Calcium Dynamics in Land Gastropods[1]

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Synopsis. In land gastropods, calcium is precipitated in the shell, in connective calcium cells which are largely distributed through the whole connective tissue, in epithelial calcium cells of the digestive gland, and in the calcium gland cells of the skin and the mantle collar. Calcium is taken up from the external medium by food and by absorption through the sole skin. To adapt to terrestrial life, these animals have to eliminate appreciable amounts of calcium for their protection and their reproduction. During the egg laying period, a calcium flux occurs through the epithelium of the reproductive tract in order to supply the egg shell and the egg fluids. This egg calcium is taken up by the embryo. The maintenance of a positive calcium balance between its uptake and the loss is due to an important reservoir of easily mobilizable calcium in the form of calcium carbonate. This reservoir consists of the connective calcium cells which are constantly able to accumulate or release calcium as long as calcium is locally available or required. The epithelial calcium cells of the digestive gland are loaded with calcium phosphate; they are not a major calcium storage compartment, but have an essential function in detoxification. All of the calcium movement occurring across cell membranes and through epithelia concerns only calcium ions. All calcium movement can be regarded either as on-off systems or as reversible systems, both of which are certainly controlled by complex processes.

INTRODUCTION

The land gastropods have successfully colonized the terrestrial environment. For these animals, calcium appears to be one of the most limiting factors when the great mass of calcium carbonate they have to extract from the medium is considered. Even if we consider only the body covering shell or the egg shell that several species have to elaborate, this remains true.

Both intracellular and extracellular mechanisms of calcification are found in land gastropods. The deposition of the body shell is one of the more investigated processes of extracellular calcification and a great wealth of details has been exposed about shell structure, formation and regeneration (Wilbur, 1964, 1972; Gregoire, 1972; Wilbur and Simkiss, 1968). In land gastropods intracellular calcification occurs in calcium cells located in the digestive gland and in several parts of the connective tissue.

In order to understand the physiological significance of these calcified structures, an interesting approach may be not to consider them separately, but consider them as special compartments involved in the general dynamics of calcium, separated by selective barriers and with many possibilities of exchange.

First, all the compartments concerned with calcium will be reviewed. We will then examine the behaviour of each in the whole organism and the possible interactions between them when the organism is submitted to various ecological and physiological conditions.

THE COMPARTMENT OF PRECIPITATED CALCIUM

The shell

The shell is the most studied of the calcium compartments in land gastropods, although it has been investigated less than the lamellibranch and marine gastropod shell. The land gastropod shell is characteristically thinner, less heavily mineralized and more proteaceous than the lamellibranch or marine gastropod shell. Nevertheless, the general features concerning the mechanisms of molluscan shell deposition, summarized by Wilbur (1976), can be applied to the land gastropod shell as well. These general mechanisms include

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periostracum formation, nucleation of calcium carbonate crystals on the organic matrix of the shell and growth of these crystals. However, one subject remains poorly defined at this time in land gastropods: it concerns the extrapallial fluid which exists between the mantle epithelium and the shell; this "fluid" contains the mineral and organic components produced by the mantle epithelium before their deposition on the inner shell surface.

An important point in understanding the physiological significance of the shell is the variability of its development among the various families of land pulmonates. The importance of the shell appears to be dependent on ecological conditions. Numerous families of snails have a well developed external shell which provides protection for the animal against evaporation and desiccation. With this external shell, the snails can live at some distance from the soil, i.e., on plants, trees or walls. In some slugs, e.g., Testacella, an external but reduced shell is observed; other slugs have no external shell and so, no protection against water loss; they generally live not far from the soil, in a wet environment. In some families such as Limacidae, the shell has a typical molluscan structure with a periostracum produced by the mantle edge and calcified layers. Such a shell has been described in Deroceras reticulatum (syn. Ariolimax reticulatus Fournié, 1979). The Arionidae have either an atypical shell such as in Agriolimax columbianus (Mead, 1943; Meenakshi and Scheer, 1970), or possess only some calcified granules as is the case in Arion rufus.

So, in land gastropods, the shell is not a constant feature and, when present, its degree of calcification is of lesser importance than in lamellibranchs or marine gastropods.

The digestive gland

The digestive gland in pulmonates is the most developed organ of the body; it consists of many branched blind-ending tubules embedded in connective tissue; this gland empties into the stomach through large ducts (Runham, 1975).

Calcium cells have been described in all species of land gastropods whose digestive glands have been investigated (for review, see Fournié and Chétail, 1982a). It seems most likely that their existence is a general feature in Stylommatophora. These calcium cells belong to a particular type of epithelial cells lying between the digestive and excretory cells of the acinar epithelium of the gland. Ultrastructural studies of these calcium cells (Bani, 1962; Abolins-Krogis, 1965; Walker, 1970) indicate that they are pyramidal in shape with closely adjacent microvilli on the apical surface; calcium salts are precipitated in membrane bound vesicles at the basal part of the cell. The composition of the mineral precipitate has been investigated by Fretter (1952), Abolins-Krogis (1960), Burton (1972) and Simkiss (1980). Calcium, magnesium, phosphate and carbonate ions have been found in the spherules. Burton (1972) indicates in Helix pomatia a molar ratio of 1/0.6 for calcium/magnesium and of 1/0.1 for phosphate/carbonate. It appears certain that phosphate is the main form of calcium precipitate in the digestive gland of Stylommatophora.

