XXXV. CHANGES IN THE AMINO-ACIDS IN THE PROTEINS OF THE HEN'S EGG DURING DEVELOPMENT.

BY ROBERT HENRY ADERS PLIMMER AND JOHN LOWNDES.

From the Chemical Department, St Thomas's Hospital Medical School, London.

(Received January 10th, 1927.)

OF the various changes which take place in the hen's egg during development into the chick those occurring in the protein have not yet been thoroughly investigated. The excellent review of all the work on "The Metabolism of the Developing Egg" by Needham [1925] does not refer to any investigation of the proteins. Several papers have been published upon changes in the ammonia and nitrogen content, and Needham [1926, 1] has published data upon the urea content of the developing egg, and he has also studied the uric acid and non-protein nitrogen content [1926, 2]. Fiske and Boyden [1926] have also worked upon the nitrogen metabolism of the chick embryo.

Sendju [1925] has determined the tyrosine and tryptophan content of the developing egg by colorimetric methods and the diamino-acid content by Kossel's method. A decrease was found in the tyrosine and tryptophan content, but no definite changes in the diamino-acid content.

With the improvements made in the Van Slyke method by Plimmer and Rosedale [1925] it was hoped it would be possible to detect changes in the amino-acid content which would indicate the origin of the ammonia, urea, uric acid and other simpler compounds.

EXPERIMENTAL.

Previous experiments upon the changes in the constituents of eggs during development had shown that no marked alteration was noticeable until about the 11th day of incubation. No attempt was therefore made to analyse the amino-acids at daily intervals. The fresh egg, the egg at the stage of 15 days' incubation and the hatched chick were used so as to secure information at the beginning, at the middle and at the end of the metamorphosis. The eggs in these experiments were from Light Sussex hens fed upon a diet of vitamealo, middlings and maize. Three eggs were used at each stage of the development. Sufficient material was thus obtained to allow of several analyses of the amino-acids by the Van Slyke method.

The contents of the fresh eggs, the eggs at 15 days, and the chicks after chloroforming and cutting up into small pieces were placed in 400 to 500 cc. of alcohol and treated in a similar way to that described by Plimmer and Scott [1909] in order to separate fats and lecithin and to coagulate the protein. The material after standing some days in alcohol was warmed and extracted first with alcohol and then with ether in a muslin bag suspended in a special extractor. The amounts of coagulated protein so obtained were:

From fresh eggs	17.0 g.
„ eggs at 15 days' incubation	20.6
„ hatched chicks	19.3

The alcohol and ether extracts were made up to 1000 cc. and the nitrogen in them determined by Kjeldahl's method. The amounts were:

From fresh eggs	0.1288 g.
„ eggs at 15 days' incubation	0.1344
„ hatched chicks	0.1190

These figures, if calculated per 100 g. of protein, show a decrease in the amount of alcohol- and ether-soluble nitrogen, but they probably have no relation to any changes in the proteins.

The coagulated protein at each stage was hydrolysed with 200 cc. of 25 % hydrochloric acid for 36 hours. The solution was evaporated *in vacuo* to remove hydrochloric acid as far as possible and the residue dissolved in water and made up to 520 cc.

The amino-acids were determined in 100 cc. portions of these solutions by the Van Slyke method as modified by Plimmer and Rosedale. Four analyses were made at each stage. The residual diamino- and monoamino-acid solutions were then combined and the analyses repeated. Sulphur estimations were also made by direct determination, as shown possible by Plimmer and Lowndes [1927].

The series of analyses has been repeated with one egg at each stage. These eggs were also from Light Sussex hens, but fed upon white rice, dried yeast, fish meal and cod-liver oil, from group XLV of birds in other experiments on nutrition.

The amounts of protein obtained were:

From a fresh egg	5 g.
„ an egg at 15 days' incubation	5
„ a hatched chick	7

After hydrolysis the solutions were made up to 250 cc. and two analyses of the amino-acids were made. The residues were combined for another estimation and a sulphur estimation.

