METABOLISM OF L-HISTIDINE, CARNOSINE, AND ANSERINE IN SKIPJACK TUNA[1]

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The effects of some physiological conditions on the levels of L-histidine, carnosine, and anserine were examined in skipjack tuna, *Katsuwonus pelamis*. Also examined were the turnover rates of L-[U-^{14}C]histidine in skipjack blood and skeletal muscle, the metabolic interrelationship among several organs, and interconversion between histidine and the dipeptides. In white muscle of control tuna, these compounds in total occurred at a mean ± SD concentration of 147.4 ± 3.5 μmol/g muscle (n = 5) (histidine, 93.5 ± 9.2; carnosine, 2.90 ± 1.66; anserine, 51.1 ± 10.2 μmol/g muscle), but in red muscle the total concentration was only 21.3 ± 2.1 μmol/g muscle. Exhaustive burst swimming did not lead to any large changes in these concentrations, whereas starvation markedly affected them. In white muscle, histidine decreased to 30% and 4% of control levels after 5 and 12 days of starvation, respectively; carnosine increased 3.7- and 8.6-fold, while anserine decreased in the initial stage but recovered to control levels during sustained starvation. A similar pattern also was observed in red muscle. The metabolic half-life of ^{14}C-labeled histidine was 0.72 ± 0.124 h (n = 3) in skipjack blood, and 66 and 52.5 h in white and red muscle, respectively. The label was incorporated into muscle carnosine and anserine, though the incorporation was fairly slow. The label was transported rapidly from blood into liver and muscles and also from white muscle into red.

INTRODUCTION

Tuna are considered to be the "ultimate teleosts" (Hochachka 1980) in terms of their ability to reach high burst-swimming speeds (Waters and Fierstine 1964; Dizon, Brill, and Yuen 1978) and maintain muscle temperatures as much as 10 degrees C above ambient temperature (Stevens, Lam, and Kendall 1974). The tuna's ability to engage in burst swimming is sustained by elevated capacities for anaerobic glycolysis in the white muscle, typically represented by the activity of lactate dehydrogenase, which in tuna is the highest so far found in any animal (Guppy and Hochachka 1978). During burst swimming, large amounts of lactate, nearly 100 μmol/g muscle, are accumulated as an end product in white muscle (Guppy, Hulbert, and Hochachka 1979), accompanied by proton production (Hochachka and Mommsen 1983), which may have harmful effects. Not surprisingly, buffering capacity is typically high in tuna compared to other fishes (Castellini and Somero 1981).

Intracellular buffering capacity in vertebrate muscles is dominated by the imidazole group of L-histidine, which occurs (1) as histidine residues on protein, (2) as free L-histidine, and (3) as histidine-containing dipeptides, such as carnosine (β-alanyl-L-histidine), anserine (β-alanyl-N$^{\pi}$-methyl-L-histidine), and balenine or ophidine (β-alanyl-3-methyl-L-histidine). This free L-histidine and related dipeptides are widely distributed in vertebrate muscle (Crush 1970) at levels roughly proportional to anaerobic metabolic capacity (Hochachka and Somero 1984). Generally, most terrestrial mammals and amphibians contain large amounts of carnosine (2–20 μmol/g muscle). Aves and some fishes, in contrast, have higher levels of anserine than of carnosine in their muscle. Large amounts of balenine

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are found only in the muscle of snakes and whales (Crush 1970). One of these dipeptides, anserine, was reported to be synthesized from histidine via N^{π}-methyl-L-histidine (Harms and Winnick 1954) or via carnosine (McManus 1962). Among the scombrioids, species such as tuna and marlin are known to have large quantities of these compounds in their white muscle (Lukton and Olcott 1958; Suyama and Yoshizawa 1973; Abe 1981, 1983). A recently discovered clue to their physiological roles comes from studies of marlin white muscle, which contains 125 μmol/g muscle of histidine and related dipeptides (mainly anserine); this pool accounts for ~60% of total buffering capacity, whereas in trout white muscle a smaller pool (20 μmol/g muscle) constitutes only 25% of total buffering capacity (Abe et al. 1985). This indicates that at least one of the important physiological functions of these compounds is intracellular buffering of protons that are produced during burst swimming, though other possible roles have been proposed; for example, when copper complexed, they may serve as oxygen transporters for cytochrome oxidase (Brown 1981) or as direct activators for some enzymes (Parker and Ring 1970).

Despite this interesting background on these compounds, there is literally no information on the metabolism of histidine and related dipeptides in tuna or marlin. For this reason, we decided to investigate the turnover patterns of L-histidine in tuna muscle and blood, the interrelationship of histidine metabolism and exchange in different tissues and organs, and the potential interconversions between histidine and related dipeptides.

