FLAVINS IN CHICK EMBRYO

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Summary During incubation of fertilized hen egg, flavin in egg white migrated into the embryo. The total amount of flavins in the embryo increased with development. On the 7th day of incubation, molar ratio of riboflavin and flavin nucleotides in the embryo was already similar to that of animal tissues. Even though appreciable amounts of FMN and FAD were found in egg white, flavin nucleotides found in the embryo in its early embryonic life cannot be simply ascribed to the migration of these nucleotides originally present in egg white, because egg yolk contains no appreciable amount of flavin nucleotides; the possibility that flavin nucleotides are synthesized from riboflavin in the embryo in its early embryonic life still exists.

It had been reported (1, 2) that fertilized egg of hen contains only free riboflavin and that it is esterified to its nucleotide forms in chick embryo. It was also reported (3) that eggs of birds contain only free riboflavin and not nucleotide forms of it. Recently, however, we observed that hen egg, regardless of fertilization, contains appreciable amounts of both FMN and FAD (4). Thus, the feature of the synthesis of flavin nucleotides from free riboflavin during the development of chick embryo has to be reexamined. The present paper deals with the change in the amounts of flavins in chick embryo as well as in chick in early postnatal period.

MATERIALS AND METHODS

Fertilized eggs of the White Rock breed were purchased from a local poultry farm and incubated at 38°C under high humidity. Chicks after hatching were provided with the standard diet *ad libitum*.

The total amount of flavins and molar ratio of separated flavins were estimated according to YAGI (5). The extraction of flavins from the embryo was made by warm-water-extraction method (5). For the extraction of flavins from egg white

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or egg yolk, sample was diluted with water for 10 times, followed by adjustment of solution pH at 4.7 and then subjected to warm-water-extraction method.

The measurements were made for at least four samples (4–7 samples), and the mean $\pm$ standard deviation was given.

RESULTS AND DISCUSSION

Table 1 shows the change in the amounts of embryo and other portions of egg during incubation and that in the weight of chick after hatching. Table 2 shows the changes in the concentrations of the total amounts of flavins in these samples during incubation as well as after hatching. Table 3 shows the total amounts of flavins in these samples.

From these data, it is clear that both the total amount and the concentration of flavins of chick embryo increase with incubation. Since no appreciable change in the total amount of flavins was observed during incubation, the above-mentioned increase in flavins in the embryo is due to the migration of flavins from egg yolk and/or egg white. Data summarized in Table 3 suggest that the flavin in egg white migrated into the embryo. All these results are essentially in accord with those of previous papers (1, 2). Differing from these papers, the present study provided the data after hatching. The total amount of flavins of chick within 24 hr after hatching does not differ from those of eggs during incubation, but it decreased on

Table 1. Weight of embryo in fertilized egg during incubation and that of chick after hatching.

Age (in days)	Embryo (g)	Egg white (g)	Egg yolk (g)	Allantois and amnion (g)	Total (g)
Before incubation	—	32.0 $\pm$ 3.4	17.5 $\pm$ 1.1	—	49.5 $\pm$ 4.8
7 days incubation	0.9 $\pm$ 0.2	12.0 $\pm$ 1.6	30.2 $\pm$ 6.8	3.3 $\pm$ 0.7	46.4 $\pm$ 7.8
14 days incubation	13.0 $\pm$ 1.9	5.8 $\pm$ 1.2	17.5 $\pm$ 4.7	13.7 $\pm$ 3.4	50.0 $\pm$ 5.7
Hatching ^a	43.2 $\pm$ 3.2	—	—	—	43.2 $\pm$ 3.2
7 days after hatching	41.5 $\pm$ 3.2	—	—	—	41.5 $\pm$ 3.2

^a Within 24 hr after hatching.

Table 2. Concentration of flavins in fertilized egg during incubation and in chick after hatching.

Age (in days)	Embryo (μ g/g)	Egg white (μ g/g)	Egg yolk (μ g/g)	Allantois and amnion (μ g/g)
Before incubation	—	4.3 $\pm$ 0.2	7.1 $\pm$ 0.9	—
7 days incubation	1.4 $\pm$ 0.4	10.8 $\pm$ 2.1	3.8 $\pm$ 1.6	0.5 $\pm$ 0.5
14 days incubation	3.1 $\pm$ 0.5	11.8 $\pm$ 3.2	6.9 $\pm$ 1.9	3.2 $\pm$ 1.4
Hatching ^a	5.7 $\pm$ 1.4	—	—	—
7 days after hatching	4.4 $\pm$ 0.2	—	—	—

^a Within 24 hr after hatching.

