Ultrastructure of Postembryonic Development of the Pectoral Muscles in the Japanese Lesser Horseshoe Bat, Rhinolophus cornutus cornutus from the Standpoint of Adaptation for Flight

Yokoyama, Keiichi
Zoological Laboratory, Faculty of Agriculture, Kyushu University

Uchida, Teruaki
Zoological Laboratory, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/23695

出版情報：九州大学大学院農学研究院紀要. 24 (1), pp.49-63, 1979-08. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 24 (1), 49-63 (1979)

Ultrastructure of Postembryonic Development of the Pectoral Muscles in the Japanese Lesser Horseshoe Bat, *Rhinolophus cornutus cornutus* from the Standpoint of Adaptation for Flight

Keiichi Yokoyama and Teru Aki Uchida

Zoological Laboratory, Faculty of Agriculture,
Kyushu University 46-06, Fukuoka 812

(Received May 2, 1979)

From the viewpoint of adaptation for flight in bats, this report presented electron microscopic observations on the pectoral muscles during the growth period from the late embryo to the adult stage in the Japanese Lesser horseshoe bat, *Rhinolophus cornutus cornutus*. Evidence obtained by examining the muscle fibers at different growth stages strongly indicated that their number was determined before birth, and that they remarkably increased in size to the flying stage probably because of the longitudinal splitting of the myofibrils when they attained a certain size. Furthermore, in the late embryo and newborn stages, a small number of longitudinally oriented T tubules occurred along the margin of the A-band although the predominant orientation of T tubules was transverse. In the flying stage which gave the first indication of differentiation into the adult muscle fiber types, however, almost all the T tubules ran in transverse direction near the A-I junction. Judging from the facts that the completion in orientation of the T tubules and the earliest sign of muscle-type differentiation were recognized in the flying stage, it was ultrastructurally concluded that the flying stage was the most important stage of the postnatal period *in* the bat from the standpoint of survival potential.

INTRODUCTION

It is well known that the ability of the Japanese Lesser horseshoe bat, *Rhinolophus cornutus cornutus* (Rhinolophidae) to execute quick turns and to fly at low speed through dense vegetation during foraging time is remarkable. Recently, the authors (Yokoyama *et al.*, 1975; Yokoyama and Uchida, 1979) reported the correlation between morphological changes on growth of the wings and ecological aspects in the bat from the standpoint of adaptation for flight. Moreover, the relationship between ecological information on growth and biochemical properties of the pectoral muscles with the growth in the bat also was discussed (Yokoyama *et al.*, 1979) : i.e., it was revealed that there was a remarkable increase in B subunit containing isozymes in pectoral muscle LDH during the transition period from the flapping (and eye-open) to flying stage, which was regarded as an adaptation for a highly manoeuvrable flight at the isozyme level.

Consequently, it seems very important to investigate ontogenetically how the above-mentioned biochemical adaptation of the pectoral muscles during the postnatal period is reflected in the ultrastructure of their postembryonic development. In this paper, thus, we deal with the ultrastructural changes of the pectoral muscles from the late embryo to the adult stage, focusing our attention on the fine structure of the muscles at the fiying stage which marks an important epoch in the mode of life during the postnatal period.

MATERIALS AND METHODS

From late June to early September a maternity colony, nursery colonies (including mother colony, infant colony, flapping colony and flying colony) and a young colony are formed in a cave in the vicinity of Tôno City, Iwate Prefecture in northern Japan.

The whole pectoral muscles of two embryos, four newborn young, two infants in each of the colony-forming and flying stages, and an adult pregnant female collected at the cave from June through July of 1976-7 were carefully dissected. The muscles were first fixed in 4 % paraformaldehyde with 0.05 M phosphate buffer (pH 7.4). Postfixiation was achieved with 1 % osmium tetroxide, after which the tissue was dehydrated in acetone and embedded in Epon 812. Thin sections for electron microscopy were cut with glass knives on a Porter-Blum MT-l microtome. After staining with uranyle and lead acetate, the sections were examined with a Hitachi HS-9 electron microscope.

RESULTS

Late embryo stage (about one week before birth)

The forearm length and body weight are 14.0 mm and 2. Og, respectively. At this stage the myotube vanishes already. As shown in Fig. 1, the myonuclei of the pectoral muscles in the late embryo have already migrated to the periphery of the cells and have taken their position beneath the sarcolemma with caveolae. In each of the embryo muscle fibers a few myofibrils are seen within an individual muscle fiber, and the fiber is accordingly small in diameter. The interstitial space between the muscle fibers is considerably broad.

As seen in Fig. 2, although the T tubules usually run in transverse direction near the A-I junction, a small number of longitudinally oriented T tubules also are observed at the margin of the A-band facing to the interfibrillar sarcoplasm. The cristae of mitochondria with a less dense matrix are less closely packed, and darkly stained glycogen particles are abundant in the interfibrillar sarcoplasm. The sarcoplasmic reticulum network of diverging and converging tubules forms a loosely woven lacework around the myofibril that extends throughout each sarcomere. But, differentiation of the network at the M-line does not occur at this stage.

As indicated in Fig, 1, on the other hand, the satellite cells are conspicuous, and usually rest on the surface of the muscle fibers without forming a

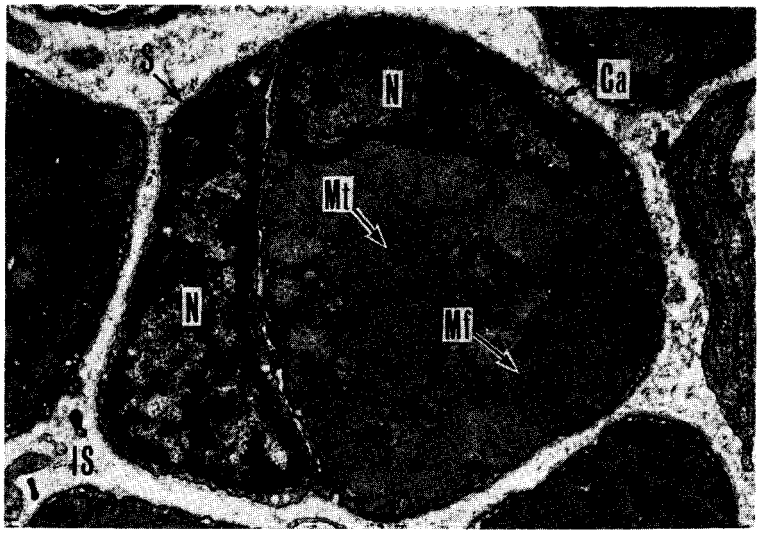


Fig. 1. Cross-section of the pectoral muscle fibers in the late embryo stage, showing a satellite cell (S) present on the surface of a muscle fiber. The fiber is small in diameter and the interstitial space (IS) between the muscle fibers is considerably broad. Ca, caveola ; Mf, myofibril ; Mt, mitochondrion ; N, nucleus. x 10,000.

