Influence of Interposition of Pink Muscle Fiber into Dorsal Ordinary Muscle on Increasing Rate of K-value in Carp (Cultured)

Osamu YADA, * Mutsuhide TSUCHIMOTO, * Mutsuyosi TSUCHIMOTO, * Qin WANG, * Paula Andrea Gomez APABLAZA, * Abdul JABARSYAH, * and Katsuyasu TACHIBANA*

In order to clarify the influence of the interposition of pink muscle fiber into the dorsal ordinary muscle on the post-mortem temporal change of K-value, using carp (cultured) *Cyprinus carpio*, the dorsal muscle was divided into five muscle parts toward depth with the naked eye, as follows: the dark muscle part (P-1), the intermediate muscle part (P-2), and three ordinary muscle parts (P-3, P-4, P-5). The muscle fiber types in these parts were discriminated by the inactivation of actomyosin ATPase activity for both acid and alkaline preincubations, and the temporal changes of ATP related compounds in these parts were then measured at a kept temperature of 32°C. Five muscle parts were organized from the muscle fiber types as follows: P-1 was organized from only red muscle fiber type, P-2 from only pink muscle fiber type in a thin layer and from two muscle fiber types of not only pink muscle fiber, but also white muscle fiber of the IIa or IIb subtype in a region of mosaic pattern, and all of P-3, P-4, and P-5 were from two muscle fiber types of white muscle fiber (IIa or IIb subtype) and pink muscle fiber, respectively. The temporal changes of K-value were remarkably faster in order of the dark muscle part of P-1, the intermediate muscle part of P-2, three ordinary muscle parts of P-3, P-4, and P-5, although its change did not exhibit a remarkable difference among three ordinary muscle parts. On the other hand, the temporal change of K-value among muscle fiber types was considerably different and remarkably faster in the order of: red muscle fiber, pink muscle fiber, and white muscle fiber. From these results, it was considered that the interposition of pink muscle fiber into the dorsal ordinary muscle might accelerate the temporal change of K-value.

Key words：Muscle fiber type, Pink muscle fiber, White muscle fiber, K-value, ATP related compounds, Carp (cultured)

We have already reported that the temporal changes of K-values (a freshness index) in various fish species, collected from tropical, subtropical, and temperate water fishing grounds ranging from 28°C to 9°C in bottom temperature, were attributed to the differences in habitat water temperatures of the respective fishing grounds.[1,2] However, in spite of fishes being from the same fishing ground at a habitat water temperature, there was also considerable variance among the fish species. As for this difference, Tomioka and Endo have reported that it was due to differences of IMP degrading activity in ordinary muscles of various fishes.[3] We also reported that the increasing rate of K-value at the kept temperature of 32°C was significantly related to IMP degrading activity in ordinary muscles of various fishes including fishes from the tropical, subtropical, and temperate waters at different habitat temperatures.[4] Furthermore, we reported that IMP degrading activity was different between two groups of carp (cultured) adapted at 10°C and 30°C, and this influenced the temporal change of K-value.[5] However, the reason that the IMP degrading activity is different among fish species in spite of living in the same habitat water temperature, has not been fully elucidated.

On the other hand, it is generally known that the dorsal skeletal muscle in fish is arranged in three distinct layers of dark muscle, intermediate muscle, and ordinary muscle, and these muscles are composed of red muscle fibers, pink muscle fibers, and white muscle fibers, respectively.[6] Therefore, most histochemical studies in fish muscle have been involved in these distinct layers,[7−12] and only a few studies have dealt with the difference of muscle fiber types in ordinary muscle among various fishes. Very recently, in our serial studies on muscle fiber types of dorsal ordinary muscle in fish, we have reported that the dorsal ordinary muscle in many fish species is composed of not only white muscle fibers, but also pink muscle fibers being in a mosaic pattern, and carp (cultured) is one such species.[13] Furthermore, regarding the muscle fiber types among parts toward depth of dorsal ordinary muscle in carp (cultured),

* Laboratory of Fishery Nutritional Sciences, Graduate School of Marine Science and Engineering, Nagasaki University, Bunkyo-machi, Nagasaki 852−8521, Japan

we reported that the ordinary muscle part near a layer of intermediate muscle was organized from many pink muscle fibers and IIa and IIb like subtypes of white muscle.[13] The pink muscle fibers, however, were decreasing gradually as the position of ordinary muscle becomes deeper, and then the pink muscle fiber became unrecognizable in the deepest position.[13]

In regard to the relationship between each of three muscle fiber types (red, pink, and white muscle fiber types) and the temporal change of K-value, we reported that the temporal change of K-value in carp was remarkably faster in red muscle fiber and, to the contrary, it was distinctly slower in white muscle fiber than in pink muscle fiber.[14] Furthermore, it is known that IMP degrading activity in dark muscle is higher than that in ordinary muscle.[15, 16] These findings suggest that IMP degrading activity may be different among muscle fiber types of red, pink, and white muscle fibers, and then the interposition of pink muscle fiber into dorsal ordinary muscle might influence the temporal change of K-value.