Table 3. Total amount of flavins in fertilized egg during incubation and in chick after hatching.

Age (in days)	Embryo (μg)	Egg white (μg)	Egg yolk (μg)	Allantois and amnion (μg)	Total (μg)
Before incubation	—	138.9 $\pm$ 10.1	125.3 $\pm$ 20.0	—	264.2 $\pm$ 28.8
7 days incubation	1.2 $\pm$ 0.2	129.9 $\pm$ 29.1	107.2 $\pm$ 34.6	1.7 $\pm$ 1.6	239.9 $\pm$ 54.2
14 days incubation	41.6 $\pm$ 11.5	66.6 $\pm$ 17.1	115.6 $\pm$ 26.3	46.8 $\pm$ 28.3	270.6 $\pm$ 35.2
Hatching ^a	246.8 $\pm$ 68.4	—	—	—	246.8 $\pm$ 68.4
7 days after hatching	179.9 $\pm$ 6.7	—	—	—	179.9 $\pm$ 6.7

^a Within 24 hr after hatching.

the 7th day after hatching.

The results of the separating determination of flavins in fertilized egg and in the embryo as well as in chick after hatching are shown in Table 4 and 5.

Table 4. Amounts of riboflavin and flavin nucleotides in fertilized egg.

	FAD (μg)	(%)	FMN (μg)	(%)	Riboflavin (μg)	(%)
Egg white	10.1 $\pm$ 6.7	7.2 $\pm$ 5.0	18.1 $\pm$ 2.7	13.1 $\pm$ 2.0	110.6 $\pm$ 13.2	79.5 $\pm$ 7.0
Egg yolk	N.D. ^a		trace		125.3 $\pm$ 20.0	<100

^a N.D., not detectable.

Table 5. Amounts of riboflavin and flavin nucleotides in embryo during incubation and in chick after hatching.

Age (in days)	FAD (μg)	(%)	FMN (μg)	(%)	Riboflavin (μg)	(%)
7 days incubation	0.95 $\pm$ 0.02	83.5 $\pm$ 8.9	0.11 $\pm$ 0.01	9.5 $\pm$ 1.1	0.07 $\pm$ 0.04	6.6 $\pm$ 0.5
14 days incubation	26.8 $\pm$ 6.3	65.2 $\pm$ 5.1	5.7 $\pm$ 1.9	13.8 $\pm$ 2.9	9.1 $\pm$ 4.4	20.9 $\pm$ 4.3
Hatching ^a	160.1 $\pm$ 30.5	67.2 $\pm$ 11.8	20.1 $\pm$ 22.8	7.0 $\pm$ 7.8	66.6 $\pm$ 34.9	25.7 $\pm$ 7.9
7 days after hatching	108.5 $\pm$ 11.2	60.3 $\pm$ 2.3	39.2 $\pm$ 6.1	21.8 $\pm$ 3.2	32.6 $\pm$ 1.9	18.1 $\pm$ 1.1

^a Within 24 hr after hatching.

As can be seen from Table 4, appreciable amounts of FMN and FAD were found in egg white of fertilized egg. This datum is definitely different from those of the previous researchers (1-3) who reported that egg contains only riboflavin. Further detailed examination on the occurrence of FMN and FAD in eggs of birds will be reported elsewhere (4). It is also noted that egg yolk of fertilized egg contains negligible amount of flavin nucleotides.

In Table 5, molar ratio of riboflavin and flavin nucleotides in the embryo and in chick after hatching is given. On the 7th day of incubation, the molar ratio of flavins in the embryo is similar to that of animal tissue, *viz.* the major part

is FAD. The result is not in agreement with that of KUBOTA (2) who reported that the major part of flavins in chick embryo in its early embryonic life is FMN. Also in other samples, FAD is the major part of flavins.

Since egg yolk contains negligible amount of flavin nucleotides, flavin nucleotides in chick embryo can be increased by either migration of flavin nucleotides present originally in egg white or migration of riboflavin from egg yolk and/or egg white into the embryo followed by its esterification *in situ*. To make clear this point, further investigation is needed. In any case, the increase in the amounts of flavin nucleotides in chick embryo suggests the increased amount of flavoproteins or flavin enzymes which are responsible to the increasing respiration of chick embryo.

Relatively high ratio of riboflavin found in chicks within 24 hr after hatching would be ascribed to the intake of yolk sack by the embryo before hatching.

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