Studies on the calcium uptake by teleost fishes

I. ^{45}Ca uptake by the crucian carp

By

Kikuya MASHIKO and Kenji JOZUKA

(Received March 10, 1962)

Recent studies utilizing the radioisotopes have indicated the fact that the bone-seeking elements, the calcium and strontium, are directly taken up from the environmental water by marine as well as fresh water fishes (ROSENTHAL, 1956, 1957, 1957a; BOROUGHS *et al.* 1956, 1957; TOMIYAMA *et al.*, 1956, 1956a). Before these works, however, the demonstration of this fact had been very difficult. SMITH (19291, 930) never admitted the calcium uptake by fishes across the gills, and KROGH (1938, 1939) saw only once Ca^{++} taken up with Cl^{-} by gold fish, but in most experiments Ca^{++} was not absorbed at all. More recently SCHIFFMAN (1961) showed by means of a perfusion method that both the in- and outflux of ^{85}Sr took place across the gills of rainbow trout. On the other hand, by the use of a divided chamber method, REID, TOWNSLEY and EGO (1959) concluded that ^{85}Sr can be uniformly taken up through the body surface of the fish.

The present writers have also carried out for the past several years various experiments on the calcium metabolism by fishes. The present report deals with the results obtained from the experiments on the crucian carp, including the internal distribution of ^{45}Ca taken up from the environmental medium as well as from injection, the effect of calcium concentration of the external medium on the uptake, the pathway of the uptake and output of calcium, and the types of ^{45}Ca incorporation.

The writers wish to express heartfelt thanks to Mr. H. SHIIRE, Mr. M, UESAKA, Mr. H. ANDO, Mr. S. MORITA, Mr. H. NIMURA and Mr. T. YAMABE for their careful laboratory assistance during the different phases of the work reported here.

Materials and methods

In the present study the crucian carp, *Carassius carassius* (L.), which were collected from Lake Kahoku-gata, a brackish water lake in the vicinity of the city of Kanazawa, were used. The salinity of the said lake water is, because of the inflow and stagnation of the sea water, variable, sometimes reaching 10 g/l or more at some localities. But the crucian carp, which is one of the commonest fishes found in Japanese fresh waters, is usually found in the areas of lower salinity of this lake (less than 500 mg/l in chlorinity).

In most experiments, diluted artificial sea water was used as a balanced solution in which the fish were placed, since the effects caused by the change of the calcium content of the medium had to be avoided as much as possible. The temperature of the aquarium was maintained at 20°±0.5°C. As a rule the fish weighing about 80 g were used in order to avoid the influence of age difference.

For analysis of radioactivity, the fish were washed with tap water before they were sacrificed and dissected. The intestine was opened and all the materials remaining in it were washed out. The tissues were dried at 110°C and weighed to 0.1 milligram. Then the tissues were ashed in nitric acid and the ashes were dried slowly, repeatedly diluted with distilled water in the course of drying. The radioactivity was then counted in a gas-flow counter or with G-M counter. The decay correction was made when necessary.

In most cases, the radioactivity is represented with the term of "relative concentration" (r.c.) which means

$$\frac{\text{cpm per 100 mg tissue in dry weight}}{\text{cpm per 1 ml of the medium in which the fish is placed}},$$

because the specific activity of ^{45}Ca used was variable and the counter employed was not always the same.

The ^{45}Ca used was obtained from the Oak Ridge National Laboratories in the form of $^{45}CaCl_2$.

Distribution of ^{45}Ca taken up from the external medium

Accumulation by various tissues. The experiment was made by placing the fish in 1/50 artificial sea water containing $^{45}CaCl_2$, 100 ml of the water per one fish being used as a rule. At the predetermined time intervals, the fish were killed, dissected and then the radioactivity of various tissues was determined.

In the observation of which the result is shown in Fig. 1, the fish of about 25 g in weight were used, and after 1/2, 1, 2, and 4 days, the measurements were made. In the case of Fig. 2, fifteen of the fish weighing about 80 g were used, and the measurements were made after 3, 6, 10, 20, and 30 days' immersion. Each point on the curve of Fig .2 represents the average of three fish, respectively.

As seen in these figures, the accumulation of ^{45}Ca is mostly observed in osseous tissues such as scales, gills, fins, ribs and vertebrae. The rate of accumulation by the ribs and vertebrae, however, is apparently slower when compared with that by the other osseous tisues. Generally the increasing rate of accumulation is particularly large for the two or three days, but the accumulation seems to continue in nearly all the osseous tissues during the period of 30 days. The amount of ^{45}Ca found in soft tissues such as intestine, muscle, and kidney is variable as already observed by many authors, but it is distinctly small.

In the experiment of this kind, the comparison in further details may be difficult, because each value after a different time interval is of different individuals. Generally speaking, however, the accumulation of ^{45}Ca is apparently large in the case of Fig. 1 as compared with that of Fig. 2. This is considered to be due to the fact that the accumulation of Ca is larger in younger fish than in older ones. Taking this into consideration, the fish weighing about 80 g were used as a rule in the subsequent experiments.

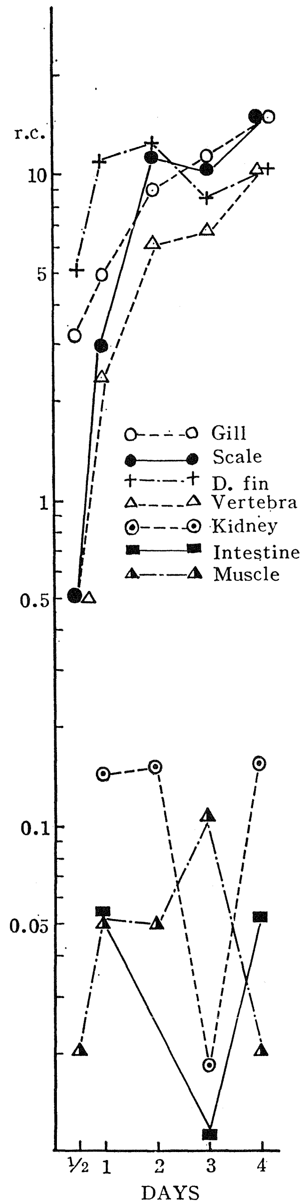

Fig. 1 Accumulation of ^{45}Ca by various tissues of crucian carp weighing about 25g. Accumulation (ordinate) is represented in the relative concentration (r. c.).