So, we have carried out serial studies to clarify the cause of the differences in the temporal change of K-value among fish species living in the same habitat temperature. As a part of these studies, in the present report, the influence of the interposition of pink muscle fiber into dorsal ordinary muscle on the temporal change of K-value was examined.

Experimental Methods

Sample Fish

The carp (cultured) Cyprinus carpio was used as the sample fish species, because it was already known that the dorsal ordinary muscle of this fish species is organized partially from three muscle fiber types; two white muscle fibers (IIa and IIb subtypes) and pink muscle fiber.[13] So, we selected the objective carps, which interposed partially pink muscle fibers in deepest ordinary muscle. The body size of sample fish is shown in Table 1. The body weight was within the range from 0.8kg to 1.0kg. The sample fish were purchased from a fish farmer in Nagasaki prefecture and were carried back to the laboratory alive. They were held in a tank at the same habitat water temperatures of 14°C for at least 24h to allow them to recover from fatigue.

Table1. The body size and habitat temperature of sample fish in carp (cultured)

Code number of fish specimen	Standard body length (cm)	Body weight (g)	Habitat temperature (℃)
C-1	28.8	902.1	14
C-2	30.2	851.2	
C-3	31.0	1000.1	
C-4	30.2	876.8	
C-5	28.2	801.4	

The sample fish of C-1-C-5 were used for the experiments of five muscle parts toword depth of dorsal muscle and three muscle fiber types of red, pink and whtie muscles, respectively.

They were then killed by cutting the hindbrain after being anesthetized in fresh water containing MS-222 (100 ppm) and immediately, two muscle blocks of dorsal muscle with scales and skin were cut off from both sides of each sample fish body. From one muscle block, muscles nearby the lateral line at two positions under the first dorsal fin ray and the last dorsal fin lay were arranged in a pillar form of about 1 cm × 1 cm in a horizontal section and the maximum cm in depth. In the carps with the code number C-1 - C-5, these were then divided into continuous five parts with the naked eye, as follows: dark muscle part (P-1) of dark red color, intermediate muscle part (P-2) of light pink color, and three ordinary muscle parts toward depth (P-3, P-4 and P-5) of relative white color, respectively (Fig. 1). Another muscle block between two positions under the first dorsal fin ray and the last dorsal fin ray was kept in water at a constant temperature of 32°C, put in a polyethylene bag and was offered for the measurement of ATP related compounds with the lapse of kept time.

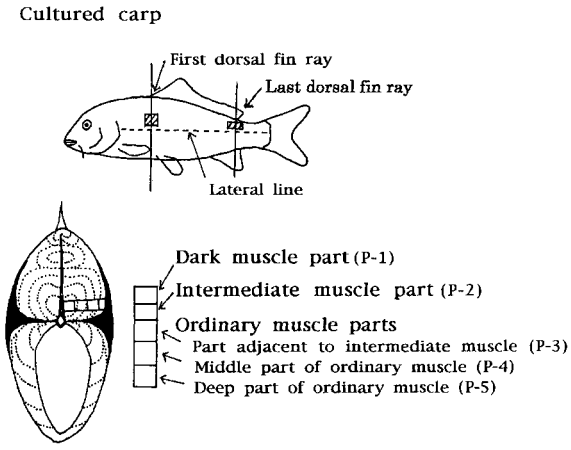

Fig. 1. The illustration of muscle parts used for the discrimination of muscle fiber types and the measurement of ATP related compounds in carp (cultured).
The sample fish of code number C-1 - C-5 (Table 1) were used for experiments of five muscle parts toward depth and three muscle fiber types of red, pink, and white muscles, respectively.

Discrimination of Muscle Fiber Types

The tissues were mounted on a cryostat chuck, turning the cross section upward and embedded in a commercial mounting medium. The mounted tissues were frozen immediately for 10 - 15 sec by immersion in isopentane, which was cooled to its melting point (-165°C) with liquid nitrogen. The frozen tissues were placed in a refrigerated cryostat cabinet at -25°C for 1 h. Then the serial cross sections were cut at a thickness of about 10 μm and mounted on glass cover slips, and dried at room temperature.