convex toward them, being unique in their position since they lie between the basement membrane of the muscle fiber and the fiber plasma membrane. The interspace between the cell and the muscle fiber is narrow and the interface does not parallel each other. The cytoplasm of each satellite cell is very sparse in comparison with the nucleoplasm, of which the greater part of the condensed chromatin is disposed along the outer rim of the nucleus, some of which being also scattered throughout the central regions of the nucleus. Furthermore, the transitional cell from the undifferentiated cell to the satellite cell, which is usually enclosed by a common basement membrane of the muscle fiber, is rarely found associated with the muscle fibers. The apposed membranes of the cell and muscle fiber, however, are separated by a clear gap.

In addition, as shown in Fig. 3a, there are a large number of morphologically undifferentiated cells lying free in the interstitial space between the muscle fibers. Not uncommonly the pseudopodium-like structures of the undifferentiated cells make close membrane contact with each other. Occasionally, the undifferentiated cells are longitudinally arrayed parallel to the muscle fibers. Centriole and kinetosome are present close to the Golgi apparatus, together with numerous free ribosomes, rough endoplasmic reticula and mitochondria (Fig. 3b).

Newborn stage (at birth)

The forearm measures 19.5 mm and the body weight weighs 3. Og. As seen in Fig. 4, many undifferentiated cells are still observed in the interstitial

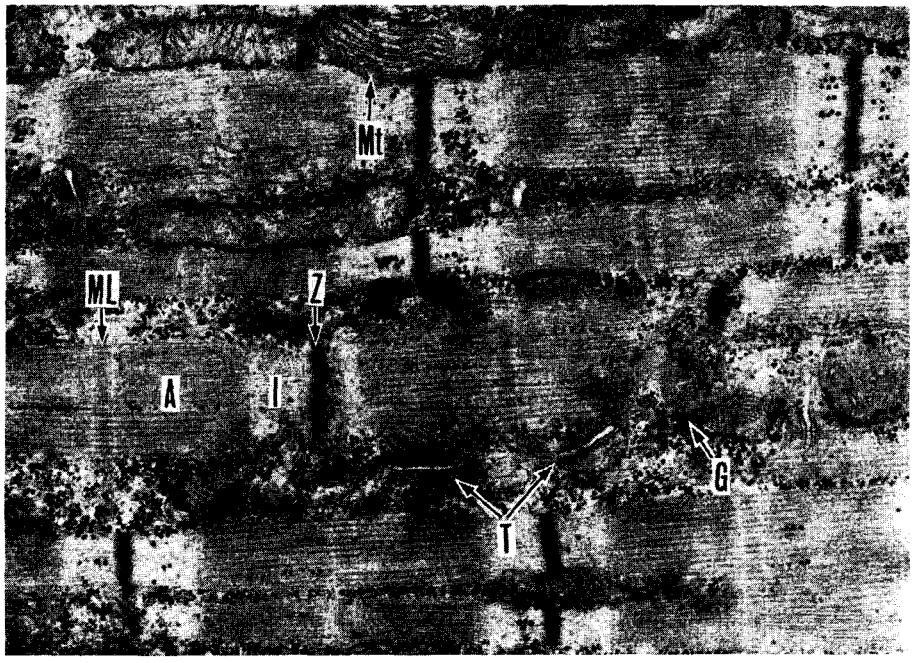


Fig. 2. Longitudinal section of the pectoral muscle fiber in the late embryo stage, showing the longitudinally oriented T tubules (T) seen at the margin of the A-band facing to the interfibrilar sarcoplasm. The cristae of mitochondria (Mt) are loosely packed in a clearly less dense matrix. A, A-band; G, glycogen particle ; I, I-band; ML, M-line; Z, Z-line. x 30,000.

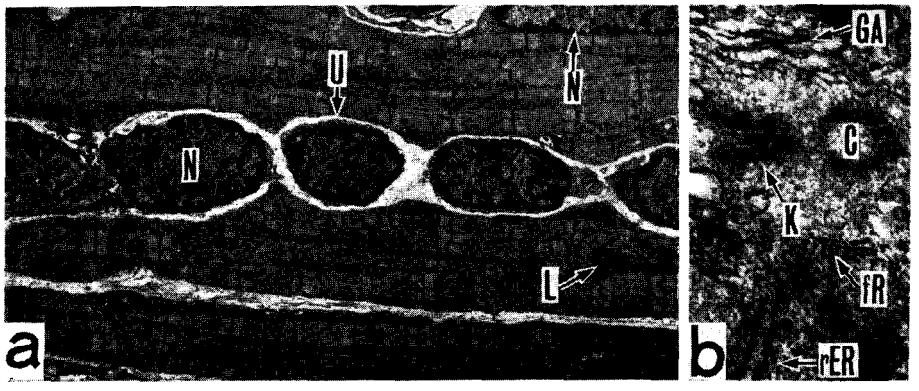
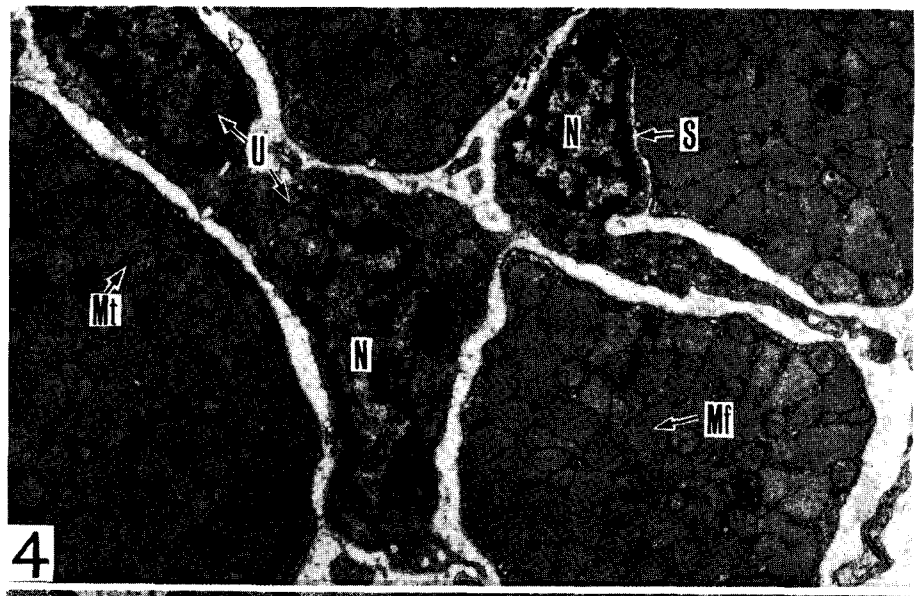