MATERIAL AND METHODS

Live skipjack tuna, *Katsuwonus pelamis* (wt 1,130–2,130 g), and kawakawa, *Euthynnus affinis* (wt 750–1,180 g), captured on hook and line were obtained from local fishermen at Kewalo Basin, Honolulu, Hawaii. They were kept in large holding tanks supplied with aerated seawater (25 C) at Kewalo Research Facility of the National Marine Fisheries Service's Honolulu Laboratory. Kawakawa used in these studies were well fed on frozen smelt daily. Unless noted otherwise, skipjack tuna were not fed and were used within 5 days of capture. Control tuna were hooked at sea on a commercial fishing vessel and had muscle samples excised immediately after capture. Exhausted fish were those sampled after burst swimming in the holding tank.

L-[U-^{14}C]histidine (348 mCi/mmol; radioactive purity 95.5%) was purchased from Amersham Corporation, Oakville, Ontario. It was dried under N_2 and brought to 40 μCi/ml with physiological saline containing 40 μmol/ml L-histidine. Other reagents were obtained from Sigma Chemical Company, St. Louis, Mo.

CANNULATION

Skipjack tuna were anesthetized with tricaine methane-sulfonate (1:15,000) and maintained by perfusing aerated seawater (22–24 C) containing the anesthetic (1:30,000) over the gills. A cannula was implanted into the ventral aorta just anterior to the heart, a procedure taking approximately 5–10 min. Cannulated fish were kept lightly anesthetized during experimentation (3–6 h). Anesthetic and surgical procedures were as described by Jones, Brill, and Mense (1985).

ADMINISTRATION OF THE TRACER

The cannulated tuna received 30 μCi of L-[U-^{14}C]histidine via the cannula, followed by several volumes of saline wash. Serial blood samples (0.5 ml each) were withdrawn at appropriate time intervals for assessing the decay in specific activity of histidine.

A second group of fish received 30 μCi of labeled histidine intramuscularly 1 day after capture and were kept in a holding tank. At appropriate time intervals muscle samples were excised from freshly killed fish.

EXTRACTION OF L-HISTIDINE AND RELATED COMPOUNDS

Blood samples were mixed with an equal volume of 10% trichloroacetic acid and centrifuged. The supernatant was kept at −20 C and used for the determination of total radioactivity and for the fractionation of histidine-related compounds by high-performance liquid chromatography (HPLC).

Muscle samples (1 g) were homogenized in 5 ml of 1% picric acid with a Polytron homogenizer (Kinematica, Zurich, Switzerland). The homogenate was centrifuged, and the pellet was rehomogenized in 5 ml of the acid. Both supernatants were combined and applied to a column (0.5 × 1.5 cm) of Dowex 2 × 8 (200–400 mesh, Cl^- form). The eluate and washings obtained with 0.01 N HCl were freeze-dried and brought to a volume of 1 ml with distilled water.

DETERMINATION OF L-HISTIDINE AND RELATED COMPOUNDS

L-Histidine and its related compounds were determined by HPLC essentially as described previously (Abe 1981). A Spectra Physics SP-8000B liquid chromatograph equipped with Zipax SCX column (0.21 × 50 cm; Shimadzu, Japan) was used. Elution was carried out under isocratic conditions for 10 min using 12 mM KH_2PO_4 at 35 C and a flow rate of 1 ml/min followed by a gradient up to 30 mM. UV detection was used at 210 nm with 0.04 absorbance units at full scale.

For the determination of specific radioactivity, the concentrated extract was injected into the HPLC column and a 2-ml fraction of the eluate was collected. The L-histidine, carnosine, and anserine fractions were evaporated to dryness in vacuo and dissolved into HPLC-grade water.

DETERMINATION OF RADIOACTIVITY

A Beckman LS 9000 liquid scintillation counter was used (1) on the blood and tissue extracts (10–500 μl) to determine total radioactivity and (2) on combined fractions (1 ml) obtained from the HPLC column to determine specific radioactivity of histidine, carnosine, and anserine fractions. As a scintillant, 10 ml of ACS II (Amersham, Oakville, Ontario) was used with plastic scintillation vials obtained from Canlab, Vancouver, B.C. Counting efficiency was >90%, and the background fell between 30 and 50 dpm.

In all cases statistical analyses were performed using Student's *t*-test—or the Cochran-Cox test when the variances were different.

