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CALCIUM ABSORPTION BY *AUSTRALORBIS GLABRATUS* AND *PHYSA ACUTA* IN CONSTANT CONCENTRATION ENVIRONMENT

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SUMMARY

Following some previous works on the calcium metabolism of fresh water mollusks, the species *Australorbis glabratus* and *Physa acuta* are studied in an environment with a constant concentration of this ion. These species presented completely distinct behaviours as far as calcium absorption is concerned. Thus, in *A. glabratus*, the curves of the element's absorption according to time — both in soft parts and shells — are always directed upwards. On the contrary, in *P. acuta* these curves rise to a peak and then decrease and tend to present stationary values.

The causes of these different behaviours are not known and should be looked for in the physiological differences of the two species.

INTRODUCTION

Several experimental and field studies have stressed the great influence that the chemical composition of the water of breading-places exerts on the life of the mollusks that live there and, accordingly, on their distribution and prevalence.

Thus, DESCHIENS [6] has proved experimentally that the survival of *Bulinus contortus* and *Australorbis glabratus* was affected according to the water content of some ions (chloride, sulphate, sulphite, bicarbonate and carbonate, nitrite, nitrate and silicate); he also demonstrated that calcium was the only well tolerated cation, even in high concentrations, while sodium chloride, in concentrations above 6 g% and 4 g%, stopped the growth of, respectively, *A. glabratus* and *B. contortus*.

It is known, kowever, that small quantities of magnesium are necessary for the snails and for the chlorophyllous micro-flora they feed on, as is the case with iron (MALEK [21]), small quantities of which are necessary for algal growth and chlorophyll production as well as for the life of the mollusks themselves, since the respiratory pigment of *Planorbids* is a ferric chromo-proteid (DESCHIENS et al. [7]), as is evidenced by the rapidity with which, at least some species, absorb the iron in the water and concentrate it in their bodies (JOHNSON et al. [20]).

According to his considerations, MALEK [21], concludes that the tolerance of *B. contortus* and *A. glabratus* to anions and cations is a follows, by order of decreasing values: carbonate, chloride, sulphate, nitrate, nitrite, calcium, potassium, magnesium and iron.

Observations performed on mollusks in Nature allow similar conclusions. Thus, ANDRADE [1] and ANDRADE et al. [2] reported that the *S. mansoni* vector planorbids in Brazil may be frequently found in the

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hardest waters, though they may also be found in waters with large ranges of pH value (4.9 to 8.8), of chloride ion (2.5 to 252.4 p.p.m.) of calcium hardness (2 to 90.0 p.p.m. Ca CO_3), of magnesium hardness (4.0 to 130.0 p.p.m., Mg CO_3), of sulphate ion (14.4 to 384.0 p.p.m.), and of other elements considered (total acidity, mineral acidity, alkalinity, orthophosphates, nitrites, ammoniacal nitrogen, iron and silica).

HARRY & ALDRICH [17] also reported that the total concentration of dissolved solids exerted a large influence on mollusks, considering that copper or zinc might be limiting agents for their dispersion. In Puerto Rico, *A. glabratus* was also found in waters with the highest concentrations of calcium, magnesium, bicarbonate, carbonate, chloride and sulphate, while high carbonate and bicarbonate contents relatively to the chloride and sulphate may explain the absence of snails in some areas, such as the rivers of limestone zones. In a precious paper, HARRY et al. [19] state that the ratio between weak acid radicals (CO_3 and HCO_3) and strong acid radicals (Cl and SO_4) is a factor of possible significance concerning the distribution of the same mollusk species in that territory. Thus, specimens of *Biomphalaria* were seldom found in waters where this ratio was "less than 3:1, when the ions are expressed in equivalents per million. In the limestone streams, where the snail has not been found, the ratio is usually between 4:1 and 6:1" [17].

As a proof of this influence, these Authors point out that calcium sulphate is employed in aquaria to feed the snails, while lime is used as a molluscicide because the former reduces the concentration of strong acid radicals and the latter reduces those of the weak acids. The Authors conclude, in accordance with FRÖMMING [16], that the problem of calcium scarcity or deficiency as a conditioning factor for the presence of fresh water mollusks in not yet sufficiently clarified. Also HARRY [18] and CUMBIE have observed that "the limestone sink ponds in Puerto Rico do not seem to be an important source of schistosomiasis, even though *Australorbis* may be present".