Although it had been found by Plimmer and Phillips [1924] that the tyrosine content could not be determined by bromination on account of the presence of cystine, or some other compound which reacts with bromine, it was of some interest to ascertain the absorption of bromine by the mono-amino-fraction at each stage of development. This estimation has been made for us by Mr L. R. Bishop.

The following are the data, all calculated in terms of nitrogen per 100 cc.:

Exp. 1.			Total N. Stage: 0						0.4578 g.			
			,, 15 days						0.4928			
			,, hatched chick						0.4788			
			Diamino N						Monoamino-N			
	Amide	Humine	Total	Amino	Non-amino	Arginine	Histidine	Ly-sine	Total	Amino	Non-amino	Arginine
Stage: 0												
(1)	0.0372	0.0073	0.1190	0.0708	0.0482	0.0651	—0.0009	0.0548	0.2954	0.3150	—	0.0224
(2)	0.0378	0.0067	0.1162	0.0690	0.0472	0.0644	—0.0016	0.0534	0.2926	0.2995	—	0.0210
(3)	0.0377	0.0067	0.1176	0.0642	0.0534	0.0616	0.0108	0.0452	0.2898	0.3050	—	0.0196
(4)	—	0.0063	0.1176	0.0693	0.0483	—	—	—	0.2954	0.3050	—	0.0210
Mean	0.0376	0.0067	0.1176	0.0683	0.0493	0.0637	0.0023	0.0516	0.2933	0.3061	—	0.0210
Residual solution	—	—	0.1190	0.0672	0.0518	0.0678	0.0014	0.0498	0.2926	0.3102*	—	0.0196
Stage: 15 days												
(1)	0.0435	0.0092	0.1302	0.0733	0.0569	0.0721	0.0042	0.0539	0.3010	0.3115	—	0.0210
(2)	0.0454	0.0087	0.1330	0.0737	0.0593	0.0707	0.0094	0.0529	0.2996	0.3150	—	0.0196
(3)	0.0423	0.0092	0.1316	0.0789	0.0527	0.0742	—0.0044	0.0618	0.3024	0.3242	—	0.0168
(4)	0.0399	0.0090	0.1302	0.0796	0.0506	0.0707	—0.0036	0.0631	0.2996	0.3160	—	0.0154
Mean	0.0428	0.0090	0.1312	0.0764	0.0548	0.0719	0.0013	0.0580	0.3006	0.3167	—	0.0182
Residual solution	—	—	0.1358	0.0721	0.0637	0.0756	0.0105	0.0497	0.3024	0.3212*	—	0.0168
Stage: hatched chick												
(1)	—	0.0077	—	—	—	—	—	—	0.2912	0.2780	0.0132	0.0154
(2)	0.0442	0.0080	0.1302	0.0679	0.0623	0.0742	0.0100	0.0460	0.2884	0.2833	0.0051	0.0154
(3)	0.0364	0.0078	0.1260	0.0753	0.0507	0.0833	—0.0177	0.0604	0.2884	0.2845	0.0039	0.0154
(4)	0.0381	0.0085	0.1316	0.0676	0.0640	0.0805	0.0004	0.0507	0.2912	0.3002	—	0.0168
Mean	0.0396	0.0080	0.1293	0.0703	0.0590	0.0793	0.0006	0.0506	0.2898	0.2865	0.0033	0.0158
Residual solution	—	—	0.1330	0.0679	0.0651	0.0819	0.0055	0.0456	0.2870	0.2790*	0.0080	0.0154

* On repeating at a later date: stage 0, 0.2905; 15 days, 0.2955; hatched chick, 0.2716.