The calcium cells of the connective tissue

Cells which accumulate calcium into spherulites have been described for a long time in various parts of the connective tissue in Stylommatophora (for review, see Fournié and Chétail, 1982a). A substantial density of calcium cells was reported in the mantle subepithelial connective tissue by several authors suggesting a role of these cells in calcium supply for shell formation or regeneration. Campion (1961) observed calcium cells in the subepithelial connective tissue of the sole in Helix aspersa. Bensalem and Chétail (1982) described in the land prosobranch Pomatias elegans a continuous range of calcium cells lining the sole epithelium. We observed also the presence of such calcium cells in the sole connective tissue in Achatina fulica, Limax maximus, Deroceras reticulatum, Arion rufus and Helix aspersa (Fournié and Chétail, 1982a). Calcium cells have also been mentioned for a long time around the vascular system. Tompa and Watabe (1976) gave a partic-

ularly interesting description of the calcified arteries of *Anguispira alternata*. They reported that these arteries are very white and thick-walled; their walls are outlined by a dense layer of calcium cells containing spherules of calcium carbonate and appearing to be degenerating connective tissue cells. Sen Gupta (1977) observed also rings of calcium cells around the blood vessels of the digestive gland in *Bensonia monticola*. Connective calcium cells are also present in the interacinous connective tissue of the digestive gland. Two other interesting localizations have to be mentioned: They are relatively abundant around the nervous system, principally around the ganglia; we observed also rather numerous calcium cells around the intestine in *Deroceras*. In the land prosobranch *Pomatias elegans* numerous calcium cells were observed by Vovelle and Grasset (1979) in the connective tissue underlying the epithelium of the opercular fold.

As yet, no ultrastructural description has been given of the connective calcium cells in Stylommatophora. Identical connective calcium cells which accumulate calcium carbonate into spherules were largely described in some species of Basommatophora, i.e., *Ferrissia wauthieri* (Richardot and Wauthier, 1972; Richardot, 1976), *Pomacea paludosa* (Watabe *et al.*, 1976), *Lymnea stagnalis* (Sminia *et al.*, 1977) and in the land prosobranch *Pomatia elegans* (Vovelle and Grasset, 1979). These calcium cells represent a special way of differentiation of the pore cells described in the connective tissue of molluscs; they have the potentialities to differentiate into several cell types (for review, see Richadot, 1976). Current studies performed in *Deroceras* and *Helix aspersa* reveal that the connective calcium cells in Stylommatophora are also pore cells (unpublished data).

We obtained a general view and a quantitative idea of the distribution of the connective calcium cells by examining sections of whole animals in several species of land gastropods. Frozen sections were stained using the method of Kashiwa *et al.*, which gives a positive reaction with ionic calcium and unstable precipitates.

The calcium cells are more strongly stained with the Kashiwa's method than with the other common methods for calcium (Stoelzner or alizarin red S). The staining of the spherules with the Kashiwa's method is strongly reduced if the sections are previously dipped in distilled water. These observations suggest that the spherules are calcium carbonate. The observations of sections of whole animals reveal a great abundance of these calcium cells in all the connective tissue of the species examined; it seems that their presence is a common feature in the stylommatophoran connective tissue. The importance of this calcium compartment has been up to now underestimated. Although it is impossible to quantify the amounts of calcium stored in the whole connective compartment, we can consider that it contains at least as much calcium as the digestive gland. Lastly, we have to call attention to the fact that these connective calcium cells are principally located in areas which are particularly strategic points for the calcium exchanges.

The mantle collar and the epiphragm

The terrestrial snails may close the shell aperture with an epiphragm in order to endure cold or dry weather conditions. This epiphragm is produced by the mantle collar (Block, 1971; Rokitka, 1973) which results from the differentiation of the mantle epithelium lining the shell aperture. The epiphragm consists of mucoid material and precipitated calcium carbonate. Both are produced by unicellular glands as shown by Campion (1961); these glands lie deep in the connective tissue of the mantle collar and discharge material through pores which pass between the epidermal cells by means of distinct and unusually long ducts. The calcium glands produce and secrete spherical and rod-like granules of calcium carbonate.

The skin and the slime

The lateral skin of the foot of the land gastropods contains unicellular glands identical to those previously described in the mantle collar. These gland cells are connective mucus cells and calcium cells whose apical part is a long duct passing

between the epidermal cells of the skin, as described by Campion (1961) in *Helix aspersa*. Walker (1970) reported that the irritation of the body wall in *Deroceras reticulatum* produces a discharge of a milky white slime containing granules of calcium carbonate. The significance of these granules in the mucus is not yet well understood; Campion (1961) indicates that the viscosity of mucus could be increased by calcium ions. Our observations of mucus smears in *Deroceras* reveal that a strong discharge empties the whole cell contents. This is apparent since we observe mucus droplets, calcium carbonate granules and nuclei in the smear.

Both mantle collar and skin glands represent compartments from which appreciable amounts of calcium are definitively lost with regard to calcium balance in the land gastropod body.

Preliminary conclusions

In conclusion to this preliminary review of the compartments where calcium is precipitated, it is possible to distinguish two different kinds of compartments:

—compartments where calcium is precipitated for external use: these are the mantle collar and the skin which are secreting out of the body granules of precipitated calcium;

—compartments where calcium is accumulated with the possibility of internal relocations: these are the shell, the digestive gland and the connective calcium cells.