Meanwhile, several Authors (MEILLON et al. [22], BUTTNER [5], SCHUTTE & FRANK [26], FRAGA DE AZEVEDO et al. [41]), have noticed no significant differences in the chemical composition of waters where vector snails, live or not, while others like DESCHIENS [6] conclude that a single mineral analysis of the waters is enough to enable one to conclude whether there can exist or not any vector snails; however, this Author has experimentally verified that *A. glabratus* and *B. contortus* tolerate waters with a wide variation of the pH value (4.5 to 10) and of some chemical elements, mainly as far as the former mollusk species is concerned.

We agree with MALEK [21] when he states that the chemical extremes that limit mollusk distribution seldom occur, and that the factors that oppose the presence of snails in some waters are varied and interdependent. As a proof of the broad limits that may be observed in the chemical composition of the breeding-places, it is enough to mention that vector snails can survive for some weeks in distilled water or in water containing few dissolved solid elements (HARRY et al. [19]).

On the other hand, the fact that we divide aquatic mollusks into fresh water and salt water mollusks is a proof of the specific limitation exerted by the chemical composition of their environment.

However among all the elements that have been studied calcium has an important place because this cation affects molluscal life in several ways: a) it is one of the basic elements of shell composition; b) it affects animal metabolism and helps to regulate tissue permeability; c) it is necessary to the green algae that are beneficial to the molluscal habitat (MALEK [21]); d) it hastens the precipitation of ooze, thus contributing for the clear waters that are beneficial to mollusks (BOYCOTT [4], in WATSON [28]); e) it acts as an antidote by reducing the toxic effects of monosaline solutions of sodium and potassium (WELCH [29]).

These are the reasons why research on the influence of calcium in molluscal life has been concerned with the explanation of this element's interference in shell formation and growth, and in molluscal weight and longevity.

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The mechanism of the influence of calcium in the formation of mollusk shells was studied by ANDRADE et al. [1, 2], and by BEVELANDER [3]. These Authors have reported that calcium in the water is stored by the animal in several organs, from where it is carried by the circulation to the mantle in the ionic state, where a diastasis precipitates it as calcium carbonate in the previously formed proteic periostracum. The influence of this alkaline earth element on the size of mollusks was confirmed by PERLOWAGORA [23] who expressed the results of his observations on *A. glabratus* by employing the calcium content of the water shell diameter ratio. He also noticed that the total weight of the animals was also affected by the high calcium content of the water in which they lived, since the size and weight of that species in calcium carbonate-poor waters were less than those of witness maintained lower and mortality higher in waters with a poor calcium content. In other aquatic animal such as certain species of fishes [25] it was also proved that a "relationship exists between calcium-45 uptake from water and the concentration of body calcium and body weight".

FRANK [15], also observed a good rate of growth and a low mortality in *Biomphalaria pfeifferi* whenever the Ca CO_3 concentration was about 18 p.p.m. and the Na-Ca ratio was 1.0.

Furthermore, it has also been proved that the ionic Na-Ca ratio conditions molluscal life. Thus, SCHUTTE & FRANK [26], noticed that both *Bulinus* (*Physopsis*) and *Biomphalaria* could be found in waters in which that ratio ranged from 0.5 to 2.0; whenever it was over 2.4 the former species was always present, while *Biomphalaria* was rare.

However, in spite of these demands, the bilharziasis vector fresh-water snails may be found in calcium deficient water bodies and it is admitted that this elements merely affects molluscal population density and the thickness and fragility of the shell (SCHUTTE & FRANK [26]).

In fact, these vector mollusks may either occur in water bodies with 5.7 and 8.5 p.p.m. calcium as is the case in some regions of Sudan, or in waters where the calcium content was null (precipitation as calcium oxalate) as was the case in Mozambique, Portuguese East Africa (FRAGA DE AZEVEDO et al. [14]). It has however been proved that snails are able to concentrate in the blood the calcium they extract from diluted solutions (VAN DER BORGHT & PUYMBROECK [27]).

Nevertheless, when estimating the effect the calcium content of the water where mollusks are living, one must not neglect the influence of the bottom ooze on this element. This ooze, as was reported by ROMEIRO & AGUIAR [24] concerning *Australorbis tenagophilus*, is a more important natural factor for shell calcification than the calcium concentration of the environment water.

