

3. Agglutinins from previous vaccinations persisted in titer high enough for diagnosis (1/40) in only 21% at the end of one year, and in only 12% at the end of 2 years.

4. There is some correlation, but not marked, between the severity of reaction to inoculation and the titer developed. (Correlation coefficient is $+0.393 \pm 0.058$.)

5. There is no significant correlation between the number of previous vaccinations and the titer developed. (Correlation coefficient is $+0.154 \pm 0.092$.)

6. There is no significant correlation between the time elapsed since the last vaccination and the titer developed. (Correlation coefficient is -0.105 ± 0.093 .)

7. There was a steady rise in agglutinin titer in the group of 25 on whom 3 sets of tests were performed. (Typhoid group 1/3, 1/73, 1/276; previously vaccinated group 1/28, 1/59, 1/270; unvaccinated group 1/0, 1/50, 1/62.)

Further study of this group is contemplated. Parallel agglutination and complement fixation tests are to be run at the end of 3 months from inoculation.

4894

I. Relation of Bile Salts, Cholesterin, Sodium Citrate and Sodium Bicarbonate to Toxicity of Pneumococcus.

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, College of Medicine, Tulane University.

Experiments were performed to determine certain facts relative to the action of sodium citrate, sodium bicarbonate, and some of the constituents of the bile, namely cholesterin and sodium taurocholate, on the toxicity of the pneumococcus. These substances were used separately and in combinations and were injected intraperitoneally into the white mouse. In all, approximately 75 such mice were employed. It is thought by some that bile salts increase the toxicity of the pneumococcus and that cholesterin acts as a buffer reducing this toxicity. The results obtained by various workers^{1,2,3,4}

¹ Rosenow, E. C., *J. Inf. Dis.*, 1911, ix, 190.

² Cole, Rufus, *J. Exp. Med.*, 1912, xvi, 664.

³ Horrall, O. H., and Carlson, A. J., *Am. J. Physiol.*, 1928, lxxxv, 59.

⁴ Ravdin, I. S., Morrison, M. E., and Smyth, C. M., *Ann. Surg.*, 1929, lxxxix, 871.

have, however, not agreed in all features and for that reason it seems justifiable to place on record all experiments pertinent to this subject.

Previous workers⁴ have reported that when sodium taurocholate was dissolved in distilled water and injected into the peritoneal cavity of the mouse, the largest dose tolerated was 0.009 gm. In those experiments in which normal saline was used in place of distilled water the sodium taurocholate product* showed approximately this toxicity. We, therefore, employed one-half the lethal dose or 0.005 gm. of the product.

In comparison with sodium taurocholate dissolved in normal saline, a like solution with 1% sodium citrate added, was made up and it was found that the toxicity was practically doubled while the sodium citrate injected alone had no toxic effect. Twenty-four hour growths of the pneumococcus, Type I, on blood agar were washed down with sterile saline. Suspensions and dilutions thereof were injected into a number of mice in varying dosage in order to standardize the virulence.

To determine the relationship of the number of microorganisms to their virulence, a dilution of 1:8 was made. While 0.05 cc. of the undiluted suspension killed in 18 to 24 hours, equal amounts of the diluted suspension produced death in one-half of the animals in 48 hours. If 0.005 gm. of sodium taurocholate was added to the undiluted suspension the animals died in 8 to 12 hours, whereas if it was added to the diluted suspension the animals died in 20 to 40 hours.

A series of injections of pneumococcus I and sodium taurocholate was made. One-half cc. of the pneumococcus suspension and 0.005 gm. of sodium taurocholate caused death of the animals in 8 to 12 hours. On the other hand, if the sodium taurocholate was injected first and the pneumococcus 10 minutes later, the animals died in 10 to 14 hours; if the pneumococcus was injected 4 hours later the animals likewise died in 10 to 14 hours. Control animals injected with pneumococcus alone died in 18 to 24 hours.

In the next series it was attempted to determine the effect of cholesterin on the toxicity of sodium taurocholate and the pneumococcus. 0.005 gm. of sodium taurocholate, saturated with cholesterin, were added to the pneumococcus suspension and the injected animals died in 8 to 14 hours. However, in 2 animals in which 5% sodium bicarbonate was added to the sodium taurocholate saturated with cholesterin one animal lived for 48 hours and the other survived. Control animals injected with a suspension of cholesterin in saline

* Merck & Co., about 47% pure sodium taurocholate.

alone and with sodium bicarbonate were unaffected while animals injected with sodium taurocholate saturated with cholesterin showed practically the same degree of toxic effects as those injected with sodium taurocholate alone.

As a comparative observation 2% sodium taurocholate dissolved in normal saline was used in conjunction with the pneumococcus Types I and III on the one hand and 1% sodium citrate and 2% sodium taurocholate dissolved in normal saline in conjunction with the pneumococcus Types I and III on the other. In the latter instance death occurred in 18 hours with Type I and in 18 to 50 hours with Type III, while in the former instance death occurred in 26 hours with Type I and survival resulted with Type III.

To determine whether a toxic filtrable substance might be formed upon the addition of sodium taurocholate to pneumococcus suspension, varying dilutions of each were made and incubated from 10 minutes to an hour in order to allow any change which might be effected. In no instance did we obtain after passage of this preparation through a Berkefeld filter a filtrate which manifested any toxic effect when injected intraperitoneally into the mouse.

4895

II. Comparison of Inulin Bile Titration and Virulence of Pneumococcus.

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, College of Medicine, Tulane University.

As it might be possible to determine the variation of the virulence of the pneumococcus by means of the extent and rapidity of fermentation of inulin and the effect of different dilutions of bile salts on this reaction, a series of observations were carried out.

Sodium taurocholate was the bile salt chosen and in certain instances other substances such as cholesterin, sodium citrate and sodium bicarbonate were also incorporated in the solution. Normal saline was used as a vehicle throughout. A 24 hour culture of the pneumococcus was washed down with normal saline and transplants made from this suspension in inulin were incubated for 24 hours. Pneumococcus I was used routinely and type III merely employed at times in comparison. In all instances cultures were obtained from our laboratory stock supply.