Exp. 2.			Total N. Stage: 0					0.2912 g.				
			,, 15 days					0.2646				
			,, hatched chick					0.2464				
Stage: 0												
(1)	0.0280	0.0053	0.0700	0.0435	0.0265	0.0399	0.0051	0.0352	0.1834	0.1730	0.0104	0.0112
(2)	0.0290	0.0055	0.0700	0.0371	0.0329	0.0371	0.0076	0.0253	0.1862	0.1730	0.0132	0.0112
Mean	0.0285	0.0054	0.0700	0.0403	0.0297	0.0385	0.0012	0.0303	0.1848	0.1730	0.0118	0.0112
Residual solution	—	—	0.0728	0.0366	0.0362	0.0385	0.0110	0.0233	0.1862	0.1728	0.0134	0.0126
Stage: 15 days												
(1)	0.0251	0.0052	0.0644	0.0293	0.0351	0.0343	0.0141	0.0160	0.1666	0.1512	0.0154	0.0098
(2)	0.0251	0.0052	0.0658	0.0288	0.0370	0.0336	0.0177	0.0145	0.0166	0.1502	0.0164	0.0084
Mean	0.0251	0.0052	0.0651	0.0290	0.0361	0.0340	0.0159	0.0152	0.0166	0.1507	0.0159	0.0091
Residual solution	—	—	0.0630	0.0307	0.0323	0.0343	0.0099	0.0188	0.0166	0.1517	0.0149	0.0084
Stage: hatched chick												
(1)	0.0216	0.0045	0.0658	0.0298	0.0360	0.0357	0.0138	0.0163	0.1568	0.1360	0.0208	0.0084
(2)	0.0214	0.0042	0.0630	0.0333	0.0297	0.0357	0.0044	0.0229	0.1540	0.1385	0.0155	0.0070
Mean	0.0215	0.0043	0.0644	0.0315	0.0329	0.0357	0.0092	0.0175	0.1554	0.1373	0.0181	0.0077
Residual solution	—	—	0.0658	0.0290	0.0368	0.0357	0.0150	0.0151	0.1540	0.1353	0.0187	0.0084

The sulphur estimations, in terms of cystine N, were:

Cystine N				
Exp. 1		Exp. 2		
Diamino	Monoamino	Diamino	Monoamino	
Stage: 0	0.0084	0.0161	0.0035	0.0114
,, 15 days	0.0082	0.0159	0.0031	0.0076
,, hatched chick	0.0126	0.0147	0.0014	0.0082

The amounts of bromine absorbed, and the corresponding amounts of tyrosine nitrogen, were:

Stage:	Exp. 1		Exp. 2	
	Br	Tyrosine N	Br	Tyrosine N
0	0.516	0.0225	0.304	0.0132
" 15 days	0.466	0.0203	0.278	0.0121
" hatched chick	0.429	0.0187	0.224	0.0098

Expressed in terms of percentage of the total nitrogen the data come out as follows.

The two sets of figures giving a mean are from the mean result of the several analyses and from the result using the combined solutions which remained from the separate determinations.

	Exp. 1			Exp. 2		
	Stage			Stage		
	0	15 days	hatched	0	15 days	hatched
Amide	8.2	8.7	8.2	9.8	9.5	8.7
Humin	1.5	1.8	1.7	1.8	1.9	1.8
Diamino:						
Total	25.7 26.0 } 25.8	26.7 27.5 } 27.1	27.0 27.8 } 27.4	24.0 25.0 } 24.5	24.6 23.8 } 24.2	26.1 26.7 } 26.4
Amino	14.9 14.7 } 14.8	15.5 14.6 } 15.0	14.7 14.2 } 14.5	13.8 12.6 } 13.2	11.0 11.6 } 11.3	12.8 11.8 } 12.2
Non-amino	10.8 11.3 } 11.0	11.1 12.7 } 11.9	12.3 13.6 } 13.0	10.2 12.4 } 11.3	13.6 12.2 } 12.9	13.3 14.9 } 14.1
Arginine	13.9 14.8 } 14.3	14.6 15.3 } 15.0	16.6 17.1 } 16.8	13.2 13.2 } 13.2	13.5 13.5 } 13.5	14.5 14.5 } 14.5
Histidine	0.5 0.3 } 0.4	0.3 2.1 } 1.2	-0.1 1.1 } 0.5	0.4 3.8 } 2.1	6.0 3.7 } 4.8	3.8 6.1 } 4.9
Lysine	11.2 10.9 } 11.0	11.7 10.1 } 10.9	10.6 9.5 } 10.2	10.4 8.0 } 9.2	5.7 7.1 } 6.4	7.1 6.1 } 6.6
Cystine		1.8	1.7		1.2	0.6
Monoamino:						
Total	64.1 63.9 } 64.0	61.0 61.6 } 61.3	60.5 60.0 } 60.2	63.4 63.9 } 63.7	62.9 62.9 } 62.9	63.1 62.5 } 62.8
Amino		63.4	60.0	59.4 59.3 } 59.3	56.9 57.3 } 57.1	55.8 54.9 } 55.3
Non-amino		0.6	1.3	4.1 4.6 } 4.3	6.0 5.6 } 5.8	7.3 7.6 } 7.4
Arginine	4.6 4.3 } 4.4	3.7 3.4 } 3.6	3.3 3.2 } 3.2	3.8 4.3 } 4.0	2.9 3.2 } 3.1	3.1 3.4 } 3.3
Cystine		3.5	4.9		3.9	3.3

