The uptake of Radionuclides by Molluscs, with special reference to Limnaea species

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THE UPTAKE OF RADIONUCLIDES

BY

MOLLUSCS, WITH SPECIAL REFERENCE TO LIMNAEA SPECIES

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* Now at Manchester University.

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INTRODUCTION

The aim of this research project was to investigate the uptake of radiostrontium and radiocalcium, primarily by Limnaea species, under varying calcium ion concentrations in the external aquatic environment.

The reasons for studying the fission products 89 Sr and 90 Sr are,

(i) that almost nothing is known about the metabolism of these radionuclides by fresh water molluscs,

(ii) that these fission products are known to be potential hazards from the public health point of view,

(iii) that the possibility exists that these fission products have potential ecological effects,

(iv) that molluscs may act as biological indicators, and

(v) that strontium may act as an indicator for calcium.

90 Sr has a half life of 28 years and this means that a slow rate of turnover may result in radiation exposure for the entire life of the organism. 89 Sr has a half-life of 51 days, so that it will take almost a year (six half lives) before 98.5% of the activity has decayed. In the present work, 85 Sr and 47 Ca were used because they are gamma-emitting isotopes, and because of this, they can be measured a number of successive times. This is not so with 89 Sr and 90 Sr, which are both beta-emitting isotopes, and whose use therefore involves killing in order to extract the

isotope. It is assumed that the uptake of 85 Sr and 47 Ca is similar to that of 89 Sr and 90 Sr.

The Phylum Mollusca are ideally suitable for investigating the uptake of radionuclides, since they have two distinct parts to their organism - the shell and the body proper (head, foot, mantle and viscera). An aquatic environment provided a less variable habitat than a terrestrial environment, so water snails were chosen in preference to terrestrial species.

The molluscan species studied were Bithynia tentaculata (Linn) and Viviparus viviparus (Linn) of Prosobranchiata; and Limnaea stagnalis (Linn), Limnaea auricularia (Linn), Limnaea pereger (Mull) and Limnaea truncatula (Mull) of Pulmonata.

MATERIALS AND METHODS

The freshwater species of gastropods were obtained from the Shropshire Union Canal at Chester (396673, 396675) page 7 , except for Limnaea truncatula which was obtained from I.C.I., Wilmslow, Cheshire.

The experiments were carried out in (a) unfiltered and filtered canal water and (b) aerated and non-aerated distilled water containing amounts of calcium varying from 0-600 ppm Ca^{++}. (Figures 1a and 1b). The calcium ion concentrations were obtained by addition of suitable amounts of filtered saturated 'Analar' calcium sulphate solution, which had been made up for at least 10 days. Aeration was supplied by a Hy-Flo diffuser pump, as shown in Figure 1a. Continuous flow experiments (c) were also performed (i) using the same solution and (ii) changing the solution at known intervals of time, in each case using a range of calcium concentrations, and (d) humidity experiments were performed in a thermostatic bath at 18°C, with fed and injected snails. The initial experiments were with unfed snails but later were studied with fed as well as unfed snails.

In experiments in which the snails were fed, the source of the food was Elodea callistroides (from L. Haig, Newdigate, Surrey), Elodea canadensis (Michx) from the Shropshire Union Canal, Chester, Nasturtium officinale x microphyllum (Watercress), from a stream Ruthin (186582) which contained Limnaea pereger, and Lactua sativa (Linn) the garden lettuce.

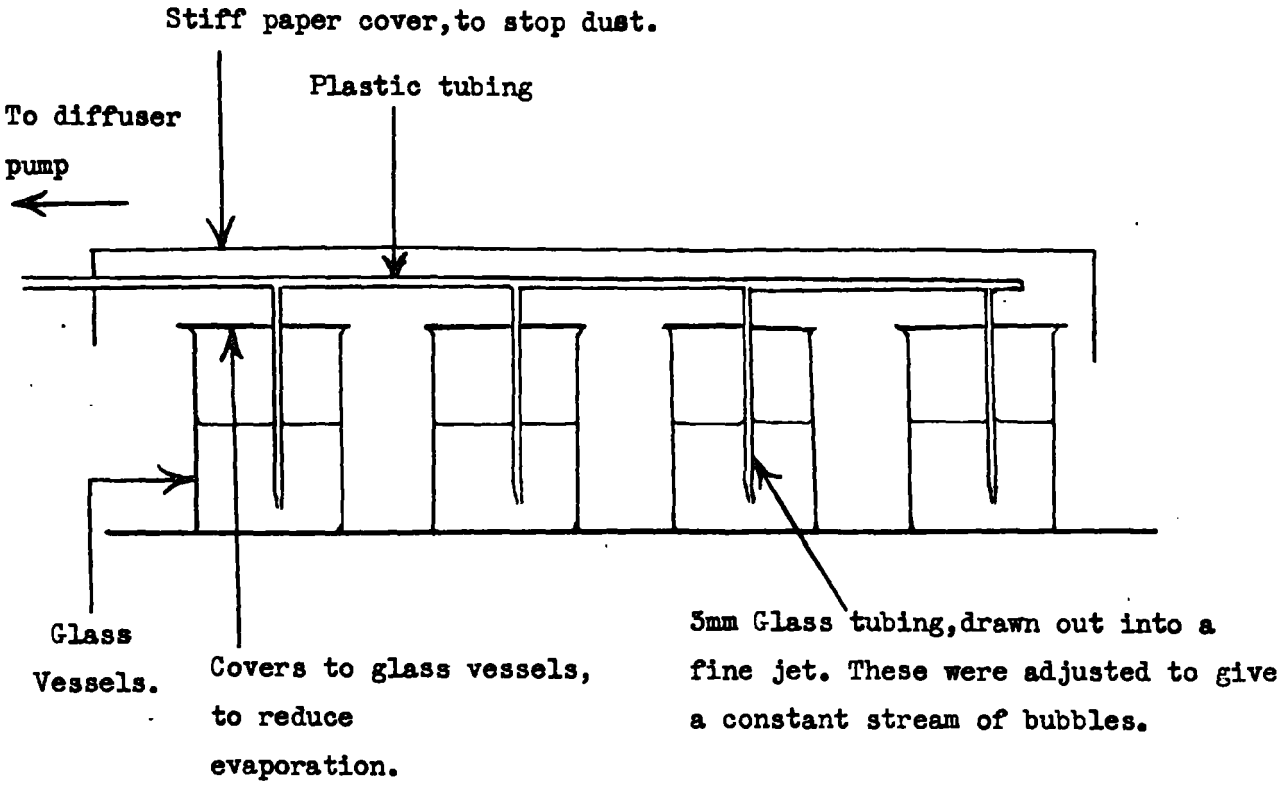


Figure. 1 a.

Arrangement of experimental vessels - Aerated condition.

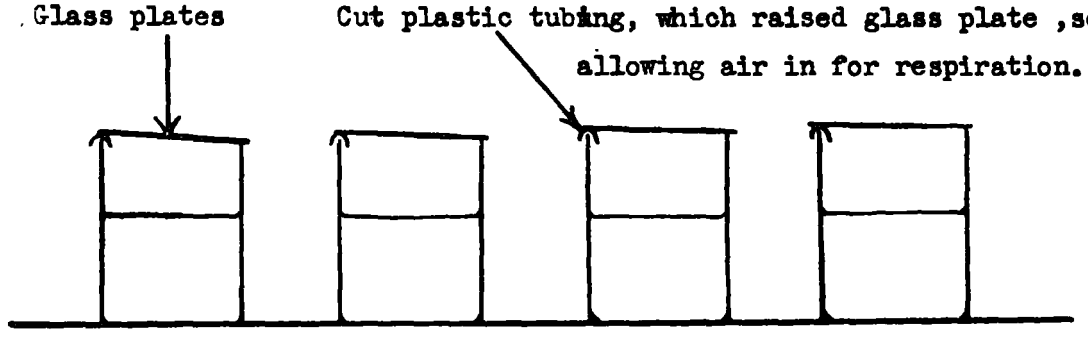


Figure. 1 b.

Arrangement of experimental vessels - Non-aerated condition.

Any dead snails or bleached or discoloured plant material in the experiments, were removed immediately and the pH of the solution determined to see whether it could be used or discontinued. The pH of the experiments was determined with B.D.H. Universal Indicator and pH papers, and the pH of the solution containing the snails or plants, was determined every time a snail or plant was sampled for breakdown. The pH varied throughout the experiments from pH 6.5-7.0.

Laboratory conditions were used with temperatures varying from 18-22°C, and the lighting was mainly diffuse daylight supplemented by artificial lighting. A series of experiments were performed in the dark to see whether uptake over a wide calcium range would be influenced by darkness (see page 180).

Borosilicate glass, silica, polythene and polystyrene apparatus was used throughout the experiments.

In addition to taking samples for radioactivity measurement, a 10 ml. sample was taken for calcium ion determination every time a snail or plant was used for breakdown, into a 250ml. flask. The calcium concentrations were determined by a standard titrimetric procedure using ethylenediamine tetraacetic acid (EDTA) solution with Patten and Reeder's (calcein) indicator. (Vogel 1961; Lucas & Pickering 1962). This indicator gave a clearly defined colour change at the end point.

The calcium ion concentration of the canal water, filtered through glass wool, was determined when snails were collected from the canal.

The radioactive isotopes used in the experiments were obtained from the Radiochemical Centre, Amersham. Initially, 89 Sr and 90 Sr were used, but later 85 Sr and 47 Ca were used. Sufficient radioactive isotope was added to the calcium solutions to give a count rate of at least 2000 cpm. The isotope was allowed to equilibriate with the glass walls of the vessels for at least three hours (non-aerated) and 1 hour (aerated) before the introduction of either snails or plants. In the case of the feeding experiments, the plants were allowed to equilibriate with the isotope solution before the introduction of the snails.

Ecology of the Shropshire Union Canal, Chester

The ecology of the Shropshire Union Canal at Chester, is summarised in Figures 2, 3, 4, 5 & 6.

The canal in this area is infrequently cleaned by British Waterways, although the banks are kept in good order. A large amount of debris is found floating in the surface waters. The canal is slow moving and is relatively shallow, so that the environment presents a fairly uniform habitat for both the flora and the fauna.

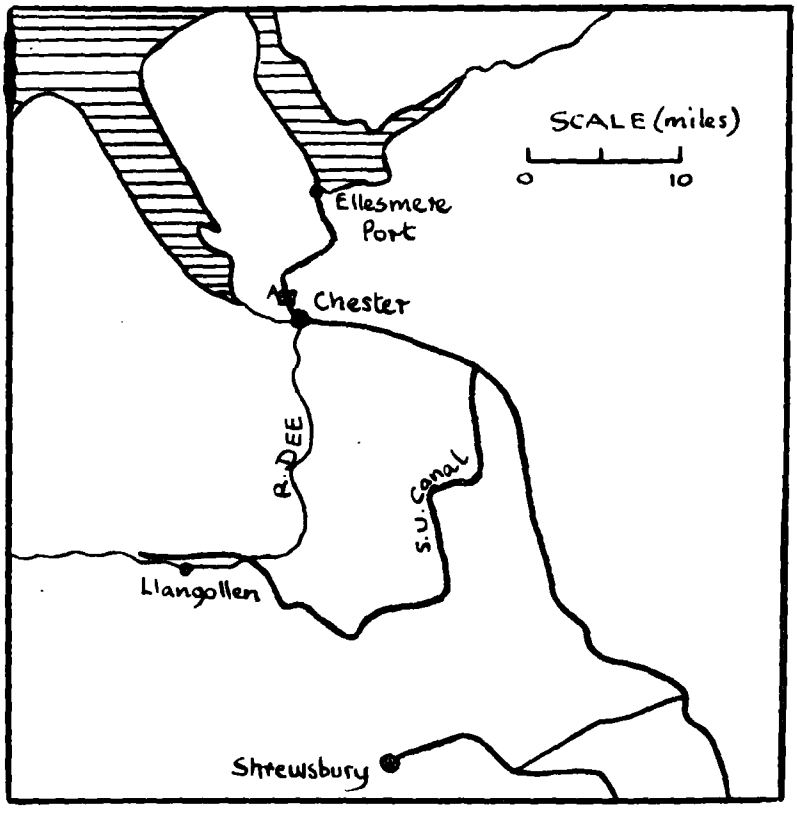


FIGURE 2.

MAP OF SHROPSHIRE UNION CANAL, TO SHOW POSITION OF AREA OF SAMPLING SNAILS (A).

(TAKEN FROM 'FIELD STUDIES,' 1. 1, 1959, Freshwater Studies in Shropshire Canal by H.M.Twigg).

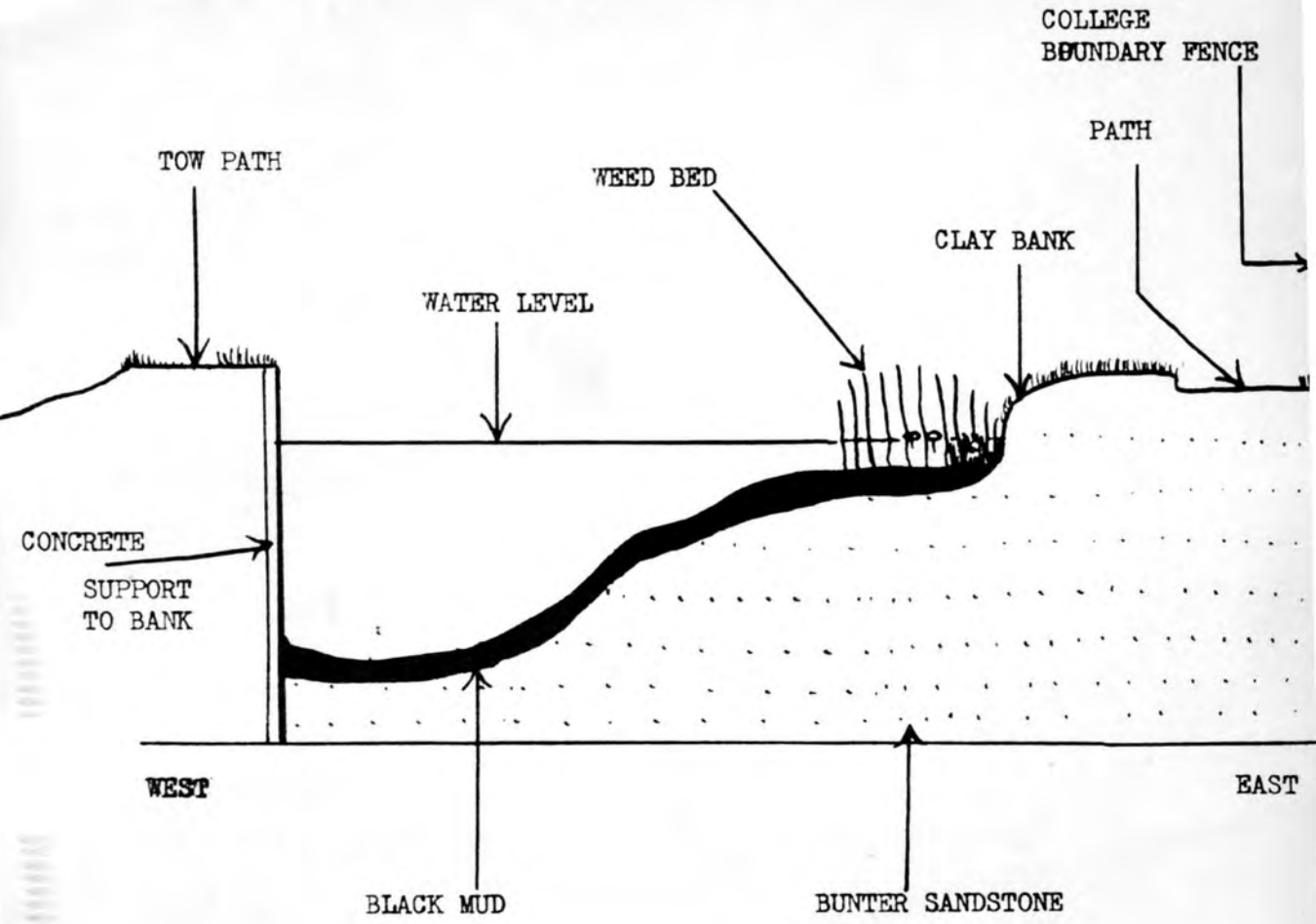


FIGURE. 3.

CROSS SECTION THROUGH THE SHROPSHIRE UNION CANAL, CHESTER COLLEGE, SHOWN IN PHOTOGRAPH FIG 4., PAGE 9.

0 3 6 9 12 15 18 feet

SCALE (Horizontal and vertical).

FIGURE 4.
PHOTOGRAPH OF SHROPSHIRE UNION CANAL, CHESTER.
SHOWING REED BEDS ON EAST BANK. GRID REF. 396673

FIGURE 5.
PHOTOGRAPH OF SHROPSHIRE UNION CANAL, CHESTER.
GRID REF. 396675.

FIGURE 6.

PHOTOGRAPH SHOWING SURFACE WATERS (CLOSE-UP) OF SHROPSHIRE UNION CANAL, CHESTER

The snails were obtained from the reed beds on the east bank of the canal, amongst Juncus articulatus, Sparganium erectum and in deeper water Glycera maxima. The close-up photograph (on page 11) of the surface waters, shows the presence of Lemna minor and Hydrocharis Morsus ranae, which forms a typical habitat for snails. Elodea canadensis and Myriophyllum spicatum are under the surface of the water and cannot be seen in the photograph.

Several pieces of wood were placed in the water and anchored to the bank by means of wire (photographs on pages 9 and 11). After a period of 1-2 months, an algal matt forms on the wood which supports a large snail population, and facilitates ease of collecting.

Viviparus species was found mainly on the surface mud in shallow water. Bithynia species was found in large numbers on the rhizomes of the reeds, and on the wood. Limnaea stagnalis was found mainly on the surface mud in shallow water, on Elodea and on the wood. Limnaea pereger and Limnaea auricaularia were found on the rhizomes of the reeds, amongst the Elodea and on the wood.

The following is an analysis of the canal water from the Shropshire Union Canal, Chester from where the snails were collected for the experiments. The analysis was performed by Mr. B.A. Brooks, in the City Analyst's Office, Chester on 23.10.62.

Alkalinity	140 ppm as Na_2CO_3
chloride	114 ppm as NaCl
carbonate	84 ppm as CO_3^{--}
Total Hardness	229 ppm
calcium	165 ppm as $CaCO_3$
magnesium	64 ppm as $MgCO_3$
pH	7.4
magnesium	15.55 ppm as Mg^{++}
calcium	66 ppm as Ca^{++}

The reasons for using Limnaea species and Bithynia species

In the initial experiments with Limnaea stagnalis, it was found that they needed a large volume of water to be active, otherwise they would die. This finding is in agreement with those of Taylor,1894-1900; Turner,1927; Crabb,1929; Boycott,1936; Robertson,1941.

There was also some difficulty in weighing the specimens of L.stagnalis, due probably to the large volume of fluid in the mantle cavity, which was difficult to remove. It was inadvisable to have large volumes of radioactive isotope, because of the risk of contamination. It was decided that smaller species of Limnaea would not need such large volumes of water in which to live, and that they would greatly reduce the

difficulty of weighing. Three species of Limnaea were selected, two of which, Limnaea auricularia and Limnaea pereger, occurred in great numbers in the Shropshire Union Canal at Chester. Limnaea truncatula was the smallest species used in the experiments.

L. pereger has been found in lakes in North Wales (Boycott 1936) with calcium concentrations of 1, 2, 5 and 8 mg Ca/l, to lakes containing 122 mg Ca/litre. L.pereger is therefore suitable for experiments since it can tolerate a wide range of calcium concentrations.

Limnaea species belong to the group of molluscs termed 'pulmonates' and are descended from land living molluscs, possess a radula and feed on organic detritus and algae. Bithynia tentaculata and Viviparus viviparus were selected as a comparison with Limnaea species, as they are ciliary feeders and possess opercula. Bithynia tentaculata and Viviparus viviparus are also found in abundance in the Shropshire Union Canal. All the snails selected for experiments were washed in distilled water to remove surface algae and detritus, and then in the appropriate calcium concentration for the experiment.

The Limnaea species were measured, from the apex of the shell to the base of the umbilicus and this measurement was called the shell length (SL). Viviparus species were measured across the most

ventriocose part of the shell, that is, the maximum diameter (MD).

The reasons for using certain plant materials as a source of food for the snails

Seven vessels containing uncontaminated filtered canal water and Limnaea pereger were set up, each containing different plant material.

In (i) pieces of lettuce, (ii) Elodea canadensis, (iii) Nasturtium officinale, (iv) Cladophora, (v) Fontinalis, (vi) Oscillatoria and (vii) artificial prepared food consisting of 50% Cow and Gate dried milk and 50% Farex.

These jars were left for 14 days and then examined, to see which plant the snails had eaten. All the lettuce had been eaten, some Elodea and some watercress. There was no sign, even under a binocular microscope that any of the Cladophora or Fontinalis had been eaten. Although Oscillatoria and the prepared food had been eaten, there was difficulty in sampling and this food therefore proved to be unsatisfactory.

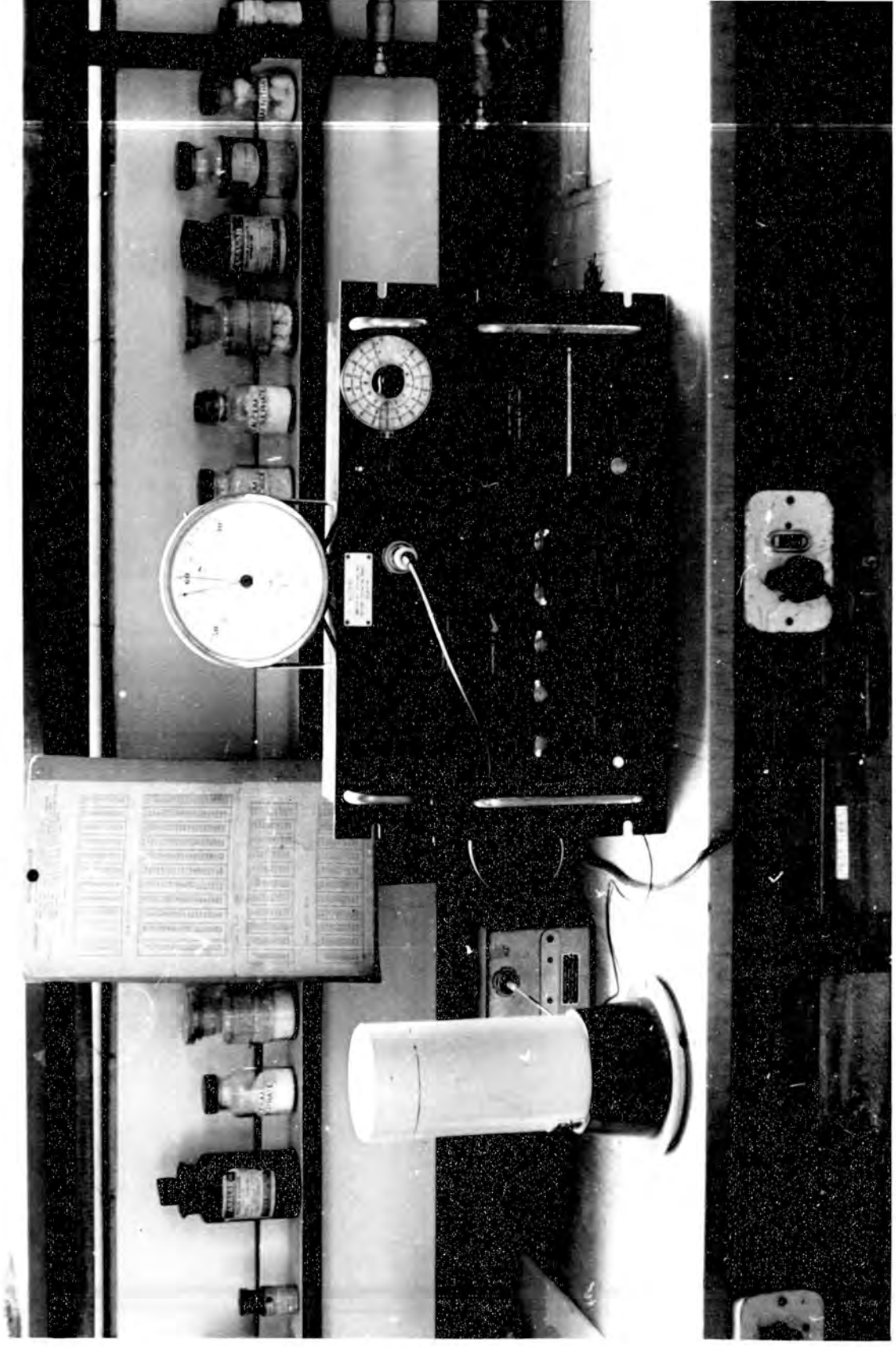


FIGURE 8
PHOTOGRAPH OF LIQUID β-COUNTER ERICSSON 1221C SCALER WITH 1221C POWER UNIT.

Calibration of the liquid β-counters

89 Sr and 90 Sr were used in the first series of experiments on the uptake of radioactive isotopes by molluscs. These two isotopes are both β- emitters (Figure 7), and measurement of the activity was performed on dual liquid β-counters. (Figures 8 & 9).

Element	half-life	beta energy	gamma energy
89 Sr	51 days	1.44 MeV (100%)	0.91 MeV (0.01%)
90 Sr	28 years	0.54 MeV (100%)	
90 Y	64·2 hours	2.25 MeV (100%)	

Figure 7.

Physical data on 89 Sr and 90 Sr from 'The Radiochemical Manual, Part One, Physical Data,' published by the Radiochemical Centre, Amersham, 1962.

The two liquid counters were standard counting apparatus. The equipment consisted of Ericsson 1221C Scalers with 1221C Power Unit or with L19.105A Power Unit. The liquid radiation counter tube employed was the 20th. Century Electronics Type M.6 (operating voltage around 1150 volts) which was accomodated in the cylindrical (Veall) type lead castle. The capacity of the M.6 is about 10ml.

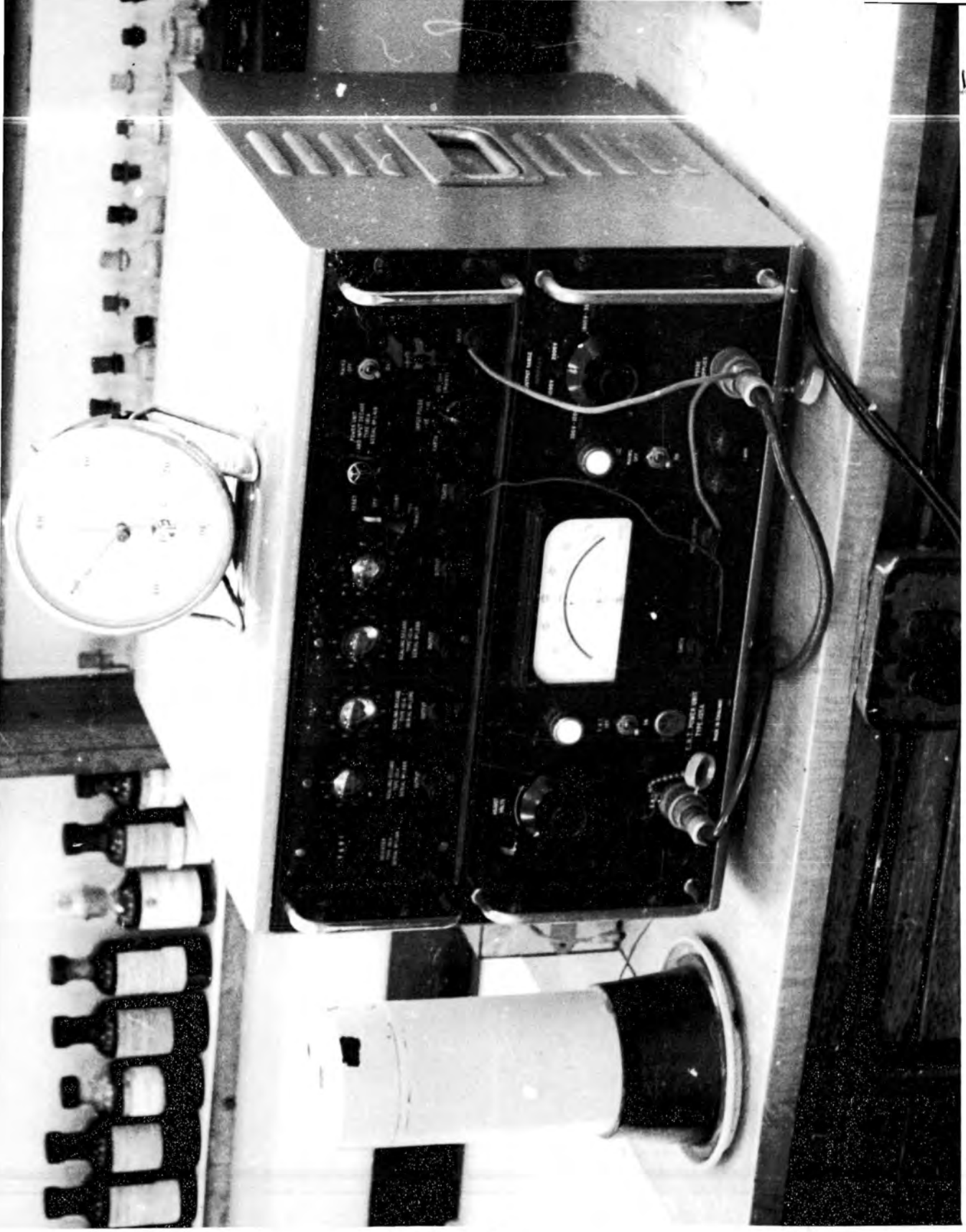


FIGURE 9.
PHOTOGRAPH OF LIQUID β-COUNTER ERICSSON 1221C
SCALER WITH L.19.105A POWER UNIT.

and the liquid should be added until the top of the tube is covered to a depth of 1/4 inch. The counter measures the concentration of the radioactive isotope not the total amount. The counting rate is independent of the weight of dissolved material in the solution, provided that the density is not significantly different from that of water.

The operational voltage of the Geiger Muller tube type M6 was determined using 10 ml. of standard 89 Sr solution and measuring the activity over a wide E.H.T voltage range.

Method of using the M6 tube.

One millilitre of 89 Sr obtained as strontium chloride from Amersham was made up to 50 ml. in a graduated flask, with aerated distilled water and made slightly acid, (pH 6.5), by external spotting. The flask was thoroughly shaken and allowed to stand for 30 minutes before a 10 ml. sample was pipetted into the M6 tube which had been previously washed out with concentrated HCl and three washes of distilled water.

Contamination of the glass walls of the M6 tube, due to adsorption or ion-exchange with the isotope solution, necessitated cleaning the tube after each determination. The tube was washed out with 2N HNO_3 and three washes of distilled water, which removed 89 Sr and 90 Sr from the walls of the tube.

Background was determined periodically throughout the experiment. This ensured that the M6 tube had no residual activity on the glass walls, and was clean.

Determination of the Geiger Muller (GM) plateau.

The 10ml. sample in the M6 tube was counted over a wide E.H.T voltage range. Since 10 ml. of strontium chloride was used in determining the activity of the strontium, the strontium chloride solution was replaced by 10 ml. of distilled water after thorough washing, to determine the background over the same voltage range.

The count rate was corrected by adding the appropriate paralysis correction which was read off from a set of tables (Ericcison pamphlet for the beta counters), before background was sub-tracted from the count. The corrected count was plotted against increasing E.H.T voltage, and the operating voltage was found to be 1100v for the particular M6 tube used, this figure being the mean of the GM plateau (Nokes, 1960).

Double checking of the count rate

The 10 ml. sample was counted on two liquid beta-counters. This was considered necessary, since there was a fluctuation in the E.H.T supply to the counters throughout the day, the mercury

contacts in the castle were lowered due to placing the M6 tube in the castle, and there was intermittent faulting in the electronic circuit of the counters. One counter was used to check the performance of the other.

Stability of the M6 tube

The stability of the M6 tube was determined on the setting above, using the formula on the Barnes Control Chart. A clean dry M6 tube was placed in the castle of the liquid β-counter and a sealed standard source of 90 Sr was placed on top of the tube , and the castle lid closed. The Barnes Control Chart gave upper and lower limits of stability and provided the calculated figure fell between these two figures, then the tube was said to be stable.

A χ^2 determination was performed at the beginning and the end of each day that the tube was used, to ensure stability.

Efficiency of the M6 tube

Although the efficiency of the M6 tube was not required in these experiments, an approximate figure has been quoted of 5.6% for 89 Sr (Carr, Harrison & Sutton, 1961), 10-11% for 90 Y and 0.5% for 90 Sr (Carr, Harrison & Sutton,1961: Sherwood & Dunster 1958). These figures vary from tube to tube.

The same M6 tube was used throughout the experiments for 89 Sr, and another tube for 90 Sr. It was extremely important that the counters were allowed to warm up for at least 1½ hours before use, to avoid fluctuation in the count rate.

Calibration of the γ-scintillation counter

Since both 85 Sr and 47 Ca are gamma emitting isotopes (Figure 10), they are measured by means of a gamma scintillation counter (Figure 11). In this case, an EKCO N529D Scaler with a thallium activated 1½ inch diameter NaI crystal contained in a lead castle, was used, which gave 2π geometry.

However, 47 Ca which has a short half-life has a daughter product, i.e. 47 Scandium, which has a similar half life (Figure 10). This situation presents the problem of separating 47 Sc from 47 Ca. The Decay or Disintegration Scheme for a particular radionuclide is a statistical means and is constant (Moore, 1963). The Decay Scheme for 47 Ca is shown in Figure 12.

Element	half life	beta energy	gamma energy
85 Sr	65 days	electron capture 100%	0.513 MeV (100%) via 0.9 usec Rb^{85}
47 Ca	4.7 days	0.66 MeV (83%) 1.94 MeV (17%)	0.48 MeV (6%) 0.83 MeV (6%) 1.31 MeV (77%)
47 Sc	3.4 days	0.45 MeV (74%) 0.61 MeV (26%)	0.16 MeV (74%)

Figure 10

Physical data on 85 Sr, 47 Ca and 47 Sc from, 'The Radiochemical Manual, Part One, Physical Data,' published by the Radiochemical Centre, Amersham, 1962.

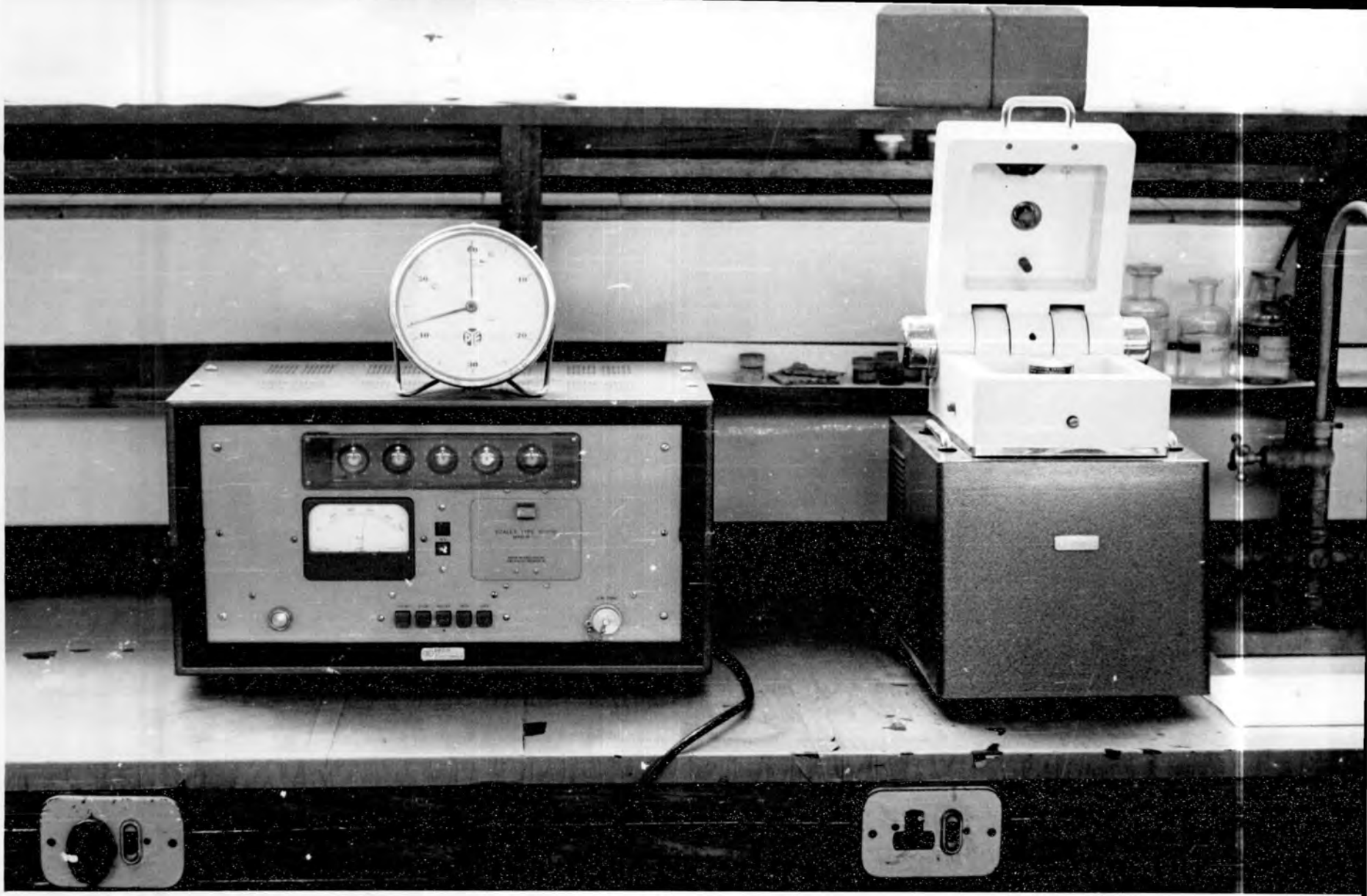


FIGURE 11.

PHOTOGRAPH OF GAMMA SCINTILLATION UNIT, EKCO N 529 D SCALER.

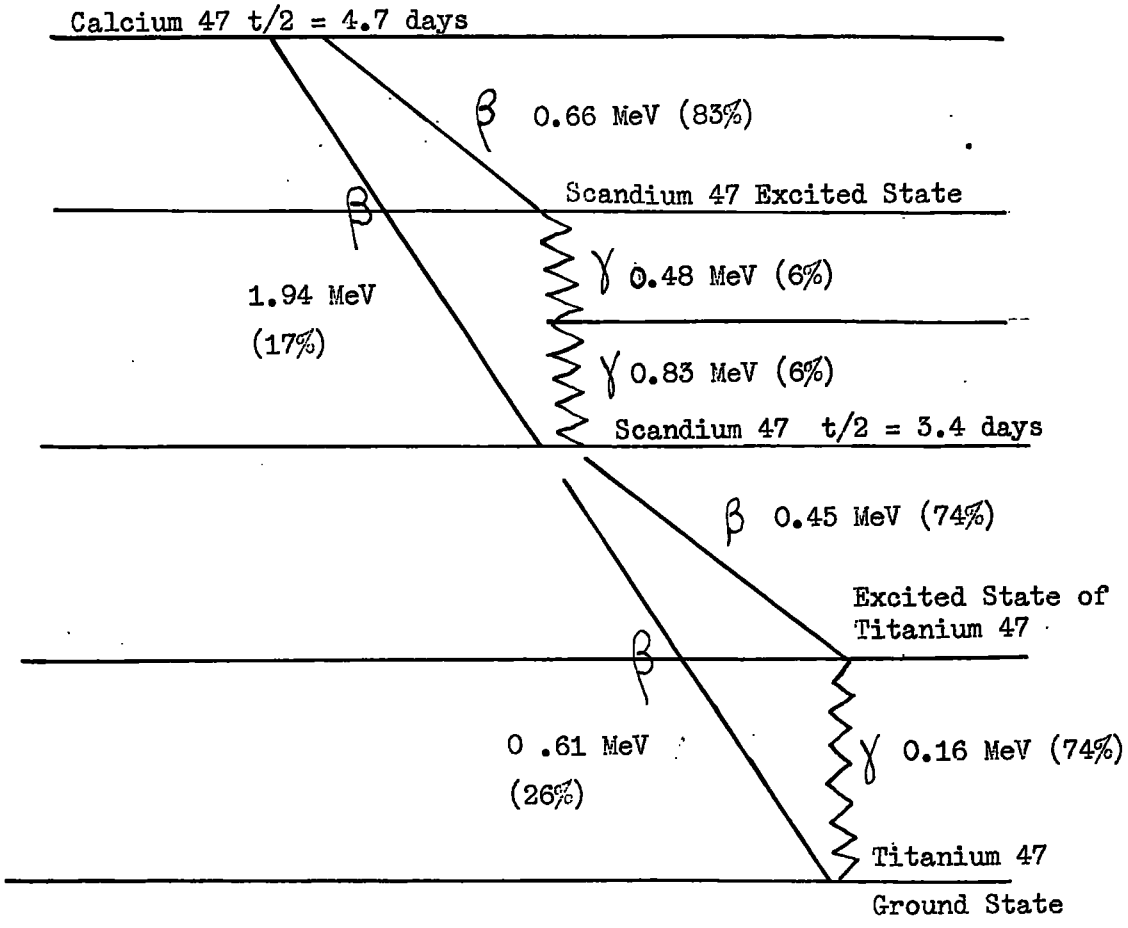


Figure 12.

Decay Scheme for 47 Ca.

Data obtained from,' The Radiochemical Manual, Part One, Physical Data,' 1962.

Note: Not all the steps in the transfer from the excited to the ground state of 47 Sc are known with any certainty.

Determination of 85 Sr.

Method 1

The discriminator was set at 5v. The 5 ml sample of a diluted radiostrontium solution was placed centrally on the NaI crystal. The lid of the castle was closed. The count rate was measured with increasing applied E.H.T from 600v to 1400v at 100v intervals. The amplification was 1000 x 0.1 = 100. The measurements were repeated with 5ml of distilled water in a similar polystyrene pot with polythene cover, in place of the isotope solution, to obtain a background count.

The experiment was repeated at 10v discrimination.

From the results, a graph of S^2/B against E.H.T was plotted on lin/lin paper, (Figure 13), and showed a maximum, representing ~~that is,~~ the optimum E.H.T voltage for the γ-emitting radiostrontium 85 and the discriminator bias voltage. (Outeridge 1954).

The operating voltage from this experiment showed that the best setting for counting radiostrontium 85 was 1100v E.H.T., 10v Disc Bias and amplification 100.

Method 2.

An energy spectrum of the sample used in Method 1 was performed. The Disc Bias was set at a maximum, and the E.H.T voltage turned down until no counts were given, i.e. 800v E.H.T. On this voltage, and amplification 100, the Disc Bias was varied from 0v to 50v and a graph plotted of corrected cpm against increasing Disc Bias (Figure 14). It can be seen that at 10v Disc Bias, that the maximum energy of the isotope is counted.

Plot: S^2/B against increasing applied voltage (E.H.T) for radiostrontium 85.

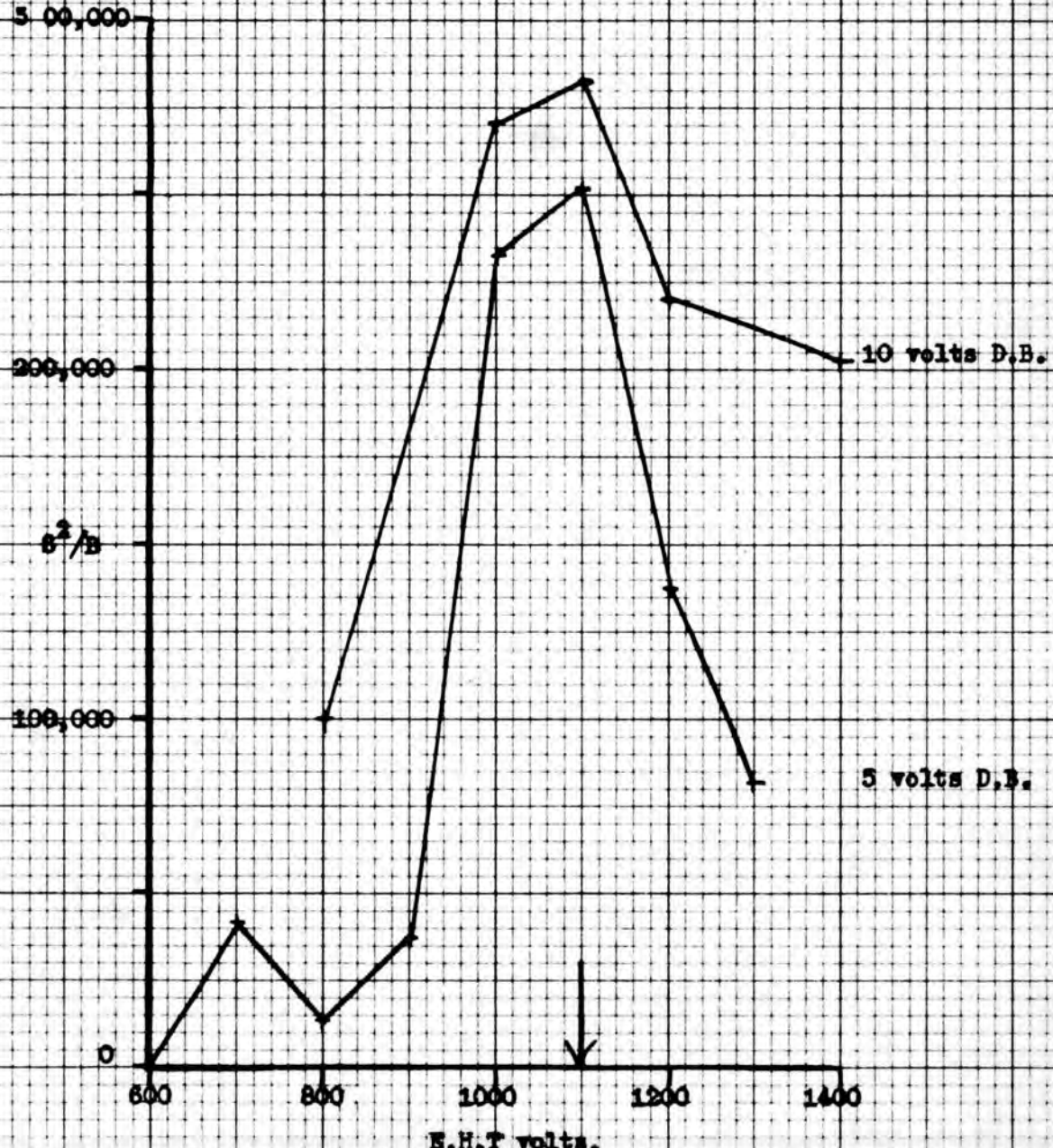
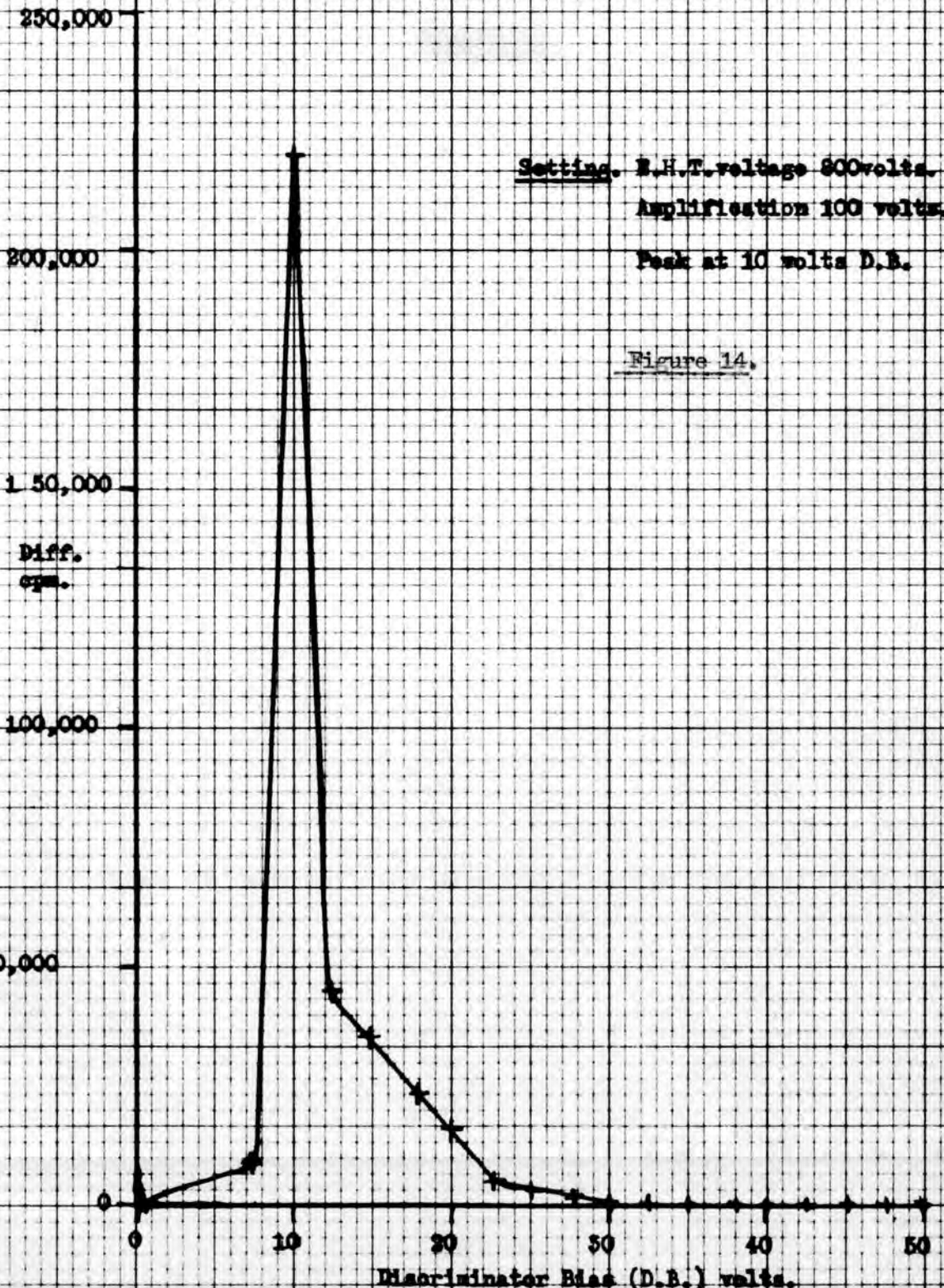


Figure 13.

Energy spectrum of Strontium 85 .

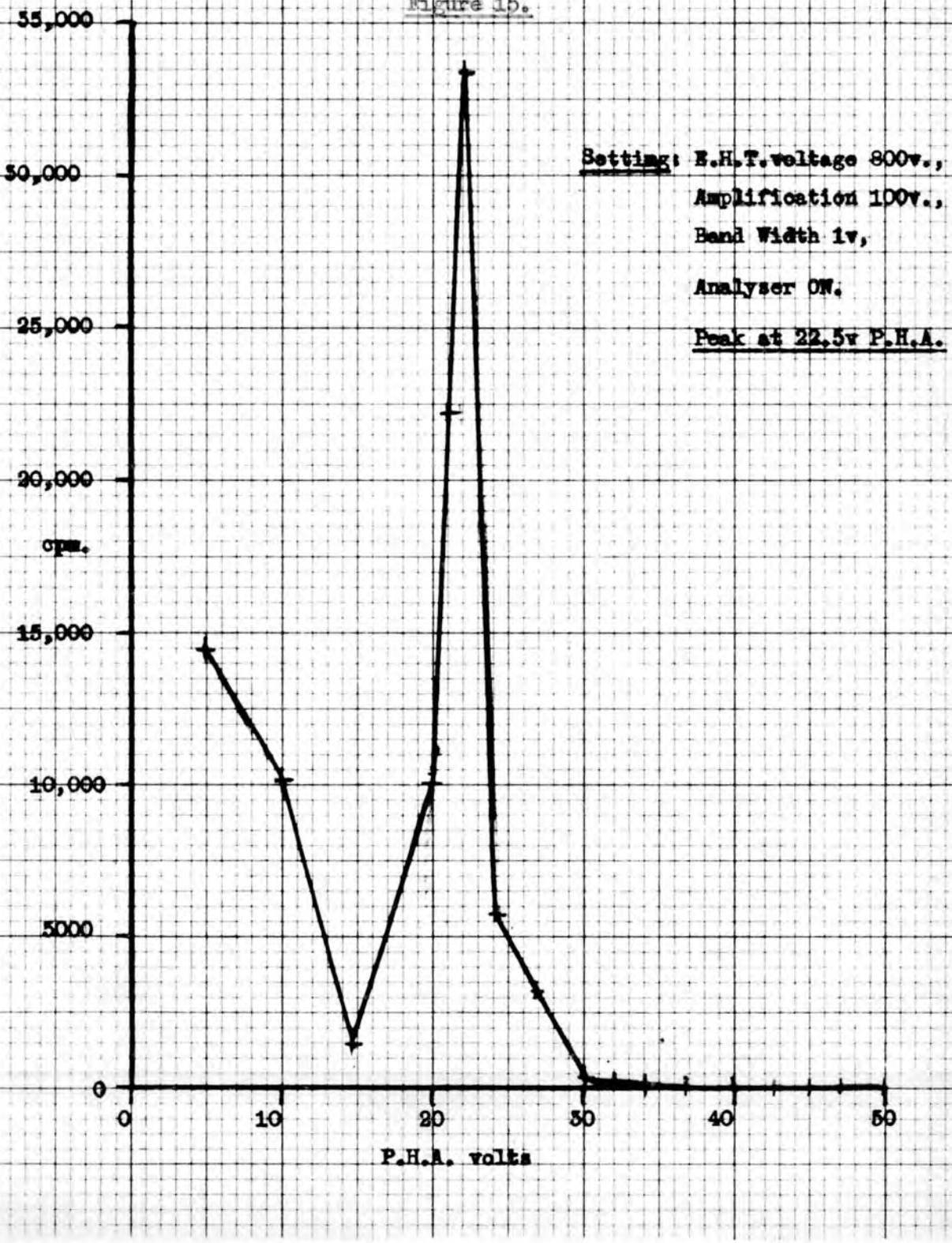


Setting. E.H.T. voltage 800volts.
Amplification 100 volts.
Peak at 10 volts D.B.

Figure 14.

Energy spectrum of Strontium 85 (P.H.A and N 529 D Scaler).

Figure 15.



Method 3

The energy spectrum of a 5 ml sample was performed with a pulse height analyser (PHA) and the N529D Scaler. The setting for counting the maximum energy was 800v E.H.T., amplification 100, band width 1v, analyser ON and PHA 22.5v (Figure 15).

Determination of radiocalcium 47

The main reason for using 47 Ca was that it was the only gamma-emitting isotope of calcium available and that whole body measurements could be made on the living snail at intervals of time. The gamma scintillation method facilitated ease of counting and handling radioactive material.

A great disadvantage of using 47 Ca was discovered during the preliminary work. This was the difficulty of obtaining a pure sample of 47 Ca. Radiocalcium 47 decays with a half life of 4.7 days into radioscandium 47 which has a half life of 3.4 days. Thus one has the situation where both parent and daughter isotopes have short half-lives. As the 47 Ca decays, the 47 Sc builds up into the solution, and at the same time decays, as shown in Figure 16. The regrowth of 47 Sc into the mixture has a maximum at 5.8 days.

Therefore when 47 Ca is used in an experiment over 14 days, the initial count must be high enough for decay, that is, 4,000 cpm. This count will give a count sufficiently high to be statistically

This graph is copied from Specification Sheet 5581, July 1964, published by The Radiochemical Centre, Amersham, CIS.1P Calcium 47.

This graph shows the decay of radiocalcium 47, and the ingrowth of radioscandium 47 due to decay of 47 Ca.

