METABOLISM OF RADIONUCLIDE IN FISH-II STRONTIUM-CALCIUM DISCRIMINATION IN GILL EXCRETION*

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Gills of fishes as well as digestive tract, play an important role in the absorption of minerals from the environmental water. Therefore, the authors have investigated the behaviour of strontium and calcium in the gill absorption of fishes and reported in the previous paper¹⁾ that strontium-calcium discrimination was different between fresh water and marine fishes.

On the other hand, it is well known that gills of fishes, besides kidneys, take a significant part in the excretion of minerals²⁾. In mammals there are many studies on the behaviour of strontium and calcium in the renal excretion.^{3), 4)} Few reports, however, have been presented in fishes, on these problems. It is necessary to investigate the behaviour of strontium and calcium in the branchial excretion for the understanding of overall behaviour of these minerals in fish body. Using the gill-perfusion technique, the authors investigated the discrimination rate of strontium and calcium in branchial excretion. Present paper is concerned with these results obtained.

Method

Japanese eel, *Anguilla japonica*, obtained through a fishmonger, was used in this study. Method for gill perfusion is illustrated in Fig. 1 and is the same substantially as described in the previous paper.¹⁾ This apparatus was modified from KEY's original and SCHIFFMAN's ones.^{5), 6)} In the following explanation, only the points differing from the previous method are described.

This apparatus consists of double perfusion system in which the internal perfusion medium enters into blood vessels at the bulbus arteriosus adjacent to the heart and is collected into a waste bottle via dorsal aorta, whereas the external perfusion medium is led into the buccal cavity and collected into a test tube via the branchial slit and polyvinyl tube which is connected to the slit. As the external perfusion medium, the artificial fresh water containing 10 mg. Ca per 1000 ml was used.^{***} As the internal perfusion medium, KEY's physiological saline solution,^{***} blood serum of horse (Ca; 9.8 mg %) or serum of eel (Ca; 13.5 mg %) were used. This serum of horse was obtained at the Tokyo Metropolitan Meat Inspector Office and that of eel was collected at Sanwa Fresh Water Fish Co. Ltd. These internal perfusion media

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*** Refer to the previous paper.¹⁾

were labelled with Sr-89 and Ca-45 (total activity; about 3×10^4 cpm/ml) and introduced into blood at the bulbus arteriosus via polyvinyl tube with the aid of pulsation of autodispenser. During the circulation in the blood vessels of gills, excretion of strontium and calcium from internal to external medium occurred at the gill membrane.

Fig. 1. Schematic view of perfusion apparatus.

The activity of Sr-89 and Ca-45 in the external perfusion medium collected successively in test tube, was measured separately using aluminium absorber. The ratios of Sr-89 to Ca-45 in test tube were compared with those in the internal perfusion medium. The strontium-calcium discrimination rate in the gill excretion ($DR_{\text{gill excretive}}$), defined as Sr/Ca in the external perfusion medium: Sr/Ca in internal perfusion medium, was calculated.

Results and Discussion

Firstly, gill-perfusing experiment was carried out on five eels, using KEY's physiological saline solution as the internal perfusion medium. The results obtained in this experiment are summarized in Table 1-A. In Fig. 2 is plotted the ratio of the activity of strontium-89 to that of calcium-45 in test tubes collected successively in one example, where the ratio of these activities (Sr-89:Ca-45) in the internal perfusion

Fig. 2. Ratio of Sr-89 to Ca-45 in test tube collected in the gill-perfusing experiment using KEY's physiological saline solution.

medium is 0.281. As shown in Table 1-A and in this Fig. 2, the values of ratio of the activity of Sr-89 to that of Ca-45 in the external perfusion medium are always below those in the internal perfusion medium. From these results, it can be said that in case of using the physiological saline solution as the internal perfusion solution, discrimination is observed between strontium and calcium and calcium is more easily excreted than strontium from the gills of fishes.

In the previous paper,¹⁾ authors reported that the strontium-calcium discrimination factor at the gill absorption was 0.74 in case of fresh water eel. Similar value was obtained in discrimination rate in the branchial excretion of these minerals. It can be stated, therefore, that calcium is absorbed and excreted more easily than strontium from gills of fishes. In this connection, the report of SINGER et al²⁾ is interesting, that calcium is more easily excreted than strontium in the endogenous excretion in large intestine of rat.

In case of using blood serum of horse, some different results were obtained. Fig. 3-A shows the result of one example among the five perfusion experiments performed with this blood serum. Discrimination rate between strontium and calcium in gill excretion in this experiment is given in Table 1-B. Mean value of this discrimination rate is 1.00 or so, in other words, no discrimination can be observed between these two minerals in gill excretion. Fig. 3-B shows one example from the results obtained in the gill perfusion experiments using blood serum of eel.

Table 1. Strontium-calcium discrimination rate in the gill excretion of Japanese eel, *Anguilla japonica*. Three kinds of media (A, B, and C) were used as the internal perfusion medium.