RESULTS

EFFECT OF PHYSIOLOGICAL CONDITIONS ON L-HISTIDINE AND DIPEPTIDES IN TUNA

Control tuna showed extremely large amounts of L-histidine and anserine and lesser amounts of carnosine in their white muscle (table 1). Although some of these values closely coincided with those previously reported for different populations (Abe 1983), anserine content was much higher in the Hawaiian samples. The concentrations of histidine compounds in red muscle (table 1) were lower than those in white muscle, the total content being only one-seventh that observed in white muscle (table 1). This is a typical distribution pattern of these compounds in fish muscle that contains them in large amounts (Abe 1983; Abe et al. 1985). Red muscle, in addition, contained small but detectable amounts of N^{π}-methyl-L-histidine, whereas white muscle did not. Individual differences in the concentration of each compound were rather large, but the combined total pool size in white muscle was similar among individuals. This was also the case with red muscle (table 1).

In white muscle of exhausted fish, anserine concentration was not different from that in controls. Histidine, on the other hand, increased significantly ($P < .05$). However, we found no significant changes in these compounds in red muscle following burst swimming. The increase of total histidine-related compounds, equivalent to ~20 μmol/g muscle in white muscle ($P < .001$) was due entirely to histidine increases.

Starvation of skipjack tuna also resulted in changes in the concentrations of these compounds (table 2). Although we found rather large individual differences in each compound, on average, histidine levels in white muscle decreased significantly ($P < .01$) from those in controls. Carnosine, on the other hand, showed a marked increase ($P < .01$), whereas anserine showed no large changes. Tunas 7 and 8, which were starved for 5 and 12 days after capture, respectively, showed an extremely large decrease in histidine and an increase in carnosine. Anserine concentrations were practically identical to those in the controls.

TABLE 1

CONCENTRATION (μmol/g muscle) OF L-HISTIDINE AND RELATED COMPOUNDS IN WHITE AND RED MUSCLE OF SKIPJACK TUNA

	WHITE MUSCLE				RED MUSCLE				
GROUP AND NO.	His	Car	Ans	Total	His	N^{π}-meHis	Car	Ans	Total
Control Fish:									
1	99.5	.672	49.2	149.4	14.0	.212	.212	7.36	21.8
2	84.2	5.00	52.7	141.9	12.6	.232	.917	7.66	21.4
3	98.5	4.50	46.1	149.1	13.3	.050	.883	6.90	21.1
4	104.2	2.74	38.2	145.1	11.7	.227	.512	5.32	17.8
5	81.0	1.57	69.1	151.7	14.7	.184	.256	9.29	24.4
Mean ± SD	93.5 ± 9.2	2.90 ± 1.66	51.1 ± 10.2	147.4 ± 3.5	13.3 ± 1.1	.181 ± .068	.556 ± .299	7.31 ± 1.28	21.3 ± 2.1
Exhausted Fish:									
1	111.8	12.7	40.7	165.2	17.2	.039	1.97	7.08	26.3
2	104.4	6.69	50.7	161.8	14.3	.041	.644	7.41	22.4
3	133.9	3.25	29.6	166.8	26.1	.046	.988	9.99	37.1
4	119.3	4.49	47.2	171.0	13.9	.172	.941	8.78	23.8
Mean ± SD	117.4 ± 10.9*	6.78 ± 3.63	42.1 ± 8.03	166.2 ± 3.3**	17.9 ± 4.9	.075 ± .056	1.14 ± .50	8.32 ± 1.16	27.4 ± 5.8

NOTE.—Control fish were obtained on a commercial fishing boat just after capture. Exhausted fish are those kept in a tank on the boat, transported to the harbor, and killed or dead after burst swimming in the holding tank. His = L-histidine; Car = carnosine; Ans = anserine; N^{π}-meHis = N^{π}-methyl-L-histidine.

* $P < .05$.

** $P < .001$.

TABLE 2

EFFECT OF STARVATION ON THE LEVELS (μmol/g muscle) OF L-HISTIDINE AND RELATED COMPOUNDS IN WHITE AND RED MUSCLE OF SKIPJACK TUNA