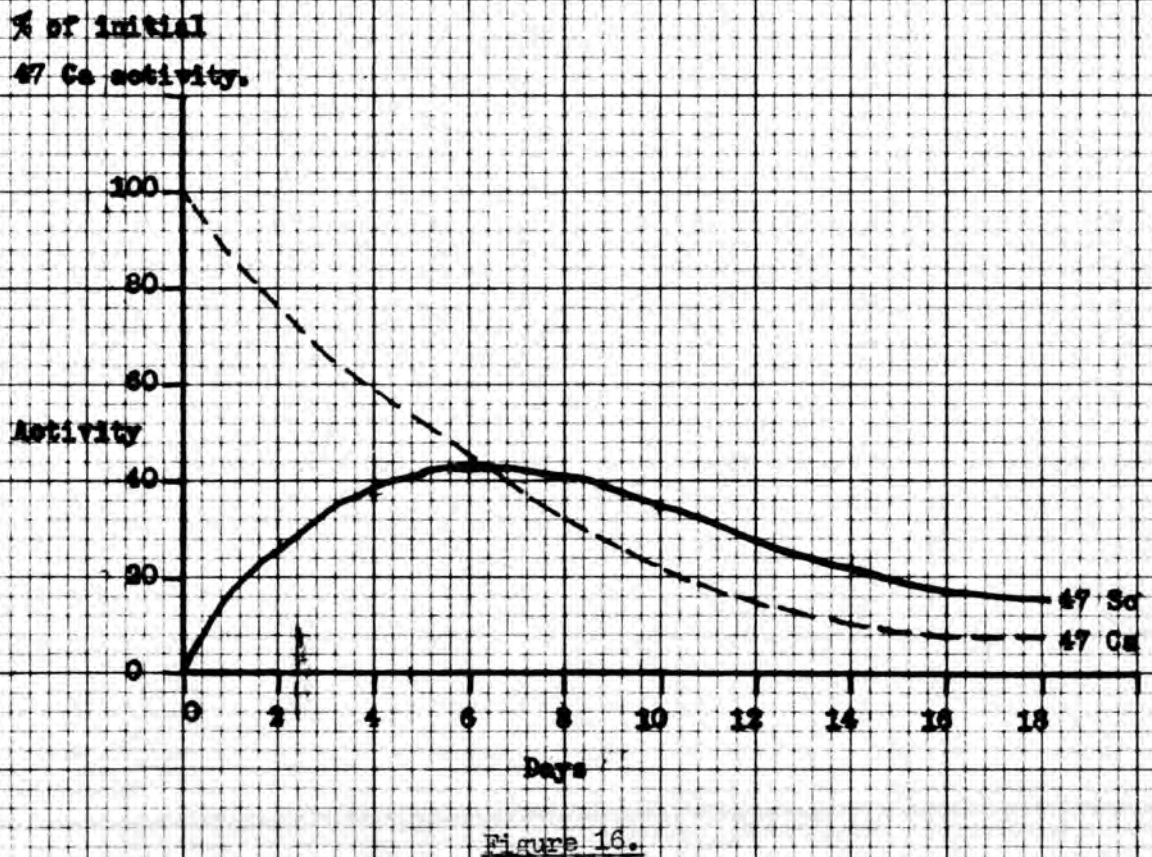


Figure 16.

correct after three half lives. The instrument must be set in such a way as to count 47 Ca and not a mixture of 47 Ca and 47 Sc.

Method 1

Radiocalcium 47 was obtained from the Radiochemical Centre, Amersham as sterilised calcium chloride in isotonic saline solution, with 0.27 ug of calcium per 22.2 uC in 0.1ml. The isotope was diluted with distilled water to give a count of 20,000 cpm per 5ml.

The operating voltage determined according to a S^2/B plot was 1100v E.H.T., 10v Disc Bias and 100 amplification.

To confirm the setting obtained by the S^2/B plot, a similar plot was performed using 5 ml of standard 60 Co solution which has gamma energies similar to 47 Ca, i.e. 1.17 and 1.33 MeV. The S^2/B plot for 60 Co gave a peak at 1125v E.H.T., with Disc Bias 10v and amplification 100. If 1125v E.H.T = 1.33 MeV, then 1100v = $\frac{1.33 \times 1100}{1125}$ = 1.3005 MeV, which is approximately 1.31 MeV, the energy of the highest gamma of 47 Ca.

There was little real value in working out the efficiency of the counter but an approximation was carried out as outlined below. A standard solution of 60 Co was diluted quantitatively with acidified distilled water and a 5 ml sample counted. This liquid sample was placed on the NaI crystal and counted over 20 minutes, and gave a corrected count of 1.2×10^4 cpm. These values were substituted in the general equation,

$$\text{Efficiency (\%)} = \frac{\text{observed cpm}}{\text{calculated cpm}} \times 100$$

$$= \frac{1.2 \times 10^4}{11.183 \times 10^4} \times 10^2$$

$$= 0.1073 \times 10^2$$

$$= \underline{10.73\% \text{ for } {}^{60}\text{Co at 1125v E.H.T., D.B.10v. Amp 100}}$$

At 900v E.H.T., Disc Bias 10v and amplification 25, the efficiency of the counter, using ^{60}Co, is 2.6% (Pickering 1964). These figures suggest that the efficiency of the counter is related to the amplification setting.

Since both ^{47}Ca and ^{47}Sc are gamma emitters, then the S^2/B setting gave the total activity of both these isotopes in the sample. ^{47}Sc has a low energy gamma, but a high percentage of gamma's (page 23) and ^{47}Ca has high energy gamma and high percentage of gamma's. Higher discrimination values tended to cut out the lower energies, thus the setting used, i.e. 1100v E.H.T., Disc Bias 10v and amplification 100, counted primarily the higher energy gammas, that is, ^{47}Ca.

Half life determinations on the sample used gave a value of 6.5 days, which is consistent with the value expected from a mixture of ^{47}Ca and ^{47}Sc.

To cut out the lower energy gamma of ^{47}Sc, the amplication was set at 25v. The E.H.T was reduced at Disc Bias 15v, until the count rate stopped, which occurred at 900 v E.H.T. The Disc

Bias was turned down, at 0v Disc Bias there was a fast count which diminished at 7v Disc Bias and there was no count at 10v Disc Bias. Thus at 10v Disc Bias, amplification 25 and 900v E.H.T. only the highest gamma's were counted, i.e. 47 Ca.

At 100v amplification, E.H.T 900v, it was not until a Disc Bias of 37v was reached that the count rate was almost nil. At 1100v E.H.T., amplification 100, the Disc Bias had no effect in reducing the number of counts over the Disc Bias range of 0-50v. i.e. it counted the maximum energy of both 47 Sc and 47 Ca. To substantiate these findings, samples of Cerium 144, which has a half life of 285 days and gamma energy of 0.13 MeV (compare 47 Sc with gamma energy of 0.16 MeV), and 60 Co with a half life of 5.27 years and gamma energies of 1.17 and 1.33 MeV (compare 47 Ca with gamma energy of 1.31 MeV) were used.

The following setting was used to determine the 47 Ca in the initial set of experiments, 900v E.H.T., Disc Bias 35v and amplification 25.

Method 2.

To ensure that the 47 Ca was counted a pulse height analyser (PHA) was introduced into the circuit which separated the 47 Ca from the 47 Sc by virtue of their different energies. The PHA was a stabilised Dynatron N/101, with modifications by Mr. R. Herbert, Principal Physicist at the Radium Institute, Liverpool. The PHA was introduced into the EKCO N 529D Scaler circuit as shown in

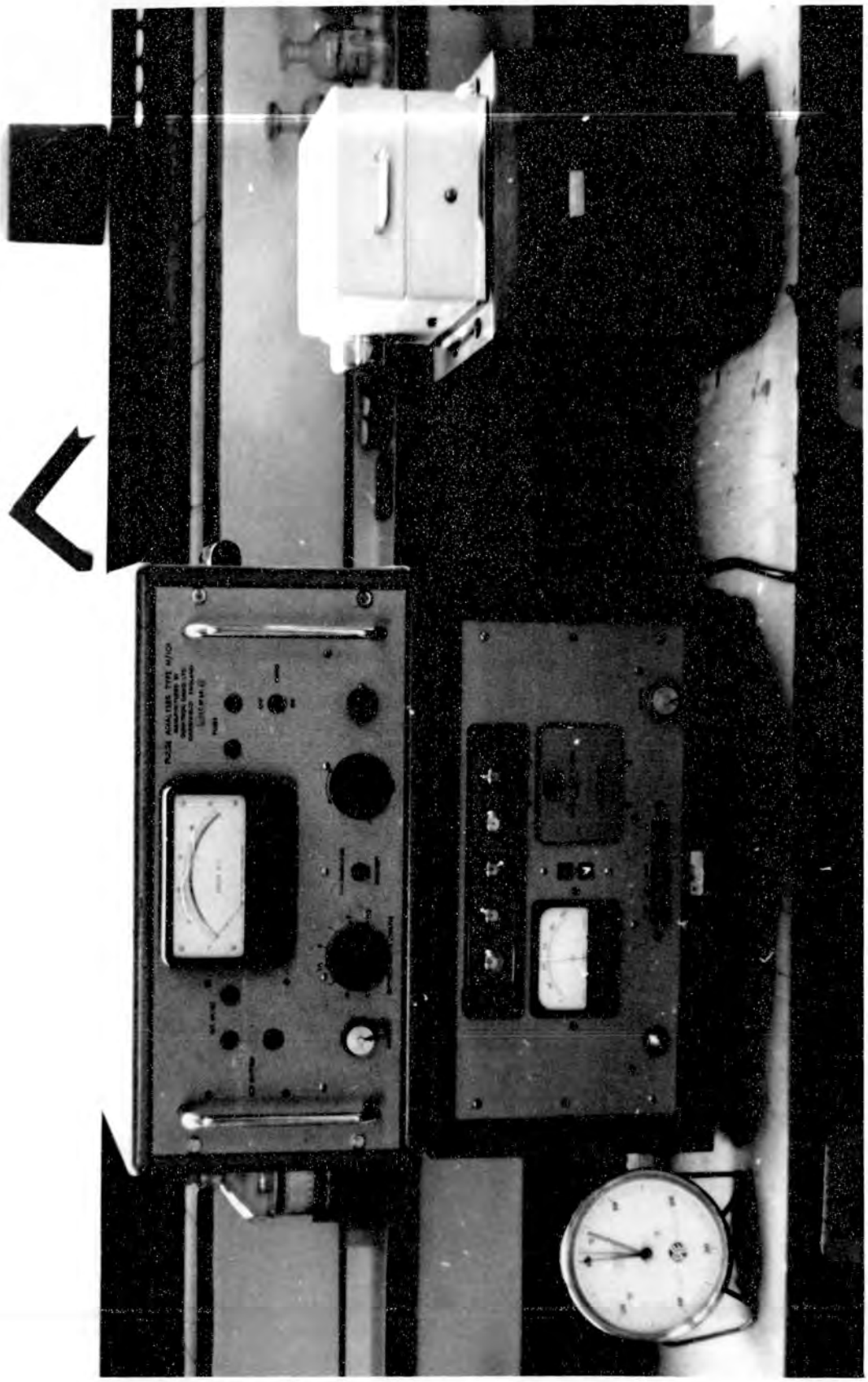


FIGURE 17.
PHOTOGRAPH SHOWING PULSE HEIGHT ANALYSER WITH EKCO N529D SCALER

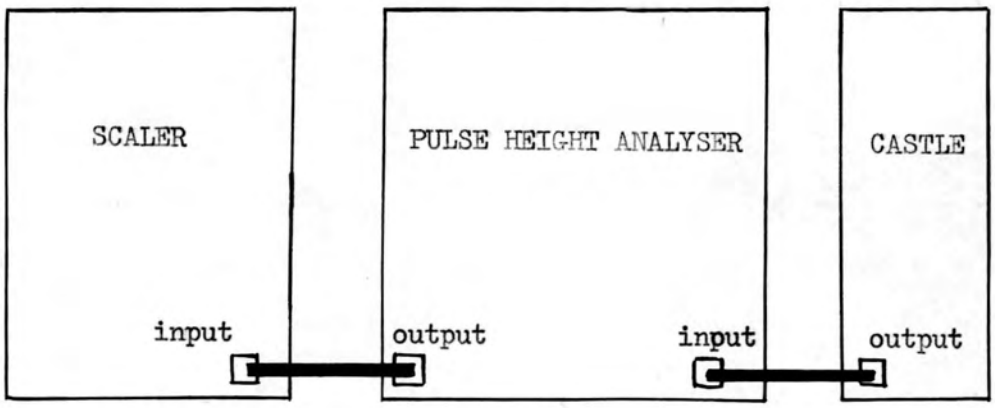


Figure 18.

Diagram to show how the PHA is introduced into the N 529 D Scaler circuit.

Figures 17 & 18.

There was no paralysis encountered with the PHA so that a more active source was used than with the scaler. A PHA used at narrow band widths, say 1v or 2v, limits the number of pulses. Since a high count rate is desirable a more active source can be used. The PHA can differentiate between the low energy gamma (0.16 MeV) of the scandium 47 and the high energy gamma (1.31 MeV) of the calcium 47. Thus one has a double counting technique.

Before one can calibrate the PHA for 47 Ca and 47 Sc, one has to work with a pure sample of 47 Ca.

The Amersham Method for the separation of radiocalcium from radioscandium. (Radiochemical Manual, Part Two 1963)

This method claims that filtration through filter paper removes the bulk of the scandium from a neutralised solution of calcium 47. Experiments were carried out to test this claim. The isotope from Amersham was diluted to give a count of 20,000 cpm per 5ml on the previously determined S^2/B plot. The diluted calcium solution was neutralised using calcium hydroxide solution, and then filtered through Green's No.791 filter paper as shown in Figure 19.

The collecting pot contained 1 ml of concentrated HCl, to prevent adsorption of the isotope onto the wall of the pot. A 5 ml sample was collected, which included the acid, and counted.

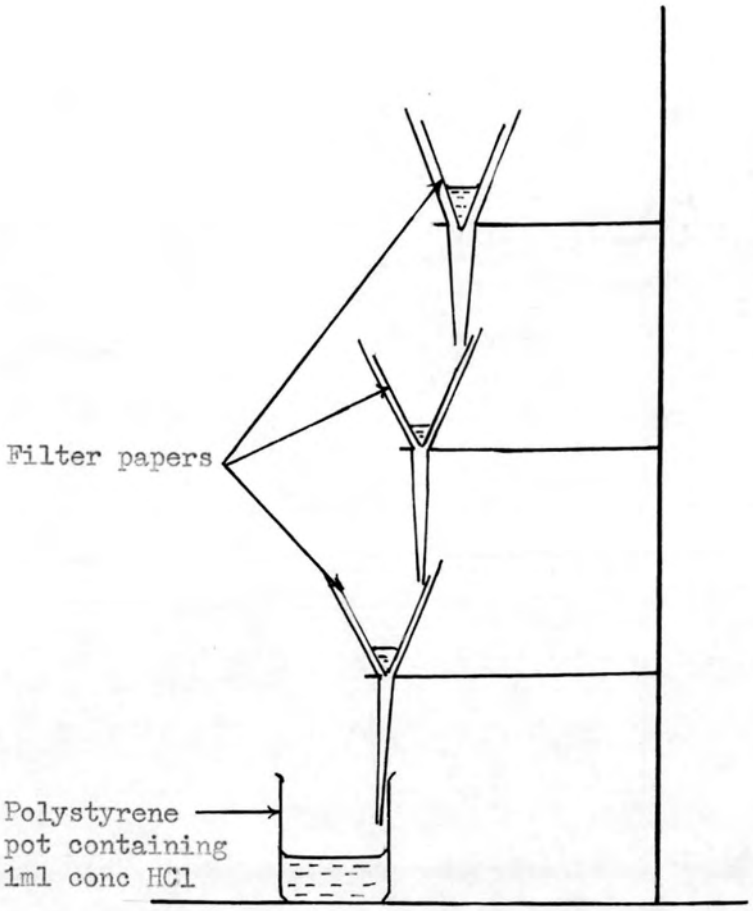


Figure 19.

Diagram showing the arrangement of apparatus for filtration of 47 Ca by the Amersham Method.

The counts were corrected by multiplying by 5/4 to give a 5ml reading, which will be comparable with other samples.

Procedure for the calibration of EKCO N 529 D Scaler with PHA in the determination of 47 Ca and 47 Sc

The 5ml sample, reputed to be pure calcium 47 by the Amersham method, was placed centrally on the NaI crystal and the castle lid closed.

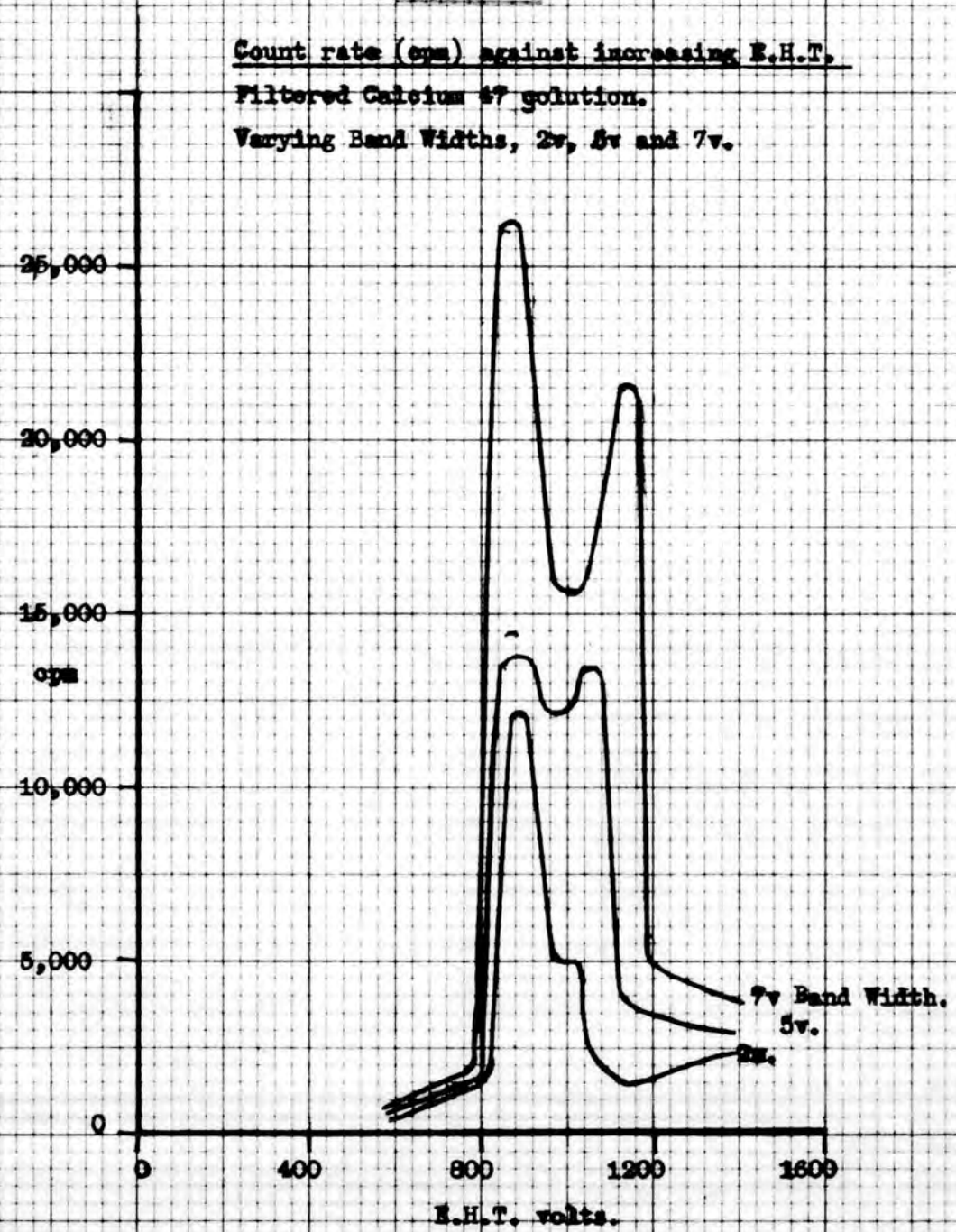
The energy spectrum of 47 Ca was determined first, since it had a high energy gamma, i.e. 1.31 MeV. With amplification set at 25 (250 x 0.1) PHA at 30v, analyser switch on and band width 2v, a table of results was constructed of count rate against increasing applied voltage (E.H.T) from 1-1600v at 50v intervals. The experiment was repeated at 5v and 7v Band Widths. These results were expressed graphically, with count rate plotted against increasing E.H.T in Figure 20.

It can be seen from Figure 20, that (i) the count rate increased with increasing band width and (ii)that the main peak was at 875v for 47 Ca. From this, one would expect the 47 Sc peak to be at 3.75v PHA, which can be increased to 15v PHA by increasing the amplification to 100, i.e. a factor of 4.

A test for scandium was performed on the same sample at this setting

The E.H.T was left at 875v and the amplification was

Figure 20.


Count rate (cpm) against increasing E.H.T.
Filtered Calcium 47 solution.
Varying Band Widths, 2v, 5v and 7v.
25,000
20,000
15,000
cpm
10,000
5,000
0
0
400
800
1200
1600
E.H.T. volts.
7v Band Width.
5v.
2v.

readjusted to 100 (1000 x 0.1), Band Width 2v, analyser switch on, and the PHA voltage was varied from 0-50 volts. A graph of count rate against PHA voltage was constructed from the data obtained, Figure 21. Figure 21, shows that (i) there was a peak at 22v PHA and (ii) that this peak indicates that 47 Sc was present in the solution. This proves that the claimed separation by filter paper does not take place. If no 47 Sc was present, then there would have been no peak. This proves that there was not complete separation and that both 47 Sc and 47 Ca were present.

The 47 Ca energy spectrum was constructed, by plotting the count rate against PHA voltage in Figure 22. The setting was 875v, amplification 25, Band Width 2v, analyser switch on.

Chemical method for the separation of pure 47 Ca

Another method for the separation of calcium and scandium was then employed (Ellison 1964). To the diluted 47 Ca solution from Amersham, 100 ug of 'Analar' ferric chloride was added until a pH of 2.5 was reached, using pH papers and B.D.H. Universal Indicator. The solution was neutralised with equal quantities of freshly prepared N/10 ammonium hydroxide which had been kept in a refrigerator, and N/10 ammonium chloride, until a brownish red precipitate of ferric hydroxide appeared. 1ml of the ammonium hydroxide and the ammonium chloride was added cautiously to the isotope solution, which darkened before clouding appeared, and then the precipitate occured. The

Figure 21.

Count rate (cpm) against increasing P.H.A voltage.

Filtered Calcium 47 solution.

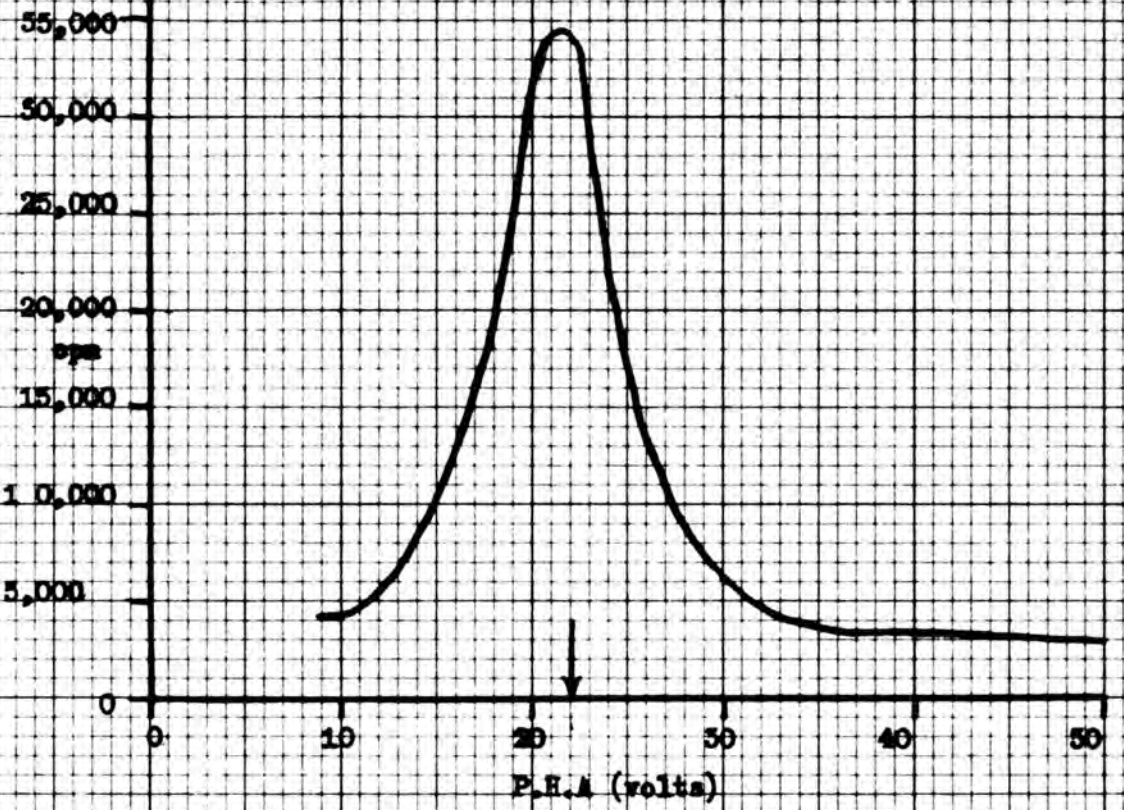
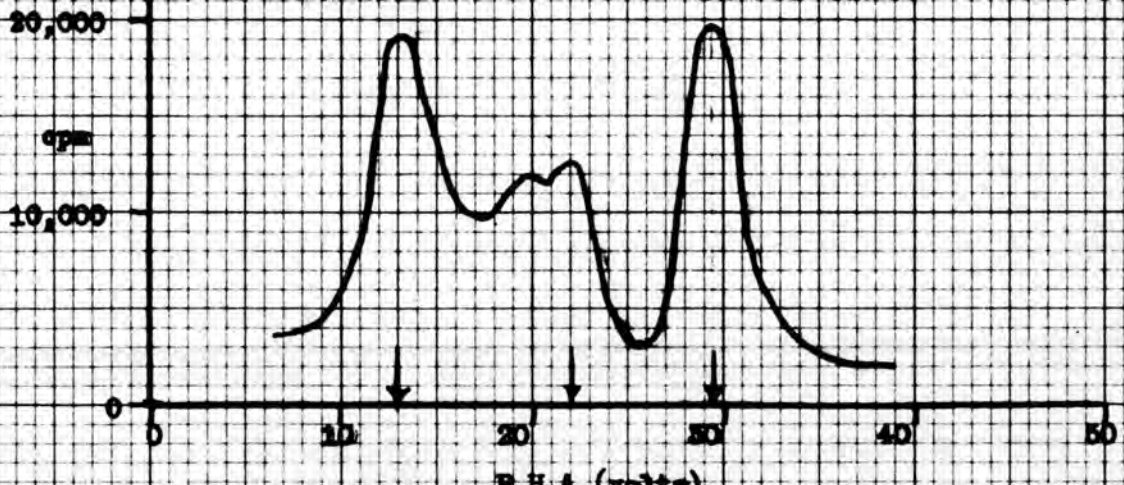


Figure 22.

Energy spectrum for Calcium 47. Count rate (cpm) plotted against increasing P.H.A voltage. Note that there are three peaks which correspond with the different gamma energies of Calcium 47, i.e. 0.48 MeV, 0.84 MeV, 1.31 MeV.



The solution was tested with pH papers and B.D.H. Universal Indicator until a pH of 7.0 was reached.

The formation of the colloidal precipitate of ferric hydroxide formed a surface, onto which the scandium was adsorbed. This separation is termed 'radiocolloid formation,' and is probably due to the adsorption of hydrated ions on suitable surfaces. (Radiochemical Manual, Part one, Radioactive Chemicals 1963).

Removal of the precipitate and the scandium was effected by filtering through Whatman No.541 filter paper. Only one filtration was found to be necessary.

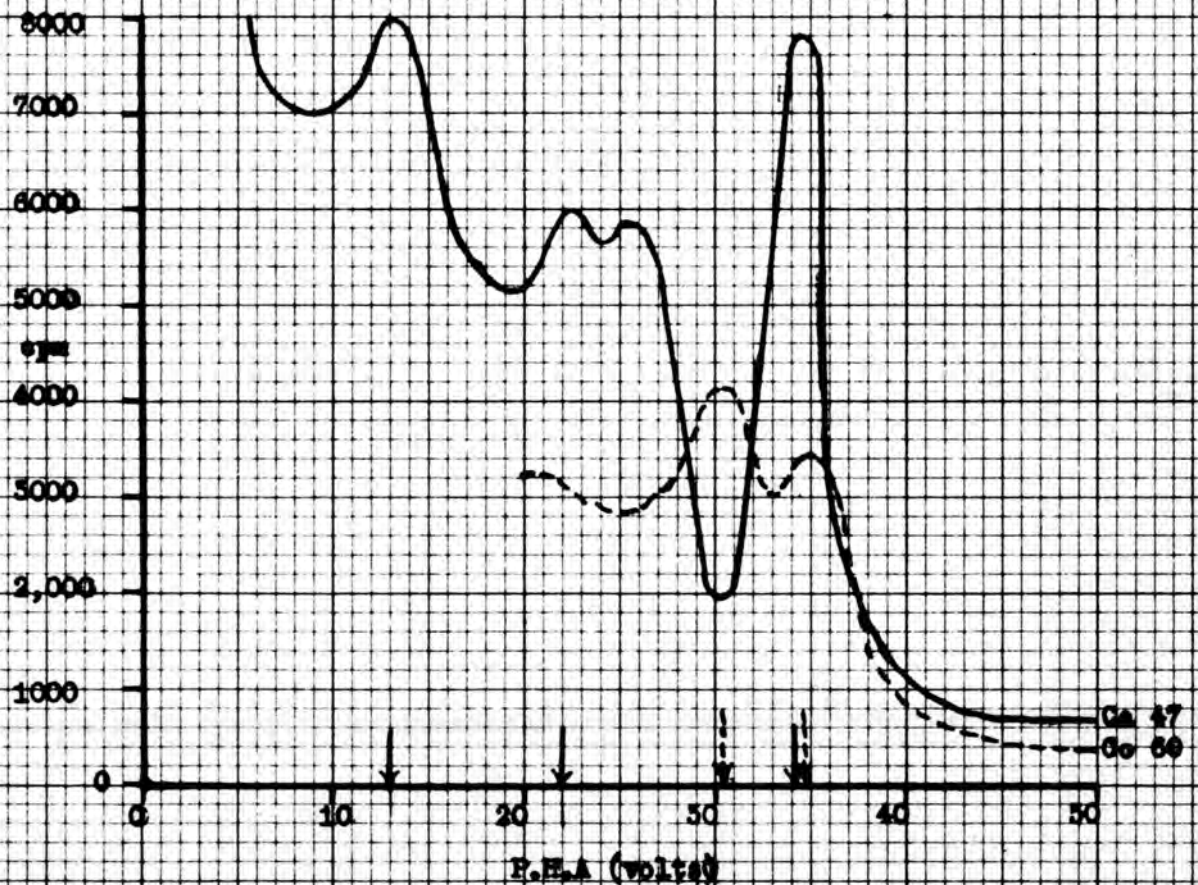
The filtrate was counted for 47 Ca and 47 Sc by the previous method. The energy spectrum was constructed as before, for the filtered 47 Ca, and this was checked using a standard 60 Co source, Figure 23. The standard 60 Co has two peaks at 30.5v and 34.6v which corresponds with the gamma energies of 1.17 and 1.33 MeV. The filtered 47 Ca has three peaks at 13v, 22v and 34v. The peak for 1.33 MeV for 60 Co was at 34.6v, the peak for 47 Ca is at 34v which will correspond with,

$$\frac{1.33 \times 34}{34.6} = 1.308 \text{ MeV}$$

The other two peaks for calcium give energies of 0.47 MeV and 0.84 MeV. These figures are closely in agreement with the figures quoted in the Radioisotope Manual published by Amersham, and which is summarised on page 24. Thus the setting of 875v E.H.T., amplification 25, band width 2v, analyser switch on and 34v PHA was the correct setting for measuring the 47 Ca in a sample.

Figure 23.

Count rate (cpm) against P.E.A (volts) for (i) filtered Calcium 47, and (ii) Cobalt 60.



The energy spectrum of 57 Co was constructed and then the filtered 47 Ca was compared to see if there was any scandium present. The setting for the 57 Co was 875v E.H.T., amplification 100, band width 1v with analyser switch on. Since the band width is 1v, then the instrument does not differentiate between two of the gamma energies of 0.122 MeV and 0.136 MeV. (Figure 24). Figure 24 shows that the peak for 57 Co was at 14v PHA and that this corresponds with 0.122 MeV. The graph also showed that the filtered 47 Ca solution at this setting was almost a straight line with no definite peak. This indicates that there is no scandium present in the sample.

The 47 Ca sample was kept for a number of days, and measured over the energy spectrum to observe the growth of 47 Sc from its parent isotope 47 Ca. This ingrowth is shown in Figure 25.

Fig. 25 shows that (i) that there was no 47 Sc present in the filtered 47 Ca solution, but as time progressed 47 Sc grew into the solution from the decay of 47 Ca.

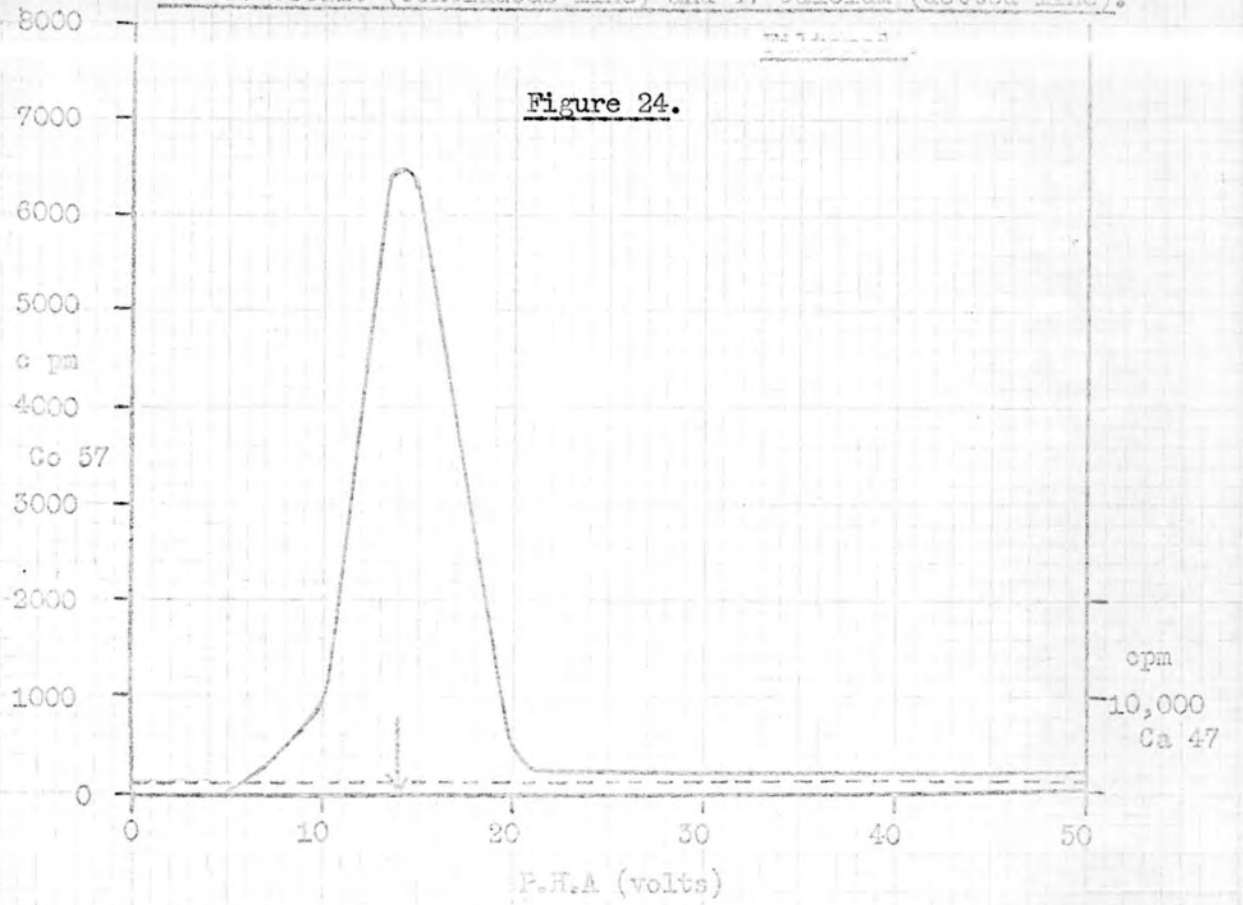
(ii) that the peak occurred at 19v PHA. If 14v PHA corresponds with 0.122 MeV, i.e. the energy of 57 Co, then 19v PHA will correspond with $\frac{0.122 \times 19}{14}$ = 0.165 MeV for scandium 47.

(iii) that the chemical separation method employed removed the whole of the scandium from the calcium solution.

From these results, one can obtain the settings to differentiate between calcium 47 and scandium 47. These settings were :-

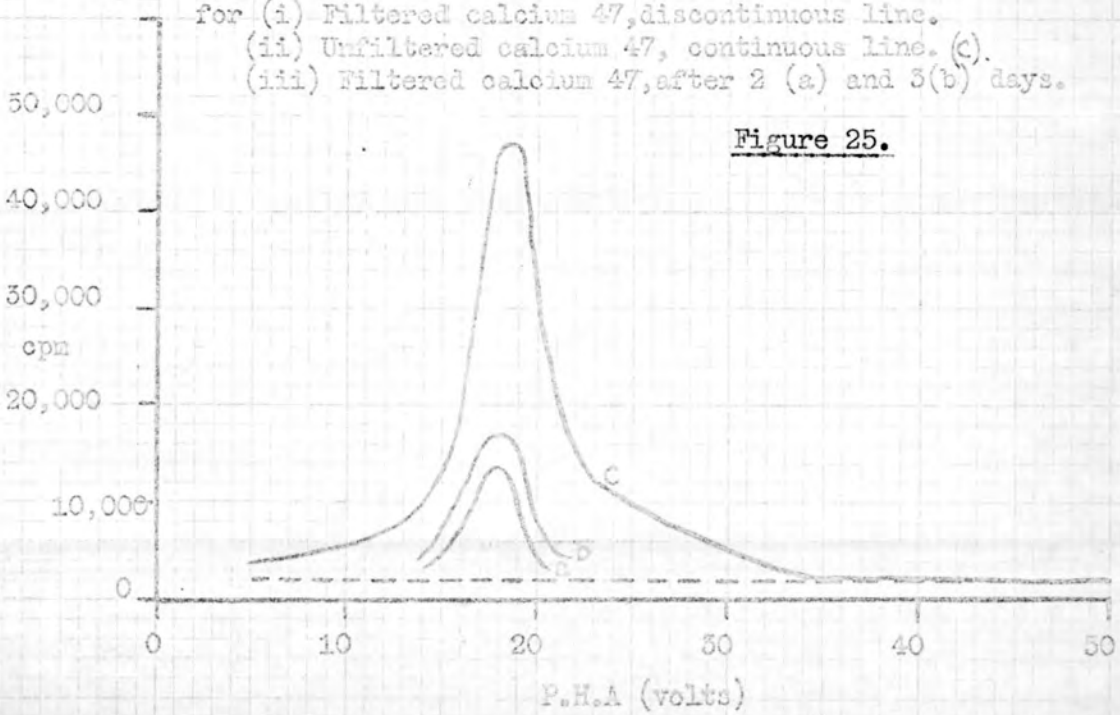
Count rate (6pm) plotted against increasing P.H.A (volts)
for 57 Cobalt (continuous line) and 47 Calcium (dotted line).

Figure 24.



Count rate (cpm) plotted against increasing P.H.A (volts)
for (i) Filtered calcium 47, discontinuous line.
(ii) Unfiltered calcium 47, continuous line. (c).
(iii) Filtered calcium 47, after 2 (a) and 3(b) days.

Figure 25.



(i) Calcium 47.

E.H.T. 875v., Amplification 25v., P.H.A. 34v., Band Width 1v., Analyser ON.

(ii) Scandium 47.

E.H.T. 875v., Amplification 100v.,P.H.A. 19v., Band Width 1v., Analyser ON.

Since there was a growing-in of the scandium into the filtered calcium solution, a factor has to be determined to give a correct value for scandium, (i.e. 'f').

Calculation of the factor 'f'

$$f = \frac{\text{count rate of Ca 47 filtered at 19v, i.e. low voltage}}{\text{count rate of Ca 47 filtered at 34v, i.e. high voltage}}$$

$$= \frac{1500 \text{ cpm}}{4600 \text{ cpm}}$$

$$= 0.3260$$

This factor was then used to correct the scandium count obtained from the PHA setting.

Let 'x' be the value at the high voltage, i.e. 34v, and 'y' be the value at the low voltage, i.e. 19v.

Then 'x' = calcium count, which was then corrected for decay, and

'y' - 0.3260 x = scandium count, which was corrected for decay.

It was found unnecessary to determine absolute values, since most of the results were expressed in the form of Accumulation Factors (A.F.), and the initial standard of at least 2000cpm per 5ml, observed at the beginning of the experiment. Since the sensitivity of the Ekco scaler and PHA was different, for counting scandium and calcium, then a ratio of the cpm's is therefore not applicable. However, a comparison of

the A.F's of scandium and calcium can be made.

The energy spectra of both Ca 47 and Sc 47 were checked at the beginning and the end of the first experiment involving the PHA unit. It was found that the peaks which corresponded with the energies of the gamma radiations, had decreased throughout the day (12 hours). This decrease in the optimum voltage on the PHA implied that there was a fluctuation in the E.H.T. supply. The resistors in the unit have a variable temperature coefficient, and the output of the 100v from the resistors compared with the output through a neon stabiliser was not constant. Thus in all experiments involving counting of Ca 47 and Sc 47, three different settings for each isotope were counted. The highest figure of the three readings was taken to be the correct figure.

These settings are as follows :-

(i) for calcium 47 ... PHA 27v, 28v, 29v and 34v. (It was found necessary to have four readings for calcium, since it varied more than the scandium.)

and

(ii) for scandium ... PHA 17v, 18v and 19v.

Method of killing and extracting the activity of β-isotopes from snails

Method 1

The snail was removed from the isotope solution with plastic forceps, and drained on filter paper. It was then placed in a measured amount of acetone, which was enough to cover the snail - normally 2 ml. - in a test-tube for 2 minutes, and then the snail was washed with distilled water.

This method proved unsatisfactory for two reasons :-

(i) the acetone caused the snail to produce excessive mucus which hindered dissection into shell and body, and

(ii) the acetone and the distilled water removed a significant amount of activity from the snail (more than 5%).

Method 2

The snail was removed from the isotope solution with plastic forceps, shaken, drained on the walls of the glass vessel and placed in a test-tube containing a measured amount of the isotope solution - (usually 2 ml) that it had been in, and placed in a warm water bath for a few seconds. The snail was removed from the test-tube very quickly, drained and placed in a weighed crucible ready for extraction. It was weighed before and after it was killed (fresh weight) and the difference which was constant, was the amount of fluid present in the mantle cavity, the activity of which was negligible.

The slight warming caused the snail's collar muscle to relax, thus

facilitating easy and rapid removal of the body from the shell.

The snail was killed, weighed in a crucible to obtain the fresh weight (FW) and placed in a hot-air oven at 100°C for 30 minutes. It was then weighed after cooling in a dessicator to give the dry weight (DW).

The crucible was heated with its cover on, to dull redness. 1ml of concentrated HCl was added to the cooled crucible, which was re-heated until nearly all the acid had evaporated. The crucible and cover were allowed to cool, and 10ml of distilled water was pipetted onto the incinerated snail. The acidic solution was allowed to stand for 30 mins. before being made up to 50ml in a graduated flask with distilled water. 10ml of this solution was pipetted into a M6 tube and counted on the liquid β-counter, and the count multiplied by 5, then corrected for background and decay.

The activity of the shell and body were extracted in the same way.

The initial count on the M6 tube of a 89 Sr and 90 Sr solution is a Yttrium-Strontium count, and it is necessary to keep the 10ml sample in a polythene tube with screw cap on, acidified with 1ml concentrated HCl to prevent adsorption by the tube, for 3 weeks. During the period of 3 weeks, any yttirum 90 (half-life 64.2 hours) would have decayed to less than 1%. At the end of 3 weeks, the 90 Y will be in radioactive equilibrium with the 90 Sr. This count is proportional to the strontium present in the solution.(Overman & Clark, 1960). At the end of 3 weeks, the 10ml sample is counted, the count multiplied by 11/10, then corrected for background and decay. (See pages 209-209c).

[redacted].

Method of extraction of β-activity from plant material

The plant material was drained, blotted lightly on Green's filter paper No.796, before being weighed. It was then treated as for the snail.

Method used to extract β-activity from the radula of fed Limnaea pereger

Six snails were placed in 250ml of distilled water containing 66 ppm Ca^{++}, after feeding on lettuce for 4 days. The calcium solution contained 90 Sr.

The faeces were collected by filtration through glass wool contained in a Gooch crucible which was attached to a filter pump. The faeces were then dissolved in 5ml 2N NaOH solution and heated in a covered crucible. This liquor was transferred to a graduated centrifuge tube and centrifuged for 1 hour. The supernatant fluid was counted for activity, and the residue was looked at under a binocular microscope for the radula. The radulas were dissolved in aqua regia - 3ml of concentrated HNO_3 being added first, then concentrated HCl drop by drop, the mixture being heated after every drop. The acidic solution was centrifuged again and placed under a binocular microscope to see if any of the radulas observed previously were still there. The solution was made up to 10ml with distilled water and counted in a M6 tube.

Since the activity expected was small, the 10ml sample was counted for 1 hour. There was no significant difference between the background and the count. This proves that defaeceation of worn radulae does not contribute a part in the elimination of the isotope. It also implies that the radulae are very little, if any, calcified, but are made of chitin and or other protein. (Runham 1962a; 1963b; 1963c.)

Method of killing and extracting the activity of γ-isotopes from the snail

The snails were removed from the glass vessels with plastic forceps, drained on the walls prior to being weighed (FW) and counted whole. The snails were placed in weighed crucibles and dried at 100°C for 30 mins. in a hot air oven. The crucible with cover on, was allowed to cool in a desiccator before being weighed (DW). The crucible and cover were placed in a muffle furnace at 550°C for 30 mins. after which, they were removed from the furnace, allowed to cool in a desiccator, and 5ml concentrated HCl added with 1 ml of distilled water. The acidic digested solution was allowed to extract activity from the walls of the crucible, by leaving it for 30 mins. before pipetting 5 ml into a polystyrene pot with polythene cover on, to count on the NaI crystal in the scintillation unit. The count rate was corrected by multiplying by 6/5, and then for background and decay.

The scaler could not differentiate between the density of concentrated HCl and distilled water, so 5 ml of conc HCl was used,

which necessitated only one extraction from the crucible; otherwise, with 1ml of conc HCl and 4ml of distilled water, it took 2 or even 3 extractions to extract all the activity from the crucible walls.

The dissection of the snails into the required portions was performed in the pot which contained the snail. The shell was transferred to another weighed pot. Thus the body pot would contain all the body fluids. The pots were tested for activity by acid extraction every time they were used, to see if any activity had become adsorbed onto the walls of the pot. If so, this activity was added to the activity of the crucible to give a corrected count.

Method of extraction of γ -activity from plants

The small amount of plant material used in the sampling process did not necessitate incineration. The plant material was drained & then lightly blotted with filter paper, and weighed before being counted in a pot (see Figure 26). Then 5ml of conc HCl was pipetted onto the plant material, and allowed to extract activity for 30 minutes, before being counted. The acid completely digests the plant material used, and therefore does not affect the geometry of counting. The count was corrected for background and decay. The filter paper was counted to see if there was any residual activity on it. If this was significant, it was added to the extracted amount.

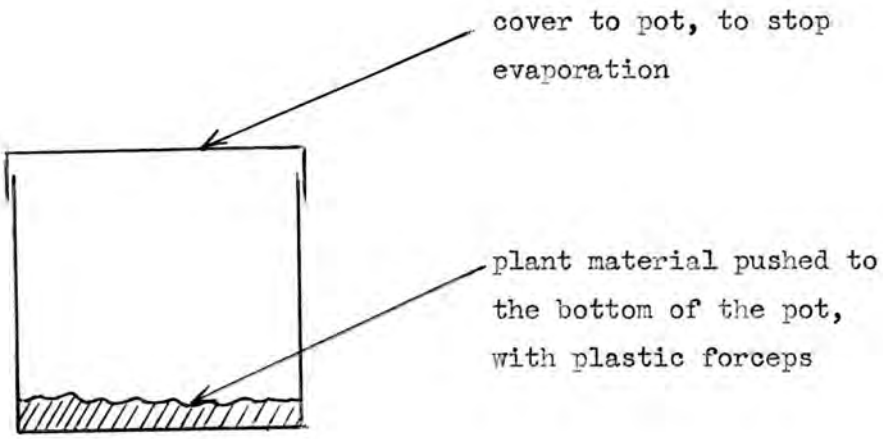


Figure 26.

Diagram to show the position of the plant material in the pot, when it is counted solid. Normally does not exceed 2 mm high.

Method of studying the initial uptake of isotope by snails

Method 1

The preliminary experiments presented two problems :-

(i) a special tube had to be designed to keep the snails in the same position in the tube while counting, and (ii) a method of aeration or means of continuous flow was necessary to accelerate uptake by the snail.

A flat bottomed tube was placed through the opening in the lid of the castle (Figure 27). The background on the tube was determined, 5ml of 85 Sr solution was added to the tube and counted. A snail was then introduced into the isotope solution and counted continuously for 6 hours to determine the initial uptake by the snail.

It was observed that after the snail had been introduced into the tube, with foot or operculum (Limnaea and Bithynia species respectively) pointing downwards at the bottom of the tube, that the count rate dropped. The snail was uncontaminated and was initially 'dead' space displacing the isotope solution as shown in Figure 28. The count rate rises when the isotope is being absorbed by the snail.

Bithynia species were content to remain in the same position for the duration of the experiment, while Limnaea species moved about the tube, thus upsetting the geometry of the counter, and therefore giving a varying count rate.

The uptake by Bithynia species in the first 6 hours was

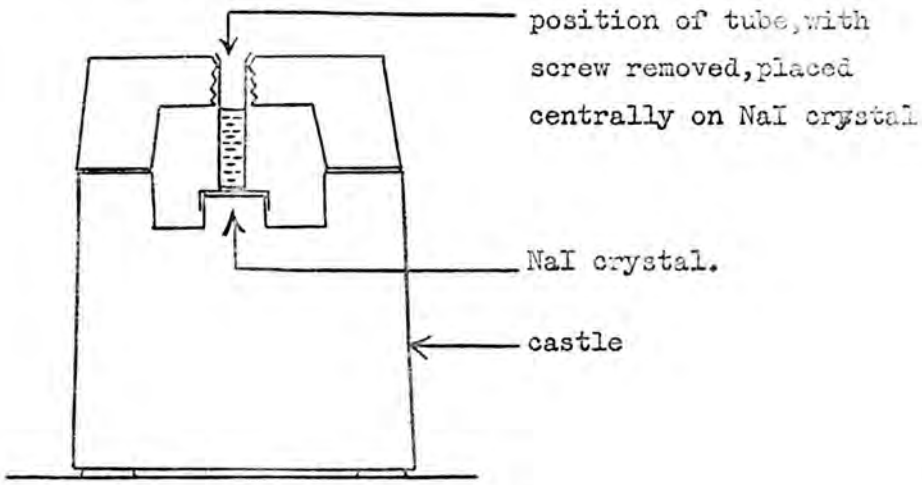


Figure. 27 .
Diagram to show the position of the tube in the castle.

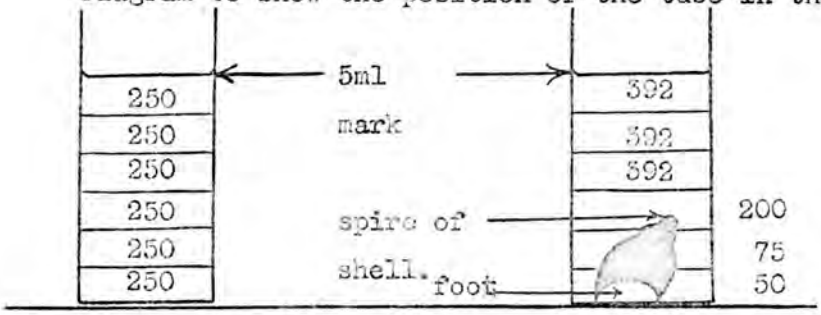


Figure 28.
Diagrams to illustrate the displacement of the isotope solution, when a snail is placed in the tube.

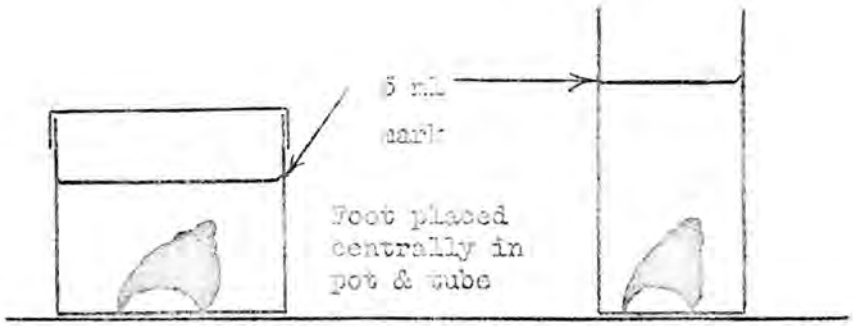


Figure 29.
Diagrams to show the relationships between the tube and the pot.

negligible, so a pipette was used to aerate the liquid. This ensured mixing of the isotope and accelerated the uptake by the snail.

The snails were also counted in 10ml polystyrene pots previously used in other experiments (page 52) with 5 ml of isotope solution and snail in, so that a comparison between two sets of experiments could be compared. The different sized pots presented different geometry arrangements to the counter and the count rate was affected. (Figure 21).

This static method proved unsatisfactory as a means of studying the initial uptake of isotopes by snails.

Method 2

As a result of the problems mentioned in Method 1, the following method was adopted.

(a) Construction of the tube. The tube was designed by the author as shown in Figure 30, and supplied by J.H. Martin, Esq., Scientific Services, Saughall Road, Chester. The inlet tube was splayed to accommodate the spire of theshell and after satisfactory trials, three tubes were designed with varying lengths of inlet tube inside the tube to accommodate different shell lengths (S.L) of the different snails used in the experiments.

(b) The apparatus used is shown in Figure 31. The Woullfe's bottle had a capacity of 1 litre and flow through the polythene tubing was started with a puff-ball. A screw clip was used to

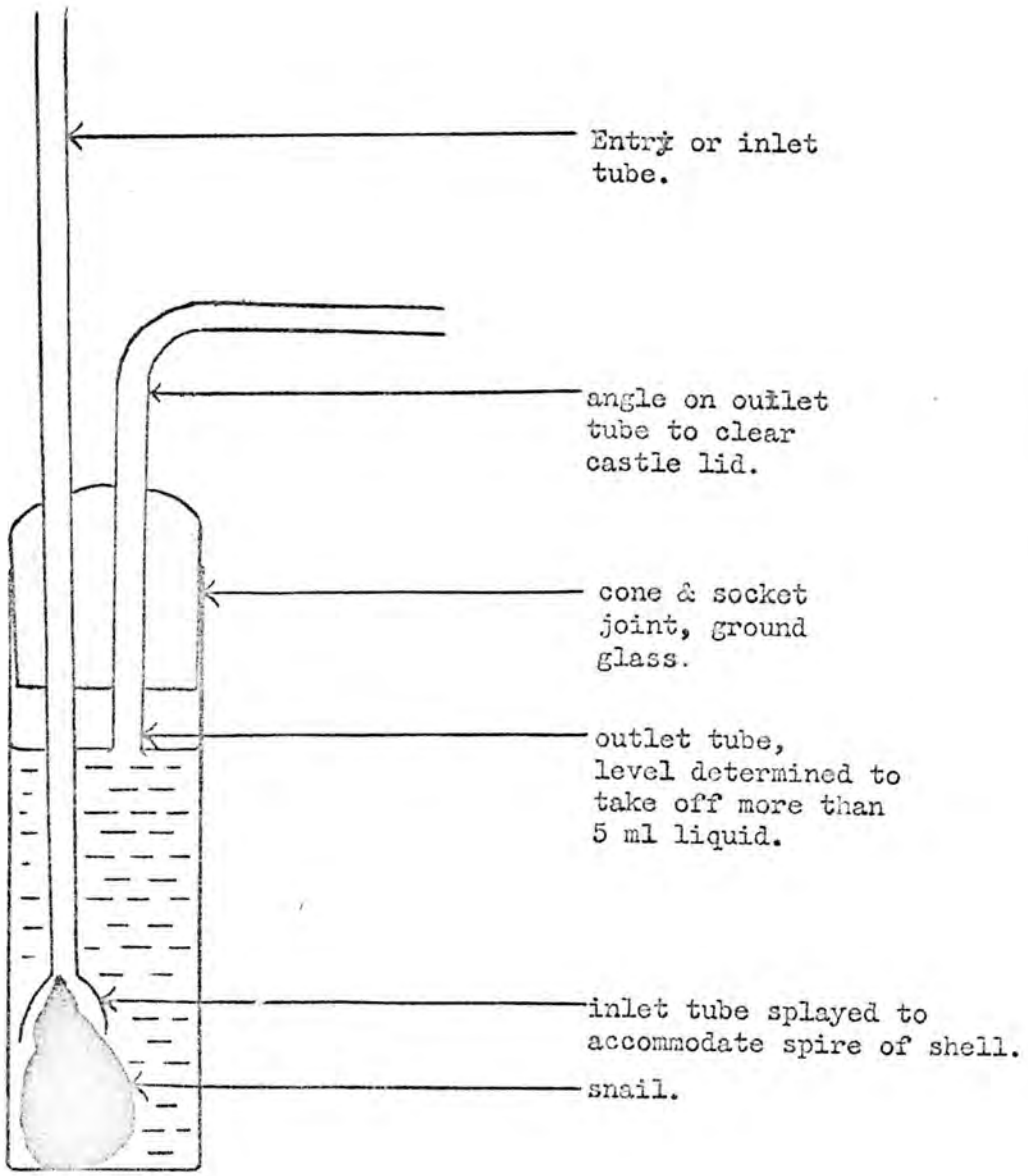


Figure. 30.