As a contribution to the knowledge of the calcium metabolism in fresh-water snails and, particulary, in bilharziasis vectors, we have already published some previous papers [8, 9, 10, 11, 12, 13] on *A. glabratus*, with the special objective of knowing the distribution of calcium between the shell and the soft parts. Autoradiographs of the soft parts allowed us to gather data concerning the distribution of calcium, labelled with the 45 isotope. Together with the calcium up take studies, we also performed some others concerning the elimination of this element.

We also made a pilot experiment with the objective of testing the application of Ca^{45} in a field study on mollusks.

All these works have a common characteristic. Calcium absorption by the mollusks was studied by placing them inside small aquaria containing a radioactive calcium solution, with an adequate concentration and within the survival limits for this species.

However, the initial concentration was gradually reduced as the snails kept absorbing the calcium from the solution and, accordingly, the results obtained concerned variable concentration solutions.

In fact, in the studies we have performed up to the present in order to measure the absorption of Ca^{2+} the snails were kept in calcium chloride solutions, from which they kept extracting the calcium ion whenever

it was convenient for them. Obviously the solutions become increasingly weaker as the animals keep consuming the respective ions. The elimination products are deposited in the solutions, but as they may be either soluble or insoluble they may or may not reenter the process described. Radioactivity measurements on samples of the solutions showed that there was indeed a calcium depletion in the course of time. On the other hand, by measuring the mollusks we were able to assess the concentration of calcium in their bodies after it had been extracted from the outside.

Meanwhile, we noticed that the snails were living in poorer and poorer solutions, as far as calcium was concerned, a fact that may very possibly influence the significance of the results obtained and oppose their generical interpretation, since experimental conditions differed from the natural environment. However, the results we obtained were adequate enough to clarify the calcium metabolism in *A. glabratus*. This knowledge, besides its intrinsic value, furnishes some data on the possibility of labelling mollusks in order to study their movements in their natural habitat.

For this purpose, we made a pilot experiment, in the laboratory, using a large aquarium where environmental conditions had been reproduced. These experiments allowed us to conclude that the radiation labelling was effective and useful for our purposes.

As far as field work is concerned, our attention was then directed to indigenous species of continental Portugal.

It became then necessary to check whether the conclusions drawn from the experiments on *A. glabratus* could be extended to include indigenous species, or to what extent had they to be altered to fit the planning of field experiments on the latter species.

The results of the experiments have an intrinsic value since they explain the calcium metabolism of these same native species.

Concretely, we have studied the indigenous *P. acuta*, concerning which we have no information on calcium metabolism and on labelling possibilities regarding a field experiment.

We then made a comparative study on the absorption of calcium by *A. glabratus* and *P. acuta*, under identical experimental conditions.

In the present work we decided to study the Ca^{2+} absorption by these two mollusks while keeping them in a constant concentration of this ion, thus coming close to natural conditions. The concentration was kept constant due to the buffering action of an excess of solid salt. As the concentration of Ca^{2+} had to be limited, we had to selected a not very soluble salt; carbonate, with a solubility of 0.014 gl^{-1}, is fairly adequate for the necessary conditions. The Ca^{2+} concentration is 0.0056 gl^{-1}, a little lower than the minimum natural figure reported in the waters usually inhabited by these species.

However, their most common habitat ranges from 17 to 70 p.p.m.. Our previous experiments, during which we kept *A. glabratus* in distilled water for long periods, prove that this factor is not relevant enough to invalidate the results now obtained. Besides, there is also a pragmatical reason: we had no salt with a convenient anion within the adequate solubility range. On the other hand, carbonate is naturally the most common calcium salt in contact with the waters where snails live and was accordingly selected.

MATERIAL AND METHODS

For each species observed, 100 glass beakers were prepared, each with 50 ml of distilled water saturated, with $Ca^{45}CO_3$ (salt in excess). The solution was obtained with mechanical stirring with an inside magnetic stirrer.

Four days were allowed for the solution to become homogenous before the snails were placed inside the containers.

One adult mollusk was placed into each beaker and was kept without any food for the time of the experiment.

