The Accumulation of Nucleic Acid and Protein in the Skeletal Muscle of Chicks During Embryonic and Post-Embryonic Development

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(Received for publication on July 30, 1966)

SIMMONDS *et al.*⁸⁾ have shown that the weight of breast muscle of chicks underwent a more rapid increase during the first 14 days after hatching than during 14 days thereafter. We also found a similar phenomenon in the previous work which was concerned with the growth of pectoral muscle of chicks⁵⁾.

The present study was undertaken to define the rapid growth of breast muscle of chicks for a time after hatching in terms of the accumulation of its nucleic acid and protein components.

Materials and Methods

White Leghorn eggs, purchased from a commercial hatchery, were used throughout these experiments. The eggs were incubated at 37.7° in a forced-draft incubator. The newly hatched chicks received water and a commercial formula feed (20% crude-protein) for starting chick at 24 hours after hatching and thereafter fed *ad libitum* the same feed throughout the experiment. Determinations were made at 2- or 3-day intervals from the 15th to the 49th day after start of incubation.

The weight of embryos was measured after washing in physiological saline and thawing out with gauze. The embryos and chicks were killed by decapitation and the breast muscle was dissected as completely as possible. After having been weighed, the muscle was finely minced with scissors. The minced muscle was then well mixed and samples were taken for determination of deoxyribonucleic acid (DNA), total N, non-protein N and N of protein components, such as sarcoplasmic-, fibrillar- and extracellular-protein N. Sixteen embryos were used on the 15th, 17th and 19th day of incubation, and 4 to 12 chicks for each day from the 21st to the 49th day. The muscles of the embryos and chicks of each day were divided into 4 groups in such a way that number of the muscles in each group was the same. The values for DNA and N presented in Tables 2 and 3 are the means calculated from determinations carried out in 4 groups.

Samples for DNA determination were homogenized and extracted three times with a mixture of alcohol and ether (1:1). The residues after extraction were dried over sulfuric acid in vacuum and stored until required for analysis. After treated with cold 2% perchloric acid, the residues were taken up in 10% perchloric acid and DNA was extracted for 20 minutes at 70°¹⁾. DNA was determined by using a color reaction with indol according to CERIOTTI's method²⁾ and represented in terms of its phosphorus content, using a standardization curve showing the relationship between color density of various dilutions of the DNA standard solution and their phosphorus content. Total N determination was carried out by the micro-

KJELDAHL method and non-protein N was determined by the method described by DICKERSON⁶⁾. Protein components were separated by the method of ROBINSON⁷⁾ and N of each separated component was determined by the micro-KJELDAHL method.

The second experiment, which was a close replicate of the first except that determinations of non-protein N and N of protein components were omitted, was carried out from the 10th to the 26th day of incubation, in order to obtain more detailed results on changes in nucleic acid and protein content of muscle during embryonic development.

Results

Body weight and muscle weight are shown in Table 1 and semilogarithmic plots of body weight and muscle weight are given in Fig. 1. The increase in body weight was continuous

Table 1. Body weight and breast muscle weight of chicks at various stages of development

Days after incubation	Experiment 1		Experiment 2	
	Body weight g	Muscle weight g	Body weight g	Muscle weight g
10			2.2±0.1	0.027±0.000
11			3.6±0.2	0.028±0.001
12			4.8±0.1	0.049±0.003
13			6.9±0.2	0.072±0.003
14			8.4±0.2	0.11 ±0.005
15	12.3±0.4	0.14±0.01	11.4±0.3	0.14 ±0.004
16			14.9±0.7	0.16 ±0.011
17	18.4±0.4	0.17±0.01	18.7±0.2	0.20 ±0.016
18			24.0±0.5	0.18 ±0.005
19	27.7±0.5	0.18±0.01	25.2±0.4	0.17 ±0.010
20			43.8±0.4	0.21 ±0.012
21 (H)*	43.3±0.8	0.21±0.01	40.1±0.9	0.18 ±0.009
22			36.5±1.3	0.24 ±0.022
23			40.9±2.2	0.23 ±0.025
24	42.7±0.7	0.31±0.03	39.4±2.0	0.26 ±0.033
25			45.3±1.8	0.31 ±0.028
26			47.9±1.8	0.50 ±0.060
27	55.6± 2.3	0.72±0.09		
30	66.8± 1.7	1.16±0.12		
33	94.2± 1.6	2.29±0.24		
36	110.3± 2.3	3.10±0.34		
39	139.0± 2.3	3.70±0.15		
42	163.6± 6.0	5.14±0.21		
49	258.5±13.6	8.58±0.47		