SPECIAL TUBE DESIGNED BY THE AUTHOR (Pyrex glass).

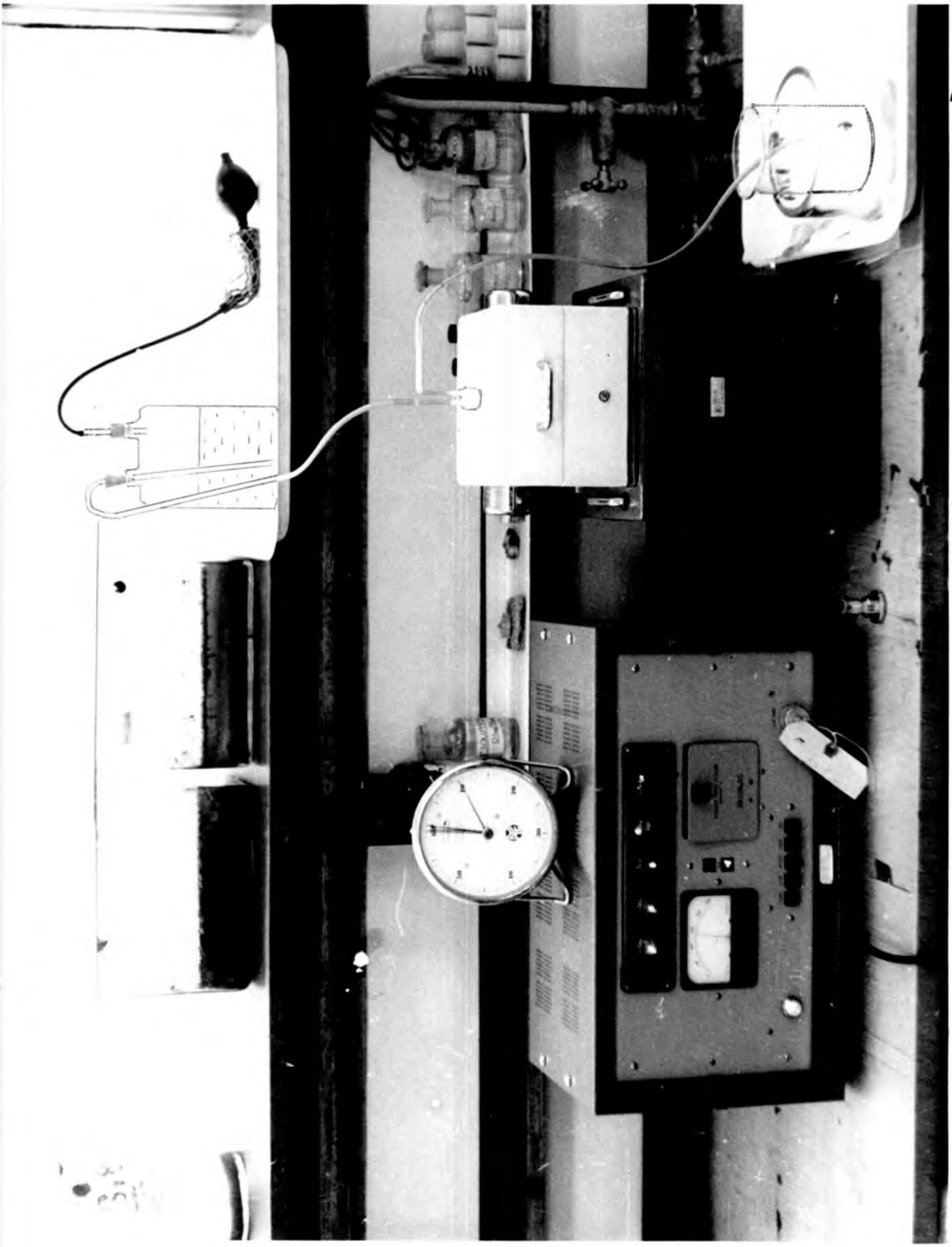


FIGURE 31.
PHOTOGRAPH SHOWING METHOD 2, USED IN INITIAL UPTAKE BY SNAILS

regulate the flow, to about 100ml per 30 mins. The background was counted with the empty tube in the castle. The initial run was without a snail, so that the isotope was adsorbed onto the walls of the glass and polythene apparatus. It took two runs of isotope solution for the apparatus to become adsorbed with isotope. The snail was introduced into the tube, and the flow started. Continuous counting of the run was recorded. 10ml samples of the solution were taken from the beaker, for calcium determinations with E.D.T.A., at the beginning and end of a run.

The same liquid was used over again and it was observed that there was an increase in the calcium ion concentration throughout the experiment, probably due to leaching from the snail.

The snail was counted (a) drained and (b) in 5ml of the isotope solution in a polystyrene pot, for direct comparison with previous experiments, (see page 72 for calculation).

A run through of the liquid, minus snail, was performed at the end of the experiment to ensure that the count rate on the liquid had not significantly altered.

The activity of the snail was extracted as on pages 52-53.

The disadvantages of this method are (i) that the 1 litre of isotope solution only lasts for 4-5 hours, and when the solution is being poured back into the Woulffe's bottle, there is a time lag, which is evident on the plotted graph of corrected cpm against time, (Figure 32), and (ii) with using the same solution over

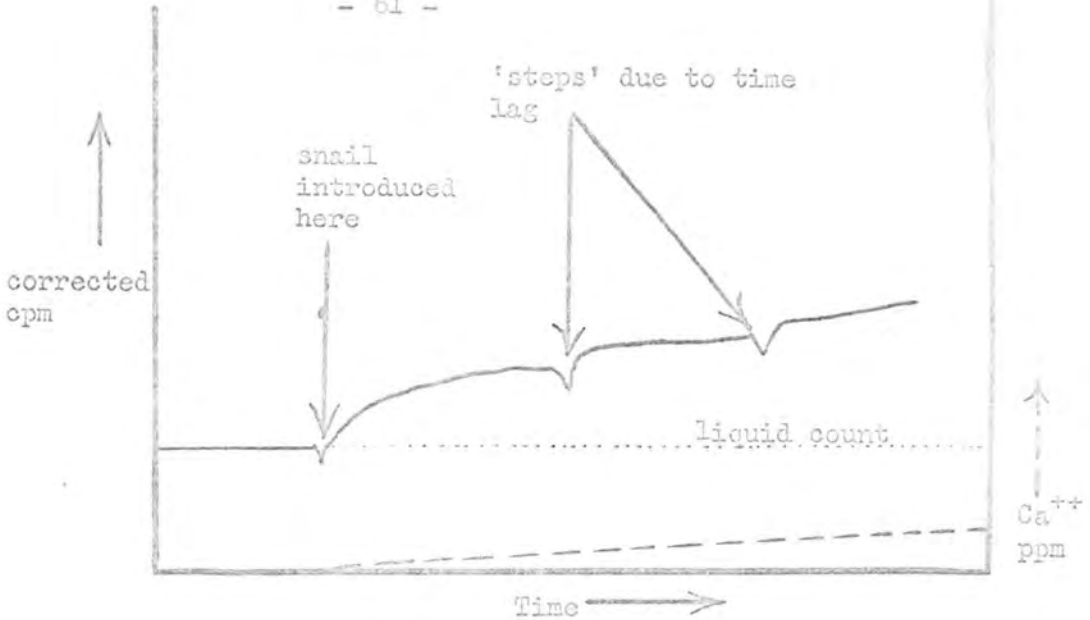


Figure. 32.

Graph to illustrate the 'step-like' phenomenon obtained using Method 2.

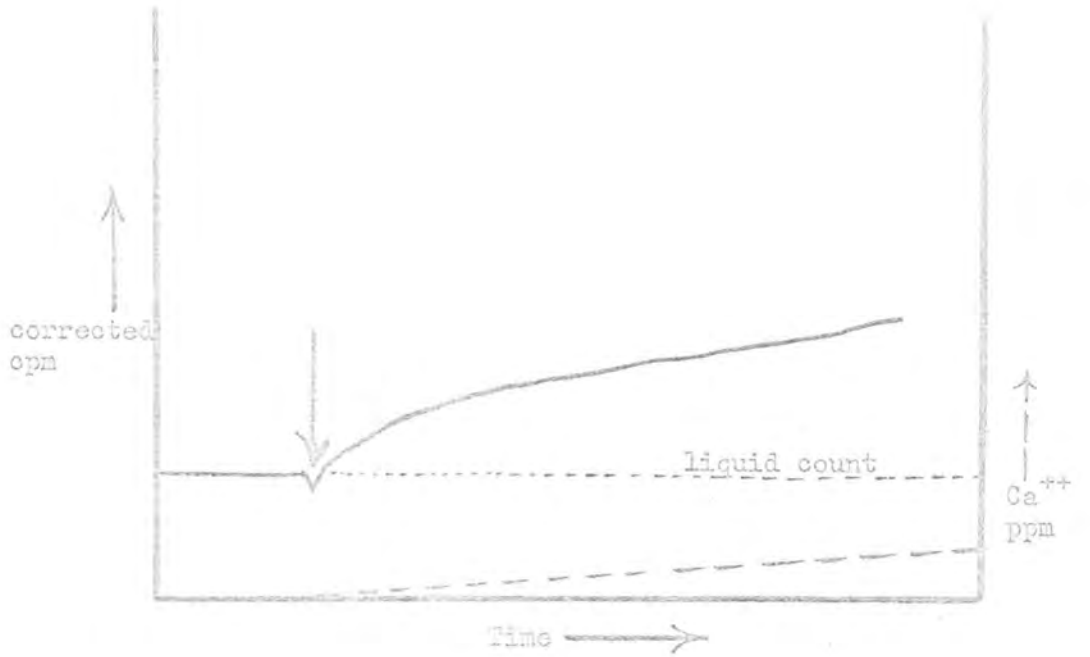


Figure. 33.

Graph to illustrate the smoothing of the 'steps' obtained using Method 4.

and over again, the calcium level in the solution increases with time, thus one cannot plot corrected cpm against time, since the calcium concentration varies.

Method 3

Here, 2 litres of isotope solution were made up and 250ml were used for each run through the apparatus used in Method 2. In this experiment, new amounts of isotope solution were used and not the same solution over and over again. The advantage was that the calcium concentration was constant, that is, the amount of leaching from the snail was constant over a definite period of time. A series of experiments with Limnaea pereger were performed at 0, 6ppm, 66ppm and 350 ppm Ca^{++} concentrations. Results were expressed as corrected cpm (or AF) against time, and also AF against calcium ion concentrations.

Method 4

The only way to correct the time lag phenomenon was by inserting a peristaltic pump into the circuit, and leaving out the beaker. Results from this method gave a more even graph of corrected cpm against time than Method 3, (Figure 33).

The disadvantage of this method with snails was that it did not ensure a constant calcium level, since the same solution was

circulating over the snail a large number of times, and therefore leaching of calcium from the snail caused an increase with time.

This method has proved successful with smaller organisms which do not have a large concentration of calcium salts which are available for ion-exchange in their bodies, e.g., the uptake of 137 Ba by the moss Fontinalis. (Pickering 1964).

All the experiments on continuous flow were with unfed snails.

Experiments in a Humid Atmosphere as an alternative to an aquatic environment

Preliminary method. (Method 1)

The aim of these experiments was to see whether the isotope is transferred from the body to the shell, and at the same time to provide evidence on survival under these humid conditions.

The first set of experiments were with unfed Limnaea auricularia and Limnaea stagnalis, in a humid (saturated aqueous) atmosphere in a thermostatic bath at 18°C, after being injected with 85 Sr solution into the highly muscular foot. Since only a small volume of the isotope solution could be injected, then the solution must have a high activity. A 50 ul Hamilton microsyringe was used to inject 0.01ml of 85 Sr solution into the foot. (Figures 34 and 35).

The snails were moving after 3 days in the conical flasks. The snails were weighed (fresh weight), counted whole, dissected into shell and body, and the activity extracted as on pages 52-53 The conical flasks were washed out with acid and counted to see whether any activity had passed from the snail to the glass when the snail had moved about the flask.

Method 2

Three sets of experiments were set up as a result of Method 1,

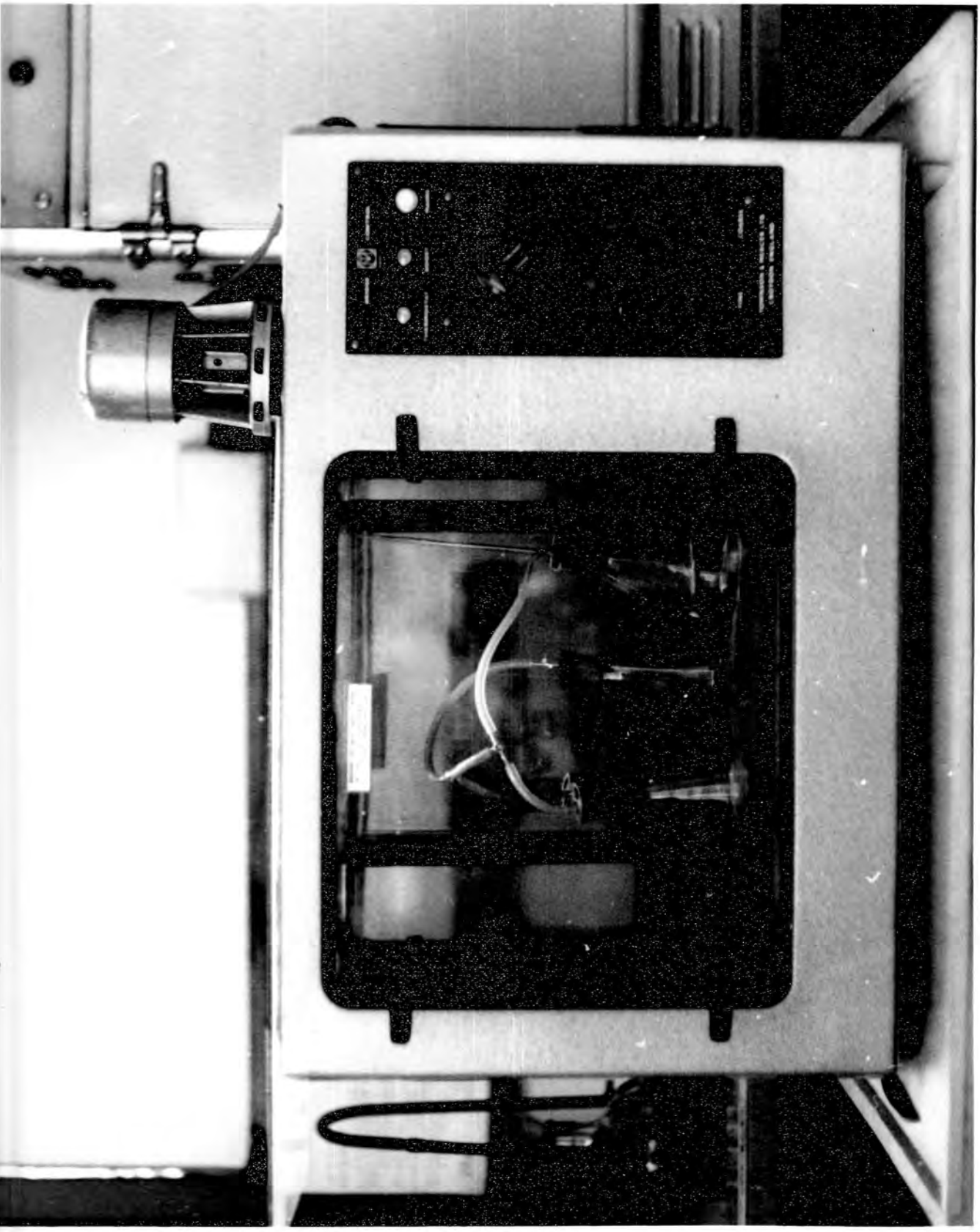


FIGURE 34.
PHOTOGRAPH SHOWING APPARATUS FOR EXPERIMENTS IN A HUMID ATMOSPHERE.

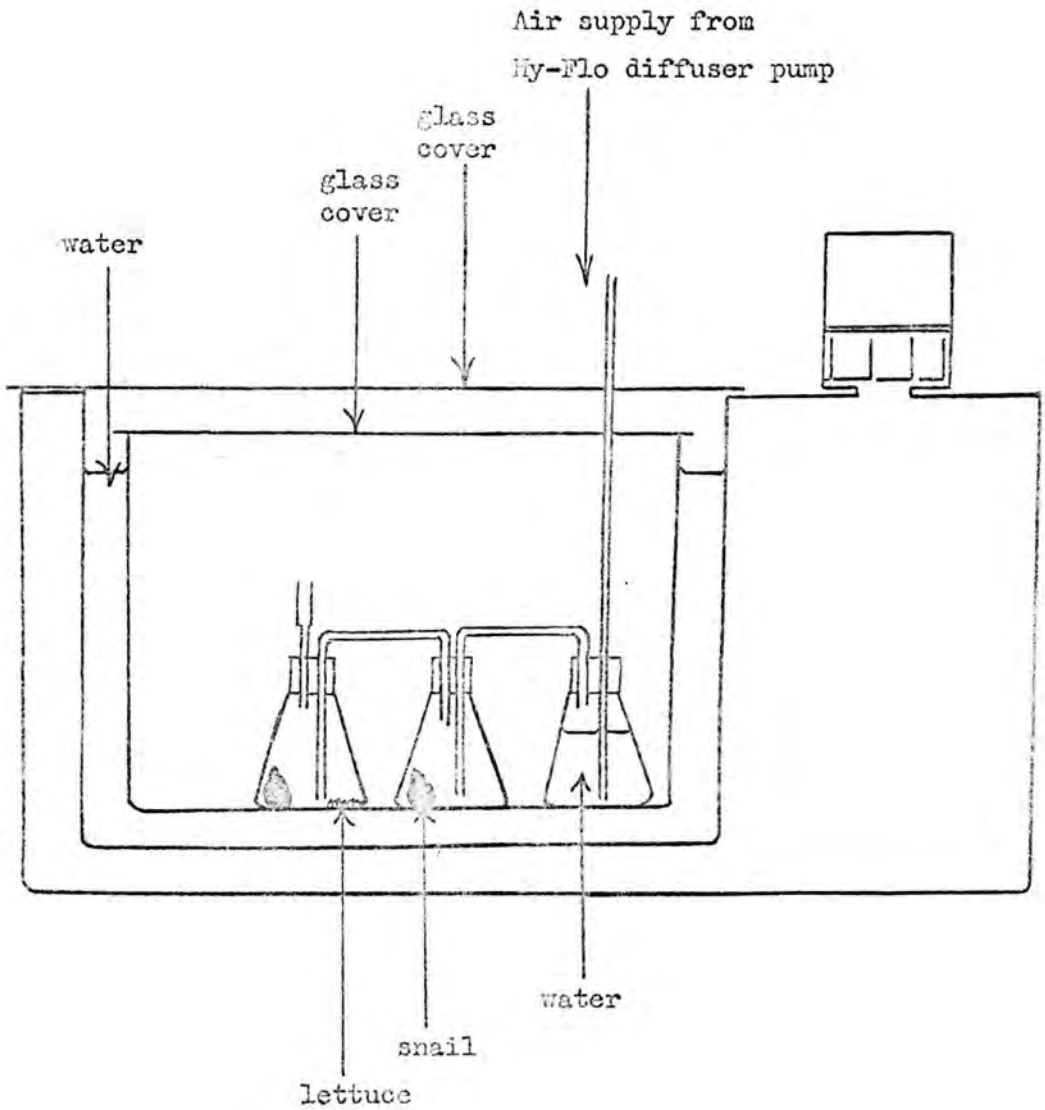


FIGURE 35.

Plan of the thermostatic bath, and arrangement of apparatus for humidity experiments mentioned on page 64.

See photograph on page 65 .

with the object of comparing movement of isotope from (a) body to shell with (b) food to shell.

(i) Injection of 85 Sr and 47 Ca solutions into the foot of Limnaea auricularia.

There was considerable difficulty in injecting isotope into the foot or body of Bithynia and Viviparus, since they possessed hard opercula which closed when stimulated by the syringe.

47 Ca was used to see whether it followed the same path as 85 Sr.

(ii) Injection of 85 Sr into the vascular bundles of lettuce.

(iii)Ingestion from the contaminated lettuce by the snails.

The isotope was injected into the mid vein (vascular bundles), since if the isotope had been placed on the surface of the lettuce, then uptake could have taken place via the foot, body or shell (by contact) as well as the alimentary canal.

In addition to counting the snail, any pieces of uneaten lettuce were weighed, counted whole and the activity extracted as on pages 52-53.

Results.

The results of these injection experiments cannot be expressed as A.F's. since there was no surrounding liquid. The results were expressed as either corrected cpm or correct cpm/fresh weight in grams against time. No reference to the external calcium concentration could be made, as there was no surrounding liquid habitat.

Expression of the results

1. Statistical corrections

Counter corrections e.g., calculation of overall standard deviation.

Background (BG) 70 counts in 10 minutes

Sample + Background 300 counts in 10 minutes

$$BG = 7 \pm \sqrt{\frac{7}{10}} = 7 \pm 0.84 \text{ cpm } (\sigma\ 1)$$

$$\text{Sample} + BG = 30 + \sqrt{\frac{30}{10}} = 30 \pm 1.73 \text{ cpm } (\sigma\ 2)$$

$$\text{Sample} = 23 \pm \sqrt{\frac{7}{10} + \frac{30}{10}} = 23 \pm 1.9 \text{ cpm } (\sigma\ 3)$$

$$= 23 + 8\%$$

One can improve precision, by extending measurements, or one can calculate the number of counts to requisite precision. In radioactivity, if the count is low, then one must count for a long period of time to obtain a significant count. (Holman 1962 : Youden 1957).

Expression of results for β -isotopes

The results from all the methods used on pages 49-53 were expressed as Accumulation Factors (A.F) and these were plotted against time or against calcium ion concentration of the surrounding solution at the time of breakdown of the snail or plant.

$$A.F. = \frac{\text{cpm of the snail or plant}}{\text{weight of the snail or plant}} \times \frac{10}{\text{cpm of the surrounding medium}}$$

To distinguish between Fresh Weight and Dry Weight determinations, the A.F's are expressed as A.F (F.W) or A.F (D.W) respectively.

Expression of results for γ-isotopes

In β- counting the material is in a liquid form, since the activity of the organism cannot be counted a number of successive times.

In γ- counting the same organism can be counted a number of successive times. Thus the solid count on the whole or dissected snail must have a correction applied to it, so that it is comparable with the liquid count obtained on acid extraction. This correction is due to the different geometry which a solid object presents to the NaI crystal, compared with a liquid sample. Therefore, the count rate of solid to liquid is determined for the snail or the plant in the 85 Sr and 47 Ca experiments.

$$\text{Geometry Factor 'f'} = \frac{\text{count (cpm) on the solid}}{\text{count (cpm) on the solid dissolved in the liquid.}}$$

$$\text{A.F} = \frac{\text{cpm of the snail or plant}}{\text{weight of the snail or plant}} \times \frac{5}{\text{cpm of the surrounding liquid}} \times \text{'f'}$$

The geometrical factors ('f') for Limnaea pereger, are listed in Figure 36, and 2 plots of (i) Shell length (SL) against 'f' in Figure 37, and (ii) log SL against 'f' in Figure 38. It can be seen that there is an exponential relationship between these two factors.

Name of snail	SL mm	'f'
Limnaea pereger	14	0.78
" "	14	0.73
" "	11-12	0.81
" "	10-11	0.83
" "	8-9	1.30
" "	7-8	1.41
" "	6-7	1.44

Figure 36.

Table of Geometrical factors for 85 Sr, and Shell Length (SL), for L. pereger. The Geometrical Factor (f) is mentioned on page 69.

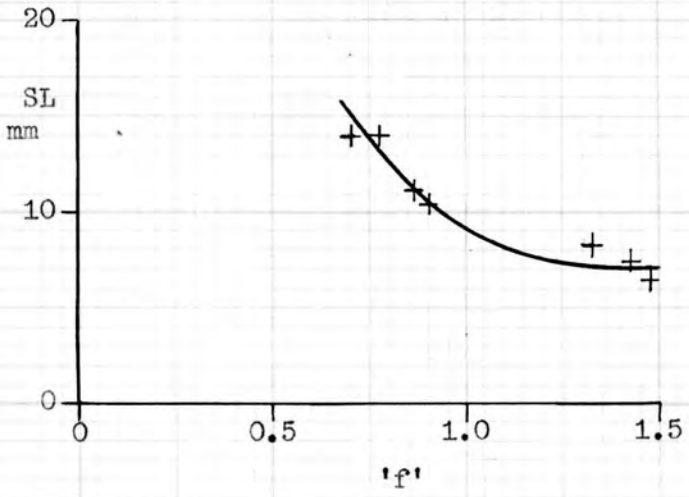


Figure 37.

Plot of Shell Length (SL) in mm against 'f'.

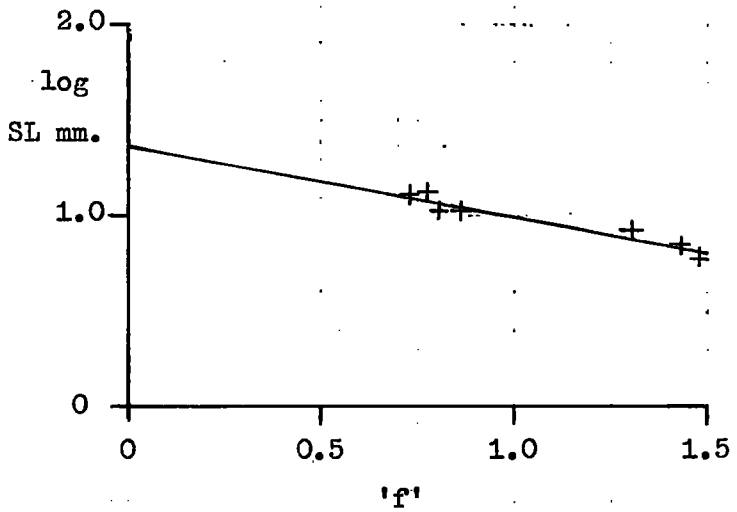


Figure 38.

Plot: log Shell Length (SL) in mm against 'f' (Semi-log plot).

Calculation of snail and liquid counts used in Continuous flow experiments using 85 Sr (page 57)

Tube	Count on liquid and snail	=	5074 cpm
	Count on liquid	=	2799 cpm
		=	3275 cpm
Pot	Count on liquid and snail	=	6374 cpm
	Count on liquid	=	3671.4 cpm
	Count on snail	=	2702.6 cpm

Ratios or Conversion factors

Snail + liquid = $\frac{6374}{5074}$ = 1.2563 Liquid = $\frac{3671.4}{2799}$ = 1.3116

Snail = $\frac{2702.6}{3275}$ = 0.8252 (Limnaea pereger)

Corrected values

(i) Corrected value for snail = $\frac{3275 \times 0.8252}{2.3537}$ = 1148.2 cpm/g

(ii) Corrected value for liquid = 2799 x 1.3116 x 1/5 = 3671.17/5 = 734.23 cpm/ml.

Note: Figures corrected for background and decay.

Accumulation Factors

$$A.F. = \frac{\text{corrected cpm of snail}}{\text{weight of snail}} \times \frac{5}{\text{corrected cpm of liquid}}$$

A.F. = 1148.5/734.23 = 1.5638 @ 2.5 ppm Ca^{++}

In this case, a correction has been made for both the snail and the liquid, since one is comparing 2 different geometrical shapes, that is, the pot and the tube.

Expression of the results.

These results were expressed as :-

(i) corrected cpm against time (@ constant calcium concentrations).

(ii) corrected cpm against time (@ varying calcium concentrations).

(iii) A.F against time (@ constant calcium concentrations).

(iv) A.F against calcium concentrations.

These results are extremely important since they show the uptake of isotope by the snail in the first hours.

EXPERIMENTAL WORK

A N D

R E S U L T S

Introduction

The aim of the experimental work was to study the influence of calcium in the surrounding medium on the uptake of 89 Sr, 90 Sr, 85 Sr, and 47 Ca by species of molluscs listed on page 2 .

The scheme of analysis is expressed diagrammatically below:-

	Whole Snail								Shell/Body								Eggs	Shells
	Unfed				Fed				Unfed				Fed					
	Sr 89	90	85	47	89	90	85	47	89	90	85	47	89	90	85	47	85	89/90
Limnaea pereger	1	2/3	4				5a 5b		7		6						18	20/
L. truncatula	8																	
L. auricularia				10 11			9	10 11			19	19			9 19			
L. stagnalis										17								/21
Bithynia tentaculata	12	13 *16		15			14					15						
Viviparus viviparus										17								

Figure 39

Table showing the number of experiments performed. The number in the table is the number of the Experiment.

Notes: * uptake in the dark; 5 a - the food source is lettuce;

5b - the food source is Elodea.

In all 89Sr & 90Sr expts. counts after 3weeks have been given. In Expts 1, 2, 7, 17, initial counts are quoted. Initial counts are not purely Strontium counts but are modified by the presence of Yttrium 90.

Experiment 1

The uptake of 89 Sr by unfed Limnaea pereger (whole snail)

The uptake of 89 Sr by L. pereger under calcium concentrations from 0-90 ppm Ca^{++}, was studied for 35 days under non-aerated conditions as shown in Figure 1b. The snails were unfed for the duration of the experiment.

The average fresh weight of the snails was 0.1080 g and their length was 2-3 mm. The snails were from an aquarium containing breeding L. pereger and the calcium concentration at the time of sampling was 50 ppm Ca^{++}.

The uptake of 89 Sr was plotted as :-

(i) Accumulation Factor (fresh weight) against time, for certain calcium ranges, as shown in Figure 40, and the results in Figure 41.

It can be seen that the uptake of isotope is greater at low calcium levels in the medium and that the initial counts[89Sr] (see page 77) are always greater than 89 Sr. Equilibrium is reached sooner at high calcium levels, i.e. 7 days at 66-75 ppm Ca^{++} compared with 14 days at 8-12 ppm Ca^{++}. The graph also shows that above 66-75 ppm Ca^{++}, further additions of calcium would not markedly affect the equilibrium level.

If, as their shape suggests, these curves are exponential growth curves, (Rosenthal, 1957, 1960) then E_t is approximately 5 times the exponential half life, i.e. $E_t/5 = t_{\frac{1}{2}}$ which is 97% accurate,

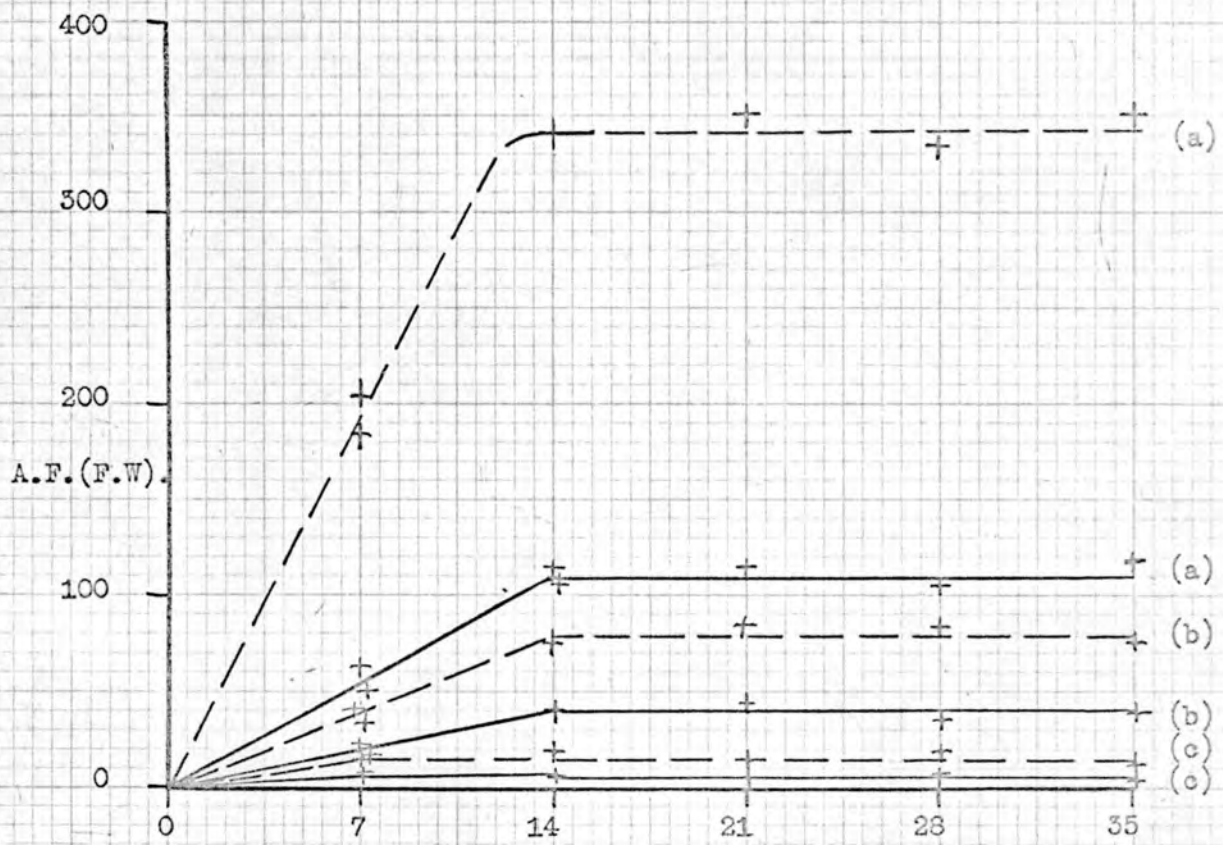


Figure 40 .

Plot: A.F(F.W) against Time,in days at calcium ranges of (a) 8- 12 ppm Ca^{++}, (b) 18 - 22 ppm Ca^{++}, (c) 66- 75 ppm Ca^{++}, for Initial uptake (dashed lines) and 89 Sr (continuous lines). Uptake of 89 Sr by Limnaea pereger (whole snails). Each reading (+) is the mean of 10 snails. 89Sr counts are 3 week counts.

(Nelson 1963). These relationships are summarised in the following equation (Dawes 1956) :-

$$A.F = \frac{k.t_{\frac{1}{2}}}{0.693}$$

where k = the rate of uptake and $t_{\frac{1}{2}}$ is the half life of the process. AF/E_t is proportional to the rate of uptake, and from Figure 41, is higher at low calcium levels. E_t or $E_t/5$ is smaller at higher calcium levels, which means that the rate of release of calcium is greater at higher calcium levels.

Ca^{++} ppm	A.F(FW) at Equilibrium	Time taken to reach Equilibrium E_t (in days)	$E_t/5$	A.F(FW)/E_t
8 - 12	Y-Sr 340	14	2.8	24.2
	89 Sr 110	14	2.8	7.8
18 -22	Y-Sr 78	14	2.8	5.5
	89 Sr 40	14	2.8	2.8
66- 75	Y-Sr 15	7	1.4	2.15
	89 Sr 9	7	1.4	1.28

Figure 41

Table of results obtained from Figure 40, which shows the relationship between A.F(FW) and Equilibrium time, E_t.
Y-Sr are A.F's based on initial counts.
89 Sr are A.F's based on three week counts.

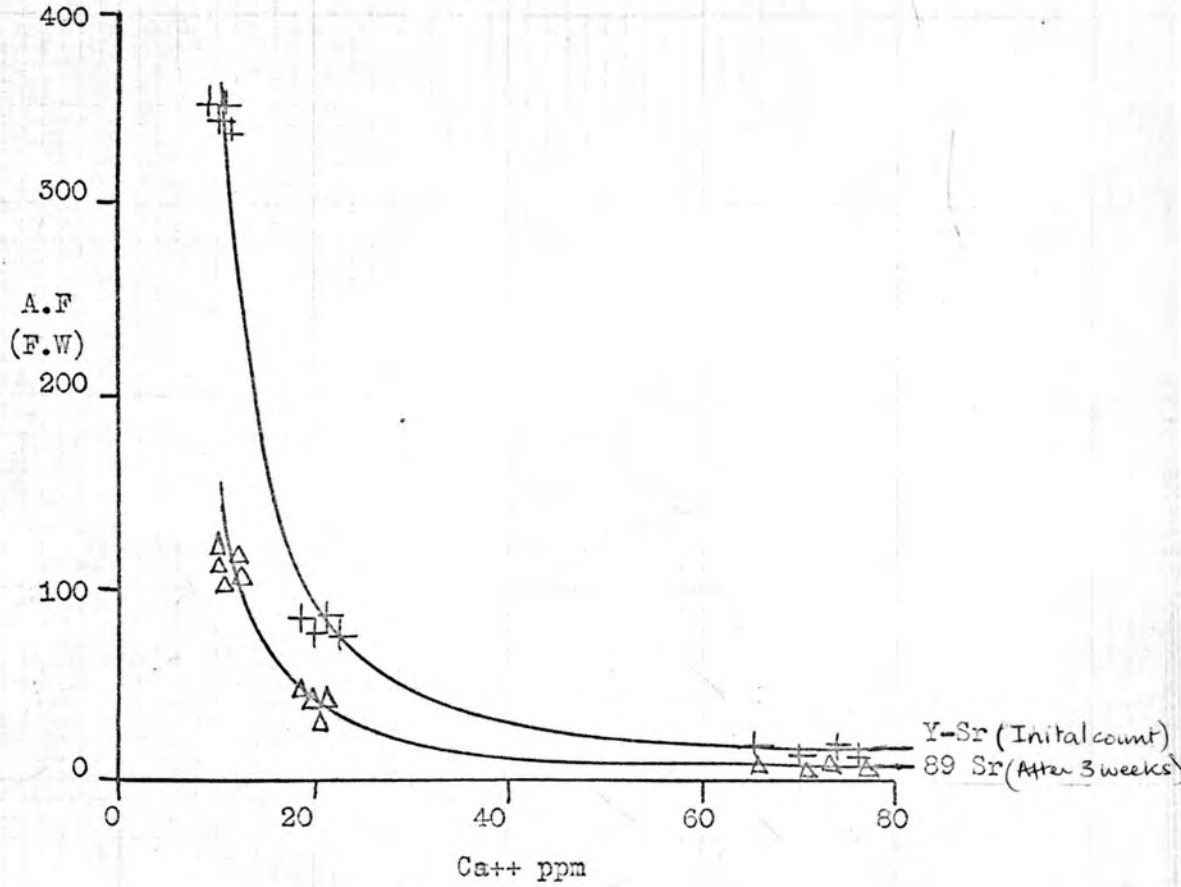


Figure. 42 .

Plot: A.F.(F.W) against calcium concentration. Uptake of Y-Sr (+) and 89 Sr (Δ) by Limnaea pereger, from 14 days to 5 weeks, at calcium ranges mentioned in Figure 40 . Readings are the mean of 10.
Y-Sr are A.F's based on Initial count.
89 Sr are A.F's based on 3 week count.

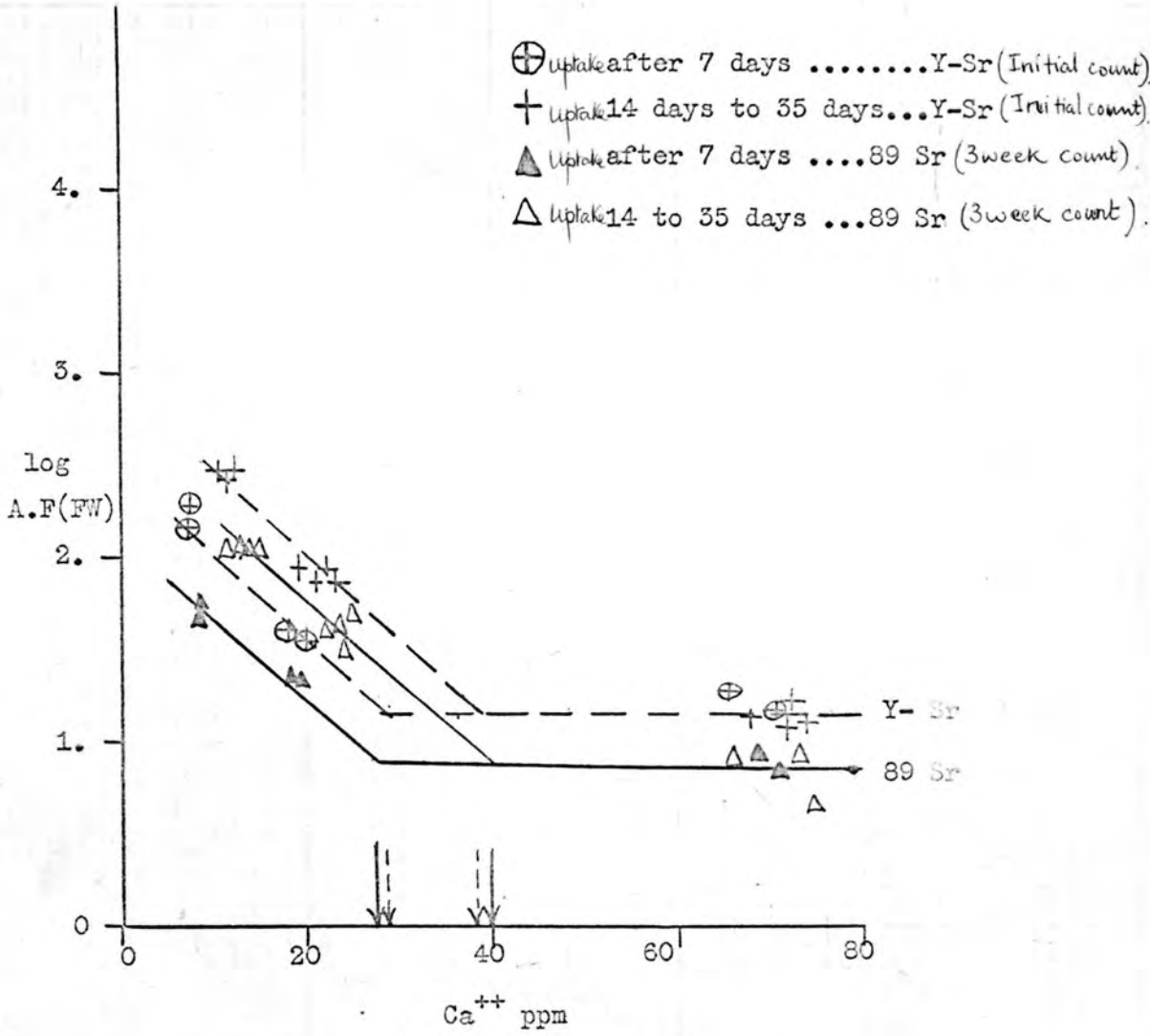


Figure 43

Plot: log A.F (F.W) against calcium concentration.

Uptake of 89 Sr by Limnaea pereger.

Readings are the mean of 10.

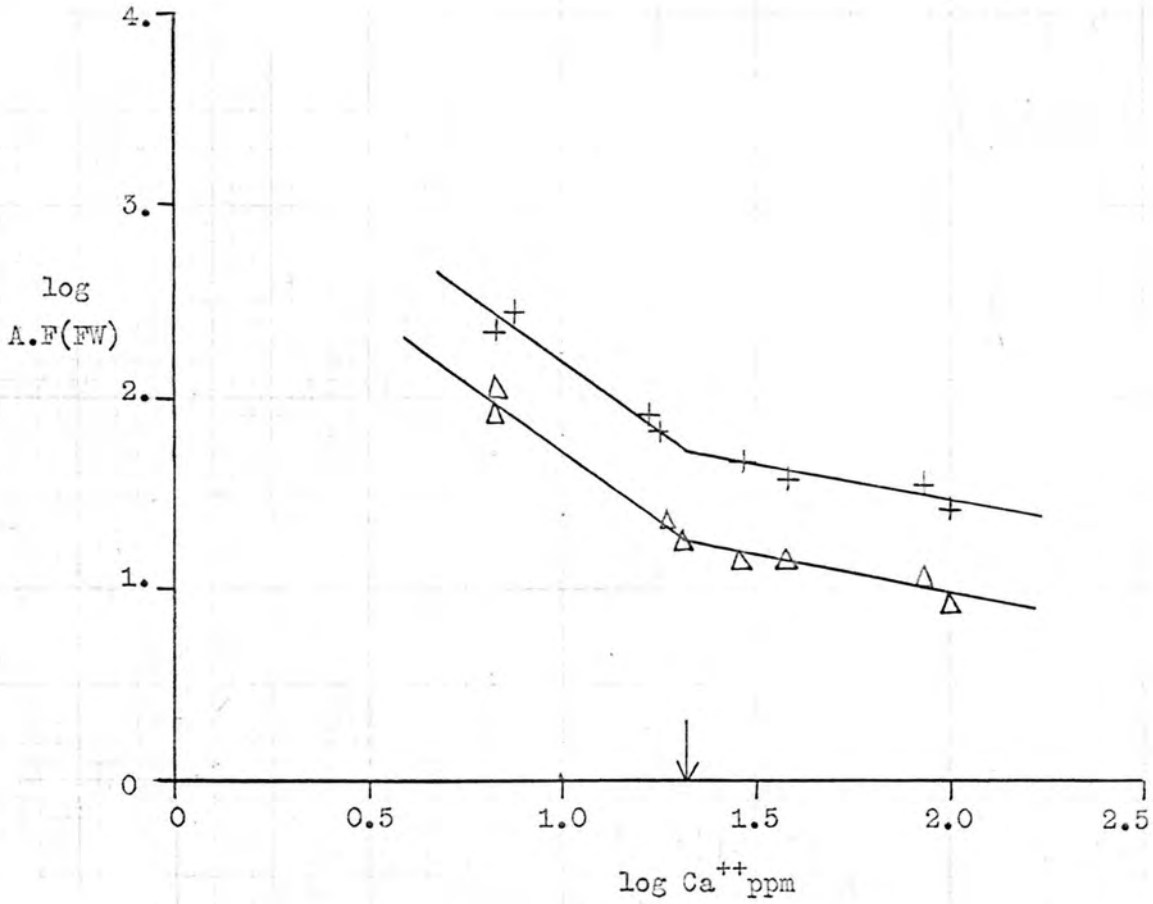


Figure 44 .

Plot: log A.F(FW) against log calcium concentration.

The uptake of 89 Sr by Limnaea pereger at 21 days.

Readings are the mean of 10 .

+ is Y-Sr and Δ is 89 Sr.

Y-Sr are A.F's based on Initial counts

89Sr are A.F's based on 3 week counts

(ii) A plot of A.F(FW) against calcium concentration at 21 days is plotted in Figure 42. At this time all the A.F(FW) at the different calcium levels will have attained equilibrium. The uptake is greater at low calcium levels, particularly below 20 ppm Ca^{++} , than at higher calcium levels. A.F's based on initial counts are higher than A.F's based on the three week count (89 Sr) at all calcium levels.

(iii) A semi-log plot of A.F(FW) against calcium concentration was plotted in Figure 43 . It can be shown that 2 lines may be drawn through the points, suggesting two processes of uptake. The lines change their slope at different calcium levels, dependent on the time. The slope changes at 26 ppm Ca^{++} for 89 Sr at 7 days, to 40 ppm Ca^{++} at 14-35 days.

(iv) A log plot of A.F(FW) against calcium concentration was plotted in Figure 44. From the expression,

$$\log \underset{\text{isotope}}{\text{A.F(FW)}} = a - b \log Ca^{++} \text{ ppm},$$

where 'a' is the log A.F(FW) at calcium value of 1 and 'b' is the slope; then the following expressions are obtained from Figure 44.

$$\log A.F(FW)_{^{89}Sr} = 1.29 - 1.0 \log Ca^{++} \text{ ppm (below 22.4 ppm)}$$
$$= 1.8 - 0.4 \log Ca^{++} \text{ ppm (above 22.4 ppm)}$$

It can be observed that the slope changes at 22.4 ppm Ca^{++}. A slope of -1.0 means that the rate of release is proportional to the total uptake at any time. This seems to be true for calcium (see experiment 10 page 142), and a slope of the same order in strontium, implies that the strontium follows the same path as calcium, i.e. at the same rate. A slope of -0.4 indicates that the strontium is not following the calcium, i.e. a lower rate, and conversely a slope greater than -1.0 means that the strontium goes through the membrane more easily than the calcium, i.e. at a higher rate.

This experiment proves that the uptake of strontium 89 is calcium dependent below 22.4 ppm Ca^{++} in the medium, and not solely dependent above this level.

Experiment 2

The uptake of 90 Sr by unfed Limnaea pereger (whole snail)

The uptake of 90 Sr by Limnaea pereger was studied in unfed snails in calcium concentrations varying from 0-80 ppm Ca^{++}, over a period of 21 days, and under the same conditions as with 89 Sr i.e. non-aeration. The snails were collected from the Shropshire Union Canal, Chester and the calcium concentration at the time of sampling was 40.52 ppm Ca^{++}. The mean fresh weight of the snails was 0.3658 g.

The uptake of 90 Sr was plotted as:-

(i) log A.F(FW) against log calcium concentration, in Figure 45.

Expressions from the graph in Figure 45 are on page 86.

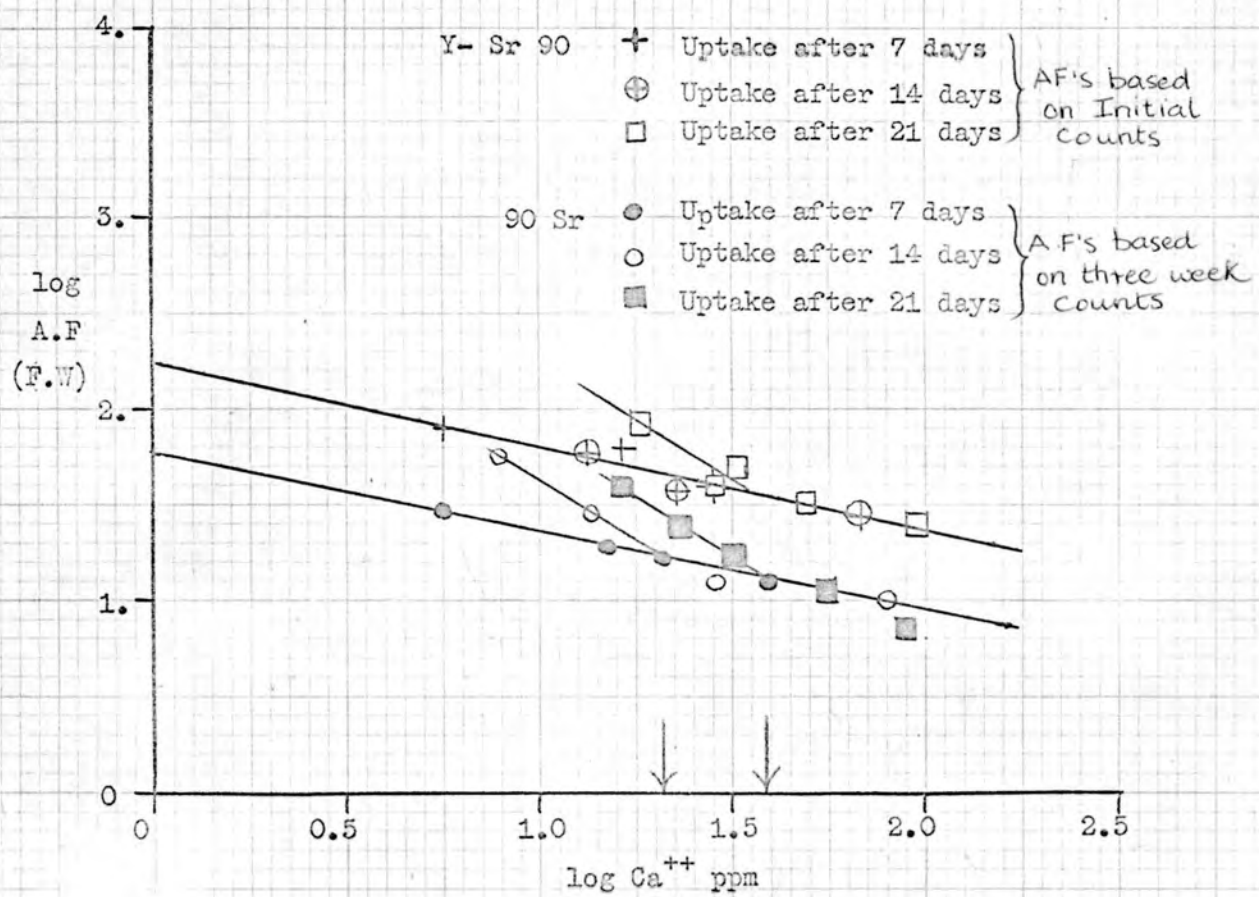


Figure 45

Plot: log A.F (F.W) against calcium concentration.

Uptake of 90 Sr by Limnaea pereger at varying calcium concentrations.

Each reading is the mean of 10.

log A.F(F.W) ^{90}Sr = 1.78 - 0.4 log Ca^{++} ppm (at all calcium levels at 7 days).

= 1.78 - 0.4 log Ca^{++} ppm (above 21 ppm Ca^{++} at 14 days).

= 1.0 - 0.85 log Ca^{++} ppm (below 21 ppm Ca^{++} at 14 days).

= 1.78 - 0.4 log Ca^{++} ppm (above 38.9 ppm Ca^{++} at 21 days).

= 1.0 - 0.85 log Ca^{++} ppm (below 38.9 ppm Ca^{++} at 21 days).

The rate of uptake above 21 ppm Ca^{++} is similar to that obtained for 89 Sr **on pages** 82 and 85 . The results below this figure show a lower slope than that obtained with 89 Sr.

(ii) A plot of slope against time in days, is plotted in Figure 46 for 90 Sr figures. This suggests that Equilibrium is attained after 14 days in the isotope at low calcium levels, and at 7 days for calcium levels above 38.9 ppm Ca^{++}.

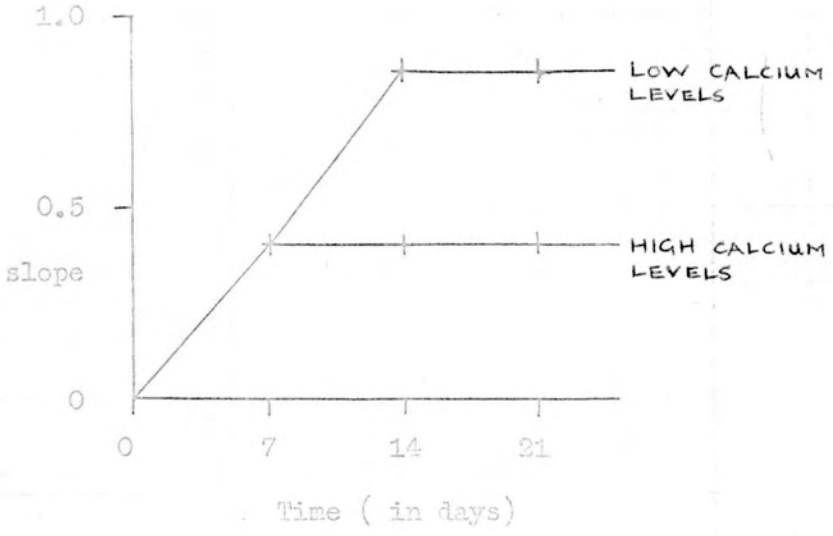


Figure 46

Plot: slope of uptake of 90 Sr by Limnaea peregeri, against Time (in days). The data was abstracted from Figure 45 .

Experiment 3

The effect of aeration on the uptake of 90 Sr by unfed Limnaea pereger (whole snails)

Aeration was supplied by means of a Hy-Flo diffuser pump and the set up shown in Figure 1a.

The uptake of 90 Sr by the snails in an aerated environment is plotted in Figure 47, as A.F(F.W) against certain calcium ranges from 0-90 ppm Ca^{++}. The mean fresh weight of the snails was 0.2564 g.

The results from Figure 47, are tabulated in Figure 48, and a comparison between aerated and non-aerated conditions are tabulated in Figure 49.

By plotting A.F (F.W)/E_t, i.e. rate of uptake, against calcium concentration in Figure 50, it can be seen that aeration does not significantly affect the uptake of 90 Sr by Limnaea pereger.

It was noted that the pH did not vary appreciably during the time of aeration, i.e. from pH 6.5 to 6.8, over 21 days.

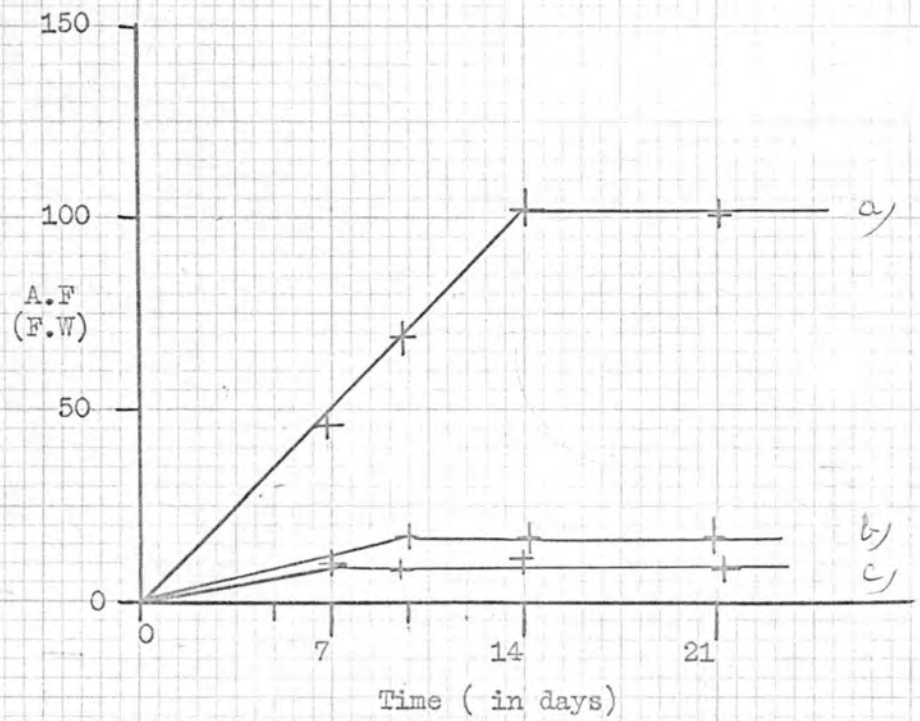


Figure. 47.