It has long been thought that calcium might be stored in the soft tissues with the only function of serving as the reserves for shell edification or regeneration. In fact, it appears that the land gastropods have a well developed digestive gland with numerous calcium cells and abundant connective calcium cells, irrespective of the presence of a shell. In order to understand the significance of such amounts of calcium stored in the connective tissue and the digestive gland, we have to take into account the importance of calcium requirements for reproduction.

CALCIUM AND REPRODUCTION

The loss of calcium from the body into the genital duct during the elaboration of the eggs is evident in land gastropods even if we consider only the two following observations:

—In several species of land gastropods, the egg shell is heavily calcified.

—In the species which have a body shell, the new born animal hatches with a well calcified body shell.

Figure 1 illustrates the general structure of the land gastropod egg and the anatomy of the genital duct. It is now generally accepted that the perivitelline fluid (*i.e.*, albumen) is produced by the albumen gland while the jelly and the egg shell are elaborated during the transit in the common duct (*i.e.*, uterus or "oviduct").

The compartments of calcium involved in reproduction

The egg shell. An interesting comparative study of the calcified eggs of land snails is given by Tompa (1976*a*) who stated that three types of land snail egg shell can be distinguished on the basis of the degree of calcification:

—uncalcified eggs without any deposit of $CaCO_3$ in the egg shell;

—partly calcified eggs with discrete individual crystals of $CaCO_3$ in the outer egg layers;

—heavily calcified eggs with a hard brittle shell of fused $CaCO_3$ crystals similar to bird eggs.

The significance of the degree of calcification of the egg shell of the land gastropods is not yet well understood. Tompa (1974) indicates that there is no ecological correlation between calcification of the egg and the habit of oviposition. It does not seem that calcification of the egg protects it against desiccation. On the other hand, he noted a correlation between the size of the egg and the degree of calcification suggesting that a function of the egg shell is to provide a structural support for the large eggs. Studying in particular the egg shell in *Anguispira alternata*, Tompa (1976*b*) demonstrated that the calcification of the shell occurs progressively during the transit of the egg in the uterus.

The egg fluids. A few details are presently known about the calcium content of egg fluids (*i.e.*, albumen and jelly) in land gastropods. Bayne (1966) reports the presence
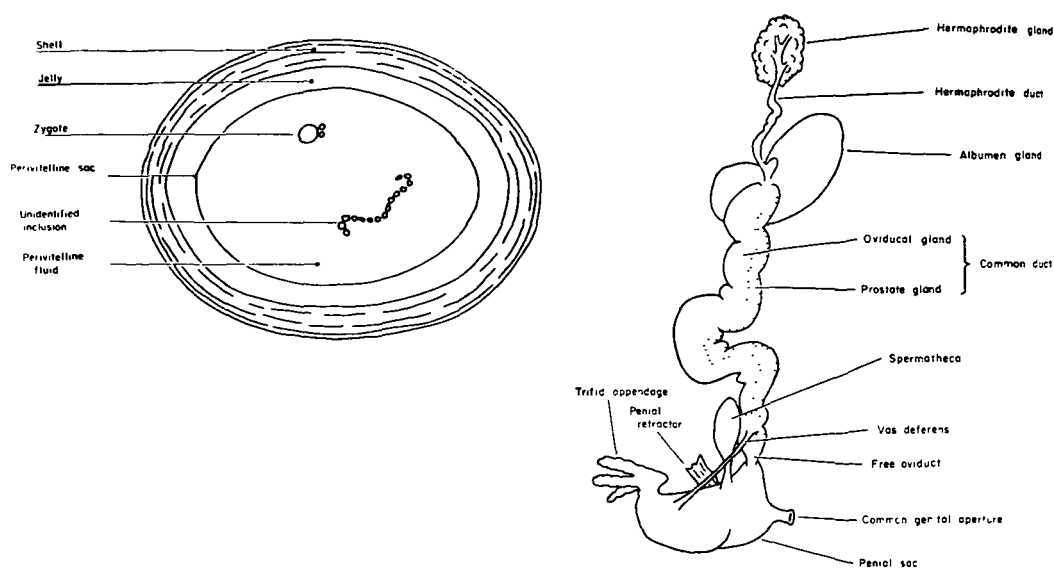
FIG. 1. Egg structure and reproductive tract anatomy in *Deroceras reticulatum*, after Bayne (1966). The albumen gland produces the perivitelline fluid (or albumen). The shell and the jelly are elaborated during the transit of the eggs in the common duct (or uterus).

of calcium in the egg shell, the jelly and the albumen in *Deroceras reticulatum*. In this species which has a partly calcified shell with discrete scattered crystals of calcium carbonate, we measured the calcium levels of the eggs; the results indicate that the shell and the egg fluids contain each about 50% of the whole calcium of the eggs. The molar concentration calculated in the egg fluids is 37 mM/liter in *Deroceras*. The presence of calcium in the egg fluids has been, up to now, underestimated because authors have investigated only eggs with heavily calcified shells. In these cases, the calcium of the egg fluids appeared negligible compared to the egg shell calcium. Several species of land gastropods lay eggs with a completely uncalcified shell (*e.g.*, *Succinea putris, Limax maximus*); it is evident that, in these species, only the egg fluids can provide embryos with calcium.

