The Origin and Destiny of Cholesterol in the Animal Organism. Part XI.—*The Cholesterol Content of Growing Chickens under Different Diets.*

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(From the Physiological Laboratory of the University of London.)

In previous papers of this series evidence has been brought forward showing that cholesterol is a substance which is strictly conserved in the animal organism, and that waste of cholesterol can be made up from the food taken by the animal. Whether cholesterol can, to any extent, be synthesised in the organism from proteins, fats or carbohydrates, it is difficult to ascertain. It seemed likely that evidence on this point might be obtained by comparing the cholesterol content of eggs and newly hatched chickens, by studying the change of the cholesterol content with growth, and also by ascertaining whether chickens could be reared and would thrive on food deprived of its cholesterol and phytosterol. Parke,* as long ago as 1867, found that the quantity of matter which could be extracted by both ether and alcohol from the yolk of hen's eggs diminished during incubation, and he stated that cholesterol changes similarly. In 1908, L. B. Mendel and Leavenworth† published the results of some experiments to determine whether cholesterol is produced during the development of the eggs, and found that no increase took place, but that the cholesterol appeared to decrease, in part, in company with the rest of the lipoid substances. In Part IV of this series Ellis and Gardner‡ concluded from a large number of analyses of eggs and chickens that in the differentiation of the ovum into the complex aggregates of cells constituting the chicken no formation of cholesterol takes place. Whether the cholesterol of the egg remained unchanged or whether some loss occurred could not be definitely decided. In this paper we give an account of our experiments on the growth of chickens under various diets, and comparisons of the cholesterol and

* Parke, 'Hoppe-Seyler's Medizinisch-chemische Untersuchungen,' 1867, p. 211; for lecithin, *cf.* Mesernitzky, 'Biochemische Centralblatt,' 1907, vol. 6, p. 784.

† Mendel and Leavenworth, 'Amer. Journ. Physiol.,' 1908, vol. 21, pp. 82–84.

‡ Ellis and Gardner, 'Roy. Soc. Proc.,' 1909, B, vol. 81, pp. 129–132.

cholesterol ester content of day-old chicks, and chicks at various stages of growth.

Method of Estimation of the Cholesterol.

The weighed chickens were minced in a mincing machine and the minced mass 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, which was powdered and then extracted with ether in a Soxhlet's apparatus for about a fortnight. The ethereal solution of the extract was made up to a known volume and suitable aliquot proportions taken for analysis. The analyses were made by a modification of Windaus' digitonin method devised by Fraser and Gardner.*

General Plan of Experiments.

Fifty-four white Wyandotte day-old chicks of the same strain were obtained from a dealer and six of these were killed straight away and analysed with the following result.

The weight of the six chickens was 212 grm., and the extract was made up to 1 litre; 100 c.c. of this extract were then taken, and by direct precipitation gave 0·3407 grm. of the compound, corresponding to 0·0828 grm. of cholesterol, whilst 100 c.c. after saponification gave 0·49 grm. of the compound corresponding to 0·1189 grm. of cholesterol.

The remaining chickens were placed in four pens A, B, C, and D, as nearly as possible under the same conditions, 10 in A, 10 in B, 14 in C, and 14 in D. Each pen consisted of a foster mother, kept at a suitable uniform temperature by electric means, and was provided with a run with a sandy floor in which was placed a trough of water, and in which the animals were fed. Three diets were made use of.

(1) An ordinary commercial chicken food, of approximately the following composition :—

	Per cent.		Per cent.
Water	9·65	Fats	2·25
Proteins	22·50	Ash.....	1·20
Carbohydrates.....	64·40		

(2) The same chicken food as the above, which had previously been freed as far as possible from fats, cholesterol and phytosterol. In this food the percentage of protein was found to be about 19·9 per cent.

(3) The same diet as in (2), but with the addition of about 2 per cent. of cholesterol.

In the case of the first two meals given to the chickens, the diet (1) was

* Fraser and Gardner, 'Roy. Soc. Proc.,' 1910, B, vol. 82, p. 560.

replaced by ordinary hard-boiled egg, pounded up and moistened with warm water.