Fig. 3. Longitudinal sections of the pectoral muscle fibers in the late embryo stage, showing the undifferentiated cells lying between two neighboring muscle fibers. a) The longitudinal arrangements of the undifferentiated cells (U). L, lipid droplet; N, nucleus. ~4,000. b) The Golgi apparatus (GA), centriole (C) and kinetosome (K) in the cytoplasm of the cell. fR, free ribosome; rER, rough endoplasmic reticulum. x 40,000.



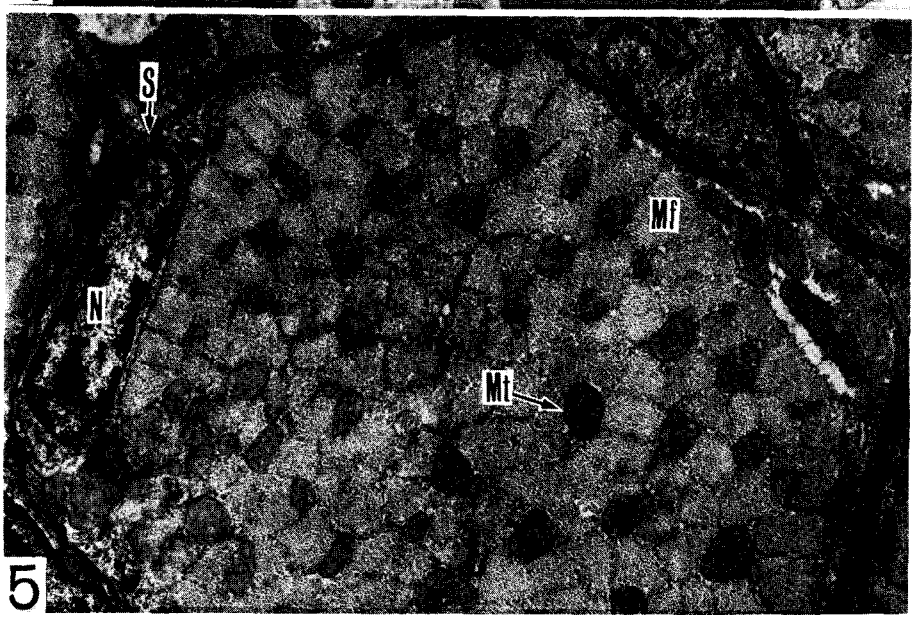


Fig. 4. Cross-section of the pectoral muscle fibers in the newborn stage showing a satellite cell. The satellite cell (S) with large pseudopodium-like structures closely applies to the surface of a muscle fiber. Mf, myofibril ; Mt, mitochondrion ; N, nucleus; U, undifferentiated cell. x 10,000.

Fig. 5. Cross-section of the pectoral muscle fibers in the colony-forming stage, showing a remarkable increase in size of the muscle fiber (cf. Fig. 4). The interstitial space between the muscle fibers cannot be observed. Abbreviations as in Fig. 4. x 10,000.

space between the muscle fibers as well as in the preceding stage. The interstitial space, however, becomes narrower and the satellite cells with large pseudopodium-like structures also are occasionally found in the space. The characteristic features of such cytoplasmic organellae of the muscle fibers at this stage as myofibrils, T tubules and mitochondria, the fiber diameter and the status of the satellite and undifferentiated cells are similar to those in the late embryo stage. The longitudinal arrangements of the undifferentiated cells, however, cannot be found in this stage.

Colony-forming stage (about 7 days after birth)

The forearm length and body weight are 20.1 mm and 4. Og, respectively. As indicated in Fig. 5, one of the most striking changes that occur during the transition period from the newborn to this stage is a growth in number of the myofibrils within an individual muscle fiber. As the result, the muscle fibers increase considerably in size during this period and the interstitial space between the muscle fibers can hardly be distinguished. Furthermore, another remarkable change that occurs during the period was a substantial increase in number of the mitochondsia with a conspicuously dense matrix. As shown in Fig. 6 at high magnification, the complicated cristae of mitochondria are disposed in longitudinal rows between myofibrils.

On the other hand, Fig. 6 shows also a myofibril that is apparently just commencing to split in two part at only one Z-line. The myofibril splitting occurs frequently in this stage. On rare occasions, however, the Z-lines are divided into three parts, A number of glycogen particles are present in the splitting portion. There is a much higher incidence of splitting in large muscle fibers, while no splitting is seen in the small muscle fibers. At this stage, although the longitudinally oriented T tubules decrease in number, a few such T tubules still occur, and the satellite cells also are frequently found (Fig. 5), although the satellite cell and muscle fiber are in close connection with each other and the interface between them becomes smooth. The undifferentiated cells also are present in the interstitial space between the muscle fibers. One can rarely find the features which show indications of the membranous fusion between both the plasma membranes of the satellite cell and muscle fiber.

