CLII. THE CHANGES IN THE LIME CONTENT OF THE HEN'S EGG DURING DEVELOPMENT.

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PROUT [1822], who was unable to detect lime in the contents of the egg before development, but observed its presence in the newly-hatched chick, concluded that the lime was derived from the shell. Though Voit [1877] could not repeat this result, it was confirmed by Vaughan and Bills [1878]. They found that the fresh egg contained an average of 0.07 g. CaSO_4 (= 0.03 g. CaO) and the hatched chick 0.38 g. CaSO_4 (= 0.16 g. CaO). The opposite was again observed by Pott and Preyer [1882]. Tangl [1908], from analyses of the shells of fresh eggs and of those from hatched chicks, concluded that both organic matter (0.4 g.) and lime (0.16 g.) disappeared from the shell. The most satisfactory data upon the lime content of the egg and of the developing chick are those of Carpiaux [1908]. Carpiaux determined the lime content of the egg at several stages of development and found that it rose from 0.04 g. to 0.20 g., the first noticeable increase occurring about the thirteenth day. His analyses of the shell both before and after development did not show any particular change in its composition. Although the increase of lime in the embryo had thus been clearly demonstrated, it was considered that some further information might still be gained. Analyses of the lime content at each day of incubation have now been made, both as regards the interior including the chick and yolk sac, the shell and the membrane. Our results confirm those of Vaughan and Bills and of Carpiaux, that lime enters the egg from the shell. The loss of lime from the shell could not be detected with certainty owing to the great variations of its lime content, but the membrane, at first almost lime-free, becomes richer in lime. An increase in lime has also been found in pigeons' eggs during development, so that it is very probable that lime passes from the shell to the contents in all birds' eggs during development. It has further been found that the membrane of soft shelled eggs contains large amounts of lime, more than sufficient for the needs of the developing chick. In all cases the shell has much more lime than is needed. Attempts to hatch soft-shelled eggs were a failure owing to the contents drying up. The function of the shell is thus partly protective against breakage, to furnish lime and to prevent evaporation.

EXPERIMENTAL.

The experiments were designed so as to determine the total lime in the contents of eggs at each day of incubation. At the later stages, at which separation of embryo and yolk sac becomes possible, the lime was to be estimated in each of these parts. With any increase of lime in the interior, a corresponding loss would be expected from the shell. This loss would be expected to involve loss of carbon dioxide, as the shell is composed of 90–95 % calcium carbonate with traces of magnesium carbonate, phosphates and organic matter. The shell was therefore also examined for lime and carbonate. Owing to the great variations which were found in the amount of lime, carbon dioxide and weight of membrane, these estimations were abandoned as it was not possible to tell if lime and carbon dioxide were actually lost by the shell. It may be pointed out that Tangl, though he used eggs from the same hen, also found variations both in weight of shell and membrane, and really arrived at his conclusion of loss of lime from the shell by calculation. In the later experiments the egg membrane was analysed since, if lime enter the egg from the shell, it must pass through the membrane.

Two dozen eggs were required for the series of analyses; two were usually analysed before incubation, the others at daily intervals, with the extra egg for a possible longer period of development with an incubator temperature lower than the normal of the hen. Three series of eggs had to be used. The first series, O, were eggs from the experimental group of Light Sussex chickens of Plimmer and Rosedale [1923], fed upon a diet of white rice, dried yeast, slaked lime and cod liver oil. These eggs were all unfertile and were thus of no value for this investigation. The second series, S, also Light Sussex, were from a dealer from hens fed upon another diet. Many of these were unfertile, and most of the embryos were dead on removal of the egg from the incubator. The third series, R, were from White Leghorn hens fed upon maize meal, middlings, bran, fish meal and dried yeast with an allowance of about 3 oz. per day of wheat. In this series the eggs with dead embryos could be discarded and replaced by other eggs which were then incubated for the necessary length of time. A complete series was thus obtained.

Preparation of the material. The eggs were weighed before incubation and on the day of removal from the incubator. The contents were separated from the membrane and shell, and the shell washed free from adhering contents with distilled water. The contents and water were put into a 700 cc. Jena glass flask, in which any small fragments of shell could be seen and removed, if necessary. In those cases in which embryo and yolk sac were separately analysed, they were cut apart with scissors and the wash water added to the yolk sac portion. The shell was allowed to dry in the air and weighed. In the earlier experiments the whole was used for carbon dioxide and lime estimations and the membrane afterwards collected, dried and weighed, so as to get the weight of shell. In the later experiments the shell was picked from the

membrane; this was a simpler operation with the eggs of the later periods of incubation; the shell then easily chips from the membrane which at the same time becomes more opaque and tougher in texture.