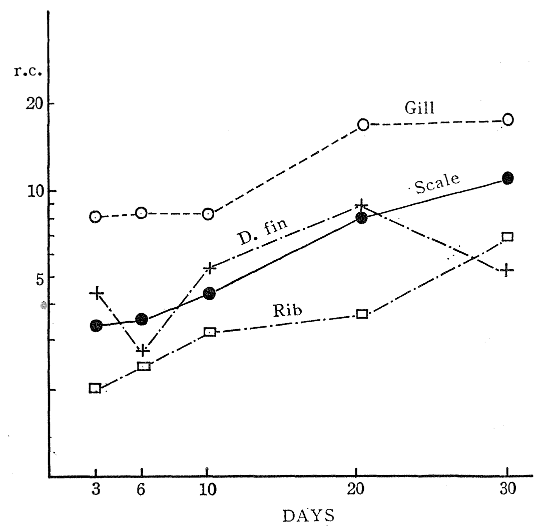

Fig. 2 Accumulation of ^{45}Ca by osseous tissues of crucian carp weighing about 80g. Accumulation (ordinate) is represented in the relative concentration (r. c.).

Accumulation of ^{45}Ca by the scale

Successive observation of the accumulation by the scale. For the purpose of avoiding individual differences, the writers made another observation on the successive ^{45}Ca accumulation by the scale from the environmental water. In this case, four fish weighing from 33.4 to 92.5 g were placed in 1/50 articicial sea water containing ^{45}Ca, and two scales, one from each side of a fish, were successively taken every 24 hours. The result of the measurements thus performed is as shown in Table 1 and Fig. 3. Each figure given in the table represents the average of two scales from one fish. The result is nearly similar to those shown in Figs. 1 and 2, except for the somewhat longer duration of the rapid increase period of the accumulation. In regard to the accumuation of ^{45}Ca by the scale, at least two types of incorporation must be considered generally to occur, *viz.*, the growth accumulation and the ion exchange occurring between the scale and the internal environment. The fact that in this experiment the ^{45}Ca accumulation is also observed to decrease with the age of fish may suggest that both the growth accumulation and the ion exchange can occur more actively in younger osseous tissues.

In addition, it may be noteworthy that the increasing rate of the ^{45}Ca incorporation appears rapidly to decrease after about five days in general as seen in Fig. 3. This phenomenon seems to suggest that the ion exchange occurring between the scale and the internal environment takes place more actively in the first several days. Further discussion will be given later.

Table 1. Successive measurement of the ^{45}Ca accumulation by the scales. Each figure in the table represents the average of two scales, one from each side of the same individuals.

No.	1		2		3		4	
Body wt. (g)	33.4		42.5		53		92.5	
Day	cpm	r. c.	cpm	r. c.	cpm	r. c.	cpm	r. c.
1	2,857	1.50	23,471	1.30	15,348	0.85	7,122	0.40
2	6,951	3.66	—	—	—	—	—	—
3	7,851	4.13	58,194	3.23	52,479	2.92	13,712	0.76
4	11,902	6.26	—	—	—	—	—	—
5	15,122	7.96	133,586	7.42	94,723	5.26	25,438	1.41
6	15,563	8.19	—	—	—	—	—	—
7	16,184	8.52	162,048	9.00	156,344	8.69	32,515	1.81
8	—	—	175,345	9.74	176,698	9.82	41,438	2.30
9	—	—	205,797	11.43	187,742	10.43	43,956	2.44
10	—	—	222,395	12.36	191,808	10.66	47,688	2.65
11	—	—	231,500	12.86	194,051	10.78	53,342	2.96
12	—	—	255,172	14.18	209,611	11.65	57,415	3.19
13	—	—	265,565	14.75	228,714	12.71	62,500	3.47
14	—	—	269,860	14.99	248,762	13.82	69,486	3.86
15	—	—	286,563	15.92	265,403	14.74	73,091	4.06
17	—	—	294,333	16.35	284,360	15.80	79,214	4.40
Medium cpm/cc	1,900		18,000		18,000		18,000	

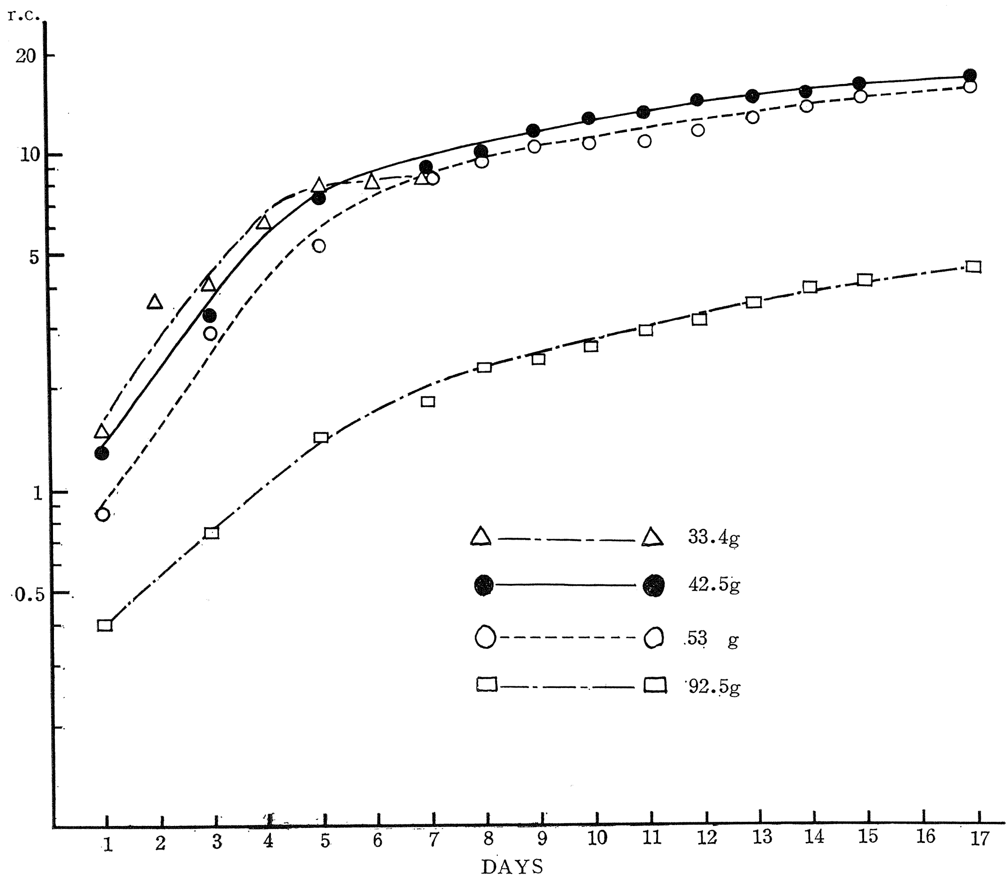

Fig. 3 Successive measurements of the ^{45}Ca accumulation by the scales. Each point in the figure represents the average of two scale, One from each side of the same individual.

Variation of accumulation by the scale. In order to see the extent of variation among the scales of a single individual, a measurement of ^{45}Ca accumulation was made of the scales collected from different positions of a fish body. The scales examined were taken from four positions, *i. e.*, a point near the operculum above the lateral line (anterior scale) on and a point near the caudal fin above the lateral line (posterior scale) on each body side. The result of measurement is as follows (in cpm per 100 mg) :

right anterior scale 5683 ; right posterior scale, 5438 ;

left anterior scale, 5525 ; left posterior scale, 5720.

