Bull. Fac: Fish. Hokkaido Univ.
38(3), 185-190. 1987.

Uptake of Asparatic Acid and Sulfate by Calcified Tissues in Goldfish and Tilapia

Yasuo MUGIYA* and Hirofumi IKETSU*

Abstract

To elucidate the metabolic aspect of organic matrix of calcified tissues, the incorporation of ^{3}H-asparatic acid and ^{35}S-Na_2SO_4 into otoliths, scales, and ribs were examined in goldfish (*Carassius auratus*) and tilapia (*Oreochromis niloticus*). Plasma and muscle were also analyzed from the comparative point of view.

A single or multiple intraperitonial injection of radioactive asparatic acid or sulfate produced a high incorporation of these substances into plasma in both fish, but the incorporated levels rapidly decreased with time. Otoliths showed a poor incorporation of isotopes, and the incorporation levels remained almost unchanged for at least several days after injection. Scales and ribs had a higher incorporation than otoliths, but their radioactivity levels gradually decreased with time. The level of amino acid incorporation into muscle was stable in goldfish, but slightly decreased on day 3 after injection in tilapia. Four weeks' administration of ^{3}H-asparatic acid via food resulted in a poor incorporation into these tissues in tilapia. Rearing tilapia in water containing sulfate-35 also resulted in a poor incorporation. These results suggest that otoliths are more inactive in matrix turnover than scales and ribs.

Calcified tissues such as bone, scales and otoliths have been used for age determination in fish. Such tissues consist of calcium phosphate or carbonate, and collagenous and non-collagenous organic matrix. Although calcium physiology in these tissues has been studied by many researchers (see Ichii and Mugiya, 1983), little attention has been paid to the metabolic aspect of organic matrix, especially in otoliths. The main component of otolith matrix is proteins containing acidic amino acids predominantly (Degens et al., 1969). Mucopolysaccharides containing sulfate also may play an important role in otolith calcification (Mugiya, 1968).

The present study was undertaken to elucidate the comparative aspect of asparatic acid and sulfate uptake by otoliths, ribs, and scales in goldfish and tilapia. Plasma and muscle were also analyzed from the comparative point of view. Because of the low incorporation of these substances into otoliths, various methods of administration were tested.

Materials and Methods

Goldfish (*Carassius auratus*) and tilapia (*Oreochromis niloticus*) were obtained from a commercial dealer and reared in our laboratory for at least one month before use. They were kept at 23℃ ±1 (goldfish) or 28℃ ±1 (tilapia) throughout the acclimation and experimental periods, and fed commercial carp food pellets, *ad*

* *Laboratory of Physiology and Ecology, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部生理学生態学講座)

libitum.

Experiment I. Goldfish weighing about 6.0 g were given a single intraperitoneal injection of ^{3}H-asparatic acid (New England Nuclear) at a dose of 2.0 μCi/g-body weight, and kept in aerated and filtrated water (50 l). They were sacrificed on days 1, 2, and 3 post-injection.

Experiment II. Goldfish weighing about 3.9 g intraperitoneally received ^{3}H-asparatic acid at a dose of 2.0 μCi/g 4 times every other day, and were sacrificed on days 3 and 8 after the last injection.

Experiment III. Tilapia weighing about 7.5 g were given a single intraperitoneal injection of ^{3}H-asparatic acid at a dose of 1.5 μCi/g, and were sacrificed on days 1 and 3 after injection.

Experiment IV. Tilapia weighing about 10.0 g were intraperitoneally injected ^{3}H-asparatic acid at a dose of 2.0 μCi/g. The injection was made twice leaving an interval of 3 days, and the fish were sacrificed 2 days later.

Experiment V. Tilapia weighing about 0.9 g were *ad libitum* fed carp food pellets ("Nitsu-pai" 2C) containing ^{3}H-asparatic acid (approx. 16.7 μCi/g-pellet). The feeding continued for 4 weeks.