The preincubation procedure of cross sections was carried out according to the method modified by Guth and Samaha[17], which is commonly used in mammalian studies connected with the

discrimination of muscle fiber types in skeletal muscle. A series of seven cross sections was preincubated at pH 4.36 and 15°C for 0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 min in an acid preincubation solution of 0.1 M potassium acetate buffer containing 18 mM $CaCl_2$. Another series of cross sections was preincubated at pH 10.50 and 20°C for 0, 5, 10, 15, 20, 25, and 30 min in an alkaline preincubation solution of 0.1 M 2-amino-2-methyl-1-propanol (AMPro) buffer containing 18 mM $CaCl_2$. Beforehand, the optimum combination of pH and time at acid and alkaline preincubation was examined to discriminate the different muscle fiber types. After preincubation at an acid or alkaline condition, the preincubated sections were rinsed with 0.1 M AMPro buffer (pH 9.4) containing 18 mM $CaCl_2$ and 50 mM KCl.

The previous two series of rinsed cross sections were reacted at 20°C for 20 min in a reaction medium of 0.1 M AMPro buffer (pH 9.40) containing 18 mM $CaCl_2$, 50 mM KCl, and 3.1 mM ATP, according to the method described by Guth and Samaha.[17] The reacted sections were rinsed with distilled water and immersed in 1% $CaCl_2$ for 3 min, then immersed in 2% $CoCl_2$ for 3 min. Finally, the production of phosphate from ATP under the remaining activity of actomyosin ATPase was displaced cobaltous sulfide by 1% $(NH_4)_2S_x$.

The cross section of intermediate muscle part (P-2) was also confirmed by PAS (periodic acid-Schiff) stain[18] because the glycogen content in pink muscle fiber was higher than that in white muscle fiber.

These cross sections after preparations of acid and alkaline stabilities and PAS stain were offered for the discrimination of muscle fiber types under the microscope.

Measurement of ATP Related Compounds

For the carp coded C-1 - C-5, the muscles of continuous five parts toward depth, which were dark muscle part (P-1), intermediate muscle part (P-2), and three ordinary muscle parts (P-3, P-4 and P-5) of the same parts as the discrimination of muscle fiber types (Fig. 1), were cut at intervals of 2 h from a large muscle block kept in water at a constant temperature of 32°C. Moreover, the kept temperature at 32°C was chosen as the same temperature in our previous studies,[1,2,5] regarding the temporal change in K-value. Of the muscle cut off from each part, 1.0 g of flesh was taken and extracted by 10% perchloric acid according to the method of Ehira *et al*.,[19]

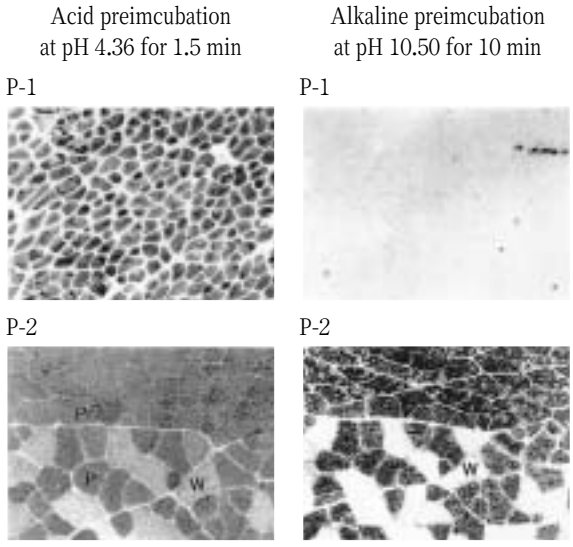

Fig. 2. The comparison of muscle fiber types between P-1 of dark muscle part and P-2 of intermediate muscle part in muscles nearby the lateral line at a region under the first dorsal fin ray of carp (cultured) for an example of code number C-1.
The photographs of cross sections express the inactivation of actomyosin ATPase activity after acid or alkaline preincubation, and the depth of color then expresses their stability. The alphabets of R, P, and W express the red, pink, and white muscle fiber types, respectively.