The albumen gland and the common duct

These observations on the egg shell and egg fluids present the problem of establishing the source of calcium which passes through the albumen gland and common duct epithelia. In the land gastropod genital duct, there is no calcium reserve in the form of precipitated mineral which could

serve as a calcium supply either for the calcified egg shell or for the egg fluids. This fact was mentioned by Tompa (1976*b*) in *Anguispira* and we observed the same lack of precipitated calcium salts in the genital duct of several other species. Nevertheless, measurements of calcium in the albumen gland and in the common duct in *Deroceras* reveal (Table 1) that these parts of the genital duct in particular are able to hold appreciable amounts of calcium, compared to the digestive gland where calcium is stored as precipitate. It is interesting to note that just before the first laying, calcium levels may be higher in the albumen gland and in the common duct than in the digestive gland. The albumen is elaborated by the albumen gland cells and packed into secretory vesicles; the jelly is produced in the same way by the jelly cells of the common duct. The concentrations of calcium observed in the egg fluids in *Deroceras* (37 mM/liter) are about 4 to 5 times higher than the blood calcium levels measured in several species of land gastropods (Schofeniels and Gilles, 1972). It is difficult to accept that the calcium is in ionic form in the egg fluids. If this were the case, the osmotic pressure of the egg fluids would be very high and the levels of free calcium

TABLE 1. *Calcium levels in the genital duct in Deroceras. Comparison with the digestive gland at different periods of the life cycle.*

	Early beginning of the ovogenesis period	Just before the first laying
Albumen gland	1.4	1.8
Common duct	1.4	1.8
Digestive gland	4	1

25 animals were used for each measurement. Values are given in 10^{-4} mM of calcium/mg of fresh tissue.

could be toxic for the embryo. So, in the egg fluids, calcium ions are probably bound to organic products and the Ca-binding process may occur in the albumen gland cells and in the jelly cells of the common duct.

The embryo. The calcium accumulated in the egg shell and in the fluids is used by the embryo (Carrick, 1939). Tompa (1975) showed that the embryo of *Anguispira* absorbs calcium from the egg shell in order to form the embryonic body shell; by 43 days of development, the embryos of *Anguispira* have absorbed up to 60% of the egg shell calcium. In *Deroceras* the development time is 25–30 days at 15°C; in this species a well calcified shell is already formed at the 9th day of development. A particularly interesting feature we observed in *Deroceras* is the differentiation of calcium cells in the embryonic connective tissue of the skin and in the connective tissue embedding the lobes of the hepatic mass; calcium gland cells differentiate also in the skin. All these calcium cells appear at 12–15 days of development. Immediately after hatching, the egg remnant is consumed by the newly hatched animal. Tompa (1976a) reports this fact in *Anguispira*. In *Deroceras*, the remnant of the egg (envelopes and fluids) which contains still 50% of the whole egg calcium is also consumed by the neonate slugs. So, in land gastropods, all the calcium deposited in the eggs is fully used:

—for the calcification of the embryonic shell; the newly hatched animal has a complete and well calcified shell;

—for the deposition of calcium reserves in the first calcium cells which differentiate during the embryonic life;

—as a nutritive element for the neonate animal.

Calcium loss by ovipositing

Calcium requirements for embryonic and neonate life in the land gastropods explain the considerable amount of calcium that the adult loses during the ovipositing period. It is interesting to give some data illustrating the amounts of calcium mobilized for the requirements of reproduction. Tompa (1976a) reports that *Anguispira* mobilizes 10–25 mg of calcium in less than a day; Wilbur and Tompa (1979) indicate that the rate of calcium transport across the uterine epithelium during egg shell formation is about 1.10^{-5} $M/cm^2/hr$ in *Anguispira* and 3.10^{-6} $M/cm^2/hr$ in *Helix aspersa*. It is also interesting to compare the amounts of calcium mobilized for reproduction with the calcium contents of the whole animal. The slug *Deroceras* lays about 100 eggs in 2–6 consecutive layings. Measurements of calcium in the laid eggs and in the slugs indicate that the sum of the calcium lost by ovipositing equals 65% of the total calcium measured in the whole animal just before the laying period. Each egg mass contains about 25 eggs; the calcium measured in an egg mass represents 70% of the average level of calcium in the whole digestive gland.

Calcium supply for reproduction requirements

Considering that the reproductive tract does not store calcium reserves, the question of the source of the reproductive calcium is stated. Tompa and Wilbur (1977a) observed in *H. aspersa* that the blood calcium rises suddenly during the time of calcification of the egg shell in the uterus and drops back to the normal level within a few hours after the last egg is released. These authors conclude that calcium is mobilized from other parts of the body and is transported to the uterus via the blood. It was suggested by many authors that the digestive gland may represent the main source of calcium for the eggs. Mead (1943) and Meenakshi and Scheer (1970) indicate that *Ariolimax columbianus* draws calcium from the body shell to supply the eggs with calcium. In a recent study (Fournié and Chétail, 1982b) we tried to investigate the possible sources of calcium for reproduction

