

to below 7 previous to heating. Conversely an alkaline pH at high temperature hydrolyses tuberculin and thereby destroys its biological activity. Third, the complete disintegration of the bacilli is a logical method in further increasing the yield of tuberculin, for the number of organisms present in most cultures grown in synthetic media is large.

Conclusion. From the results presented above it is justifiable to conclude that tuberculoprotein is suitable for the production of purified tuberculin and that a greater yield of tuberculin over a short period of time may be obtained if the organisms are treated with dilute alkali.

11857 P

Enhancing Action of Egg-Yolk on Virulence of Meningococcus for Mice.

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Nungester and his coworkers¹ found that gastric mucin of the hog exerts a great enhancing action on the virulence of meningococcus in experimental infection of mice. This observation has been widely confirmed and extended to a number of other microorganisms of low virulence. However, as Miller² and Nungester, *et al.*,³ have pointed out that different lots of mucin varied considerably in their activities and that sterilization by excessive heat might decrease their action, difficulties have been met with in our hands to secure a potent product for experimental work. Recently, in our studies on rickettsiae cultivated in the yolk-sac of the developing chick,⁴ it was accidentally noticed that rickettsia in yolk was more virulent than the same organisms contained in other tissues. It was thought that possibly yolk has an enhancing action on virulence similar to that of mucin. In order to test this supposition, meningococcus has been chosen for study and the results are herewith communicated.

¹ Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **30**, 120.

² Miller, C. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1136.

³ Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Inf. Dis.*, 1936, **59**, 11.

⁴ Pang, K. H., and Zia, S. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 76.

Materials and Method of Study. Two freshly isolated strains of meningococcus were employed for this study. Both were of low virulence when suspended in saline or Tyrode's solution. Organisms were grown on blood-agar plate for 6 to 16 hours and made up in Tyrode's solution to 500 million organisms per ml as compared with "Wellcome" opacity-tube for the standardization of bacterial vaccines. Decimal dilutions were then made also in Tyrode's solution to obtain decreasing numbers of organisms and were then mixed with 9 volumes of diluted egg-yolk immediately before inoculation.

Fresh egg-yolk was obtained aseptically and emulsified into uniform consistency by shaking vigorously with sterile glass rods. This emulsion was very sticky and its relative viscosity was found to be over 60 as determined with an Oswald's viscosimeter. Emulsions of varying degrees of viscosity were prepared by the addition of different amounts of sterile Tyrode's solution as listed in Table I.

TABLE I.
Relative Viscosities of Different Dilutions of Egg-yolk.

Relative viscosity	60	52.5	43	34	25.5	19	14.5	11	8.5	7	6.4	5
Egg-yolk in ml	5	4.6	4.4	4.2	4.0	3.8	3.6	3.4	3.2	3.0	2.8	2.5
Tyrode's in ml	0	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.5

Yolk-emulsions possessing relative viscosity between 11 to 19 have been found to be most satisfactory. They were effective in enhancing the virulence of the organisms and, at the same time, sufficiently dilute to be used for inoculation employing a 27-gauge needle.

Results. Yolk-emulsion alone in 2 ml amounts of any dilutions produced no harmful result after intraabdominal injection into white mice. On the other hand, animals receiving as few as 10 meningococci suspended in the same amount of yolk-emulsions of proper viscosity showed marked prostration, ruffling of hair, discharge from eyes, and occasional convulsion and tremor. Death, preceded by coma, usually took place in 16-48 hours. Severe bacteremia could be detected by culturing a drop of blood from the tail vein either just before or soon after death of the animals. However, when the amount of egg-yolk emulsion, irrespective of its viscosity, was decreased this enhancing action on the virulence of the organisms was also greatly reduced. If 1 ml was used instead of 2 ml to suspend the organisms, at least 1000 times more organisms were required to kill mice. As the amount of yolk-emulsion was further decreased below 0.5 ml it became practically inert.

Comment. Emulsions of egg-yolk of certain viscosity have been found to exert an enhancing action on the virulence of 2 freshly

isolated strains of meningococcus for white mice when the proper amount of yolk-emulsion was employed and the intraabdominal route was used for inoculation. As little as 10 organisms were able to kill white mice in 16-48 hours. In contrast to the varying quality of gastric mucin, egg-yolk is easily obtainable and the material has been found to be uniform in pH value and in viscosity as long as the eggs were fresh. If aseptic care is taken in handling the material, no further sterilization is required. Emulsions of the desired viscosity may be prepared according to the data given.

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Immunological Studies on Proteins of *Corynebacterium diphtheriae*.

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Previous studies¹ have shown that type-specific protein derived from the Park-Williams 8 strain of *C. diphtheriae* may be converted into a group-specific protein by heating at 56°C for 30 minutes. This finding suggests that diphtheric proteins in general may not be a single antigen. However, direct chemical methods, such as fractional precipitations with ammonium sulfate, have, thus far, not revealed any antigenic differences. Likewise the application of the sensitive complement-fixation reaction has proven impractical since rabbit sera immunized with whole organisms are markedly anti-complementary and diphtheric proteins alone failed to produce potent antiserum.¹ If it were possible to enhance the antigenic quality of the type-specific protein in rabbits by adsorbing the protein to charcoal² the usual serological methods could then be applied. The results of such a study are described below.

Method. Proteins were prepared¹ from representative strains of the serological types D41 and D43 and of group x.³ All were serologically active, giving precipitin-titers of 1:100,000 with the

¹ Wong, S. C., and T'ung, T., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 824.

² Seibert, F. B., *J. Immun.*, 1935, **28**, 425.

³ Sia, R. H. P., and Huang, C. H., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 348.