Experiment VI. Tilapia weighing about 10.6 g were given a single intraperitoneal injection of ^{35}S-Na_2SO_4 (New England Nuclear) at a dose of 1.9 μCi/g, and sacrificed on days 1, 2, 3, and 5 after injection.

Experiment VII. Tiapia weighing about 11.0 g were reared in water containing ^{35}S-Na_2SO_4 (approx. 5000 dpm/l) for 6 days, and then sampled for counting.

Blood was collected from the caudal vessels by cutting the tail of the fish and draining it into heparinized capillaries. After centrifugation, the separated plasma was stored at -20℃ for a few days, and then analyzed for radioactivity. The otolith (astriscus for goldfish and sagitta for tilapia), scale, rib bone, and muscle (dorsal) were dissected, rinsed 3 times in water, placed in individual counting vials, and dried at 90℃ overnight. In the sulfate-uptake experiments, each sample was lightly rinsed in 99% ethyl alcohol containing 10% Formalin to preserve acid-mucopolysaccharides better. After weighing, they were solubilized using the method of Mahin and Lofberg (1966), added to the Triton-X scintillant (Turner, 1968), and counted for radioactivity by a liquid scintillation spectrophotometer (Aloka LSC-673).

Results

Experiment. I. A single intraperitoneal injection of ^{3}H-asparatic acid into goldfish resulted in a high incorporation of radioactivity into plasma on day 1 after injection (Fig. 1). This radioactivity rapidly decreased to a level of about half on day 3. The incorporation of the isotope into otoliths was rather low on day 1 and the activity remained almost unchanged thereafter. The radioactivity level of ribs was about 3-4 times higher than that of otoliths on a unit-weight basis, and this activity appeared to increase slightly until day 3. The highest incorporation was found in scales (Fig. 1), but the activity level tended to decrease gradually from day 2. The level of ^{3}H-asparatic acid incorporated into muscle was a little lower than that of scales, and a small peak appeared on day 2 (Fig. 1).

Experiment. II. Multiple injections of ^{3}H-asparatic acid into goldfish induced

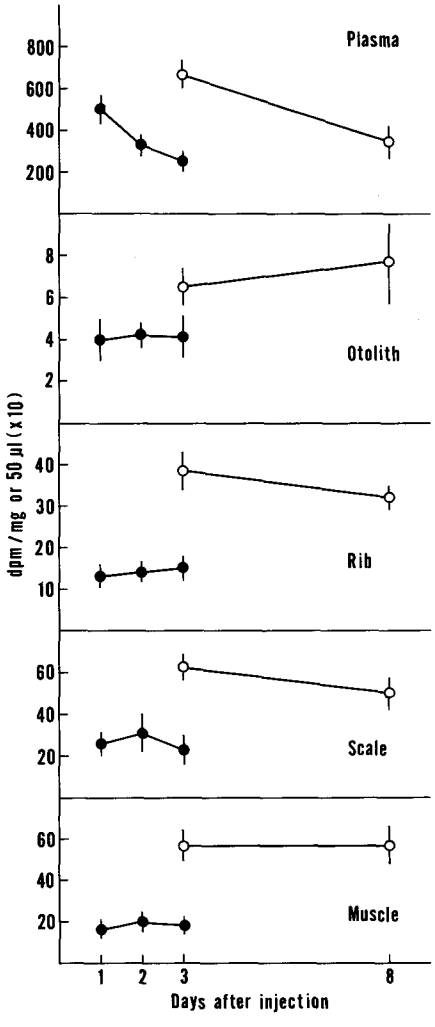

Fig. 1. Incorporation and turnover of intraperitoneally injected ^{3}H-asparatic acid in goldfish. Vertical bars indicate SE of mean for 4 or 5 fish. ●: single injection ; ○: multiple injection.

