

## AMPHIBIAN CALCIUM METABOLISM

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### Summary

Calcium is present in amphibian blood at a concentration similar to that in other vertebrates, about  $1\text{--}2\text{mmol l}^{-1}$ . The fraction of free calcium in amphibians is lower than that in other tetrapod vertebrates because about 50% of the plasma  $\text{Ca}^{2+}$  is bound to plasma proteins and perhaps other molecules. Plasma  $[\text{Ca}^{2+}]$  varies seasonally, increasing in spring and summer and decreasing in winter. Changes in plasma  $[\text{Ca}^{2+}]$  also occur during larval development, as the concentration of this ion increases in larval forms as they approach metamorphosis. Calcium is exchanged at a variety of sites in animals. There is evidence for  $\text{Ca}^{2+}$  uptake across the skin and gills of larval anurans. It is also transported into the blood from the small intestine (especially the duodenum) and reabsorbed in renal tubules from the glomerular filtrate. The possibility of  $\text{Ca}^{2+}$  absorption from urine stored in the urinary bladder has not been confirmed, however. Calcium is stored in bone and in specialized endolymphatic sacs. This  $\text{Ca}^{2+}$  can be mobilized when the need arises. There are a number of endocrine and other humoral factors that appear to be involved in amphibian calcium metabolism. These include parathyroid hormone, calcitonin, vitamin D and prolactin.

### Introduction

The study of amphibian calcium metabolism has lagged behind the study of other major ions such as  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  for several reasons. The interest created in  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  by the early work of Krogh (1937) and Ussing (1949) stimulated a large number of workers to study the transport of these ions in frog skin. Divalent ions are more difficult to work with than monovalent ions for a variety of reasons. The ease and inexpensiveness of flame photometry compared with atomic absorption spectroscopy probably caused a bias towards monovalent ions. The binding of divalent ions such as  $\text{Ca}^{2+}$  to plasma protein (Walser, 1973) complicates renal transport studies. Our knowledge of amphibian calcium transport is also poor compared with what is known about fish  $\text{Ca}^{2+}$  transport. This is probably because of the economic importance of fish and the generally larger pool of support for studies of fish biology. Nevertheless, this indifference to amphibian calcium metabolism is unwarranted. Amphibians, the first animals to make the water–air transition, occupy a pivotal place in evolution. The changes in  $\text{Ca}^{2+}$  metabolism that occurred when vertebrates began to abandon the aquatic

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habitat and a ready supply of dissolved ions at the interface between their epithelial exchange surfaces and the water in favour of a terrestrial existence may provide clues to a greater understanding of  $\text{Ca}^{2+}$  regulation in tetrapods in general and for human medicine in particular. Calcium ions, although at a low concentration in bodily fluid compared with  $\text{Na}^+$ ,  $\text{Cl}^-$  or even  $\text{K}^+$ , are critically important for a wide variety of physiological and biochemical functions, such as membrane stabilization, muscle contraction, nerve transmission, cell secretion and enzyme regulation.

### The physical state of calcium in the blood

Total  $\text{Ca}^{2+}$  concentration in amphibian blood (Table 1) is very similar to that of other vertebrate groups. Total  $[\text{Ca}^{2+}]$  does not begin to describe  $\text{Ca}^{2+}$  availability for biochemical function or even for transport of  $\text{Ca}^{2+}$  into cells where it can be used. Because of the physical nature of proteins and divalent ions, a significant portion of the calcium that exists in biological fluids is bound and therefore unavailable for ionic interactions. This binding of  $\text{Ca}^{2+}$  to plasma protein is greatest, among tetrapods, in the Amphibia (Table 2), where only half of the plasma calcium is ionized.

### Seasonal and developmental changes in calcium metabolism

It would be a mistake to conclude that, once protein binding is accounted for, the description of amphibian blood calcium is complete. Plasma  $[\text{Ca}^{2+}]$  fluctuates seasonally and during development. There are seasonal alterations in the structure and apparent function of the parathyroid glands (Dougherty, 1973). In the winter, the parathyroid cells shrink and the spaces between them enlarge. Histological evidence of secretory activity

Table 1. Plasma calcium ion concentrations in amphibians

	Plasma $[\text{Ca}^{2+}]$ ( $\text{mmol l}^{-1}$ )	Reference
Anurans		
<i>Rana pipiens</i>	1.47	Sasayama and Clark (1984)
<i>Rana catesbeiana</i>		
Larvae	1.81	Oguro <i>et al.</i> (1975)
Adults	2.55	Oguro <i>et al.</i> (1975)
<i>Rana temporaria</i>	2.50	Simkiss (1968)
<i>Bufo marinus</i>	3.30	Tufts and Toews (1985)
<i>Xenopus laevis</i>	1.39	McWhinnie and Scopelliti (1978)
Urodeles		
<i>Desmognathus monticola</i>	1.60	Wittle (1983)
<i>Notophthalmus viridescens</i>	2.13	Wittle and Dent (1979)
<i>Cynops pyrrhogaster</i>	2.45	Uchiyama (1980)
<i>Tylotriton andersoni</i>	1.93	Oguro and Sasayama (1978)
<i>Ambystoma tigrinum</i>		
Larvae	0.60	Stiffler <i>et al.</i> (1987)
Adults	1.00	Stiffler (1991)
<i>Ambystoma mexicanum</i>	1.62	Kingsbury and Fenwick (1989)

