CXXIX. THE BASIC AMINO-ACIDS OF CRYSTALLINE EGG-ALBUMIN[1].

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The extensive investigations of Sørensen [1917, 1930] have provided evidence that crystalline egg-albumin conforms more closely than almost any other protein to the criteria for a homogeneous and reproducible chemical substance. He has also shown that its solubility in aqueous solutions of neutral salts follows the laws of physical chemistry with considerable precision, and that its osmotic pressure in solution is that of a substance of molecular weight close to 34,000, a magnitude that is supported by the results of Cohn, Hendry and Prentiss [1925], and of Svedberg and Nichols [1926]. Egg-albumin is therefore an eminently suitable substance to employ in studies of proteins as electrolytes in solution; its usefulness for this purpose has been restricted, however, by the inaccuracy and incompleteness of our present knowledge of its amino-acid composition.

There is good reason to believe that the acid- and base-binding capacities of proteins can be quantitatively accounted for in terms of the proportions of trivalent amino-acids (basic and dicarboxylic amino-acids) which they yield on complete acid hydrolysis [Cohn, 1925, 1931]; accurate determinations of the tervalent amino-acids are consequently of the first importance in studies of the electrochemical behaviour of proteins. We have therefore undertaken a determination of the basic amino-acids yielded by egg-albumin and, in order to facilitate the application of our results, have employed a standard preparation of the protein upon which electrometric titrations are being conducted in another laboratory.

At the time when the present analysis was begun no determinations by modern methods of the three basic amino-acids of crystalline egg-albumin had been made. Osborne, Jones and Leavenworth [1909] employed the procedure of Kossel and Patten [1903] (see Table I) and Skraup and Hummelberger [1909], who worked with commercial egg-white, used the original method of Kossel and Kutscher [1900]. Since then a few determinations of single bases only have been recorded and these are also shown in Table I.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, Washington, D.C.

Table I. *The basic amino-acids yielded by egg-albumin.*

Analyst	Albumin prepared by	Histidine %	Arginine %	Lysine %
Authors (1st analysis)	Cannan and Shore	1·43	5·30	4·97
Authors (2nd analysis)	,,	1·25	5·31	4·69
Authors (3rd analysis)	du Vigneaud	1·48	5·56	4·78
Calvery [1932] (corrected)	Calvery	1·39	5·04	4·00
,,	,,	1·41	5·01	3·91
Calvery	du Vigneaud	1·38	5·11	3·78
Osborne, Jones and Leavenworth [1909]	Leavenworth	1·71	4·91	3·76
Skraup and Hummelberger [1909]	Commercial	1·5	2·9	3·9
Hanke [1925] (colorimetric)	—	2·3	—	—
Sakaguchi [1925] (colorimetric)	—	—	6·07	—
Fürth and Deutschberger [1927] (flavianate)	—	—	6·0	—
Hunter and Dauphinee [1930] (arginase)	Merck	—	5·02*	—

* This figure is calculated from Hunter and Dauphinee's data on the assumption that their egg-albumin contained 15·62% protein-N [Sørensen and Høyrup, 1917].

Just as we had completed the first analysis given in the table we learned, through a personal communication, that Dr H. O. Calvery of the University of Michigan had likewise conducted an analysis of crystalline egg-albumin. Dr Calvery kindly allowed us to see his results in advance of publication [Calvery, 1932]; his value of 5·03 % for arginine was in good agreement with our own, but his higher values of 2·44 % of histidine and 6·41 % of lysine induced us to repeat the analysis of our preparation of egg-albumin, and ultimately to analyse a preparation kindly furnished us by Dr du Vigneaud, of the University of Illinois, for this purpose[1]. The close agreement of the results of these three analyses was brought to Dr Calvery's attention and, on re-examining his own data, he found that an unfortunate error had been committed in his original calculations. The figures given in Table I represent the corrected results of Calvery's previous determinations of the bases of egg-albumin, and with them is given a new analysis of du Vigneaud's preparation carried out by Calvery simultaneously with our analysis of the same material.

Experimental.

First analysis. The albumin was prepared from fresh hen's eggs by one of the authors while working with Prof. R. K. Cannan at New York University and Bellevue Hospital Medical College. The method of Sørensen and Høyrup [1917] was used and the protein was crystallised three times from ammonium sulphate solutions at a reaction close to p_H 4·7. The product was dissolved in water, dialysed free from sulphate, and was then coagulated by being poured into a large volume of water heated to 70°. The coagulum was washed successively with liberal quantities of water, alcohol, and ether, and was then dried. It contained 15·95 % of nitrogen corrected for ash and moisture. The moisture content was 2·90 % and the ash 0·53 %. On distillation with mag-

[1] We are deeply indebted to Dr du Vigneaud for supplying us with this very fine preparation.

nesia the equivalent of 0·12 % of free ammonia-nitrogen was found; corrected for this the protein-nitrogen of the preparation was 15·83 %.