DISCUSSION OF RESULTS.

No change can be considered to occur in the amide and humin nitrogen, though a small decrease was observed in the amide nitrogen in the second experiment.

In both experiments a distinct increase occurred in the total diamino-nitrogen amounting to about 2 % of the total nitrogen of the egg proteins. Corresponding with this increase there was an increase in the amount of arginine nitrogen amounting to about 1 %. The figure in the first experiment with the hatched chick is probably high from error in the amide determination.

As has been previously experienced the estimation of the amino-nitrogen has given very erratic results; consequently no stress can be laid upon the figures for histidine and lysine; they probably increase, as the arginine increase only accounts for half the increase of the total diamino-nitrogen.

The total monoamino-nitrogen definitely decreased in both experiments. In the first experiment the amino-nitrogen figures were too high, but on repetition at a later date on the combined residues, they showed a decrease, as found in the second experiment. There was an increase in the non-amino-nitrogen.

Cystine nitrogen increased in both the diamino- and monoamino-fractions in the first experiment; in the second experiment the amounts of barium sulphate were so small that the figures are probably not correct.

The bromine absorption figures indicate a marked decrease during development. As the cystine nitrogen increases, this change probably indicates a decrease in the tyrosine content.

It would thus appear that in the metabolism of the developing egg the monoamino-acids are used for the formation of urea, uric acid, etc., and for furnishing energy, whilst the diamino-acids are retained for building the body protein of the chick. Loss of monoamino-acid would leave a remainder with a higher diamino-acid content. The changes run parallel with the changes which have been observed with salmon during the spawning season. These fish derive their energy from the monoamino-acids of the muscular tissue, whilst the diamino-acids pass to the reproductive organs. The eggs of the brook-trout and salamander have also been found [see Needham, 1925] to show an increase of diamino-nitrogen during development.

SUMMARY.

Estimation of the amino-acids in the hen's egg during development by the modified Van Slyke method has shown that there is an increase of about 2 % in the diamino-nitrogen, of which the arginine increase makes about 1 % of the total nitrogen of the egg proteins. The monoamino-acid nitrogen decreases by about 4 % during development with a corresponding decrease in the amino-nitrogen content.

We gratefully acknowledge a grant to one of us from the Government Grant Committee of the Royal Society for defraying the expenses of the work.

REFERENCES.

- Fiske and Boyden (1926). *J. Biol. Chem.* **70**, 535.
Needham (1925). *Physiol. Reviews*, **5**, No. 1.
— (1926, 1). *Brit. J. Exp. Biol.* **3**, 189.
— (1926, 2). *Brit. J. Exp. Biol.* **4**, 114, 145.
Plimmer and Lowndes (1927). *Biochem. J.* **21**, 247.
Plimmer and Phillips (1924). *Biochem. J.* **18**, 312.
Plimmer and Rosedale (1925). *Biochem. J.* **19**, 1015.
Plimmer and Scott (1909). *J. Physiol.* **38**, 247.
Sendju (1925). *Japan. J. Biochem.* **5**, 391.