From this result, the accumulation by the scales is considered to vary to some extent (up to 6%) among the scales of a single individual. The extent of variation, however, may not be so great as to have any significant influence on the discussions mentioned above and those that appear below.

Heavy accumulation of ^{45}Ca by the regenerating scale. In the present experiment, another noticeable phenomenon was that there was often found a number of scales which accumulated a particularly large amount of ^{45}Ca. By the use of the autoradiograph, it was clarified that such a heavy deposit of ^{45}Ca takes place in regenerating scales. Fig. 4 is an autoradiograph obtained by a regeneration experiment. The row of scales with heavy deposit seen above the lateral line shows the accumulation by the scales which are regenerating after the removal of the original scales. The scales with heavy deposit scattered in other areas seem to be those regenerating at the points where the original scales were accidentally torn off during the operation or rearing.

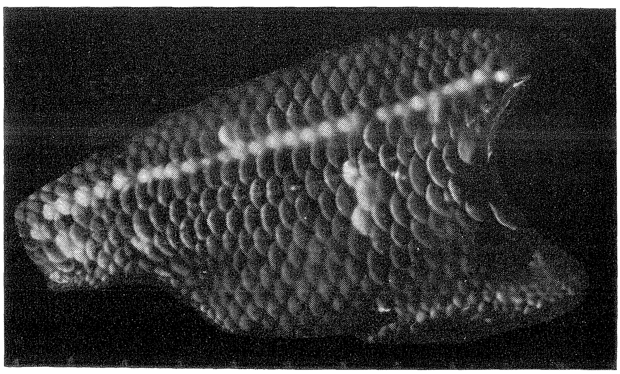

Fig. 4. Autoradiograph showing the heavy accumulation of ^{45}Ca by regenerating scales (10 days after the removal of original scales).

Patterns of ^{45}Ca accumulation by scales. The ordinary scale on the living animal, the regenerating scale, and the previously isolated scale are different from one another in the pattern of ^{45}Ca accumulation, showing their own special features respectively. In the ordinary scale on a living fish which has been placed in ^{45}Ca solution, the accumulation densely occurs in the posterior (exposed) part of the scale rather than in anterior (covered) part. In the regenerating scale of the early stage, the accumulation takes place nearly uniformly over the whole area, but, with the process of regeneration, the pattern of accumulation gradually becomes similar to that in the ordinary scale. On the contrary, when a scale is gently removed from a living fish and then immersed in the isotopic solution, the accumulation is observed to occur only in the anterior (covered) part but not in the posterior (exposed) part. This phenomenon is possibly due to the effect of the mucous layer (or cuticle) which covers the exposed part of the scale and make it impermeable to the calcium ion. The different types of the accumulation pattern may be apparently seen in the autoradiographs shown in Fig. 5.

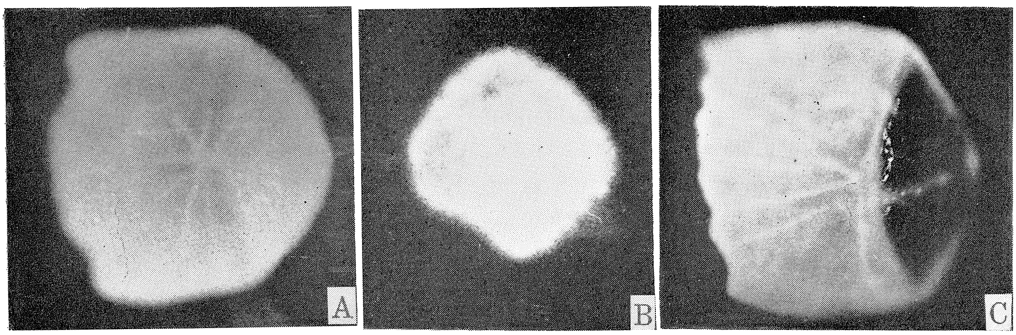

Fig. 5 Autoradiographs showing the patterns of ^{45}Ca accumulation in various scales. A, a normal scale of the fish which was placed in the isotopic solution ; B, regenerating scale of the same fish (10 days after removal of the original scale) ; C, scale immersed in the isotopic solution after isolation from a living fish.

Distribution of the ^{45}Ca injected

For the purpose of observing the behavior of ^{45}Ca in the fish body, 10 μc of $^{45}CaCl_2$ was injected into the abdominal cavity through the anus. After the injection the fish were washed with running tap water and were placed in filtered pond water which aerated and changed repeatedly throughout the experiment. After the intervals of 1, 2, 3, 6, 10 and 15 days, two or three fish were sacrificed, respectively. A total of sixteen fish were used.

The result obtained from this experiment are shown in Fig. 5. In this figure, for the convenience of eliminating the individual differences, the data are represented in percentages of radioacivity of the scales which were determined of the coresponding individuals after the first 24 hours. In this kind of experiment, the individual differences are to a certain extent unavoidable, but the differences seem to be successfully eliminated in this way as seen in the figure.

The pattern of distribution of ^{45}Ca in the fish body appears to be similar, both when the isotope was given by injection and when it was taken up from the external medium. As already pointed out by Boroughs and Reid (1958), this fact indicates that once the calcium is absorbed by the blood system, its internal distribution is the same. The amount of ^{45}Ca accumulation is seen to increase with time in all tissues nearly through the whole period of 15 days, indicating that the absorption in the addominal cavity is made gradually and continously. The rate of ^{45}Ca accumulation by osseous tissues may depend partly upon the blood supply and partly upon the accumulating power of the respective tissue. The accumulation by gill and scale is always most remarkable and that by the fin is slightly less conspicuous. The accumulation by the rib and the operculum is apparently low in its amount as compared with that by the above mentioned tissues, while the increasing rate is most remarkable in the rib and next in the operculum. In the case of the rib, for instance, the accumulation of ^{45}Ca after 15 days is as much as about seven times the value after 24 hours. This phenomennon may indicate that in these bones the accumulating power is high but the ^{45}Ca supply is low.

