METABOLISM OF RADIONUCLIDES IN FISH—I.
STRONTIUM-CALCIUM DISCRIMINATION IN GILL ABSORPTION*

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Studies on the metabolism of strontium and calcium in various food organisms can present useful knowledge for the quantitative estimation of probable deposition of strontium-90 in human body through different kinds of food chains. As for mammals, many studies have been reported already by Comar and his co-workers1)-4) in detail on the behaviour of these minerals in the important physiological processes such as absorption, secretion and excretion. Concerning the aquatic animals such as fishes, however, few studies have been made fully on these points.

On the other hand, it is well known that the gill of fish, in addition to the digestive tract and kidney, takes a great role in absorption and excretion of nutrient minerals5). In the studies on the metabolism of calcium and strontium in fishes, therefore, a great attention should be directed also to the behaviour of these minerals in the gill absorption. Taking this into consideration, a special perfusion technique was applied on the measurement of strontium absorption relative to calcium at the gill of fish, using Sr-89 and Ca-45 as tracers.

Experimental

Materials: Japanese eel, Anguilla japonica, was used in this perfusion experiment for the following reasons. The eels are suitable for the gill perfusion technique because the narrow gill slit of this fish can be connected to the circulation system of the environmental water. Secondly, as this fish is euryhaline, they can be adapted to the both environmental media of sea water and fresh water. Therefore, the behaviour of strontium and calcium both in sea water fishes and in fresh water ones can be investigated using the same species. The fresh water eels were obtained through a fishmonger, while the marine eels were collected at the Atsumi Bay of Aichi Prefecture and have been reared in sea water until used in the present perfusion experiment.

Method of gill perfusion: The eels weighing 150 to 200 gm. were anesthetized by quinaldine and perfusion experiments were carried out, using the apparatus shown in Fig. 1. This apparatus was modified from Key’s6) and Schiffman’s7) and consists of double perfusion system in which the internal perfusion medium enters the bulbus arteriosus adjacent to the heart and is collected from dorsal aorta, whereas the ex-

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ternal perfusion medium is led into buccal cavity and collected from the branchial slit.

As the external perfusion medium, sea water containing 398 mg. Ca per 1000 ml. and artificial fresh water* containing 10 mg. Ca per same volume were used for the sea water and fresh water eels, respectively. These perfusion solutions were labelled with strontium–89 and calcium–45 (total activity; about $7 \times 10^4$ cpm/ml). Being added the stable calcium contained as carrier, the final amounts of calcium in perfusing sea water and fresh water were 498 mg. and 100 mg. per 1000 ml., respectively. This labelled external medium water was poured into separating funnel fixed at the height of about 30 cm. from the fishes and led into the buccal cavity via vinyl tube and injector. After flowing round the gills, the perfusion medium was recovered through the collecting vinyl tube connected with the gill slit. To prevent the leakage, the mouth was kept closed tightly with three forceps and the part of the collecting tube inserted to the gill slit was connected with the mucous membrane of operculum by placing the rubber membrane as shown in Fig. 1.

On the other hand, the internal perfusion medium was introduced into blood at the bulbus arteriosus via vinyl tube with the aid of pulsation of autodispenser. The flow speed of the medium was about 1 ml. per minute. After circulating into the branchial arteries, capillaries of gills and branchial veins, the medium was led to the test tube at the dorsal aorta via vinyl tube. During this perfusion process, the uptake of strontium and calcium from the external to internal medium should occur at the gill membrane. At the beginning of experiment, the blood in the above mentioned vessels was flown out and replaced with the perfusion medium, after injecting heparin as anti-coagulant. As this internal perfusion medium, the Key’s physiological saline solution given in foot-note** was used.

The activity of strontium–89 and calcium–45 in perfusates collected successively in test tube, was measured separately using aluminium absorber. The ratios of strontium–89 to calcium–45 in these perfusates were compared with those in the external perfusion medium. The strontium-calcium discrimination factor at the gill absorption

* This artificial fresh water contains 10 mg. Ca, 10 mg. Na and 0.5 mg. Mg per 1000 ml. of distilled water.

** 7.86 gm.NaCl, 0.316 gm. KCl, 0.24 gm. CaCl$_2$, 0.4 gm. NaHCO$_3$, 0.9 gm. glucose and 0.5 gm. urea were dissolved in 1000 ml. of distilled water.
Results and Discussion

Five fresh water eels and other five marine ones were used in the course of experiments on the gill perfusion. As a part of results obtained, the activity of strontium-89 and calcium-45 in each perfusate collected successively in the two examples is shown in the upper half parts of the Fig. 2 and Fig. 3. In the lower half parts of these figures, the ratio between these activities (strontium-89:calcium-45) in the perfusates and that ratio in external medium are plotted. From these figures, the following conclusion can be obtained. Although the amount of strontium and calcium absorbed fluctuates somewhat with time during the perfusion experiment, the ratios...
between the amounts of these minerals absorbed show similar values among each perfusate collected, in other words, the absorption of strontium increases or decreases in proportion to that of calcium. The fact that the values of the ratio of strontium to calcium in the perfusates are found to be always below those in the external perfusion medium as shown in Figs. 2 and 3, indicates the presence of discrimination against strontium at the gill absorption of strontium and calcium. In other words, gills can absorb calcium more favorably than strontium.