Flying stage (about 20 days after birth)

The forearm measures 35.6 mm and the body weight weighs 6. Og. In this stage, as shown in Fig. 7, both of the number of myofibrils within an individual muscle fiber and the diameter of each muscle fiber increase remarkably, and the first indication of differentiation into three muscle fiber types, *I.e.*, the small (Fig. 7a), intermediate (Fig. 7b) and large (Fig. 7c) fiber types corresponding to those of the adult is discernible. The shape of myofibrils in all types is polygonal in cross section with straight sides. The myofibril size in the small and intermediate fibers is smaller than those of the large fibers. In the small fibers a number of lipid droplets are frequently observed associated with the mitochondria, while in the intermediate and large fibers the lipid droplets are absent. As seen in Fig. 8, the density of matrix and

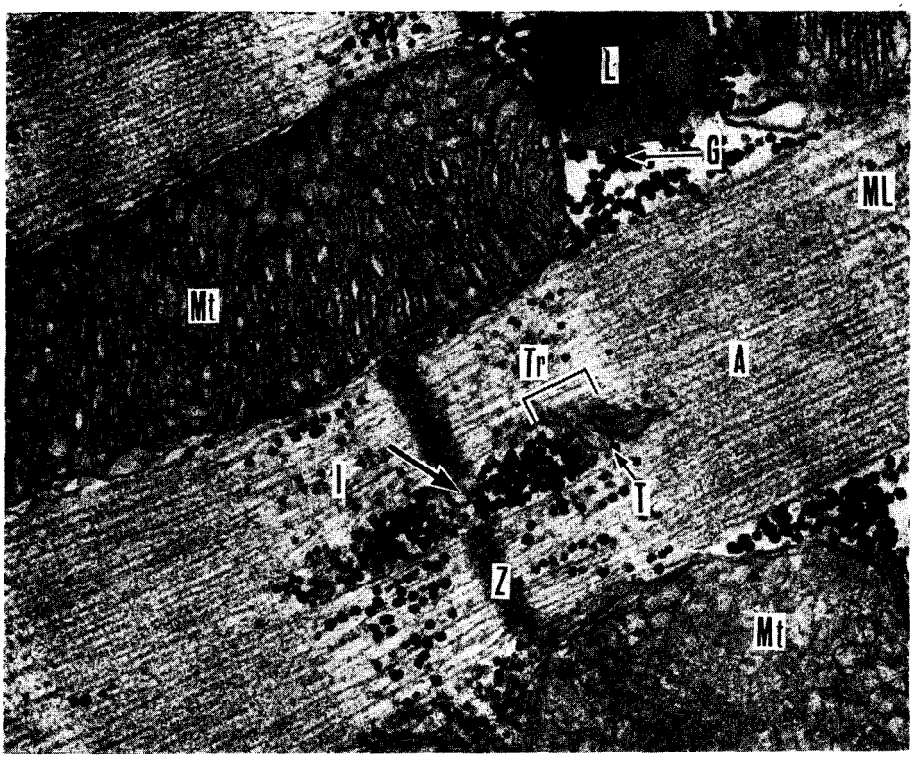


Fig. 6. Longitudinal section of the pectoral muscle fiber in the colony-forming stage, showing a myofibril splitting (an arrow) seen at the Z-line (Z). The cristae of mitochondria (Mt) with a dense matrix are much complicated in structure. A, A-band; G, glycogen particle ; I, I-band; L, lipid droplet; ML, M-line; T, T tubule; Tr. Triad. ~60,000.

morphological features of cristae in the mitochondria bear a striking resemblance to those in the preceding stage (cf. Fig. 6).

On the other hand, in this stage the longitudinally oriented T tubules are already absent and almost all the T tubules exhibit a transverse orientation near the A-I junction. The sarcoplasmic reticulum has developed into its mature form of well organized tubules, and differentiation of the network at the M-line seen in a mature muscle fiber is evident (Fig. 8 inset). The longitudinal splitting of the myofibrils is still found frequently. The number of the satellite and undifferentiated cells in developing muscles of this stage becomes fewer than those of the preceding stages.

Adult stage

The forearm length and body weight are 40.5 mm and 8.7 g, respectively. At this stage, the distinctive features of the three fiber types of which the first sign was indicated at the flying stage become established. Although the myofibrils in the small fibers (mitochondria-more rich) are polygonal in cross section (Fig. 9a) as well as in the flying stage, in the intermediate (mitochon-