Of this material 191·4 g., corrected for moisture and ash, were boiled for 31 hours with 1600 cc. of 6*N* hydrochloric acid. The hydrolysate was concentrated *in vacuo* to a syrup which was diluted and again concentrated several times to remove as much of the acid as possible. The syrup was then diluted, sulphuric acid was added, and the remaining hydrochloric acid was removed by the careful addition of an excess of silver oxide, the reaction being maintained at p_H 2–3 with sulphuric acid. The silver chloride was thoroughly extracted with hot, very dilute hydrochloric acid in order to recover any histidine that may have been precipitated, and the extract was evaporated to dryness; the chloride remaining in this was removed in the same manner. The combined solutions were concentrated to 4 litres and an attempt was made to introduce an excess of silver ion by the alternate addition of sulphuric acid and silver oxide according to the technique of Kiesel [1926]. Although silver oxide was added until silver sulphate crystallised copiously from the solution, excess of silver ion could not be demonstrated by means of barium hydroxide. A similar behaviour has been repeatedly encountered in this laboratory and, apparently, the difficulty can be surmounted only by liberal dilution of the hydrolysate or by the use of silver nitrate; in the present analysis we chose the latter alternative. After the addition of excess of silver nitrate the precipitate of silver sulphate was removed and washed, and the arginine and histidine silver compounds were thrown down by adjusting the reaction to approximately p_H 12 with warm saturated barium hydroxide. From this point the analysis was conducted as described by Vickery and Block [1931]; the data from which the yields of the three bases were calculated are assembled in Tables II–IV.

Second analysis. The material used for the second analysis had been crystallised three times and was then dialysed and coagulated as before, washed and dried in the air. It contained 15·70 % of nitrogen calculated moisture- and ash-free. The moisture content was 12·22 % and the ash was 0·25 %. The amount taken was determined both by weighing the air-dry material and by analysis for nitrogen of an aliquot part of the hydrolysate, after this had been carefully agitated in order to bring any insoluble humin into suspension. The quantity carried on for analysis, as determined from the air-dry weight, was 72·35 g., as determined from the nitrogen in the hydrolysate, was 72·41 g.; the average of these, or 72·38 g., was employed in the calculations. Hydrolysis was effected by boiling the protein with 900 cc. of 6*N* hydrochloric acid for 25 hours; the acid was then removed as before. The filtrate from the silver chloride was brought to 5 litres and an excess of silver ion was introduced by means of silver oxide and sulphuric acid. Owing to the greater dilution of this solution with respect to amino-acids no difficulty was encountered. The procedure followed was that described by Vickery and Leavenworth [1928], except that the histidine fraction was freed from cystine

Table II. *Histidine fraction.*

Analysis	Protein taken for analysis g.	Vol. of histidine fraction cc.	Nitrogen content g.	Histidine equivalent in protein %	Vol. of fraction used for diflavianate cc.	Weight of diflavianate g.	Sulphur content of diflavianate. Theory 8·17 % %	Yield of histidine corrected %
1	191·4	500	0·7735	1·49	420	11·678	8·18	1·43
2	72·38	250	0·285	1·45	230	4·219	8·14	1·25
3	64·25	250	0·2887	1·66	230	4·434	8·17	1·48

Table III. *Arginine fraction.*

Analysis	Vol. of arginine fraction cc.	Nitrogen content g.	Arginine equivalent in protein %	Vol. of filtrates from arginine silver cc.	Solubility* correction for arginine silver 0·036 g. arginine per litre g.	Vol. of fraction used for flavianate cc.	Weight of flavianate g.	Arginine equivalent in 500 cc. of fraction g.	Sulphur content of flavianate. Theory 6·56 % %	Yield of arginine corrected %
1	500	3·474	5·64	12,500	0·450	50	2·717	9·687	6·61	5·30
2	500	1·532	6·58	9,200	0·331	100	1·9705	3·513	6·64	5·31
3	500	1·475	7·14	7,700	0·277	100	1·8480	3·294	6·52	5·56

* The weight of arginine calculated from the solubility correction is added to the weight derived from the flavianate.