requirements in *Deroceras*. The short and single life cycle of this species can be divided into a juvenile period, a male stage and a female period which includes an oogenesis phase followed by the ovipositing period. The animals die a few days after the laying. Young animals collected in the field were individually bred in isolation so that it was possible to get animals whose position along the life cycle was known. The variations of calcium levels in the storage compartments (shell, digestive gland and common duct) can be summarized as follows: During the male period, calcium is progressively accumulated in all the three storage compartments. From the beginning of the ovogenesis period until the first laying, the calcium stock decreases progressively in the three compartments and reaches its lowest value at the time of the first laying. During the short period (about 8 days) between the first and the second laying, we observed a considerable increase of calcium in the storage compartments immediately followed by a sudden decrease. In the common duct, we observed an appreciable increase of calcium just before each laying, followed by a calcium drop at the time of the layings. In the albumen gland, the same sequence of calcium accumulation and release occurs but both phenomena take place earlier than they do in the common duct. These results indicate clearly that the shell, the connective calcium cells and the calcium cells of the digestive gland serve as calcium reservoirs for the requirements of reproduction. The dynamics of calcium accumulation and release appears to be very rapid and precise. Immediately after each period of calcium release, the storage compartments are immediately reloaded. The decrease of calcium in the storage compartments occurs simultaneously with an increase in the genital duct. All these observations suggest a precise correlation between calcium movement and reproductive physiology.

CALCIUM MOVEMENTS

This survey of the compartments of precipitated calcium and of their behaviour during reproduction provides further evidence of the diversity of calcium move-

ment which may occur in the land gastropod body. We will distinguish three main kinds of movements (Fig. 2):

—*uptake movements* from the external medium;

—*loss movements* which concern the calcium lost from the body for reproduction, epiphragm edification and slime extrusion;

—*internal movements* which take place through the blood; they include the precipitation of calcium in the storage compartments and the calcium release from these compartments to supply the internal needs (shell edification) and the loss movements.

Calcium uptake from the external medium

Two main sources of calcium are available for land gastropods: the food and the calcareous substratum where they live (Kado, 1960).

Percutaneous uptake. Robertson (1941) drew attention to the fact that the presence of calcium carbonate plays an important part in the distribution of land snails. Kado (1960) gave evidence of the uptake of calcium through the sole skin in *Euhadra nipponensis*. He plastered the sole of snails with a stiff paste prepared by mixing up a fine powder of calcium carbonate made from calcium chloride solution labelled with ^{45}Ca. He noticed a rapid entrance of calcium into the blood in the early phase of the experiment. The author reports that "*in vitro*" experiments with the outer layer of the sole tissue suggest an active transport of calcium through the sole skin. Recently we observed some interesting facts in *H. aspersa* (unpublished data): 50 young snails newly hatched from the same egg mass were divided into two groups; the first group (control group) was bred with the addition of hard calcareous stones in their box while the other group was bred without stones. Both groups were fed in a similar manner and bred for 8 months. In the animals bred without calcareous stones, a very characteristic general dwarfing is observed; the shell is thicker than in control snails but it is often deformed and always less mineralized. Calcium measurements at the end of the experiment indicate lower calcium levels in the shell, the sole, the lateral skin of the foot and the genital duct of the snails
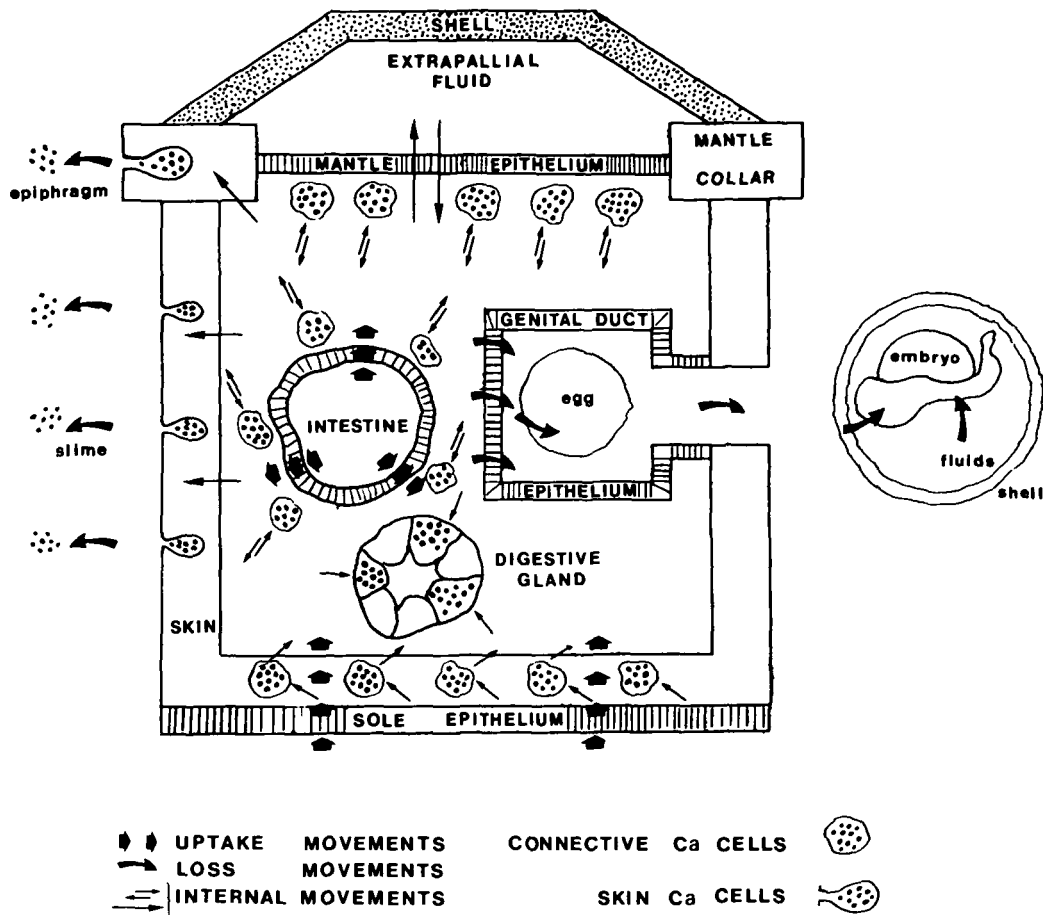
FIG. 2. Schematic representation of the calcium movements in the land gastropod body. If we except the loss of calcium carbonate granules from the mantle collar and the skin, all the other arrows concern only calcium ions. The internal compartment between the different organs represents the haemolymph.