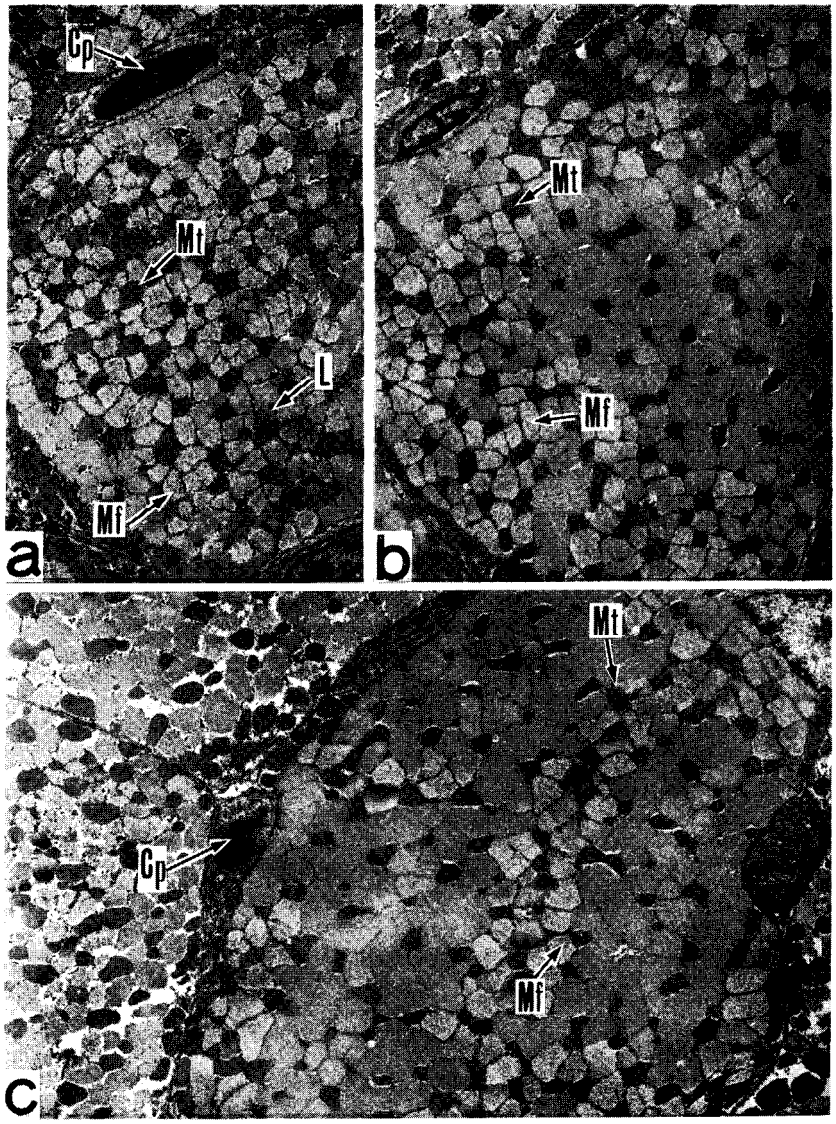


Fig. 7. Cross-sections of the pectoral muscle fibers in the flying stage. showing the first indication of differentiation into the three muscle fiber types. a) The small fiber type. b) The intermediate fiber type. c) The large fiber type. Cp, capillary; L, lipid droplet; Mf, myofibril; Mt, mitochondrion x 4,500.

dria-less rich) and large (mitochondria-moderate) fibers the myofibrils are not usually polygonal but flattened (Fig. 9b, c).

In this stage no longitudinal splitting of the myofibrils is seen. In addition, in the small fibers the large lipid droplets are often present associated

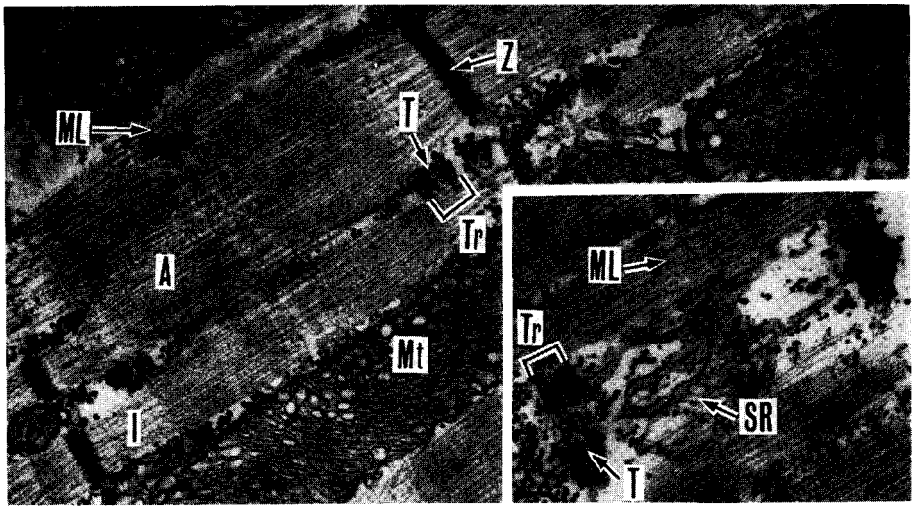


Fig. 8. Longitudinal sections of the pectoral muscle fibers in the flying stage, showing typical T tubules and sarcoplasmic reticulum. The T tubules (T) exhibit a transverse orientation near the A-I junction. Differentiation of the network of the sarcoplasmic reticulum (SR) at the M-line (ML) is visible (inset). Abbreviations as in Fig. 6. ~30,000.

with the large mitochondria as well as in the preceding stage, whereas in the intermediate fibers the lipid droplets become first recognized in this stage. The glycogen particles are abundant in the interfibrillar sarcoplasm of the small and intermediate fibers. In the large fibers, however, the lipid droplets and glycogen particles are considerably fewer and the mitochondria are very small in diameter (Fig. 9c). But, as indicated in Fig. 9d, the cristae of mitochondria become consisted of regularly parallel plates, compared with the complicated cristae in the colony-forming and flying stages.

DISCUSSION

Although considerable researches on muscle growth from various angles in Chiroptera have added to our knowledge of this aspect, few ultrastructural investigations have been made except for the report on the myogenesis of the web muscles of the fruit-bat by Church (1969). In our previous papers (Yokoyama *et al.*, 1975; Yokoyama *et al., 1979;* Yokoyama and Uchida, 1979), we reported the information on growth of *R. c. cornutus* from the standpoint of morphological, biochemical and ecological aspects, and indicated that the flying stage of the bat was the most important stage in the mode of life during the postnatal period. In this study, we will discuss here the ultrastructural changes with growth of the pectoral muscles in the bat from the late embryo to adult stage, and especially accumulate attention on the flying stage at which the first sign of differentiation into the three muscle fiber types seen in the adult stage occurs.