Diet (2) was replaced by the white of egg, and the yolk which had been extracted with ether.

Diet (3) was replaced by white of egg and extracted yolk to which about 2 per cent. of cholesterol had been added.

Pens A and B.—Ten chickens were placed in pen A, weighing altogether 336 grm. After one week the weight of these chicks was 512 grm.; 10 chickens were placed in B, weighing altogether 346 grm., and after one week their combined weight was 555 grm.

After one week two chickens were taken from pen A and three from pen B, their total weight being 260·1 grm. These were killed and analysed in the usual way, the total ether extract being then made up to 1 litre; 100 c.c. of this extract yielded by direct precipitation 0·228 grm of compound, corresponding to 0·0554 grm. of cholesterol, and after saponification 100 c.c. yielded 0·2908 grm. of the compound, corresponding to 0·0706 grm. of cholesterol.

After 14 *Days.*—The eight chickens in pen A weighed 559 grm., and the seven in pen B weighed 515·3 grm. From pen A three chickens weighing 205·5 grm. were taken, and from pen B two chickens weighing 150 grm., their total weight being 355·5 grm. These were killed and analysed with the following results:—The extract was made up to 1 litre; 100 c.c. of this gave by direct precipitation 0·271 grm. of compound, corresponding to 0·066 grm. of cholesterol, while after saponification 100 c.c. gave 0·365 grm. of compound, corresponding to 0·0887 grm. of cholesterol.

After 21 *Days.*—The five chickens in pen A now weighed 402·9 grm., and the five in B weighed 496·6 grm. From pen A two chickens were taken, weighing 199·1 grm., and from B two chickens weighing 152·5 grm., their total weight being 351·6 grm. These were killed and analysed in the usual manner, and the ethereal extract made up to 1 litre; 100 c.c. of this extract gave by direct precipitation 0·207 grm. of the compound, corresponding to 0·0505 grm. of cholesterol, whilst after saponification 100 c.c. gave 0·382 grm. of the compound, corresponding to 0·0829 grm. of cholesterol.

After One Month.—The three chickens in pen A now weighed 287·5 grm., and the three in pen B 361 grm. Two chickens were now taken from pen A, weighing 207·7 grm., and one chicken from pen B, weighing 103·5 grm., their total weight being 311·2 grm. These were killed and analysed in the usual manner, and ethereal extract was made up to 1 litre; 100 c.c. of this extract were taken, and by direct precipitation gave 0·2 grm. of the compound, corresponding to 0·0487 grm. of cholesterol, whilst 100 c.c. after saponification gave 0·2386 grm. of the compound, corresponding to 0·058 grm. of cholesterol.

In Pen C.—Fourteen chicks were in this pen, weighing altogether 766 grm. One of these died on the seventh day. Five chickens, including the dead one, were now taken and analysed in the usual way, their combined weight being 250·1 grm. The ethereal extract was made up to 1 litre, and the following results were obtained:—100 c.c. of the solution gave by direct precipitation 0·2767 grm. of the compound, corresponding to 0·0551 grm. of cholesterol, whilst after saponification 100 c.c. gave 0·3032 grm. of the compound, corresponding to 0·0737 grm. of cholesterol.

After 14 *Days.*—There were now nine chickens in pen C, weighing 657·5 grm.; four of these, weighing 295·6 grm., were killed and analysed, the ethereal extract being made up to 1 litre; 100 c.c. of this extract gave by direct precipitation 0·1646 grm. of compound, corresponding to 0·0400 grm. of cholesterol, while 100 c.c. after saponification gave 0·2341 grm. of compound, corresponding to 0·0569 grm. of cholesterol.