Values are means ±S.E. * (H) represents hatching.

throughout except for a relative small irregularity around the time of hatching, caused probably by embedding yolk in the abdominal region. The log plot of body weight during embryonic development was nearly linear and the coefficient of its linear regression was 0.088–0.117. The

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log plot after hatching was practically linear and its regression coefficient was 0.031-0.028. A comparison of both coefficients indicates a decrease in the growth rate after hatching.

Fig. 1. Semilogarithmic plots of body weight and muscle weight

* The values represent regression coefficients.

Fig. 2. Semilogarithmic plots of DNA per muscle and total N per DNA

* The values represent regression coefficients.

Table 2. DNA content, DNA concentration and total N per DNA in breast muscle of chicks at various stages of development

Days after incubation	Experiment 1			Experiment 2		
	Concentration of DNA-P μg/g of muscle	Content of DNA-P μg/muscle	Total N per DNA-P mg/mg of DNA-P	Concentration of DNA-P μg/g of muscle	Content of DNA-P μg/muscle	Total N per DNA-P mg/mg of DNA-P
10				232.1 ± 2.4	6.3 ± 0.1	34.4 ± 3.4
11				270.8 ± 19.7	7.5 ± 0.4	29.1 ± 3.4
12				286.0 ± 6.2	14.0 ± 0.6	28.9 ± 0.7
13				257.1 ± 8.9	18.2 ± 1.5	36.7 ± 2.0
14				288.9 ± 3.9	30.9 ± 1.5	34.9 ± 0.6
15	286.1 ± 7.0	41.4 ± 1.7	43.8 ± 0.6	338.7 ± 10.6	48.8 ± 0.9	32.2 ± 0.6
16				339.4 ± 15.0	60.5 ± 1.4	37.3 ± 2.6
17	318.9 ± 10.1	53.2 ± 2.9	44.3 ± 1.4	406.1 ± 21.8	60.6 ± 8.5	37.0 ± 3.1
18				333.4 ± 22.7	61.2 ± 1.8	47.2 ± 2.0
19	273.6 ± 7.4	50.1 ± 1.5	58.6 ± 2.1	339.5 ± 12.9	56.7 ± 1.8	49.9 ± 1.9
20				313.6 ± 18.2	64.3 ± 1.8	52.4 ± 2.3
21 (H)*	246.3 ± 13.2	52.6 ± 3.0	68.1 ± 5.5	317.9 ± 21.7	59.9 ± 1.8	50.2 ± 5.0
22				305.5 ± 7.9	70.8 ± 1.9	54.1 ± 1.4
23				313.1 ± 7.6	72.3 ± 10.1	58.6 ± 1.7
24	291.2 ± 14.2	88.0 ± 5.7	65.3 ± 5.0	332.2 ± 17.5	84.1 ± 9.4	58.5 ± 3.3
25				323.2 ± 22.4	100.1 ± 5.6	76.0 ± 6.6
26				263.3 ± 16.4	129.0 ± 7.4	91.9 ± 4.6
27	234.7 ± 35.5	160.8 ± 8.4	142.6 ± 3.1			
30	169.5 ± 16.3	193.1 ± 15.5	185.1 ± 13.0			
33	88.9 ± 4.4	200.6 ± 15.2	382.6 ± 18.0			
36	84.4 ± 9.1	255.5 ± 26.2	423.8 ± 36.1			
39	73.6 ± 1.4	272.1 ± 11.2	469.9 ± 6.3			
42	67.7 ± 3.2	348.0 ± 8.1	486.2 ± 16.1			
49	63.8 ± 4.4	543.1 ± 22.0	529.1 ± 32.7			

Values are means ± S.E. * (H) represents hatching.