TUNA NO.	DAYS AFTER CAPTURE	WHITE MUSCLE				RED MUSCLE				
		His	Car	Ans	Total	His	N$^{\pi}$-meHis	Car	Ans	Total
1	1	79.9	14.7	19.1	113.7	1.05	+	.439	.580	2.07
2	1	87.8	5.89	40.6	134.3	6.59	.020	.994	5.51	13.1
3	2	74.1	4.94	47.8	126.8	8.67	.101	.713	9.12	18.6
4	2	69.1	12.1	34.3	115.5	10.9	.069	2.66	7.91	21.5
5	2	40.3	7.70	38.3	86.3	7.21	.292	1.57	8.90	18.0
6	3	65.3	13.9	36.5	115.7	9.71	.075	3.04	9.00	21.8
7	5	31.5	10.6	64.0	106.1	3.92	.550	1.96	9.78	16.2
8	12	3.46	24.9	58.8	87.2	3.59	.118	4.86	13.3	21.9
Mean ± SD		56.4 ± 26.9*	11.8 ± 5.9*	42.4 ± 13.4	110.7 ± 16.0**	6.46 ± 3.16**	.153 ± .171	2.03 ± 1.37*	8.01 ± 3.46	16.7 ± 6.2

* $P < .01$.
** $P < .001$.

TABLE 3

CONCENTRATION (μmol/g muscle) OF L-HISTIDINE AND RELATED COMPOUNDS IN WHITE AND RED MUSCLE OF KAWAKAWA *Euthynnus affinis*

	WHITE MUSCLE				RED MUSCLE				
GROUP AND NO.	His	Car	Ans	Total	His	N^{π}-meHis	Car	Ans	Total
Captivity control:									
1	68.7	1.30	32.5	102.5	6.97	.037	.772	4.26	12.0
2	74.3	1.19	27.7	103.6	5.43	.054	.198	5.20	10.9
3	83.3	.780	22.8	106.9	7.14	.025	.084	3.98	11.2
Mean ± SD	76.5 ± 5.99	1.09 ± .22	27.7 ± 3.96	104.3 ± 1.87	6.51 ± .77	.039 ± .012	.351 ± .301	4.48 ± .52	11.4 ± .46
Starved fish (days):									
1 (6)	50.0	.439	22.1	72.5	4.61	.008	.068	8.25	12.9
2 (6)	73.4	2.1	33.2	108.7	15.4	.038	.297	7.13	22.9
3 (9)	65.0	.390	49.0	114.4	6.99	.303	.298	18.0	25.6
4 (13)	48.4	.372	52.8	101.6	10.0	.224	.150	15.2	25.6
5 (15)	78.1	.231	35.5	113.8	7.98	.269	.141	15.4	23.8
6 (18)	35.7	2.26	38.5	76.5	4.01	.299	.441	12.1	16.9
Mean ± SD	58.4 ± 15.0	.965 ± .863	38.5 ± 10.2	97.9 ± 17.1	8.17 ± 3.81	.191 ± .122*	.233 ± .125	12.7 ± 3.93**	21.3 ± 4.76**

NOTE.—Captivity controls are those well fed in captivity.

* $P < .05$.

** $P < .01$.

Generally during starvation, histidine appeared to decline rather markedly, with part of it probably being converted into carnosine. Anserine, however, decreased during the initial stage of starvation, but later it recovered to normal levels, probably by the conversion from histidine. As a result, the total content of these compounds per gram of white muscle decreased significantly ($P < .001$), but the decrease was not as large as the fall in histidine levels. This pattern of a significant decrease in [histidine] ($P < .001$), an increase in [carnosine] ($P < .01$), and small fluctuations in [anserine] during starvation was also observed in red muscle.

Kawakawa, a species closely related to skipjack tuna, showed similar results, though the degree of change with starvation was less (table 3). The total levels of these histidine-related compounds in well-fed, captive fish were almost 30% and 50% lower ($P < .001$) than those in skipjack white and red muscle, respectively. The composition of these compounds, however, was almost the same as in skipjack tuna, showing high histidine, high anserine (almost half the level of histidine), and low carnosine concentrations.

The levels of these compounds in blood of both skipjack and kawakawa were low, and we found no differences between these two species (table 4). It was a rather characteristic feature of the blood, however, that the level of carnosine exceeded that of anserine. Blood carnosine levels were almost identical to, or a little higher than, those in red muscle.

TURNOVER OF L-[U-^{14}C]HISTIDINE IN SKIPJACK TUNA

Three cannulated skipjack tuna received 30 μCi of L-[U-^{14}C]histidine, and decay curves were obtained for total and specific radioactivities of L-histidine (figs. 1, 2). The three specimens showed very little individual difference, as shown by the small standard deviation. Specific radioactivity of histidine was calculated (fig. 2) as dpm/μmol of histidine. To judge from such curves, the decay of histidine specific activity and the incorporation into tissues were considered to be rapid. Histidine replacement rate in the blood was calculated (according to Katz et al. 1974) as 2.60 ± 0.65 μmol/100 g body weight h^{-1} and metabolic half-life as 0.72 ± 0.124 h. This replacement rate is similar to that in rainbow trout (H. Abe and P. W. Hochachka, unpublished data). Corrected to the same temperature (assuming a Q_{10} of 2), the replacement rate is actually slower in tuna than in trout (the water temperatures for the two species were 24 and 6 C, respectively). This difference was also evident in the metabolic half-life, which was 0.72 h in tuna but 1.99 h in trout (H. Abe and P. W. Hochachka, unpublished data).