Plot: A.F (F.W) against Time (in days).

Uptake of 90 Sr by Lymnaea pereger under aerated conditions supplied by means of a Hy-Flo pump, at calcium ranges of (a) 7-14 ppm Ca^{++}, (b) 35- 54 ppm Ca^{++} and (c) 60- 75 ppm Ca^{++}, Each reading being the mean of five.

A.F of 90Sr, based on three week figures

Ca^{++} Range	A.F at Equilibrium	E_t	AF/E_t
7 - 14	104	14	7.4
35 - 54	18	10	1.8
60 - 75	10	7	1.5

Figure 48

Table of results obtained from Figure 47, page 89.

Aerated			Non-Aerated		
Ca^{++} Range	Mean of Ca^{++} Range	AF/E_t	Ca^{++} Range	Mean of Ca^{++} Range	AF/E_t
7 - 14	11	7.4	8 - 12	10	7.8
35 - 54	45	1.8	18 - 22	20	2.8
60 - 75	68	1.5	66 - 75	70	1.3

Figure 49.

Comparison of results obtained with and without aeration.

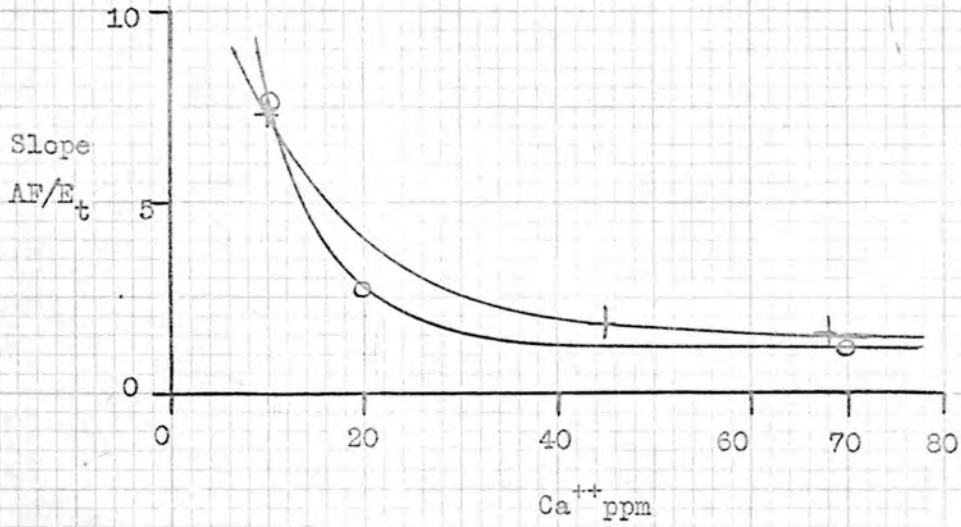


Figure. 50.

Plot of AF/E$_t$ (Slope) against calcium concentration, for aerated (+) and non-aerated (o) conditions.

Data from Figure 49 ,page 90 .
(see page 76 for explanation)

Experiment 4

The uptake of 85 Sr by unfed Limnaea pereger (whole snail)

A method of continuous flow was employed over 3 days. Since no re-circulation of solution was employed, the calcium level in the medium remained constant throughout the experiment.

In those experiments that lasted more than 3 days, the continuous flow method was not employed, but the aerated solution was changed frequently, so that the calcium level in the medium remained the same.

Calcium concentrations varied from 63-650 ppm Ca^{++}, and the experiment was over 35 days. The snails were counted at intervals of time as shown in Figure 51, which is the plot of A.F(FW) against Time in days, at constant calcium levels in the medium.

Equilibrium can be seen to vary with the different calcium levels, and the results are tabulated in Figure 52. These snails were not fed throughout this experiment, and the mean fresh weight was 0.1976 g.

The uptake of 85 Sr seems to be linear with time, which indicates the continued formation or exchange of mineral component of the snail (shell) with sequestation of the isotope with the shell matrix. (Rosenthal 1956).

The uptake of 85 Sr by whole snails (unfed) of Limnaea pereger, over 35 days at constant calcium levels. The points up to 5 days are the mean of 10 snails, and afterwards they are the mean of 5 snails.

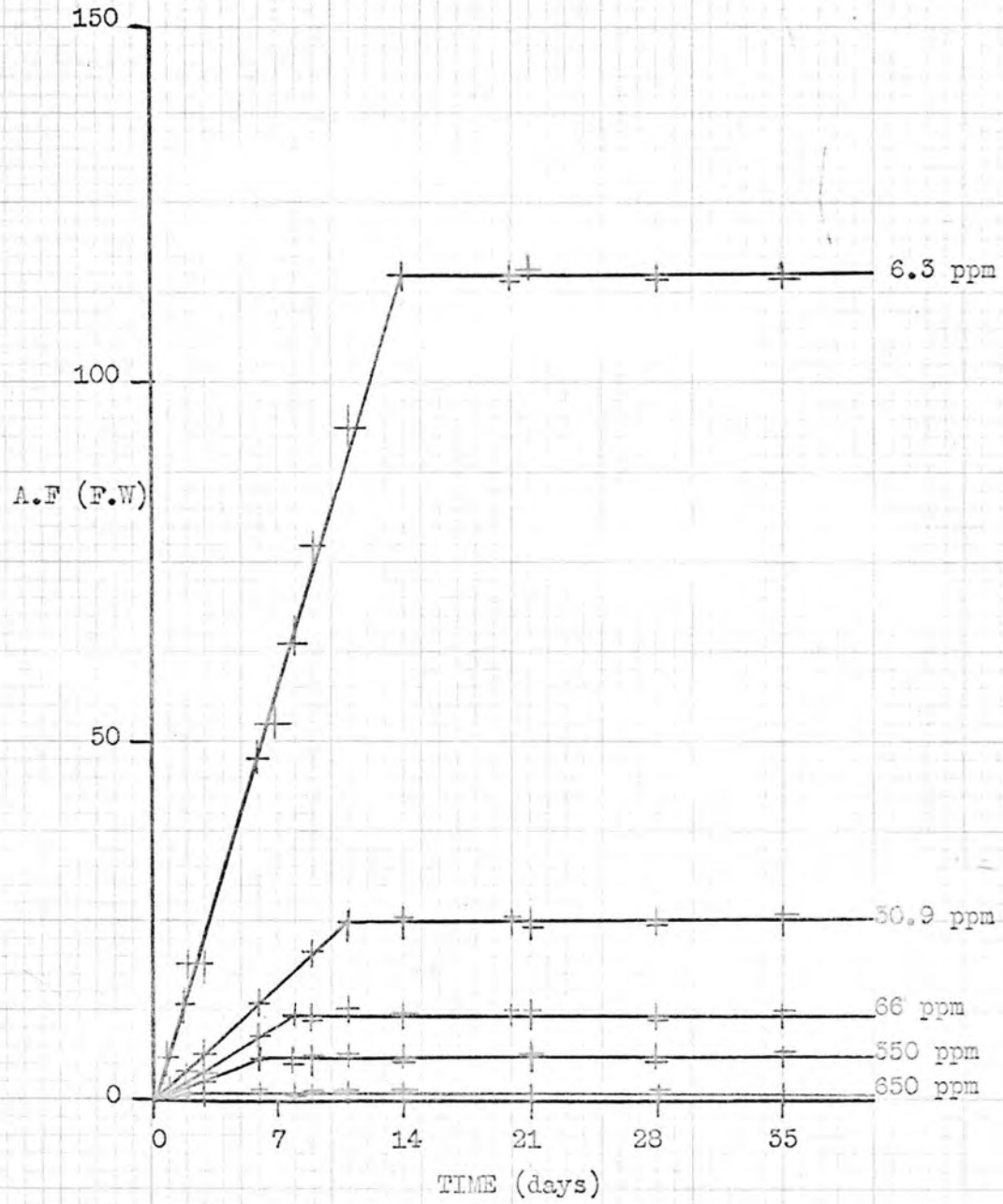


Figure 51 .

Plot: A.F(F.W) against Time (in days), at constant calcium levels - ranging from: 6.3 ppm Ca^{++}; 30.9 ppm Ca^{++} 66 ppm Ca^{++}; 350 ppm Ca^{++} and 650 ppm Ca^{++}.

Ca^{++} ppm	Equilibrium AF(FW)	log AF (FW)	Time in days of Equilibrium E_t	$E_t/5$	AF/FW/ E_t
6.3	115	2.0607	14	2.8	8.21
30.9	25	1.3979	11	2.2	2.27
66	14	1.1461	8	1.6	1.75
350	4	0.6990	6	1.2	0.83
650	0.9	$\bar{1}$.9542	2	0.4	0.45

Figure 52.

Table of results obtained from Figure 51, page 93.

Uptake of 85 Sr by unfed Limnaea pereger under aerated conditions at constant calcium levels.

The figures in Figure 52, are extremely similar to those obtained for 89 Sr on page 78, and suggest that the rate of uptake is similar for 85 Sr. Neeson, Rvonback & Roser (1963) found results obtained for 89 Sr and 85 Sr uptake by mice were in good agreement with one another.

From Figure 52, it can be seen that $E_t/5$ decreases with increasing calcium concentrations in the medium, and that $E_t/5$ approximates $A.F(FW)/E_t$ above 30.9 ppm Ca^{++}. These relationships can be expressed as:-

$$\frac{E_t}{(E_t)^2}/5 \text{ approximates } A.F(FW)/E_t = 5.A.F(FW) \quad \ldots\ldots\ldots \text{Eqn1.}$$

$$\underline{\text{or}} \qquad E_t = \sqrt{5.\ A.F(FW)} \qquad \ldots\ldots\ldots \text{Eqn. 2.}$$

Thus, one can check the Equilibrium Time if one determines the A.F (FW) or vice versa. This relationship appears to hold true for A.F(FW) determined above 30.9 ppm Ca^{++}.

If one takes the value for A.F(FW) at 6.3 ppm Ca^{++}, as 115 and substitute it in Eqn. 2, then

$$E_t = \sqrt{5.115} = \sqrt{575} = 23.9 \text{ days}$$

$\underline{\text{or}}$ if E_t = 14 days in Eqn. 1 then,

$$(E_t)^2 = 5.\ A.F(F.W)$$

$$A.F(FW) = \frac{14.\ 14}{5} = \frac{196}{5} = 39.2\ A.F(FW)$$

These results do not agree with one another, and do not agree with the equations 1 & 2. This suggests that a different process of uptake occurs below 30.9 ppm Ca^{++}.

A semi-log plot of log A.F(FW) against Time (in days) in Figure 53, results in a straight line. This would suggest that the time taken to reach equilibrium is proportional to the $\log_e$ A.F(FW).

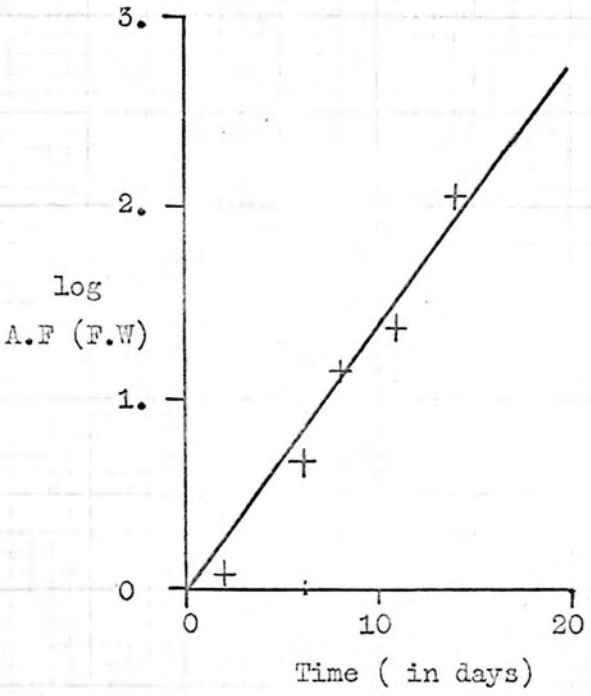


Figure 53 .

Plot: log A.F (F.W) against Time (in days). E_t.

Uptake of 85 Sr by Limnaea pereger.

Data obtained from Figure 52 page 94 .

Experiment 5(a)

The uptake of 85 Sr by fed Limnaea pereger (whole snails)

Uptake by the food

(i) The uptake of the lettuce used as food was first determined. Care was taken to avoid the mid-rib and similar pieces of lettuce were used in each experimental jar. It was found that it took 7 days for the lettuce to equilibriate with the isotope solution under aerated conditions. The calcium concentrations varied from 0-240 ppm Ca^{++}. The mean fresh weight of the lettuce was 0.2491 g.

The uptake of 85 Sr after 7 days by lettuce is shown in Figure 54, and 56, that uptake is much less above 20 ppm Ca^{++}, than below this level.

A log plot of A.F(F.W) against calcium concentrations is plotted in Figure 58. The following expression can be obtained from the graph:

$$\log AF\ (FW)_{85\ Sr} = 2.7 - 1.05 \log Ca^{++} \text{ ppm } (+ 7 \text{ days}).$$

Uptake by the fed snail

(ii) Snails were introduced into the experimental jars after the lettuce had equilibriated with the isotope solution, i.e. after 7 days. The snails were sampled at 7 and 14 days after feeding. The mean fresh weight of the snails was 0.0731 g. It was noticed that after 7 days, that the surface layers of the lettuce was eaten.

The uptake of 85 Sr by the feeding snails is shown in Figure 55. The uptake is considerably below 25 ppm Ca^{++}, but not so above

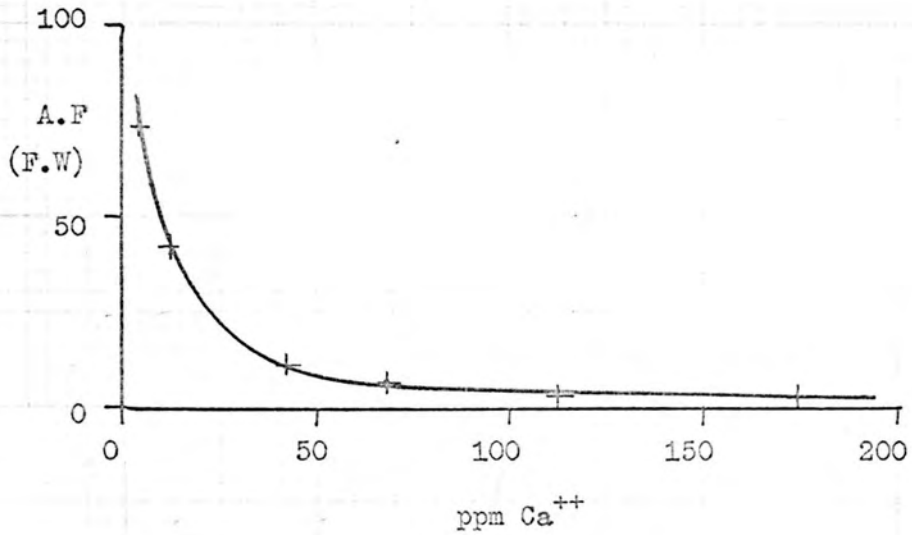


Figure 54 .

Plot: A.F (F.W) against calcium concentration.

Uptake of 85 Sr by Lettuce after 7 days aeration.

Each point represents the mean of 10 pieces of lettuce .

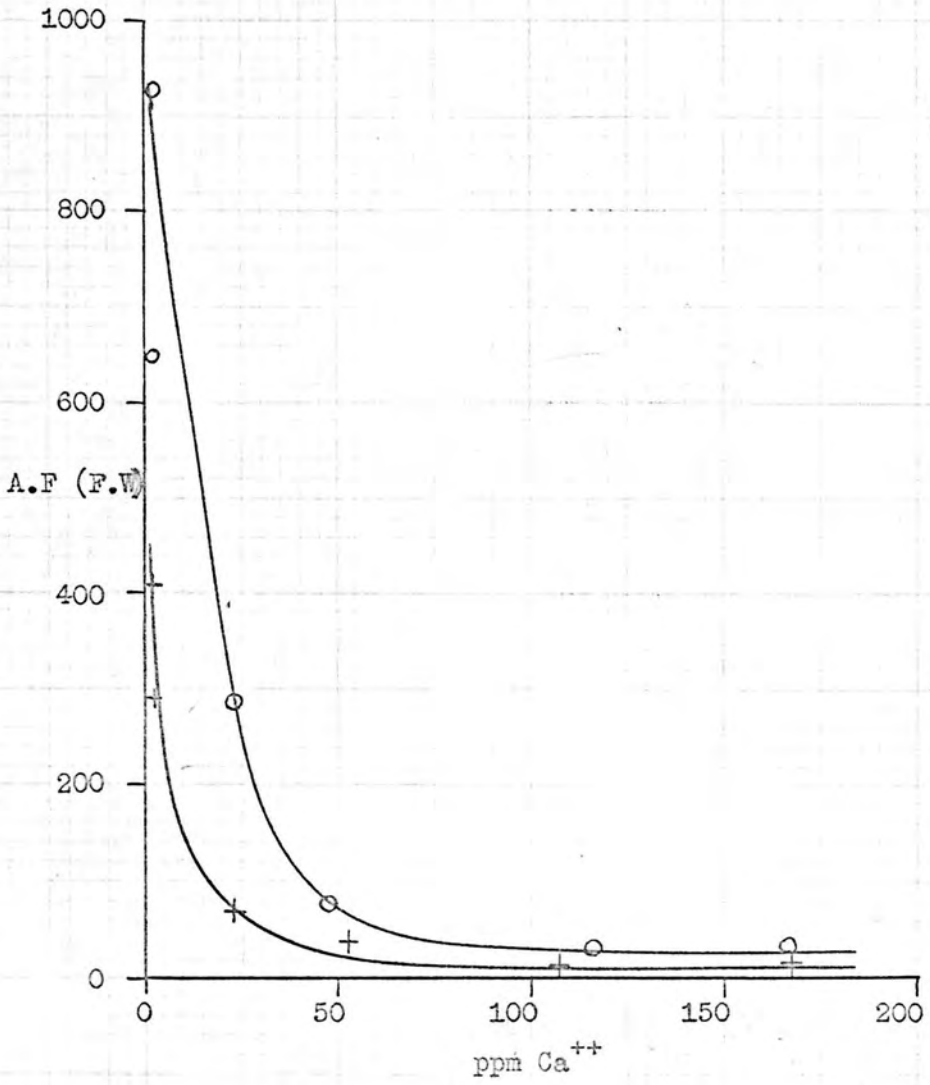


Figure 55.

Plot: A.F (F.W) against calcium concentration. The uptake of 85 Sr by Limnaea pereger, feeding on lettuce, after 7 days (+) and 14 days (o). Each reading is the mean of 7.

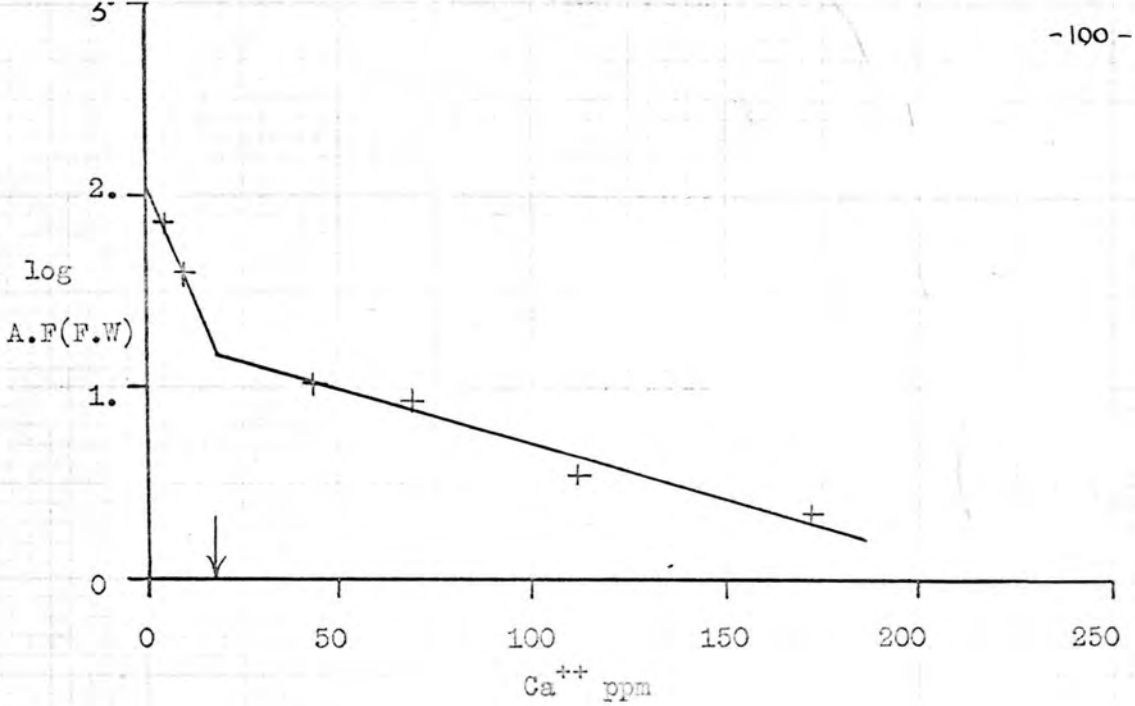


Figure 56.

Plot: log A.F(F.W) against calcium concentration.
The uptake of 85 Sr by lettuce at 7 days. Each reading is the mean of 10.

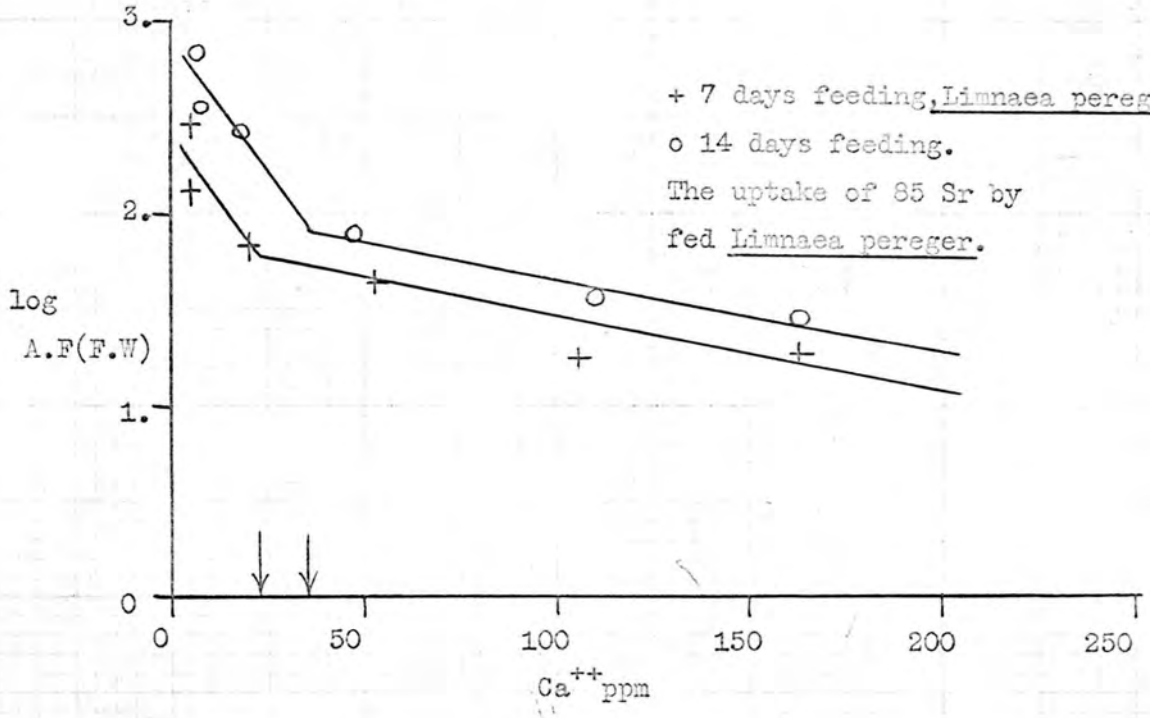


Figure 57.

Plot: log A.F(F.W) against calcium concentration.

this level. The snails were very active during their period of feeding, and their uptake of isotope increased as shown in Figure 57. A plot of log A.F(F.W) against calcium concentration for the lettuce is plotted in Figure 56, and that for the feeding snails in Figure 57. It can be seen that there are two lines in both the lettuce and snail semi-log plots, and that the calcium values at the change of slopes varies from 18 ppm Ca^{++} for the lettuce, to 23 ppm Ca^{++} for Limnaea at 7 days, and 35 ppm Ca^{++} at 14 days feeding. These calcium levels agree with the unfed snails in Experiment 2.

A plot of log A.F(F.W) against log calcium concentrations is plotted in Figure 59, for Limnaea pereger. The following expressions are obtained from Figure 59 ,

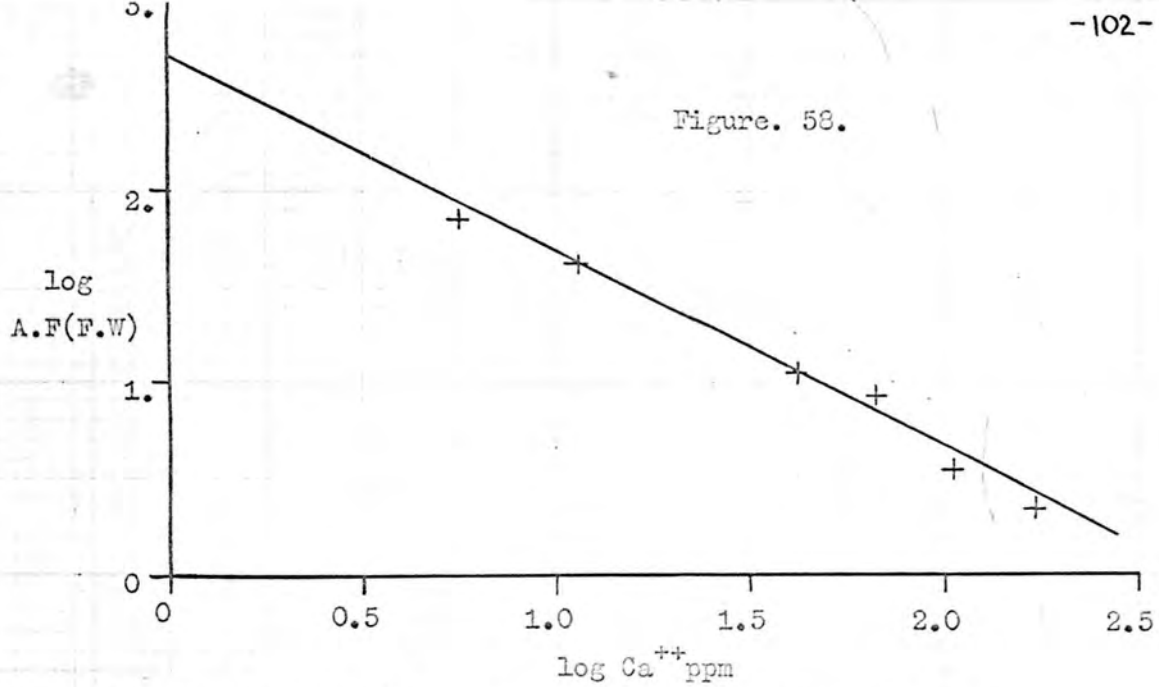
$$\log A.F(F.W)_{85\ Sr} = 3.08 - 0.85 \log Ca^{++} \text{ ppm (after 7 days feeding).}$$

$$= 3.45 - 0.85 \log Ca^{++} \text{ ppm (after 14 days feeding).}$$

Comparing these figures with those obtained in Experiments 1-4, there is only one straight line in the fed log plot in Figure 59.

Feeding does increase the maximum amount of isotope in Limnaea pereger. (Compare 3.45 at 14 days feeding with 2.25-2.40 at 14 days unfed). The slope has changed from -0.4 to -0.85, and also the 'two-line' plot of the unfed has become the 'one-line' plot of the fed. This implies that the calcium deficiency in experiments at low calcium levels in the medium, has been rectified by using

Figure. 58.

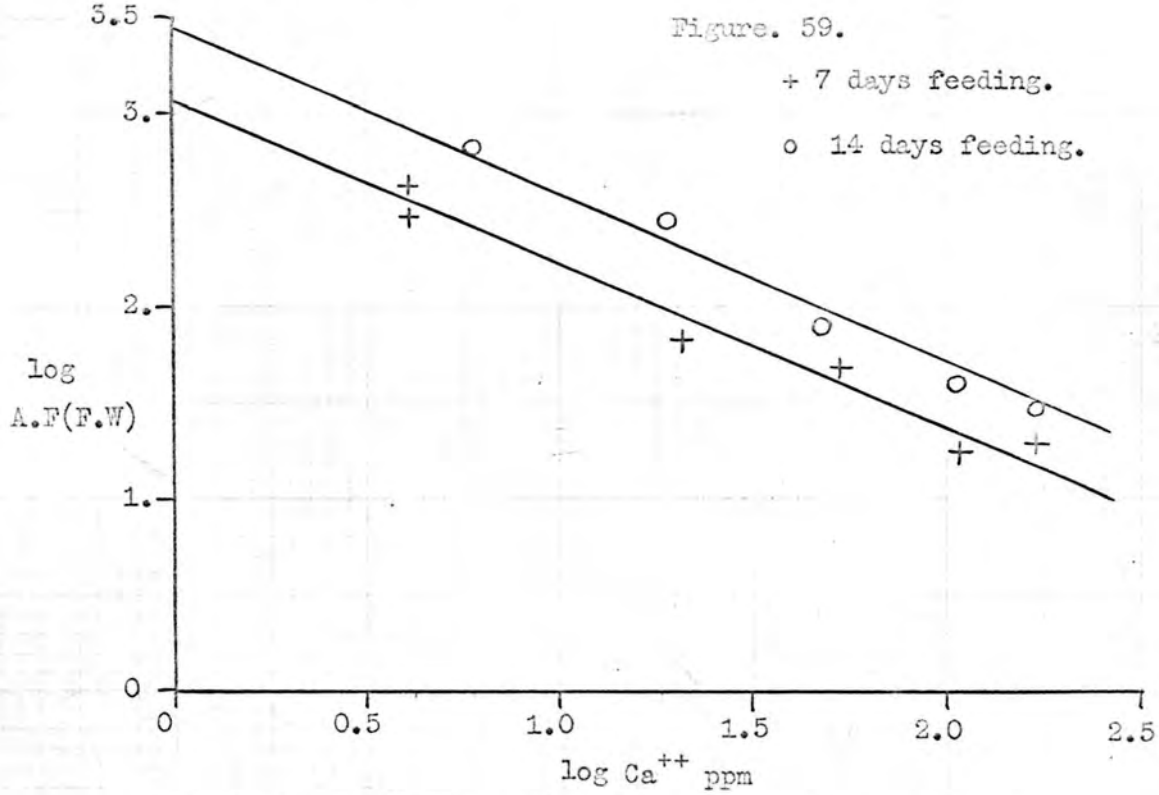


Plot: log A.F(F.W) against log calcium concentration. The uptake of 85 Sr by lettuce at 7 days. Readings the mean of 10.

Figure. 59.

\+ 7 days feeding.

o 14 days feeding.



Plot: log A.F(F.W) against log calcium concentration. The uptake of 85 Sr by Limnaea pereger at 7 and 14 days.

lettuce as a source of food.(p215).

Experiment 5(b)

The uptake of 85 Sr by fed Limnaea pereger (whole snails)

Uptake by the food

The source of the food in this experiment was Elodea canadensis. Pieces of Elodea were cut into lengths of 37.5 mm long, bearing 27 leaflets and the mean fresh weight was 0.0376 g. The experiment was aerated as in Figure 1a. Calcium concentrations varied from 0-650 ppm Ca^{++}.

The uptake of 85 Sr by Elodea was plotted as,

(i) A.F. (F.W) against calcium concentrations, in Figure 60. This Figure shows that there is greater uptake of isotope at calcium levels below 25 ppm Ca^{++}.

(ii) A.F(F.W) against log calcium concentration in Figure 61. This plot shows that the uptake before and after feeding by the snails at 7 and 14 days follows a similar pattern. The change over in slope occurs at 25 ppm Ca^{++}. Uptake below this figure is rapid compared with above this level.

(iii) log A.F(F.W) against log calcium concentration in Figure 62. It is noticed that the expression before and after feeding by the snails is different. At 7 days, in which the Elodea equilibriates with the isotope solution, it is,

$$\log A.F(F.W)_{^{85}Sr} = 2.8 - 0.8 \log Ca^{++} \text{ ppm}$$

After 7 and 14 days feeding, the expression becomes -

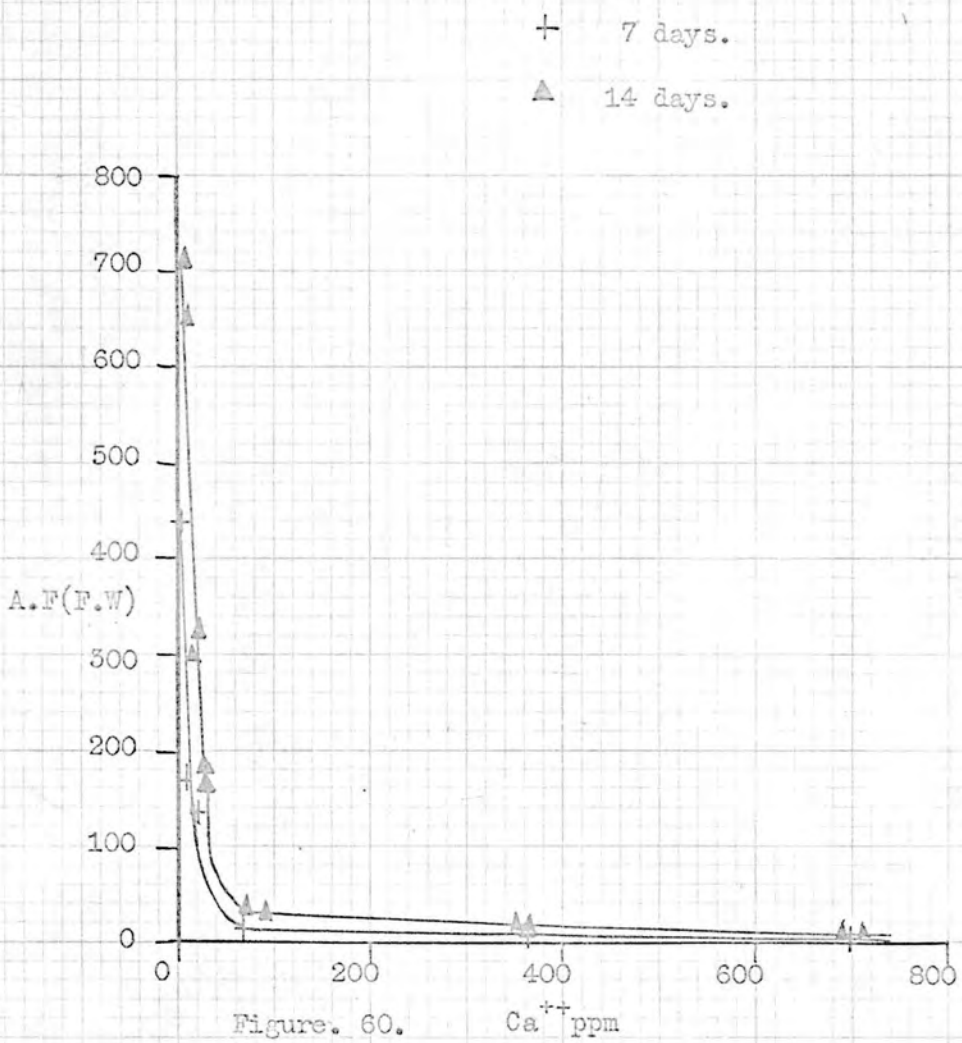


Figure. 60.

Plot: A.F(F.W) against calcium concentration.
The uptake of 85 Sr by Elodea, at 7 and 14 days.

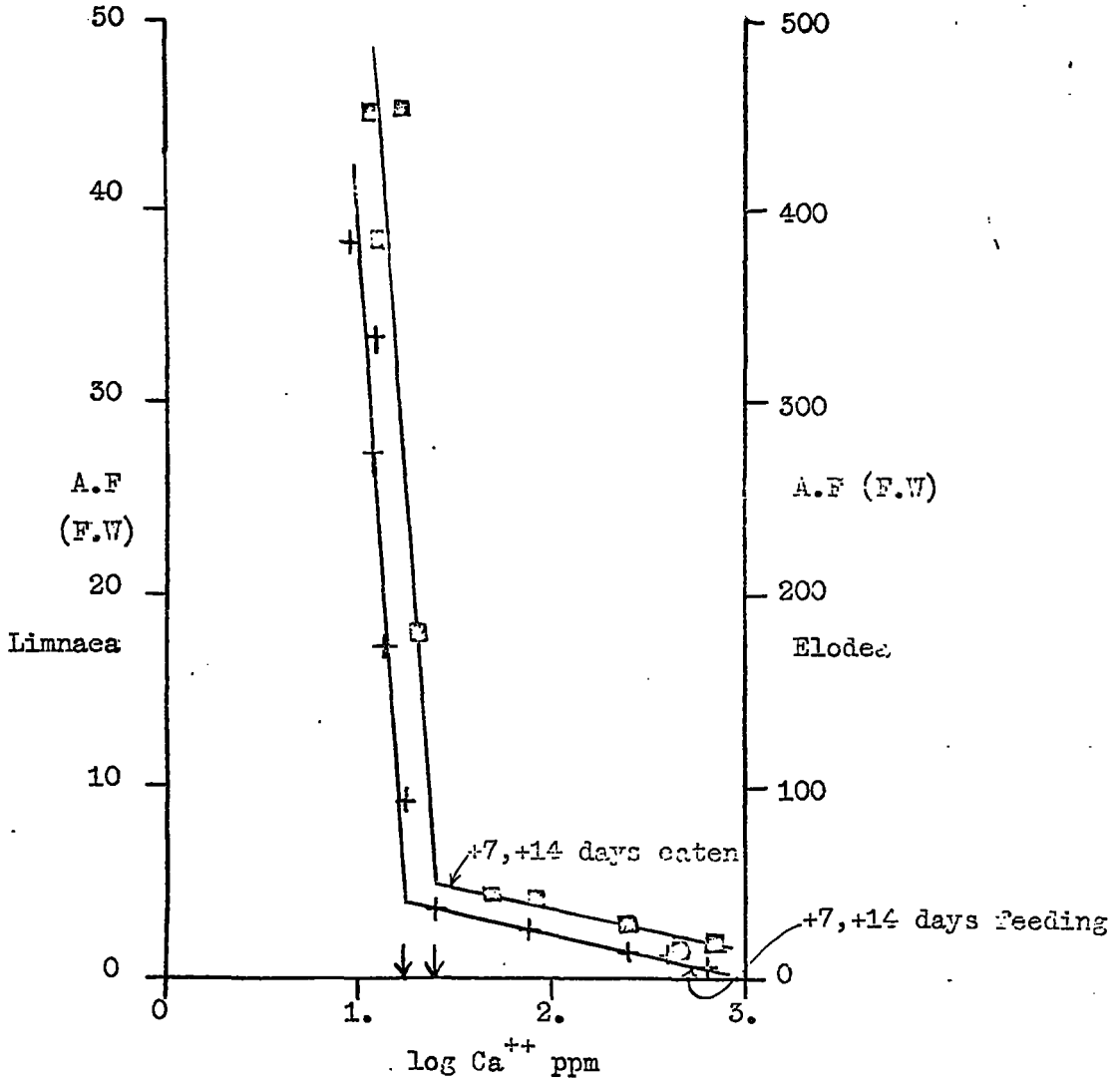


Figure . 61 .

Plot: A.F (F.W) of Limnaea and Elodea, against log Ca^{++} ppm.

Uptake of 85 Sr by Limnaea pereger and Elodea canadensis.

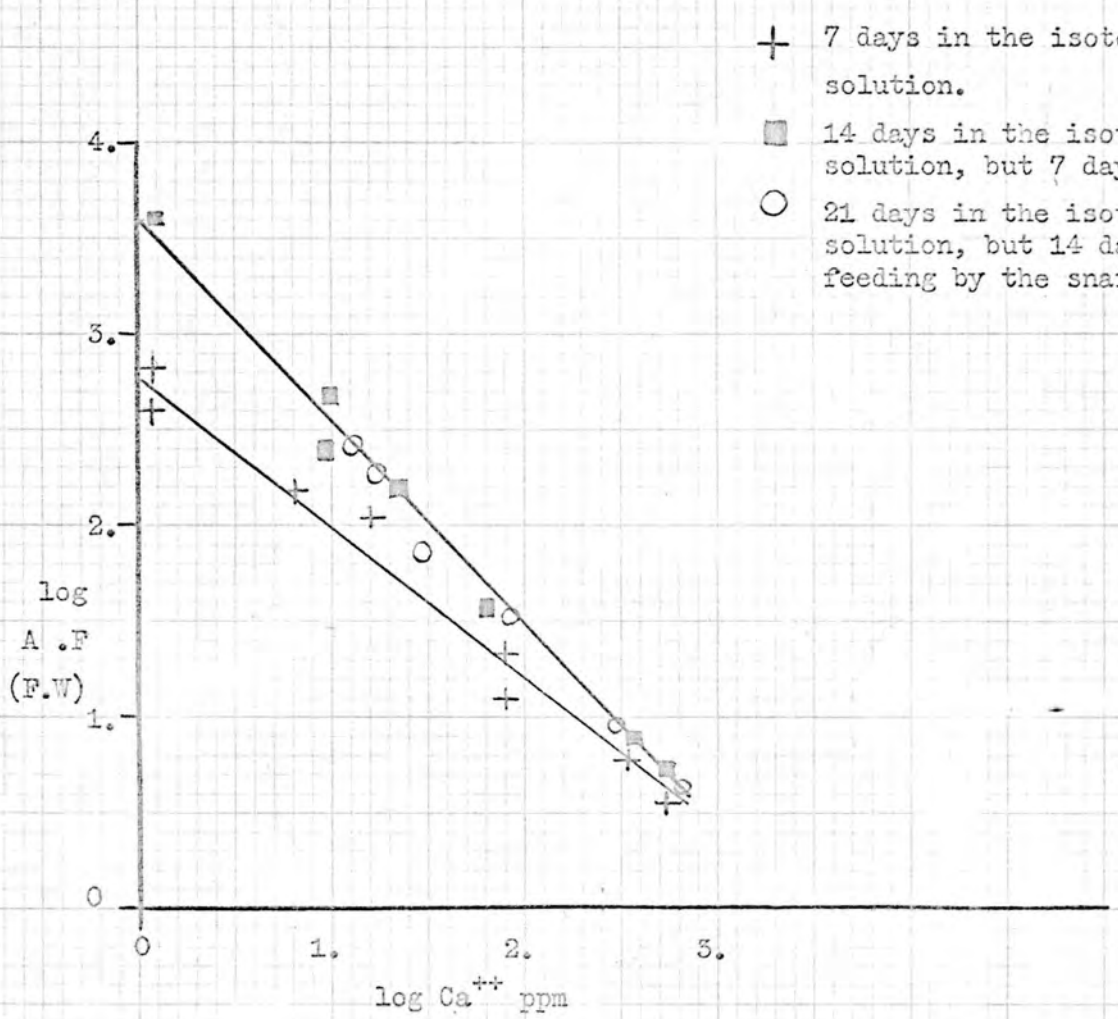


Figure 62 .

Plot: log A.F (F.W) against log Ca^{++} ppm.

The uptake of 85 Sr by Elodea canadensis, after 7 days in the isotope solution (+) , and after 7 days feeding (▩) and 14 days feeding (o) by Limnaea pereger. Each point is the mean of 5 readings.

$\log A.F(F.W)_{^{85}Sr} = 3.6 - 1.05 \log Ca^{++}$ ppm

Since both the maximum and the slope have increased, this suggests that the snails by feeding on the Elodea, have made the plant more permeable to the isotope, and it soon becomes saturated. However, the plant remained green and healthy looking throughout the experiment. The slope of Elodea is similar to that obtained for lettuce in experiment 5(a), i.e. -1.05.

Uptake by the feeding snails

The snails were introduced into the isotope solution after the Elodea had equilibriated with the isotope, i.e. after 7 days. The mean freshweight of the snails was 0.3705 g.

The uptake of 85 Sr by the snails was plotted as:-

(i) A.F(F.W) against log calcium concentration in Figure 61. This plot indicates that there are two processes or phases to the uptake dependent on the calcium concentration of the surrounding water. The change over in slope occurs at 25 ppm Ca^{++}, where the rate below this figure is much higher than above this figure. The plant and the snail are plotted on the same graph, and this semi-log graph indicates that there is a similarity in the uptake of 85 Sr. The plant figures are approximately 10 times that for the snail. The figures for the change over of slope in the semi-log plot for snails feeding on Elodea is comparable with snails feeding on lettuce, (see page 97).

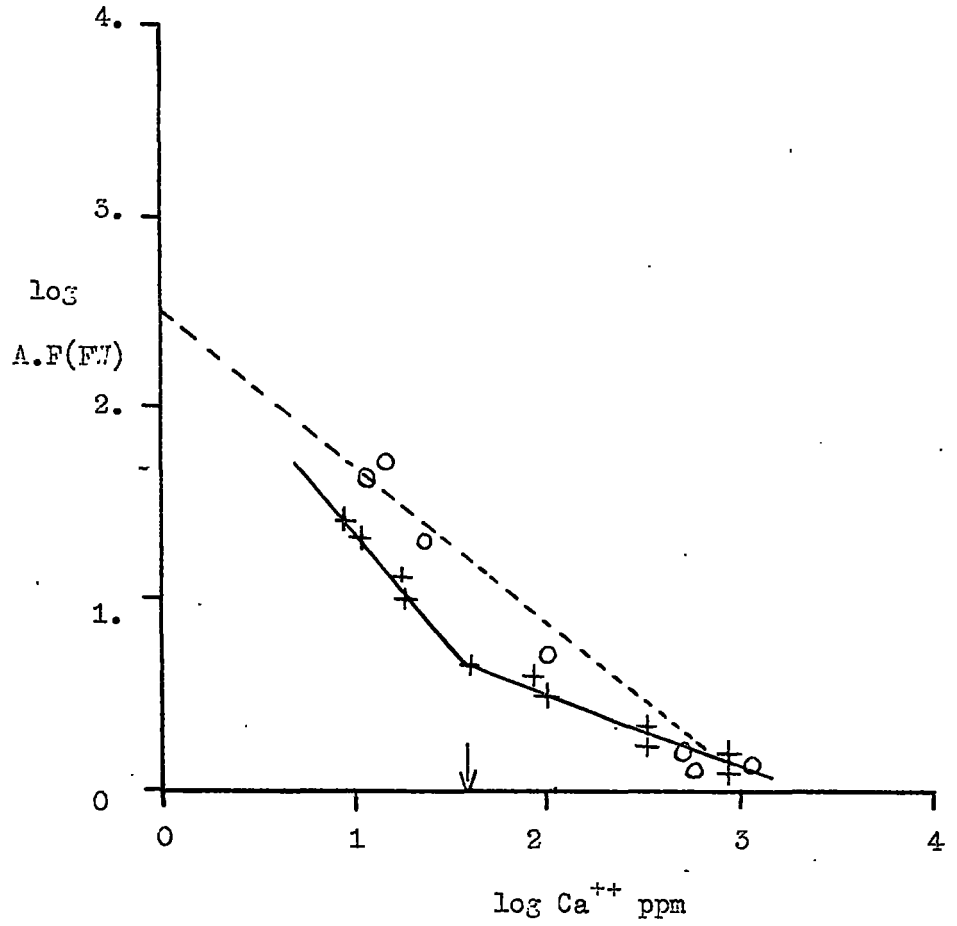


Figure 63.

Plot: log A.F(FW) against calcium concentration in the medium. The uptake of 85 Sr by Limnaea pereger, feeding on Elodea at 7 days (+) and at 14 days (o).

Each point is the mean of 10 snails.

(ii) In the log plot in Figure 63, one has a 'two-line' plot at 7 days feeding to a 'one-line' plot at 14 days feeding. This suggests that during the first 7 days, the amount of food, i.e. Elodea, does not have as much effect as lettuce, in changing the slope of the plot. It was observed that the snails did not feed as well on Elodea as they did on lettuce. At 14 days the snails had eaten sufficient food, i.e. Elodea to alter the slope of the plot. Expressions from Figure 63, are :-

$$\log A.F(FW)_{^{85}Sr} = 1.25 - 0.375 \log Ca^{++} \text{ ppm (above 39.8 ppm } Ca^{++} \text{ after 7 days feeding).}$$

$$= 1.1 - 0.7 \log Ca^{++} \text{ ppm (below 39.8 ppm } Ca^{++} \text{ after 7 days feeding).}$$

$$\log A.F(FW)_{^{85}Sr} = 2.5 - 0.85 \log Ca^{++} \text{ ppm (after 14 days feeding).}$$

The slope of -0.85 at 14 days is in good agreement with that obtained by lettuce in Experiment 5(a), page 101, although the maximum is less, i.e. 2.5 compared with 3.5. This confirms the above observation, that the snails do not feed as well on Elodea as they do on lettuce.

This experiment confirms the fact that feeding does increase the rate of uptake and accumulation of 85 Sr by Limnaea pereger.

Experiment 6

The uptake of 89 Sr by unfed/fed Limnaea pereger (shell and body) in filtered and unfiltered canal water

The uptake of 89 Sr by L.pereger was studied over 7 days in filtered and unfiltered canal water from the Shropshire Union Canal, at Chester. The mean fresh weight of the snails was 0.1334 g and that of Elodea canadensis was 0.2999 g. While the F.W:DW ratio for the snails was 3:1, that for Elodea was 12:1 The canal water was filtered through glass wool in a large Buchner funnel, which retarded the microscopic particles in suspension. The calcium ion concentration of the filtered canal water was 68 ppm Ca^{++} and its pH was 7.4.

In the experiments which had the unfiltered canal water, there was a large amount of organic detritus and floating organisms which caused polluted conditions after 2-3 days. The experiments were not aerated and this accelerated bacterial de-composition of the suspended material. This pollution caused the snails to die in the unfiltered water.

The results are tabulated in Figure 64. In the unfiltered water the AF based on initial counts is higher than the filtered solution. This is probably due to the increased surface area of the suspended particles on to which is adsorbed the particulate Yttrium ion (Y^{+++}). In jar 3, with only Elodea in, this is not so

apparent, the AF (FW) unfiltered is 30.46 compared with the filtered AF(FW) 23.73.

This experiment proved that the unfiltered canal water was unsuitable for experimental purposes, and that the filtered water contained too many variables (see page 13) for analysis of the water.

FILTERED AND UNFILTERED CANAL WATER

LIMNAEA PEREGER

AF, based on initial counts. DW	Filtered			Unfiltered		
Shell	23.4	152.1		54.8	snails died	
Body	21.8	38.8		21.2		
Elodea		669.3	286.9		85.4	253
FW Shell	19.12	29.04		50.7	snails died	
Body	1.45	13.11		16.73		
Elodea		62.12	23.23		69.8	30.5
AF's based on 3week counts DW Shell	8.4	26.6		27.7	snails died	
Body	2.0	3.8		8.6		
Elodea		409	278.7			
Shell FW	7.15	29.1		6.50	snails died	
Body	1.41	2.33		5.58		
Elodea		36.51	22.68		24.66	27.18

Figure 64.

It can be seen that the uptake by the shell is higher than that of the body, particularly so in Jar 2 when it is feeding on Elodea and probably algal material in suspension.

Experiment 7

The uptake of 89 Sr by shell and body separately of unfed Limnaea pereger

The uptake of 89 Sr by Limnaea pereger was studied over a period of 21 days and under aerated conditions. The calcium concentration varied from 0-120 ppm Ca^{++}. The snail was dissected into shell and body which were counted separately. The mean fresh weight of the snails was 0.2688 g.

Results are expressed as:-

(i) AF(FW) against calcium concentration in Figure 65. It can be seen that with time the AF(FW) increases, and that it decreases with increasing calcium levels for both the shell and the body. The change over in slope varies from 20 ppm Ca^{++} to 50 ppm Ca^{++}, as time increases from 7 to 21 days. Uptake below these levels is more rapid than above them, and the uptake for the shell is greater than that of the body. This is particularly significant, since the shell forms 14-22% of the fresh weight of the snail, and contains 33-45% liquid; whereas the body forms 60-70% of the fresh weight of the snail and has 80% liquid in its composition.

(ii) A log of AF(FW) against calcium concentration is shown in Figure 66, for the shell and the body. Expressions from the plots are tabulated in Figure 67.

Figure 33.

Plots of A.F(FW) against the calcium concentration at (A) 7 days, (B) 14 days and (C) 21 days in 89 Sr solution. Only the 89 Sr figures are plotted. Points are the mean of 5. AF(FW)'s based on 3 week counts

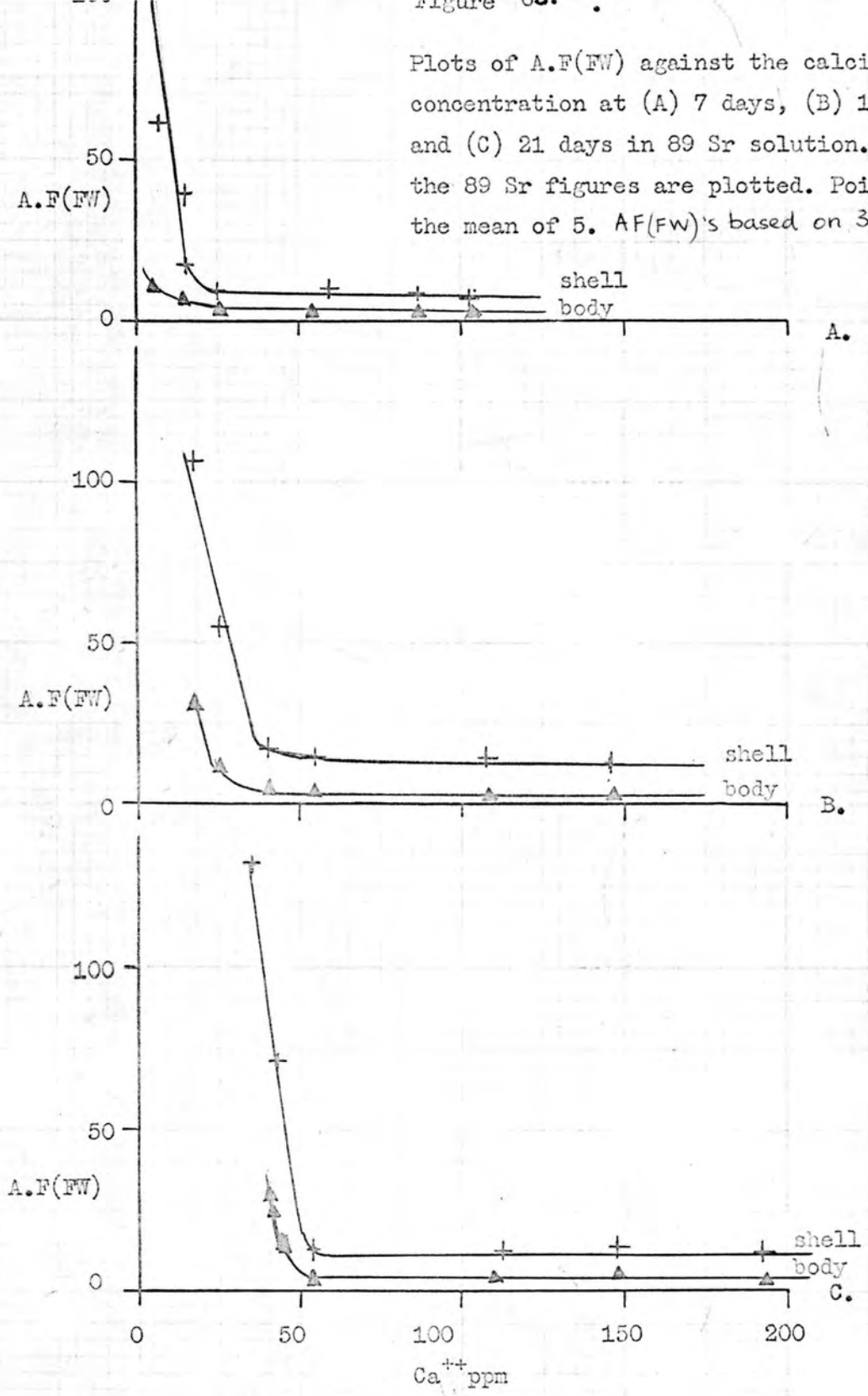


Figure 66

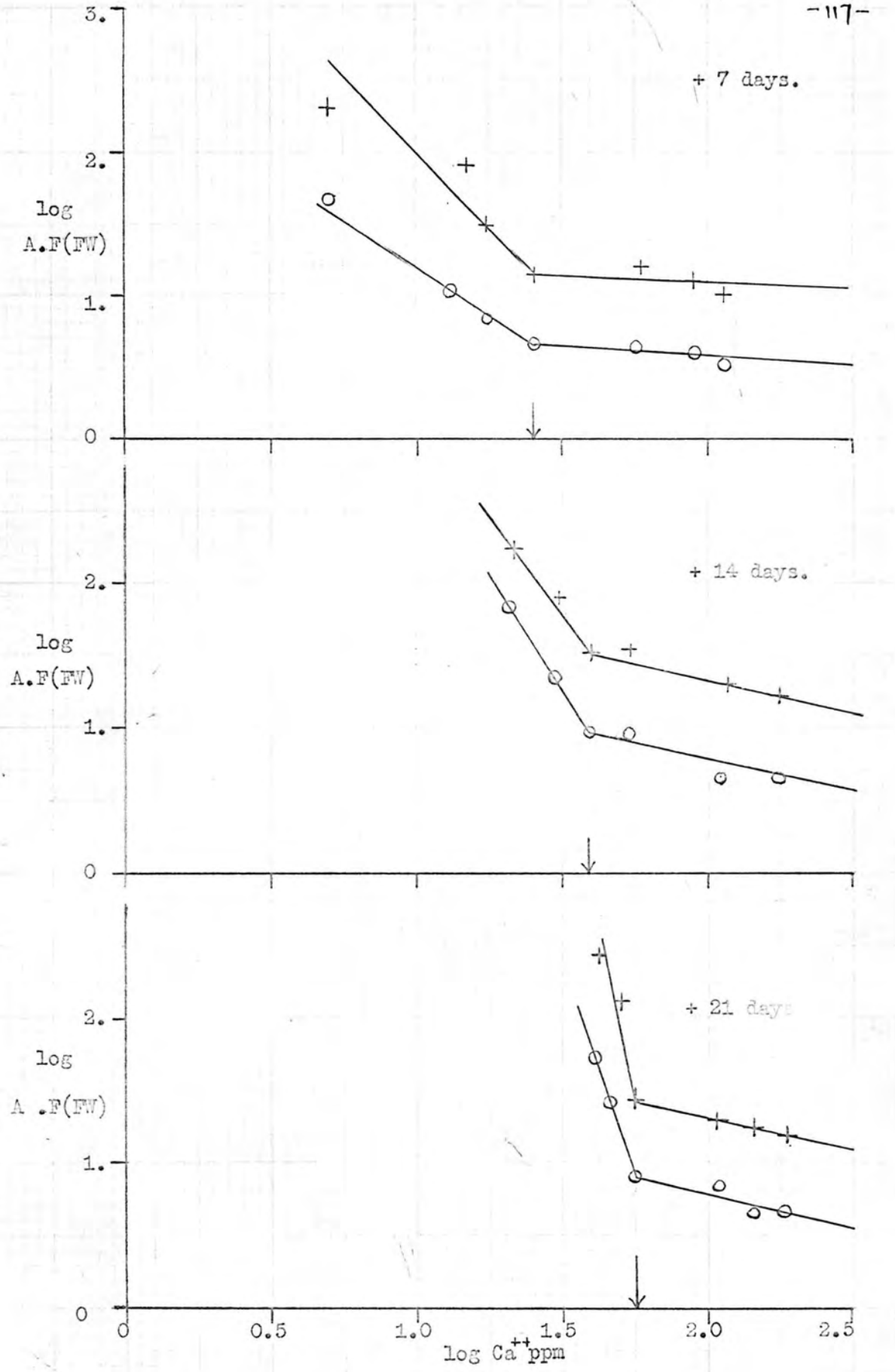
Title to the log plots on page 117 .