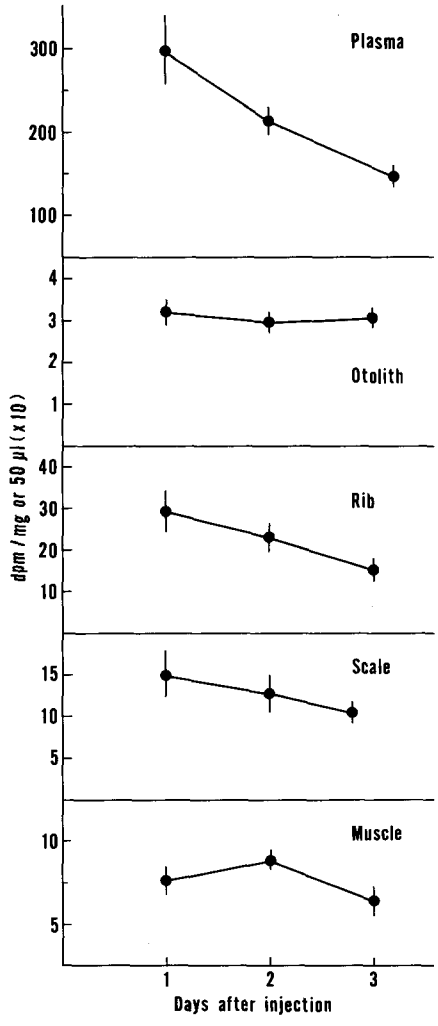

Fig. 2. Incorporation and turnover of intraperitoneally injected ^{3}H-asparatic acid in tilapia. Vertical bars indicate SE of mean for 9 fish. Single injection.

a high incorporation of the amino acid into plasma on day 3 after the last injection (Fig. 1). This activity decreased rapidly with time. A non-injected fish reared in the same experimental tank revealed 187 dpm/50 μl-plasma on day 8. Radioactivity in otoliths was about 40% higher than that of the singly injected group (Exp.1) on day 3. This activity increased slightly on day 8, but no significant difference was found between day 3 and day 8 because of great individual variations. Ribs and scales showed 2-3 times high incorporation of the isotope on day 3, compared with the results in Exp. I. These activities tended to decrease on day 8. Muscle

also showed a high incorporation on day 3, and this level remained constant until day 8 (Fig. 1).

Experiment. III. When a single injection of ^{3}H-asparatic acid was given to young tilapia, radioactivity was relatively high in plasma on day 1, but the activity exponentially decreased with time (Fig. 2). In otoliths, a low radioactivity was observed on day 1, and remained almost constant thereafter. The incorporation of asparatic acid into ribs and scales was much higher than that of otoliths on day 1, but the radioactivity levels consistently decreased with time (Fig. 2). In muscle, the radioactivity level was almost stable until day 2, and then decreased slightly (Fig. 2).

Experiment. IV. Tritium-labeled asparatic acid was twice given to tilapia intraperitoneally, and their radioactivity levels were analyzed on day 2 after the second injection. The activity of plasma was not very different from that of the singly injected group (Exp. III). However, the incorporation of asparatic acid into otoliths was much higher than that of Exp. III (Fig. 3). The other 3 tissues also showed a high incorporation of the isotope. The order of radioactivity incorporation rate was: rib > otolith > scale > muscle on a mg-basis.

Experiment. V. Four weeks' oral administration of ^{3}H-asparatic acid to tilapia failed in inducing a high incorporation of this isotope into the calcified tissues (Fig. 4). Muscle has a relatively high radioactivity, and the order of radioactivity incorporation rate was: rib > muscle > scale > otolith.

