BRIEF NOTE

Changes in Myoglobin Content during Development and Growth of Chicken

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(Received for publication March 15, 1976)

The myoglobin concentration varies widely among the animal species and various muscular tissues with different activities, and it increases with advancing age and with exercise or activity. The increase of myoglobin concentration during growth from birth to adult has been observed in bovine muscle [5, 6]. By measurements of the myoglobin content of fetal and adult muscles, Longo et al. [7] in the sheep and Kagen and Christian [2] in the human observed the extremely low myoglobin concentration in fetal muscle and the increase of its concentration with maturation. In avian muscle, the myoglobin content of immature and embryonic skeletal muscle of ducks was markedly less than that of the adult and its content in muscle rose with age [3]. The similar results were obtained in penguin muscle [10]. Blessing and Müller [1] determined the myoglobin concentrations in different muscle tissues from the chicken with a procedure combining differential spectrometric and histometric methods, and the myoglobin content of the chicken gizzard was many times higher than in the striated muscle of breast, leg and heart of the same animal.

In the present report, the variation in

the myoglobin content of various muscle tissues of developing and growing chicken was shown with the immunochemical determination according to the different antigenic properties of myoglobin. The functional implication was also discussed on the basis of these results.

Chick embryos, and newly hatched and 4-day-old posthatched chicks were obtained from the fertile eggs of White Leghorn hens maintained at National Institute of Animal Industry, and young and adult White Leghorn chickens from the Asakawa Branch, Tokyo Metropolitan Institute of Animal Industry. All the chickens after hatching were normally fed except newly hatched and 4-day-old posthatched chicks. The muscle samples from embryonic and posthatched chicken were collected from twenty to thirty for each 8- to 12-day-old embryo, ten to twenty for each 15- to 21-day-old embryo, and two to five for each newly hatched to adult chicken. They were collected together at each developmental and growing stage. All muscle portions were dissected out from leg, breast, heart and gizzard, from which fat, fascia, tendon and blood vessels were removed. These muscles were cut into small pieces in embryo and

ground twice through a meat grinder with metal plate having 45 holes of 5 mm diameter in the young and adult chicken to be homogenized completely in the glass homogenizer with one to three times in volume of distilled water to wet weight of muscle. The homogenate was then centrifuged at 14,000 rpm for 30 minutes at approximately 2°C. The supernatant obtained was subjected to measurement of myoglobin concentration.

The determination of the myoglobin concentration was carried out by the method of the single radial immunodiffusion described in the previous report [9]. Antiserum of myoglobin was prepared in two albino rabbits (about 3 kg) injected with a total of 2.5 mg of the purified chicken myoglobin emulsified in Freund's complete adju-

vants (Difco) at intervals of two weeks. The myoglobin concentration was determined using two myoglobin fractions, a major component (MbI) and a minor component (MbII) as standard antigens, which were separated by preparative disc electrophoresis [9]. The protein concentration was determined by the method of Lowry et al. [8] using bovine serum albumin crystalline as a standard.

The myoglobin content of these muscles was determined immunologically at each stage of development and growth. In the single radial immunodiffusion the characteristic precipitin lines of MbI and MbII were formed in the leg muscle, and a single ring with MbI-specific pattern was observed in heart and gizzard muscle. Although an electrophoretic MbII-pattern was observed

Table 1. Changes in myoglobin concentration of muscle tissues during development and growth of chicken (White Leghorn) (mg %)

Age	Leg			Heart	Gizzard
Embryo	Mb I	Mb II	Mb I+Mb II	Mb I	Mb I
8 days	0	0	0	Trace	0
10	0	0	0	2.6	0
12	0	0	0	3.8	0
15	0	0	0	15.2	0
18	5.6	0	5.6	45.2	17.1
21*	7.2	0	7.2	66.4	139.0
Posthatched					
4 days	7.2	2.6	9.8	126.0	499.0
2 weeks	7.7	27.3	35.0	133.2	—**
3	22.5	36.2	58.7	129.3	1022.8
6	29.8	48.8	78.4	150.5	—
8	77.7	32.1	109.8	117.9	1556.0
9	105.4	45.4	150.8	218.9	1669.6
10	97.0	35.9	132.9	265.9	—
12	86.1	40.7	126.8	245.3	1716.8
14	52.5	58.2	110.7	287.0	1516.0
16	120.8	48.5	169.3	206.3	1550.0
18	117.9	57.4	175.3	292.0	1964.0
21	111.6	60.7	172.3	290.0	2144.0
24	159.1	56.4	215.6	278.0	2543.2
27	116.6	60.5	177.1	280.9	2640.0

Remarks.

*: Newly hatched chick.