Plot: log AF(FW) against log calcium concentration.

The uptake of 89 Sr by shell (+) and body (o) of

Limnaea pereger, at 7, 14 and 21 days.

AF(FW) based on three week counts.


+ 7 days.
log A.F(FW)
+ 14 days.
log A.F(FW)
+ 21 days
log A .F(FW)
3.
2.
1.
0
0
0.5
1.0
1.5
2.0
2.5
log Ca++ ppm

L. pereger		+ 7 days	+ 14 days	+ 21 days
shell	a.	1.25 - 0.1	2.2 - 0.4	2.2 - 0.4
	b.	2.4 - 1.2		
body	a.	0.8 - 0.1	1.65 - 0.4	1.65 - 0.4
	b.	1.2 - 0.8		
Calcium level at change over Ca^{++} ppm.		26.92	39.81	56.23

Figure 67.

Table of expressions from Figure 66, at 7, 14 and 21 days in the isotope solution. Readings along 'a' are above the calcium level at the change of the slope, and 'b' below this level.

There is an initial uptake by the shell and body which is very low, and after 14 days attains the slope, found in Experiments 1 and 2. Although the slope is the same for the shell and the body, the maximum is higher for the shell than the body.

These results are in agreement with those attained for Experiments 1 (page 78) and Experiment 2 (page 86).

Experiment 8

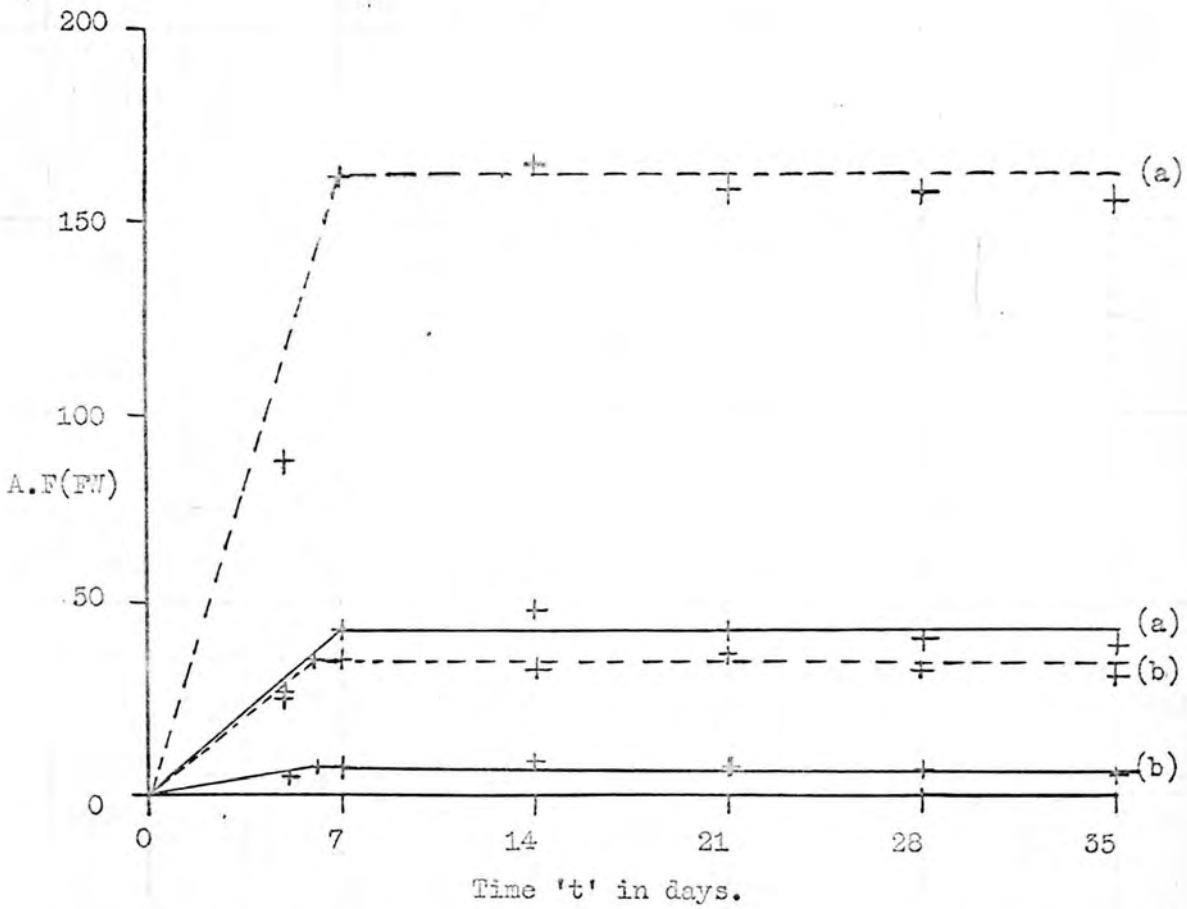
The uptake of 89 Sr by unfed Limnaea truncatula (whole snails)

The uptake of 89 Sr by L.truncatula under calcium concentrations varying from 0-103 ppm Ca^{++}, was studied for 35 days, under aerated conditions. The snails were unfed for the duration of the experiment. The mean fresh weight of the snails was 0.1717 g and were 9-11 mm long.

The uptake of 89 Sr was plotted as:-

(i) A.F(FW) against time for certain calcium ranges, in Figure 68 and the results in Figure 69.

It can be observed that equilibrium takes longer to attain at low calcium concentrations, and that there is greater accumulation of the isotope at low calcium levels. The uptake figures at equilibrium areless than for Limnaea pereger (see page 78). This difference is probably due to the difference in thickness of the shell and the mass of the snail, L. truncatula being the smaller and having a thinner shell. The amount of exchangeable calcium in the shell is less in L.truncatula than in L.pereger, and will itself become quickly saturated with isotope. L.pereger was much more active under the experimental conditions than L.truncatula, which suggests that the more active the organism the greater the amount of isotope taken up. L. truncatula is primarily air-breathing



Plot: A.F(FW) against Time, in days at calcium ranges of (a) 13.6 -27.1 ppm Ca^{++} and (b) 65 - 68.4 ppm Ca^{++}, for Y-Sr uptake (dashed line) and 89 Sr (continuous line).

Uptake of Y-Sr and 89 Sr by Limnaea truncatula.

Each reading (+) is the mean of 15 snails.

Figure 68.

Y-Sr A F(FW)'s based on initial counts

89Sr AF(FW)'s based on three week counts

and non-aquatic, whose habit results in the occasional submergence in water (Russell-Hunter, 1964), but like L.pereger is classified ecologically as a soft water species (Macan, 1960).

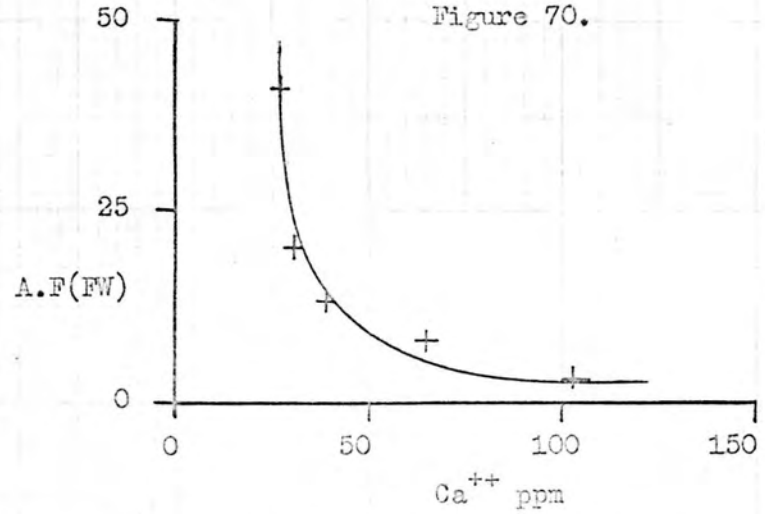
Calcium conc. ppm.	Isotope	AF.(FW) at Equilibrium	Time in days, of Equilibrium	$E_t/5$	$AF(FW)/E_t$
13.6 - 27.1	Y-Sr	165	7	1.4	23.5
	89 Sr	44	7	1.4	6.2
65.1 - 68.4	Y-Sr	33.4	6	1.2	5.5
	89 Sr	8	6	1.2	1.3

Figure 69.

Table of results from Figure 68. Uptake of Y-Sr and 89 Sr by Limnaea truncatula, at varying calcium levels.
NOTE: Y-Sr AF(FW) based on Initial counts & 89Sr AF(FW) based on 3 week counts.

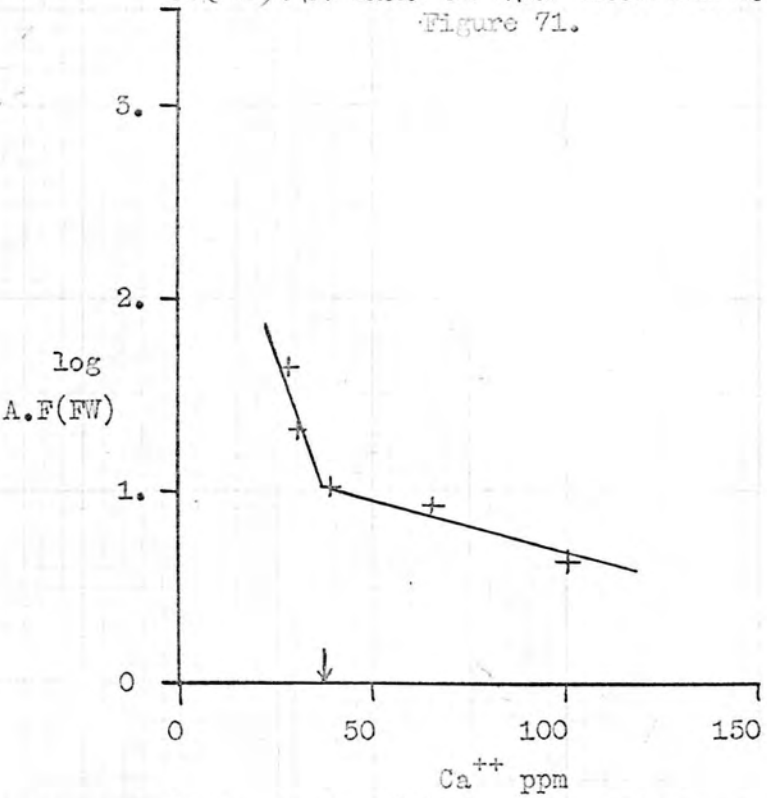
The results in Figure 69, are similar to Experiment 1 (page 78) and Experiment 4 (page 94), where $E_t/5$ is approximately equal to $AF(FW)/E_t$, except at low calcium levels. The difference between the results for Limnaea truncatula and Limnaea pereger, is that, L. truncatula reaches equilibrium quicker. A plot of AF(FW) against calcium concentration in the medium shows that uptake is greater at low calcium levels. (Figure 70).

Figure 70.

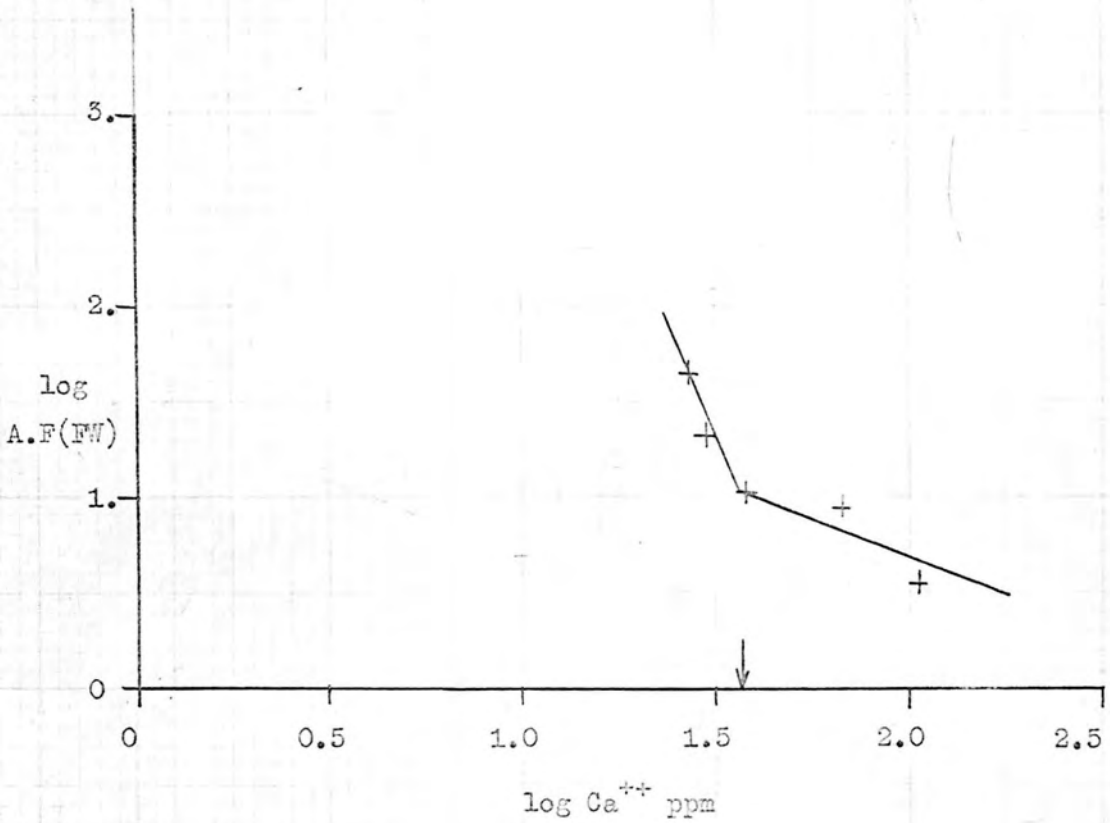


Plot: A.F(FW) against calcium concentration.
The uptake of 89 Sr by L.truncatula at
5 weeks. Readings mean of 15 snails.
AF(FW) 89Sr based on three week counts.

Figure 71.



Plot: log A.F(FW) against calcium concentration.
AF(FW) 89Sr based on three week counts.



Plot: log A.F(FW) against log Ca^{++} ppm.

The uptake of 89 Sr by Limnaea truncatula, at 5 weeks.

Each reading is the mean of 15 snails.
AF(FW)89Sr based on three week counts.

Figure 72.

(ii) log AF(FW) against calcium concentration in Figure 71, results in two lines which change their slope at 37 ppm Ca^{++}, with uptake greater below this level than above it.

(iii) log AF(FW) against log calcium concentration in Figure 72. Here the 'two-line' log plot indicates that the snails are unfed. Expressions from this plot are:-

$$\log AF(FW)_{89\,Sr} = 2.3 - 0.8 \log Ca^{++} \text{ ppm (above 37 ppm } Ca^{++})$$

$$= 5.2 - 3.3 \log Ca^{++} \text{ ppm (below 37 ppm } Ca^{++})$$

The maximum above 37 ppm Ca^{++} is similar to that obtained in Experiment 1, page 83, but the slope has increased from -0.4 to -0.8. This implies that the rate of uptake of 89 Sr is quicker in L.truncatula than in L.pereger. This would substantiate the reasoning in para.(i) page 119 .

Experiment 9

The uptake of 85 Sr by fed Limnaea auricularia

(Whole snail, shell and body)

The uptake of 85 Sr by Limnaea auricularia was studied over 7 days at calcium concentrations varying from 0-240 ppm Ca^{++}. The snails were fed with lettuce for the duration of the experiment. After counting the whole snail, it was dissected into shell and body, the activity of which were then determined separately.

Uptake by lettuce

The mean fresh weight of the lettuce was 0.3802 g. The uptake of 85 Sr was plotted in Figure 73, at 5 days aeration. The pieces of lettuce did not have any mid-vein in them. The expression from the graph is:-

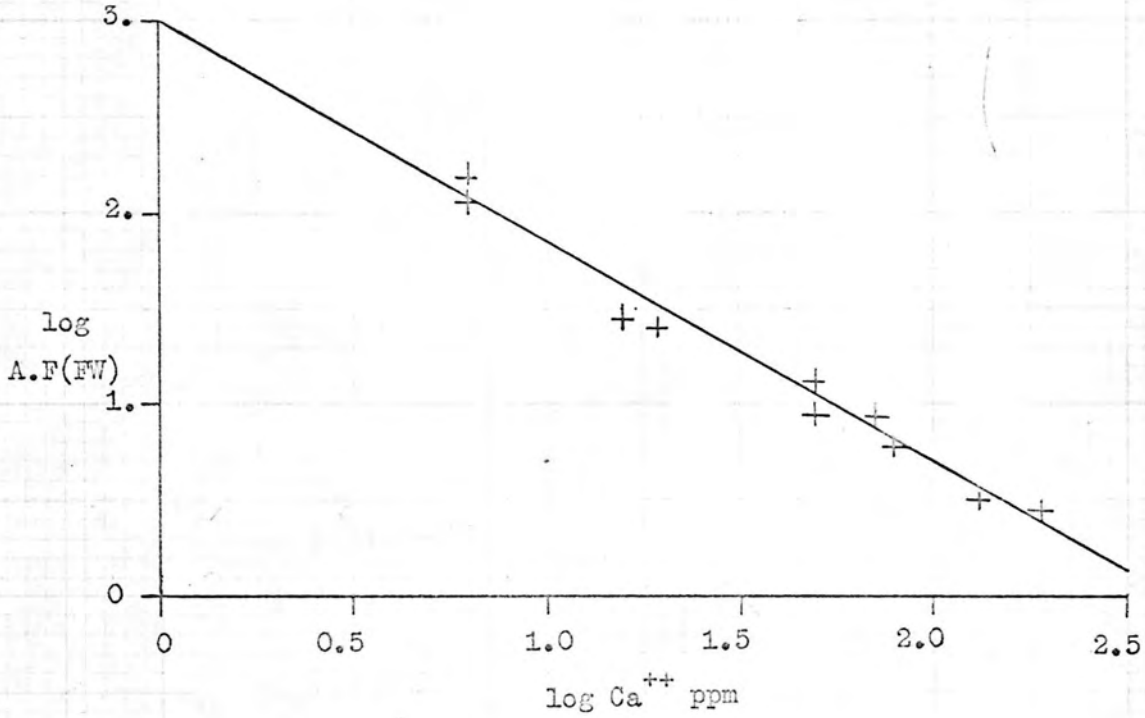
$$\log AF(FW)_{85\ Sr} = 3.0 - 1.15 \log Ca^{++}\ ppm$$

This expression is comparable with the expression obtained in Experiment 5a, page 97.

Uptake by feeding snails

The snails were introduced into the isotope solution containing the lettuce at 5 days. The mean fresh weight of the snails was 0.1168 g. and 8.5 mm long. The calcium concentration of the canal water at the time of sampling was 66.02 ppm Ca^{++}. The body weighed approximately 81% of the fresh weight of the snail, compared with up to 24% for the shell.

The results are plotted as:-



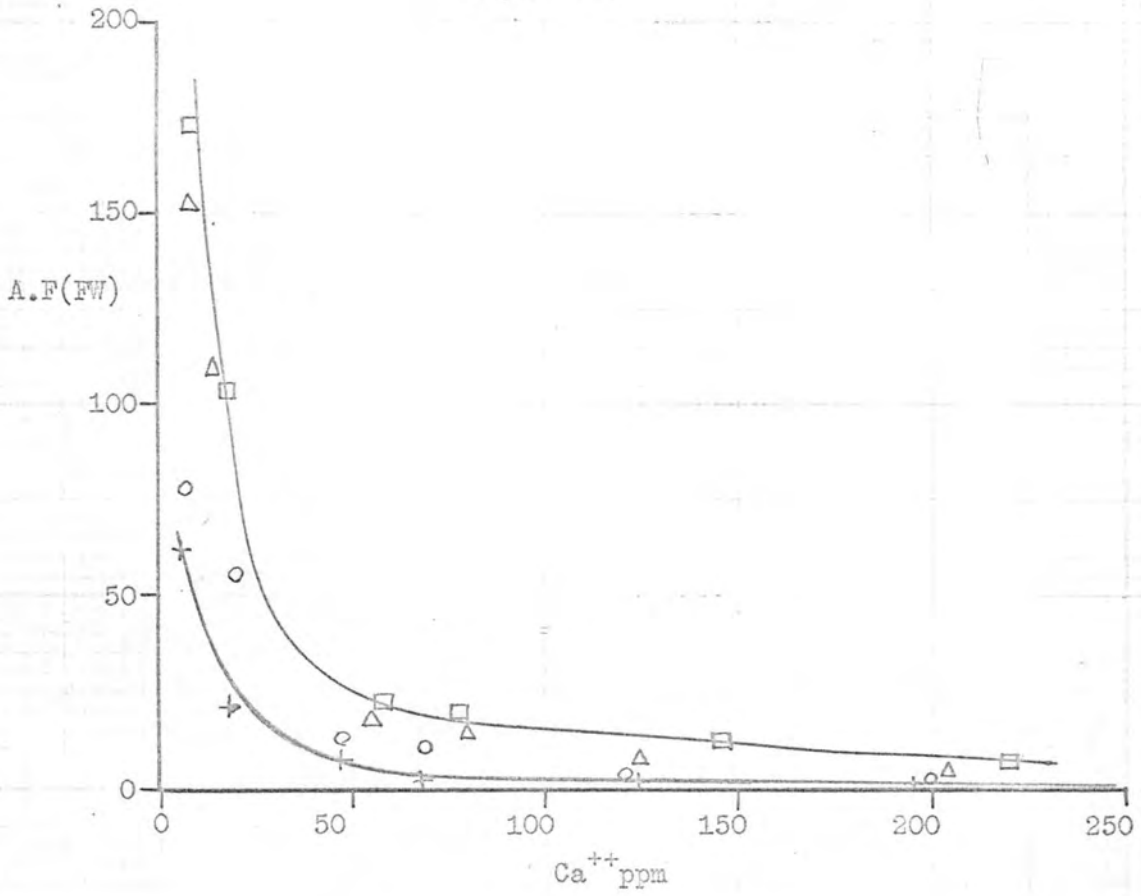
Plot: log A.F(FW) against log Ca^{++} ppm.

The uptake of 85 Sr by lettuce, at 5 days aeration.

Each reading(+) is the mean of 10.

Figure 73.

Figure 74.



Plot: A.F(FW) against calcium concentration.

The uptake of 85 Sr by Limnaea auricularia, at 1 day (+), 2 days (o), 4 days (△) and 7 days (□), feeding on lettuce.

Each reading is the mean of 5 snails.

Curves drawn at 1 and 7 days, which shows the increase in A.F(FW) due to feeding.

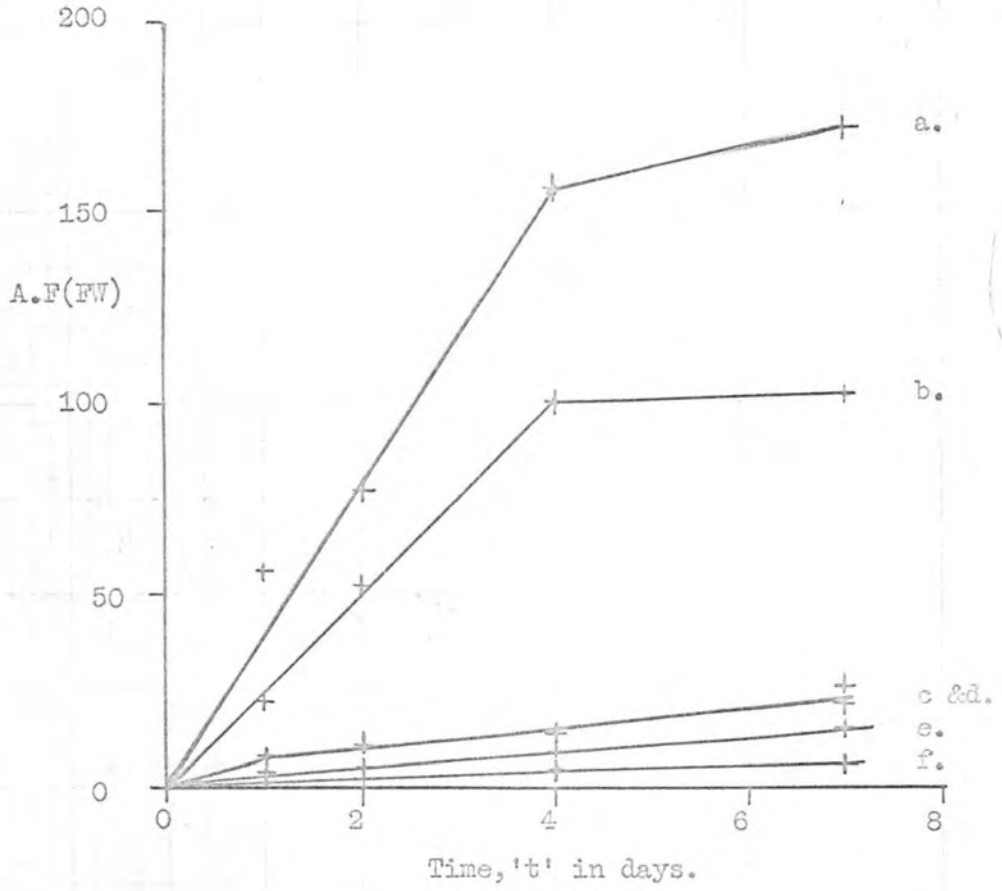
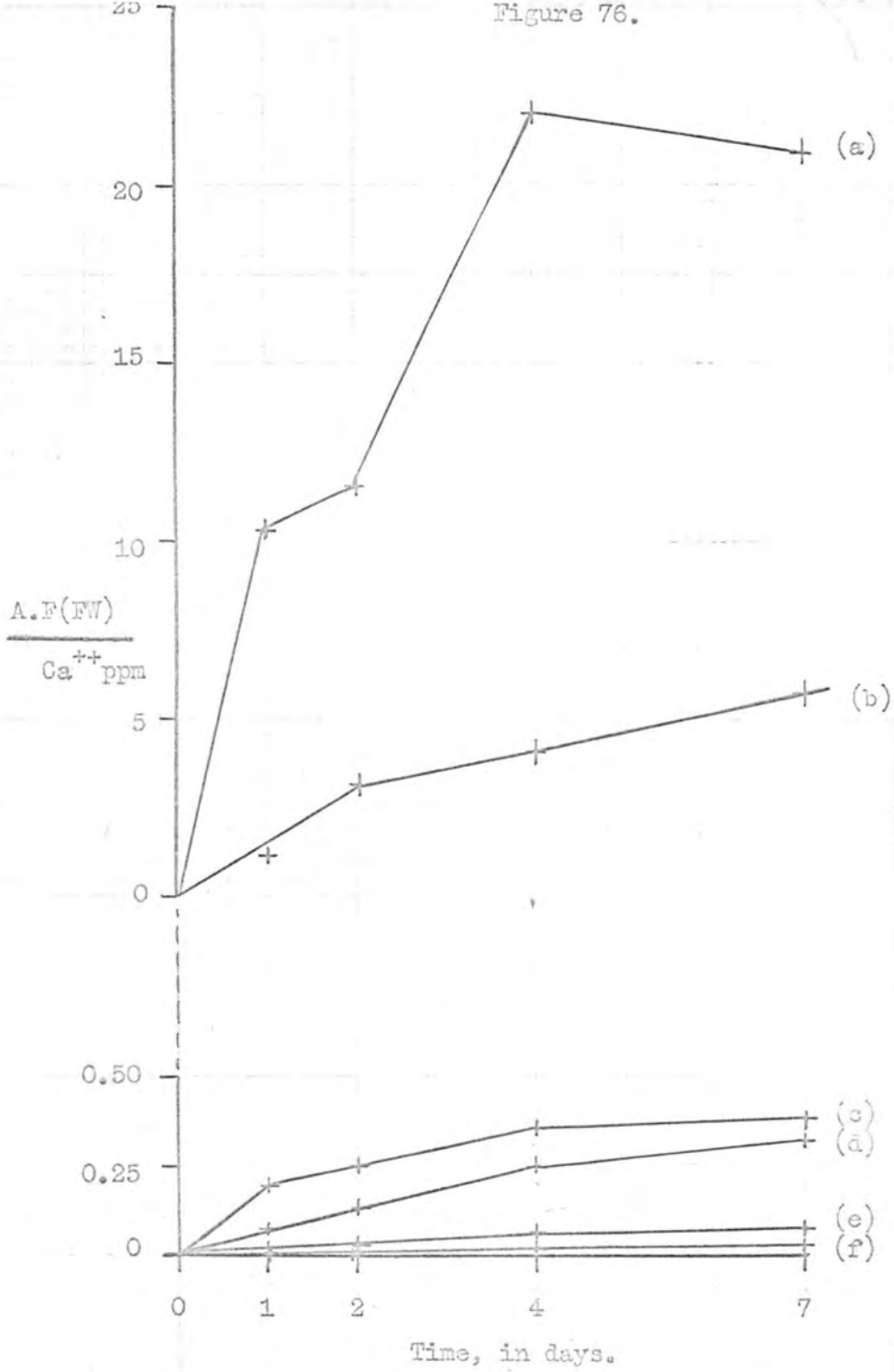


Figure. 75

Plot: A.F(FW) against Time, at calcium ranges of, (a) 0-8.6ppm Ca^{++}, (b) 17.5-18.5 ppm Ca^{++}, (c) 46.8-55.4 ppm Ca^{++}, (d) 68.1-76.5 ppm Ca^{++}, (e) 124-142.5 ppm Ca^{++} and (f) 195.1-216.6 ppm Ca^{++}. The uptake of 85 Sr by Limnaea auricularia(whole snail), feeding on lettuce.

Figure 76.



Plot: AF(FW)/Ca^{++}ppm against Time, in days.

The uptake of 85 Sr by Limnaea auricularia (whole snail) at calcium ranges of (a) 0-8.6 ppm Ca^{++}, (b) 17.3-18.5 ppm Ca^{++}, (c) 46.9-55.4 ppm Ca^{++}, (d) 68.1-76.5 ppm Ca^{++}, (e) 124-142.5 ppm Ca^{++} and (f) 195-216 ppm Ca^{++}.

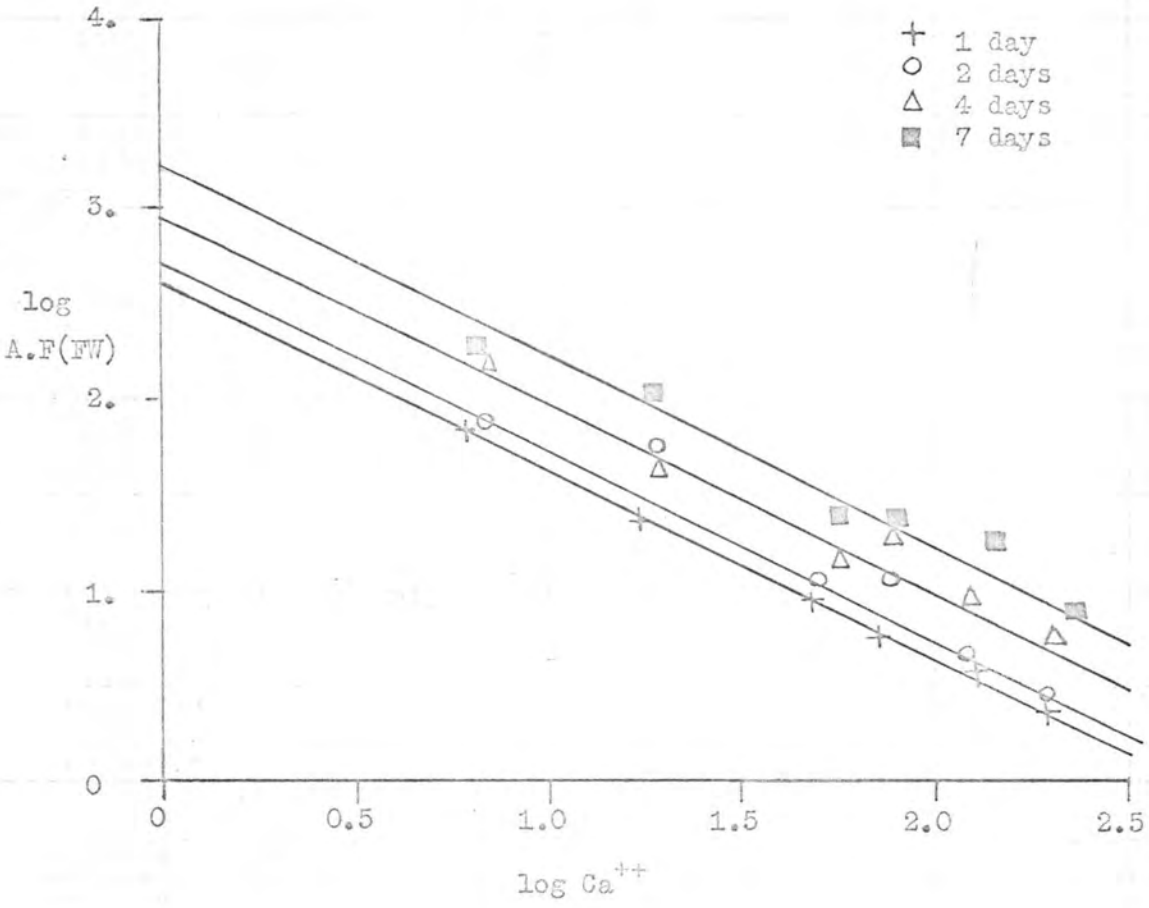


Figure 77 .

Plot: log A.F(FW) against log calcium concentration.

The uptake of 85 Sr by Limnaea auricularia (whole snail) feeding on lettuce. Each reading is the mean of 5 snails.

(i) A.F(FW) against calcium concentration in Figure 74. This plot shows that the AF(FW) increases over the whole calcium range studied, after 7 days feeding.

(ii) AF(FW) against time, in days, at varying calcium ranges in Figure 75. This graph shows that at low calcium levels, there is a sudden uptake followed by a decrease. At calcium levels above 46.8 ppm Ca^{++}, instead of two lines, there is only one line, which implies that uptake is taking place at the same rate. The experiment was only over 7 days, and it is apparent that equilibrium has not been reached.

(iii) A plot of $AF(FW)/Ca^{++}$ ppm against time is shown in Figure 76. It is only at high calcium levels that there appears to be a straight line. At lower calcium levels there are several lines which could be joined up to form a curve which suggests that at low calcium levels, that $AF(FW)/Ca^{++}$ ppm is not time dependent, or time dependent plus another factor.

(iv) log AF(FW) against log calcium concentration for the fed whole snail in Figure 77; for the shell in Figure 78 and for the body in Figure 79. The expressions from the log plots are summarised in Figure 70. The significant point about these plots is that, with the exception of the 1 day shell points, all are 'one-line' plots. This is consistent with the feeding experiments performed with Limnaea pereger (pages 97,104). The maximum is similar to that obtained in Experiment 5(a), page 101, although the slope is slightly higher approaching -1.0.

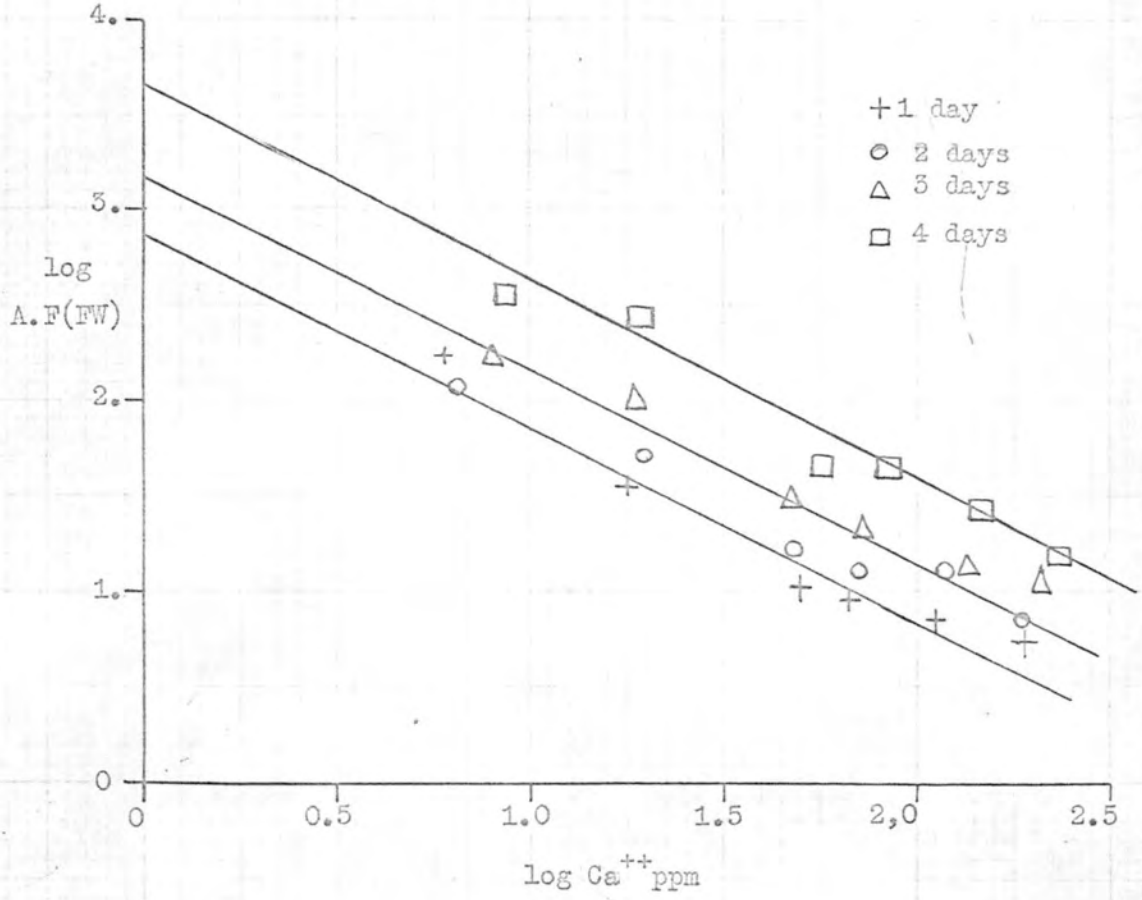


Figure 78

Plot: log A.F(FW) against log calcium concentration. The uptake of 85 Sr by Limnaea auricularia (shell); the snail having fed on lettuce. Each reading is the mean of 5.

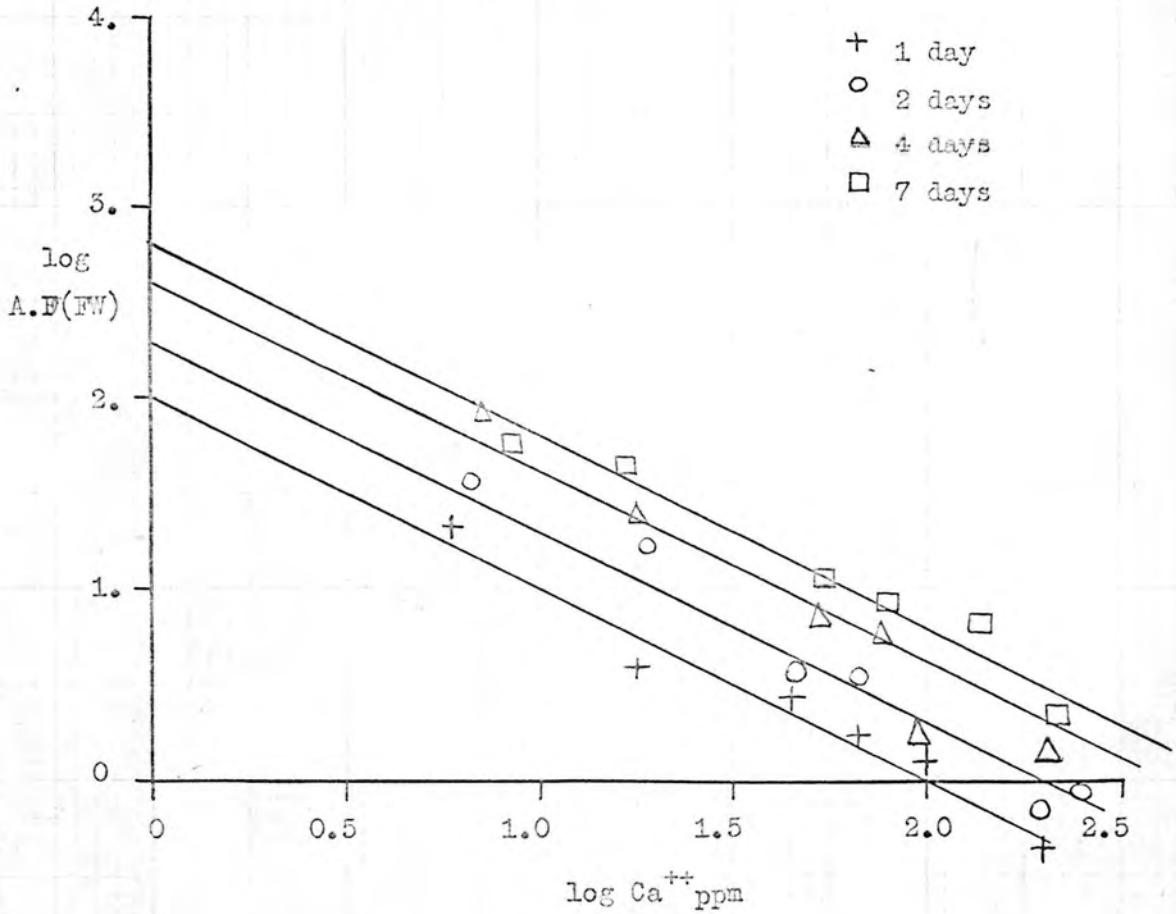


Figure 79 .

Plot: log A.F(FW) against log calcium concentration. The uptake of 85 Sr by Limnaea auricularia (body); the snail having fed on lettuce. Each reading is the mean of 5.

The slope indicates that the 85 strontium is permeating the membrane of the snail at the same rate as the calcium ion.

It can be seen quite clearly from Figure 80, that the maximum increases with time, but that the rate of uptake remains the same.

Limnaea auricularia	+ 1 day	+ 2 days	+ 4 days	+ 7 days
Whole snail	2.6 - 0.95	2.7 - 0.95	2.95 - 0.95	3.2 - 0.95
Shell	1.8 - 0.45 2.2 - 1.3	2.85 - 0.95	3.15 - 0.95	3.65 - 0.95
Body	2.0 - 0.95	2.3 - 0.95	2.6 - 0.95	2.8 - 0.95

Figure 80.

Table of expressions from Figures 77, 78, 79.

The uptake of 85 Sr by Limnaea auricularia feeding on lettuce, whole snail, shell and body.

The maximum for the shell is higher than that of the body, but the slope, i.e. the rate of uptake, is the same.

Experiment 10

The uptake of 47 Ca by fed and unfed Limnaea auricularia (whole snail)

The uptake of 47 Ca by Limnaea auricularia was studied for 9 days at calcium concentrations varying from 0-650 ppm Ca^{++}. The snails were fed with watercress- Nasturtium officinale - which was obtained from a stream near Ruthin (Grid Ref.186582) which contained Limnaea pereger and Limnaea stagnalis. Similar terminal leaflets of watercress were washed thoroughly in aerated distilled water to remove the superficial detritus and then with the appropriate calcium concentration before being placed in the isotope solution.

(a) The watercress was allowed to equilibriate with the isotope solution. By plotting log AF(FW) against log calcium concentration in Figure 81, for results obtained at 2 and 4 days aeration, it was found that equilibrium was reached at 2 days. The experession from Figure 81 is:-

$$\log AF(FW)_{47\,Ca} = 2.0 - 0.3 \log Ca^{++} \text{ ppm (2 days)}$$

The mean fresh weight of the watercress leaflets was 0.3500 g. It was determined that after the leaflets had been in the glass vessels for 4 days, that the calcium concentration in the lower calcium levels had increased, as shown in Figure 82.

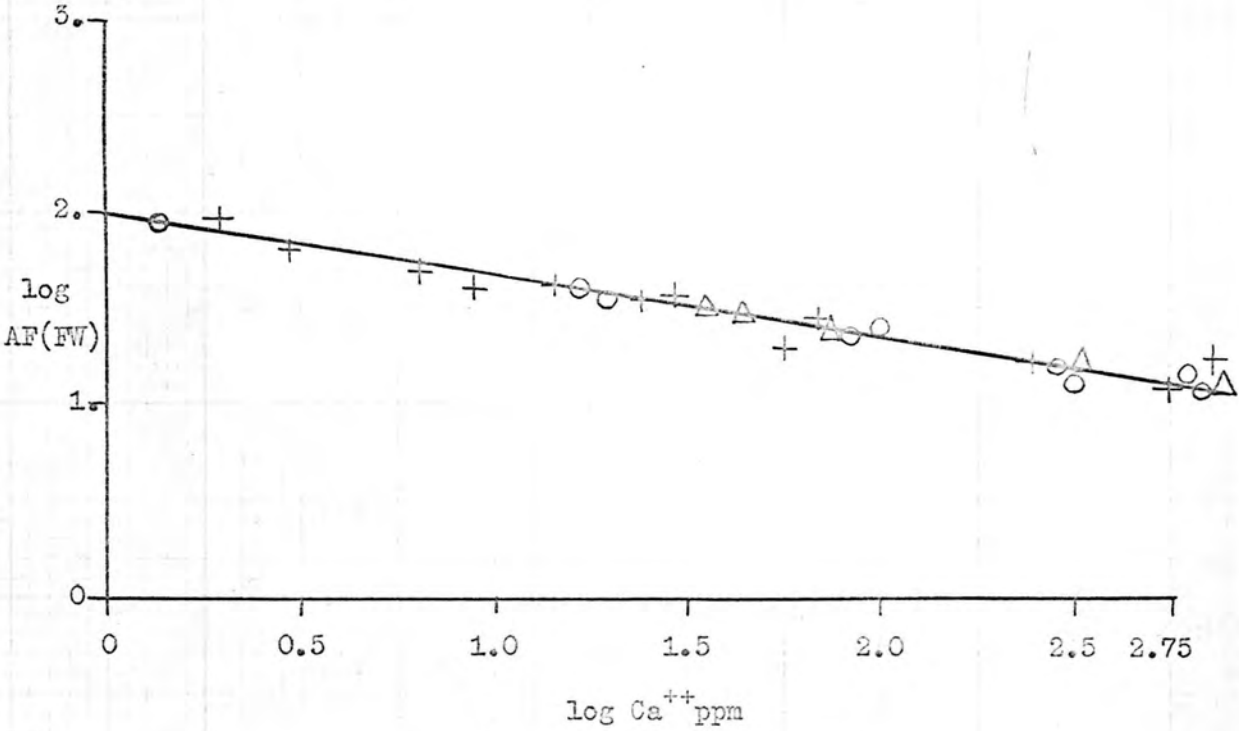


Figure 81.

Plot: log A.F(FW) against log calcium concentration.

The uptake of 47 Ca by watercress at 2days (+), 4 days (o) and 9 days (Δ).

Each reading is the mean of 15.

Jar	1	2	3	4	5	6
Initial Calcium Conc. ppm.	0	5	10	66	310	615
Final Calcium Conc. ppm.	1.3	15.4	15.4	66	310	615

Figure 82.

Table of results of the initial and final calcium concentrations, in which the watercress had equilibriated.

The rate of leaching from the watercress varied from 1.1 x 10^{-5} g/day at 5ppm Ca^{++} to 6.0 x 10^{-6} g/day at 10 ppm Ca^{++}.

(b) After 4 days aeration, the snails were introduced into the isotope solutions containing the watercress. The Ca^{++} ppm of the canal at the time of sampling was 103.2 Ca^{++} ppm and the mean fresh weight of the snails was 0.5400 g. At the same time that the snails were put into the vessels containing the watercress, snails were also placed into control isotope vessels which did not contain watercress. Thus one had a direct comparison between unfed and fed snails.

It was observed that the surface of the watercress had been eaten by the snails, but that over the period of the experiment, the watercress remained green and healthy looking.

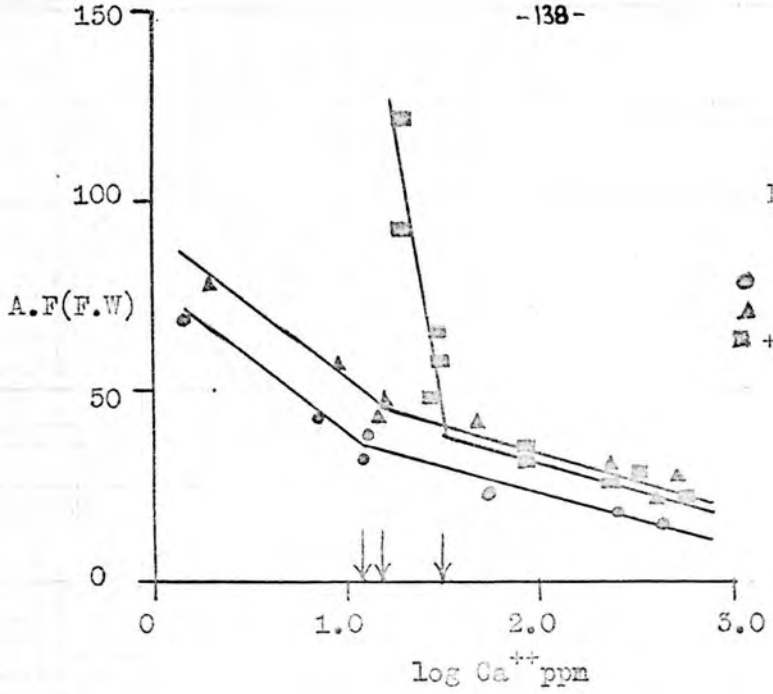
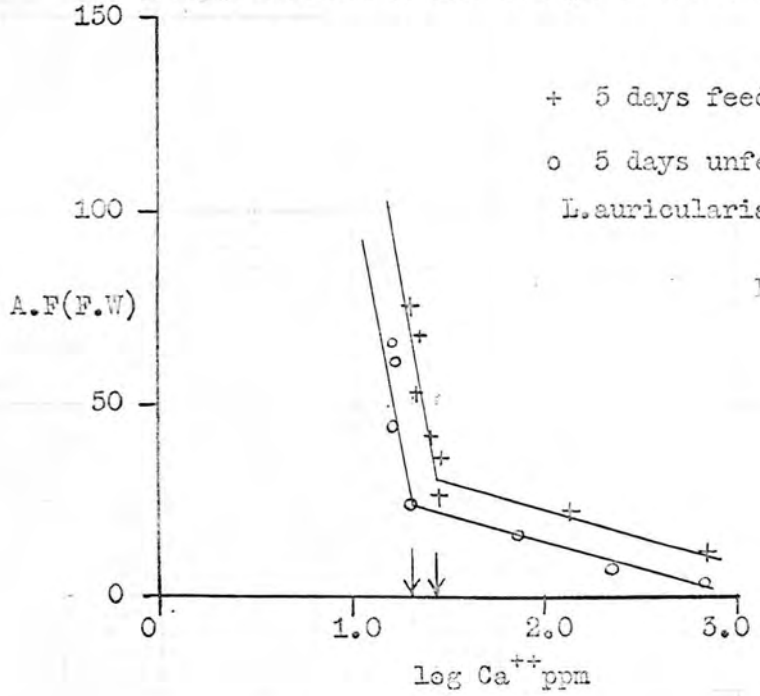


Figure 83.

○ 2 days
△ 4 days
□ +5 days

Plot: A.F(F.W) against log Ca^{++}ppm.

The uptake of 47 Ca by watercress, at 2 and 4 days in the isotope solution; and +5 days after introduction of the snails.



\+ 5 days feeding L. auricularia.

o 5 days unfed (control) L.auricularia.

Figure 84.

Plot: A.F(F.W) against log Ca^{++}ppm.
The uptake of 47 Ca by Limnaea auricularia. +5 days.

If the leaflets became discoloured, they were removed.

The results were plotted as:-

(i) AF(FW) against log calcium concentration in Figures 83 and 84 for watercress and snail respectively. The change over in slope for the watercress is 12.59 ppm Ca^{++} at 2 days, to 15.85 ppm Ca^{++} at 4 days; and after being eaten, to 31.62 ppm Ca^{++}. These figures suggest that feeding by snails increases the leaching of calcium from the plants. It was observed that the snails, which possess radulas, rasp off the surface layers of the leaves of the watercress. Consequently, the AF(FW) has increased considerably, particularly below31.62 ppm Ca^{++}.

The change over in slope for the snails, is 19.95 ppm Ca^{++} for the unfed snails, to 28.18 ppm Ca^{++} for the fed snails.

It is interesting to note that the slopes of the semi-log plots for both the watercress and the snail are similar above 31.62 ppm Ca^{++}. This suggests that the external calcium concentration is important to plants and animals in the uptake of isotopes.

(iii) log AF(FW) against log calcium concentration in Figure 85. The log plot of fed snails is compared with that of the unfed snails. This confirms other experiments, in that the unfed snail

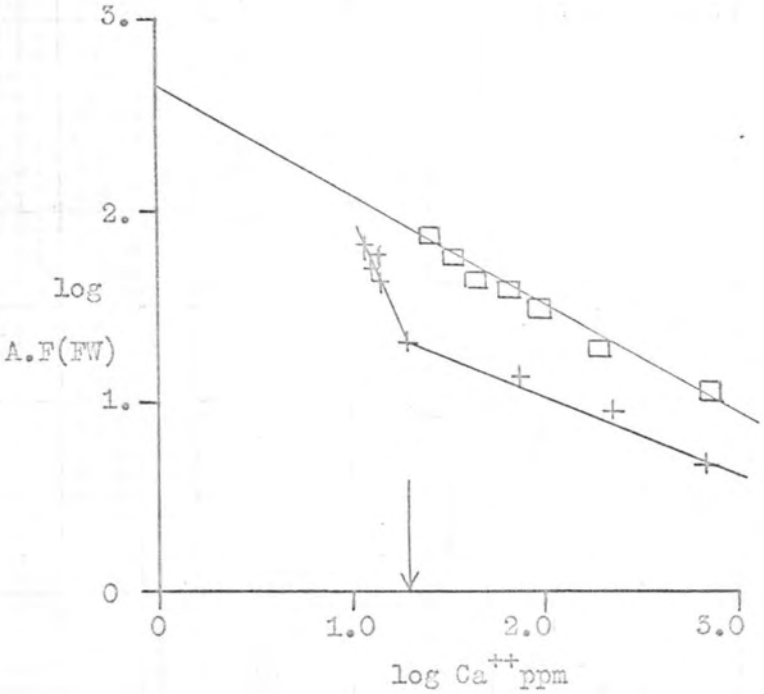


Figure 85 .