After 21 *Days.*—Pen C contained five chickens, weighing altogether 514·8 grm. None were killed this week, and

After 28 *days* the weight of the chickens was 614·5 grm. Three of these, weighing 357·7 grm., were killed and analysed, and the ethereal extract made up to 1 litre; 100 c.c. were taken, and by direct precipitation gave 0·216 grm. of compound, corresponding to 0·0525 grm. of cholesterol, and after saponification 100 c.c. gave 0·254 grm. of compound, corresponding to 0·0618 grm. of cholesterol.

Pen D.—Fourteen chickens, weighing 532·2 grm., were in this pen to start with.

After 7 *days* the weight of the chickens was 742·8 grm. Five chickens, weighing 249·8 grm., were killed and analysed with the following results:—The ethereal extract was made up to 1 litre; 100 c.c. of this extract gave by direct precipitation 0·272 grm. of compound, corresponding to 0·0661 grm. of cholesterol, while after saponification 100 c.c. of the extract gave 0·447 grm. of the compound, corresponding to 0·1097 grm. of cholesterol.

After 14 *days* the nine remaining chickens weighed 587·3 grm.; four of these, weighing 262 grm., were now killed and analysed with the following results:—The extract was made up to 1 litre, and 100 c.c. gave by direct precipitation 0·2432 grm. of compound, corresponding to 0·0511 grm. of cholesterol, while 100 c.c., after saponification, gave 0·4919 grm. of compound, corresponding to 0·1195 grm. of cholesterol.

After 21 *days* the five remaining chicks weighed 447·3 grm., and

After 28 *days* their weight was 583·5 grm.; three of these, weighing 352·5 grm., were now killed and analysed with the following results:—The extract was made up to 1 litre, 100 c.c. of this gave, by direct precipitation

0·4065 grm. of the compound, corresponding to 0·0988 grm. of cholesterol, whilst, after saponification, 100 c.c. gave 0·5518 grm. of the compound, corresponding to 0·134 grm. of cholesterol.

We do not think that the results obtained after the end of the second week are of much value for purposes of comparison, as the individual chickens showed such great variations in size and vigour. For instance, the odd chicks remaining in pens A, B, and C, at the end of the fifth week, weighed approximately 83, 159, and 115 grm., and the two chicks in pen D 258 grm.

Then, again, in pens A and B, where the animals were on the same diet and apparently under similar conditions, the growths showed considerable changes, as evidenced by the following table:—

	Pen A.	Pen B.
	100·0	100·0
First week	152·4	160·4
Second week	208·0	212·7
Third week	239·8	287·1
Fourth week	285·2	347·7

Whether this was due to the individuality of the animals, or whether it was due to differences in the amount of food taken, we are unable to say, as we gave excess of food, but had no means of measuring the amount left over.

An excess of food was given at definite times morning and evening, and the chickens were allowed to eat as much as they wished. It was impossible, however, to measure the amount of food eaten, owing to the habits of the birds, which trampled the food with the sand and excrement. It was impossible, therefore, either to estimate the unused food, or to examine the fæces, and it would have been impossible to have reared the chicks in a clean cage without sand, as they would not thrive under these conditions.

Furthermore, the laboratory conditions were not very suitable for healthy growth in prolonged experiments. The individual variations were, however, not so marked during the first two weeks, and we think that the results obtained with the various diets during this period are fairly comparable. We give, however, the later analyses for what they are worth.

In Table I we give the weights of the chickens at the various periods of growth, and the weights of cholesterol and cholesterol esters which they contain at those periods, calculated from the above data on the basis of 100 grm. of day-old chick.

Table I.

Age.	Diet of ordinary chicken food. (Animals from pens A and B.)				Diet of extracted chicken food. (Animals from pen C.)				Diet of extracted chicken food + cholesterol. (Chickens from pen D.)			
	Weight.	Total cholesterol.	Free cholesterol.	Ester cholesterol.	Weight.	Total cholesterol.	Free cholesterol.	Ester cholesterol.	Weight.	Total cholesterol.	Free cholesterol.	Ester cholesterol.
	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
1 day	100·0	0·561	0·391	0·170	100·0	0·561	0·391	0·170	100·0	0·561	0·391	0·120
7 days	156·5	0·476	0·334	0·142	148·6	0·438	0·328	0·111	139·6	0·613	0·369	0·244
14 ,, ...	210·0	0·528	0·391	0·137	198·5	0·383	0·276	0·107	171·7	0·784	0·387	0·396
21 ,, ...	263·8	0·624	0·380	0·244	279·6	—	—	—	235·4	—	—	—
28 ,, ...	319·9	0·590	0·496	0·095	333·8	0·579	0·491	0·087	306·8	1·169	0·861	0·308