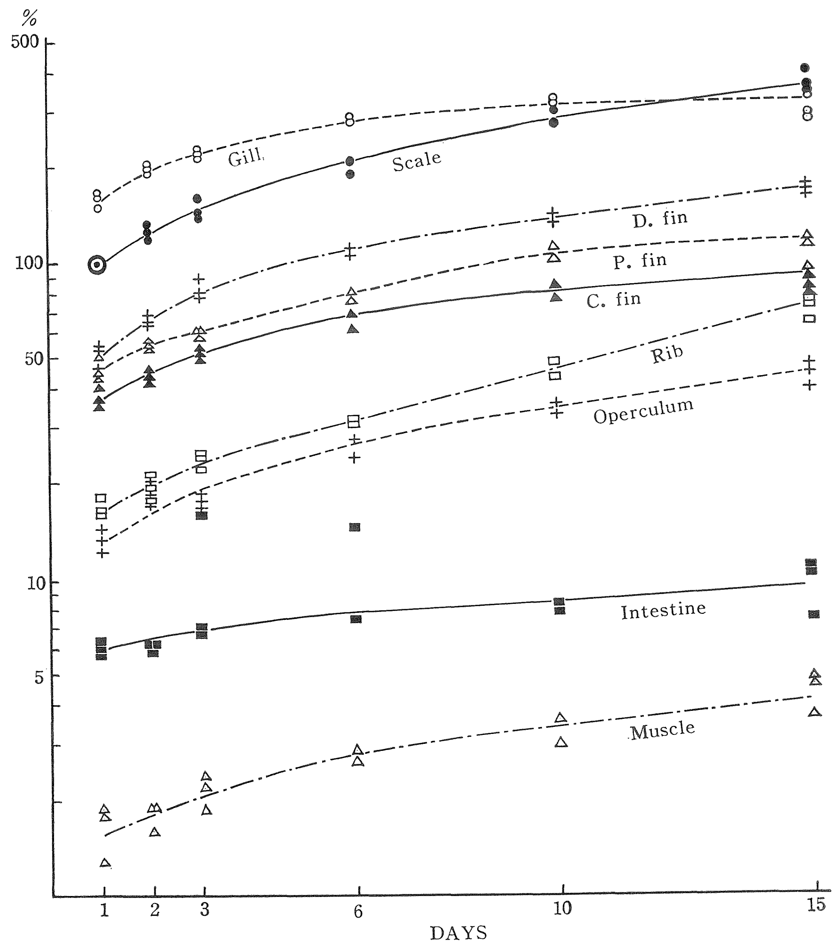

Fig. 6 Accumulation of the injected ^{45}Ca by various tissues of the fish weighing about 50 to 60 g. Accumulation of ^{45}Ca is represented in the percentage of the amount of ^{45} Ca accumulated by scales for first 24 hours.

The isotope absorbed in the abdominal cavity is considered to come first to the gills after passig the heart, and then to be distributed to the whole body through the dorsal aorta. The markedly rapid and heavy accumulation by the scales is noticeable, suggesting the fact that the scale plays as a reservoir of Ca an important rôle in the fish physiology.

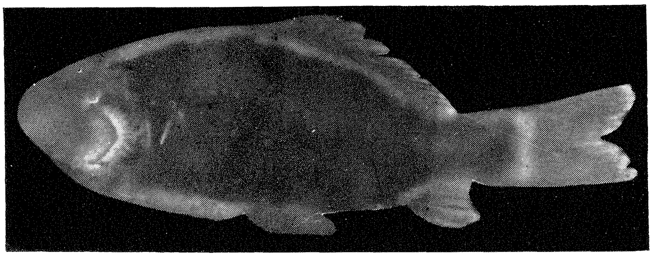

Fig. 7 Autoradiograph of a ^{45}Ca injected fish, the left side of the body being removed to expose the spine.

The effect of the Ca content of external medium on the Ca uptake

The present experiment was made for the purpose of observing the influence of calcium content of the external medium on the calcium uptake by the fish. The external media were prepared to contain various amounts of calcium, ranging from 0 to 110 mg/l, by adding $CaCl_2$ to the 1/50 Ca-free artificial sea water. Then $^{45}CaCl_2$ was added on each one so as to contain 3.5 mg/l of ^{45}Ca. Two to six fish were transferred to each medium, and after 7 and 10 days two scales were taken from each side of each fish and the radioactivity was measured. The scale was always taken at a point above the lateral line and below the anterior end of the dorsal fin.

The results obtained are as shown in Fig. 3. The ordinate and abscissa show respectively the r.c. value and the total calcium content inclucing both the radioisotope and the stable calcium. The amount of ^{45}Ca accumulation by the scale is seen to decrease rapidly with the increase of Ca content in the external medium. The accumulation of ^{45}Ca in the 110 mg/l Ca solution, for instance, is, after 7 days, about 1/14 of that in the 3.5 mg/l solution, and 1/8 after 10 days.

In this experiment, if both the calcium, the radioisotope and the stable, behave in the fish body in exactly the same way, the total amount of calcium incorporation can be calculated from the proportion of ^{45}Ca to the total calcium. The result thus obtained by the calculation is shown in Fig. 4. According to these two curves, the calcium accumulation by the scale is considered to be constant and independent of the calcium content of the external medium when the latter is more than 20 mg/l.

Rosenthal (1956) showed the data on the calcium uptake by *Lebistes* from the external water which contained various amounts of calcium ranging from about 20 to 150 ppm. He mentioned that the increase of the inactive calcium concentration decreases the rate of incorporation of ^{45}Ca by the total body of the fish in a manner consistent with a logarithmic function. Using his data, the writers made the calculation of the total calcium incorporation and obtained a result roughly similar to their own. This phenomenon seems to indicate that both the ion exchange and calcium deposit do not increase with the calcium concentration of the environmental water when the calcium content reaches a certain extent.

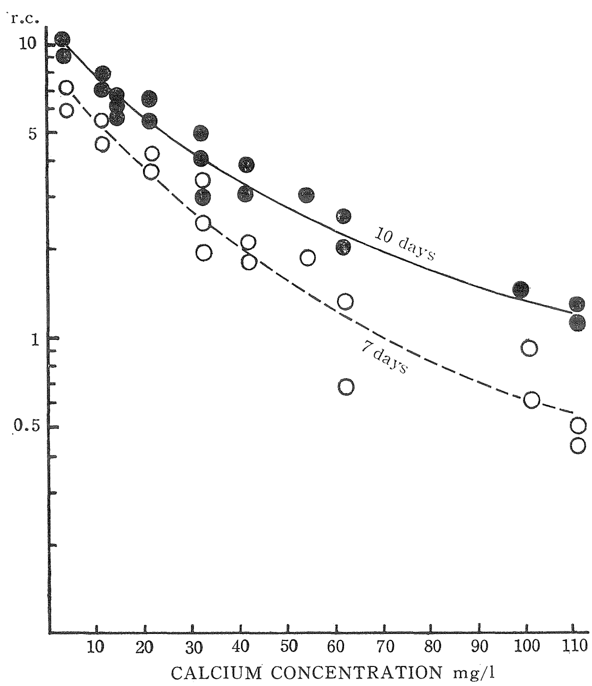

Fig. 8 The effect of the calcium content of the external medium on the ^{45}Ca incorporation into the scale. Abscissa: calcium content of the external medium including the isotope and the stable calcium, the amount of ^{45}Ca added being always 3.5 mg/1. Ordinate: relative concentration (r. c.).