Table 2. Protein binding of calcium and free ionized calcium

Group	Percentage free Ca <sup>2+</sup>	Reference
Mammalia (dog)	84	Walser (1963)
Aves (quail)	58	Clark and Sasayama (1981)
Reptilia (snake)	61	Clark and Dantzler (1975)
Amphibia (frog)	50	Sasayama and Clark (1984)

associated with, for example, Golgi complexes, is also reduced in the winter. The parathyroid glands of *Notophthalmus viridescens* (Wittle and Dent, 1979) do not regress in the winter. Paralleling the histological changes in *R. pipiens* are annual cycles in plasma [Ca<sup>2+</sup>], with [Ca<sup>2+</sup>] falling in the winter and rising in the spring and summer in *Rana pipiens* (Fig. 1: Robertson, 1977).

During metamorphosis, changes in plasma [Ca<sup>2+</sup>] also occur. In *Rana catesbeiana*, plasma Ca<sup>2+</sup> concentration increases steadily from less than 8mgdl<sup>-1</sup> in tadpoles to near 12mgdl<sup>-1</sup> in adults (Fig. 2: Oguro *et al.* 1975). Larval *A. tigrinum* have about 40% lower plasma [Ca<sup>2+</sup>] than adults (Stiffler *et al.* 1987; Stiffler, 1991). Larval amphibians have cartilaginous skeletons that do not become ossified until metamorphosis. The increasing [Ca<sup>2+</sup>] may be supplying the osteoblasts with the minerals needed to ossify the skeleton. In anurans, a specialized collection of structures known as the paravertebral lime-sacs or endolymphatic sacs (see below) appear to supply Ca<sup>2+</sup> for this purpose at metamorphosis (Pilkington and Simkiss, 1966). Larval urodeles lack parathyroid glands; these structures appear at metamorphosis (Clark, 1983).

#### Sites of calcium exchange

Amphibians possess a large number of exchange sites for calcium. These calcium exchanges occur between body fluid compartments and between the environment and body fluids. Uptake of calcium from the environment can occur across the skin

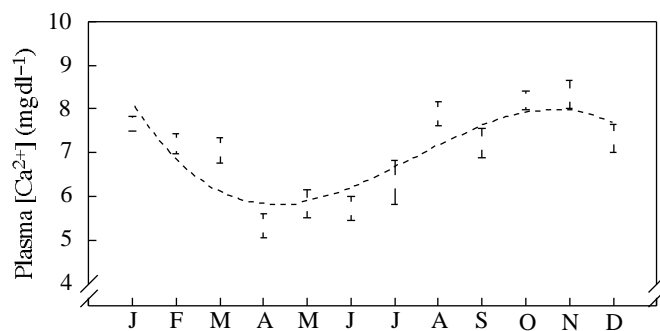


Fig. 1. Seasonal rhythm in plasma [Ca<sup>2+</sup>] in *Rana pipiens*. Data replotted from Robertson (1977). Values are mean  $\pm$  s.e.m.,  $N=15-30$  frogs at each point.

(Watlington *et al.* 1968; Baldwin and Bentley, 1981*a,b*; Kingsbury and Fenwick, 1989), across gills, at least in anuran tadpoles (Baldwin and Bentley, 1980), and at the surface of the small intestine (primarily duodenum; Robertson, 1975, 1976).

#### Skin

In isolated, short-circuited *Rana pipiens* skin with Ringer's solution bathing both sides, the influx of  $\text{Ca}^{2+}$  was consistently and significantly greater than the efflux, suggesting active transport (Watlington *et al.* 1968). These observations have not been replicated. A study by Baldwin and Bentley (1981*a*) found no significant difference between influx and efflux of  $\text{Ca}^{2+}$  in isolated *R. pipiens* skin. Seasonal differences were suggested as a possible explanation of this disparity because the latter study (Baldwin and Bentley, 1981*a*) was performed in winter; however, season was not specified in the former study (Watlington *et al.* 1968). Investigation of unidirectional  $\text{Ca}^{2+}$  fluxes across isolated *R. pipiens* cutaneous epithelial sheets also failed to demonstrate significant net fluxes (Zadunaisky and Lande, 1972). Baldwin and Bentley (1981*b*) also studied cutaneous exchanges of  $\text{Ca}^{2+}$  in two urodeles, *Ambystoma tigrinum* and *Necturus maculosus*. As with their studies on frog skin, there were no significant differences between influx and efflux in isolated short-circuited salamander skin, suggesting that active transport was not involved. Since measurements were made in both winter and summer, season did not appear to play a role. However,  $\text{Ca}^{2+}$  influxes in whole animals (larval *A. tigrinum*) were much greater than those in isolated skin, suggesting that something supporting  $\text{Ca}^{2+}$  transport in the whole animal is missing from isolated skin. Calcium uptake in *Ambystoma mexicanum* was reported to be  $0.079 \text{ mmol kg}^{-1} \text{ day}^{-1}$  in July and  $0.03 \text{ mmol kg}^{-1} \text{ day}^{-1}$  in August; however, effluxes were not given (Kingsbury and Fenwick, 1989). These conflicting results do not provide a clear indication of the possible role of amphibian skin in  $\text{Ca}^{2+}$  transport

