

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.

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

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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.

In the first trials, the inulin media used contained no peptone but it was soon found that in many instances where the virulence of the microorganism was impaired, redness but no coagulation would occur. When peptone was added it apparently afforded a nidus for the enzymatic action of the pneumococcus and caused coagulation in many of the instances wherein it had not occurred previously. One-fourth per cent peptone was used thereafter as it had been found that equally good coagulation was produced on the addition of a trace to 2% of this substance.

The time of fermentation at 37°C. was next determined and it was noted that redness began to occur within 6 hours and that coagulation was complete in 17 hours. When, however, the peptone was omitted both the rapidity and the intensity of the reactions were decreased.

Observations were made with relation to the effect of the number of pneumococci transplanted into the inulin. It was found that coagulation ceased in dilutions of 1:48 of the prepared suspension but that redness continued in dilutions as high as 1:49,000. The point of this transition depended upon the virulence of the pneumococcus and its type, since III did not lose its ability to ferment inulin to anywhere near the degree of pneumococcus I in proportion to its loss of virulence.

Upon keeping the pneumococcus in stock for 4 months the loss of virulence of type I was accompanied by a loss of ability to ferment inulin. It was also found that pneumococcus III which lost much of its virulence was less markedly affected in its ability to ferment inulin and the relative comparison of the reactions was lost.

In the subsequent tests the addition of sodium taurocholate dilutions to transplants of pneumococcus I in inulin, employing a constant suspension, demonstrated a curve with successive dilutions of the bile salt of redness, redness and haziness, redness and milkiness, and coagulation wherever the virulence of the microorganism was not too much impaired. This curve seemed to shift to a higher dilution in accordance with the decrease in toxicity of the pneumococcus for the white mouse. The lowest dilution showing a reaction was 1:400 of the sodium taurocholate.

In addition to the sodium taurocholate solution, 1% sodium citrate in the 2% sodium taurocholate solution was used and it was found that the pneumococcus showed a result in the next higher dilution which is in accordance with the increased virulence shown by this mixture for the white mouse and the increased toxicity of this solution.

Two other substances used in solution with the sodium taurocholate were cholesterin and sodium bicarbonate. Cholesterin showed no change in the dilution at which reaction of the pneumococcus on inulin took place when sodium taurocholate alone was used. Sodium bicarbonate even in the higher dilutions of 1:65,600 prevented a change in the inulin which was due, in all probability, to its alkalinity. This change was out of proportion to the slight protective effect it exerted on the white mouse.

Pneumococcus II of our stock was run as a comparison and was found to possess no virulence and to show no reaction on inulin.

Pneumococcus III, as stated previously, did not lose its ability to ferment inulin in the presence of bile dilutions in proportion to its loss of virulence. An interesting point, however, presented itself with regard to its coagulation in that it started in the bottom of the tube while that of pneumococcus I started in the top of the tube. In this type of pneumococcus sodium citrate in sodium taurocholate also shifted the reaction to the next higher dilution.

In summarizing the results, it appears that the ability of pneumococcus I to ferment inulin, especially when the bile salt dilution is added, varies with its virulence for the white mouse and its numbers, while that of pneumococcus III maintains its ability to ferment inulin on loss of virulence to a much greater degree than type I. There is also demonstrated an inhibiting effect on pneumococcus inulin fermentation, if the sodium citrate is added to the sodium taurocholate solution which is in accordance with the increased toxicity of this solution for the white mouse.

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A Comparative X-Ray Study of Passage of Foodstuffs Through Gastro-intestinal Tract of Rats.

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For many years previous to the discovery of the Roentgen ray, studies of the emptying time of various foodstuffs by the stomach and intestines were made by many investigators. These early investigations were usually conducted on animals with artificial fistulas. It was impossible at that time to visualize the viscera of these ani-