Experiment. VI. A single intraperitoneal injection of sulfate-35 resulted in a

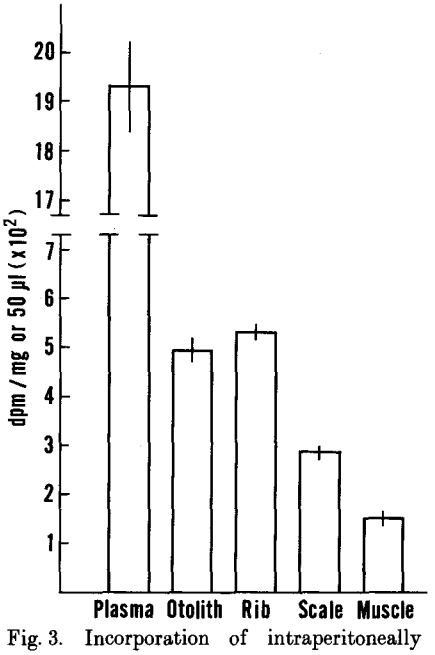

Fig. 3. Incorporation of intraperitoneally injected ^{3}H-asparatic acid into tilapia on day 2 after the second injection. Vertical bars indicate SE of mean for 12 fish.

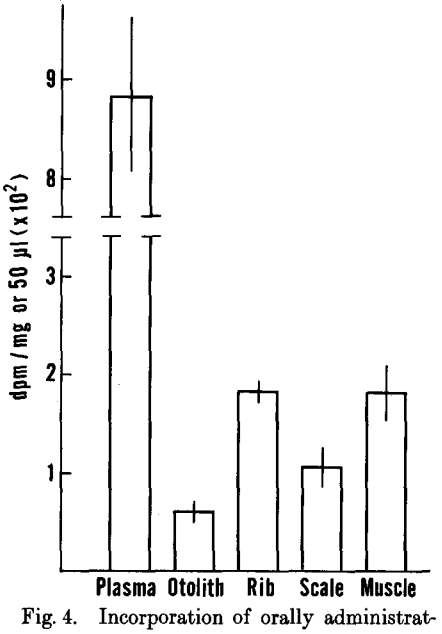

Fig. 4. Incorporation of orally administrated ^{3}H-asparatic acid into tilapia. Vertical bars indicate SE of mean for 5 fish.

high incorporation into plasma and other tissues (Fig. 5). Plasma showed a very high radioactivity on day 1, but the activity rapidly decreased to a level of one-fifteenth on day 5. The radioactivity incorporated into otoliths appeared to increase until day 2 and thereafter remained unchanged. On the other hand, ribs showed about a 10 times higher incorporation of sulfate than otoliths on day 1, but this activity rapidly decreased with time (Fig. 5).

Experiment. VII. Tilapia reared in water containing sulfate-35 for 6 days resulted in a very poor incorporation of sulfate into otoliths and ribs. The radioactive count in plasma was 427 ± 86 (mean $\pm$ SE) dpm/50 μl, while the counts in otoliths and ribs were only 6.6 ± 0.4 dpm/mg and 31.5 ± 2.1 dpm/mg, respectively.

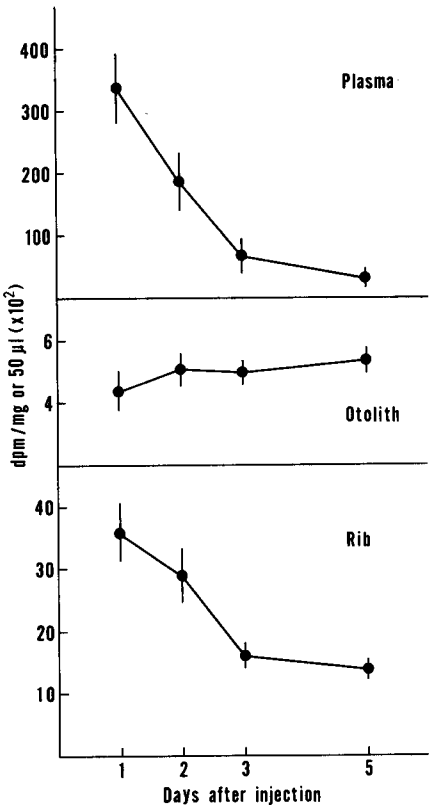

Fig. 5. Incorporation and turnover of intraperitoneally injected sulfate-35 in tilapia. Vertical bars indicate SE of mean for 6 or 7 fish. Single injection.