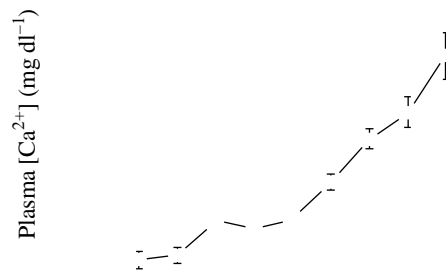


Fig. 2. Changes in plasma  $[\text{Ca}^{2+}]$  in *Rana catesbeiana* during metamorphosis. Data replotted from Oguro *et al.* (1975). Values are mean  $\pm$  S.E.M.  $N=9-61$  at each point.

(Table 3). The studies of isolated skin were performed with Ringer's solution bathing both sides of the skin. This non-physiological situation is known to obscure net  $\text{Cl}^-$  uptake in frog skin (Kirschner, 1983). Obviously, further study of cutaneous  $\text{Ca}^{2+}$  exchange will be necessary before it is certain whether  $\text{Ca}^{2+}$  is, or is not, transported in net quantities across amphibian skin.

### Gills

*Rana catesbeiana* tadpoles take up significant amounts of  $\text{Ca}^{2+}$  from the bathing medium ( $0.13\text{mmol g}^{-1}\text{ day}^{-1}$ ) and about 75% of this can be shown to cross gill epithelia (Baldwin and Bentley, 1980). The gills of *A. tigrinum* and *N. maculosus*, however, appear not to support significant  $\text{Ca}^{2+}$  uptake (Baldwin and Bentley, 1981b).

Table 3. Cutaneous  $\text{Ca}^{2+}$  fluxes

Species	Direction of flux	Size of flux ( $\text{nmolcm}^{-2}\text{ h}^{-1}$ )	Reference	
<i>In vitro</i>				
<i>Rana pipiens</i>	Influx	0.178	Watlington (1979)	
	Efflux	-0.077		
	Net flux	0.091		
<i>Rana pipiens</i>	November	Influx	Baldwin and Bentley (1981a)	
		Efflux		-0.50
	February	Influx		0.21
		Efflux		-0.59
	February	Influx		0.22
		Efflux		-0.34
<i>Necturus maculosus</i>	Influx	1.80	Baldwin and Bentley (1981b)	
	Efflux	-1.40		
<i>Ambystoma tigrinum</i>	Neotene	Influx	Baldwin and Bentley (1981a)	
		Efflux		0.80
		Adult		Influx
		( $\mu\text{molkg}^{-1}\text{ h}^{-1}$ )		
<i>In vivo</i>				
<i>Rana pipiens</i>	Influx	3.33	Baldwin and Bentley (1981a)	
	Efflux	6.67		
<i>Necturus maculosus</i>	Influx	4.58	Baldwin and Bentley (1981b)	
	Efflux	2.91		
<i>Ambystoma tigrinum</i>	Neotene	Influx	Baldwin and Bentley (1981b)	
		Efflux		4.17
	Adult	Efflux		Not detected
		Influx		2.08
		Efflux		13.75
<i>Ambystoma mexicanum</i>	July	Influx	Kingsbury and Fenwick (1989)	
		Efflux		3.28
August	Influx	1.27		

*Intestine*

The final site of uptake for calcium in amphibians is the small intestine, which absorbs dietary  $\text{Ca}^{2+}$ . In whole-gut preparations from *Rana pipiens*, the everted gut sac is able to concentrate  $\text{Ca}^{2+}$  2.7-fold in the serosal medium (Robertson, 1975). When the duodenum is examined in isolation from the jejunum and ileum, the concentration ratio of that segment is approximately 3.0 while the jejunum–ileum combination produces a ratio of about 2.0 (Robertson, 1975). Duodenal  $\text{Ca}^{2+}$  transport is maximal at night (Robertson, 1976).

There are several sites where loss of calcium to the environment could occur. In addition to diffusion of calcium across the skin or the gills, urinary loss of calcium ions is potentially great.

*Kidney*

Renal tubular transport of calcium reduces  $\text{Ca}^{2+}$  loss in the urine of *Rana pipiens* (Cortelyou, 1967; Sasayama and Clark, 1984) and *Notophthalmus viridescens* (Wittle and Dent, 1979). Urinary calcium concentration is approximately 4% of plasma calcium concentration in *Rana pipiens* (Cortelyou, 1967) and 3.9% in *Notophthalmus viridescens* (Wittle and Dent, 1979). This suggests that large amounts of  $\text{Ca}^{2+}$  are reabsorbed from the filtered plasma and, after correction for bound (unfilterable) calcium in the plasma, it has been shown that 60% of filtered  $\text{Ca}^{2+}$  is reabsorbed by renal tubules in *R. pipiens* (Sasayama and Clark, 1984).