The excess of salt on the bottom of the container could lead to contamination of the snail, by contact and mechanical drag, thus altering radioactivity very significantly. To avoid this, we have put horizontal perforated plastic plates dividing the container

into two compartments. The snail was kept in the upper stage and the excess of solid salt was deposited within the lower one.

The perforated plates had about 1 cm clearance from the bottom of the container and were kept in position by means of 3 legs.

It is admitted that, under these conditions, diffusion is big enough to keep the saturation in spite of the absorption of calcium by the mollusks, as they have a relatively slow Ca^{2+} uptake. On the other hand, diffusion is also enhanced by the agitation caused by the snail's movements. Accordingly, we may suppose, as was done through all the work, that the kinetics of the process is dominated by the mollusks' Ca^{2+} absorption.

The 100 glass containers with one mollusk each, were divided into 5 lots of 20. The snails in the first lot were killed after four days, the ones in the second were sacrificed after eight days, and so on, until the limit of 20 days was attained. This limit was considered as a maximum for the experiment since it is difficult to maintain snails without food for a longer period.

The snails inside the 20 containers of each lot were taken out of the active solution according to schedule and thoroughly washed with flowing distilled water to remove the adhering solution. They were then killed by immersion in water at 80°C for 1 minute. The soft parts were immediately separated from the shells; after the heating the separating was easily performed by pulling out the soft parts with a forceps.

Samples of the soft parts and shells were collected for radioactivity measurement.

I — *Soft parts* — They were allowed to dry in the oven at 60°C for one hour, inside a weighed container which was weighted again after this operation. By this way we obtain the mass of the soft parts since most of the water is eliminated. This elimination is not complete but as it is done systematically, we can assume that it is uniform and that the remaining fraction is always the same. The material was then calcinated and the ashes were dissolved in a minimum quantity of hydrochloric acid 12 M. The excess of acid was removed by boilling. The resulting solution raised to a volume of 100 ml inside a volumetric flask. We used either the total amount or a fraction of the fluid for the final sample, depending on the specific activity of the initial Ca^{45}.

The volume of fluid employed was alkalized with ammonia 15 M, and 20 ml of a 2% solution of calcium chloride were added and used as carrier. Finally, calcium was precipitated as carbonate by the addition of 20 ml of a 5% solution of sodium carbonate.

The precipitate was filtered through adequate paper in a special device that leads directly to the preparation of the sample for the counting.

The samples thus prepared attain infinite thickness for Ca^{45} and, accordingly, counted activity is in proportion with specific activity.

II — *Shells* — The technique concerning the shells was essentially identical, though it was not necessary to add stable calcium since the content of this element in the shell is already high enough for a precipitate to be formed.

Samples were measured in a conventional system with a G.M. tube with a mica window about 1.2 to 1.5 mg/cm^2 thick.

The figures obtained in radioactivity measurements were corrected of the decay of Ca^{45} and divided by the mass of shells and soft parts, respectively, so that the results were expressed in activity per unit of mass. This eliminates any variation due to the different sizes of the snails, which cannot be avoided even when resorting, as we did, to animals of very similar ages.

RESULTS

The results correspond to Ca^{45} activity observed on the shells and soft parts of the mollusks observed and are the averages reported for each lot. The inadequately prepared samples that produced erratical results were omitted. It was decided to neglect the values whose deviation from average was above 26. To complement quantitative data, the value of the standard de-

viation from averages is indicated. Results obtained on *A. glabratus* and *P. acuta* are separately reported.

I — *Australorbis glabratus* — Tables I and II show the results obtained on this species. However, the fifth lot of samples could not be used.

TABLE I

Average activities of soft parts of *A. glabratus*

Lot	Time (days)	Average value (c.p.m.)	Standard deviation from average
1	4	54.1	3.3
2	8	77.7	6.9
3	12	88.4	6.9
4	16	110.0	6.7

TABLE II

Average activities of shells of *A. glabratus*

Lot	Time (days)	Average value (c.p.m.)	Standard deviation from average
1	4	53.7	5.5
2	8	80.0	10.0
3	12	102.3	10.6
4	16	120.1	9.7
5	20	148.1	13.0

Figures 1, 2, 3 and 4 show the values obtained for the two aspects: the plot of the activities against the time and the square root of the time. According to these latter graphs we notice that the variation of average activity is linear with the square root of the immersion time, thus agreeing with a kinetic law of the type

$$C^2 = K.t$$

because if $t = 0$, then $C = 0$. In this equation is represented the concentration of calcium fixed from the solution that is obviously proportional to the counted activity. The introduction in this equation of the value of the concentration or of the activity is simply translated by an alteration of the K constant, which would be multiplied by an efficiency factor.