METABOLISM OF L-HISTIDINE IN TUNA TISSUES

After blood sampling was completed (5.5 h), the tuna were killed, and white muscle, red muscle, and a liver and final blood sample were obtained. In these tissues, the total activity and the specific activities of histidine, carnosine, and anserine were determined (table 5). We found only small

TABLE 4

CONCENTRATION (μmol/g blood) OF L-HISTIDINE AND RELATED COMPOUNDS IN THE BLOOD OF SKIPJACK TUNA AND KAWAKAWA

Fish Type and No.	Histidine	Carnosine	Anserine	Total
Skipjack tuna:				
1	2.11	.852	.074	3.04
2	1.84	.889	.023	2.75
3	.783	.440	.054	1.28
4	.843	.618	.027	1.49
5	1.55	.555	.024	2.13
Mean ± SD	1.43 ± .53	.671 ± .173	.040 ± .020	2.14 ± .69
Kawakawa	.806	.868	.049	1.72

NOTE.—Whole blood was used for the analysis after deproteinization with trichloroacetic acid.

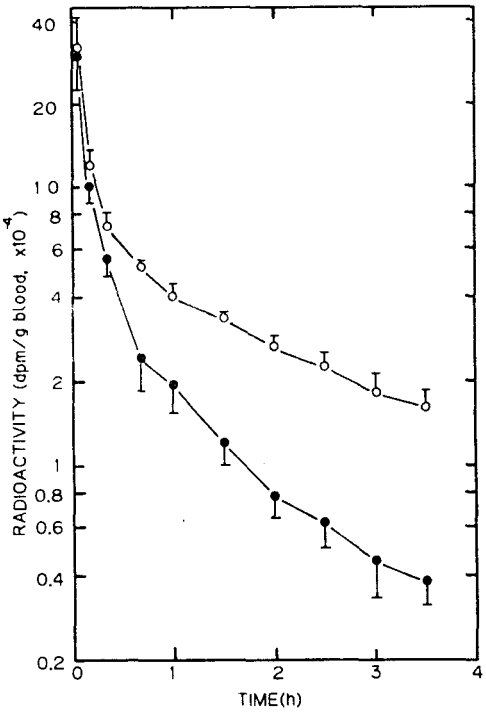

FIG. 1.—Total and specific radioactivity in the blood of skipjack tuna. A bolus of L-[U-^{14}C]histidine (30 μCi/tuna) was injected into the aorta through a cannula. ○—○ = Total radioactivity; ●—● = specific radioactivity of L-histidine. Blood samples were withdrawn ≥2 min after injection via cannula under the shallow anesthetic condition. The mean and standard deviation for three tuna are shown in dpm/g blood.

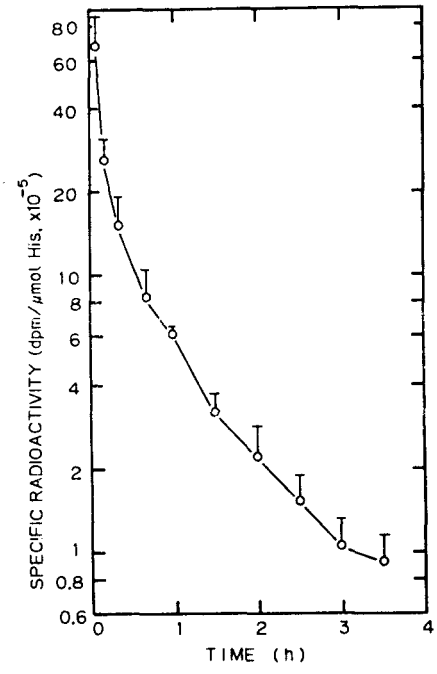

FIG. 2.—Specific radioactivity of L-histidine in the blood of skipjack tuna. The activities are shown in dpm/μmol of histidine for the same samples as those in fig. 1.

amounts of labeled histidine being incorporated into carnosine and anserine. Per gram of tissue, red muscle contained a much larger amount of total activity than did white muscle. The total of specific activities of these compounds in liver was equivalent to only 7.3% of total radioactivity in the liver, indicating a rapid catabolism of amino acids in this organ. In blood, only 36% of the radioactivity was in carnosine, anserine, or histidine, whereas in white and red muscle the values were 103% and 90%, respectively. These data suggest that only modest catabolism of histidine occurs in the muscle.