Discussion

The organic matrix of fish otoliths mainly consists of proteins dominated by acidic amino acids and of polysaccharides containing the sulfated group (Mugiya, 1968; Degens et al., 1969). However, the present experiments resulted in the low incorporation of radioactive asparatic acid and sulfate into otoliths. This primarily seems to be due to the fact that otoliths contain very little organic matrix at least in terms of weight. Therefore, to obtain the sufficient incorporation of these substances into otoliths, plasma specific activities must be kept high for a reasonably long period. A single or even multiple injection of these isotopes resulted in a rapid decrease in plasma radioactivity. The use of "an osmotic pump" is recommended to maintain a constant level of radioactive substances in plasma. Recently the *in vitro* incubation of otolith-containing sacculi produced a relatively high incorporation of ^{3}H-glutamic acid into otoliths in rainbow trout (Mugiya, 1987).

The stability of incorporated radioactivity was different according to the different tissues; i.e., otoliths, scales, and ribs. Tritium-labeled asparatic acid incorporated into otoliths seemed to be the most stable, and no practical decrease was found after incorporation, suggesting a conservative nature in otoliths. Such a conservative nature is also known in their calcium metabolism. Once deposited on otoliths, calcium remained stable even under stress (Ichii and Mugiya, 1983; Campana, 1984). On the other hand, the radioactivity of ^{3}H-asparatic acid incorpo-

rated into ribs and scales gradually decreased with time, suggesting that these tissues are matabolically more active than otoliths. Calcium turnover in bone and scales is also reported to be active in goldfish (Ichii and Mugiya, 1983).

The turnover profile of sulfate-35 incorporated into otoliths and ribs was different. Otoliths retained the sulfate without any decrease for at least the first 5 days, while ribs showed a rapid decrease in radioactivity with time, again suggesting that otoliths are more conservative than ribs. Rosenthall (1963) studied the turnover of sulfate-35 in the guppy and reported a biological half-life of about 10 days for spines. In the present young tilapia, a biological half-life of sulfate-35 was found to be about 2 days for plasma and 5 days for ribs, when calculated from day 1 after injection.

References

Campana, S.E. (1983). Calcium deposition and otolith check formation during periods of stress in coho salmon, *Oncorhynchus kisutch*. *Comp. Biochem. Physiol.* **75A**, 215-220.

Degens, E.T., Deuser, W.G. and Haedrich, R.L. (1969). Molecular structure and composition of fish otoliths. *Mar. Biol.* **2**, 105-113.

Ichii, T. and Mugiya, Y. (1983). Comparative aspects of calcium dynamics in calcified tissues in the goldfish *Carassius auratus*. *Bull. Japan. Soc. Sci. Fish.* **49**, 1039-1044.

Mahin, D.T. and Lofberg, R.T. (1966). A simplified method of sample preparation for determination of tritium, carbon-14, or sulfur-35 in blood or tissue by liquid scintillation counting. *Analyt. Biochem.* **16**, 500-509.

Mugiya, Y. (1968). Calcification in fish and shell-fish-VII. Histochemical similarities between the otolith and the macula region of sacculus in juvenil rainbow trout, with special reference to the otolith formation of fish. *Bull. Japan. Soc. Sci. Fish.* **34**, 1096-1106.

Mugiya, Y. (1987). Phase differences between calcification and organic matrix formation in the diurnal growth of otoliths in the rainbow trout, *Salmo gairdneri*. *Fish. Bull.* U.S. In press.

Rosenthal, H.L. (1963). Uptake, turnover and transport of bone seeking elements in fishes. *Ann. New York Acad. Sci.* **109**, 278-293.

Turner, J.C. (1968). Triton X-100 scintillant for carbon-14 labeled materials. *Int. J. Appl. Radiat. Isotopes* **19**, 557-563.