*Urinary bladder*

The amphibian urinary bladder is another possible site for calcium transport. This epithelium is capable of active sodium transport (Bentley, 1971) and the potential exists for active calcium transport from the urine into the blood. In a study of toad bladder (*Bufo marinus*) permeability to  $\text{Ca}^{2+}$ , Walser (1970) found that resting calcium permeability is vanishingly low ( $4 \times 10^{-9} \text{ cm s}^{-1}$ ) but that a number of factors, including the presence of  $\text{Cl}^-$  in the bathing medium, increased calcium permeability. It was suggested that calcium crosses the toad bladder as the ion  $\text{CaCl}^+$ . A second study of  $\text{Ca}^{2+}$  flux ratios across toad bladder failed to show conclusive evidence of active  $\text{Ca}^{2+}$  transport; several individual bladders showed influxes to be significantly greater than effluxes, however (Walser, 1971).

In addition to exchanges of calcium between animals and their environments, there are exchanges of this ion between compartments within the body. Calcium exchanges between bone and extracellular fluid play an important role in amphibian calcium metabolism (Yoshida and Talmage, 1962). In addition to calcium storage depots in bone, amphibians (primarily anurans) have a novel  $\text{Ca}^{2+}$  storage site. This is the endolymphatic sac, which arises from the junction of the sacculus and utriculus of the inner ear (Pilkington and Simkiss, 1966). This structure enlarges in anurans till it surrounds the brain in the cranial cavity and extends down the spinal canal, protruding as vertebral lime-sacs between the vertebrae. The name 'lime-sacs' derives from the fact that this structure is filled with calcium carbonate crystals that can be mobilized as  $\text{Ca}^{2+}$  as the need arises (Simkiss, 1968; Pilkington and Simkiss, 1966).

### Hormonal control of calcium exchanges

#### Parathyroid hormone

Parathyroid glands are found in all anurans but are lacking in larval urodeles (Baldwin, 1918). In those amphibians that possess parathyroid glands, the parathyroid hormone (PTH) is usually hypercalcaemic. Parathyroidectomy (Fig. 3 and see Fig. 5) causes significant decreases in plasma  $[Ca^{2+}]$  in *R. pipiens* (Cortelyou *et al.* 1960), larval *R. catesbeiana* (Sasayama and Oguro, 1975), *Notophthalmus viridescens* (Wittle and Dent, 1979), *Tylotriton andersoni* (Oguro and Sasayama, 1978), *Desmognathus monticola* (Wittle, 1983) and *Cynops pyrrhogaster* (Oguro, 1969). Each of the calcium exchange sites discussed above is a potential target for PTH.

Calcium uptake by the skin of *R. pipiens* showed a transient increase shortly after treatment with parathyroid hormone (Watlington *et al.* 1968). This response was not seen in a later study of this species (Baldwin and Bentley, 1981a). Similarly,  $Ca^{2+}$  uptake by the gills of *R. catesbeiana* tadpoles was not stimulated by PTH (Baldwin and Bentley, 1980).

The renal responses to parathyroidectomy and administration of PTH are not clear. Injection of the hormone increases, rather than decreases, urinary  $[Ca^{2+}]$  (Cortelyou, 1967). It was suggested that PTH mobilizes  $Ca^{2+}$  from bone and that the resulting hypercalcaemia increases the filtered  $Ca^{2+}$  load to an extent greater than can be accommodated by the tubular transport mechanism for the ion, even under the stimulus of PTH. Parathyroidectomy reduces urinary  $[Ca^{2+}]$  (Wittle and Dent, 1979) and increases tubular  $Ca^{2+}$  transport in the short term (Fig. 4: Sasayama and Clark, 1984), which would

