The Origin and Destiny of Cholesterol in the Animal Organism. Part IV.—*The Cholesterol Contents of Eggs and Chicks.*

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In a paper recently communicated to the Royal Society* the hypothesis was advanced that cholesterol is a substance which is strictly conserved in the animal organism. As it is difficult to conceive how a body of the constitution of cholesterol can be synthesised in the organism from proteids, carbohydrate or fat, it was suggested that the waste of cholesterol might be made up from the food taken by the animal. In order to test the correctness of this view we thought that evidence of fundamental importanc might be obtained by comparing the cholesterol content of eggs and newly-hatched chicks, and also by ascertaining whether chicks could be reared and would thrive on food deprived of its cholesterol or phytosterol. In this paper we give an account of our estimations of cholesterol in hens' eggs and newly-hatched chicks.

Method of Estimation.—The weighed egg or chick (including broken shell) was pounded up in a mortar with sand and sufficient plaster of Paris to cause the whole to set after a time to a dry mass. This was powdered and extracted in a Soxhlet's apparatus with ether for about twelve days. The ethereal solution of the extract was saponified in the cold by means of an alcoholic solution of sodium ethylate. After standing overnight the precipitated soap was filtered off and thoroughly washed with ether. The filtrate and washings were repeatedly shaken with water to get rid of alcohol, excess of alkali, traces of soap, etc., dried with calcium chloride and the ether distilled off. The residue was dried at 100° C. and weighed. In the case in which single eggs or chicks were analysed, the dry residue, dissolved in 10 c.c. of pyridine, was mixed with about three times its weight of benzoyl chloride also in solution in 10 c.c. of pyridine. After standing overnight the liquid was poured into water, and the precipitated cholesterol benzoate after drying was boiled with 10 c.c. of absolute alcohol and allowed to stand some hours. The crystals were filtered off, washed with a little

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absolute alcohol and weighed. In all cases these crystals were colourless, or nearly so, and melted approximately correctly. The filtrate and washings were separately measured, and corrections, which had been ascertained by previous experiments, made for the solubility of cholesterol benzoate. When cholesterol benzoate was crystallised from absolute alcohol the mother liquor at 21° C. contained 0·12 per cent. When, however, ready formed crystals were shaken for a short time with alcohol at 20° C. and filtered, the filtrate was found to contain only 0·04 per cent. When a number of eggs or chicks were analysed together, the unsaponified residue was crystallised from alcohol, and as much pure cholesterol as possible was isolated, melting at 145°—147° C. The mother liquors were then evaporated to dryness, benzoylated in a pyridine solution as described above, and the benzoate weighed. The soaps precipitated on saponification were collected together in two lots, comprising respectively the total amount obtained from all the eggs examined, and the total amount obtained from all the chicks. These were separately mixed with about twice their weight of salt, water added, and after evaporating to dryness were thoroughly extracted with ether. In neither case could any appreciable quantity of cholesterol be isolated.

In Table I the analysis of eight eggs is given, and in Table II the analysis of eight chicks.

It is obvious from these figures that no increase in the quantity of cholesterol takes place during the change from ovum to newly-hatched chick, the average percentage of cholesterol in eggs being 0·3827, and in chicks 0·3693, or in terms of the weight of the original eggs 0·3172. The same result follows, no matter whether we take the figures for crude unsaponifiable matter, or those for pure cholesterol.

At first sight it would appear that a loss of cholesterol occurs, but taking into account the facts that the difference between the average percentage of cholesterol in eggs and chicks (column *e*)—0·066—is of much the same order of magnitude as the average deviation from the mean in the two cases, viz., 0·057 for eggs and 0·075 for chicks. That individual eggs differ considerably in the loss in weight which takes place during incubation, that there is no reason to suppose that the proportion of yolk to white in different eggs is very constant, and that the method of estimation of cholesterol does not possess a very high degree of accuracy, it would seem probable that no change in the quantity of cholesterol takes place, and that all the cholesterol of the egg is contained in the newly-hatched chick. In order to obtain a more accurate value for the cholesterol content of eggs and chicks, six eggs and six chicks were analysed together, as the greater the quantity of cholesterol weighed in an analysis the more accurate is the

Table I.—Analysis of Separate Eggs.

No.	Weight of eggs in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage of cholesterol.
1	67·33	0·4024	0·2243	0·3331
2	57·57	0·3490	0·1978	0·3436
3	53·32	0·3655	0·2104	0·3946
4	58·48	0·3768	0·2162	0·3697
5	52·70	0·3265	0·1356	0·2563
6	55·40	0·3628	0·2582	0·4661
7	57·30	0·3923	0·2570	0·4485
8	55·45	0·3735	0·2514	0·4534
Total ...	457·55	2·9488	1·7509	0·3832

Table II.—Analysis of Separate Chicks.

No.	Weight of eggs in grammes.	Weight of chicks in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage referred to weight of egg.	Cholesterol referred to weight of chick.
	a.	*b.*	*c.*	*d.*	*e.*	*f.*
9	59·80	55·20	0·3490	0·2014	0·3368	0·3649
10	58·14	53·20	0·3130	0·1819	0·3129	0·3419
11	67·55	61·10	0·4415	0·1433	0·2121	0·2345
12	58·10	46·20	0·3753	0·2592	0·4461	0·5610
13	58·30	52·30	0·2965	0·2951	0·5062	0·5642
14	53·23	49·55	0·2690	0·1074	0·2018	0·2168
15	52·56	48·05	0·4815	0·1298	0·2470	0·2701
16	55·16	45·50	0·3850	0·1502	0·2723	0·3301
Total	462·84	411·10	2·9108	1·4683	0·3172	0·3693

result. In Tables III and IV the figures thus obtained are compared with the total values for the eight eggs and eight chicks dealt with in Tables I and II. As in the latter case the eggs used were taken indiscriminately from various farmers, whereas the eggs analysed together were specially selected hatchable eggs obtained from the dealer, we give in another column the total values for the eggs analysed separately after eliminating the abnormally heavy and abnormally light eggs, Nos. 1, 5, 11, and 15.

The percentages of cholesterol in eggs and chicks calculated from the data obtained by analysing a number of eggs or chicks together are nearer the truth than the averages obtained from the analyses of single eggs or chicks, as in the latter case the errors of the various estimations would be

Table III.

	Weight of eggs in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage of cholesterol.
6 eggs analysed together.....	359·00	2·4025	1·7578	0·4896
8 eggs analysed separately	457·55	2·9488	1·7509	0·3827
6 eggs analysed separately; same as above, eliminating 1 and 5	337·52	2·2199	1·3910	0·4121

Table IV.

	Weight of eggs in grammes.	Weight of chicks in grammes.	Weight of unsaponifiable matter in grammes.	Weight of cholesterol in grammes.	Percentage referred to weight of egg.	Cholesterol referred to weight of chick.
6 chicks analysed together ...	340·2	302	1·7805	1·5914	0·4677	0·5270
8 chicks analysed separately	462·84	411·1	2·9108	1·4683	0·3172	0·3693
6 chicks analysed separately; same as above, eliminating 11 and 15	342·73	301·91	1·9878	1·1952	0·3487	0·3958

accumulated. A comparison of the figures again shows that no gain in cholesterol takes place during the incubation of the chick. Whether the cholesterol of the egg remains unchanged, or whether some loss occurs, cannot be definitely decided.

Conclusions.

In the differentiation of the ovum into the complex aggregates of cells constituting the chick, no formation of cholesterol takes place. This is in accordance with our view that cholesterol is not synthesised in the organism.

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