

## 87 (2319)

## Irregular series in protein precipitation.

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The "irregular series" or "tolerance zone" in the precipitation of albumin with heavy metals has been observed by many investigators of the properties of proteins. Many theories have been advanced to account for the phenomenon but none of them have, up to the present time, given a satisfactory explanation.

The present work being conducted by the authors has linked the irregular series with the isoelectric point of the protein, thus accounting for the phenomenon by a known physical property of albumin rather than assuming the formation of a series of hypothetical compounds of varying composition and solubility.

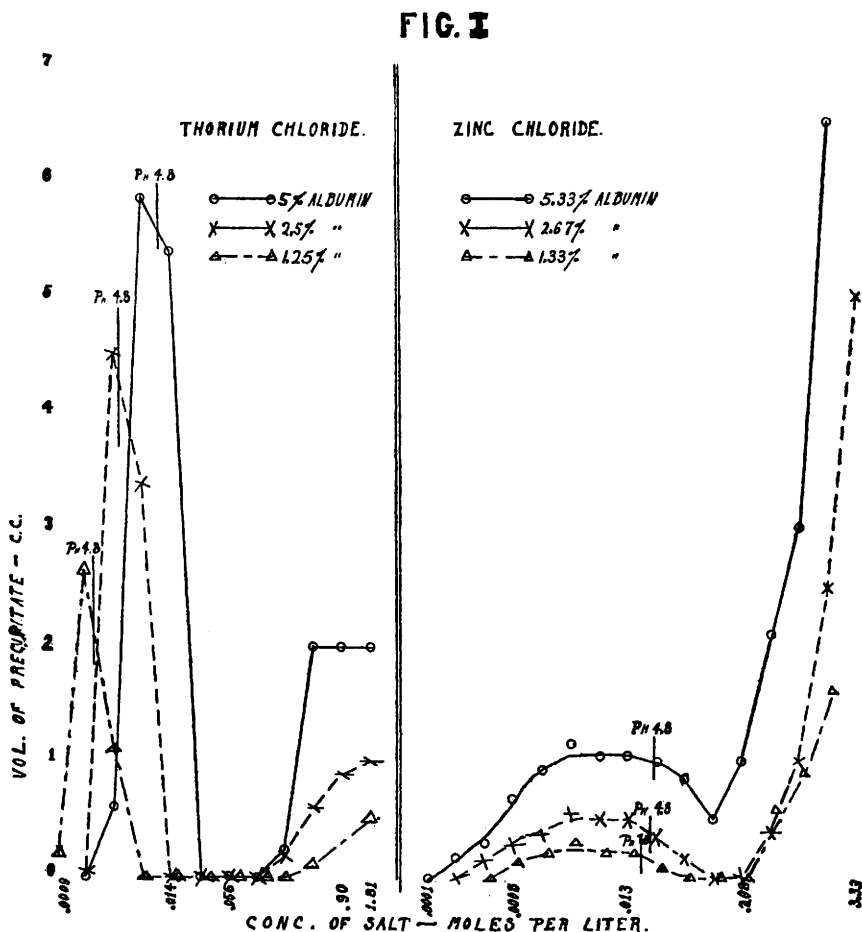
In the experiments a solution of dried egg albumin was dialyzed against distilled water until practically ash free, filtered and kept under toluene. Series were then set up in fifteen cubic-centimeter graduated centrifuge tubes, such that the concentration of albumin remained constant in the final dilution, while the concentration of heavy metal salt varied. The tubes were allowed to stand thirty minutes, centrifuged twenty minutes and the volume of precipitate measured. The hydrogen ion concentration of the supernatant liquid was determined by means of the platinum electrode.

The curves shown in Figure 1 were obtained for thorium chloride and zinc chloride.

Cupric chloride and ferric chloride give curves similar to those of zinc chloride.

The aqueous solutions of heavy metal salts are decidedly acid due to the hydrolysis of the salt. The hydrogen ion concentration increases with increasing concentration of salt. In albumin solutions containing a low concentration of heavy metal salt there is an increasing precipitation with increasing salt concentration until the hydrogen ion concentration of the solution passes the isoelectric point of the protein (pH 4.8), when there is a marked falling off and disappearance of the precipitate.

Thus an insoluble heavy metal-albumin complex is formed



when the protein is on the alkaline side of its isoelectric point, and therefore acts as an anion, as the protein passes to the acid side of its isoelectric point its properties change to that of a cation and it is not precipitated by the heavy metal in dilute solutions. The precipitate formed in dilute solutions is readily soluble in dilute acids or in solutions of hydrogen ion concentration greater than  $10^{-4.8}$ .

The precipitate formed in concentrated solutions of the heavy metal salt, was not soluble either in water or dilute acids. When the precipitate was washed with dilute acid of approximately the hydrogen ion concentration in which it had been formed until the

washings no longer gave a test for the heavy metal ion, a residue remained very similar in appearance to heat coagulated or denatured albumin and which after ignition did not give a positive test, for the heavy metal ion. The precipitation of albumin in concentrated salt solutions was not immediately complete but precipitate continued to form for a period of time which would indicate a secondary reaction or denaturing of the albumin. The precipitation with concentrated solutions of heavy metal salts is probably a salting out of the albumin followed by denaturing of the protein.

### 88 (2320)

#### Is the bacteriophage of d'Herelle volatile?

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Following the description of the phenomenon of transmissible lysis of bacteria by d'Herelle in 1917, an enormous amount of evidence has been brought forward in support of his observations.

At the same time, however, there has also been an ever-growing tendency to dispute the parasitic nature of the active principle responsible for the lysis as postulated by d'Herelle.

In this connection, recent findings by Olsen and Yasaki<sup>1</sup> appeared to offer most important evidence which we attempted to corroborate.

Subjecting 18 hour cultures of so-called "bacteriophage" to distillation at 45-50° C. under reduced pressure, Olsen and Yasaki claim to have obtained a product capable of initiating transmissible lysis of cultures of susceptible bacteria. They conclude, therefore, that the "bacteriophage" is a volatile chemical substance and not a living parasite. Following their description of the experiment, we connected three flasks in series. The first,

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<sup>1</sup> Olsen and Yasaki, *Klin. Woch.*, 1923, xli, 1879.