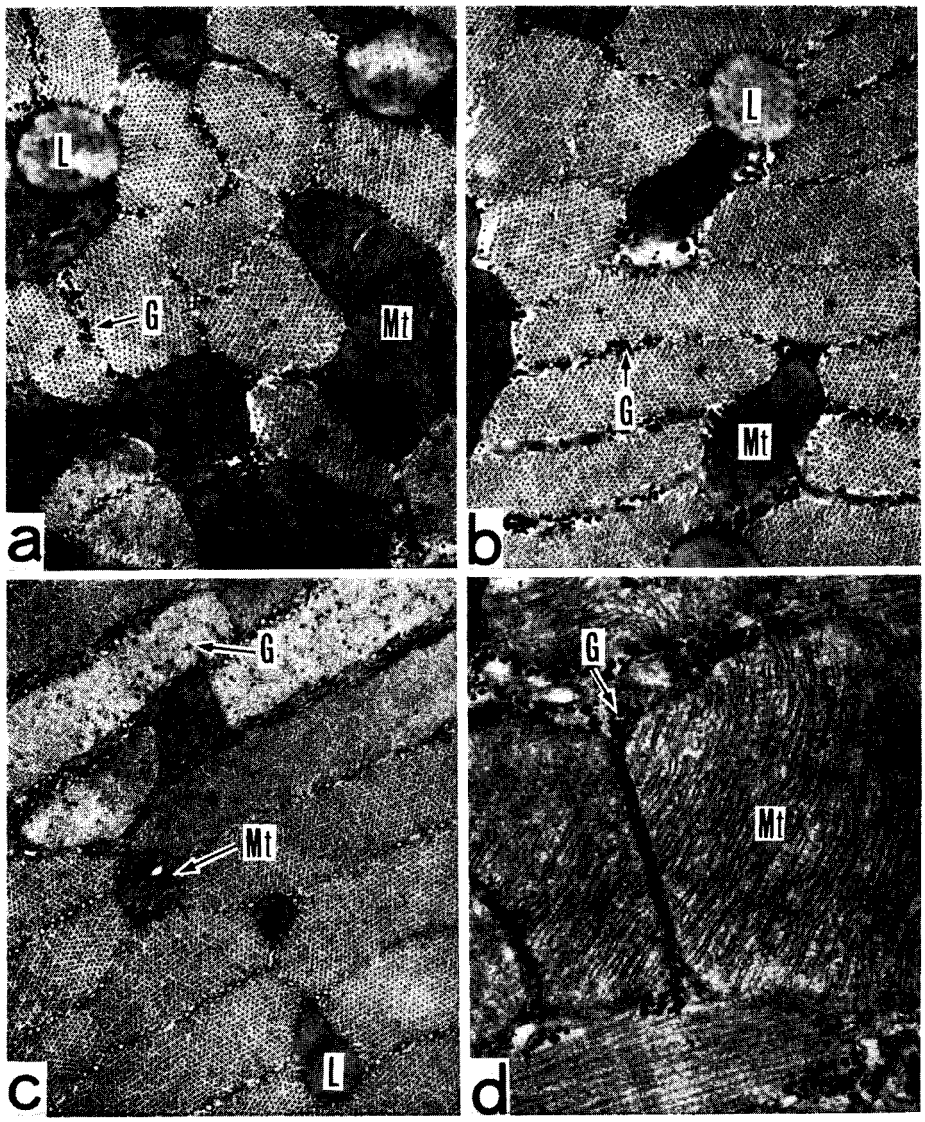


Fig. 9. Sections of the pectoral muscle fibers in the adult stage, showing the three muscle fiber types (cross-sections) and typical mitochondria (a longitudinal section). a) The myofibrils of mitochondria-more rich fiber. b) The myofibrils of mitochondria-less rich fiber. c) The myofibrils of mitochondria-moderate fiber. x 20,000. d) The mitochondria (Mt) with closely packed lamellar cristae. Abbreviations as in Fig. 6. x 40,000.

1. Ultrastructural changes in the pectoral muscle fibers during growth period

Muscle *fiber size and fate of the satellite cell*

In the late embryo and newborn stage of the bat, the pectoral muscle

tissues mainly consisted of immature muscle fibers accompanied with the satellite cells. Although the myofibrils in the immature muscle fibers were small in number, they were quite compact and myonuclei migrated to the subsarcolemmal position; even at the late embryo stage the myotubes could not be observed. Therefore, it *is* reasonable that the number of the pectoral muscle fibers is fixed before birth, and consequently does not increase during the postnatal growth. Furthermore, it is well known that the muscle fibers increase in size considerably during the growth period. The reasons are due to the increase in the amount of myofibrillar material within an individual muscle fiber. With respect to this, it is of interest to note that the longitudinal splitting of myofibrils is frequently observed at the Z-lines during the period from the late embryo to flying stage. Recently, it has been suggested that in the hind limb muscle of the mouse embryo (Platzer, 1978) and infant mouse (Goldspink, 1972), the increase in muscle fiber size is due to the proliferation of myofibrils, which is the result of longitudinal splitting in the myofibrils when they attain a certain size.

As to the status of satellite cells, in the colony-forming stage we could find indications of membranous fusion between both the plasma membranes of the satellite cell and muscle fiber. After that, in the flying stage, the number of the satellite cells became fewer than those of the preceding stages. Thus, it seems that the decrease in number of the satellite cells is due to the above-mentioned membranous fusion by a process of which the cytoplasms of the satellite cell and muscle fiber are confluent.

In this connection, the fate of the satellite cells in growing muscles has been investigated by several workers. In the rat (Enesco and Puddy, 1964; Moss and Leblond, 1971) and domestic fowl (Moss, 1968) during the postnatal life of the animals, the myonuclei considerably increased in number as development proceeds, whereas the satellite cells diminished gradually in number. Similar phenomena have been known also in the skeletal muscles of the human fetus (Ishikawa, 1966) and the mouse fetus (Schultz, 1976). Particularly, Moss and Leblond (1971) reported autoradiographically that after a mitotic division, one or both satellite-daughter cells of the rat might fuse with muscle fiber and thereby added to the myonuclear population. Furthermore, using the electron microscope, Schultz (1976) demonstrated a satellite cell which appeared to be in the process of fusion with a muscle fiber in the mouse.

On the other hand, recent and detailed morphological studies (Schultz, 1976 etc.) on the satellite cells of adult mice have supported earlier suggestions (Mauro, 1961; Ishikawa, 1966; Church, 1969) that the satellite cells represent dormant myoblasts.

Differentiation of the muscle fiber types and change in orientation of the T tubules

We indicated that in the pectoral muscles of the bat an early evidence of differentiation into the three muscle fiber types corresponding to the small (mitochondria-more rich), intermediate (mitochondria-less rich) and large (mitochondria-moderate) fibers of the adult appeared in the flying stage.