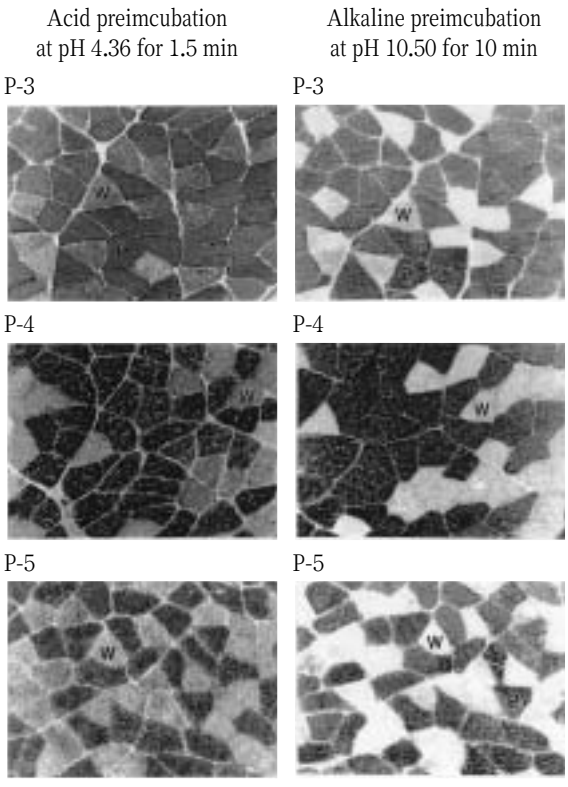

Fig. 3. The comparison of muscle fiber types among three dorsal ordinary muscle parts toward depth from P-3, P-4, and P-5 in muscles nearby the lateral line at a region under the first dorsal fin ray of carp (cultured) for an example of code number C-1.
The photographs of cross sections express the inactivation of actomyosin ATPase activity after acid and alkaline preincubation, and the depth of color then expresses their stability. The alphabets of P and W express the same muscle fiber types as shown in Fig. 2.

and the amounts of ATP related compounds were analyzed using the method of high-performance liquid chromatography by Tsuchimoto *et al.*[20]

Results

Comparison of Muscle Fiber Types among Parts toward Depth of Dorsal Muscle

Regarding the muscles nearby the lateral line in the region under the first dorsal fin ray, the muscle fiber types in the dark muscle part, the intermediate muscle part, and the three ordinary muscle parts toward depth are shown as an example of the code C-1 number in Fig. 2 and 3, respectively.

The muscle fibers in the dark muscle part (P-1) were relatively high in acid stability of actomyosin ATPase activity for acid preincubation for 1.5 min at pH 4.36, while they were very low in alkaline stability for 10 min at pH 10.50. Therefore, the fibers were organized from only the red muscle fiber type (Fig. 2). Furthermore, the size of the fibers was remarkably small in comparison with that of the other parts.

The intermediate muscle part (P-2) in acid preincubation, at the same conditions of time and pH as the case of the foregoing dark muscle part, was divided into two regions. One was a layer having high acid stability of actomyosin ATPase activity and the other was a mosaic pattern with two different acid stabilities (Fig. 2). That in alkaline preincubation, at the same conditions of time and pH as the case of dark muscle part, was also divided into two regions of a layer and a mosaic pattern, and the aspect in alkaline stability agreed with that in acid stability. One muscle fiber, which maintained the activity for both acid and alkaline preincubation, was estimated to be the pink muscle fiber type, and other muscle fibers which lost the activity for both acid and alkaline preincubation, were equivalent to the white muscle fiber type of Ⅰa or Ⅱb subtype in mammalia.[13] Furthermore, the result of the intermediate muscle part (P-2) by PAS stain is shown in Fig. 4.

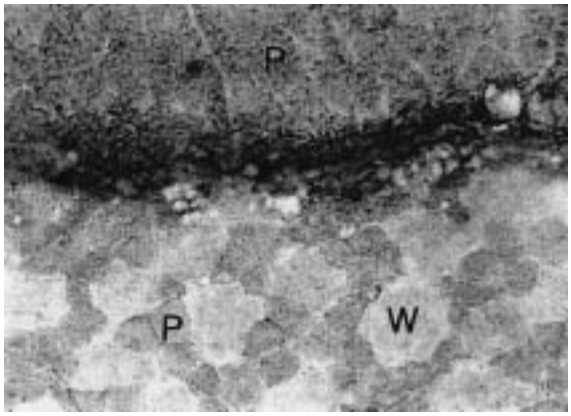

Fig. 4. The photograph of the cross section by PAS stain in P-2 of intermediate muscle part nearby lateral line at a region under first dorsal fin ray of carp (cultured) for an example of code number C-1.
The depth of color expresses the relative glycogen content and the alphabets of P and W express the same muscle fiber types as shown in Fig. 2.