A) Physiological saline solution

Fish No.	Sr-89:Ca-45 in internal perfusion medium	Sr-89:Ca-45 in external perfusion medium	DR _{gill excretive}
1	0.281	0.195	0.69
2	0.226	0.181	0.80
3	1.080	0.741	0.69
4	0.711	0.533	0.75
5	0.701	0.548	0.78
Mean			0.74±0.05

B) Blood serum of horse

1	0.647	0.615	0.95
2	0.612	0.630	1.03
3	0.609	0.609	1.00
4	0.490	0.475	0.97
5	0.507	0.532	1.05
Mean			1.00±0.04

C) Blood serum of eel

1	0.475	0.437	0.92
2	0.473	0.405	0.86
3	0.648	0.575	0.89
4	0.640	0.585	0.91
Mean			0.90±0.03

Fig. 3. Ratios of Sr-89 to Ca-45 in test tube collected in the gill-perfusing experiments using blood serum of horse (A) and serum of eel (B).

Each 50 ml of this serum was used to one experiment and four perfusion experiments were carried out. The results obtained are listed in Table 1-C. In these experiments, mean values of $DR_{\text{gill excretive}}$ is obtained as 0.90, namely, calcium is a little more easily excreted from the gills of eels in case of using eel serum as the internal perfusion solution. Generally speaking, it is very difficult to obtain large amount of eel blood. Prior to this experiment, about 200 ml of blood serum of eel was collected through the kindness of Sanwa Fresh Water Fish Co., Ltd.

As already mentioned above, different values were obtained for the discrimination rate between strontium and calcium, in accordance with the difference of internal perfusion solution used. This difference seems to be ascribable to the different nature of calcium and strontium contained in each medium. In physiological saline solution, these two minerals are solved in ionic state. On the contrary, in the blood serum large part of these minerals is bound to plasma protein. According to SAMACHSON and LEDERER,⁸⁾ more calcium is bound to protein than strontium, i.e., more strontium remaining in a free form. Therefore, the result obtained by using blood serum of eel seems to be rather natural.

No report seems to be available on the behaviour of strontium and calcium in the gill excretion of fishes as well as in the renal excretion. As for the discrimination in the excretion of mammalian kidneys, however, many studies were reported. In the urinary excretion of rat, for example, strontium-calcium discrimination factor was reported by COMAR et al.³⁾ as 0.68 and 0.34 in animals fed with milk and commercial diet, respectively. Moreover, this discrimination factor (DF_{urinary}) in man was obtained by same authors⁴⁾ as 0.87. These values were calculated indirectly from the following formula.

$$DF_{\text{urinary}} = OR^*_{\text{body-diet}} : DF_{\text{absorptive}}$$

* Observed Ratio which is defined as Sr/Ca in body : Sr/Ca in diet. Refer to the previous paper¹⁾ for detail.

Its contribution to overall discrimination in these animals is important as much as that in intestinal absorption.

On the other hand, in fishes, as reported in the previous papers,^{9),1)} "Observed ratio" between environmental fresh water and fish body ($OR_{\text{fresh water-fish body}}$) was 0.4 in case of rainbow trout, *Salmo gairdnerii irideus*, and discrimination factor at the gill absorption was 0.74 in eel, *Anguilla japonica*. Therefore, it is conceivable that there are excretory organs in which large discrimination may occur in the behaviour of strontium and calcium, and strontium is excreted more abundantly than calcium. In the gill excretion, however, calcium is a little more easily excreted than strontium. Hence, calling attention to the kidney excretion should be important. According to BLACK,¹⁰⁾ in fish body the bivalent minerals such as calcium and magnesium are excreted mainly via kidney, whereas the monovalent minerals such as sodium and potassium are excreted through the gill membranes. Further investigation is being undertaken on the behaviour of strontium and calcium in the excretion from piscine kidneys.

Summary

Gill-perfusing experiment was carried out, using Japanese eel, *Anguilla japonica*, to investigate the behaviour of strontium and calcium at the gill excretion in fishes. As the internal perfusion solution, various media were used, such as KEY's physiological saline solution, blood serum of horse or that of eel, and the following results were obtained.

1) In case of using the physiological saline solution, the discrimination was observed between strontium and calcium, and calcium is more easily excreted than strontium at the gill excretion of these minerals. The discrimination rate ($DR_{\text{gill excretive}}$) was 0.74 in this case.

2) In case of using the blood sera of horse and eel, this rate of discrimination was 1.00 and 0.90, respectively. In the former case, in other words, no discrimination was observed between the behaviour of strontium and calcium. In the latter case, which corresponded to the natural condition of eel, calcium was excreted a little more easily than strontium.

3) Discussion was made on these various discrimination rates obtained in the perfusion experiments of gill excretion. Strontium-calcium discrimination in gill excretion does not seem to contribute much to overall discrimination between water and fish body.

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* Dr. ROSENTHAL found the Sr-Ca discrimination in fish in his recent work (*Proc. Soc. Exptl. Biol. Med.* **104**, 88-91, 1960). Therefore, the description in this report, that he found 'no discrimination, should be corrected.