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Preparation of Crystallized Egg Albumin (Ovalbumin).

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Sörensen[1] indicates that crystallized egg albumin, in contrast to most common proteins, may be considered to be a distinct chemical individual. Since this property makes it especially suitable for studies on the physico-chemical and immunological behavior of proteins, a simple, dependable method for its preparation is desirable.

In all the standard methods for the preparation of crystallized egg albumin, fresh egg white is first treated with an equal volume of saturated ammonium sulphate solution, to precipitate the so-called egg "globulin". The filtrate from this sticky, dough-like precipitate of globulin is then ordinarily brought to the neighborhood of the iso-electric point of the ovalbumin, where crystallization takes place, by the addition of the required amounts of acetic, hydrochloric or sulphuric acids. Hektoen and Cole[2] pointed out that the voluminous precipitate of "globulin" obtained from egg white is not a true globulin, but a mixture or compound of all the proteins in egg white. When extracted with distilled water, the greater portion of it dissolves, leaving behind a clear, gelatinous residue which has the physical properties, at least, of a mucin. The water soluble portion yields crystals of ovalbumin, and contains large quantities of the non-crystallizable conalbumin as well as some ovomucoid.

In the present method, fresh egg white is beaten lightly to break up the membranes, strained through cheese cloth and then acidified by the gradual addition, with constant stirring, of one volume of N/1 acetic acid for each 9 volumes of egg white. Measurements made with the quinhydrone electrode on numerous samples of fresh egg white indicate that this amount of acid is sufficient to bring the material to a pH of 5.0 or less, where, as shown by Sörensen and Höyrup[3] crystallization of the ovalbumin readily takes place. Acetic acid is used in preference to a stronger acid because it may be used in this concentration with less danger of passing beyond the crystallization zone. This acidification of the egg white causes the sep-

[1] Sörensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 18. No. 5, 1930.

[2] Hektoen, L., and Cole, A. G., *J. Infect. Dis.*, 1928, **42**, 1.

[3] Sörensen, S. P. L., and Höyrup, M., *Compt. rend. trav. Lab. Carlsberg*, 1917, **12**, 213.

aration of a comparatively small, gelatinous precipitate (probably ovomucin) which may be removed easily by straining the material through cheese cloth. The filtrate obtained in this manner no longer exhibits the sliminess and viscosity characteristic of egg white, but may be readily filtered through ordinary filter paper. The clear filtrate is then brought to 40% saturation with ammonium sulphate, and filtered from the slight precipitate formed at this point. To the clear filtrate, saturated ammonium sulphate solution is now added, a little at a time, with constant stirring, until a highly opalescent solution is formed. This solution is then permitted to stand at room temperature, with frequent stirring, until crystallization takes place. Crystallization ordinarily begins in a few hours, and within 24 hours a voluminous precipitate of needle-like crystals usually separates out. The crystallization process is permitted to continue for 5 to 7 days, the mixture being stirred occasionally to facilitate the establishment of equilibrium between the crystals and the mother liquor. At the end of this time, the crystals are filtered off and permitted to drain as completely as possible from the mother liquor. They are then washed by covering them, on the filter paper, with a solution of ammonium sulphate which just fails to give a turbidity when treated with a small portion of the mother liquor. After this wash liquid has drained off, the bulk of the precipitate is scraped off the filter paper and transferred to a clean beaker. The filter paper is then extracted several times by maceration with small quantities of distilled water and the extracts, after removal of the filter paper by straining through cheese cloth, are added to the main portion of precipitate. The mixture is then stirred gently, with addition of more distilled water if necessary, until completely dissolved.

For purposes of recrystallization, this solution of ovalbumin is filtered and the clear filtrate treated with a buffered solution of saturated ammonium sulphate. This latter solution is prepared by mixing one volume of acetate buffer (N/1 acetic acid + N/1 sodium acetate) having a pH of approximately 4.7 with 9 volumes of saturated ammonium sulphate solution and then saturating the mixture with solid ammonium sulphate. This buffered ammoniun sulphate solution serves to maintain the ovalbumin, without further manipulation, at the proper pH for crystallization. It is added to the ovalbumin solution, a little at a time, with constant stirring, until a highly opalescent solution is again formed. The procedures for crystallization, washing and re-solution of the crystals are now the same as described above, and the crystallizations may be repeated as many times as desired. Hektoen and Cole,[2] using the precipitin

reaction, confirmed Sörensen and Höyrup's[4] observation that at least 3 such crystallizations and washings are required to free the ovalbumin completely from conalbumin. After the final crystallization and washing, the ovalbumin is dissolved in distilled water, placed in collodion or cellophane bags under toluol and dialyzed against distilled water until free from salts.

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Meteorological and Menstrual Reflections in Nail Growth.

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When daily measurements of nail growth is recorded in the normal person or in patients, distinct periods of expansion and retraction of the nail can be observed, as well as variations in the rate of growth.

Periods of cold and increasing barometric pressure (cyclonic cold fronts) are associated with cessation of growth and retraction of the nail, periods of increasing temperature and barometric decline with increase in the rate of growth. However, similar fluctuations occur in subjects kept constantly at normal room temperature (68 to 72°F). The periods of increase in the rate of growth apparently reflect periods of general stimulation that follow the anoxemia.

In the graph which illustrates observations made on the same individual described in a preceding note,[1] the actual growth curve of the nail is recorded. Arrows have been carried up from periods of high barometric pressure which, it will be observed, correspond to a time of retraction of the nail or slowing of its rate of growth; expansion or increase in the rate of growth occurs with diminution of the barometric pressure.

Premenstrually an acceleration in the rate of growth is observed and an unusual sensitiveness of the nail to the minor meteorological disturbances of the premenstrual period (February 27-March 6).

The rate of nail growth apparently reflects very accurately the meteorological environmental influences.

[4] Sörensen, S. P. L., and Höyrup, M., *Compt. rend. trav. Lab. Carlsberg*, **1917, 12**, 12.

[1] Petersen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1145.