The equation presented results from the integration of the differential equation

$$\frac{dC}{dt} = K' \frac{1}{C}$$

Thus, we see that the rate of the variations of the calcium concentration, both in shells and soft parts, is inversely proportional to the already existing concentration. The K value — constant of the rate of the process — of the soft parts differs from the one observed on the shells. There is, accordingly, a kinetic difference between calcium absorption by the soft parts and by the shell of *A. glabratus*.

II — *Physa acuta* — As we have previously reported, we studied this indigenous species of continental Portugal by the same technique employed for *A. glabratus*. The snails were likewise kept without food for the duration of the experiment. However, unlike *A. glabratus*, *P. acuta* hardly lasts for 20 days without being fed. The last lot already included some dead specimens which were not used for activity counts. Tables III and IV show the results obtained with the observed specimens of this species.

TABLE III

Average activities of soft parts of *P. acuta*

Lot	Time (days)	Average value (c.p.m.)	Standard deviation from average
1	4	217.3	13.7
2	8	421.2	47.2
3	12	346.4	55.5
4	16	159.3	28.4
5	20	219.7	36.0

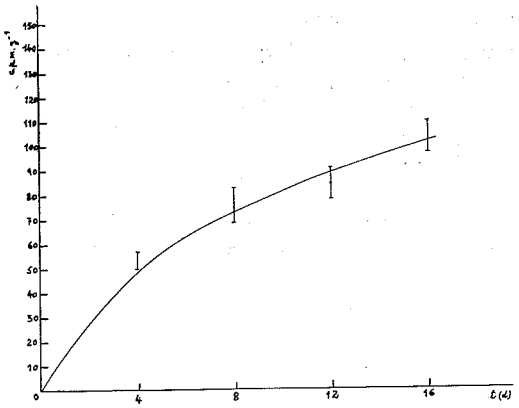

Fig. 1 — Average activities of soft parts of *Australorbis glabratus*

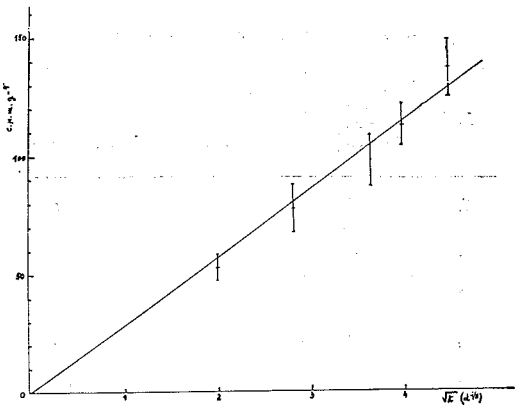

Fig. 4 — Average activity with the square root of the immersion period, of shells of *Australorbis glabratus*

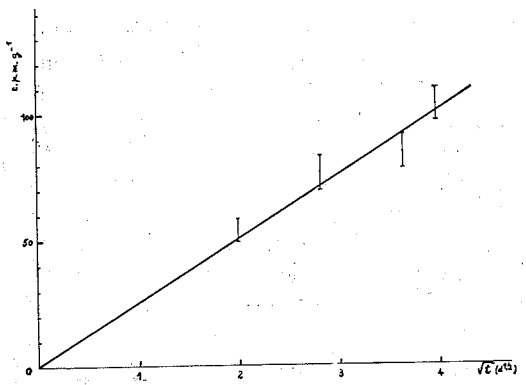

Fig. 2 — Average activity with the square root of the immersion period of soft parts of *Australorbis glabratus*

