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Enhancement of Pathogenicity of Human Typhoid Organisms by Mucin.

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It is recognized that experimental work on typhoid fever has been handicapped by the lack of a small susceptible laboratory animal. Even with highly virulent strains of *B. typhosus,* several million organisms are required to kill mice when inoculated intraperitoneally. With such numbers, the fate of the animal is due not only to virulence and invasiveness but also to toxicity from products of disintegration of the organisms. Recently Norton and Dingle[1] have been able to increase the virulence of *B. typhosus* 20,000 times by intracerebral inoculation. By combining the typhoid organisms with mucin, as used for other organisms,[2-6] their virulence for mice can be increased a million times.

The method for the preparation of mucin has been given elsewhere.[4, 6] Twelve- to 15-hour cultures of the organisms on plain agar have been used throughout. Thirteen cultures of *B. typhosus* and 2 each of Paratyphoid A and B have been tested. Five *B. typhosus* cultures were isolated within 9 months and of these, 3 required only 10 organisms to kill while 2 required 100. One strain was 2 years old and required 1,000 organisms to kill; while the other 7 were old stock strains and all failed to kill with 100,000 organisms (the largest number tested) save one, the so-called Rejuvenated Rawlins strain, which required only 10 organisms.* The Paratyphoid strains were old stock cultures. Three failed to kill with 100,000 organisms, but in the fourth 100 organisms killed. This strain had been kept frozen and dried without sub-culture for years. In the case of the stock strains that failed to kill, Rough, Normal and Smooth, motile and non-motile forms were noted. All of the

1 Norton, J. F., and Dingle, J. H., *Am. J. Pub. Health,* 1935, **25**, 609.

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

3 Miller, C. P., *Science,* 1933, **78**, 340.

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

5 Rake, G., *J. Exp. Med.,* 1935, **61**, 545.

6 Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1175.

* This strain and others were obtained from the Army Medical Center at Washington through the kindness of Colonel J. F. Siler.

virulent strains were smooth and motile. Comparative tests with the same virulent strains suspended in broth and mucin showed an increase in virulence of from 100,000 to over 1,000,000 times. It will be noted that the virulence of the stock strains was not enhanced to a like degree, suggesting that a great part of their activity is due to the breaking down of the large numbers of organisms. Attempts to enhance the virulence of strains by growth in media containing mucin have not been successful.

Protection tests have been carried out using human sera. These results have been compared with the Widal reaction of the same sera. In the protection test the serum has been inoculated intraperitoneally into mice 30 minutes before the intraperitoneal inoculation of organisms. Each mouse receives 0.5 cc. of a 2:5 dilution of serum in saline. For the Widal reaction, O and H antigens have been prepared from the freshly isolated strain of *B. typhosus* which has been used throughout for the protection tests.

Twenty-six sera have been tested. Of these, 5 came from individuals who had received typhoid vaccine from 2 to 12 years before. All sera protected against at least 10,000 killing doses (the maximum tested) and none of the mice showed any sign of sickness. The other 21 sera, from unvaccinated persons with no history of typhoid fever, can be divided into 2 groups: the first, children up to 15 years; and the second, adults above 20 years. Of the 9 children, 8 showed little or no protection while one protected against 1,000 killing doses. Of the 12 adults, 3 showed little protection, 3 protected against 100, and 6 against 1,000 killing doses. It should be pointed out, however, that the serum of unvaccinated individuals, even when it protected against death, did not prevent the mice from becoming sick save in the highest dilutions.

The Widal reaction of the serum did not run parallel with the protective power of the serum. It was never positive except with serum containing protective antibodies. On the other hand, 2 sera which protected against 1,000 killing doses failed to show any agglutination with the antigens used. It would seem, therefore, that the protection test as described is more delicate for discovering slight serological changes than is the Widal reaction.

In brief, a method for increasing the pathogenicity of typhoid cultures for mice by the use of mucin has been determined, which offers a way of selecting cultures for the preparation of vaccines and a delicate measure of antibody changes in human sera.