CXXIV. ON THE PREPARATION OF CRYSTALLIZED EGG ALBUMIN METAPHOSPHATE

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IN carrying out the reaction between metaphosphoric acid and egg albumin one may obtain slowly separating, slightly soluble crystals instead of an amorphous precipitate.

Crystallized egg albumin has already been prepared by Hopkins [1900]; Willcock [1908]; Sørensen & Høyrup [1917]; Sørensen & Palitzsch [1923] by means of different electrolytes (sodium, potassium, magnesium sulphate, mixtures either of primary and secondary ammonium phosphate, arsenate or citrate). The characteristics of our method are: (1) the use of an acid to obtain crystallization, (2) the small concentration of the acid and (3) the fact that the crystallizing agent is metaphosphoric acid, which is one of the ordinary precipi-

Fig. 1. Crystals of egg albumin metaphosphate. ($\times 1000$.)

tants of proteins. It has generally been supposed that the action of precipitating reagents is somehow connected with denaturation. The possibility of carrying out the crystallization in this way is evidence that the precipitated protein is

not in a denatured state, since up to the present denatured proteins have not been crystallized. Further discussion of this subject will be found in the preceding paper, in which the solubility of egg-albumin metaphosphate complexes is compared with that of denatured proteins.

Egg albumin crystallizes from a metaphosphoric acid solution in a *pH* region of 4.5–4.0. The amount of phosphorus in the crystals varies by 9% according to the *pH* at which crystallization is carried out. Microscopically the crystals do not differ from the ordinary egg albumin obtained from saturated ammonium sulphate solution. They are needle-shaped and appear often in clusters or as well developed prisms. Under favourable circumstances we obtained crystals as large as 1 mm. The double refraction is extremely small. The crystallized product is very insoluble in water but can easily be dissolved in salt or buffer solutions just as the amorphous precipitate can.

Experimental

Our starting material was an egg albumin solution prepared from fresh eggs by crystallization with saturated ammonium sulphate according to Sørensen & Høyrup [1917]. A solution from the thrice recrystallized products was dialysed until it gave no barium sulphate and Nessler tests.

To the egg albumin was added an amount of metaphosphoric acid solution just sufficient to give a precipitate which did not entirely redissolve. Then the liquid was centrifuged and the supernatant clear solution removed and allowed to stand at room temperature. After 12 hr. it became turbid and started depositing crystals.

After 2 days the crystals were filtered off and washed on the filter with distilled water until the washings were free from protein and phosphorus, then the crystals were dried and the nitrogen determined by the micro-Kjeldahl method and the phosphorus after Lohmann & Jendrassik [1926].

As the crystals contain a large amount of water, which can only be removed with difficulty, we found it necessary to dry at least 24 hr. *in vacuo* at 90° to obtain a N content equal to the value found by Sørensen & Høyrup [1917]. If we dried to constant weight at room temperature *in vacuo* or at 110° under atmospheric pressure the N content was as low as 13.3%.

Sample	Nitrogen		Phosphorus		Hydrogen ion concentration of the filtrate
	mg.	%	mg.	%	
264.2	41.0	15.5	2.24	0.85	3.98×10^{-5}
—	41.6	15.7	2.20	0.84	—

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