deprived of calcareous stones than in the control snails. A histological study of the sole reveals numerous calcium cells in the subepithelial connective tissue of the foot in the control snails and an almost complete absence of these calcium cells in the snails bred without calcareous stones. So, in *H. aspersa*, a normal growth occurs only if calcium uptake by the sole from the calcareous substratum can take place. The mechanism of calcium uptake through the sole epithelium is not yet well understood. The mucus secreted by the mucocytes of the sole certainly plays an important role, due to its calcium combining capacity (Fretter, 1953; Kado, 1960).

Digestive uptake. If it is accepted that food provides calcium to the land gastropods, the question of the route of calcium from the digestive duct up to the storage compartments remains unanswered. Fretter (1953) reported interesting results of experiments with ^{90}Sr in *Arion hortensis*. This author suggests that strontium (and so calcium) could enter the calcium cells of the digestive gland from the haemocoel through the basal plasma membrane, as well as from the lumen of the gland. The author points out the permeability of the intestine epithelium to ions which pass from the intestine lumen into the blood. In Fretter's experiments, slugs which have been starved

for a week after feeding on the isotope show that the element is retained in the connective calcium cells surrounding the anterior aorta; the mantle calcium cells may contain the tracer within 12 hr after its entry into the gut. Kado (1960) showed in *H. aspersa* that ingestion of radioactive calcium carbonate is rapidly followed by the appearance of the radioactivity in the blood. In *Deroceras* we observed numerous calcium cells in all the periintestine connective tissue.

It seems from these data that the digestive gland can no longer be considered as the only storage compartment for alimentary calcium. The ultrastructural data on the calcium cells in the land pulmonates' digestive gland (Bani, 1962; Abolins-Krogis, 1965; Walker, 1970) indicate that the calcium spherules are formed in the basal area of the calcium cells, the luminal membrane of these cells having only a few sparse microvilli. Runham (1975) discussed the problem of the functions of the digestive gland. The gland has, at the same time, an enzyme secretory function and a role in intracellular digestion; the author pointed out the difficulties that may be encountered for the stomach juice to ascend to the glandular tubules. It is certain that the intestine provides a large surface area for ionic exchange with the blood. All these observations lead us to think that the blood could be the major means of distribution of alimentary calcium not only towards the digestive gland, but also towards the connective calcium cells. The more rapidly accessible calcium cells are the periintestine and the perivascular ones.

Calcium loss out of the body

Due to the terrestrial life, the land gastropods have to lose large amounts of calcium towards the external medium: the neonate must be able to endure stressful ecological conditions which may prevail at the hatching time; in this order, the eggs are provided with sufficient calcium levels so that the newly hatched animal may have a well calcified body shell and tissue calcium reserves. The land gastropod has to maintain, throughout its life, a sufficient store of calcium in the mantle collar and in the skin to build up an epiphragm and to extrude a protective slime as soon as is necessary.

The calcium gland cells of the mantle collar and the skin elaborate calcium granules in membrane bound vesicles. These cells are certainly provided with ionic calcium from the haemolymph, but the mechanism of uptake still remains unknown.

Concerning the calcium loss for the eggs, it appears from the review given on pages 860–863 that the calcium is mobilized from the storage compartments and transported by the blood to the reproductive duct. Wilbur and Tompa (1977, 1979) indicate that in *A. alternata* and *H. aspersa* two kinds of cells line the uterus; one type secretes the jelly and the other cell type has well developed infoldings of the basal plasma membrane strongly suggesting a role in ion transport. These authors demonstrate that the potential across the uterus is reversibly depressed by DNP; it is reversed by increasing the calcium concentration of the outer medium. They observed *in vivo* rise in free blood calcium during the egg laying. All these arguments suggest that an active transport of calcium takes place across the uterine epithelium.

Internal movements

Most of the land gastropods are nocturnal, and are consequently able to take up calcium only by night or in wet weather and have to be resting often for an extended time, when it is too dry or too cold. So, they have to maintain a permanent reservoir of calcium in order to be able to constantly and rapidly supply calcium, even if calcium uptake is inadequate at the time when calcium is needed. The maintenance and the functioning of such an important reservoir implies the existence of internal calcium movements; they take place through the blood and consist of accumulation and release movements towards and from these compartments. Because of this important reservoir, the calcium balance between the uptake and the requirements is always positive in spite of the hazards of terrestrial life.