In addition to the 5.5-h sample, the incorporation of the label into tissues was also examined in tuna sampled at several shorter time intervals (fig. 3). The incorporation

TABLE 5

TOTAL RADIOACTIVITY AND SPECIFIC RADIOACTIVITY OF L-HISTIDINE, CARNOSINE, AND ANSERINE IN SEVERAL TISSUES OF SKIPJACK TUNA

TISSUE	RADIOACTIVITY (dpm/g tissue, ×10⁻³)			
	Total Activity	Histidine	Carnosine	Anserine
White muscle	8.02	7.16	.250	.815
Red muscle	35.6	31.5	.160	.436
Liver	51.9	2.51	.718	.562
Blood	8.64	2.54	.342	.233

NOTE.—Tissues were collected 5.5 h after L-[U-^{14}C]histidine injection (30 μCi/tuna) into the aorta.

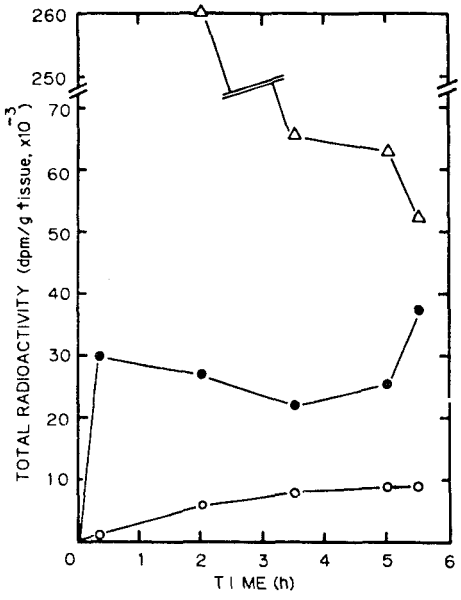

FIG. 3.—Incorporation of total radioactivity into white and red muscle and liver after bolus injection of L-[U-^{14}C]histidine (30 μCi/tuna) into the aorta. Tissue samples at 3.5, 5.0, and 5.5 h were obtained from the tuna shown in fig. 1. O —— O = White muscle; ● —— ● = red muscle; △ —— △ = liver. Each point represents one tuna sample.

into the liver was extremely high compared to that in skeletal muscle. The radioactivity taken up into red muscle was always higher than that taken up into white muscle.

In free-swimming fish, if the label was administered into white muscle, the decay of the radioactivity from the muscle was slow, whereas the label was taken up by red muscle promptly after injection (fig. 4). The decay curve was sharper for red than for white muscle. From these curves, the metabolic half-lives of histidine in white and red muscle were calculated as being 66 h and 52.5 h, respectively. During these studies, the label was apparently incorporated into anserine (fig. 5) both in white and in red muscle. This incorporation was much higher in white than in red muscle.

DISCUSSION

Several scombrioids (suborder; Scombrioidei) are known to contain extraordinarily large amounts of histidine and histidine-related dipeptides in their white muscle; for instance, some scombrids (mackerel and tuna) contain 30–100 μmol/g of histidine and 10–50 μmol/g of anserine (Lukton and Olcott 1958; Suyama and Yoshizawa 1973; Abe 1983). Some istiophorids (marlin) contain 16–54 μmol/g of histidine and 15–120 μmol/g of anserine (Suyama and Yoshizawa 1973; Abe et al. 1985). Of these fishes, skipjack tuna shows the highest histidine content (table 1), whereas Pacific blue marlin (*Makaira nigricans*) shows the highest anserine concentration (90–120 μmol/g muscle; Abe et al. 1985) of all vertebrates thus far examined. These species all belong to a fast-

