

A second inoculation probably ought to be given some weeks or months after the first. 2. The Widal Reaction is much less reliable as a test for typhoid fever among those who have had more than one inoculation with vaccine than among those who have had only a single inoculation.

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Effects of Sodium Citrate on Bile Salt Hemolysis.

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In a previous report¹ we observed an increase in toxicity of bile salts* injected intraperitoneally into the white mouse when sodium citrate was added. In the present study the effects of varying dilutions of bile salts and sodium citrate on human blood cells *in vitro* have been observed.

When bile salts are added to red blood cells of different individuals and allowed to stand at room temperature for 18 hours, hemolysis occurs through dilutions of 1:200 to 1:6400. The bloods of 100 undiagnosed individuals were examined in order to determine their susceptibility to bile salt hemolysis. The large variation in ability of bile salts to hemolyse different bloods is independent of changes due to age of cells, and independent of the temperature and humidity under which the cells are kept.

When sodium citrate is added to bile salts, hemolysis of red cells takes place in higher dilutions of the bile salts than when the bile salts *per se* are employed and the dilution at which hemolysis takes place depends upon the concentration of the sodium citrate added. If concentrations of less than 0.032% (1:3200) of sodium citrate are added there is no increase in the hemolytic power of bile salts; however, as the concentration of sodium citrate added is increased above 0.032% the hemolytic power of the bile salts is likewise increased. For example, if 1% (1:100) sodium citrate is added to the bile salts dilutions, hemolysis takes place in the 1:400 dilution, while if 4% (1:25) sodium citrate is added hemolysis takes place in the 1:1200 dilution of bile salts. We see by these figures the gradual increase in hemolytic power of bile salts produced by addi-

¹ Williams, J. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, xxvii, 637.

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

tion of sodium citrate from the 0.032 to 1% concentrations and the rapid increase from the 1 to 4% concentrations. This increase in hemolytic power of bile salts rises further as concentrations of sodium citrate greater than 4% are added. The above is an example of the hemolytic reaction of one individual. It is typical of the reactions of all individuals with the exception that susceptibility of different bloods to bile salt hemolysis varies and the reactions would vary in accordance. Sodium chloride also increases the hemolytic power of bile salts but to a less marked degree. In all cases normal saline was used as a vehicle and dilutant since with this solution the controls of citrate, saline and glucose showed no hemolysis, whereas hemolysis did occur in the higher dilutions when distilled water was employed as a solvent. Beef serum was also used as a vehicle and dilutant of bile salts and sodium citrate which were titrated against beef red cells suspended in beef serum. In the later titrations with the serum vehicle, sodium citrate increased the hemolytic power of bile salts but to a considerably less marked degree than when normal saline was used as vehicle and dilutant for the beef cells, sodium citrate and bile salts. It can be said that bloods of different individuals show a variation in susceptibility to bile salt hemolysis.

Sodium citrate and to a lesser degree, sodium chloride in proportion to their concentrations increase the extent of the dilution of bile salts in which hemolysis of red cells takes place while glucose manifests no such effect. When beef serum is used as a dilutant and vehicle in place of normal saline, the hemolytic activity manifested on beef cells by dilutions of bile salts with different concentrations of sodium citrate added is similar but less active than that manifested when normal saline is used as a vehicle and dilutant. This protective mechanism afforded the body by its serum against hemolytic agents has long been recognized.

The varying susceptibility of different bloods to hemolysis by bile salts *per se* and the increased hemolytic effect manifested on adding sodium citrate to bile salts offers a means of determining red cell fragility. With known concentrations of bile salts and sodium citrate and with red cells of known fragility, it should be possible to determine the amount of bile salts in an unknown serum. Since under certain conditions the bile salt content of the blood varies, a means of perfecting the above determinations should serve as an index to the potential danger in blood transfusions of reaction from citrated blood due to the high bile salt content of the blood of the donor or recipient or both.