VITELLIN OF HEN'S EGG

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Vitellin, the chief protein of egg yolk, has for a long time been of great interest to biologists because of its close relationship to the developing avian embryo. For this and other reasons it has also been of interest to chemists. It has been analyzed by four sets of investigators with very different results. The values obtained by Levene and Alsberg (1) and by Hugounenq (2) do not agree with those of Abderhalden and Hunter (3) and Osborne and Jones (4). The values obtained by Osborne and Jones are probably the most accurate since they are the most recent.

Because of our interest in the chemical changes that occur during embryonic development, and since we have large quantities of vitellin available, we decided to investigate its amino acid content by some of the more modern methods. We also decided to determine the distribution of the nitrogen by the method of Van Slyke (5) since it had never been reported, although Plimmer and Rosedale (6) made such a study on the combined egg yolk proteins.

EXPERIMENTAL

Preparation of Vitellin

The vitellin was prepared essentially according to the method of Osborne and Jones (4). The yolks of twenty-four eggs were separated from the whites and washed thoroughly with saline solution and running water without rupture of the yolk membrane. They were then broken into an equal volume of 10 per cent sodium chloride solution and extracted with ether containing about 2 per cent ethyl alcohol until no more lipids could be obtained in the ether layer. The water solution was then strained through cheesecloth to remove the yolk membranes which are insoluble in sodium chloride solutions. After removal of the yolk membranes the

solution was poured into 20 volumes of water and allowed to stand overnight. The supernatant liquid was siphoned off and the remaining precipitate and fluid centrifuged. Without washing, the precipitate was dissolved in 10 per cent sodium chloride and again precipitated by pouring into 20 volumes of water. The suspension was allowed to stand overnight and the supernatant fluid removed by means of a siphon. The remaining material was centrifuged and the precipitate washed once with water. It was then suspended in 5 liters of 80 per cent alcohol, heated to

TABLE I

Ash, Moisture, Nitrogen, Phosphorus, Sulfur, and Some Amino Acids of Vitellin

Substance	Author's values by special methods	Osborne and Jones	Hugounenq	Levene and Alsberg
	per cent	*per cent*	*per cent*	*per cent*
Ash	0.32	3.19		
Moisture	5.86	10.50		
Nitrogen	15.03	15.50		
Phosphorus	0.92	0.94		
Sulfur	0.95	1.02		
Tyrosine	5.01	3.37	2.00	0.40
Tryptophane	1.24			
Cystine	1.19 (1.59)*			
Arginine	7.77 (8.05)*	7.46	1.00	1.20
Histidine	1.22 (0.92)*	1.90	2.10	Trace
Lysine	5.38 (8.73)*	4.81	1.20	2.40

* These values were calculated from the per cent of nitrogen obtained in the Van Slyke distribution study recorded in Table II.

boiling, and kept nearly at the boiling point by means of a good steam bath for several hours. The precipitate was filtered as dry as possible with suction and the process repeated twice, once with 95 per cent alcohol and once with absolute alcohol. After filtering the precipitate as dry as possible on a Buchner funnel, it was washed several times with ether and finally dried in a vacuum desiccator over sulfuric acid for several days. The yield was 35 to 40 gm. from twenty-four eggs. In this manner several hundred gm. of vitellin were prepared which had the ash, moisture, phosphorus, nitrogen, and sulfur content shown in Table I.

Distribution of Nitrogen

The study of the distribution of nitrogen was made by the method of Van Slyke (5). It was modified for the determination of arginine according to the suggestion of Plimmer and Rosedale (7) and the modified apparatus suggested by Koehler (8) was used. The results of three experiments are given in Table II. Since this is the first time the nitrogen distribution has been studied, the values cannot be compared with others. However, from the percentage of nitrogen obtained by this method the percentages of the amino acids in the protein were calculated and compared (Table I) with those obtained by other methods in this investigation.

TABLE II

Distribution of Nitrogen in Vitellin Determined by Van Slyke Method

The values are expressed in per cent of the total nitrogen.

Experiment No.	I	II	III
	per cent	*per cent*	*per cent*
Acid melanin N	0.51	0.47	0.47
Amide N	9.19	9.25	9.21
Humin "	1.45	1.02	1.23
Arginine N	16.58	16.64	16.53
Histidine "	1.76	1.76	1.50
Lysine N	11.04	11.30	11.11
Cystine "	1.23	1.26	1.24
Amino N filtrate	55.31	55.50	55.00
Non-amino N filtrate	3.34	2.62	3.08
Total regained	100.41	99.82	99.37

Individual Amino Acids

All values for the amino acids reported in this investigation are calculated on an ash- and moisture-free basis. Before analysis the vitellin was ground to a powder and passed through a 40 mesh sieve. It was analyzed for cystine by the method of Folin and Marenzi (9) and for tyrosine and tryptophane by the method of Folin and Ciocalteu (10). An average of the values obtained is reported in Table I.

Two samples were used for the estimation of the basic amino acids by a method previously described (11). In the first analysis

of a sample equivalent to 36.9 gm. of ash- and moisture-free vitellin the arginine flavianate obtained was equivalent to 7.64 per cent of the protein as arginine. The nitrogen content of the flavianate was 17.36 per cent; theoretical, 17.21 per cent. The histidine was lost before the flavianate was obtained. The lysine picrate had a melting point of 262° and was equivalent to 5.36 per cent of the protein as lysine.

The second sample of vitellin used was equivalent to 100.1 gm. of ash- and moisture-free protein. The arginine flavianate obtained in this run was equivalent to 7.91 per cent of the protein as arginine. The nitrogen content was 17.31 per cent, while the theory requires 17.21 per cent. The amount of histidine flavianate obtained was equivalent to 1.22 per cent of the protein as histidine. The nitrogen content of the histidine flavianate was 12.54 per cent, while 12.52 per cent is required by theory. The amount of lysine picrate obtained was equivalent to 5.43 per cent of the protein as lysine. The melting point of the picrate was 260°, while the melting point of pure lysine picrate is 267°. An average of the values obtained for the basic amino acids is recorded in Table II.

DISCUSSION

It is interesting to note that although our method of preparation of vitellin was very similar to that of Osborne and Jones, there is a marked difference in the amounts of moisture and ash present and also a distinct difference in the nitrogen content of the two preparations. This is not true of the sulfur and phosphorus, however, for these values are easily within experimental error for duplicate determinations, even in the same laboratory.

Our value for tyrosine is much higher than any of the other values recorded in Table I, undoubtedly due to the fact that it was obtained by a colorimetric method and the others are values obtained by isolation methods. It agrees closely with the value (5.02 per cent) obtained by Folin and Denis (12) in their early investigations of the tyrosine content of various proteins by a colorimetric method.

The values for arginine, histidine, and lysine are not at all in agreement with the values of Hugounenq (2) and Levene and Alsberg (1), which are undoubtedly incorrect. In consideration of

the fact that Osborne and Jones (4) used the Kossel and Kutscher (13) procedure, there is good agreement between their values and ours.

SUMMARY

1. The preparation of vitellin is described and a sample was analyzed for ash, moisture, nitrogen, phosphorus, and sulfur.

2. The nitrogen distribution in vitellin was determined by the method of Van Slyke.

3. The tyrosine, tryptophane, and cystine contents of vitellin were determined by colorimetric methods.

4. Arginine, histidine, and lysine were determined by isolation as well characterized crystalline derivatives and the results contrasted with those of previous investigators.

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