One muscle fiber, which strongly maintained the actomyosin ATPase activity for both acid and alkaline preincubation in both a layer and mosaic pattern, was stained strongly a purple color and was confirmed to have a higher glycogen content than the other muscle fibers which lost the activity for both acid and alkaline preincubation. Therefore, the muscle fibers in this layer were organized from only the pink muscle fiber type. The muscle fibers in the mosaic pattern were organized from two muscle fiber types of pink muscle fiber and white muscle fiber of Ⅰa or Ⅱb subtype. Namely, the intermediate muscle part was organized from not only a very thin layer of only the pink muscle fiber type, but also a mosaic pattern of two muscle fiber types of pink muscle fiber and white muscle fiber of Ⅰa or Ⅱb subtype. In other words, the intermediate muscle part (P-2), which was cut off by us, was arranged in not only a layer of intermediate muscle but also a part of ordinary muscle, which interposed many pink muscle fibers in a mosaic pattern. Therefore, a layer of only pink muscle fibers was very hard to cut off by its pink color with the naked eye.

In a part (P-3) of ordinary muscle close to the intermediate muscle part, the mosaic pattern with two muscle fiber types was recognized for both acid and alkaline preincubation at the same conditions of time and pH as the cases of the foregoing muscle parts, and these mosaic patterns for acid and alkaline agreed with each other. Therefore, the muscle fibers in the ordinary muscle part of P-3 were organized from two muscle fiber types of white muscle fiber of Ⅰa or Ⅱb subtype and pink muscle fiber, and the area percentage of pink muscle fibers was similar to that of a region in a mosaic pattern of the intermediate muscle part (P-2). These aspects were similarly recognized in the other ordinary muscle parts of P-4 and P-5 (Fig. 3).

The organizations of muscle fiber types in five muscle parts were very similar not only between the two positions under the first dorsal fin ray and the last dorsal fin ray in the same specimen, but also among five sample fish specimens. Namely, the muscle fiber types in dorsal ordinary muscle were organized from two muscle fiber types of white muscle fiber of Ⅰa or Ⅱb subtype and pink muscle fiber, and were slightly different among three ordinary muscle parts toward depth.

Comparison of ATP Related Compounds among Muscle Parts toward Depth of Dorsal Muscle

The changes of ATP, ATP+ADP+AMP, IMP, and Hx+HxR mean levels in five dorsal muscle parts toward depth with the lapse of kept time at 32°C, are shown in Fig. 5 and 6, respectively. The ATP level just after killing was significantly lower ($p<0.001$ by *F*-test) in the dark muscle part (P-1) than in the other four muscle parts, and a lower tendency was exhibited in the intermediate muscle

part (P-2) than in the other three ordinary muscle parts, although it was not remarkably different among the three ordinary muscle parts (Fig. 5). The temporal change of ATP level was decreasing straight in all muscle parts until it was disappearing, and the decreasing speed was also very similar among the five muscle parts. The disappearance of ATP level was early at near 4 h in the dark muscle part (P-1), while it was late at near 6 h in the other four muscle parts. From these results, it was thought that the disagreement of disappearance time was due to the difference of ATP content just after killing among the five muscle parts.

In comparison of ATP+ADP+AMP level among the five muscle parts (Fig. 5), all of the level just after killing, aspects of temporal change, decreasing speed, and disappearance time exhibited very similar tendencies to these results of the foregoing ATP level, although the level just after killing and disappearance time was higher and later in ATP+ADP+AMP than in ATP, respectively. From the ATP and ATP+ADP+AMP results, the degrading speed of each of ATP, ADP, and AMP did not appear to be remarkably different among the five muscle parts.

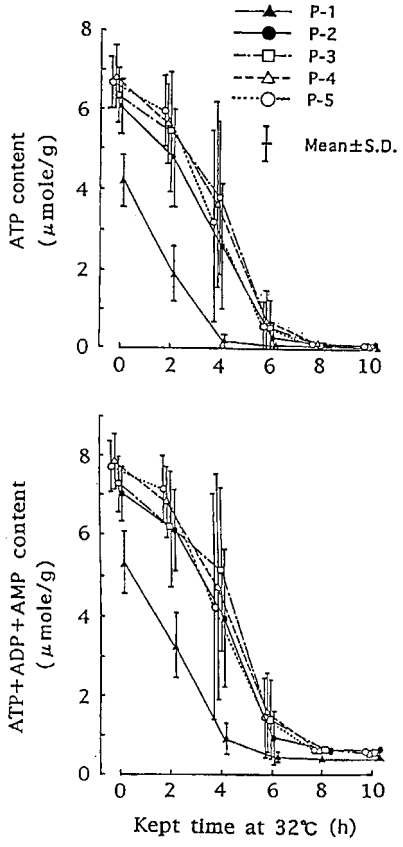

Fig. 5. The changes of ATP and ATP+ADP+AMP mean levels in each of five muscle parts from P-1- P-5 with the lapse of time at kept temperature of 32°C in carps (cultured) of code number C-1 - C-5.
The symbols from P-1 - P-5 express five muscle parts toward depth of dark muscle part, intermediate muscle part, and three ordinary muscle parts, respectively.