Table IV. *Lysine fraction.*

Analysis	Vol. of lysine fraction cc.	Nitrogen content g.	Lysine equivalent in protein %	Vol. used for picrate cc.	Weight of picrate. Crop 1 g.	Weight of picrate. Crop 2 g.	Solubility correction, 0·54 g. picrate per 100 cc. mother-liquor g.	Explosion-point of picrate. Crop 1 °C.	Explosion-point of picrate. Crop 2 °C.	Nitrogen content of picrate. Crop 1. Theory 18·67 % %	Yield of lysine corrected %
1	250	11·84	6·18	246	21·252	2·445*	0·351	266	257	—	4·97
2	500	1·012	7·28	480	7·658	0·600†	0·110	264·5	256	18·50	4·69
3	500	0·846	7·3	480	7·194	0·283	0·089	265	250	18·49	4·78

* This weight includes 0·899 g. of pure lysine picrate obtained from the mother-liquor of the crude picrate after carrying the material through another phosphotungstic acid precipitation.
† This weight includes 0·137 g. of pure lysine picrate obtained as above.

by treatment with copper hydroxide [Vickery and Leavenworth, 1929], and the precipitates of histidine silver were decomposed with hydrogen sulphide rather than with hydrochloric acid. The full data are shown in Tables II–IV.

Third analysis. The material analysed had been crystallised four times from ammonium sulphate solutions and was then dialysed and subsequently coagulated by being poured into a large volume of alcohol. The coagulum was washed with absolute alcohol and ether and was dried in the air. It contained 15·61 % of nitrogen calculated moisture- and ash-free. The moisture content was 6·85 % and the ash was 0·22 %. Dr du Vigneaud's label declared the presence of a trace of sulphate. Distillation with barium hydroxide *in vacuo* showed that the contamination with ammonium sulphate was of the order 0·003 % and could therefore be neglected. The corrected weight of the quantity taken for analysis was determined from the weight of the dry material and by analysis of an aliquot part of the hydrolysate. The two results were 64·39 g. and 64·10 g., and the average value of 64·25 g. was employed in the calculations.

The protein was boiled for 30 hours with 900 cc. of 6*N* hydrochloric acid and the subsequent procedure was exactly the same as that of the second analysis. The data obtained are given in Tables II–IV.

Discussion.

The data collected in Table I show five closely agreeing determinations of the proportion of histidine yielded by crystalline egg-albumin. These were conducted by three different operators and three different preparations of the protein were employed; furthermore the procedures used differed in minor points. The conclusion seems justified therefore that the average of these five determinations, which is 1·42 %, represents very closely the correct yield of histidine from this protein. If the molecular weight of egg-albumin be taken at 34,000, and it be assumed that the protein contains 3 histidyl radicals per molecule, the proportion of histidyl should be 1·21 %. The yield of 1·42 % of histidine is equivalent to 1·25 % of histidyl and this agrees closely with the assumption. There seems little reason to doubt, therefore, that the molecule of egg-albumin contains three histidyl radicals.

The three arginine determinations carried out in this laboratory are all somewhat higher than those of Calvery [1932], the average being 5·39 %. They represent the results of two operators working independently and employing somewhat different procedures. Calculated as arginyl, the result is equivalent to 4·83 %. The theoretical requirements for 10 arginyl radicals per molecule of egg-albumin is 4·59 %, that for 11 radicals is 5·05 %. Our highest figure for arginine, 5·56 %, when calculated as arginyl, is equivalent to 4·98 % and the agreement between this figure and the requirement for 11 arginyl radicals is strikingly close. Our results are best interpreted, then, by the assumption that the molecule of egg-albumin contains 11 arginyl radicals.

The determination of lysine is probably the least trustworthy of that of any of the bases, and it is in this determination that the advantage of working on a large scale becomes manifest. We regard the result of our first analysis, 4·97 %, as probably nearest the truth, the somewhat lower figures of the other analyses serving to confirm the order of magnitude. Calculated as lysyl, this is equivalent to 4·36 %; the theoretical requirements of 11 and 12 lysyl radicals are 4·14 % and 4·52 % respectively. The most probable value for the lysyl content of egg-albumin, according to the present analysis, is therefore 12 radicals.

Summary.

The proportions of the basic amino-acids yielded by crystalline hen's egg-albumin, after acid hydrolysis, have been found to be histidine 1·42 %, arginine 5·39 %, and lysine 4·97 %. These results are in close agreement with the assumption that the molecule of egg-albumin, the weight of which is probably close to 34,000, contains 3 histidyl radicals, 11 arginyl radicals, and 12 lysyl radicals.

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