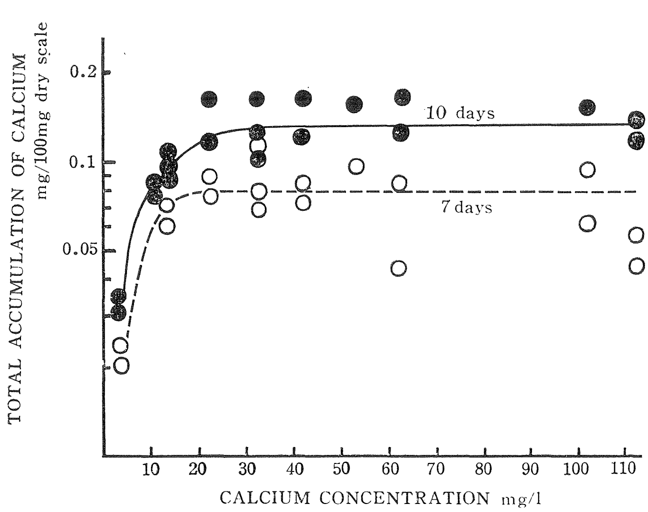

Fig. 9 The effect of the calcium content of the external medium on the total calcium incorporation into the scale. Abscissa: calcium content of the external medium, including the isotope and the stable. Ordinate: total calcium incorporation obtained by the calculation (per 100 mg dry weight of the scale).

Pathways of the Ca influx

The gills have been generally regarded as the most important of the pathways of calcium influx. Recently, SCHIFFMAN (1961) showed by means of a perfusion method that both the in- and outflux of ^{85}Sr took place across the gills of rainbow trout. As the chemical behavior of strontium is nearly similar to that of calcium in the animal body, the fact shown by SCHIFFMAN may also be true in the case of calcium. This is not, however, the whole picutre on the calcium influx in fish, because in his experiment the accumulation in fish body is not taken into account and some pathways other than the gills can be considered. The writers made the present experiments so as to observe some possible ways for calcium influx other than the gills.

Experiment A. The experiment was made by the use of a kind of divided chamber. As shown in Fig. 10, the posterior half of the fish body was separated from the anterior half, the former being enclosed with a rubber sac. The rubber sac was filled with 100 ml of 1/50 Ca-free artificical sea water containing 25 μc of ^{45}Ca, and then the fish was immersed in 2000 ml of 1/50 Ca-free artificial sea water to which $CaCl_2$ was added in order to bring the Ca content to 10 mg/l. Then the posterior half of the fish body was directly exposed to the isotope. After the immersion of 3, 6, and 24 hours, the fish were killed and the radioactivity of various tissues was determined.

Experiment B. The same apparatus was used and 250 μc of ^{45}Ca was added to the external medium. Consequently, the anterior half of the fish body was exposed to ^{45}Ca solution. After 6 hour immersion the radioactivity of tissues was measured.

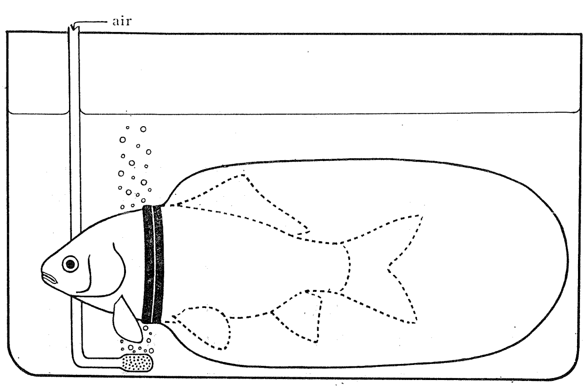

Fig. 10 Diagram showing the experimental fish with a rubber sac which separates the posterior medium from the anterior.

The results obtained are as shown in Table 1 and Figs. 10 and 11. In the Exp. A, a remarkable accumulation of ^{45}Ca is observed in the dorsal and caudal fins which were directly exposed to the ^{45}Ca solution, whereas the accumulation in the scale of the posterior body (posterior scale) is rather small in spite of the direct exposure. The uptake by the scale of the anterior body (anterior scale) is still poorer or even unrecognizable, and those by the gills and the pectoral fin are also apparently small.

In Exp. B, a marked accumulation is observed in the gills and that by the pectoral fin is also remarkable. It may be noteworthy that the accumulation by the scale is remarkably large as comparared with those of Exp. A, not only in the anterior part but also in the posterior part which was not directly exposed to the ^{45}Ca solution in this experiment.

These results suggest the following facts: 1) the major influx of calcium takes place across the gills and the calcium thus taken up is transported by the blood to other tissues such as scale, fins, bones, etc. ; 2) the fin areas also permit the Ca influx, which, however, is smaller in amount as compared with that across the gills ; 3) practically all of the ^{45}Ca found in scales may be that transported by the blood system from the gills, fins, or some other limited areas.

The fact that the accumulation of ^{45}Ca by the posteior scales in Exp. A is very small, in spite of the direct exposure, may be explained by the smaller supply of ^{45}Ca coming only through the dorsal, caudal and ventral fins. On the other hand, far larger amounts found in both the anterior and posterior scales in Exp. B may prove the fact that the ^{45}Ca supply through the gills is large enough to be distributed nearly over the whole surface of the fish body. The results obtained by the injection method described above may also indicate such a transportation.

Table 2. Accumulation of ^{45}Ca by various tissues through the posterior half of the crucian carp. Figures in the table represent the r. c. value.

Exposure to ^{45}Ca	Anterior		Posterior						
Time of exposure (hr.)	6		3		6		24		
No. of specimen	1	2	1	2	1	2	1	2	3
Anterior scale	0.17	0.30	0.003	0.005	0	0	0.007	0.06	0.03
Gill	0.78	1.37	0.006	0.003	0.006	0.06	0.05	0.14	0.04
Operculum	—	—	0.001	0.001	0	0	0	0.01	0.01
Pectoral fin	0.43	0.29	0.004	0.002	0	0	0.02	0.01	0.004
Posterior scale	0.02	0.11	0.01	0.01	0.03	0.02	0.08	0.15	0.04
Dorsal fin	0.01	0.11	0.06	0.11	0.22	0.23	3.30	2.39	3.93
Ventral fin	—	—	0.04	0.03	—	—	—	—	—
Anal fin	—	—	0.01	0.05	—	—	—	—	—
Caudal fin	—	—	0.004	0.02	0.12	0.20	0.07	0.82	0.11
Rib	0.003	0.06	0	0.002	—	0	—	—	—
Intestine	0.06	0.07	—	—	—	—	—	—	—