Calcium movements towards and from the shell

The data given about reproduction indicate that the shell may be considered as a reservoir compartment. Nevertheless, in the species which have an external well developed shell, this shell has an essential protective function and the animals have to maintain it with a sufficient degree of calcification; they must also be able to regenerate it rapidly after injury. Some authors have mentioned that in such species (*H. aspersa, Otala lactea*) the calcium is mobilized from some parts of the body shell to be used to repair another injured part (Wagge, 1951; Chan and Saleuddin, 1974). So, when external, the shell has mainly a protective function but it may also be a storage compartment. When internal, the shell has only a storage function; this certainly explains the presence of an inner spherulitic layer of labile and easily mobilizable calcium carbonate in the shell of *Deroceras* (Fournié, 1979*a, b*). The movement of calcium from the haemolymph towards the extrapallial compartment is not yet well known. Several authors have pointed out the presence of connective calcium cells and amoebocytes loaded with calcium spherules under the mantle epithelium either in normal animals or in shell-regenerating snails (for review, see Fournié and Chétail, 1982). These cells locally provide the mantle epithelium cells in ionic form. The structure of the mantle epithelial cells is very complex as shown by Saleuddin (1970) in *H. pomatia*. This epithelium transports calcium and bicarbonate ions but also forms the organic compounds of the shell. It is possible that a Ca-binding process occurs in the mantle epithelium. The extrapallial fluid represents a compartment of calcium bound to organic products. This binding allows the epitactic nucleation of calcium carbonate crystals at the inner surface of the shell. The movement of calcium from the shell into the blood was clearly demonstrated by examining the sources of calcium for the egg. The conditions under which this movement occurs remain to be studied.

Calcium movements towards and from the connective calcium cells

The land pulmonate calcium cell is a particular type of pore cell, as has been extensively described in Basommatophora (for review, see Fournié and Chétail, 1982*a*). It is certain that these cells accumulate and release calcium but the mechanism of calcium uptake and discharge remains unknown. The role of the pores on the plasma membrane of these calcium cells needs to be determined in regard to calcium entry or exit. Richardot (1976) suggested that under the pores, endocytosis vesicles are formed which coalesce with endoplasmic reticulum vesicles and Golgi vesicles to give rise to the vacuoles which precipitate calcium carbonate. This means of calcium uptake of the calcium cells could explain the association of calcium carbonate with organic compounds; furthermore, blood calcium could reach the precipitation vesicles this way without having to cross through hyaloplasma, thus avoiding any risk of toxicity. Sminia *et al.* (1976) suggest that the pores could allow the intracellular granules to dissolve directly into the haemolymph.

Calcium movements towards and from the digestive gland

In examining the problem of the digestive calcium uptake, we concluded that calcium may enter the calcium cells of the digestive gland from the haemolymph through the basal plasma membrane and that the calcium is precipitated in membrane bound vesicles. The digestive gland cells differ from the connective calcium cells in that they are glandular epithelial cells and that they elaborate essentially calcium phosphate. It is likely that the digestive gland has a storage function and that a part of its calcium reserves is mobilized for reproduction requirements.

In *Deroceras*, we measured the participation of each storage compartment in supplying calcium for reproduction: about 48% for the shell, 35% for the skin and only 17% for the digestive gland. It appears that the digestive gland is not the essential

calcium reservoir. We measured in *Achatina fulica* the levels of carbonate and phosphate of the digestive gland in normally fed animals and in animals starved for 3 weeks. We observed in the starved animals the same phosphate levels as in the control animals. Carbonate levels, on the contrary, were strongly depressed. It appears therefore that calcium carbonate represents the more easily mobilizable part of the calcium salts precipitated in the digestive gland calcium cells. It seems likely that, when calcium is mobilized from the digestive gland, the mobilization concerns only the carbonate fraction. Concerning the digestive gland, an essential feature has been underestimated: a significant number of connective calcium cells exist in the interacinar connective tissue of the digestive gland in pulmonates (McGee-Russel, 1957). So, when calcium is mobilized from the digestive gland, this mobilization could concern only these connective calcium cells containing $CaCO_3$ granules. The insolubility of the calcium granules of the digestive gland compared with the granules of the connective calcium cells has been clearly demonstrated by Simkiss (1980) in *H. aspersa*. What is then the significance of the epithelial calcium cells of the digestive gland and of stable calcium phosphate in these cells? Runham (1975) discussed this problem and reported two main suggestions to explain these facts. Firstly, the calcium granules could function to buffer the digestive juice but this function is not clearly demonstrated. Secondly, the granules of stable precipitate could represent a means of accumulation of toxic material (*e.g.*, ions). Simkiss (1980) demonstrated that zinc can be incorporated into the spherules of the digestive gland in *H. aspersa*.

It is concluded from these data that the connective calcium cells represent the more important reservoir of easily mobilizable calcium. This connective compartment is certainly the more useful safety device between calcium uptake ability and calcium requirements. The digestive gland cannot be considered either as the major

storage compartment for alimentary calcium, or as an essential reservoir of mobilizable calcium. The mobilization of calcium from the digestive gland occurs only in its interacinar connective calcium cells; the epithelial calcium cells of this gland undoubtedly play a role of detoxification.