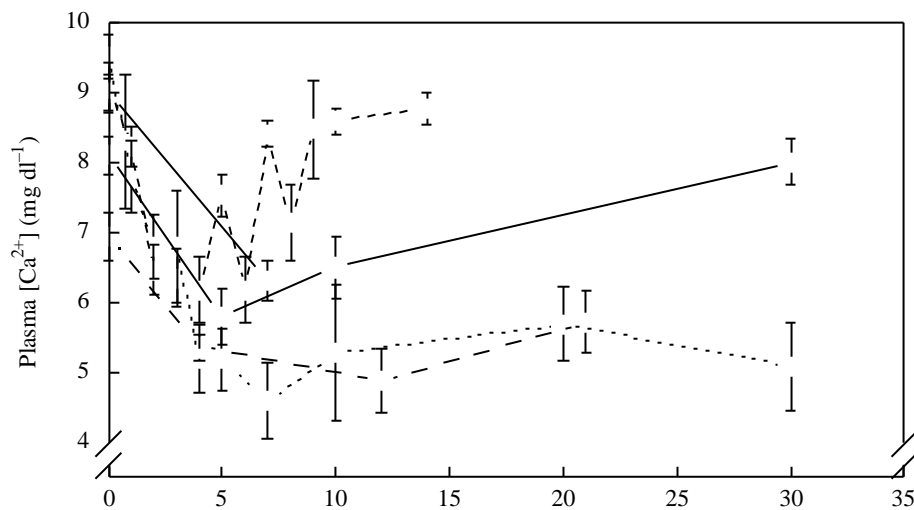


Fig. 3. Changes in plasma  $[Ca^{2+}]$  following parathyroidectomy in *Rana catesbeiana* ( $\Delta$ , data plotted from Sasayama and Oguro, 1975), *Notophthalmus viridescens* ( $\circ$ , data replotted from Wittle and Dent, 1979), *Tylotriton andersoni* ( $\bullet$ , data plotted from Oguro and Sasayama, 1978), *Desmognathus monticola* ( $\blacksquare$ , data plotted from Wittle, 1983) and *Cynops pyrrhogaster* ( $\blacktriangledown$ , data plotted from Oguro, 1969). Values are mean  $\pm$  S.E.M.

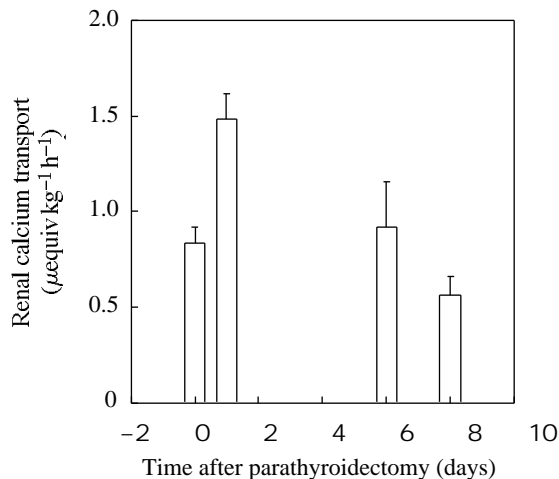


Fig. 4. Renal tubular  $\text{Ca}^{2+}$  reabsorption in *Rana pipiens* ( $\mu\text{equiv kg}^{-1} \text{h}^{-1}$ ) before and after parathyroidectomy. Data replotted from Sasayama and Clark (1984). Values are mean + S.E.M.  $N=4-13$  in each group. An asterisk indicates a value significantly different from the control value ( $P < 0.05$ ).

imply an inhibition of tubular  $\text{Ca}^{2+}$  transport by PTH. In the latter study, there was no significant change in the calcium clearance ratio, indicating that fractional  $\text{Ca}^{2+}$  reabsorption did not change and that the increased rate of  $\text{Ca}^{2+}$  transport might be explained by an increased glomerular filtration rate, which did occur. The paradoxical dilution of urine calcium concentration following parathyroidectomy in *N. viridescens* (Wittle and Dent, 1979) may be resolved by the possibility that parathyroidectomy leads to a transient hypercalcuria, which becomes reversed as other regulatory systems come on line to compensate for the loss of PTH. In support of this, there was an increase in urinary  $[\text{Ca}^{2+}]$  following parathyroidectomy in *R. pipiens* that reversed after several days (Fig. 5: Cortelyou *et al.* 1960). The hypocalcuria in *N. viridescens* was apparent 1 day after parathyroidectomy whereas the hypercalcuria in *R. pipiens* began on day two, suggesting that there may be species differences in the time courses of the possible compensatory responses. It is difficult to unravel these inconsistencies and paradoxes from whole-animal data; studies of responses of isolated tubules to PTH may be required to clarify the role of this hormone in renal tubular  $\text{Ca}^{2+}$  transport.

The role of parathyroid hormone in intestinal  $\text{Ca}^{2+}$  transport is similarly obscure. Five days after parathyroidectomy, there was no significant change in duodenal or jejunal-ileal  $\text{Ca}^{2+}$  transport in isolated preparations of *R. pipiens* intestine (Robertson, 1975).

The response of bone (and possibly other calcium deposits) to parathyroid hormone is much clearer (Yoshida and Talmadge, 1962). The approach of these workers was to perfuse (lavage) the body cavity with  $\text{Ca}^{2+}$ -free Ringer's solution to stimulate parathyroid hormone release and then to study the responses of bone to this treatment in intact and parathyroidectomized frogs (*Rana catesbeiana*). Microscopic examination of bone cells showed a large increase in osteoclast frequency after 10h of  $\text{Ca}^{2+}$ -free lavage that was