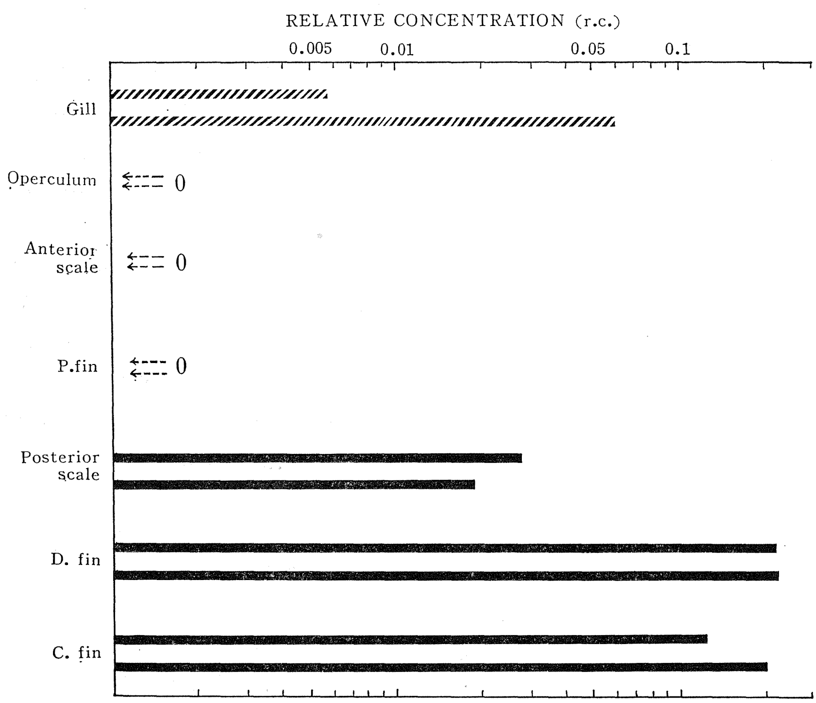

Fig. 11 Accumulation of ^{45}Ca by various tissues through the posterior half of the fish body (6 hours). The accumulation is represented in the logarithm of r.c. value.

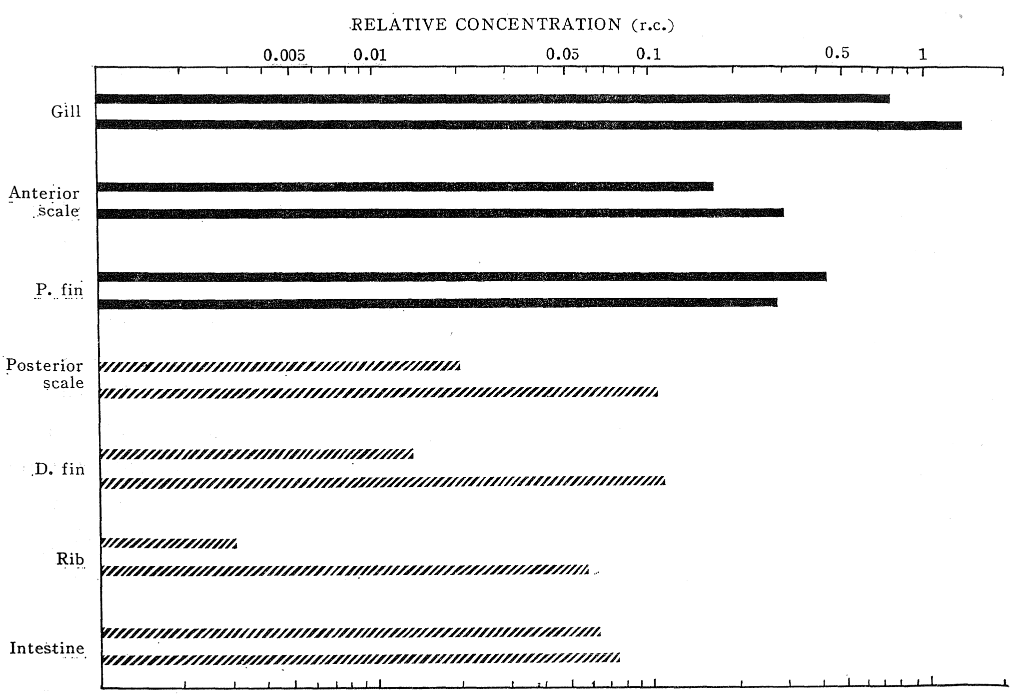

Fig. 12 Accumulation of ^{45}Ca by various tissues through the anterior half of the body (6 hours). The accumulation is represented in the logarithm of r.c. value.

Excretion of ^{45}Ca

The same method as shown in Fig. 9 was used. Three experimental fish were previously immersed for three or seventeen days in 1/50 artificial sea water containing ^{45}Ca. The fish which thus had taken up ^{45}Ca from the surrounding water were transferred into the tap water for 24 hours, the water being renewed repeatedly. Then the poserior half of each fish was enclosed with a rubber sac filled with 100 ml of 1/50 artificial sea water, and the fish was placed in 2000 ml of the same solution. In one of the three cases, the medium was prepared to contain Ca of 60 mg/l by adding $CaCl_2$ in order to observe the effect caused by different contents of calcium in the environmental medium. After 20 hours, 5 ml of the medium was taken from the inside and the outside of the sac, respectively, and the radioacivity was determined. The result thus obtained is shown in Table 3.

Table 3. Excretion of ^{45}Ca by anterior and posterior halves of fish (represented in count per minute).

No.	1	2	3
Body wt. (g)	44.7	42.5	53
Time of immersion in ^{45}Ca (day)	3	17	17
Ca conc. of medium (mg/1)	60	7.6	7.6
Total amount of ^{45}Ca excresed			
Ant. chamber	30,900	165,200	212,400
Post. chamber	37,260	154,170	193,560

In this table, a part of calcium is seen to be excreted through the anterior half of the fish body. According to the mean value in this table, the anterior excretion reaches 50% of the total excretion. No recognizable effect on the excretion caused by the different concentrations of calcium in the environmental media is to be found. In the present experiment, the difference of volume between the internal and the external media of the sac is considerably large, so that a certain extent of error is to be expected for the results obtained by the calculation. However, it cannot be doubted that considerable amount of calcium is excreted through the anterior part of the body. As already mentioned, Schiffman demonstrated the in- and outflux of ^{85}Sr across the gills of a rainbow trout. Accordingly, the excretion of calcium across the gills of crucian carp may also be expected to take place in natural condition. In addition, possibly, the outflux of calcium across the fins can also be expected.

By means of the divided chamber method, Smith (1929) found in his experiment on a large carp of 1.5 kg that the loss of calcium for 6 hours was 153 μM in the front chamber and 323 μM in the back chamber. (The amount of calcium excreted has been recalculated to total quantities by the writers.) It can be seen that there is not a wide discrepancy in proportion between the data shown by Smith and the writers. Although SMITH did not yet believe the calcium escapes in significant quantity from the body by way of the gills, KROGH (1939) mentioned that the results given by SMITH show that in a starving fish a loss of salt must take place continuously not only through the urine, but also by diffusion through the surface including the gills.

