

A PRELIMINARY NOTE ON THE FRACTIONAL PRECIPITATION OF THE GLOBULIN AND ALBUMIN OF NORMAL HORSE'S SERUM AND DIPHTHERIC ANTITOXIC SERUM, AND THE ANTITOXIC STRENGTH OF THE PRECIPITATES.

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It has been shown by investigators that the globulin of tetanus and diphtheria antitoxic sera, when separated from the sera, carries with it the antitoxic power of the sera. It has not been proved, however, that the "antitoxin" is a globulin. My work of the past two years has been directed to the study of the proteids of different antitoxic sera, especially diphtheria antitoxic serum.

An article in the *Zeitschrift für Hygiene und Infektionskrankheiten* (1889, xxxi, p. 513) by Dr. W. Seng, entitled "Ueber die qualitativen und quantitativen Verhältnisse der Eiweisskörper im Diphtherieheilserum," suggests to me that it would be well to publish a preliminary note on my work.

When the globulin, which has been separated from horse's serum by means of magnesium sulphate and purified by dissolving in water, reprecipitating and washing with a saturated magnesium sulphate solution, is dissolved in water, and this solution of purified globulin is saturated with sodium chloride, a precipitate is formed. This I will call the room temperature precipitate (R° ppe.). If, after the removal of the R° precipitate by filtration, the temperature of the filtrate be raised to 40° C., a little more salt having been added to insure saturation, a turbidity appears in the fluid and when the temperature of 44° or 45° C. is reached the precipitation is complete for this temperature.

Proceeding in the same manner one obtains a third turbidity at 49°

C. and complete precipitation at 53° C., a fourth turbidity at 57° C. and complete precipitation at 62° C., and finally a fifth turbidity at 67° C. and complete precipitation at 72° C. These precipitates dissolve in water, except a very small quantity of that obtained at 72° C., which is insoluble in water but redissolves immediately when treated with weak sodium hydroxide.

Exactly the same reactions occur when diphtheric antitoxic serum is employed instead of normal horse's serum. In both sera the final filtrates fail to give the biuret reaction, and on boiling and subsequent addition of a little acetic acid show no turbidity. Each one of the separate fractions of the antitoxic globulin possesses an antitoxic power while the final filtrate is free from antitoxin.

After the globulin precipitated by the magnesium sulphate in both the normal and diphtheric antitoxic sera has been filtered off, the albuminous filtrate is saturated with sodium chloride. One then finds that an albuminous precipitate is not formed at room temperature. A double salt which is formed by the magnesium sulphate and sodium chloride is removed by filtration and the filtrate is raised in temperature to 56° C. when a turbidity is formed. At 61° C. the precipitation is complete. At 68° C. a second turbidity appears and can be filtered off at 72° C. At 73° C. a third turbidity appears and at 76° C. this precipitation is complete. Finally at 77° C. a slight turbidity appears and at 81° C. the precipitation is complete. The final filtrates from the 81° precipitation fail to give the biuret reaction, and on boiling and subsequent addition of acetic acid show no turbidity.

The albumin precipitated at 56° C. is soluble in water. The other three precipitates are partially soluble in water, and completely so in sodium hydroxide.

In a paper which will shortly appear I shall give the quantitative relations between the corresponding fractions of the normal and antitoxic globulin and albumin, and the antitoxic value of the fractions of the antitoxic globulin.