It is well known that adult skeletal muscles are heterogeneous, being

composed of different fiber types, of which at least three are recognized as red, intermediate and white fibers with the aid of histochemical methods (Edgerton and Simpson, 1969). Furthermore, on the bases of morphological and biochemical properties, Ishikawa (1975) stated that red muscle containing a large number of mitochondria was composed predominantly of smaller dark fibers with high capacity for oxidative metabolism, while white muscle containing a small number of mitochondria consisted principally of larger pale fibers with high glycolytic enzyme activities.

It is very interesting to determine when the first indication of characteristic differentiation into the three muscle fiber types appears during the growth period. From this point of view, the histochemical studies of developing muscle have been carried out by many workers. According to recent papers, it has become clear that there is a striking difference in maturation of skeletal muscles in various mammals. Namely, in the rat and mouse (Dubowitz, 1965), pig (Cooper et ***al.,*** 1970), cat (Cody and Richardson, 1976a, b) and rabbit (Lobley et ***al.,*** 1977), the differentiation of skeletal muscle fiber types could not be discerned at birth. Especially, in the pig (Cooper et *al.*, 1970) when the infants are weaned from their mothers (28-35 days after birth), and in the rabbit (Lobley et ***al.,*** 1977) by the time the infants have opened their eyes and are beginning to show increased mobility (8-12 days after birth), the adult pattern of fiber types is established. In contrast to these, even in the fetus of rhesus monkey (Beatty et ***al.,*** 1967), in the newborn young of the guinea pig (Dubowitz, 1965) and human (Fenichel, 1966), the skeletal muscles have already shown full differentiation into adult fiber types. With respect to this, Dubowitz (1965) suggested that there was some correlation between the presence of differentiation in skeletal muscles at birth and general maturity and mobility of animals, and also the length of gestation.

As shown clearly in this study and other works mentioned above, in mammals with no differentiation of muscle fiber types at birth, it is worthy of note that the first sign of differentiation into the adult fiber types appears in such developmental epochs as flying (bats), eye-open (rabbit) and weaning (pig) stage during the postnatal growth period.

As to the orientation of T tubules, from our electron microscopical study on the pectoral muscles of the bat, it became obvious that although the predominant orientation of T tubules in the late embryo and newborn stage was transverse near the A-I junction, a small number of longitudinally oriented T tubules also were present at the margin of the A-band. In the flying stage, however, few longitudinally oriented T tubules were found and the orientation of almost all the T tubules was transverse as seen in the adult stage.

The morphogenesis of the sarcoplasmic reticulum and triads in rat skeletal muscle has been examined in detail by Walker and Schrodt (1968), Schiaffino and Margreth (1969) and Edge (1970). Especially, from the observations on the skeletal muscle fibers from the fetal and newborn to 35-day rats, Edge (1970) showed that the predominantly longitudinally oriented triads of immature muscle fibers changed orientation, and that the change from predominantly longitudinally to transversely oriented triads was complete in fibers from

10- to 15-day rats. In this connection, Chaplin et al. (1970) indicated, using the rat skeletal muscles, that the time between the beginning of the action potential and the beginning of the recorded development tention (excitation-contraction latency) declined from 6 msec to 2.8 msec during the first 10–15 days after birth. From the above facts, as stated by Edge (1970), it is presumed that the change in triad orientation might be related to the observed decrease in E-C latency. Taking the above into consideration, it seems likely that the completion in orientation of the T tubules in the flying stage of the bat is regarded as an adaptation *to* contractile mechanism of the flight muscles.

2. **Relationship among ecological aspects, changes in morphology of the wings, and in biochemistry and ultrastructure of the pectoral muscles with growth**

It is well known that *R. c. cornutus* having a relatively low wing loading (Kuramoto, 1972) and a predominance of anodal LDH isozymes of the pectoral muscles (Kitahara *et al., 1974;* Yokoyama *et al.,* 1979) is capable of doing highly manoeuvrable flight with low speed, like a butterfly. In a previous paper (Yokoyama *et al.,* 1975), we pointed out, on an analysis of the aspect ratio in five growth stages from the newborn to self-supporting (adult size) stage of the bat, that the characteristic of the short-broad wing-type was gradually completed with the lapse of time. Furthermore, it became clear that the bat was able to fly in a short period (about 22 days) after birth owing to the low wing loading at the flying stage (Yokoyama and Uchida, 1979), although the wings in the stage did not yet attain to its adult size and synostosis did not occur in the wing bones (Yokoyama *et al.,* 1975). It seemed that the above-mentioned morphological features of the wings at the flying stage apply to many members of the short-broad wing-type bats (Yokoyama and Uchida, 1979). Recently, Yokoyama *et al.* (1979) reported that in the pectoral muscles of *R. c. cornutus* there was a reversal of activity toward more anodal side of the spectrum (cardiac muscle type) during the transition period from the flapping to flying stage, which was considered as an adaptation to metabolic requirement at isozyme level of the pectoral muscles for an adult flying ability of a slow and delicate flight in the species.

Besides such ecological, morphological and biochemical changes during the postnatal period, another factor such as ultrastructural change in the flight muscles must have an important bearing on the abilities of young bats to fly. With respect to this, this ultrastructural research on the pectoral muscles of the bat from the late embryo to the adult stage revealed that the change from longitudinally to transversely oriented T tubules was almost completed in the flying stage which gave the first indication of differentiation into the three muscle fiber types in the adult, too. Accordingly, it is supposed that the above-mentioned phenomena seen in the pectoral muscles during the postnatal period are regarded as an adaptation to the contractile and metabolic requirements at ultrastructural level of the pectoral muscles for an adult flight.

As stated above, when the bat infants reached the flying stage at about *22* days of age, the area of wing membranes became adequate for their limit-

ed flight, and the LDH isozyme pattern of the pectoral muscles showed the cardiac muscle type as well as in the adult, and the earliest sign of muscle-type differentiation was recognized. It is thus concluded, from the viewpoint of survival potential, that the flying stage marks an important epoch in the mode of life during the postnatal period of the bat not only ecologically, morphologically and biochemically but also ultrastructurally, as a precursor stage of the self-supporting stage when the bat establishes the flying ability and species specific Right mode, etc.

