CXLIV. ANALYSIS OF PROTEINS.

VI. DISTRIBUTION OF NITROGEN IN THE PROTEINS OF EGGS.

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THE distribution of nitrogen in the proteins of eggs has not yet been ascertained by the method of Van Slyke. These data are of interest to us in connection with other experiments upon egg proteins and in connection with their value in nutrition. Their determination also gives us a further opportunity of testing our simplified and direct procedure mentioned in the previous paper. A complete series of analyses has been carried out by each of us simultaneously and independently. Concordant results, showing distinct differences in the egg proteins, have been obtained by this procedure.

EXPERIMENTAL.

The proteins used in these experiments were prepared from eggs with white yolks from our hens on a diet of white rice, fish meal, dried yeast and cod-liver oil.

The yolks were separated from the whites and cleaned from adhering white by washing, whilst held in the palm of the hand, under a gentle stream of water. They were then spread upon glass plates and exposed to the air for about 12 hours. The material, thus dried, was scraped off the plates and extracted with cold absolute alcohol, hot absolute alcohol and ether. The fat-free residue, so obtained, formed a slightly reddish-brown powder after grinding in a mortar. This material constituted the egg-yolk protein. No separation into constituent proteins was made for these analyses.

The whites, free from yolk, were broken away from the membranes and poured into $2\frac{1}{2}$ volumes of boiling water containing 0.4 % of acetic acid, and heated for 10 to 15 minutes. The coagulated protein was filtered off, washed with boiling water, placed in two changes of absolute alcohol for dehydration and finally extracted with ether. On grinding in a mortar, a perfectly white powder resulted and formed the egg-white protein.

The acid filtrate from the coagulated white was evaporated to a small volume and poured into two volumes of absolute alcohol. The precipitate of

ovomucoid was filtered off, washed with alcohol, redissolved in water and reprecipitated with two volumes of alcohol. It was dehydrated with alcohol and extracted with ether; it formed a white material easily pulverised.

The egg-membrane was prepared from the shells by first treating with very dilute sodium hydroxide to remove adhering white and then with dilute hydrochloric acid to dissolve the calcium carbonate. The residue was freed from acid and treated with absolute alcohol and ether. On drying, the material had an opaque white appearance.

The average yields of these proteins *per egg* and their composition are given below:

	Average yield per egg g.	Moisture %	Ash %	Nitrogen %
Egg-yolk	2.5-3	6.63	5.15	11.43
Egg-white	2.4-2.8	8.68	0.11	12.88
Ovomucoid	0.1-0.3	7.74	1.75	11.23
Egg-membrane	0.2	6.87	0.29	13.16

In each case, 30 g. of protein were hydrolysed by boiling for 36 hours under a reflux condenser with 250 cc. of 25 % hydrochloric acid. The solution was evaporated *in vacuo* to a paste, the paste dissolved in water and the solution again evaporated *in vacuo* to remove hydrochloric acid as completely as possible. The residue was dissolved in water and the volume made up to 1250 cc. The solutions were preserved with thymol.

Total nitrogen was determined in 10 cc. portions and the nitrogen distribution in 100 cc. portions.

The nitrogen distribution was carried out by our simplified and direct procedure without reference to the content of cystine. This procedure may, for convenience, shortly be described.

The 100 cc. portion was measured with a pipette into a double necked distilling flask of 750 to 1000 cc. capacity with side piece bent at right angles. It was made slightly alkaline by the addition, in small quantities at a time, of 1 to 1.5 g. of finely powdered lime, according to its acidity as estimated by titration of a separate 10 cc. portion using phenolphthalein as indicator. After solution of the lime, 100 cc. of absolute alcohol were added and the distilling flask connected to a short double surface condenser set up in a 1500 to 2000 cc. filter flask with Folin tube and bulb attached to the end of the condenser and reaching to the bottom of the filter flask. The filter flask contained excess of *N*/10 acid and sufficient water to cover the bulb amply. Distillation *in vacuo* was carried out at 45 to 50° until the volume in the distilling flask was reduced to 25-50 cc. The excess of acid in the filter flask was then titrated.

The contents of the distilling flask were filtered through a 9 cm. filter paper into a flask with 50 cc. marks up to 200 cc. and the flask and filter washed until the volume reached about 150 cc. Humins adhering to the distilling flask was dissolved in the conc. sulphuric acid used for the Kjeldahl

operation and washed out with water. The humin and the filter paper were only added to the sulphuric acid after removal of the water.

