D	D	S
10^{-5}	D	D	S
10^{-6}	D	S	D
10^{-7}	D	D	S
10^{-8}	D	D	S

Legend: D = Died; S = Survived for 2 days.

"222", and one lot from culture "Sumori". These vaccines were made with a turbidity corresponding to a one billion standard.

Immunity Tests of Mice Receiving Various Typhoid Vaccines. In this experiment four groups of mice were immunized subcutaneously with the four vaccines just described. The course of treatment was the same as for humans; however, the dosage was only one-tenth as much; i. e., 0.1 c.c. injections were made instead of 1 c.c. injections. Twelve days after the last dose of vaccine, these four groups of mice, along with a fifth group of normal controls, were subjected to a series of injections of living mucin-coated virulent culture "Sumori". The results in Table V show that the "Sumori" vaccine mice were best protected. In this group it is noted that certain of the weakest doses of test culture killed vaccine-prepared mice presumably of the same degree of immunity as those surviving the larger doses of living culture. No explanation is offered for this.

TABLE V

B. Typhosus vaccine mouse protection test

Test Culture Sumori cc.	220 Vaccine Lot No. 1 Mice	220 Vaccine Lot No. 2 Mice	222 Vaccine Mice	Sumori Vaccine Mice	Normal Controls
10^{-1}	D	D	D	S	D
10^{-2}	D	S	D	S	D
10^{-3}	D	D	D	S	D
10^{-4}	D	D	D	S	D
10^{-5}	D	D	D	D*	D
10^{-6}	D	D	D	D	D
10^{-7}	D	D	D	D	D
10^{-8}	D	D	D	D	D

Legend: D = Died; S = Survived for 2 days. *Accident.

Summary

The results herewith presented corroborate the previous reports by Nungester et al. and Miller and Rake and an unpublished report of Wade. It has been found possible by the mucin technique to increase the virulence of the meningococcus and typhoid bacillus for mice roughly

a million times in comparison with previous tests. In most, but not all, cases the newer and fresher cultures were the most likely to be mucin-virulent.

In addition, it has been found that several horse antimeningococcus sera protect mice against mucin-virulent meningococci. In experiments as yet unpublished, we have found that some, but not all, concentrated antimeningococcus sera protect in the same manner, and this agrees with the observations of Miller, as expressed in a personal communication.

In parallel experiments with the typhoid bacillus, we have noted in a comparative way that vaccine made from bacilli of high potential mucin-virulence is most effective in immunizing mice against mucin-virulent typhoid bacilli.

Finally, although the mucin technique may be considered highly artificial, it serves in some instance to differentiate hitherto unrecognized capacities of one culture from another and to assay active immunity by protection against great multiples of a fatal dose of cultures previously considered of a low degree of virulence.

Conclusions

1. Great enhancement of bacterial virulence through use of mucin, has been noted.
2. Serum protection against mucin-virulent meningococci and typhoid bacilli can be measured readily.
3. Conclusions 1 and 2 corroborate and extend the observations of Nungester, Miller, and Rake.
4. It has not been determined what relation, if any, may exist between antibacterial immunity and serum protection against mucin-virulent pathogens.

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