Plot: log A.F(FW) against log calcium concentration. The uptake of 47 Ca by Limnaea auricularia, fed (□) and unfed (+) at 5 days . The food in this experiment being watercress. Each reading is the mean of 10 snails.

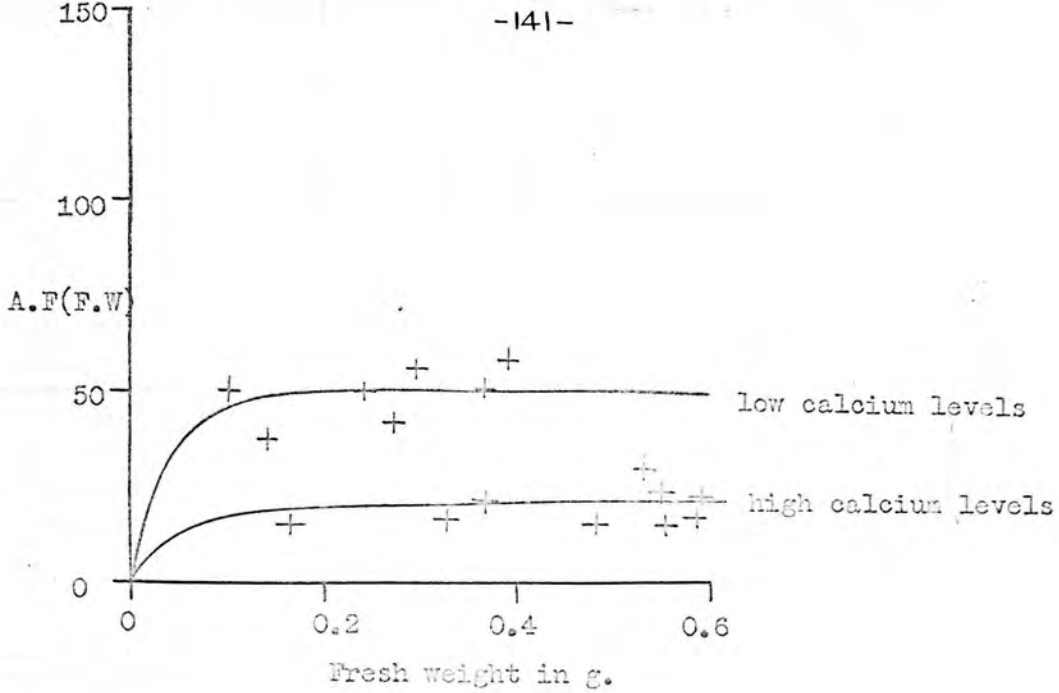


Figure 86 .

Plot: A.F (F.W) against the Fresh weight in grams - watercress leaflets.

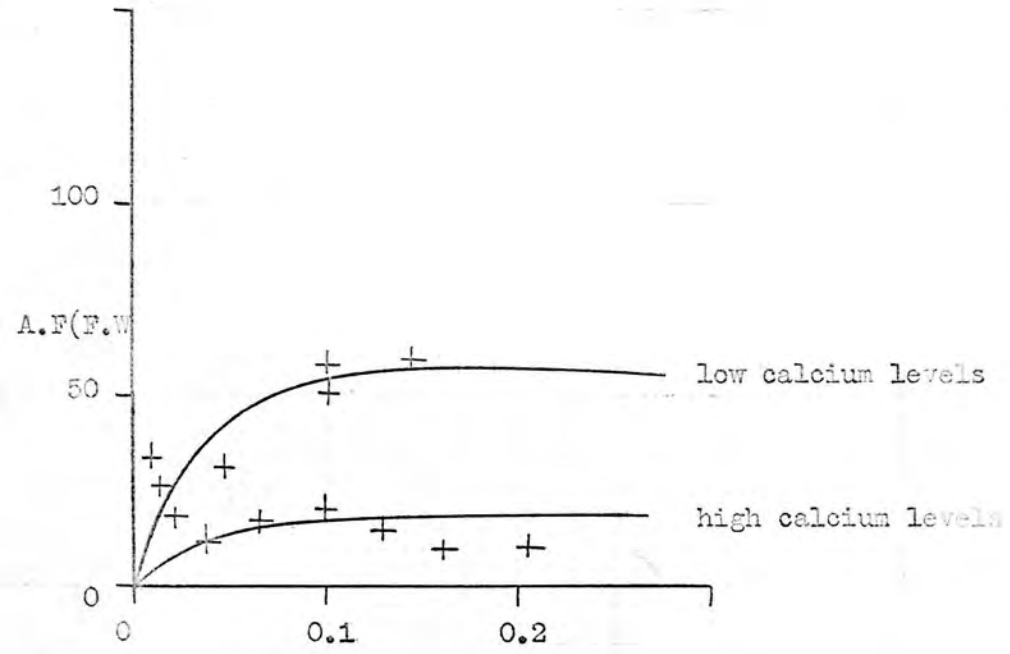


Figure 87.

Plot: A.F(F.W) against the Fresh weight in grams - Limnaea auricularia, whole snails.

is characterised by a 'two-line' plot, and the fed snail by a 'one-line' plot.

Expressions from Figure 85 are:-

$\log AF(FW)_{47\ Ca}$ = 1.85 - 0.40 log Ca^{++} ppm above 31.62 ppm,

= 2.65 - 2.15 log Ca^{++} ppm below 31.62 ppm (UNFED).

$\log AF(FW)_{47\ Ca}$ = 2.65 - 0.55 log Ca^{++} ppm (FED).

(iii) By plotting the fresh weights of (a) the watercress and (b) Limnaea auricularia against the AF(FW), in Figures 86 and 87, respectively; it can be seen that there is a divergence over a wide range of calcium concentrations. Two curves can be drawn, relating to the calcium concentration of the surrounding habitat, with higher AF(FW) at low calcium concentration. These plots suggest that the AF(FW) - weight relationship is calcium dependent.

Experiment 11

The uptake of 47 Ca and 47 Sc by unfed and fed Limnaea auricularia (whole snail, shell and body)

The uptake of 47 Ca and 47 Sc by unfed and fed Limnaea auricularia was studied over a period of 11 days, at calcium levels in the medium from 0-240 ppm Ca^{++}, under aerated conditions.

The source of food was lettuce in the fed snail experiments. Fed snails were broken down at 2, 4 and 7 days. The unfed snails were broken down at 4, 6 and 11 days. The mean fresh weight of the snails was 0.1537 g. and the shell length varied from 7-11 mm. The body weighed approximately 68% of the fresh weight of the snail, while the shell weighed approximately 27%.

The whole snail was initially counted, then dissected into shell and body which were then counted separately.

The calcium concentration of the canal water at the time of sampling was 37.03 ppm Ca^{++}. This low value was due to the heavy rains over the previous week.

a. Unfed snails

In all the jars there was an increase in the calcium concentration in the medium, the mean being 17.93 ppm Ca^{++} per jar over 11 days, which is equivalent to a rate of leaching of

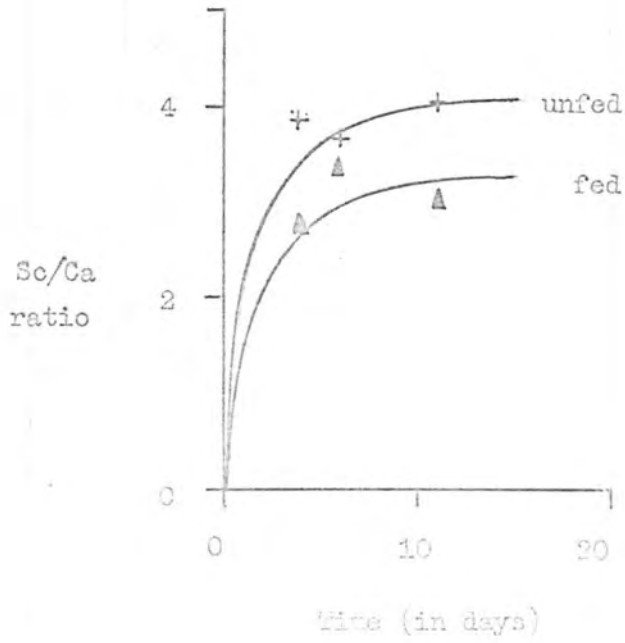


Figure 88 .
Plot: Sc/Ca ratio against Time (in days). Uptake of 47 Sc and 47 Ca by unfed and fed Limnaea auricularia.

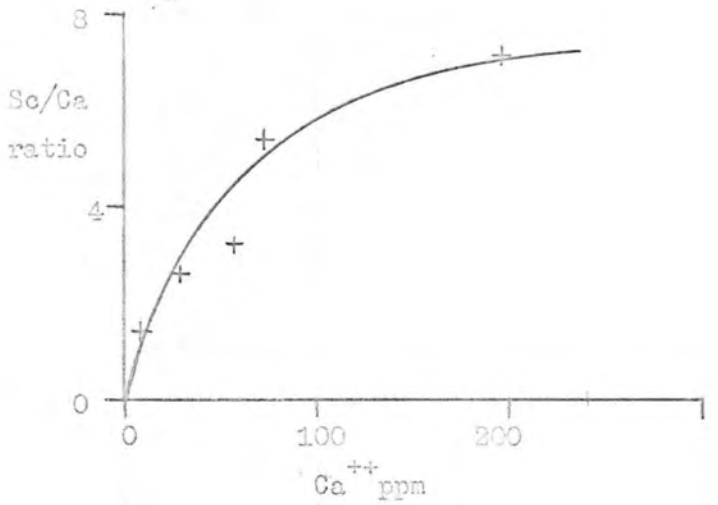


Figure 89

Plot: Sc/Ca ratio against the calcium concentration in the medium. Uptake of 47 Sc and 47 Ca by unfed Limnaea auricularia. Each point is the mean of 216.

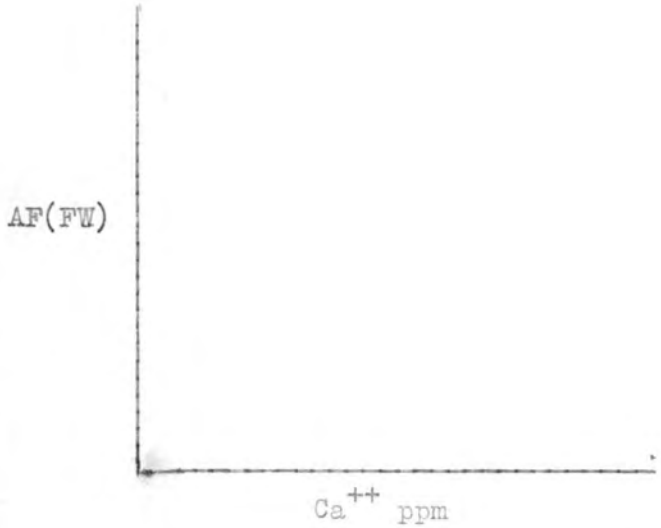
1.7×10^{-5} g Ca^{++}/ day.

The general pattern of the AF(FW)'s of the whole snails was that the Sc:Ca ratio increased with the calcium concentration in the medium and is always greater than 1, (see Figure 88).

These figures indicate discrimination in favour of scandium. With increasing calcium concentration in the medium with time, the AF(FW)'s for 47 Ca and 47 Sc increase for the whole snail as shown in Figure 89. Above 175-220 ppm Ca^{++} in the medium, there is very little effect on the Sc/Ca ratio by increasing the calcium concentration in the medium.

The results are expressed as:-

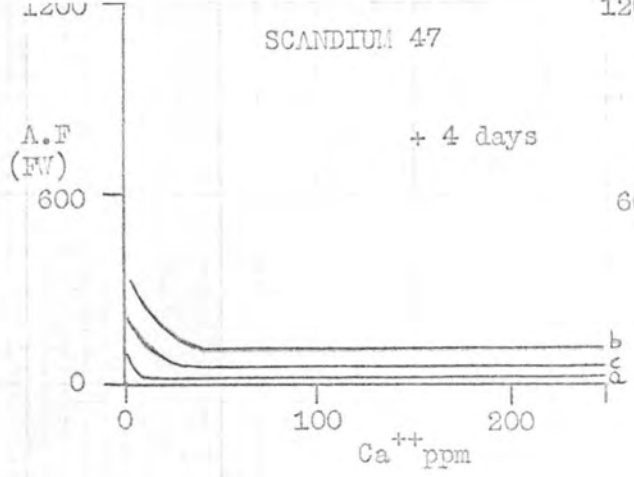
(i) AF(FW) against the calcium concentration in the medium in Figure 90. There is an increase of AF(FW) with time for both 47 Sc and 47 Ca. The increase in 47 Sc seems to come from the decay of 47 Ca. There is an apparent decrease in the uptake of 47 Ca, i.e. the uptake is either reduced, or the uptake has remained the same and the amount of active excretion increased. Although the AF(FW)'s below 65-70 ppm Ca^{++} are similar, above 70 ppm Ca^{++}, the snail absorbs 47 Sc in preference to 47 Ca. Since the AF(FW)'s are similar it does suggest that there is a similar mechanism of uptake.

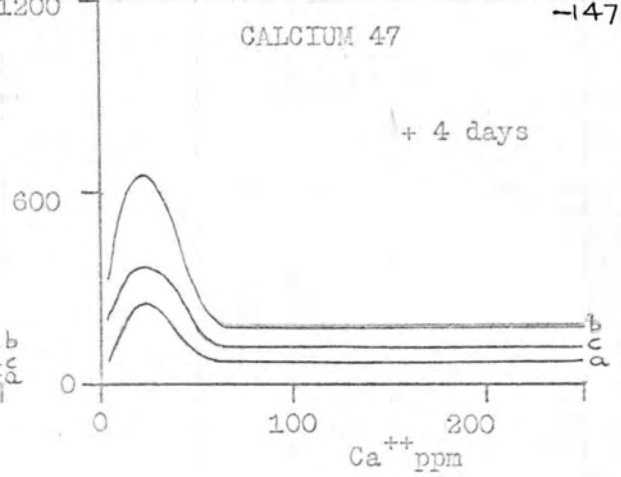


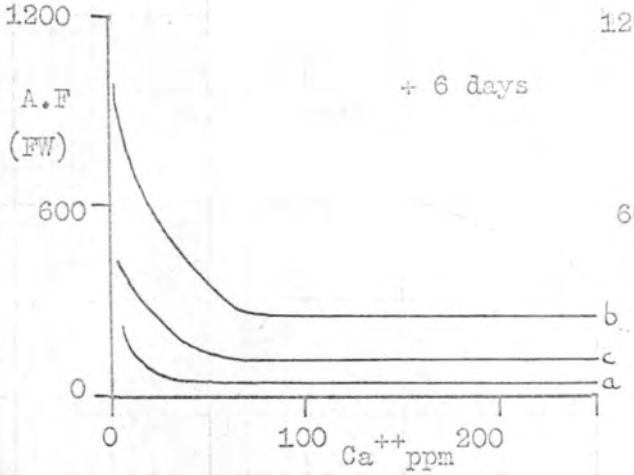
Title to Figure 90, page 147 .

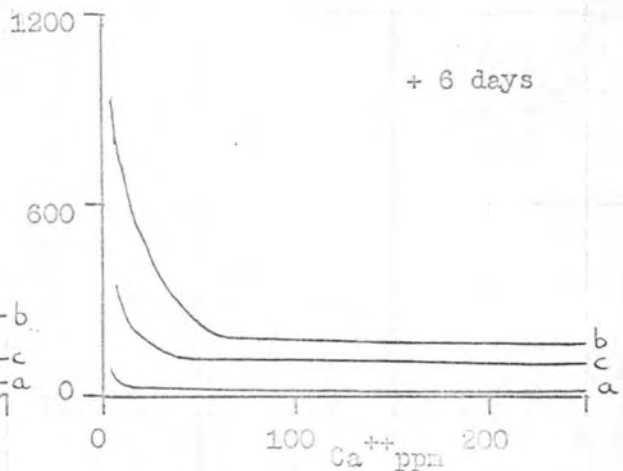
Plot: AF(FW) against calcium concentration in the medium. The uptake of 47 Sc and 47 Ca by unfed Limnaea auricularia, at +4 days, + 6 days and +11 days, for (a) body, (b) shell and (c) whole snail.

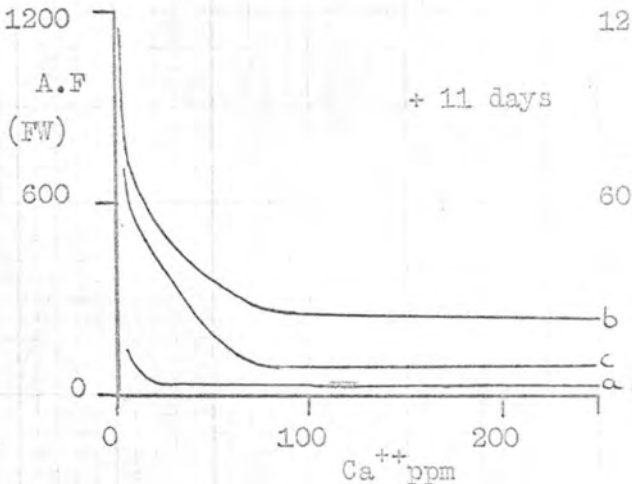
The points have been omitted on these graphs, and the curves have been drawn with the aid of either a flexicurve or french curves through the points.

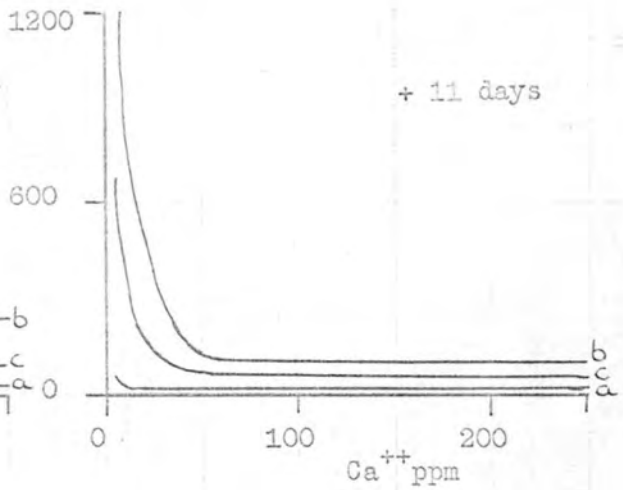

SCANDIUM 47
A.F
(FW)
+ 4 days
1200
600
0
b
c
a
0
100
200
Ca++ ppm

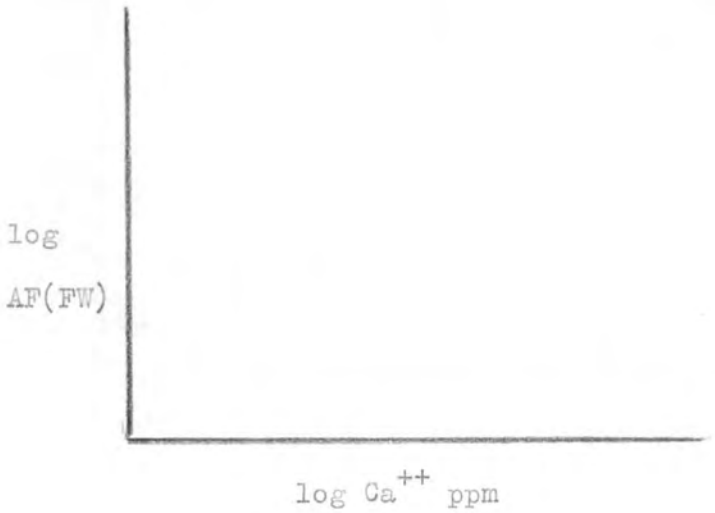

CALCIUM 47
+ 4 days
1200
600
0
b
c
a
0
100
200
Ca++ ppm


A.F
(FW)
+ 6 days
1200
600
0
b
c
a
0
100
200
Ca++ ppm


+ 6 days
1200
600
0
b
c
a
0
100
200
Ca++ ppm


A.F
(FW)
+ 11 days
1200
600
0
b
c
a
0
100
200
Ca++ ppm

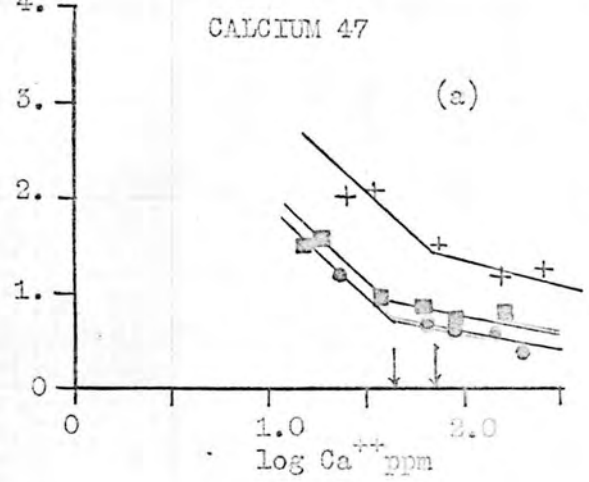

+ 11 days
1200
600
0
b
c
a
0
100
200
Ca++ ppm

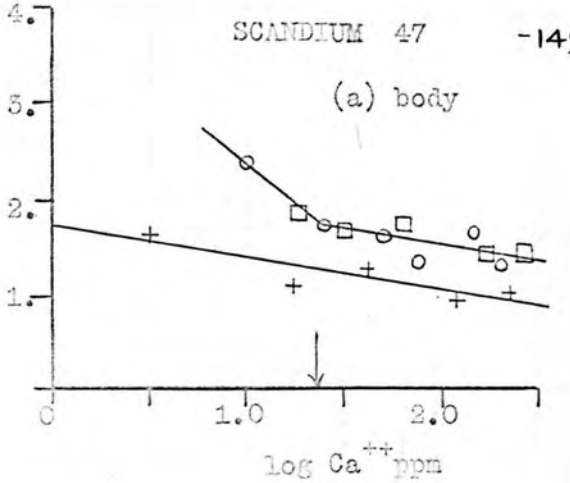


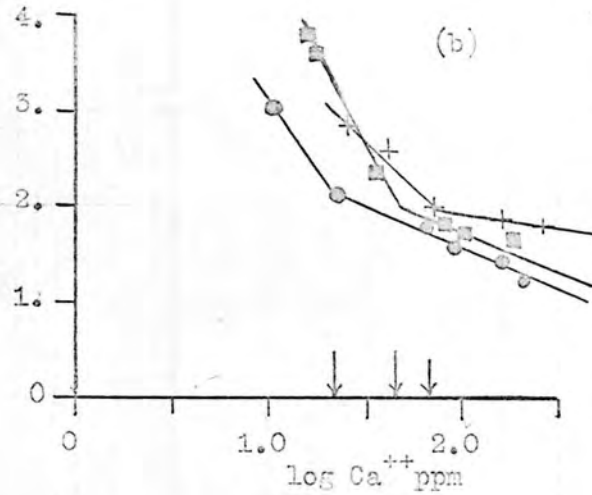
Title to Figure 91, page 149 .

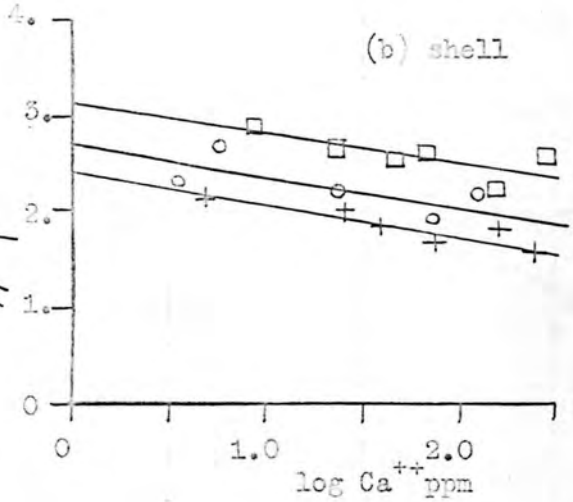
Plot: log AF(FW) against log calcium concentration of the medium. The uptake of 47 Sc and 47 Ca by unfed Limnaea auricularia, for (a) body, (b) shell and (c) whole snail, at (i) 4 days, + (ii) 6 days o , and (iii) 11days. □ .

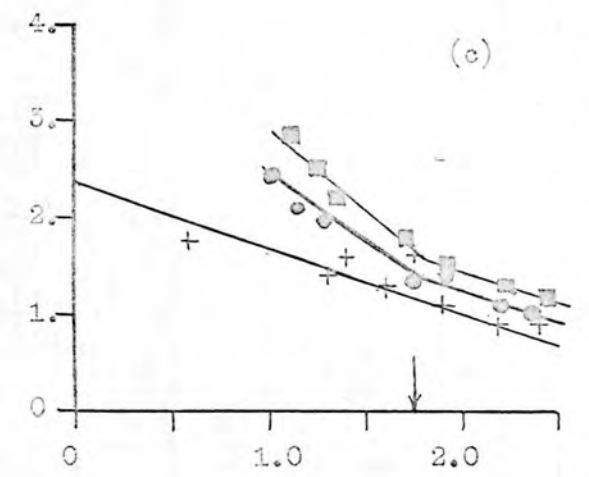
Each point is the mean of 8.

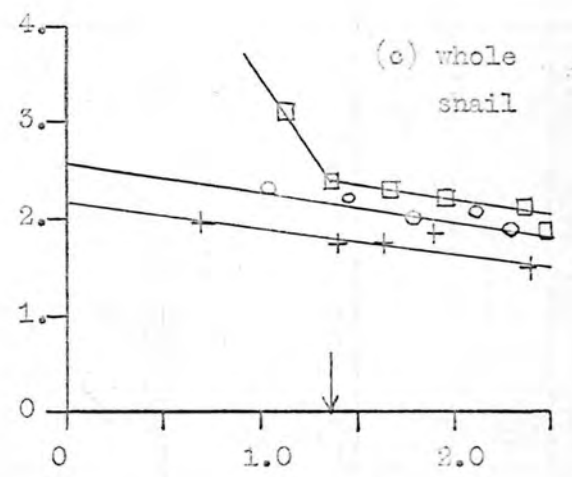

CALCIUM 47
(a)
4.
3.
2.
1.
0
0
1.0
2.0
log Ca++ ppm


SCANDIUM 47
(a) body
4.
3.
2.
1.
0
0
1.0
2.0
log Ca++ ppm


(b)
4.
3.
2.
1.
0
0
1.0
2.0
log Ca++ ppm


(b) shell
4.
3.
2.
1.
0
0
1.0
2.0
log Ca++ ppm


(c)
4.
3.
2.
1.
0
0
1.0
2.0


(c) whole
snail
4.
3.
2.
1.
0
0
1.0
2.0

(ii) log AF(FW) against log calcium concentration in the medium in Figure 91, for the unfed snail and the results in Figure 92, page 151. The slope of 47 Sc is shallower than 47 Ca, and this suggests that 47 Sc is less dependent than 47 Ca in the external calcium concentration in the medium.

The rate of uptake of 47 Sc by both body and shell is the same, but there is a higher maximum in the shell. This is probably due to the fact that the shell presents a greater surface area to the medium on which is adsorbed the particulate Sc^{+++} ion.

The rate of uptake of 47 Ca is more in the shell than the body. One would expect this, since there is more exchange calcium in the shell than in the body.

In all the log plots for the unfed snails, there are 'two-line' plots with the exception of the shell. This is similar to that obtained with radiostrontium in previous experiments. The 'two-line' plots are more definite for 47 Ca than 47 Sc and this indicates that the uptake of 47 Sc is less dependent on the calcium concentration in the medium than 47 Ca.

The slope of the unfed snails for 47 Ca and 47 Sc is -0.4 which is the same as that obtained for Limnaea species on pages 83, 86, 110 and 142. Since both the slopes for the uptake of radiostrontium and radiocalcium are the same, then it could be

		Unfed		Fed	
			Days		Days
Ca^{47}	body	2.4 - 0.4	+4	2.9 - 1.05	+2
		1.5 - 0.4	+6	3.1 - 1.05	+4
		1.7 - 0.4	+11	3.3 - 1.05	+7
	shell	2.4 - 0.2	+4	3.3 - 1.05	+2
		3.4 - 0.85	+6	3.6 - 1.05	+4
		3.5 - 0.85	+11	3.8 - 1.05	+7
	whole snail	2.4 - 0.3	+4	3.0 - 1.05	+2
		2.6 - 0.4	+6	3.1 - 1.05	+4
		2.8 - 0.4	+11	3.2 - 1.05	+7
Sc^{47}	body	1.7 - 0.4	+4	1.4 - 0.4	+2
		2.2 - 0.4	+6	1.6 - 0.4	+4
		2.2 - 0.4	+11	1.6 - 0.4	+7
	shell	2.4 - 0.4	+4	2.4 - 0.3	+2
		2.7 - 0.4	+6	2.4 - 0.3	+4
		3.1 - 0.4	+11	2.4 - 0.3	+7
	whole snail	2.2 - 0.4	+4	2.4 - 0.3	+2
		2.6 - 0.4	+6	2.3 - 0.3	+4
		2.8 - 0.4	+11	2.1 - 0.3	+7

Figure 92.

Table of regression plots for 47 Ca and 47 Sc, for fed and unfed Limnaea auricularia, at different time intervals. The expressions are given of the slope above the critical calcium level in the medium.

stated that radiostrontium follows the same path as radiocalcium through the membranes of the unfed snail, i.e. Limnaea species.

b. Fed snails

Uptake by lettuce

The mean fresh weight of the pieces of lettuce was 0.2649 g and these were allowed to equilibriate with the isotope solution before the introduction of the snails - Limnaea auricularia. It took 4 days for the lettuce to equilibriate with the isotope under aerated conditions.

The expressions from the log plot in Figure 93, are:-

$$\log AF(FW)_{47\,Sc} = 3.85 - 1.15 \log Ca^{++} \text{ ppm}$$

$$\log AF(FW)_{47\,Ca} = 3.0 - 0.95 \log Ca^{++} \text{ ppm}$$

Uptake by the feeding snails

The results are plotted in Figures 94, and 95 and the expressions from the log plot are recorded in Figure 92.

The expressions for 47 Sc are similar to those obtained with the unfed snails only that the maxima are slightly higher. Since the slope of the fed approximates that of the unfed, then the higher maxima could be due to the activity of the isotope in the food in the digestive tract. This proves

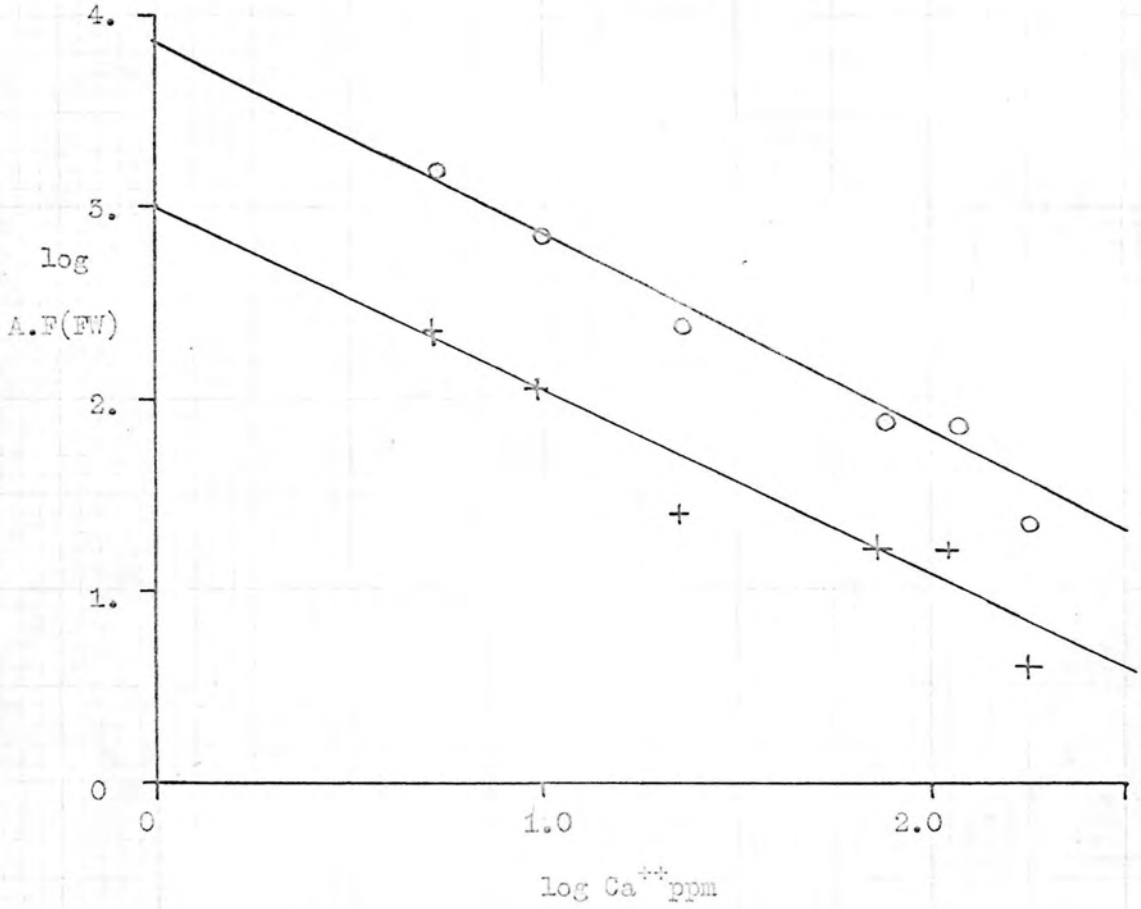
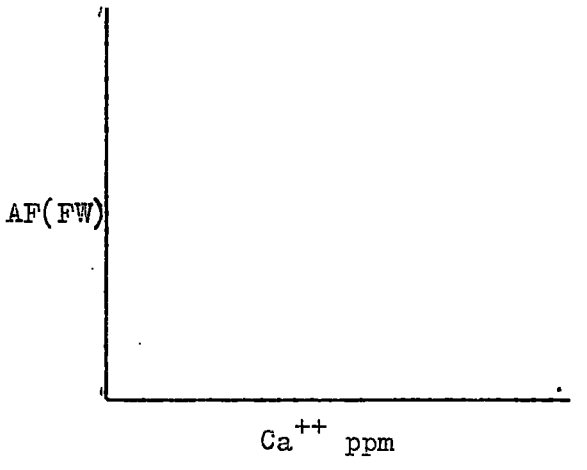


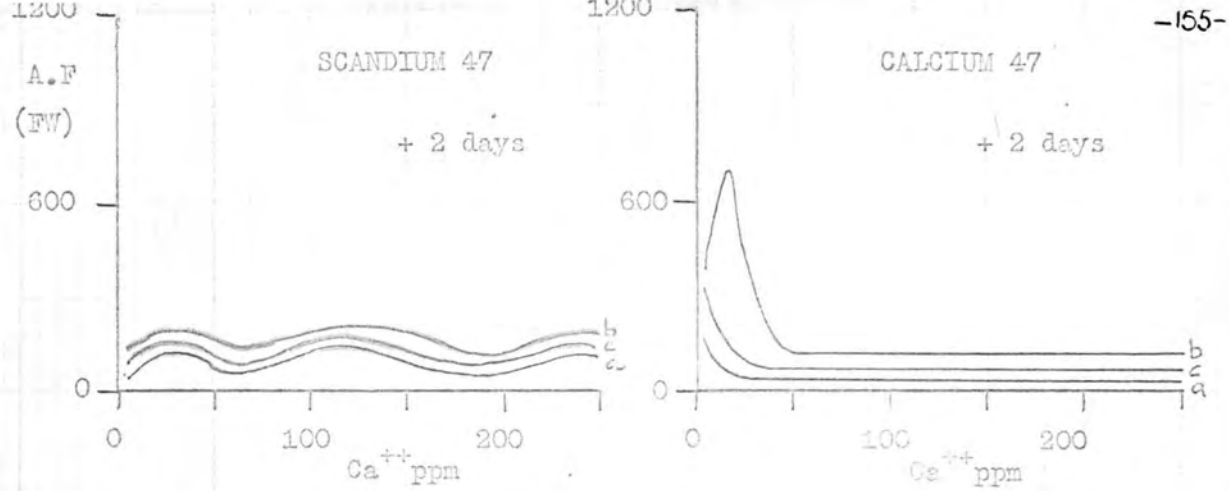
Figure 95

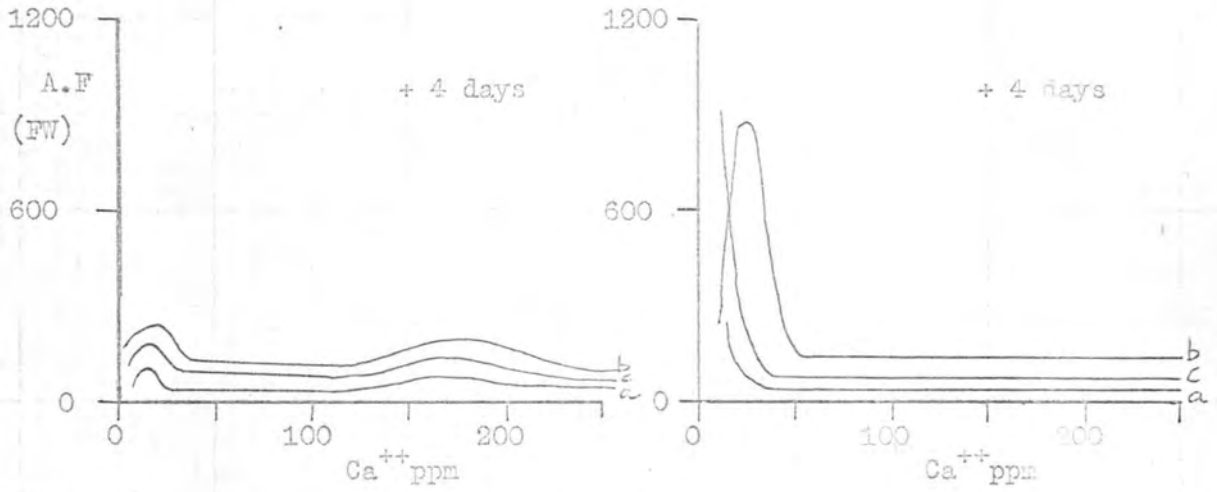
Plot: log A.F(FW) against log calcium concentration of the medium. The uptake of 47 Sc and 47 Ca by lettuce. Each point is the mean of 10.

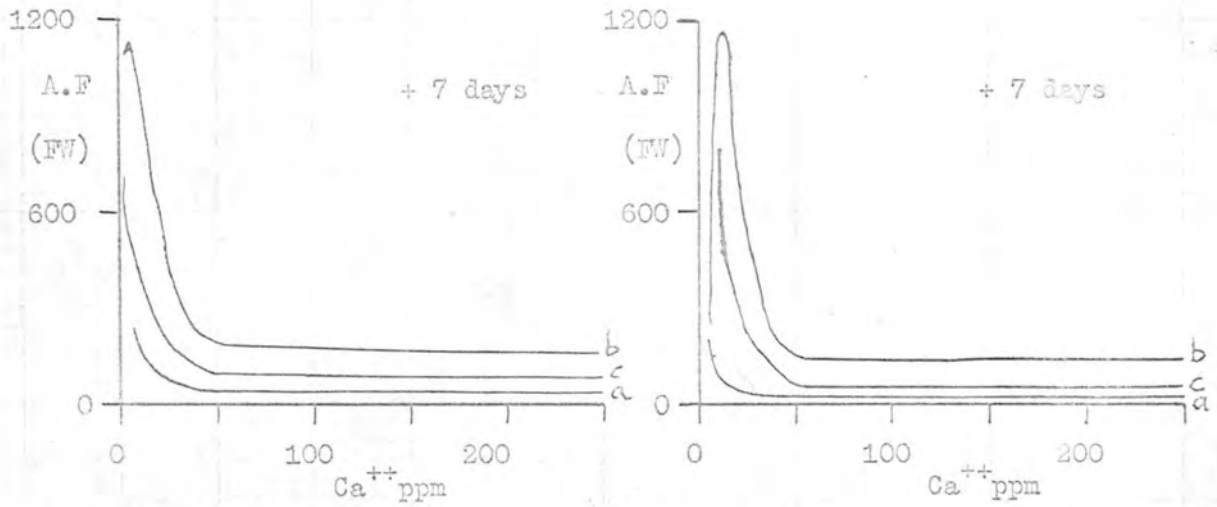
+ = 47 Ca ; o = 47 Sc.

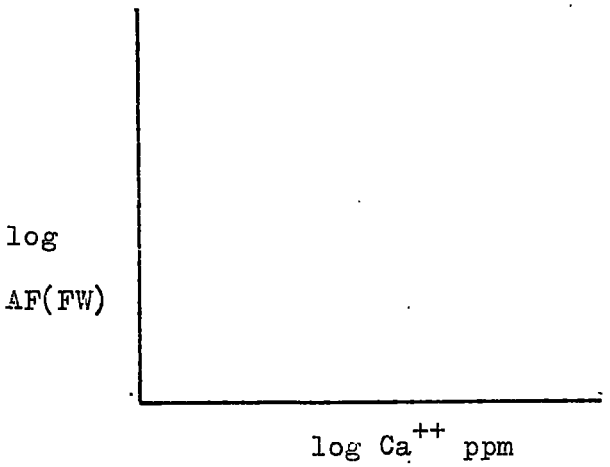


Title to Figure 94, page 155.
Plot: AF(FW) against the calcium concentration of the medium. The uptake of 47 Sc and 47 Ca by fed Limnaea auricularia, at 2 days, 4 days and 7 days; for (a) body, (b) shell and (c) whole snail. Each point is the mean of 8.


1200
A.F
(FW)
SCANDIUM 47
+ 2 days
600
0
b
c
a
0
100
200
Ca++ ppm
1200
CALCIUM 47
+ 2 days
600
0
b
c
a
0
100
200
Ca++ ppm


1200
A.F
(FW)
+ 4 days
600
0
b
c
a
0
100
200
Ca++ ppm
1200
+ 4 days
600
0
b
c
a
0
100
200
Ca++ ppm

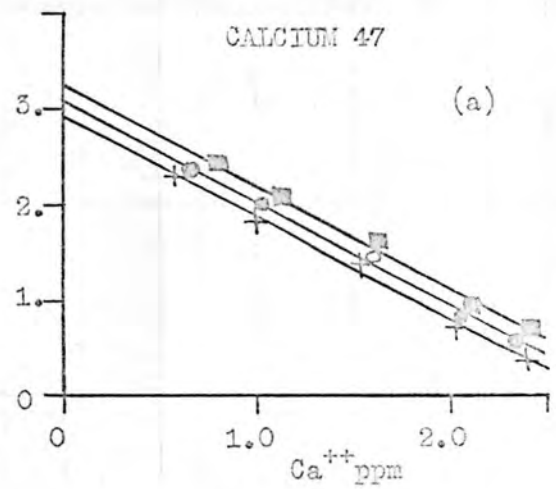

1200
A.F
(FW)
+ 7 days
600
0
b
c
a
0
100
200
Ca++ ppm
1200
A.F
(FW)
+ 7 days
600
0
b
c
a
0
100
200
Ca++ ppm

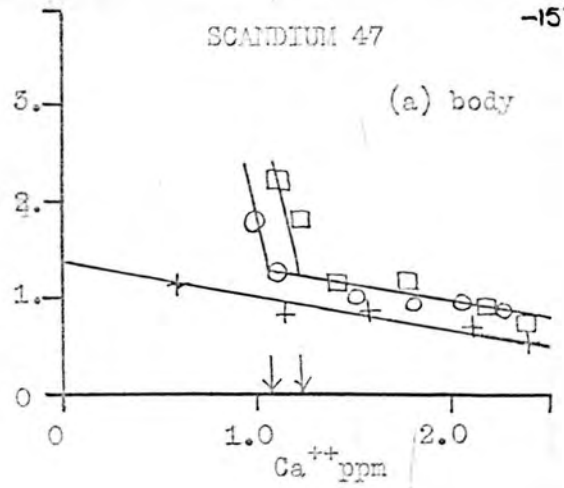


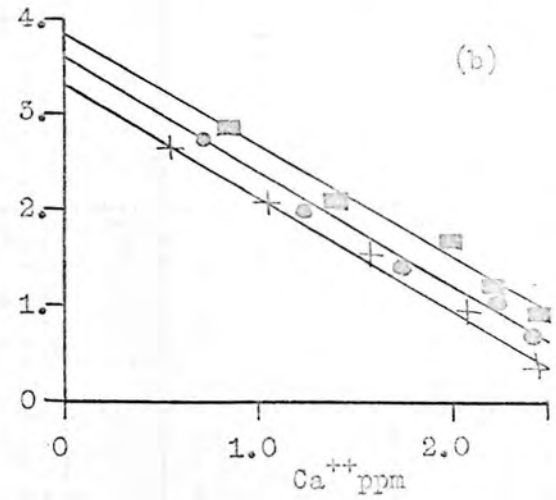
Titles to Figure 95, page 15Ø.

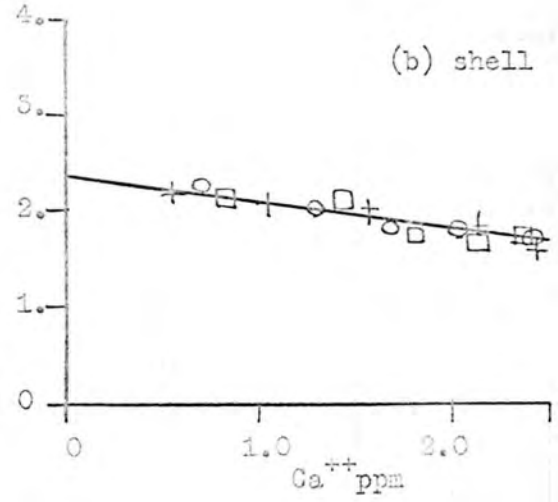
Plot: log AF(FW) against log calcium concentration of the medium. The uptake of 47 Sc and 47 Ca by fed Limnaea auricularia, for (a) body, (b) shell and (c) whole snail; at 2 days +, 3 days - o and 7 days - □.

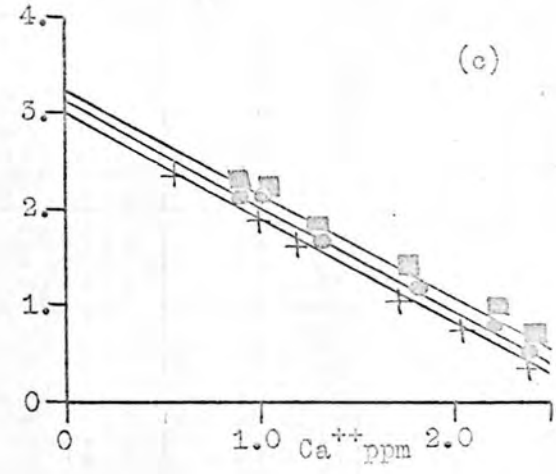
Each point is the mean of 8.

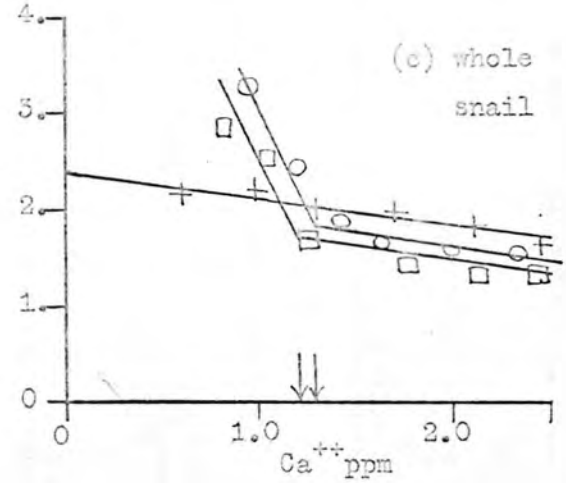

CALCIUM 47
(a)
3.
2.
1.
0
0
1.0
2.0
Ca++ ppm


SCANDIUM 47
(a) body
3.
2.
1.
0
0
1.0
2.0
Ca++ ppm


(b)
4.
3.
2.
1.
0
0
1.0
2.0
Ca++ ppm


(b) shell
4.
3.
2.
1.
0
0
1.0
2.0
Ca++ ppm


(c)
4.
3.
2.
1.
0
0
1.0
Ca++ ppm
2.0


(c) whole
snail
4.
3.
2.
1.
0
0
1.0
2.0
Ca++ ppm

that the gut discriminates against the particulate scandium ion, in favour of the ionic calcium ion.

The other factor which confirms that feeding has little if no effect on the uptake of 47 Sc, is the fact that in the body and the whole snail, there are 'two-line' plots, which are characteristic of non-fed snails.

The slope of the 47 Ca has increased to -1.05. This means that feeding has increased the rate of uptake and accumulation of 47 Ca in the snail. The other factor is that there is no evidence of a 'two-line' plot. The 'one-line' plot for 47 Ca by Limnaea auricularia feeding on lettuce, confirms the evidence obtained with 85 Sr, in that feeding does influence the rate and accumulation of the isotope.

The rate of leaching of calcium from the snails is 1.2 x 10^{-5} g Ca^{++}/ day, which is less than in the unfed experiments by approximately 50%. The amount of body fluids in the fed snails is higher than in the unfed snails as shown in Figure 96.

The body weight (fresh weight of the snail) increases with time, while the shell decreases due to the leaching of calcium from its structure. The mean fresh weight of the fed snail was 0.1363 g and were 7-12 mm long, compared with a mean fresh weight of 0.1537 g for the unfed snails and 7-11 mm long.

The plot of Sc/Ca ratio against time in Figure 88, shows that feeding reduces the Sc/Ca ratio, although the AF(FW) for

Time in days	+ 2	+ 4	+ 6	+ 7	+ 11
% FW of Snail		65%	73%		75%
Body. Unfed					
Fed	68%	67%		75%	
Shell - Unfed		27%	26%		24%
- Fed	27%	27%		21%	
Body Fluids - Unfed	5%	6%		4%	
- Fed		8%	1%		1%

Figure 96.

Data on the % fresh weights of (a) body, (b) shell and (c) body fluids of Limnaea auricularia, for fed and unfed, at different time intervals.

* * * *

the fed snails are higher than the unfed snails. This is indicated by the lower rate of leaching of calcium in the fed snails.

Experiment 12

The uptake of 89 Sr by unfed Bithynia tentaculata (whole snail)

The uptake of 89 Sr by Bithynia tentaculata was studied for 35 days in calcium concentrations varying from 0-80 ppm Ca^{++}, under aerated conditions.

The mean fresh weight of the snails was 0.1591 g and 8-11 mm long. The ratio of FW:DW::3:1.

The uptake of 89 Sr by the snail was plotted as:-

(i) AF(FW) against time, for certain calcium ranges, in Figure 97, and the results in Figure 98.

Calcium Range ppm	AF(FW) at Equilibrium	Time taken to E_t Equilibrium (days)	$E_{t/5}$	AF(FW)/ E_t
0-10	32.5	7	1.4	4.6
65-68	7	5	1.0	1.4

Figure 98

Results obtained from Figure 97, on the uptake of 89 Sr by whole snails of Bithynia tentaculata. AF(FW) 89 Sr based on three week counts.

The results show that the uptake is less than for Limnaea pereger (page 78), Limnaea truncatula (page 121) and Limnaea auricularia (page 128). The uptake at calcium levels above 66 ppm Ca^{++}, is similar to the uptake by Limnaea species;

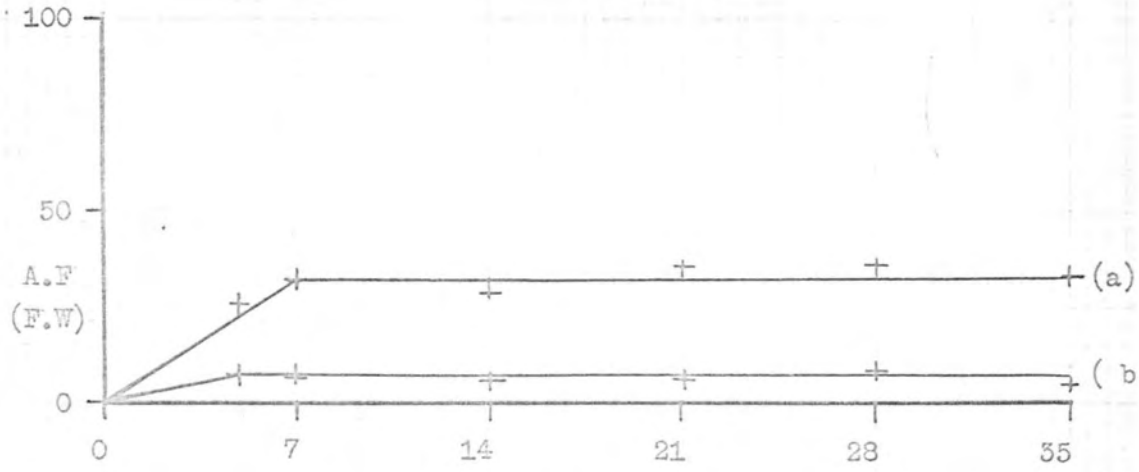


Figure 97 .

Plot: A.F (F.W) against Time 't' in days.
The uptake of 89 Sr by Bithynia tentaculata, at calcium ranges of (a) 0- 10 Ca^{++} ppm and (b) 65-68 ppm Ca^{++}.
Each reading is the mean of 8.
A.F (FW) 89Sr based on three week counts.

while below this figure the uptake by Bithynia is considerably less than Limnaea species. This is probably due to the fact that Bithynia has a chitinous operculum which seals the entrance to its shell when it is not moving, so preventing rapid exchange or uptake of the isotope by the body. Snails were sampled throughout the year, and it was found that there was less dimineralisation from the snail in October than in April, which is probably due to the lower temperature stabilising the solubility product of $CaCO_3$. In April, the rate of dimineralisation or leaching was 1.08 to 1.5 x 10^{-5}g Ca^{++}/day, while in October it was 8.8 x 10^{-6} Ca^{++}/day. This seasonal difference, affected the uptake only slightly, less uptake taking place in October than in April, e.g. in April the AF(FW) at 66 ppm Ca^{++} was 15.26 compared with 12.00 in October. This confirms the point that the calcium concentration of the environment is important in determining the uptake of isotope.

(ii) log AF(FW) against log calcium concentration in Figure 98. Expressions for 28 days and 35 days are as follows:-

$$\log AF(FW)_{^{89}Sr} = 2.2 - 0.65 \log Ca^{++} \text{ ppm (4 weeks)}$$

$$= 2.0 - 0.65 \log Ca^{++} \text{ ppm (5 weeks)}$$

The significant point about this plot, is that there is only one line, and not two lines which is characteristic of non-

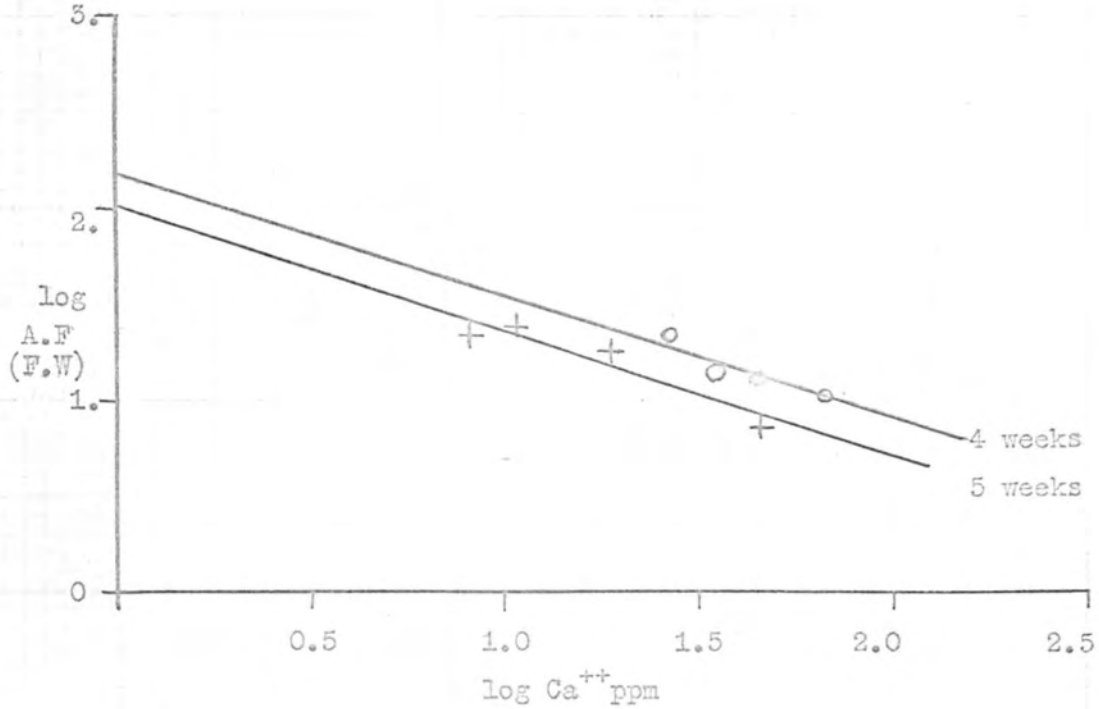


Figure 98 .