The filtrate was acidified with 18 cc. of conc. hydrochloric acid and 15 g. of phosphotungstic acid (prepared by Wu's method), dissolved in a little water, added. The flask was heated until the precipitate had nearly gone into solution, and allowed to cool and stand for 2 to 4 days.

The phosphotungstate precipitate was filtered off on a 5 cm. funnel. For this purpose the newly introduced filters of Jena glass are more advantageous than the Buchner funnel. The precipitate was washed 5 times with 10 cc. of dilute hydrochloric acid (1 : 10), the material being stirred up with the wash liquid and sucked dry each time. The filtrate and washings were passed in turn through a 7 cm. paper and collected in a 250 cc. flask and made up to volume.

The precipitate was dissolved on the filter in 30 to 40 cc. of approx. *N* NaOH, which was first used to dissolve any particles remaining in the flask. This solution was passed through the same 7 cm. filter and collected in a 100 cc. flask. Flask, filter and paper were then washed with changes of water till the volume reached the 100 cc. mark.

The several estimations were then made on the solutions directly. No bumping occurred in the Kjeldahl operation, unless the heating was too rapid. Arginine was estimated by boiling 40 cc. with an equal volume of 40 % sodium hydroxide under a reflux with a Folin tube attached and distilling the ammonia into the bulb after running the water from the condenser. Amino nitrogen was determined in the di-amino fraction by reaction for 1 hour, in the mono-amino fraction for half an hour.

The remains of the solutions from several experiments were mixed together and the above estimations repeated.

Cystine estimations were not made, as the method of estimation is still under investigation.

The results are shown in the following tables. Five separate experiments were made by each of us, but only one series of data is given in order to save space. The two sets were exactly comparable.

These data are followed by calculation of the mean result in terms of nitrogen in g. per 100 cc. and in percentages of total nitrogen.

The tables show the good agreement of the data given by our simplified and direct procedure for a series of analyses on the same solution. The greatest variations are in the estimations of the amino nitrogen; these data should only be taken to tenths of a milligram. In calculating the percentages no allowance has been made for solubilities of the hexone bases.

The individual figures for the several proteins show distinct differences. Egg-white proteins contain more mono-amino nitrogen than the egg-yolk, which has more di-amino nitrogen. It is remarkable that egg-membrane has the highest di-amino figure and has most histidine, and that the histidine is so low in the egg-white and ovomucoid. The lysine nitrogen is very similar in the four proteins.

EGG-YOLK. T.N. = 19.1 cc. N/10 per 10 cc.

Di-amino 100 cc.					Mono-amino 250 cc.		
Amide N 100 cc. cc. N/10	Humin N 100 cc. cc. N/10	T.N. 10 cc. cc. N/10	Arg. N 40 cc. cc. N/10	Amino N 10 cc. mg. N	T.N. 25 cc. cc. N/10	Amino N 10 cc. mg. N	
17.2	4.6	5.3	5.6	3.7510	11.8	6.4161	
17.2	4.5	5.1	5.8	3.7971	11.9	6.6222	
17.4	4.4	5.2	5.5	3.6155	12.1	6.4470	
17.4	4.5	5.1	5.7	3.7516	11.8	6.4714	
17.4	4.8	5.1	5.5	3.9707	11.8	6.5124	
Mean	17.3	4.6	5.2	5.6	3.7772	11.9	6.4138
Remains of solutions		5.3	5.7	3.8930	12.0	6.4267	

EGG-WHITE. T.N. = 21.3 cc. N/10 per 10 cc.

19.5	4.3	4.5	5.2	3.7508	14.3	7.6055	
19.5	4.1	4.6	5.2	3.9486	14.4	7.6177	
19.3	3.9	4.8	5.0	3.9684	14.3	7.5192	
19.8	4.4	5.0	5.0	3.8355	14.6	8.2888	
19.6	4.2	4.7	4.8	4.0577	14.5	8.3168	
Mean	19.5	4.2	4.7	5.0	3.9122	14.4	7.8696
Remains of solutions		4.6	5.2	4.3524	14.5	7.9840	

EGG-MEMBRANE. T.N. = 22.5 cc. N/10 per 10 cc.