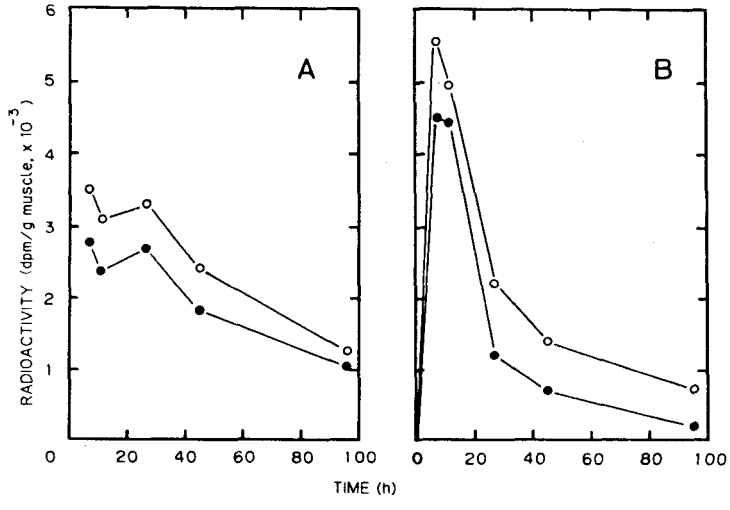

FIG. 4.—Total and specific radioactivities in white and red muscle of skipjack tuna after injection of L-[U-^{14}C]histidine (30 μCi/tuna) into white muscle. Each point represents one skipjack tuna. *A*, White muscle, *B*, red muscle. O —— O = Total activity; ● —— ● = specific radioactivity of L-histidine.

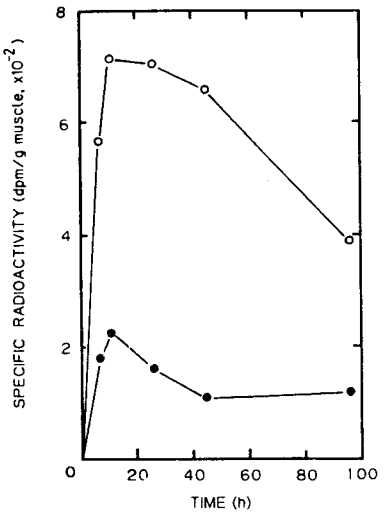

FIG. 5.—Incorporation of the label into anserine in white and red muscle of tuna after intramuscular injection of 30 μCi of L-[U-^{14}C]histidine into white muscle. The same muscle samples as shown in fig. 4 were used for anserine-specific radioactivity. O —— O = White muscle; ● —— ● = red muscle.

swimming group of fishes that have adapted both for endurance and for burst anaerobic exercise. Red muscle of these fishes shows much lower contents of these compounds than does white muscle, which is consistent with these compounds playing a proton-buffering role correlated with anaerobic metabolism. Our control fish, captured at sea, are considered to have been sampled during high-speed, steady-state swimming associated with feeding; thus, in such high-activity states, tuna apparently maintain high total contents of these compounds, although there are some individual differences in how each compound contributes to the total pool.

In tuna exhausted after burst swimming, the histidine content of white muscle increases by an average of 20 μmol/g. Since histidine is considered nutritionally to be an essential amino acid in all fish species thus far examined, this increase in histidine concentration may be derived from the degradation of body proteins or from dietary proteins eaten just before capture. These data therefore are equivocal and may not mean that an interconversion between these compounds necessarily occurs during burst swimming.

Starvation, on the other hand, markedly affects the composition of this pool of histidine-related compounds (table 2). In the initial stage of starvation, both histidine and anserine levels decrease with a concomitant rise in carnosine concentration. The decline in histidine concentration may mean that this amino acid is utilized as an energy source via the Krebs cycle or as a glucose source via the Cori cycle during starvation. If used as an energy source directly, a mole of histidine oxidation could yield 21 mol of adenosine triphosphate. Although this metabolic fate is possible in principle, in practice it does not seem to occur. Instead, during sustained starvation the decomposition of anserine is arrested, whereas free L-histidine appears to be converted into carnosine and anserine, suggesting that the biosynthetic pathway of anserine in tuna may be via carnosine, as occurs in trout (H. Abe and P. W. Hochachka, unpublished data).

This interconversion may have an important implication, namely, that white muscle "defends" the total pool size of these compounds in order to maintain its proton-buffering capacity. This is also supported by the fact that the total concentration of these compounds was rather similar among individuals, though a rather large difference was found in the level of each compound (table 1). As a result of this arrangement, even if histidine is depleted almost completely during starvation, the total pool size of these compounds decreases by only 30%–40%. This is also true for red muscle. These effects of starvation are also supported by the results obtained from the starvation of

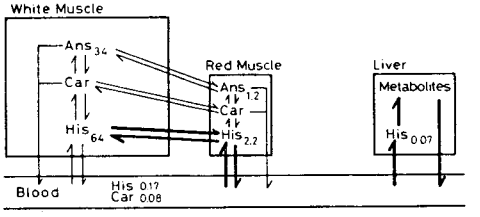

FIG. 6.—Proposed interrelationship of the metabolism of L-histidine and related dipeptides among several tissues and organs of skipjack tuna. Values indicated are total amounts of L-histidine and anserine in the tissue (mmol/tissue). The heavy lines show the main and fast processes.

kawakawa (table 3), though the changes observed in the latter were smaller.