As for mammals, many studies have been reported on the strontium-calcium discrimination factor at various important physiological processes such as the gastrointestinal absorption, urinary excretion and placental transfer. According to Comar et al.12, for example, strontium-calcium discrimination factor at the time of intestinal absorption is 0.34 in rat and 0.78 in man, whereas this factor in the urinary excretion is 0.86 and 0.87 in rat and man, respectively. In mammals strontium and calcium are taken from their food only, but in fishes these minerals are taken not only from the food but directly from the environmental water through the gill membrane. Tomiyama et al.8) reported that fishes took the most part of nutrient calcium directly from environmental water, showing the experimental result that gold fish feeding on the worm absorbed calcium directly from the environmental water about 50 times as much as from diet. Schiffman9) reported also about this fact with the result that fishes took strontium from water about 10 times as much as from diet.

Table 1. Strontium-calcium discrimination factor in the gill absorption of Japanese eel, Anguilla japonica

A) Fresh water fishes

<table>
<thead>
<tr>
<th>Fish No.</th>
<th>Sr-89:Ca-45 in artificial fresh water</th>
<th>Sr-89:Ca-45 in the perfusate collected from dorsal aorta</th>
<th>DF_{gill absorptive}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.895</td>
<td>0.670</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>0.834</td>
<td>0.641</td>
<td>0.77</td>
</tr>
<tr>
<td>3</td>
<td>0.518</td>
<td>0.372</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>0.518</td>
<td>0.380</td>
<td>0.73</td>
</tr>
<tr>
<td>5</td>
<td>0.517</td>
<td>0.367</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Mean 0.74 ± 0.02

B) Marine fishes

<table>
<thead>
<tr>
<th>Fish No.</th>
<th>Sr-89:Ca-45 in sea water</th>
<th>Sr-89:Ca-45 in the perfusate collected from dorsal aorta</th>
<th>DF_{gill absorptive}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.884</td>
<td>0.611</td>
<td>0.69</td>
</tr>
<tr>
<td>2</td>
<td>0.946</td>
<td>0.642</td>
<td>0.68</td>
</tr>
<tr>
<td>3</td>
<td>0.945</td>
<td>0.627</td>
<td>0.66</td>
</tr>
<tr>
<td>4</td>
<td>0.784</td>
<td>0.533</td>
<td>0.68</td>
</tr>
<tr>
<td>5</td>
<td>0.887</td>
<td>0.583</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Mean 0.67 ± 0.01
The values of $\text{DF}_{\text{gill absorptive}}$ obtained in the present experiments using ten examples of eels are shown in Table 1. In the fresh water eels, $\text{DF}_{\text{gill absorptive}}$ is: $0.74\pm0.02$, whereas this factor is $0.67\pm0.01$ in the marine eels. Therefore, in the gill absorption of these minerals, the marine eels discriminate against strontium at higher rate than the fresh water eels do. This remarkable difference in the discriminating rate observed at the gill absorption between marine eels and fresh water ones seems to be caused from the different amount of calcium and strontium in the external perfusion medium.

Rosenthal\textsuperscript{10,11) reported using fresh water fishes such as zebra fish (Danio), white cloud mountain fish (Tanichthys) and guppy (Lebistes), that strontium-calcium "observed ratio"* in water and fish body was almost 1.0, namely, no discrimination was observed between these minerals. On the other hand, Borogus \textit{et al.}\textsuperscript{12,13) obtained 0.5 as this "observed ratio" in case of \textit{Tilapia} reared in sea water.

In the previous report of the authors\textsuperscript{14), the rate of discrimination between Sr and Ca taken from diet and environmental water was investigated using rainbow trout, \textit{Salmo gairdnerii irideus}. And 0.4 as the "observed ratio" between water and fish body was obtained when fish took these two minerals from water. Furthermore, the strontium-calcium observed ratio between marine fish and sea water was estimated using strontium-calcium ratio in several marine fishes and the values 0.2 to 0.3 were obtained in disregard of uptake from food. Consequently, the value of strontium-calcium observed ratio between fish body and environmental water also seems to be higher in case of fresh water, in other word, rate of discrimination is less in fresh water. This result coincides with the result of present investigation on the discrimination factor above discussed.

The amount of strontium and calcium, and the ratio in fresh water are quite variable according to the type of water such as ground water and tap water, and also to the sampling places.\textsuperscript{15) In the present experiment, accordingly, no stable strontium was added to the artificial fresh water except for that contained (0.016%) in the chemical reagent, CaCl\textsubscript{2}·2H\textsubscript{2}O. Further investigations, however, are being carried out on the behaviour of strontium and calcium in the gill absorption in case of fresh water eels, using various artificial fresh water medium containing different amount of strontium and calcium. It is necessary, furthermore, for the metabolic studies on strontium and calcium to investigate the behaviour of these minerals in such other important physiological processes as intestinal absorption, gill excretion, and urinary excretion. These investigations will be conducted in near future in our laboratory.

* Sr-Ca."observed ratio" is defined as below;

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O_A \text{ water-fish} = \frac{\text{Sr/Ca in fish}}{\text{Sr/Ca in water}}
\]

while, "Discrimination factor" is applied to a single physiological process such as intestinal absorption, renal excretion etc.
Summary

To investigate the behaviour of strontium and calcium at the gill absorption of fishes, gill-perfusing experiments were conducted using the fresh water or sea water adapted eel, *Anguilla japonica* and following results were obtained.

1) Discrimination against strontium in favour of calcium was observed at the gill absorption of these minerals.

2) The strontium-calcium discrimination factor (*DF<sub>gill absorptive</sub>*) expressing this discriminating rate was 0.74 in case of fresh water eel. In this case, the fresh water of external medium contained 100 mg. per 1000 ml. of medium.

3) In marine eels, on the other hand, this *DF<sub>gill absorptive</sub>* was found as 0.67 and this higher rate of discrimination than that observed in fresh water eels seems to be caused by the pretty high concentration of calcium and strontium in sea water. In this case the sea water of the external medium contained 498 mg. Ca and 12 mg. Sr per 1000 ml. of medium.

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References