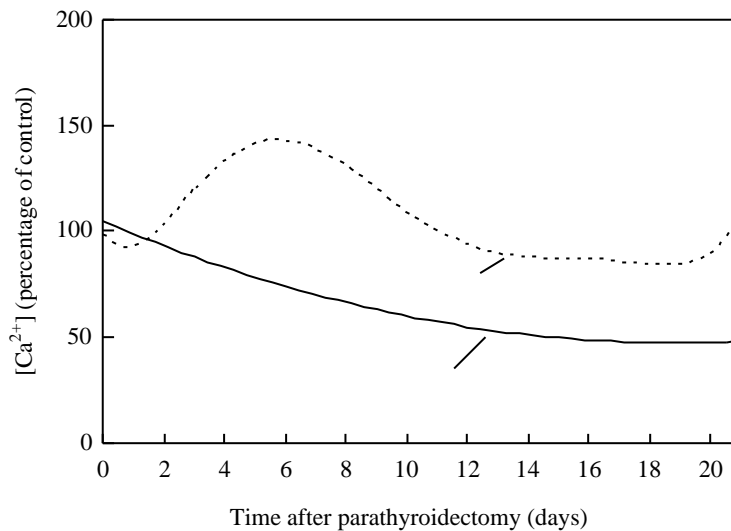


Fig. 5. Plasma and urine  $[Ca^{2+}]$  following parathyroidectomy in *Rana pipiens*. Data replotted from Cortelyou *et al.* (1960).

abolished by parathyroidectomy (Fig. 6). Since osteoclasts are known to be the bone cell type that mobilizes  $Ca^{2+}$ , these data suggest that parathyroid hormone stimulates the development of osteoclasts which, in turn, dissolve bone to liberate  $Ca^{2+}$ .  $Ca^{2+}$ -free lavage also increased the liberation of  $^{45}Ca^{2+}$  from calcareous deposits in this study and the response was abolished by parathyroidectomy (Fig. 7). Parathyroid extract causes the appearance of osteoclasts in bone of *N. viridensens*; these cells could not be located in control animals (Wittle and Dent, 1979).

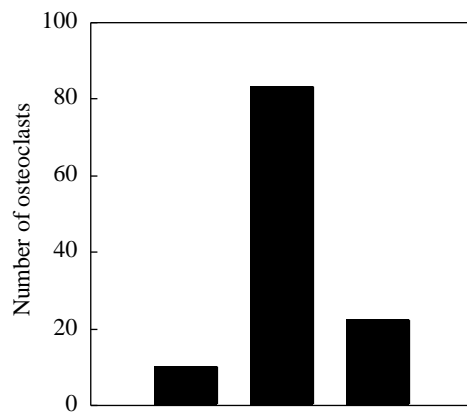


Fig. 6. The response of *Rana catesbeiana* bone cells (osteoclasts) to peritoneal lavage with  $Ca^{2+}$ -free solutions. The number of osteoclasts (number/microscopic field  $\times 100$ ) increased during lavage in control animals; it did not in parathyroidectomized (PTX) animals. Data plotted from Yoshida and Talmage (1962).

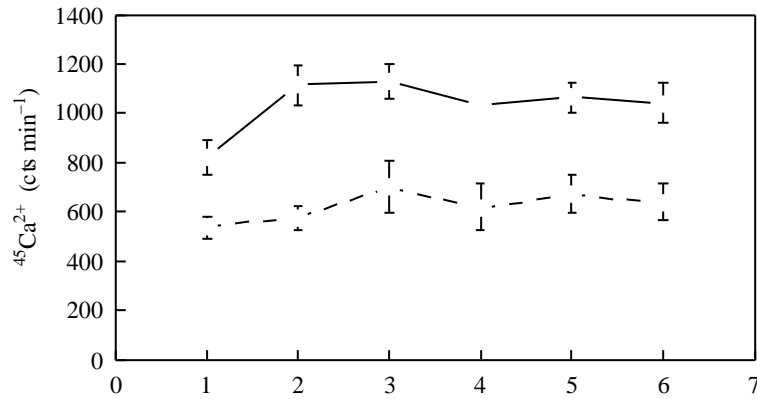


Fig. 7. Effects of irrigation of the peritoneal cavity (lavage) with  $\text{Ca}^{2+}$ -free solutions on mobilization of previously loaded  $^{45}\text{Ca}^{2+}$  from bone. In control animals (*Rana catesbeiana*), there appears to be a faster rise in radioactivity in the blood, at least initially, than in parathyroidectomized (PTX) animals. Data plotted from Yoshida and Talmage (1962). Values are mean  $\pm$  S.E.M.  $N=14$  (controls); 8 (PTX).

### Calcitonin

In amphibians, calcitonin is produced by the ultimobranchial glands, which are the evolutionary anlage of the C-cells of the mammalian thyroid. Calcitonin or ultimobranchial gland extracts cause decreases in plasma  $[\text{Ca}^{2+}]$  in *Rana pipiens* (McWhinnie and Scopelliti, 1978), *Cynops pyrrhogaster* (Uchiyama, 1980), *Bufo boreas* and *B. marinus* (Boschwitz and Bern, 1971) and *Rana catesbeiana* tadpoles (Sasayama, 1978), but not *Xenopus laevis* (McWhinnie and Scopelliti, 1978) or *Ambystoma mexicanum* (Kingsbury and Fenwick, 1989). Ultimobranchialectomy (UBX; removal of the ultimobranchial glands) results in significant increases of the plasma  $[\text{Ca}^{2+}]$  in *R. pipiens* (Robertson, 1975).