A common metabolic response to starvation among tuna thus appears to be the defense of the total pool size of histidine-related compounds. This implies that the physiological reason for large accumulations of free L-histidine specifically in tuna white muscle is the same as that obtaining for large accumulations of the related dipeptides: in effect, histidine is an intracellular buffer, equivalent mole for mole, with either dipeptide. Its disadvantage is that it is more metabolically reactive. That is presumably why the conversion of histidine into much more metabolically inert forms (i.e., carnosine and anserine) is necessary. In this view, these dipeptides are a "histidine sink" that can keep the total histidine-related buffering capacity at high values. This end is not so easily achievable with histidine per se because it can be utilized easily in the other metabolic pathways according to the physiological conditions. Our data and interpretations are in agreement with studies on starved eels, trout, and Japanese dace, which show no decrease of the dipeptide pool but a notable decrease in histidine levels during long-term starvation (H. Abe, unpublished data). Similarly, during spontaneous starvation accompanying upstream spawning migration in the sockeye salmon, histidine decreases to about one-tenth normal levels, whereas anserine levels remain unchanged (Mommsen, French, and Hochachka 1980).

The turnover of L-histidine in skipjack tuna blood is rapid, and almost the same as that in trout blood (H. Abe and P. W. Hochachka, unpublished data); however, it is much slower when comparisons are corrected for temperature differences. In white muscle, the metabolic half-life of L-histidine is 66 h, which implies much faster turnover in tuna than in trout muscle (half-life of 7.5 days). However, this difference disappears if one makes a simple Q_{10} correction of ~ 2, in which case the half-life of histidine in tuna white muscle would be 11 days at 4 C. However, since skipjack tuna maintain muscle temperatures significantly above ambient temperatures, this fast turnover in white muscle suggests that histidine metabolism is probably quite rapid under the normal conditions of this species at sea. To put this in perspective, it should be noted that the metabolic half-life of L-histidine in rat gastrocnemius muscle is ~ 3.6 h (Tamaki et al. 1980); so the above data indicate a rather slow absolute turnover rate for this amino acid in tuna muscle.

Although some conversion of histidine into carnosine and anserine occurs following injection of histidine into the ventral aorta (table 5), following intramuscular injection the label is also incorporated into anserine, especially in white muscle (fig. 5). The appearance of only a small amount of the label in anserine is taken to indicate that the rate of anserine biosynthesis is slow in skipjack as it is in trout (H. Abe and P. W. Hochachka, unpublished data), possibly because of limited β-alanine availability. In white muscle, β-alanine occurred at 0.016 ± 0.005 μmol/g in the control tuna ($n = 5$) and at 0.054 ± 0.0115 μmol/g in red muscle. These values are lower than those in trout white muscle, where β-alanine's availability seems to determine rates of dipeptide synthesis (H. Abe and P. W. Hochachka, unpublished data). Thus it is reasonable to conclude that the level of β-alanine also may be a factor controlling the rates of biosyntheses of these dipeptides in tuna muscle.

The uptake of the label from blood into red muscle was much faster than that from blood into white muscle (fig. 3), and the label was also transferred efficiently into red muscle from white muscle in which it was initially administered (fig. 4). These data are consistent with a direct transfer of L-histidine from white to red muscle, possibly via exchange at the rete (as suggested for lactate movement during recovery from exercise [P. W. Hochachka, unpublished data]). Red muscle, with its high capillary density, is then the main site for equilibrating muscle and blood pools of histidine.

According to the above considerations, although exact rates and pathways are not known, the main movements of L-histidine and related compounds in skipjack tuna may be summarized as shown in figure 6. The incorporation and decomposition of L-histidine by liver (possibly also by kidney) are very fast, indicating that most of the histidine derived from dietary proteins is

used as a carbon and energy source in the liver. However, there occurs a constant incorporation and accumulation of the amino acid into white muscle, and the biosyntheses and degradations of carnosine and anserine appear to proceed both in white and in red muscles. Both L-histidine and anserine are accumulated in white muscle, the former being slowly metabolized to the latter in muscle in order to stabilize a large pool of imidazole-based intracellular buffering capacity even under conditions of starvation.

These conclusions raise interesting problems about the turnover of carnosine and anserine in white muscle; about why anserine, not carnosine or balenine, has been selected by this species; and about interorgan transfer and metabolism of these metabolites.

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