The IMP level just after killing was very low in all five-muscle parts and did not exhibit any remarkable difference among the five muscle parts (Fig. 6). The temporal change was increasing in concert with the decrease in ATP+ADP+AMP level and after it reached a peak level, was decreasing contrary in all of the five muscle parts. Namely, the temporal change of IMP level exhibited a mountainous pattern in all five-muscle parts. The peak level, however, was different among the five muscle parts and was lower in the order of dark muscle part (P-1), intermediate muscle part (P-2), and the three ordinary muscle parts (P-3, P-4, and P-5) although it did not exhibit any remarkable difference among the three ordinary muscle parts. Furthermore, the time in which a peak level was reached was remarkably faster in the dark muscle part than in the other four muscle parts, in agreement with the disappearance time of the foregoing ATP+ADP+AMP level.

The Hx+HxR level just after killing was also very low in all five-muscle parts and there was no difference among the five muscle parts (Fig. 6). The temporal change was increasing until the disappearance of the IMP level in all five-muscle parts. The increasing speed was remarkably faster in the dark muscle part (P-1) than in the other four muscle parts, although it exhibited no

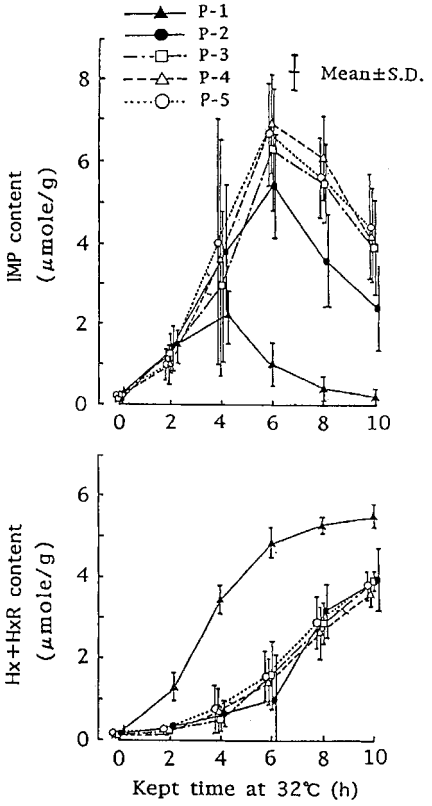

Fig. 6. The changes of IMP and Hx+HxR mean levels in each of five muscle parts from P-1- P-5 with the lapse of time at kept temperature of 32°C in carps (cultured) of code number C-1 - C-5.
The symbols from P-1 - P-5 express the same muscle parts as shown in Fig. 5.

difference not only between the intermediate muscle part (P-2) and the three ordinary muscle parts (P-3, P-4, and P-5) but also among the three ordinary muscle parts.

The K-value (%) just after killing was also very low in all five-muscle parts and exhibited no difference among five muscle parts (Fig. 7). The temporal change was increasing in a similar aspect to that of the Hx+HxR level. The increasing speed, however, was faster in the dark muscle part (P-1) and intermediate muscle part (P-2) than in the other ordinary muscle parts (P-3, P-4, and P-5), although it did not exhibit a remarkable difference among three ordinary muscle parts.

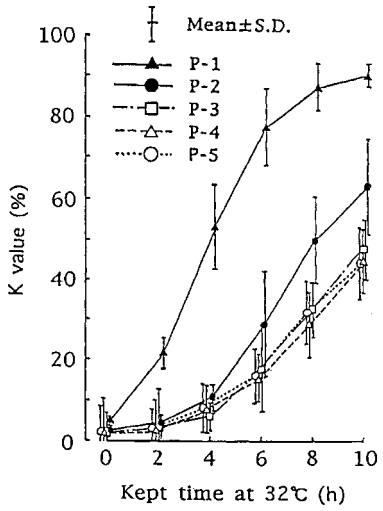

Fig. 7. The change of K-value in each of five muscle parts from P-1 -P-5 with the lapse of time at kept temperature of 32 ℃ in carps (cultured) of code number C-1 - C-5.
The symbols from P-1 - P-5 express the same muscle parts as shown in Fig. 5.

This result of the intermediate muscle part differed from the foregoing result of the Hx+HxR level. Regarding this disagreement, it was thought that this might be caused by the difference of ATP content just after killing among the muscle parts. These results suggest that the degrading speed of IMP might be remarkably faster in the dark muscle part than in the other muscle parts.
From this result, it was suggested that the interposition of pink muscle fiber into dorsal ordinary muscle might considerably accelerate the post-mortem temporal change of K-value.