** : Not determined.

Table 2. Changes in protein content of water extracts from muscle tissues and in percentage of myoglobin in the soluble muscle protein during development and growth of chicken (White Leghorn)

Age	Leg		Heart		Gizzard	
Embryo	Protein	Mb I + Mb II	Protein	Mb I	Protein	Mb I
	g%	%	g%	%	g%	%
8 days	3.38	0	2.72	Trace	3.14	0
10	3.42	0	2.60	0.10	3.38	0
12	3.80	0	2.30	0.17	3.76	0
15	3.24	0	2.82	0.54	4.58	0
18	4.58	0.12	3.48	1.30	4.56	0.38
21*	3.68	0.20	3.56	1.87	6.04	2.30
Posthatched						
4 days	4.66	0.21	4.76	2.65	6.12	8.15
2 weeks	4.02	0.87	3.80	3.51	—**	—
3	6.60	0.89	4.88	2.67	6.24	16.39
6	4.42	1.77	3.24	4.65	—	—
8	4.44	2.47	3.32	3.55	5.59	27.86
9	4.78	3.16	3.84	5.70	4.98	33.53
10	4.51	2.95	4.44	5.69	—	—
12	5.13	2.47	4.18	5.87	5.42	31.68
14	4.88	2.27	4.48	6.41	7.14	21.23
16	4.40	3.85	4.36	4.73	7.18	21.59
18	4.33	4.05	4.42	6.61	5.06	38.81
21	4.29	4.02	3.92	7.40	4.76	45.04
24	4.68	4.96	5.61	5.61	6.15	41.35
27	5.00	3.58	4.74	5.93	6.38	41.38

Remarks.

*: Newly hatched chick.

** : Not determined.

in both heart and gizzard, it was not measured by the immunodiffusion method in these tissues due to a considerable small amount of MbII compared with MbI.

The changes in the myoglobin content of individual muscle tissues during development and growth are shown in Table 1. These results indicate that myoglobin was already detected in the heart muscle from 8-day-old chick embryos, and that it appeared in leg and gizzard muscle at 18-day-old embryonic stage. In the heart muscle the concentration of myoglobin increased gradually with development from two to three times by 2 weeks after hatching as much as in later embryonic stage, whereas approximately five to six times increment

was observed in the leg muscle from day 18 of incubation to 2 weeks after hatching. The myoglobin concentration of the gizzard muscle, compared with leg and heart muscle, remarkably increased to 8 times at hatching and to 30 times by 4 days after hatching as much as in 18-day-old embryo. The myoglobin concentration of leg, heart and gizzard muscle was of 1 : 8 : 3 ratio in the 18-day-old embryo and of 1 : 9 : 19 ratio in the newly hatched chick, respectively. After hatching the myoglobin concentration in the heart increased approximately from 130 to 280 mg% with advancing age, and it was only twice in the mature heart muscle as much as in the immature chick (Table 1). On the other hand, in leg and gizzard

muscle nearly 18 and 5 times increase of the concentration, respectively, occurred at maturation ranging from 10 to 180 mg% in the leg muscle and from 500 to 2,500 mg% in the gizzard muscle (Table 1). The value of the myoglobin concentration of the chicken gizzard finally tested at 27 weeks was approximately 10 times higher than in the heart muscle and also 15 times higher than in the leg muscle at the same age. The proportion of the myoglobin content for these three tissues in this study was similar to that estimated from the values determined spectrometrically by Blessing and Müller [1] in the chicken dried muscle.

In the leg muscle, MbII was detected only in chicken leg muscle and it was contained in higher amount than MbI until 6 weeks after hatching, but after 8-week-old MbI became predominant over MbII (Table 1). The chemical and functional differences between MbI and MbII have not yet been known, but Weber et al. [10] reported that the oxygen affinity of MbI was distinctly higher than MbII in penguin myoglobin.

As shown in Table 2, since the protein concentration of the water extracts from the muscle tissues slightly increased during development but little during growth, the changes in the myoglobin content for the soluble protein in various muscles, expressed as a percentage of the soluble protein, were parallel to those in its content of the fresh muscle tissues (Table 1). These results suggest that there may be no correlation between the quantitative changes in myoglobin and in soluble protein in the muscle tissues during development and growth.

These findings show that the chicken gizzard composed of smooth muscle cells contains myoglobin like skeletal and heart muscle composed of striated muscle cells and that in extremely higher concentration.

It is presumed that the myoglobin in the gizzard may act as an oxygen reservoir for the powerful contraction at gastric digestion. On the other hand, the heart muscle appears to reach adult levels earlier in growth than the other two muscles tested and in the lower level than the gizzard muscle. The results of the myoglobin levels in leg and heart muscle during development and growth of the chicken were in agreement with the findings in the duck by Kagen and Linder [3] and in the heart of the chick embryo by Kagen et al. [4]. In general, the gizzard muscle which consumes temporarily a large amount of oxygen by the powerful muscular activity may require myoglobin in much higher level than the heart muscle beating continuously since early stage of development or the skeletal muscle composed of the muscles with various activities. It is an interesting finding, however, that the considerable increase of myoglobin content occurred in the gizzard muscle of the newly hatched and 4 days old chicks before oral food or water intake.

Acknowledgments: The author is most grateful to Dr. Motoo Naito, Professor of Animal Breeding at University of Tokyo, for the valuable advice and for reading the manuscript. Sincere appreciation is extended to Dr. Tsuneo Abe, Department of Animal Breeding, National Institute of Animal Industry, for his kind advice and cooperation in the preparation of antiserum and the immunological technique, and to the generous supply of fertile eggs from the stock in National Institute of Animal Industry.

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