The increase in muscle weight was not continuous since the weight accumulation hardly occurred during about the last 5 days before hatching. The accumulation of muscle weight from the 10th to the 15th day of incubation was an exponential one since the log plot for this period was linear and its regression coefficient was 0.161, which was a value somewhat higher than that of body weight for the same period (Exp. 2). The log plot of muscle weight from the 21st to the 49th day showed a curve which included two linear parts, one from the 21st to the 33rd day and the other from the 33rd to the 49th day. The regression coefficient of the former linear part was about 2.5 times as high as that of the latter, indicating that muscle weight increased more rapidly during about the first 12 days after hatching than thereafter.

Table 2 shows DNA content per muscle, DNA content per unit of muscle weight and total N/DNA ratio. Figure 2 represents semilogarithmic plots of DNA content per muscle and total N/DNA ratio. The log plot of DNA content per muscle from the 10th to the 15th day of incubation was linear and its regression coefficient was 0.183, which was a value close to that of muscle weight for the same period. Similar to the accumulation of muscle weight, no further increase in DNA content per muscle occurred for about 5 days after the 16th day. After the 21st day the DNA content showed a marked increase which diminished after about the 27th day. Both of the log plots of the DNA accumulation from the 21st to the 27th day and from the 27th to the 49th day were linear and their regression coefficients were 0.081 and 0.023 respectively (Exp. 1). The value of the regression coefficient for the former period was close to that of muscle weight for a period from the 21st to the 33rd day. DNA content per unit of muscle weight reached a maximum on the 17th day of incubation and then decreased continuously except for a moderate rise around the 24th day.

Total N/DNA ratio probably did not increase during a period from the 10th to the 17th day of incubation. A statistical analysis showed no significant difference in the ratios obtained for this period. After the 17th day the increase in the ratio occurred, but it was not continuous until the 24th day since a slight drop occurred on the 24th day in Experiment 1 and

Table 3. Protein composition of breast muscle of chicks
at various stages of development

Days after incubation	Amount of nitrogen, mg/mg of DNA-P			
	Non-protein N (%)	Sarcoplasmic-protein N (%)	Fibrillar-protein N (%)	Extracellular- protein N (%)
15	5.9±0.2 (13.6)	17.2± 1.0 (40.0)	16.7± 0.6 (38.6)	3.4±0.1 (7.8)
17	5.0±0.3 (11.2)	17.3± 0.5 (39.2)	18.8± 0.9 (42.5)	3.2±0.1 (7.2)
19	7.5±0.8 (12.7)	19.2± 0.6 (32.7)	26.6± 0.9 (45.3)	5.4±0.3 (9.2)
21 (H)*	6.8±0.2 (10.0)	19.3± 2.2 (28.4)	33.6± 2.6 (49.4)	8.4±0.8 (12.3)
24	7.7±0.7 (11.7)	17.2± 1.4 (26.2)	33.8± 2.5 (51.7)	6.8±0.2 (10.4)
27	15.9±1.1 (12.9)	33.1± 3.5 (26.9)	68.1±10.7 (55.4)	5.9±0.1 (4.8)
30	25.6±2.0 (13.9)	56.7± 3.6 (30.6)	95.7± 6.7 (51.7)	7.1±0.8 (3.9)
33	55.2±2.6 (14.4)	120.1± 9.3 (31.4)	194.5± 8.0 (50.8)	13.3±1.0 (3.5)
36	54.7±2.1 (13.1)	131.6±10.0 (31.6)	217.4±11.2 (52.2)	13.2±1.0 (3.2)
39	69.2±0.6 (14.8)	141.6± 1.3 (30.2)	242.3± 3.2 (51.7)	15.4±1.4 (3.3)
42	79.5±6.2 (16.3)	150.5±10.0 (30.9)	244.5± 8.2 (50.3)	12.0±1.3 (2.5)
49	81.8±6.9 (15.5)	165.6±13.1 (31.3)	267.7±19.6 (50.6)	14.0±1.4 (2.6)

Values are means ±S.E. * (H) represents hatching.

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on the 21st day in Experiment 2 respectively. The log plots of the ratio from the 24th to the 33rd day and from the 33rd to the 49th day were nearly linear and their regression coefficients were 0.081 and 0.008 respectively (Exp. 1). A comparison of both coefficients indicates that the rate of the increase in the ratio markedly decreased after about the 33rd day of incubation.