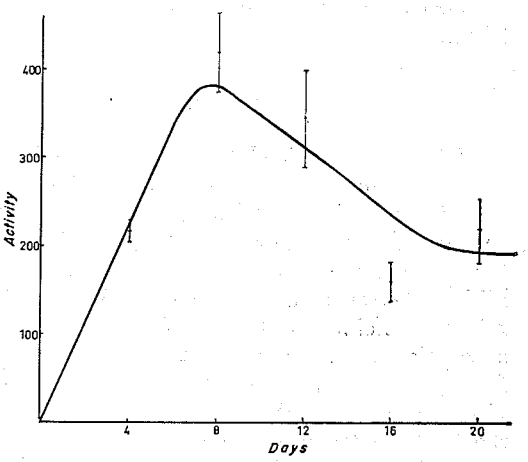

Fig. 5 — Average activities of soft parts of *Physa acuta*

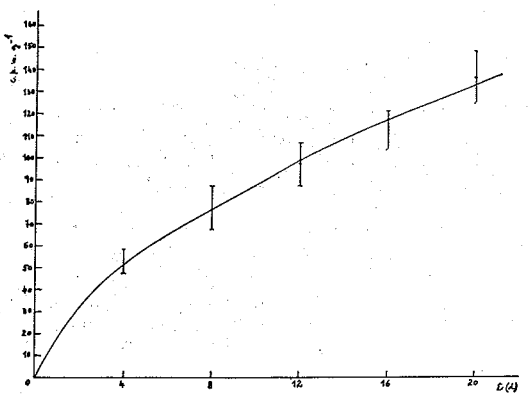

Fig. 3 — Average activities of shells of *Australorbis glabratus*

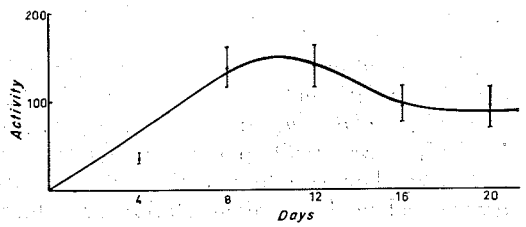

Fig. 6 — Average activities of shells of *Physa acuta*

TABLE IV

Average activities of shells of *P. acuta*

Lot	Time (days)	Average value (c.p.m.)	Standard deviation from average
1	4	33.6	4.0
2	8	136.8	31.2
3	12	139.9	24.1
4	16	80.6	24.6
5	20	80.4	20.0

Figures 5 and 6 show average activities according to time, as was done with *A. glabratus*. The evolution of the activity absorption is completely different from the formerly observed one, as may be seen by a comparison of Figures 1 and 2 with Figures 5 and 6.

DISCUSSION

The results obtained on *A. glabratus* lead us to assume that calcium absorption by this mollusk is conditioned by the Ca^{2+} content of their environment. Accordingly, the natural metabolic balance that is established between the absorbed and the eliminated calcium must be conditioned by the absorption capacity, which in turn depends from the calcium concentration of the environment; the higher the concentration of the Ca^{2+} in the water, the greater the Ca^{2+} absorption. Thus, when snails are transferred from a poorer environment to a richer one, they absorb calcium until a constant equilibrium is attained. When they are moved from a richer environment to a poorer one, they loose calcium until an equilibrium is attained between absorption and elimination, the later being now higher.

The evolution observed on *P. acuta* is completely different, as said before. In the case of *A. glabratus*, the curves keep rising in both cases (soft parts and shell). In *P. acuta*, they rise to a peak and then decrease and tend to present stationary values. The peak of the soft parts curve occurs a little earlier than the one of the shells, in accordance with the general idea that calcium is absorbed by the soft parts and then passes on to the shell.

Accordingly, concerning calcium absorption, there is a considerable difference in the behaviour of the species observed. The reason of this significant difference should be looked for in the biological differences of the two species and may constitute an element for physiological differentiation among the several species, as a contribution to their characterization.

RESUMO

Absorção de cálcio pelo Australorbis glabratus *e* Physa acuta *em ambiente de concentração iônica constante*

Em continuação a alguns trabalhos anteriores sôbre o metabolismo do cálcio em moluscos de água doce, estudamos as espécies *Australorbis glabratus* e *Physa acuta* em ambiente com concentração constante dêste sal. Estas espécies apresentaram comportamento inteiramente distinto no que se refere à absorção de cálcio. Assim, em *A. glabratus* as curvas de absorção do elemento segundo o tempo — tanto nas partes moles como nas conchas — têm sempre orientação ascendente. Em *P. acuta*, ao contrário, estas curvas se elevam a um pico, decrescem e tendem a valôres estacionários.

As causas dêstes comportamentos diversos não são conhecidas e deveriam ser procuradas nas diferenças fisiológicas das duas espécies.

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