The problem of the amoebocytes

Several authors have mentioned that amoebocytes can elaborate calcified spherulites; they have been observed in the interacinar connective tissue of the digestive gland, and in the mantle subepithelial connective tissue principally in areas of shell regeneration (for review, see Fournié, 1979a, b; Fournié and Chétail, 1982a). The question of their significance has existed and been debated for a long time. Our opinion is the following: amoebocytes do not carry precipitated calcium from one part of the body to another; they are able to accumulate calcium locally after arriving at the site of action. Their disruption liberates the spherules which can dissolve. This could explain the observation of free calcium granules in the connective tissue or in the epithelium intercellular spaces. This ionic calcium, if rapidly liberated, could supply a particular local requirement. So, all the internal calcium movements and the movements across epithelia (mantle, reproductive tract) concern only ionic calcium or calcium ions bound to organic products.

CONTROL OF THE CALCIUM DYNAMICS

The movement of calcium from the haemolymph into the calcium cells leads to intracellular calcification. We have shown in the connective calcium cells and, to a lesser degree, in the digestive gland calcium cells, the occurrence of both precipitation and release. In this view, Simkiss (1980) concludes that the connective calcium cells are candidates for the distinction between calcification and mineralization. All the theories, principles and dogmas established for intracellular calcification can be applied to the land gastropod calcium cells (Simkiss, 1976, 1980; Wilbur,

1976, 1980). Enzymes such as ATPases, alkaline phosphatases and carbonic anhydrase are involved in this mechanism. Carbonic anhydrase and alkaline phosphatases have been observed in the land pulmonate calcium cells (Fournié and Chétail, 1982a).

Another kind of calcium movement is that occurring across epithelial selective barriers. The movement from the haemolymph into the extrapallial compartment through the mantle epithelium leads to extracellular calcification. Transporting and calcification-inducing enzymes participate in this mechanism. Inhibitors of carbonic anhydrase have been shown to retard shell regeneration in H. aspersa (Stolkowski, 1951) and H. pomatia (Abolins-Krogis, 1958). But the mantle epithelium is also the site of calcium relocation from the shell into the haemolymph and so a site of a mineralization process. The movement of calcium from the haemolymph into the uterus leads to extracellular calcification only when calcium carbonate crystals are precipitated in the egg shell. In the species which lay eggs with uncalcified egg shells the uterus has only the function of supplying the jelly with calcium. A flux of calcium occurs intermittently through the albumen gland epithelium. In this case, calcium is probably taken up at the basal part of the cell, and bound to organic compounds which are packed into secretory vesicles.

During this review, it has appeared that most of the calcium movements could be considered as internal on-off movements, according to the name given by Wilbur (1980) to such intermittent processes. The intermittency of calcium accumulation and release in the calcium cells, and the reversibility of calcium movements across the mantle epithelium, the periodicity of the flux of calcium through the reproductive tract epithelia, suggest that these movements occur under precise control mechanisms. Concerning the control of shell formation and regeneration, the studies were done later in land gastropods than in Basommatophora (Kunigelis and Saleuddin, 1983). Some data have recently emerged concerning the control of the calcium mobilization for the egg production. In H. aspersa, blood calcium rises suddenly with the onset of egg laying (Tompa and Wilbur, 1977a). In Deroceras, the calcium increase in the reproductive tract is synchronized with a decrease in the storage compartments before each laying. After the last laying, the calcium flux across the reproductive tract stops and is followed by a calcium overload in the storage compartments. All of the connective tissue is being invaded by numerous heavily loaded calcium cells. This overload, particularly important in the skin, is probably the cause of the death of the slugs after the last laying. In recent experiments (unpublished data), we observed in slugs gonadectomized before the laying period, that calcium levels in both storage compartments and the reproductive tract remained comparatively lower than in the control slugs. In gonad regenerating slugs these calcium levels are higher than in gonadectomized slugs. So, a precise correlation exists between calcium dynamics and reproductive physiology. The consequences of deprivation of the calcareous substratum observed in H. aspersa suggest a possible connection between calcium dynamics and the growth factors.

CONCLUSIONS

We attempted here to clarify a complex problem. We tried to give the most general features of the dynamics of calcium through the land gastropod body. At this time, many basic questions remain unanswered and this enormous problem is far from being exhausted. Nevertheless, we will present the following main ideas:

—The particular behaviour of land gastropods towards calcium has to be related to the particular conditions of the terrestrial life. Starvation and dormancy restrict the capacity of calcium uptake. Nevertheless the animals have to spend considerable amounts of calcium for their protection and their reproduction. The maintenance of a positive calcium balance is possible due to an important reservoir of easily mobilizable calcium in the form of calcium carbonate. In all species of land gastropods, this reservoir consists of calcium cells distributed in the whole connective tissue in areas which are particularly strategic points in the view of calcium dynamics; these cal-

cium cells are constantly able to accumulate or release calcium as long as calcium is locally available or required.

—Two main kinds of compartments have to be considered in the land gastropod body: a compartment of precipitated calcium (shell and calcium cells) and a compartment of calcium bound to organic products including the blood, the extrapallial fluid and the egg fluids. All the internal calcium movements and the movements across epithelia (sole skin, intestine, mantle and reproductive duct) only involve ionic calcium or calcium ions bound to organic compounds.

—In three systems where a calcification process is observed (body shell, connective calcium cells, egg shell), decalcification may always occur involving a translocation of ionic calcium towards another compartment. In this view, the whole land gastropod organism can be regarded as a complex mineralization system.

—All the internal calcium movements between the compartments of the land gastropod body can be considered either as internal on-off systems or as reversible systems, and the epithelia or cell membranes where these movements take place are certainly targets for complex and precise control processes.

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