Table 3 shows the composition of total N. The increase in non-, sarcoplasmic-, fibrillar- and extracellular-protein N/DNA ratio followed a course similar to that of total N/DNA ratio. For a period from the 15th to the 24th day the increase in sarcoplasmic-protein N/DNA ratio hardly occurred and non- and fibrillar-protein N/DNA ratio increased only 1.3- and 2.0-fold respectively. After the 24th day these three ratios showed a marked increase for a period of about 9 days and thereafter the rate of the increase in the ratio decreased. The increase in extracellular-protein N/DNA ratio from the 17th to the 27th day was not continuous since the ratio decreased for about 6 days after the 21st day. Sarcoplasmic- and fibrillar-protein N made up the greatest part of total N. Fibrillar-protein N increased to a maximum from 38.6% of total N on the 15th day to 55.4% on the 27th day and then decreased slightly. Sarcoplasmic-protein N, on the contrary, decreased from 40.0 to 26.2% for 9 days after the 15th day and thereafter increased to about 77% of the original value by 6 days. Extracellular-protein N increased from 7.8 to 12.3% during embryonic period and after hatching decreased gradually, whereas non-protein N slightly decreased until the time of hatching and thereafter increased to a somewhat higher level than originally. The distribution of N as percentage of total N seems to reach a constant value by about 21 days after hatching.

Discussion

The accumulation of muscle weight and DNA content per muscle from the 10th to the 15th day of incubation followed closely an exponential course and the coefficients of their linear regressions were 0.161 and 0.183 respectively. A comparison of both regression coefficients shows that muscle weight and DNA content increased at a rate similar to each other for this period. The increase in total N/DNA ratio, on the contrary, hardly occurred for the same period. HERRMANN *et al.*⁴⁾ suggested that the increase in number of cell nuclei in muscle may be equivalent to the production of new nucleo-cytoplasmic unit, on the basis of the consideration that it is the quantity of cytoplasm per cell nucleus which is comparable to the histologically discernible protoplasmic units of cell, since a nuclear multiplication in muscle occurs without a corresponding typical division of the cytoplasm. Furthermore, they suggested that in chick embryo the ratio of cytoplasm to nucleus in nucleo-cytoplasmic unit remained constant for a period from the 12th to the 16th day of incubation and that the increase in muscle weight for this period was due to proliferation of the unit. In agreement with the results reported by HERRMANN *et al.*, the present data that for a period from the 10th to the 15th day total N/DNA ratio remains constant and muscle weight and DNA content per muscle increase at a rate similar to each other indicate that the increase in muscle weight for this period is due to the accumulation of the nucleo-cytoplasmic unit without change in a value of cytoplasm per nucleus.

For a period from the 16th to the 21st day, no further accumulation of muscle weight occurred, although body weight continued to increase. For this period DNA content per muscle also did not increase and total N/DNA ratio showed a slight increase after the 17th day. The suppression of the accumulation of muscle weight, therefore, may be accounted for by the suppression of the increase in nucleo-cytoplasmic unit. HERRMANN *et al.*⁴⁾ and KONIGSBERG⁶⁾ indicated

that the DNA accumulation in leg muscle of chick embryo did not occur for a period from the 18th to the 29th day of incubation. It is of interest to study why the DNA accumulation of skeletal muscle of chick embryo is suppressed around the time of hatching.

Total N/DNA ratio was initiated to increase on about the 17th day, but the rate of its increase was so slow until the 24th day that a only 1.5-fold accumulation was observed to take place. For this period, a marked change occurred in the composition of total N. Fibrillar-protein N increased from 38.6% of total N on the 15th day to 51.7% on the 24th day, whereas sarcoplasmic-protein N decreased from 40.0 to 26.2%. This change is caused by the rapid accumulation of fibrillar-protein N, the increased amount which corresponds to 80% of that of total N increased for the same period. DICKERSON⁸⁾ reported that the composition of total N in pectoral muscle of chicks resembled that of an adult by 4 weeks after hatching. In this experiment, the distribution of total N reached a constant value by about 21 days after hatching.