Furthermore, plasma calcitonin titres increase when *R. pipiens* are adapted to high- $\text{Ca}^{2+}$  environments (Robertson, 1987). These results, taken together, suggest that calcitonin is a hypocalcaemic hormone, as it is in other vertebrate groups. Potential target organs for calcitonin are, of course, the same as those discussed above.

The skin, as a calcium uptake organ, is one potential site of calcitonin action, but experiments have given varying results. Calcium influx was unaffected by calcitonin in a study of *R. pipiens* skin (Baldwin and Bentley, 1981a). Experiments on intact *A. mexicanum* showed significant inhibition of  $\text{Ca}^{2+}$  influx (Kingsbury and Fenwick, 1989). Calcitonin was effective in decreasing branchial  $\text{Ca}^{2+}$  uptake by *R. catesbeiana* tadpoles (Baldwin and Bentley, 1980).

Urinary excretion of  $\text{Ca}^{2+}$  is also responsive to calcitonin. Ultimobranchialectomy increases renal calcium loss (Robertson, 1975), indicating that calcitonin stimulates renal tubular  $\text{Ca}^{2+}$  transport. This would be counter to all expectations, however, and it is possible that the UBX-induced hypercalcuria is an indirect effect of the increased plasma

[Ca<sup>2+</sup>] under these conditions and of the consequent increase in the filtered load of Ca<sup>2+</sup> in the nephrons.

Intestinal Ca<sup>2+</sup> transport in *R. pipiens* is unaffected by UBX (Robertson, 1975) in vitamin-D-deficient preparations and in preparations containing vitamin D<sub>3</sub>. Intestinal segments from ultimobranchialectomized frogs did increase Ca<sup>2+</sup> absorption when dihydrotachysterol, a vitamin D analogue, was present, however (Robertson, 1975).

#### Vitamin D

Vitamin D, or cholecalciferol, is known to be intimately involved in calcium metabolism in the vertebrates. *Rana pipiens* exhibits a small, but significant, increase in plasma [Ca<sup>2+</sup>] when treated with 2.5mg of vitamin D (Fig. 8: Robertson, 1975). Again several potential sites of Ca<sup>2+</sup> exchange have been investigated as possible targets for vitamin D. The intestine of *Rana pipiens* clearly responds to vitamin D (Fig. 8: Robertson, 1975) as does the kidney (Fig. 9: Robertson, 1975). Baldwin and Bentley (1981a) could find no significant effect of vitamin D in *Rana pipiens* cutaneous Ca<sup>2+</sup> exchange; however, they did find a stimulatory effect of this vitamin on *Rana catesbeiana* gill Ca<sup>2+</sup> uptake (Baldwin and Bentley, 1980).

#### Prolactin

The anterior pituitary hormone prolactin has a hypercalcaemic effect in teleost fish (Pang *et al.* 1971). This peptide has been tested for calcium regulatory activity in a few species of amphibians. An early study of prolactin activity in Ca<sup>2+</sup> regulation was unable to find a statistically significant response in *Bufo boreas* and *B. marinus* (Boschwitz and Bern, 1971). Later studies in *Rana catesbeiana* tadpoles (Sasayama and Oguro, 1982) and adults (Baksi *et al.* 1978) revealed a hypercalcaemic response to this peptide.

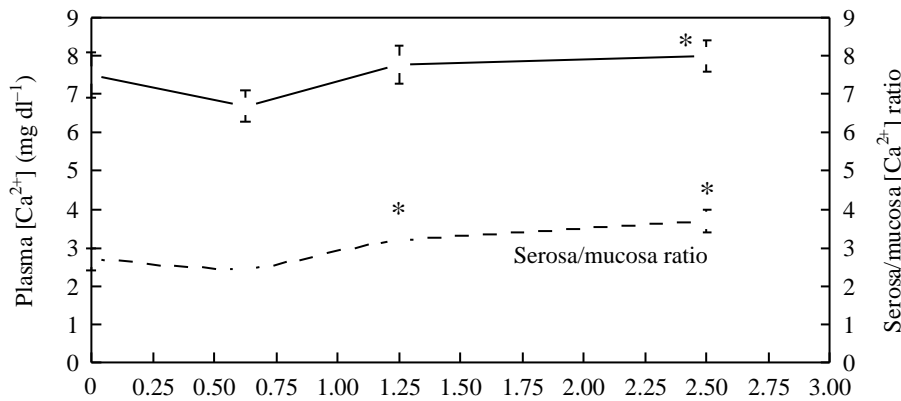


Fig. 8. Responses to vitamin D of *Rana pipiens* plasma [Ca<sup>2+</sup>] and serosal/mucosal [Ca<sup>2+</sup>] ratios in intestinal sac preparations. Data plotted from Robertson (1975). Values are mean  $\pm$  S.E.M.,  $N=6-10$  frogs in each group. Asterisks denote a significant difference from the control value ( $P<0.05$ ).

Hypophysectomy causes a hypocalcaemia in *Necturus maculosus* that is reversed by prolactin (Fig. 10: Pang, 1981). It may be that aquatic amphibians rely more on prolactin than on parathyroid hormone. Indeed, larval urodeles, which are aquatic, lack parathyroid glands (Clark, 1983).