15.4	3.5	7.1	7.5	4.7109	14.0	7.0344	
15.4	3.4	7.0	7.8	4.8142	14.0	6.9424	
15.4	3.2	6.9	7.5	4.9015	14.1	6.7937	
15.2	3.4	6.9	7.6	4.6799	14.0	7.0707	
15.2	3.8	6.7	7.6	4.7031	14.0	6.8348	
Mean	15.3	3.5	6.9	7.6	4.7619	14.0	6.9352
Remains of solutions		6.7	7.7	5.0575	14.0	7.1443	

OVOMUCOID. T.N. = 21.6 cc. N/10 per 10 cc.

	25.8	3.6	4.9	4.5	4.4605	13.9	7.7719
	25.6	3.5	4.7	4.7	4.7943	13.7	7.9421
	25.8	3.7	4.9	4.7	4.6539	13.9	7.9618
	25.8	3.6	5.0	4.6	4.7574	14.0	8.0051
	25.5	3.9	4.8	4.9	4.4818	13.8	8.1423
Mean	25.7	3.7	4.9	4.7	4.6296	13.9	7.9646
Remains of solutions			4.9	4.9	4.5528	13.7	7.9278

MEAN RESULTS IN G. N PER 100 CC.

	Total N	Amide N	Humin N	Di-amino						Mono-amino		
				T.N.	Argi- nine N	Amino N	Non- amino N	Histi- dine N	Lysine N	Total N	Amino N	Non- amino N
Egg-yolk	0.2681	0.0242	0.0064	0.0728	0.0392	0.0378	0.0350	0.0064	0.0252	0.1666	0.1624	0.0042
„ white	0.2982	0.0273	0.0059	0.0658	0.0350	0.0391	0.0267	0.0007	0.0301	0.2016	0.1967	0.0049
„ membrane	0.3150	0.0214	0.0049	0.0966	0.0532	0.0476	0.0490	0.0137	0.0297	0.1960	0.1734	0.0226
Ovomucoid .	0.3024	0.0360	0.0052	0.0686	0.0330	0.0463	0.0223	-0.0037	0.0356	0.1946	0.1991	-0.0045

MEAN RESULTS IN PERCENTAGES OF TOTAL N.

	Di-amino								Mono-amino			Total
	Amide N	Humin N	T.N.	Argi- nine N	Amino N	Non- amino N	Histi- dine N	Lysine N	T.N.	Amino N	Non- amino N	
Egg-yolk	9.0	2.4	27.1	14.5	14.1	13.1	3.1	9.4	62.1	60.6	1.6	100.6
Egg-white	9.2	2.0	22.1	11.7	13.1	8.9	0.2	10.1	67.6	66.0	1.6	100.9
Egg-membrane	6.8	1.6	30.7	16.9	14.8	15.5	4.3	9.4	62.2	55.0	7.2	101.3
Ovomucoid	11.9	1.7	22.7	10.9	15.3	7.4	-1.2	11.7	64.3	65.5	-1.4	100.6

From these data the content in hexone bases has been further calculated in terms of moisture- and ash-free protein, for comparison with the figures by isolation. In these figures are included data for arginine from the mono-amino fraction (see next paper).

	G. of amino acids per 100 g. of moisture- and ash-free protein				
	Arginine di-amino	Arginine mono-amino	Arginine total	Histidine	Lysine
Egg-yolk	5.7	1.9	7.6	1.4	6.0
Egg-white	5.1	1.8	6.9	0.1	7.4
Egg-membrane	7.4	1.2	8.6	2.2	6.9
Ovomucoid	4.2	1.5	5.7	0	7.6

By isolation [see Plimmer, 1917]:

Egg-yolk vitellin	—	—	7.5	1.9	4.8
Egg-white crystallised albumin	—	—	4.9	1.7	3.8
Egg-membrane keratin	—	—	—	—	—
Ovomucoid	—	—	—	—	—

The two sets of figures are not in agreement. This is partly accounted for by the different proteins used, though no marked difference has been found between the vitellin and livetin constituting egg-yolk protein, and the crystalline albumin and conalbumin of egg-white. The nitrogen distribution method always gives higher values for lysine. Other data than those given above show 0 or 0.7 % of histidine in egg-white, figures more in agreement with those by nitrogen distribution.

SUMMARY.

The nitrogen distribution in the proteins of eggs has been determined by a shortened and simplified modification of the Van Slyke method. The results are given in a series of tables and comparison is made with the figures obtained by other workers by isolation.

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REFERENCE.

Plimmer (1917) Constitution of Proteins. Part I. Analysis. (Longmans, Green and Sons, London.)