Discussion of Results.—Dealing only with the results of the first two weeks, it will be seen from the table that, on the ordinary diet, the total cholesterol decreases by about 15 per cent. during the first week, and then increases again during the second week to within about 6 per cent. of the value in the day-old chick; the free cholesterol shows a similar decrease during the first week, but at the end of the second week has increased again to the original value. The ester cholesterol also decreases by about 17 per cent. during the first week, but the decrease is much less marked during the second.

In the case of animals fed on the extracted diet, there is a decrease in the total cholesterol during the two weeks, but the decrease during the first week is nearly double that in the second. This variation in the rate of decrease appears to be due to the ester cholesterol, which shows a marked decrease during the first week, but only a slight change during the second. The free cholesterol goes down fairly steadily.

In the case of the animals fed on extracted food + cholesterol, the total cholesterol increases during the two weeks, more markedly in the second than in the first. The cholesterol thus stored up is mainly in the form of esters; these show a marked increase during the two weeks, the increase in the second being double that in the first. The free cholesterol does not appear to be much affected; the figures are of much the same order as in the case of the animals on ordinary diet, the main difference being that the decrease noticed during the first week is less marked.

It will be noticed that the rate of growth of the animals in the earlier weeks on the cholesterol diet was slower than in the other cases, but we have no means of forming an opinion as to whether this is due to the diet, or to the amount of food eaten, or to the individuality of the animals. The day-old chicks, however, in this case were smaller than in the others.

What is the meaning of the high free cholesterol content in the fourth week of the experiment with extracted food we are unable to say, but we cannot attach much value to this particular result for the reasons already stated.

The cholesterol content of the chickens thus appears to depend on the sterol content of the diets, but there is nothing in the figures of the first two weeks to indicate that in the growing animal the organism can synthesise cholesterol. We have not carried out this investigation in as detailed a manner as was intended at the outset, as the animals did not prove very suitable for the investigation of problems of growth, partly owing to the habits of the birds, rendering it very difficult to control the amount of food taken, and to estimate the excrement, and partly owing to the difficulty of

getting strictly comparable sets of birds. The laboratory conditions with strict control of diet and proper collection of excrement are not very suitable for keeping birds in health for a prolonged period. One of us is, however, extending his observations to the study of growing rats, which can be much more suitably reared and controlled, and hopes shortly to be able to publish an account of his experiments.

The results given in this paper, however, taken in conjunction with the fact that, in the differentiation of the ovum into the complex aggregate of cells constituting the chicken, no formation of cholesterol takes place, appear to support the view formerly expressed that cholesterol is not readily synthesised in the organism.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

Contributions to the Biochemistry of Growth.—On the Lipoids of Transplantable Tumours of the Mouse and the Rat.*

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The following observations were carried out with the object of obtaining information concerning the presence of lipoid substances in rapidly growing cells. Our observations were made on transplantable tumours of mice and rats, which, as we have pointed out in previous papers of this series, are especially suitable for the study of the biochemical problems of growth. So far as we are aware, neither qualitative nor quantitative observations on the presence of lipoids in these tumours have as yet been made.

The analytical method employed was the same as the one used in our observations on normal and degenerating nerves.† The fact that by means of this method 99 to 101 per cent. of the total lipoids present in normal

* This research is in continuation of papers in 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 263; 1910, vol. 82, pp. 307 and 316; 1913, vol. 86, p. 302.

† Cramer, Feiss and Bullock, 'Proc. Physiol. Soc.,' 1913, p. li, 'Journ. Physiol.,' vol. 46.