The Metabolism of Radionuclides by Marine Organisms. III. The Uptake of Calcium$^{46}$ in Solution by Marine Fish$^{1,2}$

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ABSTRACT

To determine whether marine fish required calcium in their diet, or whether they could take up an adequate amount from sea water, non-feeding fish (Tilapia mossambica) were put in artificial sea water with calcium$^{46}$. The uptake was rapid at first, then continued at a slow rate for the duration of the experiment (21 days). The normal concentration ratio of calcium by marine fish is about 20. At 21 days, the ratio of Ca$^{46}$ in the fish to the Ca$^{46}$ in an equal weight of sea water was about 0.6. The readily exchangeable calcium in the fish is therefore only a few per cent of the total. The concentration ratio of 0.6 would seem to preclude the necessity of feeding captive marine fish calcium in order to have an adequate diet. The skeleton contained about 43 per cent of the accumulated Ca$^{46}$, the integument 24 per cent, the viscera 21 per cent, the gills 8 per cent, and the muscle 4 per cent. The Sr/Ca ratio in marine fish as a whole is about 1:400, but the ratio in normal sea water is about 1:40. This means that marine fish discriminate against strontium either by excreting it faster or by absorbing it less.

During some studies on the metabolism of radiostrontium by marine fishes (Boroughs, Townsley, and Hiatt 1956) it occurred to us that our results may have been influenced to some extent by feeding captive fish an artificial diet which may have been deficient in both calcium and strontium. Although marine fish live in an environment rich in both calcium and strontium, no published information could be found concerning the availability of these elements to fishes directly from the sea water. Because freshwater fishes, such as brook trout and guppies, can accumulate calcium directly from their environment (Lovelace and Podoliak 1952, Rosenthal 1956), we designed a simple experiment to test the hypothesis that marine fish can take up and accumulate calcium from sea water.

MATERIALS AND METHODS

Artificial sea water was made up according to Lyman and Fleming (1940). The total calcium was adjusted so that the low specific activity Ca$^{46}$ which was obtained from Oak Ridge plus the added calcium were just equal to the calcium of sea water. Tilapia mossambica, the fish used for these experiments, were kept in carboys containing 946 microcuries of Ca$^{46}$ in 20 liters of water. The water temperature was about 22°C throughout the experiment. The extremes were 20.5° and 23.2°C. The water was aerated, but the fish were not fed during the entire experiment. Six fish were kept in each carboy and three fish were removed at each time interval from four hours to 21 days. The fish were then dissected, ashed, and counted according to the procedure described elsewhere (Boroughs, Townsley, and Hiatt 1956). An aliquot of the dose was counted to correct for decay, and appropriate corrections were made for self-absorption.

RESULTS AND DISCUSSION

Figure 1 shows that Tilapia, a sluggish fish, took up the calcium from solution rather rapidly, and that the uptake continued throughout the duration of the experiment. Although the three fish in the three-day carboy were very small in comparison with the other fish used (average weight: 72 g, while the average of all the fish was 102 g), size alone cannot account for the dip in the curve. The same data were plotted in Figure 2 in order to allow for the weight of each
fish, and the dip still occurs. A measurement of the radioactivity present in the carboy at the beginning of the experiment showed that this carboy received about 20 per cent less Ca45 than did the other carboys. At any rate, we attribute no biological significance to the dip at three days.

Figure 2 also indicates that the concentration ratio achieved during the 21 days is about 0.6. The concentration ratio is here defined as the microcuries/g fresh weight of fish divided by the microcuries/g of sea water. In other words, the internal concentration of calcium had not reached the external concentration.

Figure 3 shows amounts of Ca45 recovered in five different organ-systems of Tilapia. As one would expect, most of the radioactivity appeared in the skeleton. At 21 days, 43 per cent of the Ca45 was in the skeleton, 24 per cent in the integument, 21 per cent in the viscera, 8 per cent in the gills, and 4 per cent in the muscle. This distribution does not correspond to the distribution of non-radioactive calcium in these tissues. On the basis of an elemental analysis of tuna fish by Goldberg (unpublished), and an analysis of the percentage of the total ash contributed by each organ of tuna fish made in this laboratory (Boroughs, Townsley, and Hiiatt 1956) it is apparent that about 70 per
cent of the total calcium is in the bones, 15 per cent in the gills, 6 per cent in the integument, 3 per cent in the viscera, including the blood, and 1-2 per cent in the muscle.