Estimation of lime. The lime in the contents of the eggs was estimated by Aron's process as adapted by Plimmer and Page [1913] as calcium sulphate, by precipitation, after oxidation with sulphuric and nitric acids, with two volumes of alcohol. The same method was used for the estimation of the lime in the egg shell and membrane, the former after the estimation of the carbon dioxide.

Estimation of carbon dioxide in the shell. The method adopted for this purpose was that of Van Slyke [1918], but it required some modification. The method consists in liberating the carbon dioxide by dilute hydrochloric acid in an evacuated flask and absorbing the gas in baryta water, which is afterwards titrated. In the first place it was necessary to use the whole shell on account of variations in its thickness and for the recovery of the whole membrane as well as for the subsequent estimation of the lime. The dried shell was placed in a tall beaker of 550 cc. capacity and covered with an inverted funnel without stem. A second and larger funnel with short stem was placed on the top of the beaker with the stem passing into the inverted funnel below. The beaker was put into a large crystallising dish containing 100 cc. of *N* sodium hydroxide. The whole was then put into a large vacuum desiccator with an opening at the top. This was fitted with a rubber stopper holding a tap funnel and a tube with rubber tubing and screw clip. The desiccator was then exhausted and closed by tightening the screw clip. Very dilute hydrochloric acid was now allowed to drop in from the tap funnel at first slowly on account of the rapid reaction and frothing. After this had ceased stronger acid was allowed to run in until the whole of the carbonate was decomposed. The contents were usually allowed to stand for 24 hours to complete the absorption of the carbon dioxide by the sodium hydroxide. Air was then admitted, the contents of the crystallising dish titrated with *N*/2 hydrochloric acid, using the B.D.H. Universal Indicator instead of methyl orange and phenolphthalein. The first reading was taken when all the blue colour was discharged and a sharp green appeared without trace of yellow. At this point the p_H is 8-8.5, the same as the neutral point of phenolphthalein, and corresponds to conversion of carbonate into bicarbonate. The second reading was taken when all orange colour was discharged and the solution became red. The reaction corresponds here to p_H 3, the neutral point of methyl orange. Both end points were quite sharp and distinct. The difference between the reading corresponds to half the carbon dioxide in terms of *N*/2, from which the carbon dioxide in the whole shell was easily calculated.

The solution containing the lime as calcium chloride was separated from the undissolved membrane by simply picking this out and washing it, filtered from particles of pigmented matter and made up to 1000 cc. The membrane was dried and weighed. The lime was estimated in 100 cc. by adding 20 cc.

Egg Series O.

The first four were from one hen, the next two from another, the last two from a third.

Contents			Shell				Lime in shell-less membrane %
Weight g.	Lime g.	Lime %	Weight g.	Membrane g.	CO ₂ g.	Lime g.	
44.6	0.0367	0.08	6.4	0.2	2.18	2.76	44.8
43.4	0.0374	0.09	5.6	0.2	2.28	2.71	49.9
45.1	0.0377	0.08	5.9	0.1	2.37	3.03	52.6
45.9	0.0370	0.08	6.1	0.2	—	3.1	52.3
49.4	0.0412	0.08	5.6	0.3	2.04	2.6	49.1
50.0	0.0360	0.07	5.0	0.2	1.84	2.51	52.2
50.6	0.0364	0.07	5.4	0.1	—	2.75	52.2
50.4	0.0400	0.08	5.6	0.2	2.09	2.81	52.0

Egg Series S.

Days of incuba- tion	Contents			Shell				Lime in shell-less membrane %
	Weight g.	Lime g.	Lime %	Weight g.	Membrane g.	CO ₂ g.	Lime g.	
0	57.4	0.0463	0.08	5.4	0.4	1.90	2.65	53.4
0	49.8	0.0328	0.07	6.0	0.1	2.30	2.82	48.1
9 unfert.	50.9	0.0450	0.09	5.3	0.3	2.09	2.65	52.8
13 "	56.6	0.0443	0.08	6.3	0.2	2.37	3.06	49.8
19 "	55.4	0.0534	0.09	4.7	—	—	—	—
0	50.5	—	—	—	0.4	2.05	2.25	47.8
7? fert.	49.2	0.0419	0.09	5.7	0.3	2.28	2.73	50.8
8? "	55.0	0.0462	0.08	6.1	0.3	2.41	3.04	52.7
14? "	60.6	0.0400	0.07	5.6	0.3	2.11	2.77	52.8
15? "	55.8	0.0514	0.09	6.2	0.3	2.29	2.97	50.0
20 live	49.4	0.1427	0.29	5.6	0.1	2.42	2.88	51.6
? dead	57.0	0.1282	0.22	6.0	—	—	—	—
? "	57.8	0.1381	0.24	6.0	Chick	Lime g. 0.0726	Yolk sac	Lime g. 0.0655
? "	52.4	0.1756	0.35	5.9	—	0.1016	—	0.0740
? "	53.8	0.1448	0.27	4.9	—	0.0622	—	0.0826
? "	—	0.0649	—	—	—	0.0170	—	0.0479