For a period from the 21st to the 33rd day, the muscle weight increased rapidly and thereafter the rate of the weight accumulation decreased. For discussion of this rapid increase in muscle weight, the period from the 21st to the 33rd day was divided in the following three phases; the 21st to 24th day, 24th to 27th day and 27th to 33rd day. For the first period DNA content per muscle showed an about 1.7-fold increase, whereas total N/DNA ratio decreased slightly. This result suggests that the accumulation of muscle weight for this period is initiated by the increase in nucleo-cytoplasmic unit with a slight decrease in the ratio of cytoplasm to nucleus. The increase in muscle weight for the second period is caused by the increase in both nucleo-cytoplasmic unit and cytoplasm per nucleus, since DNA content and total N/DNA ratio increased about 1.8- and 2.2-fold respectively. For the third period, total N/DNA ratio showed an about 2.7-fold increase although DNA content increased only round 1.2-fold, indicating that the accumulation of muscle weight for this period was accounted for by the increase in cytoplasm per nucleus rather than in nucleo-cytoplasmic unit.

Summary

The present study was undertaken to define the increase in weight of breast muscle of chicks for a period from the 10th to 49th day of incubation in terms of the accumulation of its nucleic acid and protein components. The results were as follows:

1. For a period from the 10th to the 15th day, muscle weight and deoxyribonucleic acid (DNA) content per muscle increased at a rate similar to each other, whereas the increase in total N/DNA ratio hardly occurred.
2. After the 16th day, no further accumulation of muscle weight and DNA content occurred for a period of about 5 days. Total N/DNA ratio was initiated to increase on about the 17th day, but the rate of its increase was slow until the 24th day.
3. A marked change occurred in the composition of total N for a period from the 15th to the 24th day. Fibrillar-protein N increased from 38.6 to 51.7% of total N, whereas sarcoplasmic-protein N decreased from 40.0 to 26.2%. A distribution of N as percentage of total N reached a constant value by about 21 days after hatching.
4. After hatching the marked accumulation of muscle weight occurred for about 12 days and thereafter the rate of its accumulation decreased. DNA content per muscle also showed a considerable increase during 6 days after hatching, which diminished thereafter. Total N/DNA ratio rapidly increased between 3 and 12 days after hatching and thereafter the rate of

its increase lowered.

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ふ化前後における雛骨格筋の核酸量ならびに蛋白質成分量の変動

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雛の胸筋重量は、ふ化時から2週令にかけ著しく増加するが、その後増加の度合は、低下することが報告されている。本実験は、このような胸筋重量の速かな増加を、その核酸ならびに、蛋白質含量の変動から究明するために、実施したものである。

白色レグホーンの Embryo および雛を用い、浅胸筋について、筋重量、デオキシリボ核酸 (DNA)、蛋白質成分を、ふ卵開始後 10 日から 49 日 (28 日令) にわたり測定した。結果は次の通りである。

1) ふ卵開始後 10 日から 15 日までは、筋重量ならびに筋当りの DNA 量は、ほぼ似た速度で増加するが、DNA 当りの Total N の増加は、ほとんど起らなかった。

2) ふ卵開始後 16 日からふ化時にかけ、筋重量および筋当りの DNA 量は、ほとんど増加を示さなかった。これに対し、DNA 当りの Total N は、17 日ごろから

増加し始めたが、24 日 (3 日令) まで、その増加の度合はゆるやかであつた。

3) ふ卵開始後 15 日から 24 日にかけて、Total N の組成に、著しい量的変化が認められた。すなわち、15 日における Fibrillar-protein N は Total N の 38.6% に相当していたが、24 日には 51.7% に増加した。これに対し、Sarcoplasmic-protein N は 40.0% から 26.2% に低下した。なお、Total N の成分割合は、ふ化後 21 日ではほぼ一定の値に達した。

4) ふ化後約 12 日間、筋重量は著しい増加を示したが、その後その増加の度合は減少した。これに対し、筋当りの DNA 量は、ふ化後 6 日間のみ顕著な増加を示すにすぎなかった。DNA 当りの Total N 量は、ふ化後 3 日から著しく増加し、12 日以後増加の度合は低下した。