Discussion

The pathways of calcium uptake. REID *et al.* (1959) observed the internal distribution of ^{85}Sr accumulated by *Tilapia mossambica* from the external media, using a compartmentalized tank which made possible the separation of the posterior body from the head and the gills. *T. mossambica* used is a euryhaline fish which can adapt itself to marine as well as fresh water environments. In their paper they concluded as follows: "The rate of accumulation of isotope taken in through the integument exposed to the isotope in the front compartment equals that taken in through the posterior integument. The total area of all epithelia, *i.e.* skin, mouth, gills, exposed in the front compartment measured approximately the same as the area of the skin exposed in the rear compartment. This means that the integument of *Tilapia* can be considered as being uniformly permeable to strontium and calcium ions, contrary to the findings of CHIPMAN with the tuna skin *in vitro.*"

The conclusion of these authors does not agree either with the result obtained by the present writers. It may be, however, very difficult to consider that the integument of fish is uniformly permeable to strontium and calcium ions as concluded by REID and others. Most of ^{45}Ca accumulated by scales must be considered to be supplied through the blood system, but not to be taken up directly across the cuticle of the body surface. As already mentioned above and also as pointed out by BOROUGHS and REID (1958), the pattern of distribution of ^{45}Ca in the fish body appears to be similar, both when the isotope was given by injection and when it was taken up from environmental medium, but no particular accumulation by scale can be seen in the case of uptake from envionmental medium as compared with that in the case of injection.

The result of the writers' experiment using the rubber sac may also show that the ^{45}Ca influx practically does not take place across the general body surface. In addition, as already mentioned, the pattern of accumulation by the scale of intact animal is apparently different from that by the previously isolated scale (Fig. 5). Considering from these facts, the present writers are of the opinion that the uptake of ^{85}Sr and ^{45}Ca through the posterior body as observed by Reid and others is probably that which takes place across the fin areas.

There is another very noteworthy fact which is found in the data given by

Reid and others. Seeing the table shown by them as the data of the representative fish, an apparent difference is found between the fresh water adapted fish and the sea water adapted one. In the fresh water adapted fish, the amount of accumulation through the front body is seen to be roughly three times as much as that through the posterior body, while both are nearly the same in the sea water adapted one. The present writers have also made an experiment on the ^{45}Ca uptake by a marine teleost, *Duymaeria flagellifera*, using the same method as that shown in the present paper (Mashiko and Jozuka, 1961). In that case, the ^{45}Ca accumulation was nearly the same in amount, both when the posterior half was exposed to ^{45}Ca solution and when the anterior half was exposed to it. It is a very interesting fact that the result obtained by the writers for this marine fish is similar to the data of Reid and others for the sea water adapted *Tilapia*. Unlike the conclusion of these authors, however, the present writers are of the opinion that this difference between the fresh water fish and the marine fish may be due to the difference of permeability of the gill surface and probably also of the fin.

Types of incorporation of ^{45}Ca into the osseous tissues. In regard to the incorporation of ^{45}Ca into the osseous tissues of fishes, four types may be considered. i) Ion exchange. As already mentioned, when a scale is placed in ^{45}Ca solution, a rapid incorporation of ^{45}Ca takes place. This is obviously caused by the ion exchang. It is naturally expected that such an ion exchange should occur between the osseous tissues and the internal environment. The rate of incorporation appears to be remarkably large in the early period of exposure to ^{45}Ca solution and gradually to fall with time. ii) Growth accumulation. The growth accumulation of calcium can be theoretically calculated by the use of the following representative data in the writers' study : the specific activity of ^{45}Ca being 1.3 mc/g, 1 μc corresponding to 100,000 count per minute in the gas-flow counter. Assuming that the scale increases the weight by two times a year, and the calcium content of scale is 10 per cent. of the total weight, the increase of calcium content per day is approximately 27 μg per 100 mg of scales. The isotopic solution used was prepared to contain ^{45}Ca and stable Ca in proportion as 13.7 : 7.2. Hence, the growth accumulation is approximately 25,000 cpm/day/100 mg of scales. This value seems to be more or less too larger as compared with results obtained by the writers in the above mentioned experiments. As a matter of course, however, the accumulation by the scale does not always take place uniformly, because there are various physiological factors affecting the calcium accumulation. It must also be taken into consideration that all of the writers' experiments have been carried out under the starving condition, because the dissolution of the scale calcium caused by starvation has been observed in the carp and the gold fish (Ichikawa, 1953 ; Yamada, 1956, 1961). The result of the above calculation, therefore, shows only the calcium accumulation under the ideal condition, indicating that the growth accumulation reaches a considerable amount. iii) Regeneration. As shwon in Fig. 5, a particularly heavy deposit of ^{45}Ca is observed in the regenerating scale. The results obtained from regeneration experiments indicate that the rate of accumulation is most remarkable about 16 days after the removal of the original scale. iv) Calcium reserve. The scale can be considered to play an important rôle as a reservoir of calcium as bones generally do. The remarkably rapid accumulation of ^{45}Ca and the formation of concentric ridges by scale may suggest the possibility of this function. The above mentioned findings of Ichikawa and Yamada concerning the dissolution of the scale calcium caused by starvation seem also to support this assumption.

Of the four types mentioned above, the ion exchange is an inorganic incorporation accompanied with no calcium increase, and it is not directly affected by the physiological conditions. The other three types are the biological accumulation, and their significance may vary according to conditions. As seen in Fig. 3, the incorporation of ^{45}Ca into the scale is observed to change its rate about five days after immersion. It seems, in consequence, to be probable that the ion exchange plays especially an important part during the first several days. In the same figure, we can see that the younger the fish, the greater the rate of calcium accumulaiton. This may suggest that both the ion exhange and the growth accumulation take place more readily and actively in younger fish than in older ones.

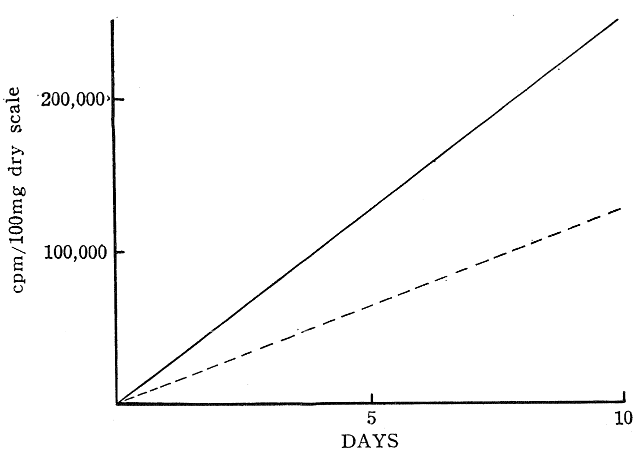

Fig. 13 Theoretical accumulation of calcium caused by the growth of scale.

——, indicates the increasing of calcium in the case of growth by 2 times a year;
······, indicates that in the case of growth by 1.5 times a year.