Five other unfertile eggs had an average lime content of 0.0397 g.

Egg Series R.

Days of incuba- tion	Weight of contents g.	Lime in contents				Weight of shell g.	Weight of mem- brane g.	Lime in membrane g.
		Chick g.	Yolk sac g.	Total g.	%			
0	52.3	—	—	0.0536	0.10	5.1	0.2	0.0002
1	53.4	—	—	0.0508	0.09	5.0	0.3	—
5	59.8	—	—	0.0509	0.09	4.2	0.2	—
6	66.0	—	—	0.0400	0.06	4.5	0.2	0.0008
7	63.9	—	—	0.0487	0.08	3.9	0.3	—
8	62.5	—	—	0.0394	0.06	6.0	0.5	0.0025
9	63.0	—	—	0.0514	0.08	4.0	0.2	0.0008
10	56.4	trace	0.0492	0.0492	0.09	5.6	0.2	0.0020
11	58.8	0.0021	0.0544	0.0565	0.10	5.4	0.1	0.0025
	55.0	—	—	0.0579	0.10	5.5	0.2	0.0054
12	48.4	0.0015	0.0385	0.0400	0.08	4.2	0.1	0.0008
	49.5	—	—	0.0443	0.09	6.3	0.3	0.0019
13	51.2	0.0035	0.0566	0.0601	0.11	5.3	0.3	0.0029
14	52.4	0.0063	0.0422	0.0485	0.09	5.8	0.2	0.0050
	53.9	—	0.0509	0.0509	0.09	4.6	—	—
15	54.9	0.0192	0.0590	0.0782	0.14	6.4	0.1	0.0038
16	56.6	0.0331	0.0600	0.0931	0.16	6.4	0.2	0.0080
17	48.8	—	0.0700	—	—	5.7	0.2	0.0086
18	51.1	0.0782	0.0522	0.1304	0.25	5.0	0.2	0.0062
19	57.7	0.0988	0.0727	0.1715	0.30	6.0	0.2	0.0073
20	52.0	0.0629	0.0531	0.1160	0.22	5.2	0.2	0.0082
21	54.5	—	—	0.1816	0.33	4.5	0.2	0.0058
	58.7	0.1262	0.0646	0.1908	0.33	5.6	0.3	0.0073

Light Sussex hatched chick, 0.2407 g.

Pigeons' eggs.

	Weight of contents g.	Lime			Shell weight g.	Membrane	
		Chick g.	Yolk sac g.	Total g.		Weight g.	Lime g.
Unfertile	16.3	—	—	0.0111	1.2	0.1	0.0003
"	17.3	—	—	0.0107	1.2	0.1	0.0004
"	12.2	—	—	0.0074	1.3	0.1	trace
"	12.8	—	—	0.0103	1.0	0.1	"
"	13.3	—	—	0.0080	1.2	0.1	"
"	14.7	—	—	0.0109	1.3	0.1	"
"	15.9	—	—	0.0092	1.3	0.1	0.0004
"	20.1	—	—	0.0174	1.4	0.2	trace
Fertile	17.9	—	—	0.0091	1.6	0.1	0.0005
"	17.8	—	—	0.0100	1.4	0.1	0.0005
"	15.2	0.0114	0.0132	0.0246	1.3	0.1	0.0008
"	16.2	0.0077	0.0128	0.0205	1.5	0.1	0.0011

Distribution of lime in egg contents and membrane.