ACKNOWLEDGEMENT

The authors are much indebted to Mr. T. Mōri of the Zoological Laboratory, Faculty of Agriculture, Kyushu University for his technical advices. Our gratitude is expressed to Associate Professor S. Shiraishi and graduate students of the same Laboratory for their encouragement, and to Professor E. W. Jameson, Jr, of the University of California for comments on the manuScript.

REFERENCES

Beatty. C. H., G. M. Basinger and R. M. Bocek 1967 Differentiation of red and white fibers in muscle from fetal, neonatal and infant rhesus monkeys. ***J. Histochem. Cytochem., 15: 93-103***

Chaplin, E. R., G. W. Nell and S. M. Walker 1970 Excitation-contraction latencies in postnatal rat skeletal muscle fibers. *Exp. Neurol.*, ***29: 142-151***

Church, J. C. T. 1969 Satellite cells and myogenesis; a study in the fruit-bat web. *J. Anat.*, 105 : 419-438

Cody, F. W. and H. C. Richardson 1976a Application of histological techniques to developing and adult jaw muscles. *J. Physiol.*, ***Lond.. 257: 4***

Cody, F. W. and H. C. Richardson 1976b Development of histochemical profiles in cat jaw muscles. J. ***Physiol., Lond., 258: 83-84***

Cooper, C. C., R. G. Cassens, L. L. Kastenschmidt and E. J. Briskey 1970 Histochemical characterization of muscle differentiation. ***Devl.*** *Biol.*, ***23: 169-184***

Dubowitz, V. 1965 Enzyme histochemistry of skeletal muscle-1 and II. *J. Neurol. Neurosurg. Psychiat., 28 : 516-524*

Edge, M. B. 1970 Development of apposed sarcoplasmic reticulum at the T system and sarcolemma and the change in orientation of triads in rat skeletal muscle. *Devl. Biol.*, ***23 : 634-650***

Edgerton. V. R. and D. R. Simpson 1969 The intermediate muscle fiber of rats and guinea pigs. ***J. Histochem. Cytochem.. 17: 828-838***

Enesco, M. and D. Puddy 1964 Increase in the number of nuclei and weight in skeletal muscle of rats of various ages. Am. *J. Anat.*, 114: 235-244

Fenichel, G. M. 1966 A histochemical study of developing human skeletal muscle. Neurology, **16 :** 741-745

Goldspink, G. 1972 Postembryonic growth and differentiation of striated muscle. *In* "The Structure and Function of Muscle," Vol. I, Structure Part 1, ed. by G. H. Bourne. Academic Press, New York and London, pp. 179-236

Ishikawa, H. 1966 Electoron microscopic observations of satellite cells with special refer-

ence *to* the development of mammalian skeletal muscles. *Z. Anat. Entwickl.-Gesch.*, ***125: 43-63***

Ishikawa, H. 1975 Skeletal muscle. I. Structure. ***Metabo. Dis., 12: 1‘243-1338*** (in Japanese)

Kitahara, H., T. A. Uchida and ***F.*** Hamajima 1974 LDH isozymes from the standpoint of adaptation for flight in Chiroptera. *Zool. Mag.*, ***Tokyo, 83: IO-17*** (in Japanese with English abstract)

Kuramoto, T. 1972 Studies on bats at the Akiyoshi-dai Plateau, with special reference to the ecological and phylogenic aspects. ***Bull. Akiyoshi-dai Sci.*** *Mus.*, ***(8)*** :7–119 (in Japanese with English abstract)

Lobley. G. E., A. B. Wilson and A. S. Bruce 1977 An estimation of the fiber type composition of eleven skeletal muscles from New Zealand White rnhhits between weaning and early maturity. *J. Anat.*, 123: 501-513

Mauro, A. 1961 Satellite cell of skeletal muscle fihers. *J. Biophys. Biochem. Cytol., 9:* 493–***495***

Moss, F. P. 1968 The relationships between the dimensions of the fibers and the number of nuclei during normal growth of skeletal muscle in the domestic fowl. ***Am. J. Anat., 122: 555-563***

Moss, F. P. and C. P. Leblond 1971 Satellite cells as the source of nuclei in muscles of growing rats. *Anat.* ***Rec., 170: 421-436***

Platzer, A. C. 1978 The ultrastructure of normal myogenesis in the limb of the mouse. *Anat.* ***Rec.,*** 190: 639-658

Schiaffino, S. and A. M. Margreth 1969 Coordinated development of the sarcoplasmic reticulum and T system during postnatal differentiation of rat skeletal muscle. *J.* Cell *Biol.*, ***41: 855-875***

Schultz, E. 1976 Fine structure of satellite cells in growing skeletal muscle. ***Am. J.*** *Anat.*, ***147: 49-70***

Walker, S. M. and G. R. Schrodt 1968 Triads in skeletal muscle fibers of 19–day fetal rats. ***J. Cell Biol., 37: 564-569***

Yokoyama, K. and T. A. Ucida 1979 Functional morphology of wings from the standpoint of adaptation for flight in Chiroptera. II. Growth and changes in mode of life during the young period in ***Rhinolophus*** *cornutus cornutus.* ***J.*** *Fac. Agr.*, ***Kyushu Univ., 23:*** 185-198

Yokoyama, K., R. Ohtsu and T. A. Uchida 1979 Growth and LDH isozyme patterns in the pectoral and cardiac muscles of the Japanese Lesser horseshoe bat, ***Rhinolophus*** *cornutus cornutus* from the standpoint of adaptation for flight. *J. Zool., Lond.*, ***187: 85-96***

Yokoyama. K., T. A. Uchida and S. Shiraishi 1975 Functional morphology of wings from the standpoint of adaptation for flight in Chiroptera. I. Relative growth and ossification in forelimb, wing loading and aspect ratio. *Zool. Mag., Tokyo,* ***84: 233-247*** (in Japanese with English abstract)