Plot: log A.F (F.W) against log calcium concentration.
The uptake of 89 Sr by Bithynia tentaculata .

Each reading is the mean of 8.
AF (FW) 89Sr is based on three week counts.

feeding Limnaea species. The 'one-line' plot is characterised by fed Limnaea species. There must be a different mechanism of uptake or that Bithynia can tolerate lower calcium levels than Limnaea pereger.

Experiment 13

The uptake of 90 Sr by unfed Bithynia tentaculata (whole snails)

The uptake of 90 Sr by unfed Bithynia tentaculata was studied over 21 days at calcium concentrations from 0-650 ppm Ca^{++} under aerated conditions. The mean fresh weight of the snails was 0.2335 g and 9-11 mm long. The ratio of FW:DW::3:1.

The uptake of 90 Sr by the snails was plotted in Figure 99. The expression from the log plot,

$$\log AF(FW)_{90\ Sr} = 2.1 - 0.65 \log Ca^{++}\ ppm$$

is comparable with the expression for 89 Sr on page 162. The results confirm the 89 Sr results.

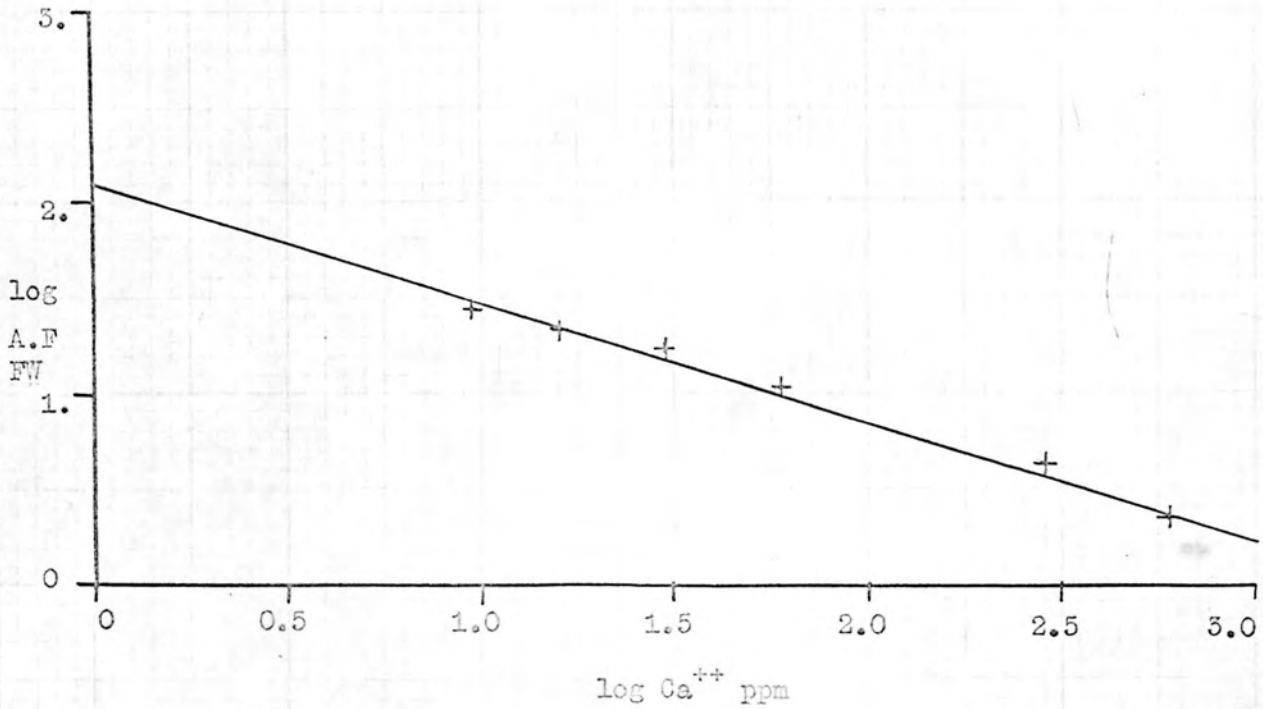


Figure 99 .

Plot: log A.F(FW) against log calcium concentration.

The uptake of 90 Sr by unfed Bithynia tentaculata (whole snails).

Each reading is the mean of 10.

AF(FW) 90Sr based on three week counts.

Experiment 14

The uptake of 85 Sr by fed Bithynia tentaculata (whole snail)

Uptake by food

The uptake of 85 Sr by fed Bithynia tentaculata was studied for 21 days, in calcium concentrations varying from 0-120 ppm Ca^{++}, and under aerated conditions.

The food in this experiment was lettuce. The mean fresh weight of the lettuce was 0.3568 g, and was allowed to equilibriate with the isotope solution before the introduction of the snails, i.e. after 7 days. The uptake by the lettuce is plotted in Figure 100, which gives the following expression:-

$$\log {}^{85}AF(FW)_{Sr} = 2.9 - 1.0 \log Ca^{++} \text{ ppm.}$$

This expression is in good agreement with that attained by lettuce in the Limnaea experiments on pages 97 and 125.

Uptake by snails

The snails were introduced into the isotope solution which contained the lettuce after 7 days. The mean fresh weight of the snails was 0.1473 g.

The results of the uptake of the fed snails is plotted in Figure 101. Expressions from Figure are:-

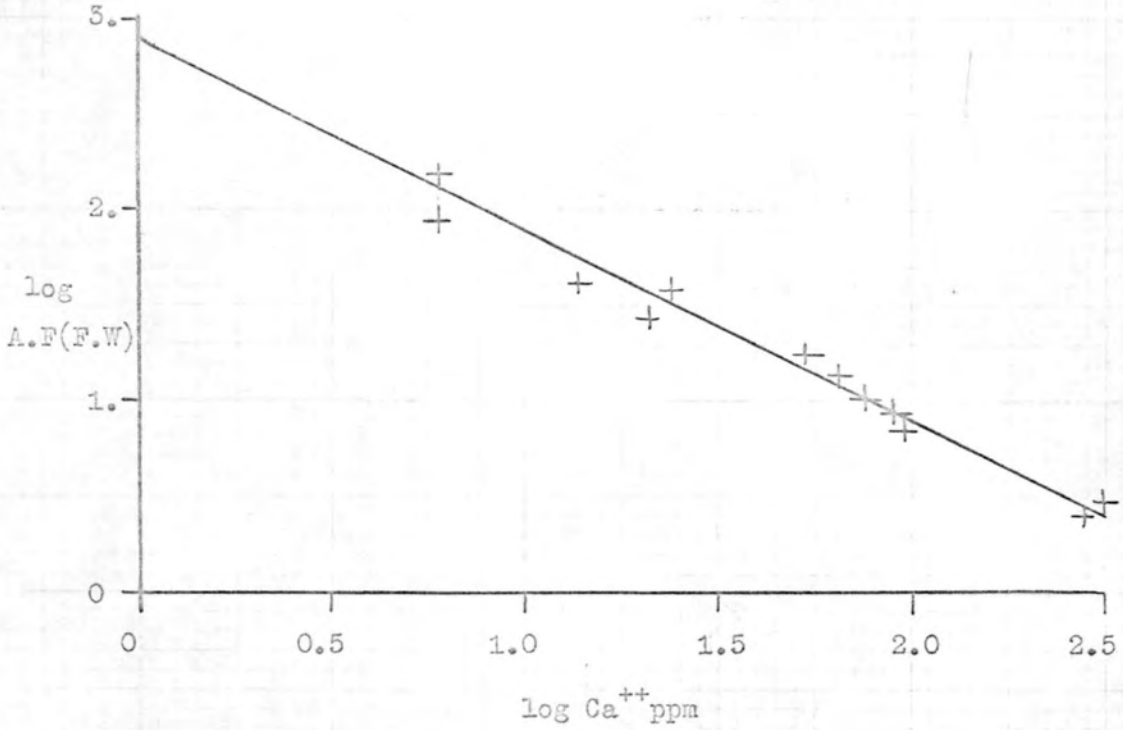


Figure 100 .

Plot: log A.F(F.W) against log Ca^{++}ppm.
The uptake of 85 Sr by lettuce, infeeding experiments with Bithynia tentaculata, at 7 days in the isotope solution, and before feeding by the molluscs.

Each reading is the mean of 10.

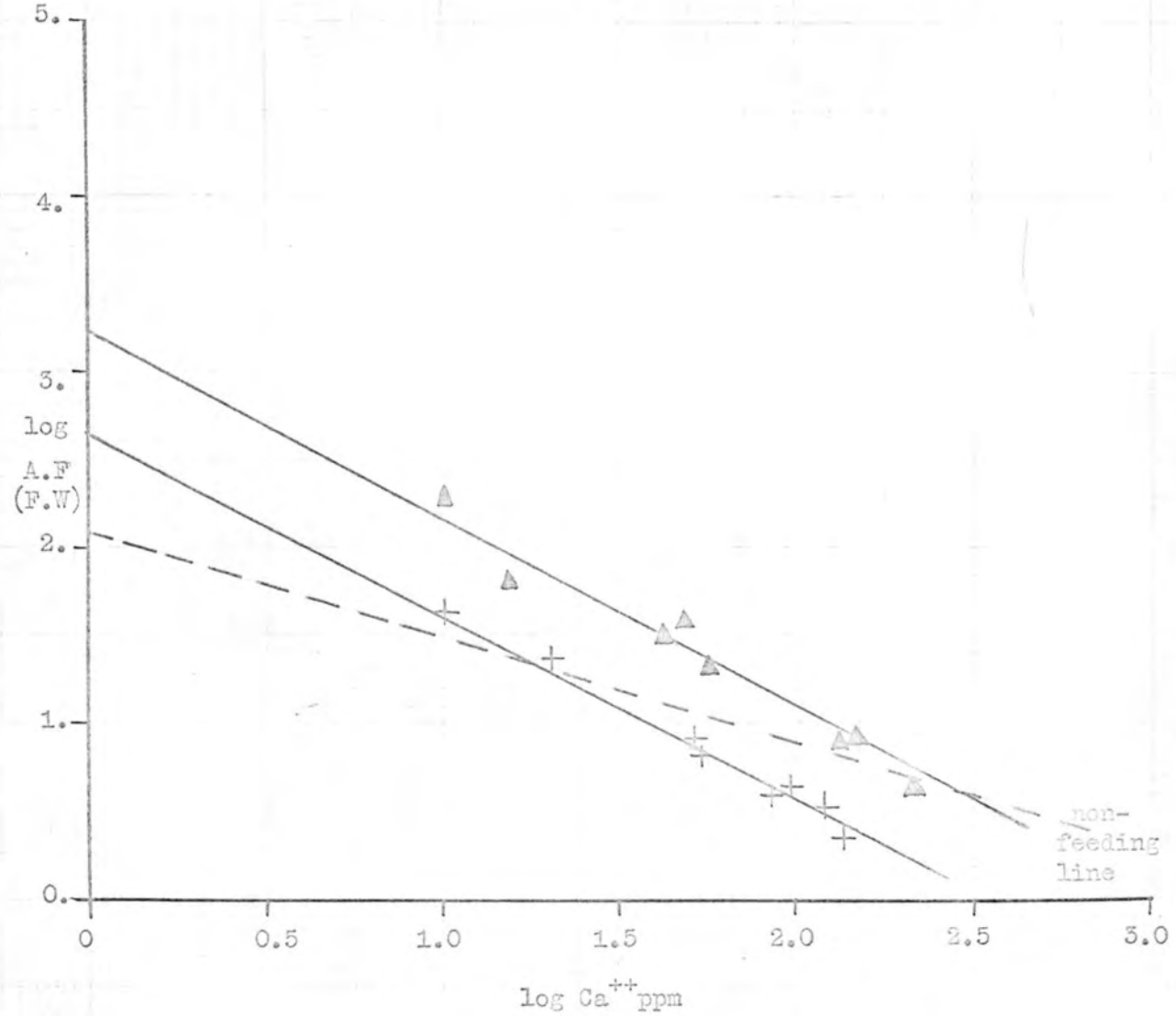


Figure. 101

Plot: log A.F (F.W) against log Ca^{++}ppm.
The uptake of 85 Sr by Bithynia tentaculata , at 14 days(+)and 21 days(▲) feeding on lettuce.

Each point is the mean of 5.

$$\log AF(FW)^{85} = 2.65 - 1.05 \log Ca^{++} \text{ ppm } (+ 14 \text{ days})$$

$$= 3.25 - 1.05 \log Ca^{++} \text{ ppm } (+ 21 \text{ days})$$

The slope of the fed snail is -1.05 compared with -0.65 for the unfed snails. This implies that the lettuce has increased the rate of uptake of the isotope, and in this case the maximum has increased from 2.2 to 3.25.

Experiment 15

The uptake of 47 Ca by unfed Bithynia tentaculata (whole snail, shell, body and operculum)

The uptake of 47 Ca by unfed Bithynia tentaculata over a calcium range of 0-50 ppm Ca^{++}, was studied for 5 days under aerated conditions.

The mean fresh weight of the snails was 0.0640 g and 8-11 mm long. The calcium ion concentration of the canal at the time of sampling was 66.8 ppm Ca^{++}.

In this experiment, the snail was counted whole, then dissected into shell, body and operculum. The weights of the various parts are summarised in Figure 102.

B.tentaculata	% F.W of snail	Ratio of F.W : D.W
body	45	6:1
shell	37	1.2:1
operculum	6.8	2:1
body fluids	11.2	-

Figure 102.

Table of percentage weights of the various parts of the dissected Bithynia tentaculata.

Mean of 90 snails.

The figure in Figure 93, shows that there is little fluid in the shell and that the skeletal structures, i.e. the shell and the operculum, nearly weigh as much as the body.

The uptake of 47 Ca by E.tentaculata are plotted as:-

(i) log AF(FW) against calcium concentrations for the body, shell, operculum and the whole snail in Figure 103.

The uptake by the body is constant after 3 days aeration and is related to the calcium concentration of the external environment, i.e. the AF(FW) decreases with increasing calcium concentration.

The uptake by the shell increases to the 5th day, and is higher than the body.

The uptake by the operculum is initially high, then decreases and at the 5th day, increases again to similar figures to the shell.

The uptake for the whole snail is constant after the 3rd day.

(ii) log AF(FW) against log calcium concentration in Figure 104, for the body, shell, operculum and the whole body. The expression,

$$\log AF(FW)_{\text{isotope}} = \text{maximum at } \log 1 - \text{slope of } \log Ca^{++} \text{ ppm}$$

are summarised in Figure 105.

Title to Figure 103, page 174.

Plot: log AF(FW) against the calcium concentration of the medium. The uptake of 47 Ca by Bithynia tentaculata, (a) whole snail, (b) body and (c) shell, (d) operculum, at 3(+), 4 (o) and 5 (□) days in the isotope solution. The snails were unfed.
Each point is the mean of 15.

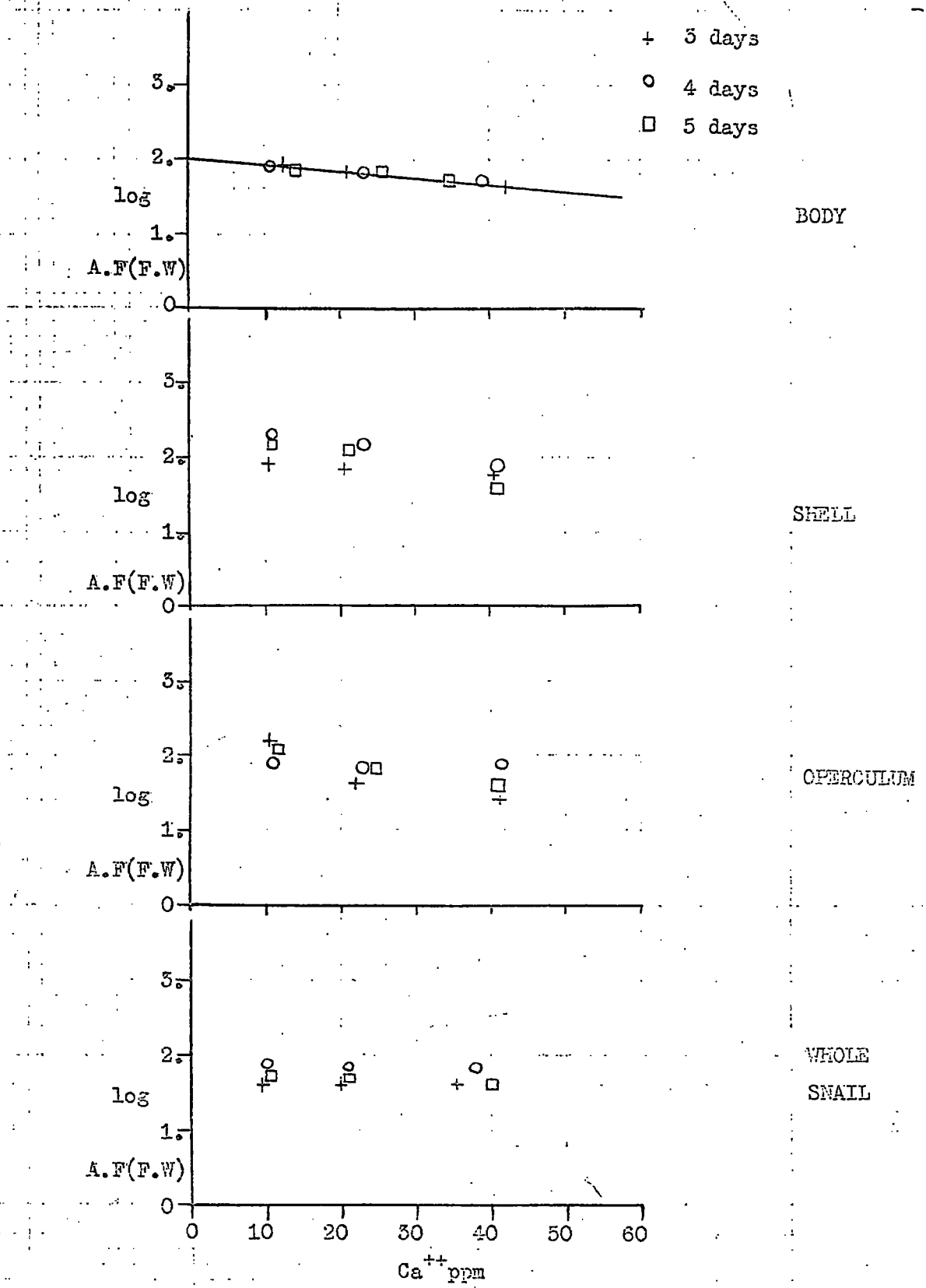

+ 3 days
o 4 days
□ 5 days
log
A.F(F.W)
BODY
SHELL
OPERCULUM
WHOLE
SNAIL
0
1
2
3
0
10
20
30
40
50
60
Ca++ ppm

Figure 105.

Title to Figure 104, page 176.

Plot: log AF(FW) against log calcium concentration of the medium. The uptake of 47 Ca by unfed Bithynia tentaculata, (a) whole snail, (b) body and (c) shell, (d) operculum. at 3(+), 4 (o) and 5 (□) days in the isotope solution.

Each point is the mean of 15.

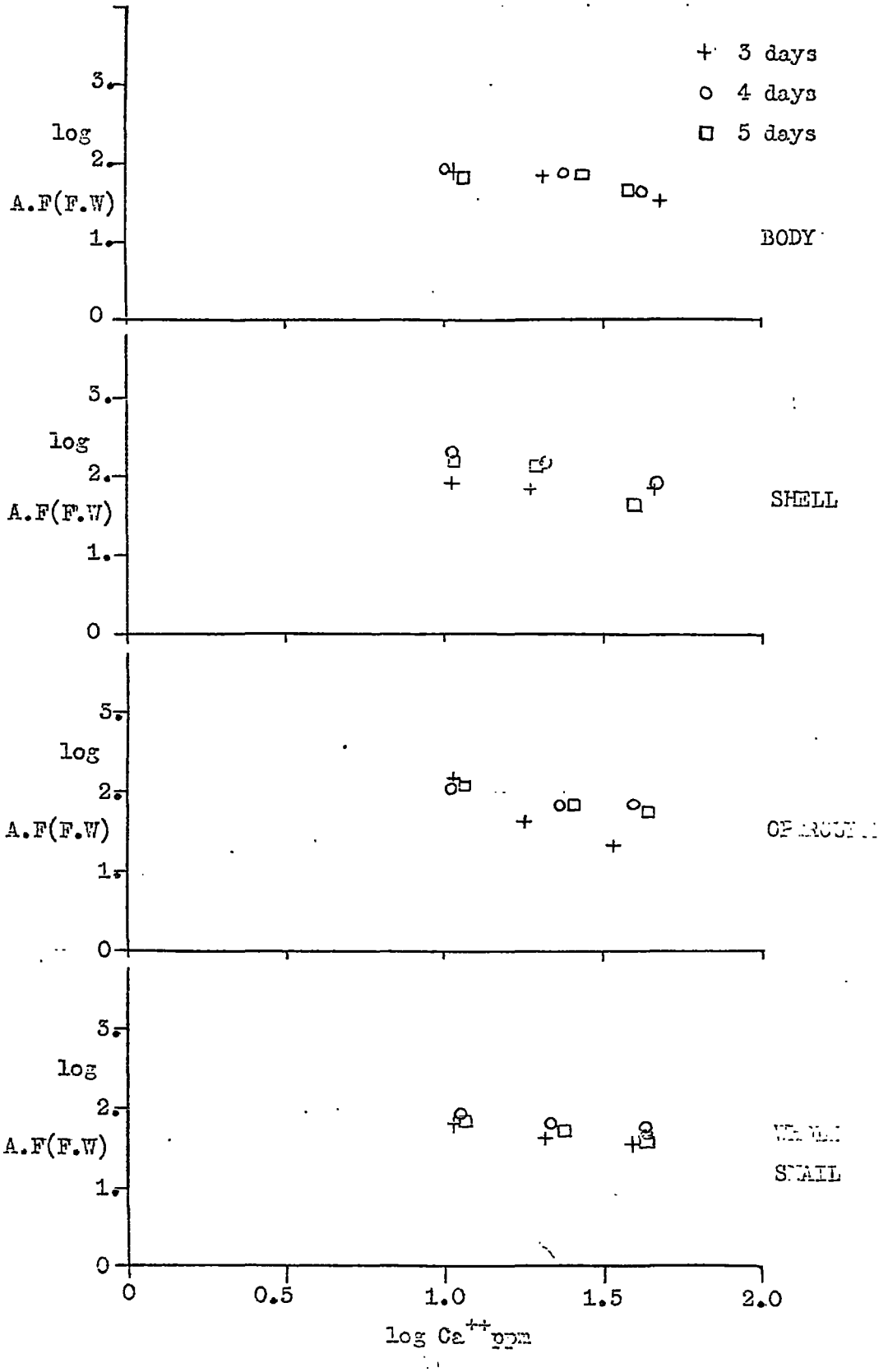

+ 3 days
o 4 days
□ 5 days
log A.F(F.W)
BODY
SHELL
log Ca++ ppm
0
0.5
1.0
1.5
2.0
1
2
3
5

Figure 104.

Days in isotope solution	Whole snail	Body	Shell	Operculum
3	2.3 - 0.2	2.3 - 0.45	2.1 - 0.15	3.55 - 1.45
4	2.5 - 0.05	2.5 - 0.4	2.95- 0.6	2.25 - 0.25
5	2.5 - 0.15	2.4 - 0.45	2.6 - 0.6	2.60 - 0.05

Figure 105

Table of expressions obtained from the log plots in Figure 104.

These figures show that the operculum has the highest initial uptake but that this diminishes rapidly, probably because the operculum only weighs 0.012 g fresh weight, and is mainly composed of chitin with a little calcium. Thus it is quickly saturated with isotope - it presents a considerable surface area to the isotope in the aquatic environment - and after a period of time, it forms an exchange system with the body, which has considerably more calcium in its calcium pools than is found in the operculum.

The shell and body have greater mass than the operculum and it will take longer for them to become saturated with isotope. The shell weighs 0.0600 - 0.1100 g fresh weight according to the length of the snail. The body weighs between 0.07400 - 0.1700 g fresh weight. Besides the mass the chemical composition has a profound effect on the uptake

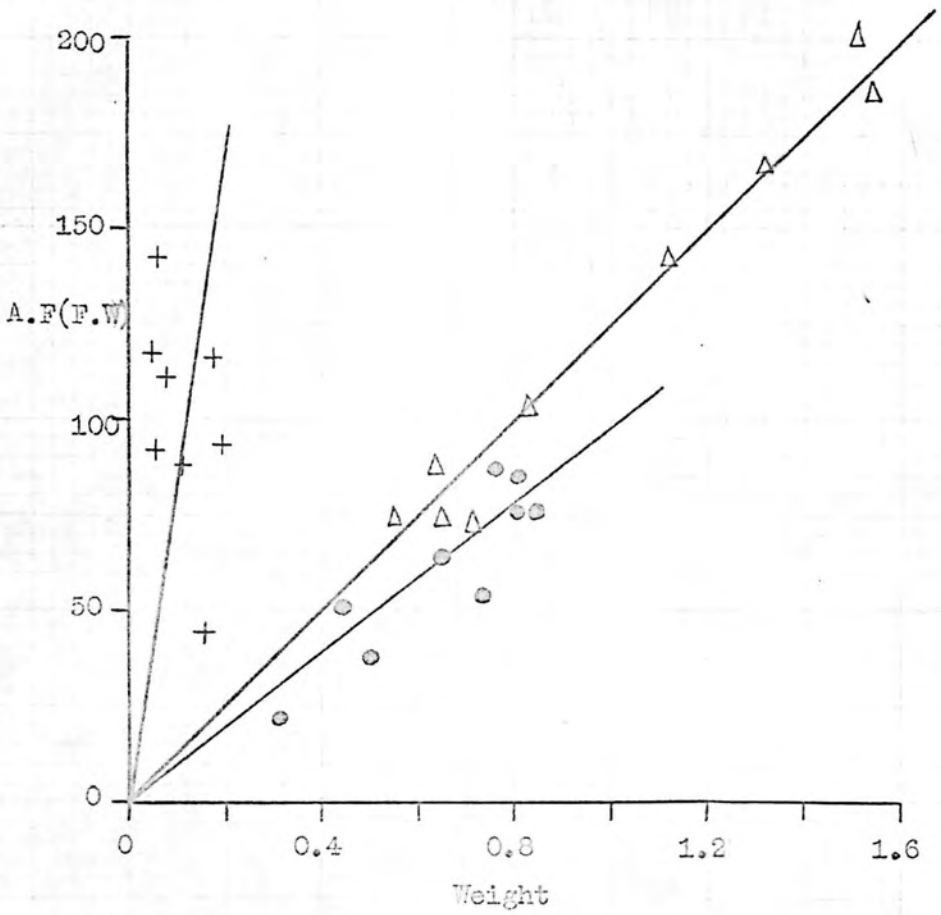


Figure 106 .

Plot: A.F.(F.W) against the fresh weight of (i) the operculum (+), (ii) the shell (Δ) and (iii) the body (⊙). The uptake of 47 Ca by Bithynia tentaculata.

of isotopes, and also the rate of metabolism of the organ or organism concerned.

(iii) The AF(FW)'s of the dissected parts were plotted against the fresh weight in Figure 106.

Since three straight lines can be drawn through the various points, then this plot suggests that there is a weight uptake relationship. Since the weight is a function of the age of the snail, then uptake is dependent on the age of the snail. All these snails are from the same habitat. Probably the calcium concentration of the environment influences the thickness or deposition of the shell, so that, there would be a greater variance in the points if the snails were from a variety of habitats.

Experiment 16

The effect of darkness on the uptake of 90 Sr by unfed Bithynia tentaculata (whole snail)

The uptake of 90 Sr by unfed Bithynia tentaculata was studied for 5 weeks in calcium concentrations varying from 0-80 ppm Ca^{++}. The experiments were non-aerated and were kept in a well ventilated but dark cupboard for the duration of the experiment.

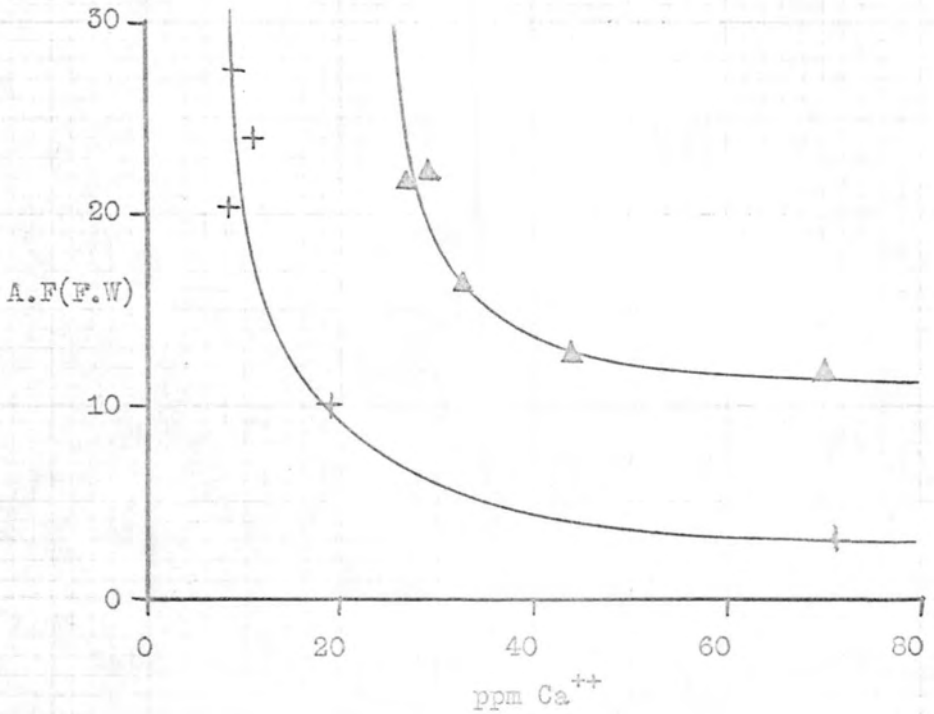
The mean fresh weight of the snails was 0.2601 g and the FW:DW ratio was 3:1.

A control experiment was set up in the light, i.e. normal laboratory conditions.

The results are plotted as AF(FW) against calcium concentration in Figure 107. It can be seen that the uptake in the light is higher than in the dark. This suggests that darkness has a retarding effect on the uptake of 90 Sr by snails.

The temperature of the solutions inside the cupboard was 15° to 16°C.

Figure 107.



Plot: A.F(F.W) against calcium concentration.
The uptake of 90 Sr by Bithynia tentaculata,
in darkness (+) and in the light (Δ).
Each point is the mean of 8 readings.
AF(FW) ^{90}Sr based on three week counts.

Experiment 17

The uptake of 90 Sr by organs of unfed (a) Viviparus viviparus and (b) Limnaea stagnalis

(a) The uptake of 90 Sr by various organs of Viviparus viviparus after 24 hours in the isotope is summarised in Figure 108, at 8.22 ppm Ca^{++}.

(b) The uptake of 90 Sr by various organs of Limnaea stagnalis at 24 and 48 hours in the isotope is summarised in Figure 109, at 2.19 ppm Ca^{++} and 2.74 ppm Ca^{++} .

The results of (a) show a marked distribution in favour of the organs containing calcium . The marked significance being that the operculum has the highest 90 Sr A.F(FW). Organs furthest away from the aquatic environment have low AF(FW) e.g. digestive gland, while organs having areas in direct contact with the surrounding medium have fairly high AF(FW) e.g. mantle and reproductive openings leading to their respective organs. The AF(DW) and activity/g were calculated, since some research workers use these figures. Since the activity of the aquatic environment is continually changing due to ion-exchange with the walls of the glass vessels and the water, the activity /g measurement often,

	FW Fresh Weight g.	DW Dry Weight g.	% of F.W of Snail	cpm Sr^{90}	Activity /g.	Sr^{90} Activity cpm	Act/gm	AF.FW
Operculum	0.0316	0.0156	0.89	278	2887.5	104.2	3297.5	20.7 ①
Head & foot	0.7237	0.01229	20.59	528	733.3	388.3	536.5	3.4 ⑤
Digestive gland	0.3914	0.0780	11.15	288.7	740.2	182.9	467.3	2.9 ⑥
Shell	1.2079	1.0466	34.4	2834.1	2361.7	1361.8	1541.4	9.7 ②
Kidney Mantle & Heart	0.0185	0.0055	0.53	32.6	1811.1	10.6	572.9	3.6 ④
Reprod. System & Sac	0.2436	0.0587	6.94	354.8	1478.3	224	919.5	5.8 ③
Body Fluids	0.8978		25.54	197.8	219.7			4:92
Total - Fresh Weight of Snails. g.	3.5145							

Figure 108.

Uptake of 90 Sr by various organs of Viviparus viviparus. Readings, mean of 5 snails. A.F. determined after 24hrs. immersion in isotope, & 0.22 ppm Ca^{++}. Numbers in circles are rank order of uptake.

Figures for 90Sr are three week figures.

fluctuates and is not a reliable figure to compare results. The AF(DW) is erroneous, since the % water possessed by the various organs varied considerably, e.g. the shell has a low % of water, while the body has a high %. Since the organism lives in an aquatic environment, it is more reasonable to express results in the form of AF(FW), which takes into consideration the activity of the environment, and that this must be quoted in relation to the calcium concentration of the surrounding medium.

(c) The results show that the shell takes up more than any other organ in the body. The digestive gland has a high initial AF(FW) which rapidly diminishes to that of the body. The mantle region presents a great surface area to the isotope solution and would therefore favour the adsorption/absorption of the particulare Y^{+++} ion rather than the ionic Sr^{++} ion. The digestive gland of Limnaea has calcareous granules in it which aid digestion, and the figures suggest that the calcium pool in the body of Limnaea is continually changing - a high AF(FW) denoting a low calcium concentration and vice versa.

	+ 24 hours	+ 48 hours
	90 Sr AF(FW)	90 Sr AF(FW)
Shell	23.9	31
body	6.6	6.5
digestive gland	8.9	5.5
mantle (heart & kidney)	6.5	10.2
Ca^{++} ppm	2.19	2.74
mean wt. of 3 snails (g)	2.831	2.0583

Figure 109.

Uptake of 90 Sr by various organs of Limnaea stagnalis. Readings, mean of 3 snails. AF(FW) 90 Sr based on three weeks figures.

Experiment 18

The uptake of 85 Sr by egg cases of Limnaea pereger

The egg cases were from a breeding aquaria containing L. pereger. They were placed in varying calcium concentrations for a period of 28 days, under aerated conditions. The results are expressed in Figure 110 and the uptake of 85 Sr in Figure 111.

It can be seen that from Figure 110, that there is a decrease in AF(FW) with increasing calcium concentration, particularly above 74.7 ppm Ca^{++}.

The expression from the log plot in Figure 111, is :-

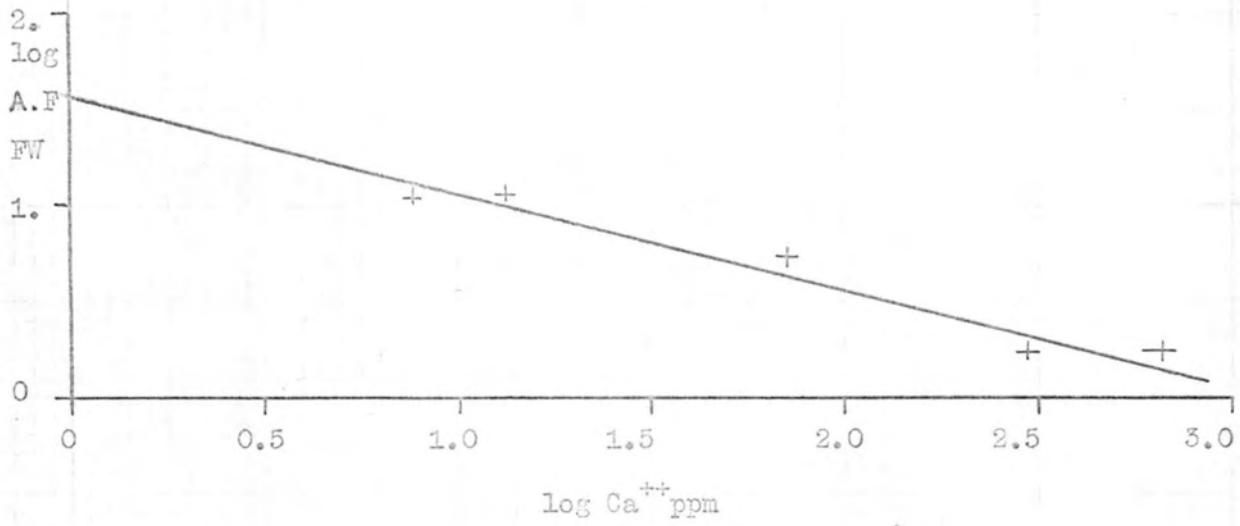
$$\log AF(FW)_{85\ Sr} = 1.6 - 0.5 \log Ca^{++} \text{ ppm.}$$

AF(FW)	11.25	11.35	5.08	1.75	1.82
Calcium level Ca^{++} ppm	7.59	13.9	74.7	303.2	645.8
Fresh weight of egg cases g	0.8819	0.4408	0.5907	0.4576	0.9383

Figure 110.

Table of results, on the uptake of 85 Sr by the egg cases of Limnaea pereger after 28 days, at varying calcium levels.

Figure 111.



Plot: log A.F(FW) against log calcium concentration.
The uptake of 85 Sr by egg cases of Limnaea pereger, after 28 days.
Each point is the mean of 6.

Experiment 19

Injection Experiments

These experiments were designed to find out if the isotope moved from the body to the shell, and whether the food contributed to the isotope uptake in humidity experiments.

(a) The uptake of 85 Sr by Limnaea auricularia, by injection into the foot

The results are summarised in Figure 112. It can be concluded from these experiments that after 2 days, that a substantial amount of the activity of the isotope is found in the shell, i.e. 10 times as much in the shell as in the body. This is significant, since the isotope could only have get to the shell through the body, and not by uptake from the normal surrounding aquatic medium.

Limnaea Auricularia	Fresh Weight g	% of FW of snail	Activity/g (solid count)	Activity (acid count)	Ratio Solid: Acid
snail	0.3942	-	687.5	-	-
shell	0.1182	30	1835.3	823.8	2.2:1
body	0.2176	55.2	17 3.9	86.5	2.0:1
			Ratio of shell:body	Ratio of shell:body	
			10.6:1	9.5:1	

Figure 112.

Uptake of 85 Sr by Limnaea auricularia, by injection method in a atmosphere, at 2 days. Readings are the mean of 6 snails.

Experiment 19b

The uptake of 47 Ca and 47 Sc by Limnaea auricularia, by injection into the foot, in a humid atmosphere

The results are summarised in Figure 113.

These results show that scandium 47 & calcium 47 are taken up rapidly by the shell. The Sc:Ca ratio for the body being a half that for the shell after 2 days.

The activity per gram of 47 Ca in the shell is comparable with the uptake of 85 Sr on page 188. This would suggest that strontium follows the same path as calcium.

L.auricularia	Fresh Weight in g.	% FW of snail	Activity/g Sc	Activity/g Ca	Ratio Sc:Ca
snail	0.1485	-	3866.6	1156	3 : 1
shell	0.0796	57	2375	837.5	2.8:1
body	0.0586	41.8	6440.6	1264.1	5 : 1

Figure 113.

Uptake of 47 Sc and 47 Ca by Limnaea auricularia, by injection method into the foot, after 2 days. Figures are the mean of 4 snails.

Experiment 19 c.

The uptake of 85 Sr by Limnaea auricularia feeding on contaminated lettuce in a humid atmosphere

The isotope was injected into the mid-vein of the lettuce, and the snails were carefully placed on the lettuce with foot resting on the lettuce, after making sure that no isotope had leaked onto the surface of the lettuce.

The results are summarised in Figure 114. It can be seen that over 3 days, that there is an increase in the activity of the snail, shell and body. The uptake by the shell at 2 days being 6 times that of the body, and at 3 days 8 times as much as the body.

The effect of feeding in the transference of isotope from the body to the shell, is less effective than injection, (see page 188).

The results from the injection experiments prove that :-

(i) the isotopes 85 Sr, 47 Ca and 47 Sc are transferred from the body to the shell of Limnaea auricularia, and

(ii) from contaminated food to the body and then to the shell.

The difference in accumulation suggest that the molluscan membranes function as a 'sieve', i.e. there are active sites or pores where similar elements of similar ionic radius, i.e. calcium and strontium, compete for passage through the membrane. The element with the smaller ionic radius or hydrated

Time in days	Organism	Fresh Wt in g.	% of F.W of organism	Activity/g.A (solid count)	Activity/g.B (acid extracted)	Ratio A : B
+1	Lettuce	0.9417	-	2725.5	870.56	3:1
+ 1	Limnaea auricularia	0'1401		274.31	19[illegible]9	1.4:1
+ 2	Snail	0.1425	-	-	635.6	-
	Shell	0.0401	28.14	1350.5	648.3	2.1:1
	Body	0.1020	71.5	210.4	121	1.7:1
	Ratio shell:body			6:1	6:1	
+ 3	Snail	0.1203	-	-	722.8	
	Shell	0.0320	27.2	1131.4	1155.5	1.0:1
	Body	0.0760	63.1	224.3	149.5	1.5:1
	Ratio shell:body			9:1	7.7:1	

Figure 114.

Uptake of ^{32}P by lettuce and snails feeding on contaminated lettuce.
Figures are mean of 8 snails.

ionic radius passes through the membrane quickest and therefore the rate of uptake, activity per gram or A.F. is highest.

Experiment 20

The uptake and release of 89 Sr by dead shells of Limnaea pereger

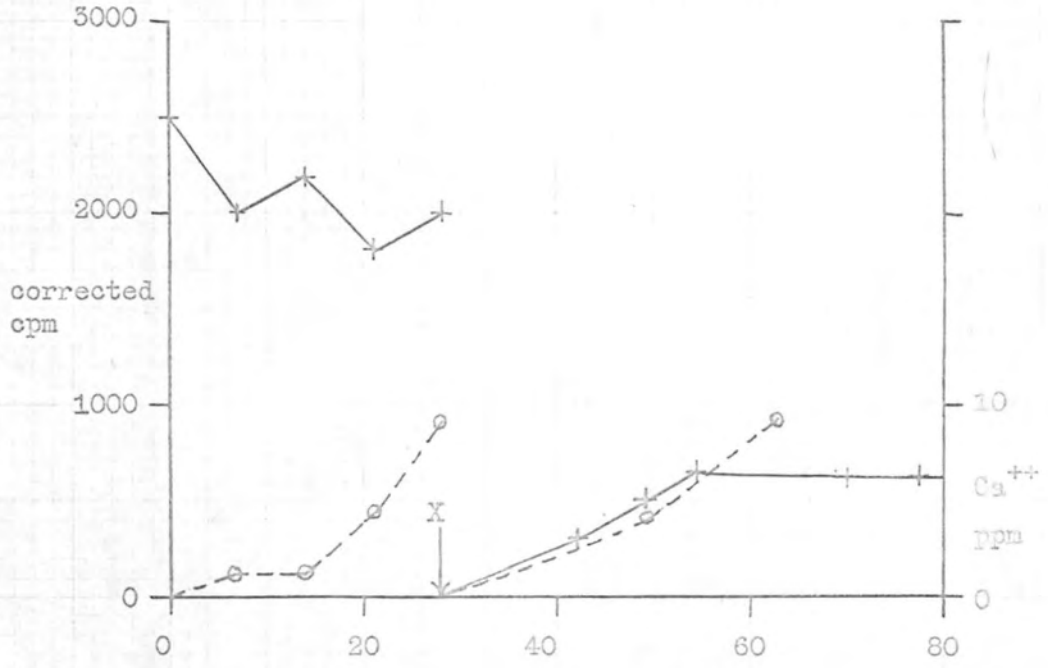
The uptake of 89 Sr by shells of dead Limnaea pereger was studied over a period of 28 days in distilled water. The shells were drained before being placed in fresh distilled water for a further 42 days.

The mean fresh weight of the shells was 0.4330 g. The isotope was allowed to equilibriate with the glass walls of the vessel for 4 days before the snails were put into the isotope solution.

The results were plotted as :-

(i) corrected cpm of liquor against time in Figure 115. It can be observed from this plot that, over 28 days there is a loss of 500 cpm per 10 ml sample. The shells were placed in 100 ml of isotope, so that there is a drop of 5000 cpm after 28 days. After 28 days in fresh distilled water there is leaching of 89 Sr from the shell into the water, in the order of 497 cpm per 10 ml, or 4970 cpm per 100ml. This means that nearly all the isotope that the shell took, is being leached back into the water.

At the same time, there is calcium being lost by the shell into the external environment at the rate of 3.1×10^{-6} g/day during the period of uptake. During the period of release of isotope, there is still calcium leaching from the shell, but at



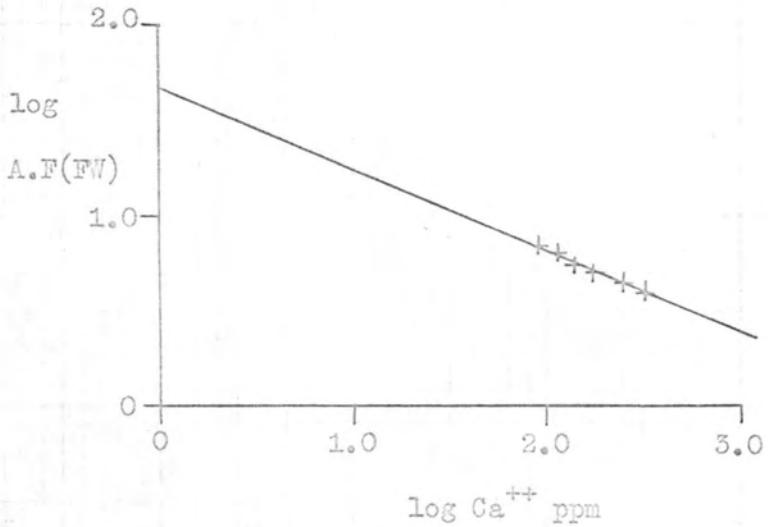
Time, in days

Figure 115.

Plot: corrected cpm of the liquor against Time, in days. The uptake and release of 89 Sr by L.pereger (+). The leaching of calcium is shown by the dotted line joining the 'o' points. 'X' is when the shells are placed in fresh distilled water.

Each point is the mean of 8.

89Sr figures are based on 3 week counts.



Plot: log A.F(FW) against log calcium concentration. The uptake of 89 Sr by dead shells of L.pereger. Each point is the mean of 8. AF(FW) 89Sr based on three week counts.

Figure 116.

a rate of 2.7×10^{-6} g/day.

(ii) a log plot of log AF(FW) of the shell was plotted against the log of the calcium concentration of the surrounding medium in Figure 117.

The expression from the plot in Figure 116, is :-

$$\log AF(FW)_{^{89}Sr} = 1.65 - 0.4 \log Ca^{++} \text{ ppm}$$

At the end of the experiment, there is still isotope present in the shell, despite leaching of calcium from the shell for 42 days. The AF(FW) at the end of 77 days was 4.54 compared with the AF(DW) of 21.47.

These results suggest that 89 Sr is incorporated in two forms in the shell of L.pereger, i.e. in a combined state with other compounds present in the shell which does not leach, and in an uncombined state which does leach.

These experiments prove that dead shells play an important part in the exchange system in an aquatic environment for isotopes as well as for normal calcium enchange. It is important to realise that in these experiments on dead shells, that there are no dynamic processes involving energy relations characteristic of living organism, e.g. ATP production. The shells behave as a physical ion-exchange system.

Experiment 21

The uptake of 90 Sr by dead shells of Limnaea stagnalis

The uptake of 90 Sr by shells of L.stagnalis was studied over 126 days in varying calcium concentrations, shown in Figure 117.

AF (FW)	343.1	335.9	297.2	148.6
Initial Calcium level Ca^{++} ppm	0	5	10	66
Final Calcium level	21.5	31.7	37.9	126.6
Difference in Ca^{++} ppm	21.5	26.7	27.9	60
Mean rate of loss of calcium	17×10^{-6} g/day	21.2×10^{-6}	22.14×10^{-6}	47.61×10^{-6}

Figure 117.

Table of results, of the uptake of 90 Sr by shells of L.stagnalis, at 126 days. AF(FW)90Sr based on three week counts.

The results were plotted as a log plot of AF(FW), in Figure 118, against calcium concentration. The expression from the plot is:-

$$\log AF(FW)_{90\,Sr} = 3.25 - 0.45 \log Ca^{++} \text{ ppm (at 126 days).}$$

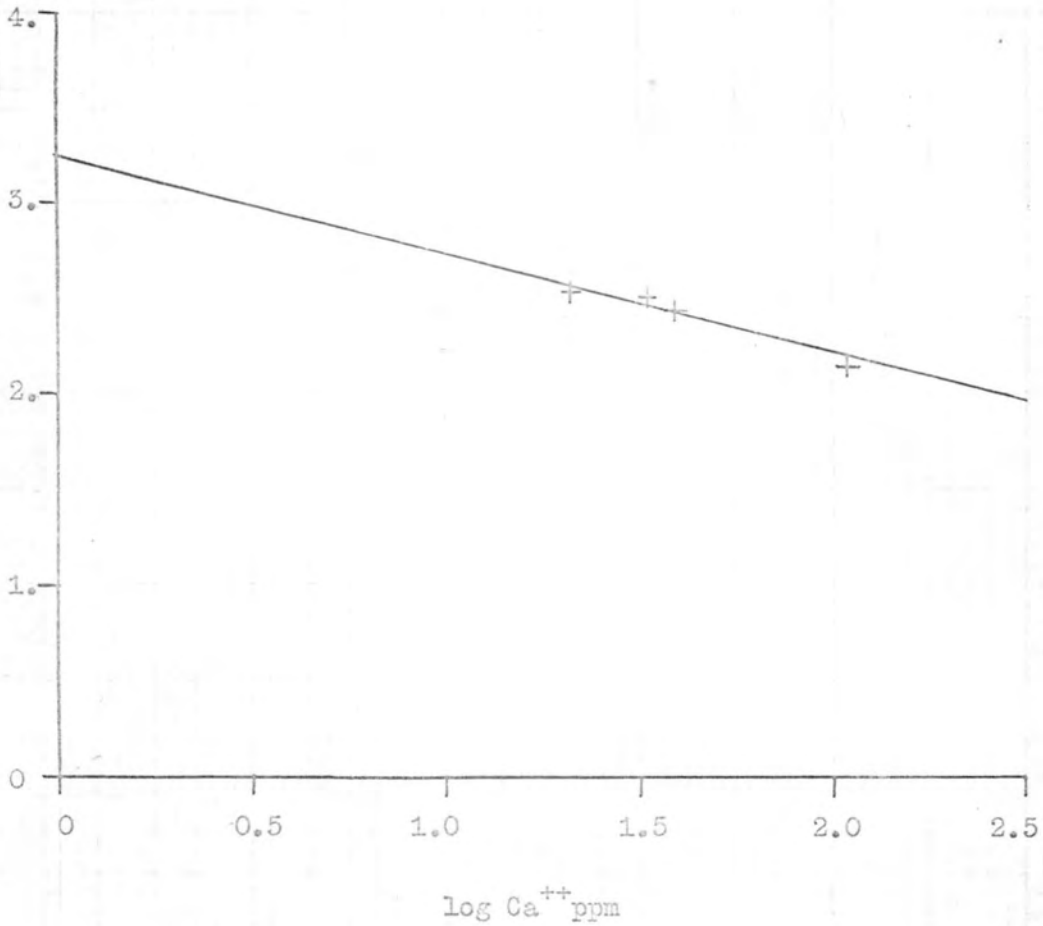


Figure 118.

Plot: log A.F(FW) against log calcium concentration. The uptake of 90 Sr by shells of Limnaea stagnalis, at 126 days. AF(FW)90Sr based on 3week counts. Each point is the mean of 5.

The slope of uptake is similar to that obtained for L. pereger, see page 197 , which suggests that the shells have the same chemical composition.

The shells were left in for a long period of time to see if the accumulation of the isotope would be greater than in Experiment 20 . This is so, since the maximum from the log plot has increased from 1.65 to 3.25. This could be accounted for by the increased size and thickness of the shell of L.stagnalis. This is shown, when one compares the rate of leaching of calcium in these two species, L. stagnalis leaches at a rate of 17 x 10^{-6} g Ca^{++} / day at 21.5 ppm Ca^{++} compared with L.pereger's rate of 3.1 x 10^{-6} g Ca^{++} / day below 10 ppm Ca^{++}.

D I S C U S S I O N

Introduction

The concentration of strontium in natural waters has been found by Lohammer,(1938), Thompson and Chow, (1955a, 1955b, 1963), Odum, (1955, 1957a, 1957b), Ichikawa, (1960, 1961, 1962), and Templeton and Brown, (1964), to be related to the amount of calcium present. The relationship is expressed as a Sr:Ca ratio, in units of number of atoms of each element present. The Sr:Ca ratio for sea water is 9.0:1000 compared with 2.45:1000 for fresh waters. Thus the amount of strontium present in fresh water is small. Sidgwick, (1962) states that calcium is the commonest metal in the earth's crust (3.63%) after aluminium (8.8%), and iron (5.1%). Strontium forms 0.042% of the earth's crust, i.e. 1% of that of calcium. Robertson, (1941) quotes values of 0.002 g/l of calcium for soft water (fresh water), 0.064 g/l of calcium for hard water (fresh water) and 0.403 g/l for sea water, of waters in and around Great Britain. Odum, (1957b) quotes values of 0.1 to 0.5 mg/l of strontium for a variety of fresh waters in North America.

The relationship between calcium and strontium in natural waters, is illustrated by analyses of the shells of a wide variety of fresh water, marine and terrestrial molluscs. The shell of molluscs is mainly calcium carbonate, which can exist

in three crystalline states - calcite, aragonite and vaterite - in a protein framework of conchiolin. It has been found that the aragonite crystal type shell contains more strontium than the other types. (Fox & Ramage, 1931; Trueman, 1944; Thompson and Chow, 1955a; 1955b; Wilbur, 1964). Furthermore, the aragonite type shell is characteristic of the marine molluscs, and the calcitic type shell of the fresh water molluscs. There are, however, intermediate type crystals - calcite-aragonite mixtures, which overlap both salt and fresh waters. In marine waters, the aberrant group of radiolarians, the Acantharia, have a predominance of celestite ($SrSO_4$) in their skeletons (Odum, 1951).

The calcitic shell of Limnaea stagnalis has 99.6% of $CaCO_3$ in the inorganic structures of the shell, and 3.04% of conchiolin in the organic structures (Taylor, 1894). Although certain ions influence the various crystalline states of $CaCO_3$, e.g. the presence of strontium influences the formation of aragonite crystals, it does not mean that it will be included within the crystal itself (Kitano, 1962, Kitano et al, 1962). The snails studied in this research work are mainly calcitic types. The reason for strontium being present in the water in trace quantities may be that a high concentration of it is toxic to fresh water organisms, (Thompson and Chow, 1955b).

The importance of radiostrontium is today extremely great

due to its presence as a fission product from atomic explosions and nuclear reactions. It enters the earth through (i) the atmosphere and (ii) rainfall which cause pollution of rivers, canals etc., and the ground. Among the 300 isotopes produced by nuclear fission, 90 Sr - 90 Y forms 5.9% of the fission yield and 89 Sr forms 4.8%, so that radiostrontium forms altogether 10.7% of the fission yield, (Bowen, 1959). Besides the fission products, neutron rich elements are formed, e.g. 45 Ca. The fission products are normally trace elements, while the neutron rich elements are essential for both plants and animals. Although radiostrontium may constitute the most serious direct hazard to man, because of its long half life - 28 years for 90 Sr - and its accumulation in skeletal structures, the effects of fission products on shorter lived biota, e.g. Mollusca, may also ultimately be of importance to man because of the food chains involved. Townsley (1963) and Townsley, Reid, Ego (1959) have determined that 10 times as much water passes through the epithelia of fresh water fish compared with marine fish. Therefore, the quantity of radionuclides in fresh water presents a greater contamination than the same quantity in sea water in fish. This is probably true for fresh water molluscs, especially those which have a gill system for respiration.

Discussion of experimental conditions

(a) Temperature

The temperature of the experiments was kept between 18-22°C. Imai (1937) and Wilbur (1963) found that although there was rapid growth of the shell of Limnaea pereger between 22-28°C, the final shell size was greatest at 18°C. Wilbur & Owen (1964) state that feeding ceases below a given temperature for molluscan species, when the digestive tract becomes empty and growth is slow or absent. Bacq & Alexander (1961) showed that the temperature affects the radiosensitivity of biological systems, i.e. that warm blooded organisms are more sensitive to radiation than cold blooded organisms.

