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Enhancement of *H. pertussis* Virulence with Starch and Use of Starch Cultures in Mouse Immunity Tests.

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We have dealt[1] with the enhancement of virulence of meningococci and typhoid bacilli for mice, and the effect of both active and passive immunization upon resistance of mice to infection with mucin-coated cultures. This work followed that of Nungester, Wolf and Jourdonais,[2] Miller[3] and Rake,[4] and in turn was followed by a similar report by Mishulow and Melman.[5] These reports show that the intraperitoneal mouse-virulence of certain strains of meningococci and typhoid bacilli may be multiplied a thousand- to a million-fold through the use of the proper gastric mucin, and that this procedure may be of use in the selection of bacterial vaccine strains and in turn in the evaluation of antiserum.

Subsequently we have found the "mucin-coating" technic applicable to *B. coli,* gonococci, and *Streptococcus viridans.*[6] In the following experiments we applied similar methods of virulence-enhancement to *H. pertussis.*

We have utilized 41 freshly isolated smooth strains, as described by Shibley and Hœlscher,[7] which fulfill Phase 1 characteristics as stated by Leslie and Gardner.[8] Twenty-four-hour growths on Bordet-agar slants enriched with 25% human or sheep blood, planted from the second of a series of two 48-hour transplants, were washed off with 1 cc. of infusion broth and further dilutions prepared from this as indicated.

Mucin-virulence: The mucin-coating technic has been applied with several minor variations to 36 pertussis cultures injected into

1 Henderson, R., Jamieson, W. A., and Powell, H. M., *Proc. Ind. Acad. Science,* in press.

2 Nungester, W. J., Wolfe, A. A., and Jourdonais, L. F., Proc. Soc. Exp. Biol. and Med., 1932, **30**, 120.

3 Miller, C. P., *Science,* 1933, **78**, 340; Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1136, 1138, 1140.

4 Rake, G., *J. Exp. Med.,* 1935, **61**, 545; Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1175, 1523.

5 Mishulow, L., and Melman, M., *J. Lab. and Clin. Med.,* 1936, **21**, 406.

6 Unpublished experiments.

7 Shibley, G. S., and Hoelscher, H., *J. Exp. Med.,* 1934, **60**, 403.

8 Leslie, P. H., and Gardner, A. D., *J. Hyg.,* 1931, **31**, 423.

530 mice. None of the changes in technic affected the outcome of the tests. Only an occasional mouse died, and these few deaths bore little or no relation to the dose of mucin-coated culture injected. Similar results were obtained in 2 series of tests in 12 guinea pigs. Obviously mucin is of relatively little use in attempts to enhance the virulence of *H. pertussis.*

Starch-virulence: Of 9 different grades of starch† obtained from the Corn Products Refining Company through the courtesy of Mr. R. H. DeWaters, the starch called "145-60 thick boiling starch" prepared in a 4% solution with 0.5% glycerin proved most useful in coating *H. pertussis* with resultant increase in virulence for mice. The results of several tests of 6 different cultures with starch are shown in Table I. A large percentage of mice were

TABLE I.
Range of Mouse-Virulence of Several Pertussis Cultures Coated with Starch at Different Times.

Culture dose (cc.)	No. of mice	
	Died	Lived
10^{-1}	42	0
10^{-2}	33	9
10^{-3}	14	27
10^{-4}	10	31
10^{-5}	0	11

killed with doses of 10^{-4} cc. Two quantitative active immunity tests in mice were tried.

Active immunity in mice: 12 mice received 3 weekly subcutaneous injections each of Pertussis Vaccine (Sauer) lot 921499 Lilly; 12 more received Pertussis Undenatured Bacterial Antigen (Krueger), lot 915013 Lilly. Two weeks after the last dose, 5 mice of each group and 5 controls were given intraperitoneal injections of decimal dilutions of starch-coated culture. The virulence of the test culture at this time only reached 10^{-2} cc.; however, both groups of vaccinated mice were protected against one or more fatal doses. These results are shown in test number 1 in Table II.

†No. 55 Hercules gum thin boiling starch.
No. 106 Alkali starch.
No. 107 " "
No. 126 Thick boiling starch.
No. 131 Thin " "
No. 140 " " "
No. 142 Thick " "
No. 145-30 " " "
No. 145-60 " " "

TABLE II.
Tests of Active Immunity of Mice After Immunization Against Pertussis.

Immunity-test dose of starch-coated *H. pertussis* (in cc.)	Pertussis Vaccine (Sauer) No. 921499 Mice		Pertussis Undenatured Bacterial Antigen (Krueger) No. 915013 Mice		Control Mice	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
10^{-1}	D	DD	D	DD	D	DD
10^{-2}	S	DD	S	DS	D	DD
10^{-3}	S	S	S	S	S	D
10^{-4}	S	S	S	S	S	D
10^{-5}	S	S	S	S	S	S

D—A mouse dead within a week.
S—A mouse living after a week.

Four weeks after the last dose of antigen, the remaining 7 mice in each group, and 7 controls, were given an immunity test as before. The virulence of the test culture at this time was 10^{-4} cc. Both groups of vaccinated mice exhibited protection against 10 or more fatal doses. These results are shown in test number 2 in Table II. It is obvious that additional tests should be conducted for exact quantitative measurements, however the procedure appears to be of use in view of the meager laboratory criteria of virulence and antigenicity of *H. pertussis*.

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Vitamin G from Different Sources and Coccidian Infection.*

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The writers[1, 2] have previously shown that a diet deficient in vitamin G has a pronounced limiting effect on the numbers of oocysts discharged by rats infected with the coccidium *Eimeria miyairii*. The present work was undertaken to ascertain the effect of vitamin G from sources other than yeast.

Experiment I. Thirteen rats were kept for 14 days on a balanced growing ration (Steenbock's) containing corn meal, linseed

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1 Becker, E. R., and Morehouse, N. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 114.

2 Becker, E. R., and Morehouse, N. F., *ibid.*, 1936, **33**, 487.