Contents			Membrane				
Yolk g.	White g.	Total g.	Yolk %	White %	Weight g.	Lime g.	Lime %
0.0418	0.0010	0.0428	97.7	2.3	0.12	0.0002	0.2
0.0375	0.0011	0.0386	97.1	2.9	0.11	0.0006	0.5
0.0304	0.0035	0.0339	89.7	10.3	0.15	0.0006	0.4
0.0310	0.0056	0.0366	84.7	15.3	0.18	0.0005	0.3
0.0384	0.0026	0.0410	93.7	6.3	0.18	0.0010	0.6
0.0281	0.0008	0.0289	98.9	1.1	0.16	0.0001	0.1

Lime in double eggs and shell-less eggs.

Double	Contents	Lime	Shell-less	Contents	Lime	Membrane	Lime
g.	g.	g.	g.	g.	g.	g.	g.
77.5	—	0.0788	—	—	—	0.98	0.3142
74.3	—	0.0793	—	48.1	0.05	1.03	0.399
			—	—	—	4.0	0.581
			—	3.0	0.0317	0.67	0.1604
			—	—	—	3.25	0.75 (from goose)

of sulphuric acid and 200 cc. of alcohol, collecting the precipitated calcium sulphate, drying and weighing.

The figures of analysis are given in the tables. Those of the S series of eggs are curtailed and many referring to unfertile eggs of the R series are not included.

Though some eggs have been found to have a slightly higher lime content, the mean value for unincubated and unfertile eggs from the large number here examined may be taken as 0.04 g. A distinct increase in this amount was first shown at the 11th day of incubation (0.06 g.); the increase became quite definite at the 15th day (0.08 g.). Subsequently there was a daily increase amounting to 0.02 g. A chick about to hatch had 0.2 g. and a hatched chick 0.25 g. The final total is five to six times the original content. The gradual increase is clearly observed in the content of the chick, the amount in the yolk-sac remaining nearly constant, being scarcely double that of the original egg. As the yolk sac is in contact with the shell during development, this structure must play the active part in the transference of the lime; as

soon as lime passes from it into the chick, it must take up fresh lime from the shell. The mechanism of the absorption of lime from the shell is difficult to prove. It is most simple to think that the carbon dioxide and water which are given off during incubation produce calcium bicarbonate. Egg membranes are excellent dialysers and allow calcium bicarbonate to pass through. The passage of the bicarbonate would thus be in the opposite direction to that of the escaping carbon dioxide. In the work of Plimmer and Scott [1909] on the changes in the phosphorus compounds in the developing egg, inorganic phosphoric acid was detected at the 13th day and was present in increasing amounts during the rest of the development. The two processes seem to run parallel. It would therefore seem more likely that the phosphoric acid circulating in the yolk sac, which is in contact with the shell membrane, becomes neutralised by solution of lime from the shell. The passage of lime through the membrane is definitely shown by the increase in its lime content. There are traces only at the beginning whilst during the last seven days the amount is considerable. The presence of lime salts in the membrane is probably the cause of its opaque appearance during the last week of incubation.

The corresponding loss of 0.2 g. from a total of 2.5-3.0 g. in the shell, an amount of lime corresponding to 1/10 of the total, was not discovered. The analyses of the shell were discontinued after the great variations were found in the series O and S in which so many eggs were unfertile, or the chicks dead. If they had been continued, the loss from the shell would most probably have been found.

The results of the increase in lime in the egg are confirmed by the analyses of pigeons' eggs; no data were available as to their exact age, the eggs being deserted by the parents. The increase was up to twice the original figure.

Shell-less eggs have been unexpectedly found to contain large amounts of lime, much more than is required for the growth of the embryo. It was found impossible to incubate these eggs, as they invariably dried up in the course of a week. The shell has thus probably the function of preventing evaporation of water from the egg, in addition to supplying lime and generally acting as a protective covering. The large excess of lime in the shell of eggs points to non-hatchability as being due to some cause within the egg.

It may finally be noted that double eggs contain twice the normal amount of lime and the lime is present mainly in the yolk; the lime content of the yolk varied from 85 to 99 % of the total.

SUMMARY.

The lime content of eggs has a mean value of about 0.04 g.

The lime content of eggs increases to 0.20-0.25 g. during development of the chick. The first increase is noticeable at the 11th day of incubation and the subsequent increase amounts to about 0.02 g. per day until the chick is hatched.

A similar increase occurs in pigeons' eggs during development.

The lime and carbon dioxide content of the egg shell were found to be very variable.

The membrane of the shell also shows an increase of lime during incubation, which shows that lime passes through the membrane.

Shell-less eggs contain more than sufficient lime for the needs of the developing chick. The shell will thus act in preventing loss of water as well as in supplying lime for the chick.

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