We have no data on the calcium content of *Tilapia*, but Goldberg's analysis of the anchoveta, a small fish roughly comparable to *Tilapia* in form, indicates that the calcium content is approximately the same in anchovetas and tuna. The calcium content of *Tilapia* is not likely to be very different from these other fish, and one may infer, therefore, that continued exposure to radioactive calcium would result in the continued accretion of this element in the bones and gills, but a comparative loss in the integument, viscera, and muscles.

It is interesting to note that a similar experiment on the uptake of radiostrontium in solution by the same species of fish (Boroughs, Townsley, and Hiatt 1956) indicates that the concentration ratio reached in 21 days was only about half of that reached with radiocalcium even though the concentration of radioactive strontium atoms was about twice that of the radioactive calcium atoms. A summary of several experiments on the comparative metabolism of these two elements in various mammals (Norris and Kisielkski 1948) shows that in both the rat and mouse, and by either oral or intravenous administration, the percentage retention of strontium is about half that of calcium. Lecher (1941) found that the intravenous injection in mice of 0.8 mg of Ca as lactate resulted in the retention of 58 per cent of the dose within 24 hours. The injection of 1.6 mg of Sr as lactate, however, resulted in only 33 per cent retention. Results such as these are difficult to understand.

The authors are aware of no Sr/Ca ratio of 1:2 in any organ of those marine fish whose elemental composition has been reported. If both elements were accumulated

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**Fig. 3.** The internal distribution of calcium$^{46}$ taken up by *Tilapia mossambica* from solution.
on an equimolar basis, the percentage of strontium in the ash of the organism would be about twice that of calcium, because this is approximately the ratio of their atomic weights. One finds, on the contrary, that the ratio of strontium to calcium is marine fish is of the order 1:400 or less. This means that for each mole of Sr there are approximately 800 moles of Ca. If these same ratios of Sr/Ca obtain in mammals, one might be tempted to ascribe the 1:2 ratio actually found for radiostrontium and radio calcium to a transient state. In the mammal it is conceivable that a surge of strontium not ordinarily present in the diet might give rise to very high transient ratios. In marine fish, however, such a situation would not be possible unless the fish were dosed with strontium far in excess of the concentrations used in our experiments. These concentrations were in each instance below the concentration of strontium in sea water. The Sr/Ca ratio in sea water is about 1:40 or 5:200. This means that fish discriminate against strontium, in comparison with calcium, either by excreting it faster, or by absorbing it less. The same discrimination against strontium occurs in other animals and in plants as well (Libby 1956; MacDonald, Noyes, and Lorick 1956). This discrimination, coupled with the large concentration of calcium and the relatively large concentration of strontium in sea water, is a fortunate combination which reduces the radiation hazard of this important fission product to some marine organisms and to man as well.

According to various analyses of the calcium content of marine fishes (Vinogradov 1953) there is approximately 20 times as much calcium in a whole fish as there is in an equal weight of sea water. The concentration ratio is therefore 20. This amount of course is quite variable, and depends for the most part upon the age and species of fish. Assuming a concentration ratio of between 0.5 and 0.7 for the 21 day fish, it is evident that only 2-3 per cent of the total calcium in the body is readily exchangeable under the conditions of the experiment. In general, it is considered that the turnover time for bone calcium is very long. Once the calcium is incorporated into some sort of matrix, only a small amount is involved in metabolism under normal conditions, and most of it remains in situ for the duration of the animal's life.

The rapid uptake of both calcium and strontium by marine fish, the continuous uptake over at least a 21-day period, and the noteworthy concentration ratio for strontium and calcium of about 1:2 indicate strongly that the artificial diet fed captive tuna during experiments on the uptake, accumulation, and loss of radiostrontium would have had little effect or no effect on the results of the experiments.

**SUMMARY**

*tilapia mossambica*, sluggish marine or brackish water fish, were put in artificial sea water with calcium so that the total calcium concentration was equal to that of sea water. The fish were not fed during the experiment which lasted 21 days. During this time, the fish as a whole took up the Ca to the extent of about 60 per cent of the Ca concentration in the water. Most of the radioactivity was recovered in the skeleton and integument, but the values obtained do not correspond to the elemental analysis of various fish for calcium. Only a few per cent of the body calcium is apparently readily exchangeable. It is concluded that marine fishes can take up calcium directly from sea water, and do not need a dietary source for this element. In comparison with a similar experiment using radiostrontium, it is further concluded that marine fishes discriminate against strontium in favor of calcium.

**REFERENCES**


Goldberg, Edward. Scripps Institution of Oceanography, Department of Chemistry. Personal communication.