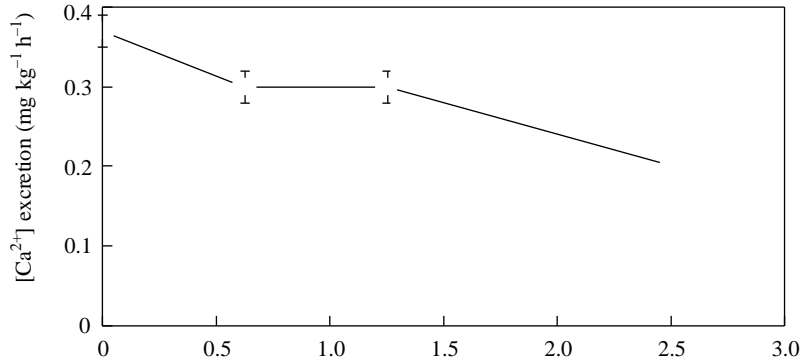


Fig. 9. Responses of urinary Ca<sup>2+</sup> excretion to vitamin D in *Rana pipiens*. Data plotted from Robertson (1975). Values are mean  $\pm$  S.E.M.,  $N=6-10$  at each point. Asterisks denote a significantly different value from the control value ( $P<0.05$ ).

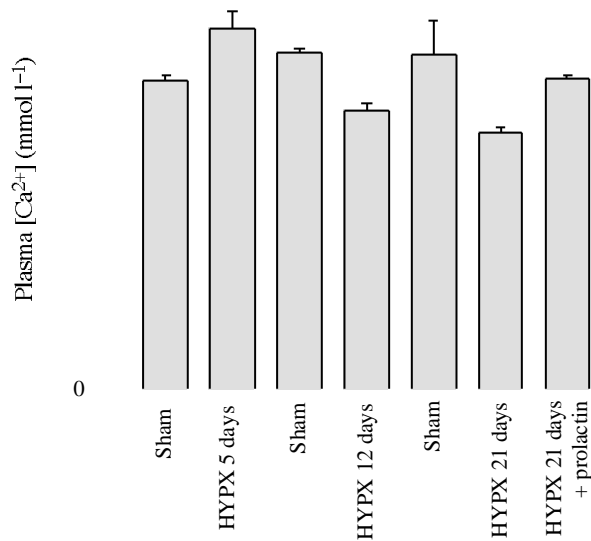


Fig. 10. Effects of hypophysectomy (HYPX) and replacement with prolactin on plasma [Ca<sup>2+</sup>] in *Necturus maculosus*. Data plotted from Pang (1981). Values are mean  $\pm$  S.E.M.,  $N=6-11$  at each point. Asterisks denote values significantly different from the sham-operated value ( $P<0.05$ ).

### Oestrogen

Oestrogen is known to affect calcium metabolism in mammals so it is interesting to know whether similar effects occur in amphibians. Baksi *et al.* (1978), working with *Ranapipiens*, demonstrated oestradiol-induced increases in plasma  $[Ca^{2+}]$  and  $1,25(OH)_2$  vitamin D<sub>3</sub>; oestradiol was given at  $5mgday^{-1}$  for 6 days.

### Interactions between calcium and acid–base balance

Amphibians, especially anurans, have significant stores of calcium phosphates and calcium carbonates in their bones and endolymphatic sacs. These salts, especially the carbonates, are potentially effective buffers during times of acidotic stress. Simkiss (1968) demonstrated that urinary  $Ca^{2+}$  excretion increases in *Rana temporaria* during respiratory acidosis, although there was no significant change in plasma  $[Ca^{2+}]$ . Tufts and Toews (1985), working with *Bufo marinus*, reported increased plasma and urinary  $[Ca^{2+}]$  during respiratory acidosis. Robertson (1972) showed that parathyroidectomized *Rana pipiens* have reduced acid–base compensatory capability during respiratory acidosis, suggesting that parathyroid hormone is involved. Parathyroid hormone stimulates  $H^+$  secretion in toad urinary bladder (Frazier, 1976). All of this has prompted Dacke (1979) to suggest that the primitive function of the parathyroid might be in acid–base balance.

### Conclusions

After a period of considerable activity through the 1970s, study of amphibian calcium metabolism has diminished significantly. From the earlier studies, we have a reasonable idea about which organs and tissues are involved in calcium exchange. We know little, however, of the magnitudes of exchange. Many of the elementary forms of analysis that have been applied to the study of other ion-transporting systems, such as Michaelis–Menten kinetic analysis, have not been applied to calcium transport in any amphibian tissue. A similar situation exists with respect to our knowledge of the stoichiometry of calcium exchanges in amphibian tissues. Although a great deal of information relating to mechanisms of calcium exchange at the cell membrane level in fish tissues has accumulated (see Flick and Verbost, 1993), there has been no such progress in amphibian physiology. Finally, our knowledge of the role played by endocrine regulatory systems in  $Ca^{2+}$  metabolism is fragmentary at best. While considerable effort has been expended to understand the involvement of a number of humoral agents, the results have often been inconclusive and inconsistent.

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