Discussion

It was already known that the dorsal ordinary muscle of carp (cultured) was organized partially from three muscle fiber types of two white muscle fibers (IIa and IIb subtypes) and pink muscle fiber, and the pink muscle fibers were decreasing gradually as the position of ordinary muscle becomes deeper, and then the pink muscle fibers became unrecognizable in the deepest position.[13] The objective carps, was organized from two muscle fiber types of white muscle fiber (I a or II b subtype) and pink muscle fiber in muscle parts toward depth of dorsal ordinary muscle. The pink muscle fiber was interposed in a mosaic pattern among the white muscle fibers from the ordinary muscle part close to the layer of intermediate muscle until the deeper part of ordinary muscle (Fig. 2 and 3). Regarding the difference of muscle fiber types among the sample fish specimens in spite of the same fish species, Jabarsyah *et al.*[13] have already reported that the muscle fiber types of dorsal ordinary muscle in cultured red sea bream were considerably different among fish specimens, while these in wild red sea bream were the same types among specimens. The causes of these differences, however, are still unknown.

In conclusion, the degrading speed of each of ATP, ADP, and AMP was not considerably different among the five muscle parts toward depth of dorsal muscle (Fig. 5). This result differed from the finding of Johnston *et al.*[21, 22] that myofibrillar Mg^{2+}-ATPase activity was significantly different in the ratio 1: 2: 4 among red, pink, and white muscles. Regarding this point, it was considered that the cause of this disagreement might be due to the difference between enzyme activity and the degrading speed in muscle by influences of ATP content, glycogen content, Ca^{2+}-concentration around myofibril, lactate dehydrogenase activity, and the other. The degrading speed of IMP, however, was remarkably faster in the dark muscle part of P-1 and the intermediate muscle part of P-2 than in three ordinary muscle parts of P-3, P-4, and P-5 although it did not exhibit a remarkable difference among three ordinary muscle parts (Fig. 6). Furthermore, the increasing speed of K-value also exhibited the same aspects as the degrading speed of IMP.

In previous study,[14] we selected the objective carps, which were composed partially of only white muscle fiber types in ordinary muscle, and compared the temporal change of ATP related compounds among the red, pink, and white muscle fibers. As the results, we found that the temporal change of K-value was remarkably faster in order of red muscle fiber, pink muscle fiber, and white muscle fiber. Regarding this point, it was already reported that the IMP degrading activity in dark muscle was higher than that in ordinary muscle.[15,16] From these findings, it was considered that the IMP degrading activity might be higher in the order of red muscle, pink muscle, and white muscle, and the interposition of pink muscle fiber into the deepest ordinary muscle might considerably accelerate the post-mortem temporal change of K-value.

References

1) Tsuchimoto M., Misima T., Utsugi T., Kitajima S., Yada S., Senta T., and Yasuda M.: The speed of lowering in freshness of fishes in several waters and the effect of the habitat temperature on the speed. *Bull. Japan. Soc. Sci. Fish.*, **52**, 1431-1441 (1986).
2) Tsuchimoto M., Misima T., Utsugi T., Kitajima S., Yada S.,

Senta T., and Yasuda M: Resolution characteristics of ATP related compounds in fishes from several waters and the effect of habitat temperatures on the characters. *Nippon Suisan Gakkaishi*, **54**, 683-689 (1988).

3) Tomioka K. and Endo K.: K value-increasing rates and IMP-degrading activities in various fish muscles. *Bull. Japan. Soc. Sci. Fish.*, **50**, 889-892 (1984). : in Japanese with English abstract

4) Tsuchimoto M.: The degradation of freshness in fish, in "Food science and management of fisheries under competition in Japan" (ed. By Nagasaki University), Ministry of Finance Printing Bureau, Tokyo, 1989, pp. 24-75. : in Japanese.

5) Tsuchimoto M., Tanaka N., Uesugi Y., Misima T., Tachibana K., Yada S., Senta T., and Yasuda M.: The influence of rearing water temperature on the relative thermostability of myofibrillar Ca^{2+}-ATPase and on the lowering speed of freshness in carp. *Nippon Suisan Gakkaishi*, **54**, 117-122 (1988).

6) Johnston I. A.: Structure and function of fish muscles. *Symp. Zool. Soc. Lond.*, **48**, 71-113 (1981).

7) Johnston I. A., Patterson S., Ward P., and Goldspink G.: The histochemical demonstration of myofibrillar adenosine triphosphatase activity in fish muscle. *Can. J. Zool.*, **52**, 871-877 (1974).