Summary

1. Using the radioisotope ^{45}Ca, the writers pursued their studies on the accumulation of calcium by the crucian carp, *Carassius carassius* (L.), including the pathways of calcium flux

2. The calcium uptake from the environmental medium by various tissues was

measured during the period from 12 hours to 30 days. The ^{45}Ca taken up is rapidly accumulated by osseous tissues such as scale, gill, and fin. The accumulation by bones such as rib, vertebra, and operculum is relatively small in amount, but the increasing rate is greater when compared with that of the above mentioned tissues. It may be explained that the ^{45}Ca supply by the blood system in these bones is not so sufficient as in the osseous tissues of scale, gill, and fin, but the capacity of accumulation is even greater.

3. The accumulation by scales of the same individual was observed continuously for the periods of seven and seventeen days. The accumlation is found to change its rate about five days after the immersion in ^{45}Ca solution.

4. The ^{45}Ca injected into the abdominal cavity is found to be transported by the blood system and mainly distributed in osseous tissues in quite a similar manner to that taken up from the external medium.

5. The regenerating scale accumulates a remarkably large amount of ^{45}Ca both in the case of uptake from the environmental medium and in the case of injection.

6. The incorporation of ^{45}Ca into the osseous tissues decreases with the increase of the concentration of stable calcium in the external medium. On the other hand, the incorporation of calcium as a whole, including the isotope and the stable, is caluculated to be constant and independent of the total concentration in the external medium when the latter becomes more than 20 mg/l. At least two types of processes, the ion exchange and the growth accumulation, may be considered to occur in this case. At present, it is difficult to distinguish the one from the other.

7. Beside the gills, the fins are also found to play an important rôle as the pathway of calcium influx. The experiment was made by means of enclosing the posterior half of the fish body with a rubber sac. Most of the body surface of the fish, excluding the gills and fins, is considered to be practically impermeable to the calcium ion.

8. A considerable amount of calcium, possibly about half of the total excretion, is excreted through the anterior part of the fish body.

9. In regard to the ^{45}Ca incorporation into the osseous tissues, four types may be considered, *i.e.*, ion exchange, growth accumulation, regeneration, and calcium reserve.

10. The ion exchange seems generally to occur most actively during the first five days or so. Both the ion exchange and the growth accumulation seem to take place more readily in younger fish than in older ones.

ADDENDUM While the present paper was in press, the writers performed further experiments on the absorption and excretion of ^{45}Ca by the crucian carp. 1) The caudal fin was enveloped with a rubber sac filled with medium containing ^{45}Ca.

Three hours later, the evident accumulation of the isotope was found in various osseous tissues such as the gills, the fins, and the scale. 2) $^{45}CaCl_2$ solution was injected into the dorsal muscle of a fish whose caudal fin was similarly enveloped with the rubber sac. After 24 hours, a considerable amount of ^{45}Ca was detected in the inner medium of the rubber sac. 3) When $^{45}CaCl_2$ was injected into the dorsal muscle, the isotope was remarkably found after 2 hours in the content of the alimentary canal, and after 6 hours much larger amount was measured.

These results indicate that both the in- and outflux of calcium undoubtedly take place across the fins, and that through the intestinal epithelium the excretion of calcium is actually done as well.

References

BLACK, V. S., 1957 : Excretion and osmoregulation. M. E. BROWN, ed. *The physiology of Fishes.* I : 163–205. N. Y., Acad. press.

BOROUGHS, H., 1957 : The metabolism of radionuclides by marine organisms. III. The uptake of calcium^{45} in solution by marine fish. Limnol. and Oceanogr., 2 : 28–32.

BOROUGHS, H., S. J. TOWNSLEY and R. W. HIATT, 1956 : The metabolism of radionuclides by marine organisms. I. The uptake, accumulation, and loss of strontium^{89} by fishes. Biol. Bull., 111 : 336–351.

BOROUGHS, H., and D. F. REID, 1958 : The role of the blood in the transportation of strontium^{89}–yttrium^{90} in teleost fish. Biol. Bull., 115 : 64–73.

ICHIKAWA, R., 1953 : Absorption of fish scale caused by starvation. Rec. Oceanogr. Works in Japan, 1 : 101–104.

KROGH, A., 1938 : The active absorption of ions in some fresh water animals. Z. vergl. Physiol., 25 : 335–350.

——, 1939 : *Osmotic regulation in aquatic animals.*, Cambridge Univ. Press, London.

MASHIKO, K. and K. JOZUKA, 1961 : Pathway of Ca uptake and excretion by teleost fishes. Jap. Journ. Linmol., 22 : 217–224.

REID, D. F., W. T. EGO, and S. J. TOWNSLEY 1958–1959 : Ion transfer through epithelia of fresh and sea water adapted *Tilapia mossambica.* Ann. Rep. Univ. Hawaii U. S. Atomic Energy Comm. of Contract No. AT (04–3)–56. 27.

REID, D. F., S. J. TOWNSLEY and W. T. EGO, 1959 : Ion exchange through epithelia of fresh and sea water adapted teleost studied with radioactive isotopes. Anat. Rec., 134 : 628.

ROSENTHAL, H. L., 1956 : Uptake and turnover of calcium-45 by the guppy. Science, 124: 571–574.

——, 1957 : Uptake of calcium-45 and strontium-90 from water by fresh water fishes. Science, 126 : 699–700.

——, 1957 a : The metabolism of strontium-90 and calcium-45 by *Lebistes.* Biol. Bull., 113 : 442–450.

——, 1960 : Accumulation of strontium-90 and calcium-45 by fresh water fishes. Proc. Exp. Biol. Med., 104 : 88–91.

SCHIFFMAN, R. H., 1961 : A perfusion study of the movement of strontium across the gills of rainbow trout (*Salmo gairdnerii*). Biol. Bull., 120 : 110–117.

SMITH, H. W., 1929: The excretion of ammonia and urea by the gills of fish. J. Biol. Chem., **81**: 727–742.

——, 1930: The absorption and excretion of water and salts by marine teleosts. Ann. J. Physiol., **93**: 480–505.

TOMIYAMA, T., S. ISHIO and K. KOBAYASHI, 1956: Absorption of dissolved ^{45}Ca by *Carassius auratus*. Res. Eff. Infl. Nuc. Bomb Test Expl., 1151–1156.

——, 1956a: Absorption of dissolved ^{45}Ca by marine fishes. *ibid*., 1163–1167.

TOMIYAMA, T., K. KOBAYASHI and S. ISHIO, 1956: Absorp tion of ^{90}Sr (^{90}Y) by carp. *ibid*., 1181–1187.

YAMADA, J., 1956: On the mechanism of the appearance of the scale structure. VI. some observations associating with the absorption of scale in the gold fish. Bull. Fac. Fish. Hokkaido Univ., **7**: 202–207.

——, 1961: Studies on the structure and growth of the scales in the gold fish. Mem. Fac. Fish. Hokkaido Univ., **9**: 181–226.