(b) Hydrogen Ion concentration (pH)

The pH in the experiments with allthe species of snails was found to be between pH 6.5-6.8. Boycott (1936) states that there are virtually no fresh water snails found in waters below pH 6 or less in Great Britain, using B.D.H. Universal Indicator. Limnaea pereger will die at pH 5.6.

The pH of the isotope solution must be kept slightly acid, i.e. pH 6.5-6.8, since at higher pH values the isotope is adsorbed onto the surfaces of the glass vessels. (Wilford et al, 1962; Pickering,1964). If the alkali solution is acidified,

then the activity of the isotope returns to the solution from the walls of the glass vessels.

(c) Darkness

The experiment on the effect of darkness on the uptake of radiostrontium by unfed Bithynia tentaculata, (page 180), indicates that darkness retards uptake. The temperature in the cupboard is lower than in the laboratory, so that the lowered temperature could have reduced the solubility product of the calcium carbonate, which would limit ion-exchange and would result in lower uptake.

Goreau (1959) found that darkness decreased the uptake of 45 Ca by corals, and stated that there was an increase in the calcification in the light.

Brown (1963) reports that constant darkness reduces the level of calcification in the tissues in fish. Felitchenko (1962) reports that higher light intensities increases the metabolism and therefore increases accumulation of 90 Sr by fish.

Collander (1930, 1941) studied Chara and other higher plants and found that the uptake of radiostrontium was stimulated by light, with the new alkaline earth elements being concentrated in the growing cells.

(d) concentration of the isotope in the surrounding medium

The question to what extent the accumulation of radioisotopes by organisms is related to the concentration of the isotope in the surrounding medium is a matter of controversy in the literature. Hiyama & Ichikawa (1957), have reported that marine fish in 10 uc 90 Sr/litre accumulate 10 times as much isotope as fish in 1 uc 90 Sr/litre. Prosser (1945) however, states that the uptake of 90 Sr and 137 Ba by goldfish is independent of the dose of 90 Sr. Townsley (1959,1963) found that in the euryhaline teleost -Tilipia mossambica- the uptake of 89 Sr in the tissues did not correspond to the multiplies of 1, 10 and 100 of the dose in the medium. Rosenthal (1956,1957a, 1957b, 1958, 1961) states, that in the fish, Lebistes, the rate of incorporation of 45 Ca and 90 Sr appears to be a function of the concentration of the nuclide in fresh water, and that the rate of uptake of 45 Ca, 90 Sr and 35 S is logarithmically related to the activity in the medium.

The concentration of isotope in the present work was approximately 0.25 uc per 250 ml of medium. Since the concentration of the isotope in the medium in the experiments was similar, then the results are comparable.

If the activity of the snails increased in proportion to the activity of the medium, then the Accumulation Factor (AF) is independent of the activity of the medium. (Kevern,1964; Polikarpov, 1958).

The concentration of the isotope in the solution, also

determines the dose rate i.e. the absorbed dose of ionising radiation. This is the energy imparted to matter by ionising particles per unit mass of irradiated material at the place of interest, and termed the rad (Taylor, 1957).

Organisms vary in their sensitivity to radiation, much more widely than the cells of which they are constituted. In general, the more complex the organism, the more vulnerable it is. It takes about 10,000- 20,000 r to kill a snail (Lindop,1961) and 600-700 r to kill man (Bacq & Alexander, 1961). These figures, are the dose of radiation which kills 50% of the organisms within 30 days, and is known as the $L.D._{50/30}$. The dose which molluscs can withstand is very high, and because of this property, are suitable organisms to study the uptake of radioactive isotopes.

(e) Radioisotopes used in the experiments

(i) β -emitting isotopes

Both 89 Sr and 90 Sr are β-emitting isotopes (page 17). 89 Sr cannot be obtained as a pure sample, since in the production of it from 235 U , some 90 Sr is formed as an impurity. (Radiochemical Manual, Part II, 1963).

The half life of 90 Sr is longer than 89 Sr, and over a period of time, the percentage of 90 Sr in the total activity of the mixture of 89 Sr and 90 Sr, increases. The amount of 90 Sr impurity in the 89 Sr can be determined by a self absorption plot and the amount expressed as a percentage. The 90 Sr present as an impurity decays by β-emission to 90 Yttrium, which has a half life of 64.2 hours, and this decays to stable 90 Zirconium (Lucas, Pickering, 1958). Thus the initial count on the acid extraction of 89 Sr is a mixture of 89 Sr, 90 Sr and 90 Y , as shown in Figure 118a. The efficiency of the β-counters will be greater for the higher energy beta radiations i.e. 90 Y (2.25 MeV) and 89 Sr (1.44 MeV).

As time proceeds, the activity of the 89 Sr decreases due to decay to 89 Zr, and the 90 Sr present decays to 90 Y. After 3 weeks any Y 90 originally present in the snail coming either from the 90 Sr in the snail or from 90 Y in the water will have decayed, and will be replaced by an amount of 90 Y in radioactive equilibrium with the 90 Sr in the snail. The final count i.e. at three weeks, represents th 89 Sr and 90 Sr in the snail at the time of separation from the water.

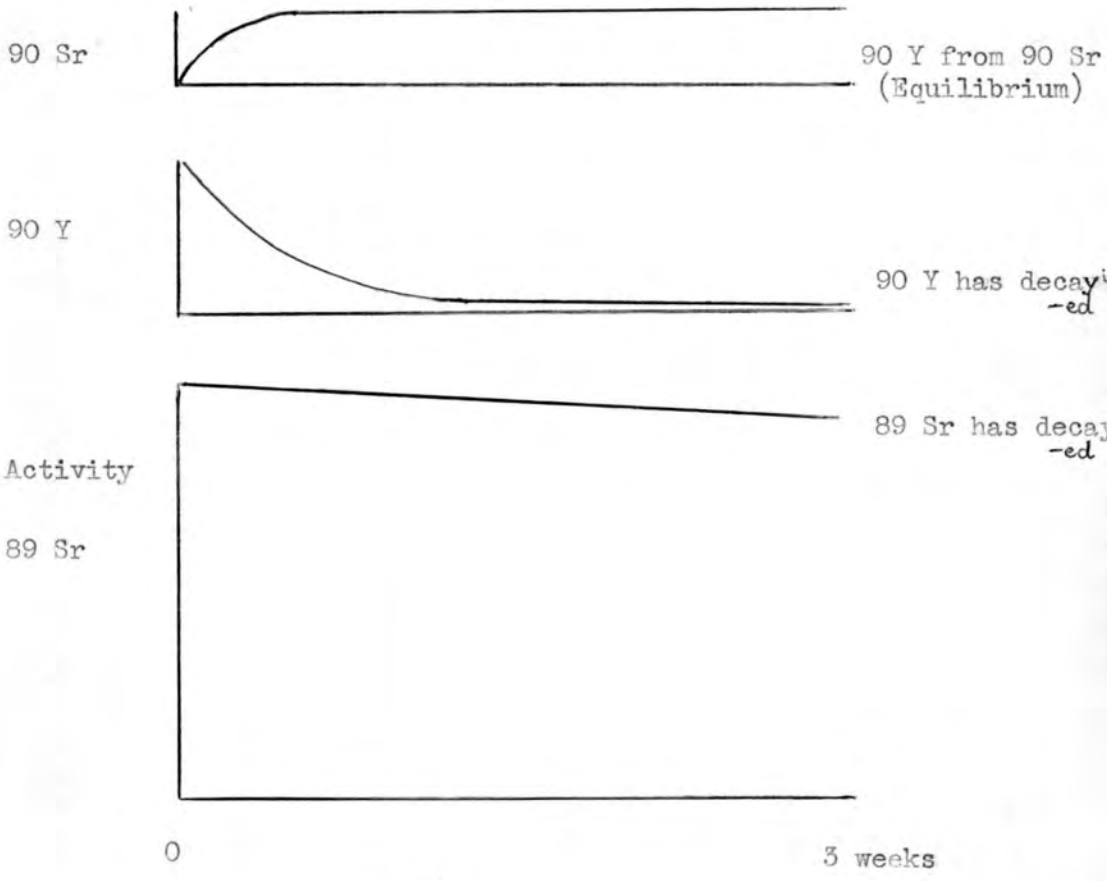


Figure 118a.

The composition of a solution of 89 Sr at time of extraction is compared with that at the end of three weeks. Plot of the Activity of the isotope against Time.

The value which one attaches to the initial count i.e. the yttrium strontium count, which need not be in radioactive equilibrium, must be treated carefully. The initial counts were plotted to see if there was any correlation with the strontium counts i.e. the final counts at three weeks. The initial counts are always higher than the final counts, and this does suggest that the yttrium is being taken up from the water and does not all originate from the decay of 90 Sr within the snail.

In 90 Sr solutions, the initial count is again a yttrium-strontium count, which must be treated carefully. The final count i.e. at three weeks, represents the strontium count. From Experiments 1 & 2, the results for 89 Sr and 90 Sr are similar, and suggest that 89 Sr behaves in a similar way to 90 Sr.

(ii) γ-emitting isotopes

85 Sr is produced in the following way,

$$^{84}Sr + ^{1}n = ^{85}Sr^{*} + \gamma$$

85 Sr is a γ-emitting isotope (page 23) and decays to a very short lived daughter isotope, 85m Rubidium.

$$^{85}Sr \xrightarrow[t/2 = 51 \text{ days}]{} {}^{85m}Rb \xrightarrow[t/2 = 0.9 \text{usecs}]{} {}^{85}Rb$$

metastable form of Rubidium

Since 85m Rubidium is such a short lived isotope, the acid extraction of a snail may be counted after 7.2 usecs i.e. 8 half-lives. The corrected count rate is therefore a strontium count. Since 85 Sr is a γ-emitting isotope, it may be counted in living snails. Uptake may be measured in the same snail at intervals of time and this cannot be done with 89 Sr and 90 Sr, since the snails have to be killed before counts can be made.

The difficulties due to geometrical shapes of the snails were taken into account and minimised (see pages 69-71).

The results obtained with 85 Sr are in good agreement with those of 89 Sr and 90 Sr. The difference in mass between the strontium isotopes does not seem to affect their uptake by snails.

47 Ca is also a γ-emitting isotope (page 23), and as in the case of 85 Sr, one has the advantage of being able to measure the activity in a snail at intervals of time. However, 47 Ca has a much shorter half-life than 85 Sr, and its daughter isotope 47 Sc has a half-life similar to its parent (page 25). Thus one has the complication of separating the calcium count from the scandium count (pages 30- 48).

(f) Surfacing of the snails

During the experiments it was observed that the molluscs, especially Limnaea truncatula, came to the surface of the medium, and in some cases climbed out of the medium. This was immediately noted and the snail pushed back into the medium by means of a plastic rod. Ophel and Judd (1962) found that there was no significant difference between the concentration of 90 Sr by partially or totally immersed goldfish under the same experimental conditions.

Discussion of the experimental results

Templeton and Brown (1964) and Lucas and Pickering (1962) in their papers on the uptake of radiostrontium by trout and fresh water algae respectively, state that many investigators have reported data on the degree of accumulation by biota, but few have related their results to the calcium and the strontium of the medium or the food supply.

Odum (1957) states that, "the Sr/Ca ratio is characteristic of a species of a taxonomic group only when grown with a characteristic Sr/Ca ratio in the external medium or food supply." He also states that plants had a similar ratio to that found in the medium around the cells or the roots.

The experiments were in the main with snails from the same habitat and that the uptake of radiostrontium and radiocalcium

was related to the calcium concentration in the medium. The stable strontium was not determined owing to insufficient apparatus.
(i) Provided that all the strontium in the fresh water molluscs is exchangeable, then it is possible to measure the amount by allowing it to exchange with radioactive strontium added to the surrounding medium until an equilibrium A.F is reached.

Timofeev-Resovskij (1958) states that equilibrium is reached in 14 days, with 90 Sr uptake by Limnaea stagnalis; but there is no mention of the calcium concentration in the medium.

It can be seen from the uptake of radiostrontium by Limnaea pereger (pages 78 & 94), that equilibrium takes longer to attain at low calcium concentrations. Above 20 ppm Ca^{++} the AF(FW) is related to the time taken to reach equilibrium.
(ii) The plot of the slope, i.e. of the log regression of AF(FW) against calcium concentration, against the calcium concentration of the environment is shown in Figure 119. The straight line that is drawn through the points shows that the previous history of the snail, in this case, the environment where the snail was bred, is extremely important in determining the amount of radioisotope that it takes up.
(iii) The results show that the uptake of radiostrontium and radiocalcium, as measured by the AF(FW) of Limnaea and Bithynia

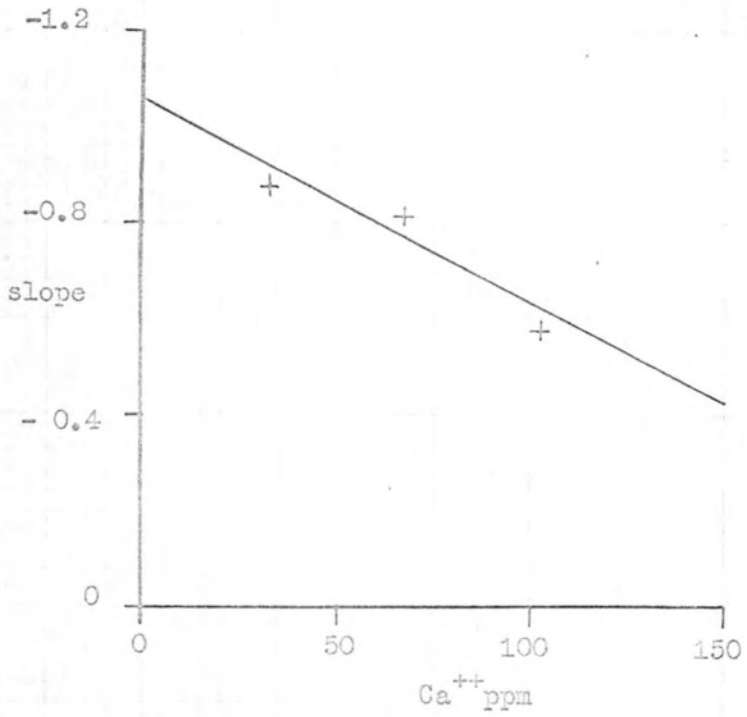


Figure 119.

Plot: slope of the log plot- A.F(FV) against calcium concentration; against the calcium concentration of the natural habitat of Limnaea auricularia, i.e. the Shropshire Union Canal, at Chester. Each reading is the mean of 150 snails.

species, is higher at low calcium levels than at high calcium levels. This dependence of uptake on the concentration of calcium in the medium has been observed by investigators in different aquatic organisms; Prosser (1945), Kirpichikov (1956), Rosenthal (1956, 1957a, 1957b, 1958, 1960), Oguri (1961), Ophel (1963) and Brown & Templeton (1964) in fresh water fish; Boroughs, Townsley & Hiatt (1956, 1957) and Schiffman (1959) in marine fish; Fretter (1953), Odum (1957), Ichikawa (1961), Machline & Templeton (1963) in marine molluscs; Schoffeniels (1951), Asano (1956), Bevelander (1952), Gong (1957), Timofeev-Resovskij (1958, 1959), Van der Borght (1962, 1963a, 1963b), Nelson (1963), Van der Borght & S.van Puymbroeck (1964) in fresh water molluscs; Gilera (1960), Lucas & Pickering (1962), Jayson & Pickering (1962) in fresh water plants, and Glaser (1962) in plankton.

(iv) In the semi-log plots of AF(FW) against calcium concentrations in the medium for both unfed and fed, the uptake of radiostrontium and radiocalcium is calcium dependent, particularly below 56 ppm Ca^{++}, dependent on the mollusc species and whether it is fed or unfed. The change in slope in the semi-log plots would seem to suggest a lower limit to the concentration of calcium which the species can withstand in its natural environment.

Prosser et al (1945) noticed such a change, at 36 ppm Ca^{++}, occurred in the uptake of radiostrontium by goldfish, and Van der Borght (1963) noted 30 mg/l in L.stagnalis (unfed).

(v) Uptake by whole snails

In the log plots of AF(FW) against the calcium concentration of the medium, two significant results have been noted.

(a) That in Limnaea species, there is a 'two-line' plot for the unfed snail, which changes to a 'one-line' plot for the fed snail. This implies that the food which the snails eat is having an effect on the rate and accumulation of radiostrontium and radiocalcium. It was noticed that lettuce was more effective than Elodea, in bringing about this change in slope in Limnaea pereger (see pages 97 and 104). This is probably due to the differential availability of calcium from the plant foods, (Hugot & Causeret 1957).

The change in slope can be interpreted in Figure 120. The experiment with Elodea on page 104, shows that there is a change occuring, since it takes longer for the Elodea to have an effect, than the lettuce, (see page 110). The effect of lettuce with radiocalcium and radiostrontium is the same, since the difference in the slope of the regression plots for the fed

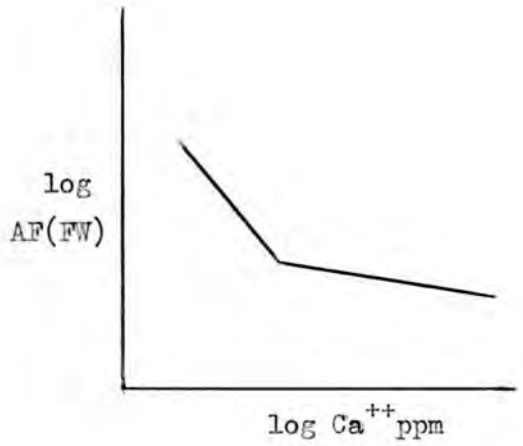


Figure 120a.

Uptake of radiostrontium or radiocalcium by unfed Limnaea species.

(Not drawn to scale)

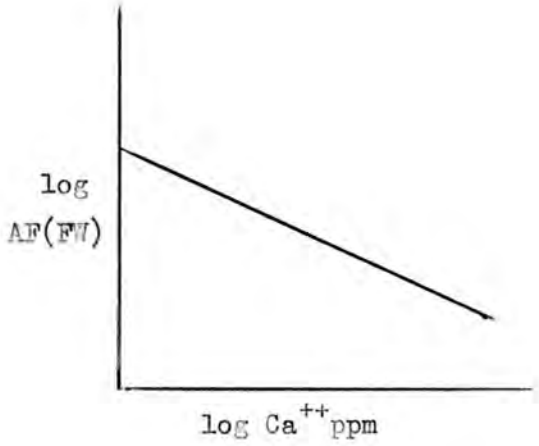


Figure 120b.

Uptake of radiostrontium or radiocalcium by fed Limnaea species.

(Not drawn to scale)

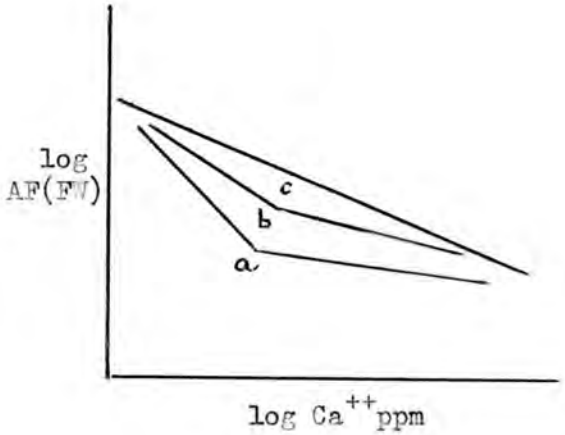


Figure 120c.

Plot of log AF(FW) against calcium concentration, to show how the uptake changes from the unfed state to the fed state in Limnaea species. (a) unfed state, (b) started to feed, effect on the two-line slope starting to show and (c) the fed state.

(Not drawn to scale)

and unfed snails is approximately the same, (Figure 121).

Slope	85 Sr	47 Ca
Fed snails	-0.85	-1.05
Unfed snails	-0.40	-0.65
Difference	-0.45	-0.40

Figure 121.

Slopes of the regression lines for fed and unfed Limnaea species for radiostrontium and radiocalcium.

The change from the 'two-line' plot to the 'one-line' plot is also seen on page 142 , on the effect of feeding on Limnaea auricularia. The food source being watercress.

The plant material contains calcium which is leached into the water, and is taken from the water by the snail as well as abstracting it from the ingested food. This additional source of calcium will generally lower the AF(FW) at low calcium levels, but at the same time there will be increased uptake by the body and the shell of the mollusc. Another source of calcium is from the digestive gland, as increasing food uptake causes secretion of calcium from this gland. (Gran 1960).

Wagge (1951) states that for calcium to be deposited the food supply must contain proteins and calcium, and that calcium is not laid down in the absence of the other. This protein is found in the food together with the calcium, and could stabilise the leaching of calcium from the snail.

(b) The rate and accumulation of radiostrontium and radiocalcium by whole snails of Bithynia tentaculata in the unfed and fed state is a 'one-line' plot. There is no evidence of another line in the log plot of AF(FW) against the calcium concentration of the medium.

The uptake of radioisotopes by Bithynia is much less than that of Limnaea species at low calcium concentrations in the medium. This was observed by Timofeev-Resovsky (1957, 1960) and Glaser (1962), but there was no mention of the calcium concentration of the medium. This fact seems to indicate some fundamental physiological difference between the two genera.

The chitinous operculum present in Bithynia, but not in Limnaea, closes when there are adverse environmental changes, i.e. low calcium concentrations in the medium, thus preventing access of radioisotopes to the body, (Potts & Parry 1964).

Scheer (1957) states that Bithynia species has a low protein concentration in its body fluids, and therefore unlike Limnaea, does not need much protein in its diet for the

deposition of calcium in the shell.

Robertson (1963) states that Bithynia can withstand lower salinities, i.e. 6°/oo, than Limnaea pereger 10-11°/oo. Limnaea is probably iso-osmotic with the medium at these low salinities, which could account for the high AF(FW) found at these low calcium levels, since there would be rapid exchange of isotope for the calcium present in the snail. An indication of the osmotic effect is shown by the decrease in the amount of body fluids in Limnaea pereger at high calcium levels in the medium, which is shown in Figure 122.

The increased uptake in Limnaea species is probably associated with the richly vascularised gill-less mantle cavity, and the adsorbed isotope is quickly distributed via the thin walled blood vessels to the rest of the body.

It is probable that the snail requires some time for the internal environment of the snail to become adjusted to the change in the environment. It is likely that the snail adjusted to an environment of low calcium levels and low osmotic pressure would show a more rapid rate of accumulation of radioisotope during the early stages of an experiment. After the initial period there is a constant accumulation of isotope from the medium until equilibrium is reached.

The high concentration of calcium (more than 50 times the quantity of strontium in an environment) would give dilution

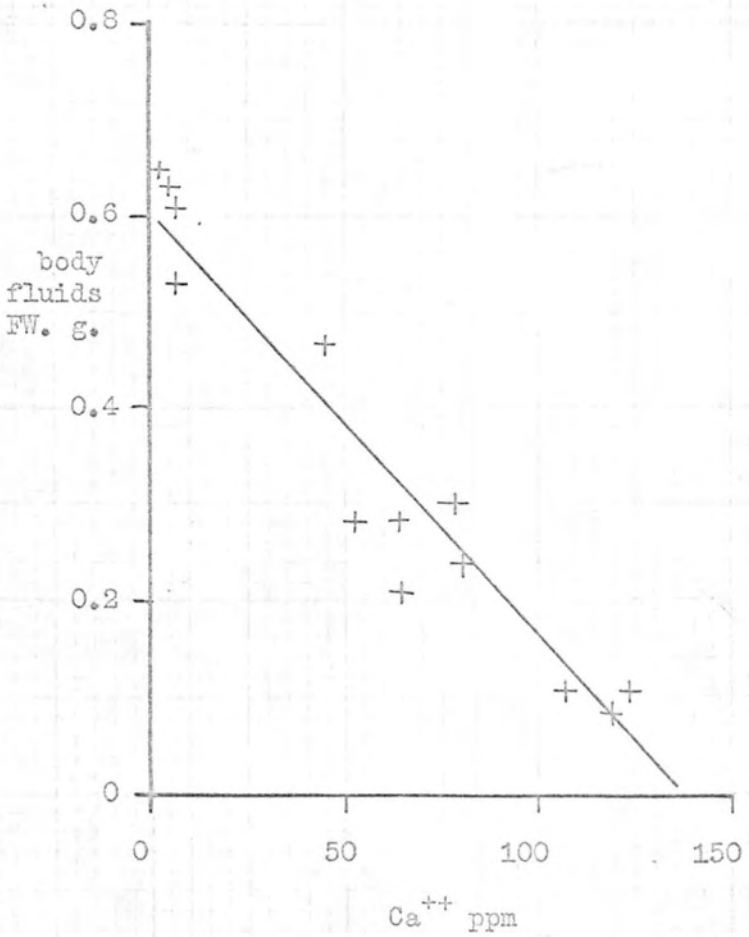


Figure 122.

Plot: fresh weight of body fluids in g. against the calcium concentration of the surrounding environment. Only the body fluids of Limnaea peregeг are plotted.

Each point is the mean of 10 snails.

effects on strontium and calcium absorption. Townsley (1963) reports that fish do not discriminate against strontium when the strontium ion and calcium ion concentrations are low.

It seems therefore, that Bithynia tentaculata exercises greater control than Limnaea species, at low calcium levels in the medium.

(vi) The distribution of radiostrontium and radiocalcium in the various structures of Limnaea species, Bithynia tentaculata and Viviparus viviparus

(a) Distribution in the shell and body

Both radiostrontium and radiocalcium were found to be accumulated to a greater extent in the shell of these molluscs than in the body. This was found to be so in Limnaea stagnalis by Van der Borght (1962, 1963), Timofeev-Resovsky (1957, 1958), Odum (1958) and in Anodonta by Schoffeniels (1951). The operculum in Bithynia and Viviparus, which are both operculates, had an initial higher AF(FW) than the shell, which decreased with time to below the value of the body.

The rate and accumulation of these isotopes in the shell and body are higher in fed than unfed molluscs, particularly if lettuce is used as the source of food.

The availability of calcium in the medium plays an important part in the uptake of these isotopes, since in the

unfed Limnaea species, uptake below 20 ppm Ca^{++} is more calcium dependent than above this level.

The difference in the uptake between radiostrontium and radiocalcium for the shell, and body, is that there is discrimination against strontium by the fed snail. This is shown in Figures 123a and 1.23b. These results show the same trend as Brown & Templeton's work on the uptake of radiostrontium and radiocalcium on the bone and muscle of trout (1964).

The uptake of dead shells of Limnaea pereger gives a regression line of:-

$$\log AF(FW)_{^{89}Sr} = 1.65 - 0.4 \log Ca^{++} \text{ ppm}$$

while the unfed shell is,

$$\log AF(FW)_{^{89}Sr} = 1.8 - 0.4 \log Ca^{++} \text{ ppm (above } 22.4 \text{ ppm).}$$

Since there is activity left in the dead shells after a long period of time, this does suggest that the strontium is in two forms in the shell, i.e. an ionisable form which can be readily exchanged with cations in the environment, and secondly, a non-diffusible fraction which is probably complexed with the protein framework of the shell, (MacDonald, 1951; Rosenthal, 1958; Eisenberg & Gordon, 1961; Williams & Pickering, 1961; Dolphin & Eve, 1963; Ophel, 1963; Pearson, 1965).

The higher maximum suggests that uptake by living cells is a dynamic process rather than a purely passive physical process.

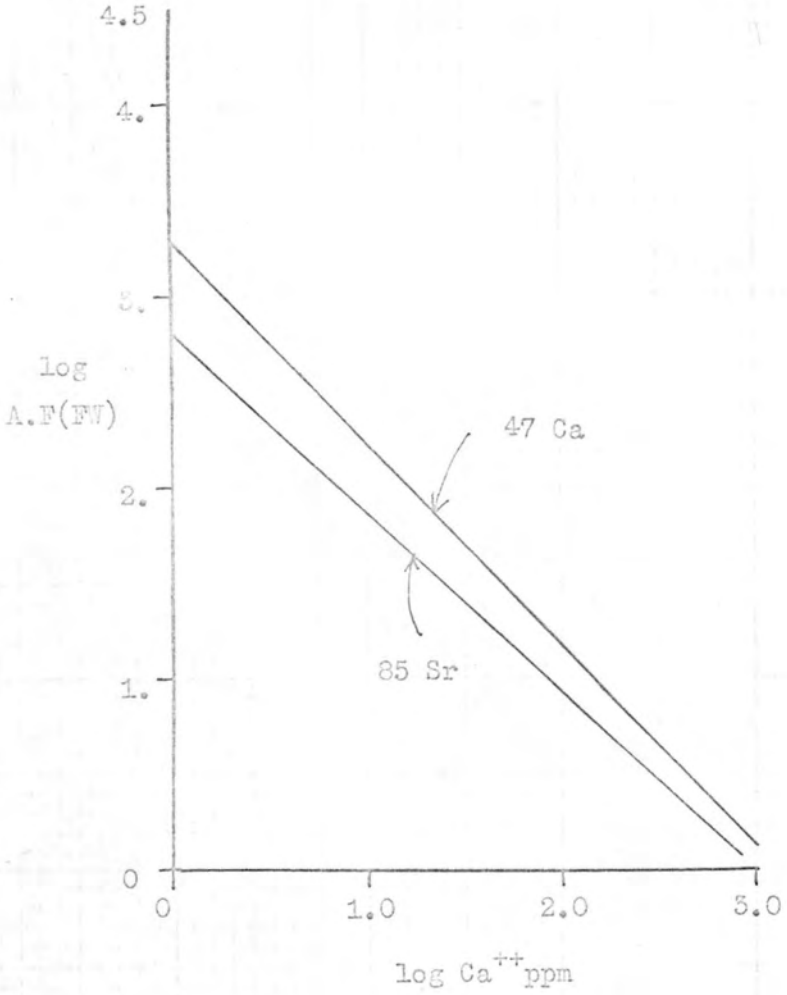


Figure 123 a.

Plot: log A.F(FW) against log calcium concentration in the medium. The uptake of 47 Ca and 85 Sr by fed Limnaea auricularia - BODY, at 7 days feeding on lettuce.

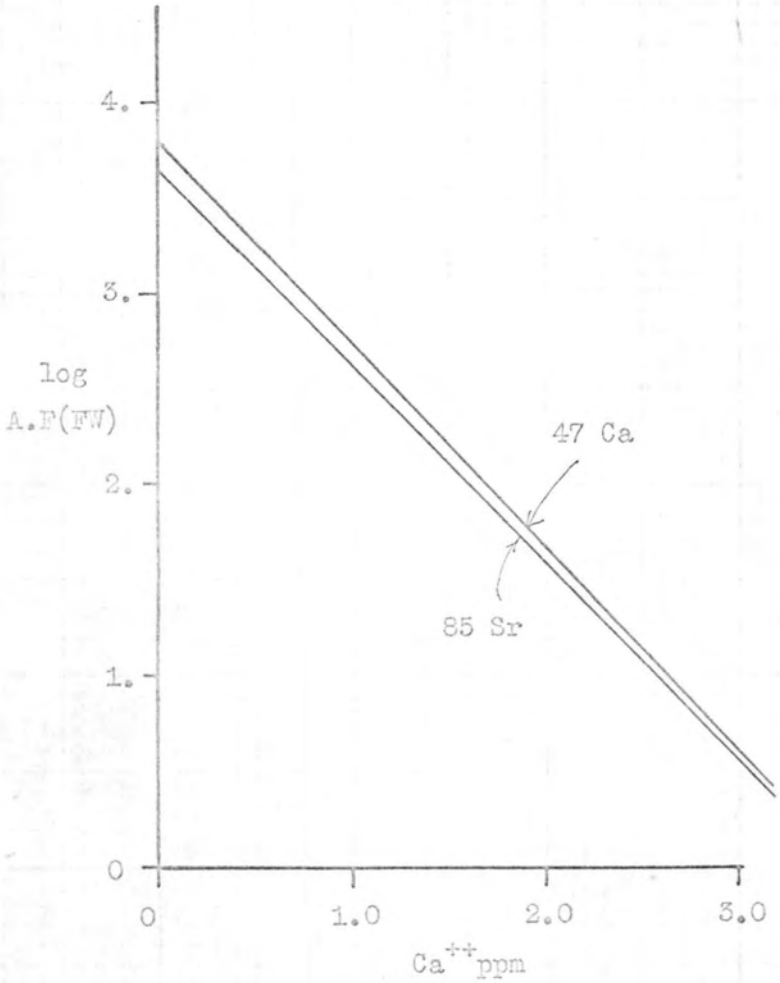


Figure 123 b.

Plot: lof A.F(FW) against log calcium concentration in the medium. The uptake of 47 Ca and 85 Sr by fed Limnaea auricularia - SHELL, at 7 days feeding on lettuce.

Gong (1957), Wilbur & Jodrey (1952, 1955), Horiguchi (1960), and Wilbur (1964) state that the transference of ions to the shell is determined by the concentration of ions in the extra-pallial fluid. Van der Borght (1962, 1963), Bevelander (1952), and Rao & Goldberg (1954) found that the newly formed border of the shell in Limnaea stagnalis and Anodonta had the highest AF(FW) of the shell. Johnson et al (1962) observed the same feature in Australorbis (Planorbidae).

The regression line of fed Limnaea pereger is,

$$\log AF(FW)_{85\ Sr} = 3.45 - 0.85 \log Ca^{++} \text{ ppm},$$

at 14 days feeding on lettuce. The significant point here is, that the rate of uptake has increased by 100%, and that the maximum has increased by approximately 100%. This stresses the importance of food in the diet of fresh water organisms, and its effect on the contamination of those organisms which feed on it. These results imply, that while a large amount of radio-isotope is adsorbed by the shell directly from the water, an appreciable amount of radioisotope is transferred to the shell from the body via the extra-pallial fluid.

The higher maximum of the regression line for dead shells of Limnaea stagnalis is associated with the greater amount of calcium available for exchange. The rate of leaching of calcium from the shell in L.stagnalis is 17×10^{-6} g Ca^{++} /day at 21.5ppm Ca^{++}, which is considerably more than the rate of 3.1×10^{-6} g Ca^{++}/

day below 10 ppm Ca^{++}. This depletion of calcium from the shell would account for the increased amount of radiostrontium in the shell.

Schiffman (1959, 1960, 1961) compared the rate of uptake of 90 Sr by trout, from food in a gelatine capsule which was inserted into the stomach of the fish and from the surrounding medium. He found that food was the main route of uptake rather than the water in which the fish swam. When he changed the food source, to that of the natural food and did not insert the contaminated food into the stomach, he found that the water was 10 times as important as a source of radiostrontium than the natural food. Gros (1953) noted that the deposition of 90 Sr in rats obtained by drinking was more than from contaminated lettuce. Fretter (1953) states that marine molluscs and polycheates obtain 90 Sr directly from the water as well as the food. Boroughs et al (1957) state that unfed marine fish can take up calcium (45 Ca) directly from sea water and do not need a dietary source for this element. Townsley (1963) found that 45 Ca and 90 Sr were directly absorbed by marine fish from the surrounding water. Toniyama et al (1956) found that goldfish feeding on worms absorbed 45 Ca directly from the water, 50 times as much as from the diet.

Injection methods into the foot of Limnaea auricularia prove that radiostrontium and radiocalcium are transferred from

the body to the shell. The activity/g of 85 Sr in the shell after 2 days, is approximately equal to that for 47 Ca, but the 47 Ca in the body is approximately 15 times the amount of 85 Sr present. This means that the body discriminates against strontium.

When Limnaea auricularia were fed on contaminated lettuce in humidity experiments, the amount of 85 Sr in the shell in 2 days is less than the injected snail, but more activity is recorded for the body. This means that the uptake of 47 Ca through the gut is more efficient than the uptake of 85 Sr, or that the gut discriminates against strontium. These results which were not in an aqueous medium, show that the presence of such a medium, increases the accumulation of isotopes in the shell. Rao & Goldberg (1954) found that the mucus sheet on the gills of Mytilus californancus provided an absorbing surface for radiostrontium which is in a constant state of renewal.

The high uptake by the shell in anaqueous medium is significant since the shell in Limnaea species only forms 14-22% of the fresh weight of the snail, and contains 33-45% liquid, whilst the body forms 60-70% of the fresh weight of the snail, of which 80% is liquid.

(b) Distribution of radiostrontium in the tissues of Limnaea stagnalis and Viviparus viviparus

The results as measured by the AF(FW) show that the greatest accumulation of 90 Sr is in the skeletal structures. The rank order for the uptake of 90 Sr at 24 hrs. by Viviparus viviparus is:-

1. operculum
2. shell
3. Reproductive system
4. mantle, kidney and heart
5. head and foot
6. digestive gland

This is in agreement with Machline and Templeton's (1962) statement, that tissues in contact with the medium are more heavily contaminated than the internal tissues. The reproductive system, mantle, kidney and heart have openings into the mantle cavity, where the water is changed frequently due to the uptake of oxygen by the gills.

The rank order for the uptake of 90Sr by Limnaea stagnalis is:-

@ 24 hours	@ 48 hours
1. shell	1. shell
2. digestive gland	2. mantle
3. mantle	3. body
4. body	4. digestive gland

This implies that there are calcium pools in the body, and in this case supports the statement on page 216, that the digestive gland has a supply of calcium which can be utilised by the body at low calcium levels in reducing the amount of radiostrontium uptake by increasing the level of calcium in the medium.

The distribution of 90 Sr by L.stagnalis is similar to that obtained by Brown & Templeton (1964) on the uptake of 90 Sr and 45 Ca by trout:-

1. skeleton
2. gills
3. integument
4. muscle
5. viscera

The gills would correspond with the mantle region, and the digestive gland with the viscera. Further work is needed on the detailed uptake of radioisotopes over long periods of time and at different calcium concentrations in the medium, which would help to elucidate the pathways of the radioisotopes ...

as well as the equilibria for the respective tissues.

(c) The uptake of 85 Sr by egg cases of Limnaea pereger

The uptake of 85 Sr by the egg cases of Limnaea pereger is similar to the regression line obtained with unfed snails.

$\log AF(FW)_{^{85}Sr} = 1.6 - 0.5 \log Ca^{++}$ ppm (at 28 days, egg cases)

$\log AF(FW)_{^{85}Sr} = 1.8 - 0.4 \log Ca^{++}$ ppm (unfed snails)

These results indicate that the uptake by the egg cases behaves like the unfed rather than the fed snails which had a slope of $-0.85 \log Ca^{++}$ ppm. Raven (1946; 1956, 1958, 1964) have observed that the layer which carries the cortical field in the Limnaea egg, consists of a complex colloid composed of proteins phosphatides, and probably ribonucleic acid. Calcium ions, adsorbed onto this complex, are important for maintaining its stability and physiological properties. An exchange of calcium ions in the membrane with other cations, e.g. strontium, in the medium may occur up to a certain concentration without altering the physiological properties of the membrane. Additional substitution may cause differential permeability of the membrane.

Jura & George (1958) found that in the jelly enclosing the pulmonate egg, calcium as well as protein, mucopolysaccharides, and fats is present. Although the jelly is essentially

protective, the calcium can be utilised by the young snails. The presence of protein as well as calcium in the jelly of the pulmonate egg could account for the 'one-line' plot by the egg cases of Limnaea peregra.

It seems therefore that the calcium concentration in the medium is important in controlling the uptake of radio-strontium in the egg cases as well as the developing and adult snails.

Fresh water molluscs as indicators of contamination of natural waters by radioisotopes

Templeton and Brown (1964) statethat, 'Studies on the metabolism of calcium and strontium have indicated that although strontium follows calcium closely, there is a degree of differentiation. In mammals, for instance, strontium is discriminated against calcium in absorption from the gut, whilst in the kidney, calcium is resorbed preferentially to strontium.'

Bauer (1955, 1962) states that, 'to date no one has been able to demonstrate any difference between calcium and strontium in the rate of skeletal clearance of these elements from the circulation.' Bauer found that 90 Sr was retained in young rats to a lesser extent that 45 Ca owing to preferential excretion (or failure of reabsorption) of the former element by the kidneys as well as by the gut.

Experimental evidence had proved that the uptake of radio-strontium

and radiocalcium by Limnaea species, Bithynia and Viviparus species follow a similar path, but that there is discrimination against strontium in the fed snails.

Van der Borght (1962) noticed that 90 Sr was accumulated in a similar manner to 45 Ca by Limnaea stagnalis, and dis-criminated against strontium in favour of calcium. Below 10 ppm Ca^{++} there was more strontium taken up by the unfed snail than calcium. This is also true with Limnaea pereger, Limnaea truncatula, Limnaea auricularia at low calcium levels, in the unfed snails. The discrimination in favour of strontium varies from 0-56 ppm Ca^{++}, dependent on the molluscan species. Above this level, the unfed snails discriminate against strontium, in favour of calcium. Since the snails discriminate against strontium above 56 ppm Ca^{++}, then in their natural habitat (66 ppm Ca^{++} to 120 ppm Ca^{++}), they will reduce the radiation hazard to themselves.

The mean Sr/Ca ratio for fresh water gastropods in fresh waters in the U.S.A. is 0.75 compared with a ratio of 2.34 for sea water (Odum 1958). Thus strontium may be taken up as a calcium replacement more in fresh water than in sea water.

The life cycle of fresh water pulmonates is mainly annual, (Comfort, 1957; Yonge, 1958; Russell Hunter, 1961, 1963; Berrie, 1963; Fenwick, 1964); and therefore Limnaea species can

only be regarded as short term indicators of radioactive pollution in fresh waters.

The main reason that they can be regarded as biological indicators, is that they accumulate radiostrontium and radiocalcium to a level higher than that of the medium. The radiostrontium is still retained in the shell after the snail has died. Limnaea pereger is an ideal choice as a short term biological indicator as it almost has a world wide distribution (Russel Hunter, 1963), and it can tolerate a diversity of fresh water habitats.

Van der Borght (1962, 1963, 1964) has suggested that Limnaea stagnalis can be used as a biological indicator, but it is not as widespread as L.pereger.

Nelson (1963) has suggested that the fresh water clam could be used as an indicator, since it is relatively immobile on river bottoms, and as the shell is increased in thickness over the years, the radiostrontium is concentrated in the nacreous layer (Thompson & Chow 1955). Also, the Sr:Ca ratio is independent of the age of the mollusc. The clam also discriminates against strontium relative to calcium in shell deposition.

Ravera and Merline (1960) have suggested that Viviparus ater could be used as a biological indicator since it is the largest prosobranch found in the Italian lakes, and is widely distributed

in Italy.

There is a strong need for a short term biological indicator, such as, Limnaea pereger or Bithynia tentaculata, which will measure the radioactive deposition over a limited period of time.

Theory of the uptake of radiostrontium and radiocalcium by molluscs

The structure of the cell membrane and the theories of cellular permeability are summarised by Wassermann (1963). The effect of secretion of H^+ and Ca^{++} by the membranes of organisms in altering the permeability of cells has been noted by several investigators in both plant and animal cells. (Meyer, 1914; Solomon, 1961; Pantin, 1931; Ellis, 1947; Kinizula & Koketsu, 1962; Abood, 1963; Jura & George, 1958; Steffensen, 1958; Ussing, 1960; Thomason & Schofield, 1961; Jayson & Pickering, 1962; Jayson et al, 1964: Raven, 1963).

The effect seems to be that the calcium affects the viscosity of the lipo-protein layer, and at low calcium levels, the permeability of the cell is increased. This would account for the increased uptake of radiostrontium at low calcium levels. Martonosi & Feretos (1964) state that the presence of strontium has no effect on the transport of calcium. Abood (1963) also states that polyvalent cations have the same effect, but

monovalent cations work the other way. This could account for the uptake of Yttrium 90 and Scandium 47. Vickery (1960) states that scandium does not exist in aqueous solution, but because of its amphoteric nature readily forms complexes of considerable stability. This could account for the fact that the uptake of scandium 47 by Limnaea auricularia is similar in both fed and unfed snails. Yttrium (Vickery 1960) is normally found in nature as a replacement of calcium, e.g. in apatite crystal. This could explain that the uptake of yttrium 90 is similar to the uptake of radiostrontium, since it replaces calcium in the snail. However, yttrium tends to be in the particulate form (see Figure 124), and therefore the uptake of yttrium would follow the path of strontium in being adsorbed onto the shell rather than be taken up by the cellular membranes. The other important point, is that yttrium 90 is the daughter product of strontium 90, and that probably the activity measured is yttrium from strontium decay. Palumbo (1963) states that the rare earths to which yttrium and scandium belong, usually occur in the insoluble form and are not readily available for uptake by living systems.

Yttrium	% Ionic	% Colloidal	% Particulate
sea water	o.4	2.2	98
distilled water	1.6	1.3	86

Figure 124

Physical characteristics of yttrium and scandium (Vickery 1960)

* * *

Yttrium occurs in the earth's crust in small amounts e.g. 31g/ton and scandium 5g/ton . (Goldschmidt, 1954).

Palumbo (1963) found that yttrium and scandium were not readily available for uptake by living organisms. He found that phytoplankton concentrated these elements 10 times as much as fish, and concluded that this selectivity was due to the external surfaces of the organisms. He found that the primary site of deposition was the skeleton in both mollusc and fish. The insolubility of these particulate isotopes at the physiological pH of the digestive tract made their absorption difficult.

It was observed in Limnaea truncatula that the initial counts of the faeces are higher than the three week counts (90 Sr), which implies that the gut discriminates against yttrium in favour of strontium. Also, the count rate decreases

with increasing calcium concentration in the medium as shown in Figure 125. (Boroughs, Townsley, Hiatt, 1956).

Experimental Jars	1	2	3	4	5
90Y-90Sr cpm.	1261.6	821	740	720	581.8
90Sr cpm.	710	450	345	310	138
Calcium conc. Ca^{++} ppm.	13.6	13.6	13.6	18.9	66

Figure 125.

Table of uptake 90Y-90Sr and 90Sr by faeces of Limnaea truncatula at 14 days, at different calcium concentrations. Figures are the mean of 6. 90Y-90Sr are initial counts; 90Sr are 3 week counts.

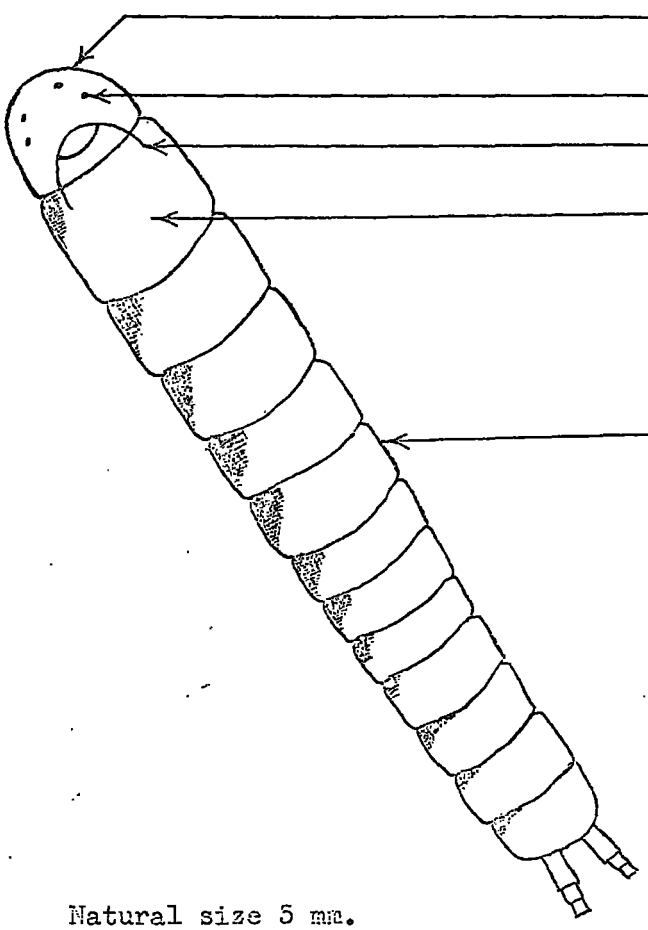
The physical properties of calcium and strontium suggest that they follow the same pathway through biological membranes, since the passage through the pores in the membranes is determined by the hydrated atom or molecule rather than the ionic radius of the element. (Harris, 1960). Although the unhydrated ionic radius of calcium is 1.06 Å and strontium is 1.27 Å , (Vickery, 1960; Sidgwick, 1961), they have the same mobility of 59.8 which suggests the same hydrated ionic radius. Nightingale (1959) states that the hydrated ionic radius of

calcium and strontium is the same ,i.e. 4.12 Å .

A new habitat of chironomid larvae

Whilst investigating the uptake of 90 Sr by Limnaea pereger, it became necessary to dissect out the different parts under a binocular microscope. It was observed that 20 out of 30 dissected snails contained stages of a developing chironomid larva (15 of Stage I and 5 of Stage II), as shown in Figures 126a and 126b respectively. The chironomid larvae were found in the mantle cavity of the snails, which were obtained from the Shropshire Union Canal, Chester in July 1963. The calcium concentration of the canal was 66 ppm Ca^{++}, and the pH of 7.4 .

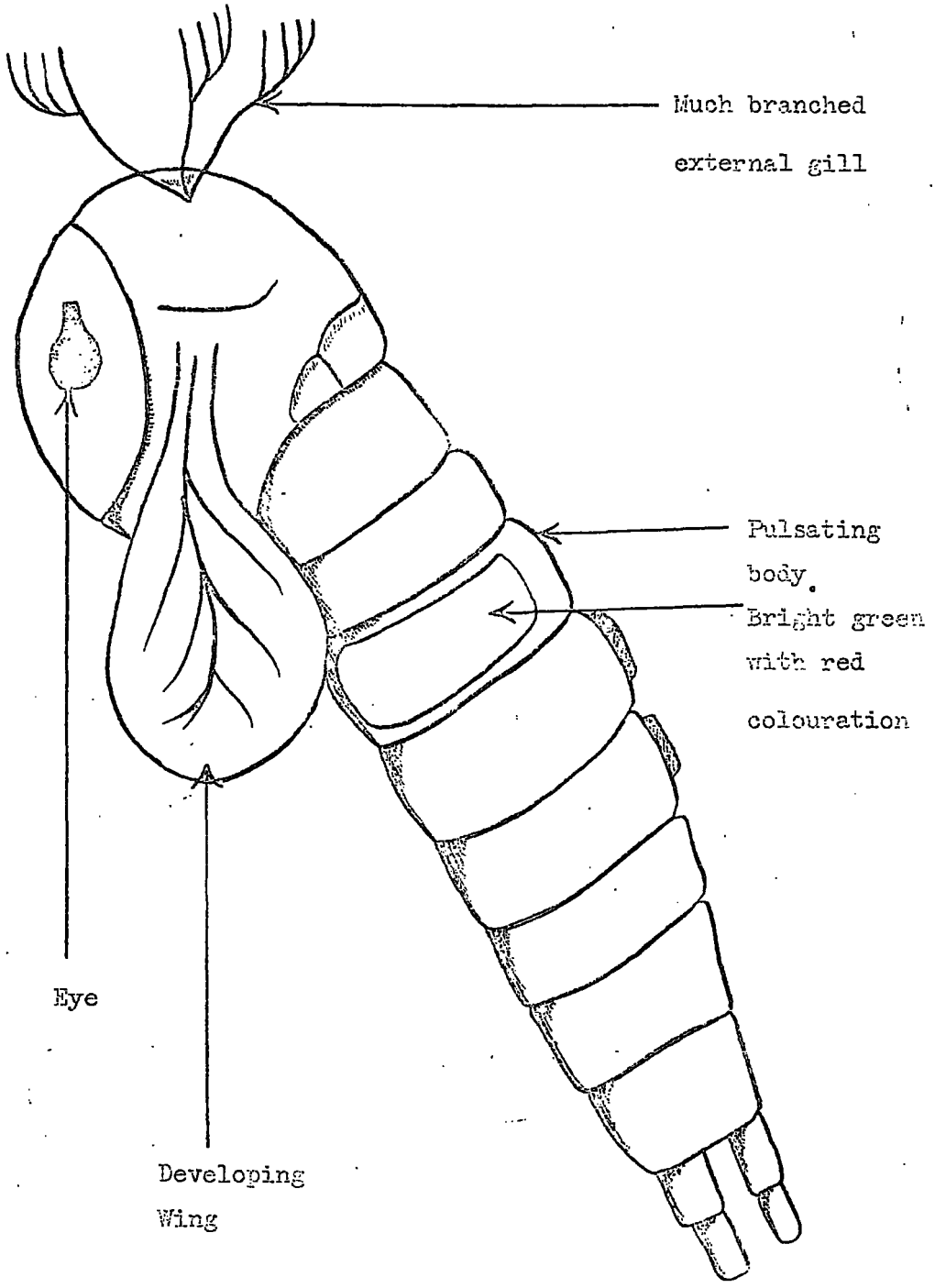
This habitat is not listed in reference books on the subject.(Taylor, 1894; Ellis, 1926; Boycott, 1934; 1936a.b; Caullery, 1952; Macan, 1959; Step, 1960; Morton, 1963). The activity of the chironomid larvae was extracted as for the snail, and is shown in Figure 127. The results in Figure 127 show that (i) the development of chironomid larvae is favoured by high calcium levels, (ii) that radioactive strontium is concentrated by the larvae, (iii) that accumulation of this isotope is calcium dependent and (iv) there is a possibility of further concentration



Natural size 5 mm.

Stage I.

Figure 126 a.



Natural size 6.8 mm

Stage II

Figure 126 b.

of this isotope in the food chains, since the chironomid larvae forms the diet of many animals.

It is highly probable that the chironomid larvae are not parasitic on the snail, but only gain shelter from living in the mantle cavity.

Isotope Count	A.F.	No. of larvae	A.F.	No. of larvae
Y-Sr	15	10	8.7	10
90 Sr	9	10	5	10
Difference in isotope counts	6		2.7	
Calcium ion conc.	8ppm		66ppm	
Larval stages		10 of Stage 1		5 of Stage I 5 of Stage II

Figure 127.

Notes: (i) Mean fresh weight of the chironomid larvae 0.1939 g

(ii) AF's at 5 days from commencement of experiment.

(iii) Y-Sr, AF, based on initial counts, &
90Sr, AF, based on 3 week counts.

Further Research

Many problems relating to the uptake of 47 Ca and radiostrontium (85 Sr, 89 Sr and 90 Sr) by molluscs remain to be solved. Some of the most interesting are :- (i) influence of increasing the activity of the surrounding water, (ii) effect of pH, (iii) effect of temperature, (iv) effect of oxygen concentrations, since all biological systems are radiosensitive in the presence of oxygen (Bacq & Alexander 1961), (v) the uptake by molluscs from different habitats, to obtain some ecological distribution, (vi) the influence of other cations, e.g. magnesium, (vii) salinity and (viii) the influence of irradiation, by means of X-rays, gamma rays or neutrons on the uptake of the above mentioned isotopes.

Summary

1. In fresh waters, the uptake of radiostrontium and radiocalcium by Limnaea species, Bithynia tentaculata and Viviparus viviparus, was influenced by the calcium concentration of the medium.

The effect of the following environmental conditions upon these snails was investigated: (i) temperature, (ii) hydrogen ion concentration, (iii) the effect of darkness, (iv) the concentration of isotope in the surrounding medium and (v) the surfacing of the snails.

2. The influence of the previous habitat had a profound effect on the uptake of radiostrontium by Limnaea pereger. The lower the calcium concentration of the habitat, the greater the uptake of radiostrontium and radiocalcium by the snails.

3. The uptake of radiostrontium and radiocalcium by unfed Limnaea species showed that the snails discriminated against calcium in favour of strontium below 56 ppm Ca^{++}, according to the species and the state of feeding; and in favour of calcium above this level.

4. In the fed Limnaea species, the snails discriminated against strontium in favour of calcium at all calcium concentrations in the surrounding medium. This proves that the food - lettuce, Elodea, watercress - plays an important part in the uptake of

radiostrontium and radiocalcium in Limnaea species.

5. In Bithynia tentaculata, the results for the fed and unfed, show that it discriminates against strontium in favour of calcium. The results also indicate that Bithynia has a greater control of the permeability of its tissues than Limnaea species at low calcium concentrations.

6. The uptake of radiostrontium and radiocalcium by the shell is greater than that of the body in all the molluscs tested, and there is greater accumulation of these isotopes by the skeletal tissues than by the visceral tissues of the body.

7. The accumulation of radiostrontium follows a similar path to that of radiocalcium, but the snails tend to discriminate against strontium. This would reduce the internal radiation dose to the snails.

8. It is suggested that Limnaea pereger is a good short term biological indicator for radioactive contamination of fresh waters, because of its widespread distribution and ability to tolerate a diversity of fresh water habitats. Bithynia tentaculata, although able to control its membrane permeability to a greater extent than Limnaea species at low calcium concentration, does not accumulate as much isotope as L.pereger, and therefore the difference in the activity between the habitat and the snail would be less in Bithynia than in Limnaea.

9. The uptake of radiostrontium and radiocalcium was studied in a humid atmosphere. The results suggest that while the food is important in the uptake and accumulation of radioactive isotopes, most of the activity taken up by the snails is taken up directly from the water. The food, however, stabilises the permeability of the snail's membranes.

10. Some information was incidently collected on the uptake and accumulation of 90Yttrium and 47Sc, which showed that these particulate radioisotopes are discriminated against by the molluscs in favour of the ionic radioisotopes 90 Sr and 47 Ca.

11. A new habitat for chironomid larvae was discovered in the mantle cavity of Limnaea pereger. This finding indicates that there is a possibility of further contamination from these isotopes in the food chain. This is particularly important with 90 Sr since it has a half-life of 28 years.

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