8) Hoyle J., Gill H. S., and Weatherley A. H.: Histochemical characterization of myotomal muscle in the grass pickerel, *Esox americanus vermiculatus* (LeSeuer), and the muskellunge, *E. masquinongy* (Mitchell). *J. Fish Biol.*, **28**, 393-401 (1986).

9) Patterson S., Johnston I. A., and Goldspink G.: A histochemical study of the lateral muscles of five teleost species. *J. Fish Biol.*, **7**, 159-166 (1975).

10) Korneliussen H., Dahl H. A., and Paulsen J. E.: Histochemical definition of muscle fibre types in the trunk musculature of a teleost fish (cod, *Gadus morhua*, L.). *Histochemistry*, **55**, 1-16 (1978).

11) Gill H. S., Weatherley A. H., and Bhesania T.: Histochemical characterization of myotomal muscle in the bluntnose minnow, *Pimephales notatus* Rafinesque. *J. Fish Biol.*, **21**, 205-214 (1982).

12) Walesby N. J. and Johnston I. A.: Fibre types in the locomotory muscles of an Antarctic teleost, *notothenia rossii*: A histochemical ultrastructural and biochemical study. *Cell Tissue Res.*, **208**, 143-164 (1980).

13) Jabarsyah A., Tsuchimoto M., Kozuru Y., Misima T., Yada O., and Tachibana K.: Discrimination of muscle fiber types in ordinary muscle by actomyosin ATPase activity and comparison of that among various fishes and regions. *Fisheries Sci.*, **65**, 292-300 (1999).

14) Yada O., Tsuchimoto M., Wang Q., Apabraza P. A. G., Jabarsyah A., and Tachibana K.: Differences of muscle fiber type and temporal change of K-value among parts toward depth of dorsal muscle in carp (cultured). *Fisheries Sci.* 2000; **66**, 147-152.

15) Obatake A., Doi T. and Ono T.: Post-mortem degradation of inosinic acid and related enzyme activity in the dark muscle of fish. *Nippon Suisan Gakkaishi*, **54**, 283-288 (1988). : in Japanese with English abstract

16) Jafri A. K. and Mustafa S.: Phosphatases & 5'-nucleotidase in the dark & white muscles of two freshwater cat-fishes *Clarias magur* (Linn.) & *Heteropneustes fossilis* (Bloch). *Indian J. Experi. Biol.*, **14**, 292-294 (1976).

17) Guth L. and Samaha F. J.: Procedure for the histochemical demonstration of actomyosin ATPase. *Exp. Neurology*, **28**, 365-367 (1970).

18) Kgeyana K. and Watanabe Y.: Staining for polysaccharide, in "Manual of histologic techniques", 5th edition, Igaku Shoin, Tokyo, 1978, pp.121-129. : in Japanese.

19) Ehira S., Uchiyama H., Uda F., and Matsumiya H.: A rapid method for determination of the acid-soluble nucleotides in fish muscle by concave gradient elution. *Bull. Japan. Soc. Sci. Fish.*, **36**, 491-496 (1970).

20) Tsuchimoto M., Misima T., Utsugi T., Kitajima S., Yada S., and Yasuda M.: Method of quantitative analysis of ATP related compounds on the rough sea-Method of high-performance liquid chromatography using reversed-phase column. *Bull. Japan. Soc. Sci. Fish.*, **51**, 1363-1369 (1985). : in Japanese with English abstract

21) Johnston I. A., Davison W., and Goldspink G.: Energy metabolism of carp swimming muscles. *J. Comp. Physiol.*, **114**, 203-216 (1977).

22) Johnston I. A., Frearson N., and Goldspink G.: Myofibrillar ATPase activities of red and white myotomal muscles of marine fish. *Experientia*, **28**, 713-714 (1972).

コイ背部普通筋へのピンク筋の介在がK値上昇速度へ及ぼす影響

矢田　修，槌本六秀，槌本六良，王　勤，
パウラ アンドレア ゴメス アパブラザ，アブドウル ジャバルシャ，橘　勝康

コイを用い，背部普通筋へのピンク筋の介在が，死後のＫ値変化に及ぼす影響を明らかにしようとした。深さ方向の筋タイプの構成は，血合筋部が赤筋のみ，中間筋部がピンク筋のみ，普通筋部が白筋（サブタイプIIa，或いはIIb）とピンク筋からなっていた。K値変化は，血合筋，中間筋，普通筋の順位で速く，ピンク筋が介在した普通筋の深さ方向の３部位では，K値変化に顕著な差は認められなかった。筋タイプの違いによるＫ値変化は，赤筋，ピンク筋，白筋の順位で速かったことから，背部普通筋へのピンク筋の介在はＫ値変化を速めるものと考えられた。